Diabetologia (2018) 61 (Suppl 1):S1–S620
https://doi.org/10.1007/s00125-018-4693-0

54th EASD Annual Meeting of the European Association for the Study of Diabetes

Berlin, Germany, 1 – 5 October 2018

Abstracts

Index of Oral Presentations

Index of Poster Sessions

PS 047 SGLT2 inhibitors around the world: evidence from clinical trials and registries
PS 048 Glycaemic and metabolic effects of SGLT2 inhibitors
PS 049 Novel aspects of SGLT2 inhibitors
PS 050 SGLT2 inhibitors: What do the CVOTs tell us?
PS 051 Tackling glucose and fat with novel agents
PS 052 Quality of nutrients and meals: How important are they?
PS 053 Dietary supplements: Which is best?
PS 054 All what you need to know for a healthy diet
PS 055 New clues on metformin, sulfonylureas and insulin
PS 056 Metabolic effects of novel, dual and triple incretin agonists
PS 057 Lipids and fatty liver: What GLP1 receptor agonists can do
PS 058 More on GLP1 receptor agonists and diabetes complications
PS 059 On the efficacy of GLP1 receptor agonists
PS 060 GLP1 receptor agonists: How good are they in real practice?
PS 061 GLP1 receptor agonists: Do age and ethnicity matter?
PS 062 Incretin-based therapies: adherence and tolerability
PS 063 DPP4 inhibitors: new regiments and new comparisons
PS 064 Incretin-based therapies: new mechanistic insights
PS 065 Beta cell function and response to incretin-based therapies
PS 066 Multiple facets of continuous glucose monitoring
PS 067 Artificial insulin delivery and insulin pump therapy
PS 068 Faster acting insulins: state of the art
PS 069 Approaches to insulin titration
PS 070 Clinical outcomes in insulin treated patients
PS 071 Combination therapy with ultra-long-acting insulin
PS 072 Clinical and patient reported outcomes
PS 073 Diabetes control around the world
PS 074 Cost effectiveness in diabetes therapies
PS 075 Psychosocial aspects in diabetes
PS 076 Education and patient/provider perceptions
PS 077 Therapeutic adherence and satisfaction
PS 078 Hypoglycaemia rates with basal insulin
PS 079 Clinical pathophysiology of insulin and hypoglycaemia
PS 080 Causes and consequences of hypoglycaemia
PS 081 Sweet mothers - big babies
PS 082 From pregnancy to breastfeeding
PS 083 Diabetic pregnancy: experimental work
PS 084 Neuropathy: prevalence and clinical impact
PS 085 Neuropathy: markers and remedies
PS 086 Autonomic neuropathy
PS 087 Foot ulcers: morbidity and mortality
PS 088 Diabetic foot ulcers: How to prevent and how to treat
PS 089 The impact of retinopathy
PS 090 Retinopathy: there is more than hits the eye
PS 091 Biomarkers of nephropathy
PS 092 Diabetic nephropathy: Predictions are hard to make
PS 093 Nephropathy: from markers to real life
PS 094 Animal studies on nephropathy
PS 095 Diabetic nephropathy: on the bench
PS 096 Of drugs and kidneys
PS 097 Unexpected comorbidities
PS 098 Factors affecting cardiovascular outcome
PS 099 Don't forget!
PS 100 Brain functionalities
PS 101 Understanding vascular complications
PS 102 The pump and the barrier
PS 103 Diabetes from childhood to adults
PS 104 Complications and treatment
PS 105 Bones and muscles
PS 106 From metabolism to vascular function
PS 107 Big vessels
PS 108 Adverse cardiovascular disease events
PS 109 Dyslipidaemia and diabetes
PS 110 Treating cardiovascular disease in diabetes
PS 111 Metabolism, inflammation in metabolic liver disease
PS 112 Unconventional aspects of cardiovascular disease in diabetes
PS 113 Epidemiology of cardiovascular disease and diabetes
PS 114 Vascular complications
PS 115 Novel diagnostic tool for NAFLD
PS 116 Clinical aspects of NAFLD
PS 117 Treating NAFLD
PS 118 Cancer and diabetes

OP 01 Inflammatory events in type 2 diabetes

1

Metabolic reprogramming of CD8$^+$ T cells regulates systemic glucose metabolism

H.-S. Yi[1], Y. Lee[1], B. Ku[2];
[1]Chungnam National University Hospital, Daejeon, [2]Chungnam National University School of Medicine, Daejeon, Republic of Korea.

Background and aims: Chronic inflammation induced by proinflammatory cytokines is closely related to metabolic diseases. However, the functional characteristics of T cells in the development of diabetes are not yet fully understood.
Materials and methods: We studied the patients visiting a hospital for routine health check-ups, who were divided into two groups: normal controls, and people with prediabetes. Gene expression profiling of peripheral blood mononuclear cells (PBMCs) from normal controls and drug-naïve patients with type 2 diabetes was undertaken using microarray analysis. We also studied the immunological characteristics and metabolic signatures of senescent T cells in the normal subject and patients with prediabetes. Moreover, liver tissues from 10 subjects who underwent hepatectomy at Chungnam National University Hospital due to hepatocellular carcinoma or metastatic liver cancer were used to isolate hepatic resident T cells, which were investigated using FACS analysis. We also investigated senescent T cells in the liver of normal or mice with metabolic deterioration caused by aging or diet-induced obesity.
Results: Patients with diabetes or prediabetes exhibit a proinflammatory response. Gene annotation enrichment analysis showed that most of the upregulated genes in the diabetes group are implicated in T cell activation, phagocytosis, and the inflammatory response. Proinflammatory cytokines and cytotoxic molecules are also highly expressed in senescent ($CD8^+CD28^-CD57^+$) T cells from patients with prediabetes. Patients with prediabetes have higher concentrations of reactive oxygen species (ROS) in their senescent $CD8^+$ T cells. the larger quantity of ROS produced by metabolically reprogrammed senescent T cells contributes to impaired glucose homeostasis. Addtionally, senescent $CD8^+$ T cells impair hepatic insulin sensitivity via production of proinflammatory cytokines. On the other hand, murine senescent ($CD153^+CD279^+$) T cells with features of inflammation are also present in larger numbers in the livers of aged compared to young mice. Feeding a high fat diet increased senescent ($CD153^+CD279^+$) T cells in the liver of mice, leading to impairment of hepatic glucose homeostasis.
Conclusion: In summary, this study defines a new clinically relevant concept of a T cell senescence-mediated inflammatory response in the pathophysiology of type 2 diabetes. We also found that T cell senescence promotes systemic inflammation and alters hepatic glucose homeostasis. The rational modulation of $CD8^+$ T cell senescence would be a promising avenue for the treatment or prevention of type 2 diabetes.
Supported by: NRF
Disclosure: **H. Yi:** None.

2

Methylglyoxal induces microglial polarisation to pro-inflammatory state

J. Lin[1], J. Wang[1], A. Schlotterer[1], M. Kolibabka[1], N. Dietrich[1], T. Fleming[2], H. Hammes[1];
[1]Medical Faculty Mannheim, Mannheim, [2]Medical Faculty Heidelberg, Heidelberg, Germany.

Background and aims: Diabetic retinopathy induces vasoregression and neurodegeneration and finally leads to blindness. Microglial activation is involved in diabetic retinopathy. Recently our study showed that exogenous AGE precursor methylglyoxal activates and recruits microglia in normoglycemic retina. Different functional properties of microglia are dependent on the state of polarization. Classical activation of M1 phenotype is pro-inflammation and alternative activation of M2 phenotype is anti-inflammation. However, the role of methylglyoxal in microglial polarization remains unclear. Hence, this study aims to investigate the role of methylglyoxal in microglial polarization.
Materials and methods: 6 week old C57Bl6 mice were intravitreally injected MG (1 μl per eye), in a dose of 10mM/L. Two days later eyes were enucleated. Retinal RNA were isolated and PCRs were performed. Microglial quantification was using retinal whole mount immunofluorescence staining. Mouse microglial cells line (BV2 cell) were incubated with 1 μM, 10 μM and 100 μM of MG for 1, 2 and 3 days. We detected the effects of MG on microglial M1/M2 phenotype markers using gene expression and immunofluorescence, *in vivo* and *in vitro* experiments, respectively.
Results: Exogenous MG increased microglial recruitment and induced pro-inflammatory gene expression of TNF-α and Galectin3 with 2 fold, 5 fold for MCP-1, Iba1 2.9 fold, ICAM1 1.7 fold in mouse retina. In vitro, MG induced RAGE translocation into nucleus in microglial cells. Immunofluorescence signals of CD74 and Galectin3 were elevated after MG treatment. MG inhibited Arginase1 and Mrc1, and the former with dose dependent manner. Oppositely, MG promoted the gene expression of CD74 at 24 hour MG treatment; RAGE, IL-1ß and MCP-1 in BV2 cells at 72 h MG treatment; TNF-α, MIP-1α in 48 h.
Conclusion: MG-induced pro-inflammation is mediated by microglia. MG induces microglial to M1 polarization state and MG shows the pro-inflammatory role *in vivo* and *in vitro*.
Supported by: SFB
Disclosure: **J. Lin:** None.

3

Metformin alleviates the negative effects of the proinflammatory environment in brown adipocytes

N. Pescador[1,2], V. Francisco[1], C. Escalona[1], L. Ruiz[1], M. Valdecantos[1], A. Calle[2], M. Obregón[1], Á. Valverde[1,2];
[1]Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, [2]CIBERDEM, Madrid, Spain.

Background and aims: Obesity-induced chronic inflammation is critical in the pathogenesis of insulin resistance and type 2 diabetes mellitus (T2DM) due to recruitment as well as activation of macrophages in adipose tissues. The pathophysiology of inflammation-induced metabolic dysfunction has been extensively documented in white adipose tissue (WAT) depots. However, little is known of the relevance of inflammation in brown adipose tissue (BAT) during T2DM linked to obesity. We have examined the impact of M1 proinflammatory signaling pathways, on insulin signaling and thermogenic gene expression in brown adipocytes and the effect of metformin in these processes.
Materials and methods: RAW264.7 macrophages were stimulated with lipopolysaccharide (LPS) (100 ng/ml) for 6 h in the absence or presence of metformin (10mM). Conditioned medium (CM) was collected and added to brown adipocytes to explore the effects on inflammation and insulin signaling pathways as well as thermogenic gene expression. Eight week-old male C57 Bl/6 mice were fed HFD for 10 weeks. Metformin (500 mg/kg) was administered daily by oral gavage for 4 weeks. Then, mice were maintained at thermoneutrality (28°C) for one week and one group was exposure to cold (4°C) for 16 h.
Results: Treatment of brown adipocytes with LPS-CM significantly decreased insulin-mediated protein kinase B (Akt) phosphorylation ($p < 0.05$). Consequently, both insulin-induced glucose transporter 4 (GLUT4) translocation to the plasma membrane and glucose uptake were decreased ($p < 0.05$). Under these conditions, early activation of the N-terminal Janus activated kinase (JNK) ($p < 0.05$), signal transducer and activator of transcription 3 (STAT3) ($p < 0.05$) and p38 mitogen-activated protein kinase (p38 MAPK) ($p < 0.05$) was also observed together with degradation of the inhibitor of nuclear factor kappa B (IκBα). ($p < 0.05$) Furthermore, LPS-CM inhibited the effect of the beta3 agonist CL316243

in lipolysis ($p < 0.05$) and uncoupling protein (UCP)-1 expression ($p < 0.05$). All these effects were prevented by the treatment of brown adipocytes with LPS-CM supplemented with metformin. In vivo administration of metformin increased *Ucp1* mRNA levels upon cold exposure.
Conclusion: Our data demonstrate that the proinflammatory environment triggers signaling cascades that interfere with insulin and CL316243 effects in brown adipocytes. Furthermore, metformin improves insulin and beta adrenergic responses by targeting the inflammatory pathways. Our results suggest the importance of brown adipocytes as sensitive cells to proinflammatory mediators released by macrophages suggesting that inflammation in BAT might play a major role in insulin resistance associated to obesity.
Disclosure: **N. Pescador:** None.

4

Hyperglycaemia epigenetically primes pro-inflammatory RELA/p65 gene in cord blood-derived CD34$^+$stem cells

V. Vigorelli, J. Resta, S. Genovese, G. Pompilio, M. Vinci;
Centro Cardiologico Monzino - IRCCS, Milan, Italy.

Background and aims: Diabetes profoundly affects multiple signaling pathways and key transcription factors that account for the systemic pro-inflammatory state and increased propensity for the patients to develop atherosclerosis. In addition, as a consequence of hyperglycemia, diabetic patients have decreased number and dysfunctional circulating CD34$^+$ cells that under normal physiological conditions contribute to the maintenance of vascular homeostasis and regeneration. Emerging evidence suggests that epigenetic mechanisms, such as DNA methylation and histone modifications, are involved in the regulation of inflammatory genes in vascular cells under diabetic conditions. Since CD34$^+$ cells are also important precursors of immune cells, we hypothesized that uncontrolled hyperglycemia might epigenetically skew CD34$^+$ cells towards inflammatory cells. We sought to evaluate epigenetic priming of inflammatory response genes by high glucose conditions in CD34$^+$ stem cells.
Materials and methods: CD34$^+$ cells were purified from cord blood of healthy donors and expanded in normal-glucose (NG; with 30 mM mannitol for osmotic control) or high-glucose (HG; 30 mM) serum-free medium plus cytokines (FLT3, SCF, IL3 and IL6) for up to 20 days. The expression of RELA/p65, KAT2B/PCAF, IL6 and TNFα genes was assessed by qPCR. Western Blot was used to evaluate acetylation of NF*k*B p65 at lysine-310. H3K9me3 and RNA polymerase II recruitment to the RELA/p65 gene promoter were evaluated by ChIP-qPCR assay.
Results: Increasing evidence links glucose metabolism to changes in chromatin. We therefore examined H3K9me3 status of RELA gene promoter encoding for the p65 subunit of inflammatory transcription factor NFκB in CD34$^+$ cells after high glucose exposure. ChIP-qPCR data showed significant reduction of H3K9me3 levels in HG-CD34$^+$ cells ($n = 5$; FC 1 ± 0.16 SE *vs* 0.4 ± 0.1 SE; $p = 0.0327$). The lowering of this repressive epigenetic modification coincided with increased recruitment of RNA polymerase II to the RELA/p65 gene promoter and a significant up-regulation of p65 gene expression ($n = 8$; FC 1 ± 0.27 SE *vs* 1.41 ± 0.3 SE; $p = 0.0034$). Interestingly, KAT2B/PCAF gene, a histone acetyltransferase implicated in NF*k*B p65 acetylation and co-activation was also overexpressed ($n = 12$; FC 1 ± 0.14 SE *vs* 1.4 ± 0.19 SE; $p = 0.0225$). This post-translational modification is critical for nuclear stabilization and full transcriptional activity of NFκB, responsible for the expression of inflammatory genes. Hence, we assessed the acetylation of NFκB p65 at lysine-310 and the expression of NFκB p65 target genes such as TNFα and IL6. The analysis revealed an increased acetylation at lysine-310 in HG-CD34$^+$ cells and significant up-regulation of TNFα ($n = 9$; FC 1 ± 0.15 SE vs 1.38 ± 0.28 SE; $p = 0.0408$) and IL6 ($n = 10$; FC 1 ± 1.25 SE vs 1.86 ± 1.09 SE; $p = 0.05$) gene expression.
Conclusion: These results suggest that elevated glucose exposure might epigenetically prime CD34$^+$ cells for a pro-inflammatory response and/or skew CD34$^+$ cell differentiation into cell lineages with deleterious properties.
Supported by: Ricerca Finalizzata, Ministero della Salute [PE-2011-02348537]
Disclosure: **V. Vigorelli:** None.

5

Crosstalk between gut and pancreas in the context of metabolic syndrome: study in a rodent model of nutritional programming

A. Sanchez-Roncero[1], P. Martínez-Oca[1], F. Escrivá[1,2], E. Fernández-Millán[1,2], C. Álvarez[1,2];
[1]Bioq y Biol Mol., Fac. Farmacia, UCM, Madrid, [2]CIBER, ISCIII, Madrid, Spain.

Background and aims: Nutritional restriction during critical periods of development has been associated with the later appearance of metabolic pathologies during adulthood, such as obesity and type 2 diabetes. The relationship between low birth weight and the appearance of these pathologies is encompassed within the thrifty phenotype hypothesis. Experimental animal models, including ours, based on perinatal nutrient restriction have supported the role of altered endocrine pancreas development during early life and insulin sensitivity as key factors for the later occurrence of dysregulated glucose homeostasis. However, the underlying mechanisms leading to this condition remain unclear. In this regard, the intestinal microbiota is an emerging factor involved in the regulation of energy metabolism by modifying intestinal permeability and, consequently glucose, lipid and immune system homeostasis. Specifically we have studied the effect of nutritional restriction-rehabilitation on the morphology and integrity of the intestinal barrier and its role on pancreas inflammation.
Materials and methods: To this end, offspring of Wistar rat dams fed ad libitum (control [C]) or 65% food-restricted during pregnancy and suckling time (undernourished [U]) were weaned onto a high-fat (HF) diet (CHF and UHF, respectively) to drive catch-up growth. The experiments were carried out in female rats aged 18 and 25 weeks (prior to or after overt metabolic syndrome establishment, respectively). Morphometric studies of gut were performed by using haematoxylin and PAS staining. Localization of tight junction proteins (occludin, ZO-1) were visualized by immunofluorescence. Circulating proinflammatory cytokines and blood lipopolysaccharides (LPS) levels were measured by ELISA and the limulus test, respectively. The inflammatory phenotype of pancreatic islets was determined by CD68$^+$-cell staining and by the in vitro response of isolated islets to LPS.
Results: Nutritional restriction altered the number and size of epithelial cells from ileum, as well as mucin-producer Goblet cells compared with C animals. Our results also indicated that both, early food-restriction (U rats) and later rehabilitation with high-fat diet (UHF and CHF rats) increased intestinal permeability causing disorganization on the tight-junction proteins of the intestinal epithelium, mainly ZO-1 and occludin. This event was correlated with increased circulating levels of bacterial LPS in female U rats even prior to high-fat feeding, enhanced levels of *Tlr4* expression and macrophage infiltration into pancreatic β-cells. Incubation of isolated islets from 25-week old CHF and UHF rats with different doses of LPS (0.1, 1 and 5 μg/ml) for 24 hours significantly induced cytokine production in both populations but with less intensity in UHF islets suggesting development of LPS resistance.
Conclusion: These data suggest that intestinal barrier dysfunction induced by inappropriate maternal nutrition contributes to the early appearance of endotoxemia in the offspring before the development of overt metabolic syndrome, therefore emerging as a causal factor.
Supported by: MINECO BFU2016-77931-R, CIBERDEM, ISCIII, CAM B2017/BMD-3684. Spain
Disclosure: **A. Sanchez-Roncero:** None.

6

Glucagon induces the expression of inflammatory markers in the liver through NLRP3 inflammasome

C. Di Fatta, R. Spiga, E. Mancuso, A. Fuoco, C. Averta, G.C. Mannino, F. Andreozzi, G. Sesti;
Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Catanzaro, Italy.

Background and aims: Hyperglucagonemia promotes an inappropriately high rate of hepatic glucose production that is the predominant cause of fasting hyperglycemia and a major contributor to the etiopathogenesis of type 2 diabetes mellitus (T2DM). T2DM and insulin-resistance are strongly associated with an increase in the production of pro-inflammatory molecules, which, in turn, nurture the onset of several invalidating complications. In this contest, the inflammasome plays a key role in the inflammatory response. It has been implicated in a broad range of common inflammatory diseases, such as T2DM. The aim of our study was to assess whether hyperglucagonemia affects the expression of inflammatory markers in the liver and to analyze the molecular mechanism underlying the synthesis of inflammatory molecules in presence of glucagon.
Materials and methods: We analyzed a cohort of 132 Italian non-diabetic subjects enrolled in the CATAnzaro MEtabolic RIsk factors (CATAMERI) study, who underwent an oral glucose tolerance test. In addition, starved HepG2 cells were treated with different concentrations of glucagon (0.05, 0.5, 1, 10, 100 nM) for 24h, in order to mimic chronic exposure. To confirm glucagon pivotal role, a specific inhibitor of the glucagon receptor was employed. Changes in FGG, C3 and CRP mRNA expression were detected through real-time RT-PCR. We evaluated the cleavage of procaspase-1 to caspase-1 by Western Blot as a marker of NLRP3 inflammasome-mediated inflammation activation.
Results: In vivo, we observed a significant ($p < 0.01$) correlation between circulating levels of glucagon and inflammatory markers, such as complement component 3 (C3, $r = 0.227$), fibrinogen (FGG, $r = 0.193$) and C-reactive protein (hsPCR, $r = 0.230$), after correction for age and sex. In vitro, glucagon induced a significant ($p < 0.05$) increase in the gene expression of C3 (~4 fold), FGG (~2.5 fold) and CRP (~2 fold) in a dose-dependent manner with a maximum effect at the concentration of 10nM. Glucagon treatment induced a significant increase in the cleavage of procaspase-1 to caspase-1 (up to 2.5 fold) in a dose-dependent manner. Pre-treatment with a specific glucagon receptor inhibitor was able to block the pro-inflammatory stimulatory effects of glucagon at both transcriptional and protein levels.
Conclusion: These data suggest that hyperglucagonemia may induce an inflammatory state stimulating directly the synthesis of inflammatory molecules (C3, FGG and CRP) in the liver via the activation of NLRP3 inflammasome pathway.
Disclosure: **C. Di Fatta:** None.

OP 02 Foot prints

7

Novel plantar pressure-sensing smart insoles reduce foot ulcer incidence in 'high-risk' diabetic patients: a longitudinal study

C.A. Abbott[1], K.E. Chatwin[1], A.N.B. Hasan[1], S.M. Rajbhandari[2], C. Sange[2], N. Musa[2], P. Foden[3], K. Stocking[3], L. Vileikyte[4], F.L. Bowling[4], A.J.M. Boulton[4], N.D. Reeves[1];
[1]Manchester Metropolitan University, Manchester, [2]Lancashire Teaching Hospitals NHS Foundation Trust, Chorley, [3]Wythenshawe Hospital, Manchester, UK, [4]University of Manchester, Manchester, UK.

Background and aims: The lifetime risk of diabetic, neuropathic, plantar first foot ulceration is 25%, whereas ulcer recurrence rates for patients with ulcer history are 50–70% within 5 years. To date, effective ulcer prevention strategies remain elusive. Foot ulcer development in the insensate foot is intimately linked to high peak plantar pressures and high pressure-time integrals during gait as patients with diabetic neuropathy cannot detect aberrant pressures and do not adjust their walking strategy appropriately. We hypothesize that an intervention providing plantar pressure feedback would reduce aberrant high pressures developed during daily activities. We aimed to test efficacy of a novel plantar pressure-sensing smart insole system, the SurroSense Rx® (Orpyx Medical Technologies Inc., Canada) in reducing DFU occurrence in 'high risk' diabetic patients. This system comprises pressure-sensing inserts worn inside patients' footwear, recording continuous plantar pressure at eight sensor locations, during day-to-day life. When critical pressure thresholds are detected, a smartwatch feeds back to the patient via an alert and encourages off-loading, to modify aberrant plantar pressures developed during daily activities.
Materials and methods: In this randomised controlled trial, patients with recent history of DFU, peripheral neuropathy, no peripheral vascular disease, and no current DFU were recruited from two hospital sites within Greater Manchester, UK. Ninety participants were consented, 58 were randomized, all being set-up with the pressure-sensing inserts and smartwatch. Intervention group (IG) received feedback alerts from the smartwatch when pressures were 'high', whereas Control group (CG) did not receive alerts. At baseline, participants received device training and a detailed foot check. Patients were reviewed monthly for a foot check and system calibration. Follow-up was for 18 months or until plantar ulceration occurred.
Results: At follow-up, there were 10 ulcers from 8,638 person-days in CG and 4 ulcers from 11,835 person-days in IG. A Poisson regression model compared the two groups on incidence of ulceration with log exposure days as offset and showed a 71% reduction in ulcer incidence in IG (Incidence Rate Ratio = 0.29, 95% CI: 0.09–0.93) relative to the CG ($p = 0.037$). Characteristics of CG ($n = 26$) vs. IG ($n = 32$) were: age, 67.1(9.6) vs. 59.1(8.5) [mean (SD)]; Type 1 diabetes, $n = 4$ (15.4%) vs. $n = 9$ (28.1%); duration diabetes, 21.2 (10.7) vs. 22.2 (14.3) years; HbA1c, 58 (41–83) vs. 65.5 (38–122) [median (range)] mmol/mol. In survival analysis, the Kaplan-Meier graph and log-rank test suggested no significant difference in treatment groups in time to ulceration (18 month ulcer-free proportion: CG - 68.4%, IG - 77.5%; $p = 0.30$). Self-reported hours of wearing the device were: CG, 4.6 (2.9) vs. IG, 5.1 (3.0) hours/day, $p = 0.63$.
Conclusion: Plantar pressure feedback and encouragement to offload throughout daily life via smartwatch alerts resulted in 71% lower DFU incidence after 18 months follow-up. We conclude that there has been a significant, positive impact of this plantar pressure feedback intervention on reducing DFU incidence in 'high risk' diabetic patients.
Clinical Trial Registration Number: ISRCTN05585501
Supported by: Primary funding years 1–3 DUK; study extension (year 4) Orpyx Med Tech Inc.
Disclosure: **C.A. Abbott:** None.

8

Ultrasound versus sharp wound debridement in healing of recalcitrant neuropathic diabetic foot ulcers: clinical and pathological study

F. Kyrillos[1], A. Albehairy[1], M. Roshdi[1], W. Elkashef[2], M. Tarshoby[1];
[1]Diabetes and Endocrinology department, Mansoura University, Mansoura, [2]Pathology department, Mansoura University, Mansoura, Egypt.

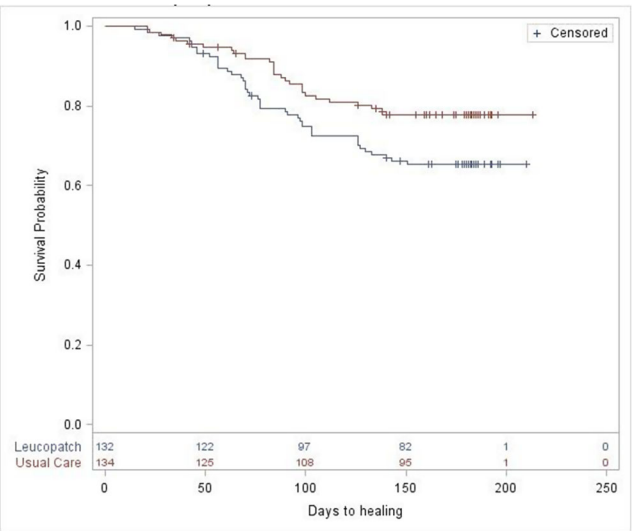
Background and aims: Debridement is the consistent mainstay for the standard care of diabetic foot ulcer. Non surgical sharp debridement is the gold standard method used with some known limitations. Researchers proposed advantages for low frequency ultrasound (LFU) debridement for healing of chronic ulcers. The aim of this study is to compare clinical outcome, pathological and immuno-histochemical effect of LFU debridement versus sharp debridement on recalcitrant neuropathic diabetic foot ulcers.
Materials and methods: 21 diabetic patients of matched age and sex with recalcitrant neuropathic foot ulcers (duration ≥6 months with standard therapy, sharp debridement and proper offloading), were recruited from Mansoura Diabetic Foot Clinic (Specialized Medical Hospital- Mansoura university). Only grade 1A and 2A ulcer (University of Texas) were included in the study. All patients continued on same ulcer management with randomization into 2 groups according to method of debridement: Sharp group; continued using scalpel (11 patients) and Ultrasound (US) group; using LFU (12 patients). Patients received 1 debridement session every 2 weeks for 2 months. Tissue biopsies were taken from the base and edge of ulcers at the first session and after 2 months of debridement. Clinical outcome was assessed by reduction of ulcers surface area after 2 months. Pathological parameters for healing were assessed blindly by the pathologist. Pathological scoring included cellularity, vascular proliferation, type of collagen, inflammatory cells and fibrosis. Immunoreactivity of Matrix metalloproteinase-1 (MMP-1) was also assessed.
Results: Greater reduction in ulcers surface area in US group (43%) versus sharp group (24.24%) ($p = 0.001$). Improvement in total ulcer pathology score was evident after each type of debridement with more improvement in US group versus sharp group (23.21% vs.6.67%, respectively) ($p = 0.004$). Significant increase in cellularity in base and edge of the ulcers, vascular proliferation of ulcer base and inflammation of the ulcer edge after 2 months of US debridement ($p = 0.04, 0.03, 0.04, 0.03$ respectively), while sharp debridement decreased the cellularity in the base of ulcers ($p = 0.04$) with no significant change in other pathological parameters. MMP-1 expression decreased significantly in both base and edge of ulcers treated by sharp debridement ($p = 0.03, 0.02$ respectively), while increased significantly in the base of ulcers after US debridement ($p = 0.037$).
Conclusion: LFU debridement is superior to sharp debridement regarding healing of recalcitrant neuropathic diabetic foot ulcers. In contrast to sharp debridement, LFU debridement increases expression of MMP-1, cellularity, vascular proliferation and inflammation in the ulcers improving the total pathology score and indicating better opportunity for ulcer healing.
Disclosure: **F. Kyrillos:** None.

9

The LeucoPatch® system in the management of hard-to-heal diabetic foot ulcers: a multicentre, multinational, observer-blinded, randomised controlled trial

F. Game[1], W. Jeffcoate[2], L. Tarnow[3], J. Jacobsen[4], D. Whitham[5], E. Harrison[5], S. Ellender[5], M. Löndahl[6];
[1]Derby Teaching Hospitals NHS Foundation Trust, Derby, UK, [2]Nottingham University Hospitals NHS Trust, Nottingham, UK, [3]Holbaek Sygehus, Holbaek, Denmark, [4]Statcon ApS, Kokkedal, Denmark, [5]University of Nottingham, Nottingham, UK, [6]Skåne University Hospital, Lund, Sweden.

Background and aims: The LeucoPatch® device uses bedside centrifugation without additional reagents to generate a disc comprising autologous platelet-rich fibrin and leucocytes which is applied to the surface of the wound. The aim of the study was to test the effectiveness of LeucoPatch® on the healing of hard-to-heal foot ulcers in people with diabetes.
Materials and methods: 595 people with diabetes and a foot ulcer consented to participate. After a 4 week run-in-period those with a reduction in ulcer area of <50% were randomised to either pre-specified good standard care alone or care supplemented by weekly application of LeucoPatch®. The primary outcome was percentage of ulcers healed within 20 weeks, defined as complete epithelialisation confirmed by an observer blind to randomisation group and maintained for four weeks.
Results: 269 people were randomised; mean age 62 years, 82% male, 82% Type 2 diabetes. In the intervention group 34.1% ($n = 45/132$) of ulcers healed within 20 weeks vs 21.6% ($n = 29/134$) of the controls (OR 1.58, 95% CI 1.06–2.35; $p = 0.02$) by intention-to-treat analysis. Time to healing was shorter in the intervention group ($p = 0.0246$) (Figure 1). No difference in adverse events was seen between groups.
Conclusion: The use of LeucoPatch® is associated with significant enhancement of healing of hard-to heal foot ulcers in people with diabetes.



Clinical Trial Registration Number: ISRCTN27665670
Supported by: Reapplix ApS
Disclosure: **F. Game:** Grants; Reapplix ApS.

10

Platelet-rich plasma plays an anti-inflammatory and cell proliferation-promoting role through miR-21/PDCD4/NF-kB pathway in vitro of a diabetic wound model

W. Deng[1], T. Li[1], D. Armstrong[2];
[1]Department of Endocrinology and Nephrology, Diabetic Foot Center, Affiliated Central Hospital of Chongqing University, Chongqing Emergency Medical Hospital, Chongqing, China, [2]Department of Surgery, Keck School of Medicine of the University of Southern California, Los Angeles, USA.

Background and aims: Infection and inflammatory disorders are two of contributing factors for non-healing diabetic foot ulcers (DFUs). Platelet-rich plasma (PRP) has an antibacterial and wound repair accelerating effect in DFUs healing. The MicroRNAs relate to wound healing are high abundance expression in platelet. MicroRNA-21(miR-21)plays a key role

in antibacterial and inflammatory through regulation of nuclear transcription factor(NF-κB) activity, but the mechanism remains unclear. This study aimed to determine the potential antibacterial, anti-inflammatory and cell proliferation-promoting mechanism of PRP on DFUs healing.
Materials and methods: Considering the common infection of DFUs caused by Staphylococcus aureus (S. aureus), HaCaT cells and S. aureus were co-cultured under high glucose conditions to serve as an in vitro model for infected cells in DFUs. Platelet-rich gel (PRG) or extract liquid of platelet-rich gel(EPG)was used to interfere with the model to observe the growth of HaCaT cells and S. aureus, and the effect of miR-21 changes in HaCaT cells on PDCD4, NF-κB and related inflammatory factors.
Results: Incubation of HaCaT cells with increasing concentrations of S. aureus induced to a dose-dependent decline of cell proliferation. Protein level of PDCD4 elevated and NF-κB activity enhanced in cells with increased IL-6, TNF-α and decreased IL-10, TGF-β1 under this condition. As an effective component of PRG, EPG had a specific anti-S. aureus activity. EPG dose-dependently protected HaCaT cells from bacterial damage and promoted cell proliferation. Meanwhile, EPG could increase intracellular miR-21, reduce PDCD4 expression and inhibit NF-κB activity to alleviate inflammation of infected HaCaT cells. Furthermore, anti-inflammatory effects to HaCaT cells were also observed in the absence of S. aureus infection.
Conclusion: In conclusion, the in vitro model we built provides a valuable tool for study of DFUs healing mechanism. MiR-21 regulates NF-κB through PDCD4 plays an anti-inflammatory and pro-cell proliferation role in the process of wound healing promoted by PRG. The results support the favorable function of PRG in treating DFUs and may provide novel therapeutic target for refractory wounds.
Supported by: National Natural Science Foundation of China (NO. 81500596)
Disclosure: **W. Deng:** None.

11

Autologous mononuclear versus mesenchymal stem cells in healing of recalcitrant neuropathic diabetic foot ulcers

A. Albehairy[1], F. Kyrillos[1], H. Gawish[1], O. State[1], H. Abdelghaffar[2], O. Elbaz[2], A.H. El-Sebaie[2], A.A. Emam[3], M. Tarshoby[1];
[1]Internal Medicine, Mansoura University, Mansoura, [2]Clinical Pathology, Mansoura University, Mansoura, [3]Mansoura University, Mansoura, Egypt.

Background and aims: Most human studies involved bone marrow mononuclear stem cells (BM-MNCs) and bone marrow mesenchymal stem cell (BM-MSCs) safety and efficacy involved treatment of ischemic wounds and critical limb ischemia, with less specific studies on neuropathic diabetic foot ulcers. Recent pilot clinical study suggested that autologous BM-MSCs is safe and promising in treatment of recalcitrant diabetic foot ulcer. **Aim of the work:** To compare the therapeutic effect of autologous BM-MNCs and BM-MSCs on the healing process in patients with recalcitrant neuropathic diabetic foot ulcers
Materials and methods: Eighteen patients with type 2 diabetes mellitus with neuropathic foot ulcer grade 2 New Texas classification were selected from Diabetic Foot Clinic, Mansoura University, Egypt from May 2016 to August 2017. Only patients who failed to respond to 12 weeks of weekly sharp debridement and proper offloading were included in the study. Patients were randomly assigned to MSCs, MNCs or control group. The study was conducted in Mansoura Regenerative Medicine Centre, Egypt. After aspiration of 20 ml of patients' own bone marrow under good aseptic technique either mononuclear stem cells (MNCs) were separated or Mesenchymal stem cells (MSCs) were cultured. The bone marrow sample was diluted with phosphate buffer saline then separation using Ficoll hybaqe harvesting the layer of MNCs then washed twice using the complete media. This process revealed $5x10^6$ to $6x10^6$ of MNCs. Mesenchymal stem cells (MSCs) were characterized by adherence, trans-differentiation and CD characterization. Cultured cells were subjected to microbiological and karyotyping testing. MSCs number ranged from $1x10^6$ to $2x10^6$. The revealed MNCs or MSCs were dissolved in 2cm saline to be used for injection in the edges of the wound at eight points once. All patients continued on same offloading and dressing and were followed for 12 weeks for the change in ulcer surface area and the presence of any local reactions
Results: Percentage reduction of Ulcer surface area was higher in both (MSC) and (MNC) groups 68% and 59% respectively compared to only 6.25% in control group after 12 weeks of follow up. (P value <0.05), However, there was no statistical significant difference between the healing rate of (MSC) and (MNC) groups. Complete healing was achieved in one patient in MSC group and in another patient in MNC group.
Conclusion: Local injection of both autologous bone marrow derived MSCs and MNCs augmented healing of recalcitrant neuropathic diabetic foot ulcers. Using both cells was well tolerated by the patients with no short term complications. The small non significant better healing associated with the use of MSCs should be weighed against the fact that MNCs separation is easier and achieved by less manipulation
Supported by: Mansoura University
Disclosure: **A. Albehairy:** None.

12

Association of diuretics use and amputations in patients with type 2 diabetes: A hypothesis driven from CANVAS warning?

L. Potier[1], R. Roussel[1], G. Velho[2], P.-J. Saulnier[3], A. Bumbu[1], O. Matar[1], M. Marre[1], K. Mohammedi[4], S. Hadjadj[5];
[1]Department of Diabetology, Endocrinology and Nutrition, Assistance Publique – Hôpitaux de Paris (AP-HP), Paris, [2]Team 2, INSERM U1138, Paris, [3]INSERM CIC1402, Poitiers, [4]Département d'Endocrinologie, Diabétologie, Nutrition, Hôpital Haut-Lévêque, Paris, [5]Department of Endocrinology and Diabetology, Centre Hospitalier Universitaire de Poitiers, Poitiers, France.

Background and aims: Recently, safety data signaled an increased risk of amputations in patients with type 2 diabetes taking SGLT2 inhibitors. If this side effect is due to drug-induced hypovolemia, the use of diuretics should also increase that risk. The aims were to analyze the association between the use of diuretics and the risk of lower limb events (LLE) in patients with type 2 diabetes.
Materials and methods: SURDIAGENE is a French prospective observational cohort including type 2 diabetes patients enrolled from 2002 to 2012. Participants were followed-up until onset of LLE, death, or December 31, 2015, whichever came first. Participants: 1459 patients with type 2 diabetes, with information on use of diuretics at baseline and available data on primary outcomes during follow-up. Exposure: Use of diuretics at baseline (thiazides, loop and/or potassium-sparing diuretics). Main Outcomes: LLE, a composite of lower-extremity amputations (LEA) (amputation at or above the metatarsophalangeal joint) and lower limb revascularizations (LLR) (angioplasty or bypass).
Results: At baseline, of the 1459 studied participants, 670 were taking diuretics (in patients with and without diuretics, mean age was 67.1 and 62.9; 55.8% and 59.8% were men, respectively). During a median follow-up of 7.2 years, LLE occurred in 85 (12.7%) and 57 (7.2%) of the users and non-users, respectively ($p = 0.001$). The hazard ratio for LLE in users vs. non-users was 2.08 (95%CI, 1.49–2.93; $p < 0.0001$). This association remained significant in multi-adjusted model (1.83 (1.27–2.67; $p = 0.0013$) and after considering death as a competing risk (subhazard ratio 1.89 (1.35–2.64; $p = 0.0002$)). When separated, LEA but not LLR were associated with the use of diuretics (2.61 (1.55–4.50; $p = 0.0013$) and 1.30 (0.84–2.02; $p = 0.24$), respectively).

Conclusion: Among patients with type 2 diabetes treated with diuretics, there was a significant and independent increase in the risk of LLE, predominantly LEA. Diuretics should be used cautiously in patients with type 2 diabetes at risk of amputations. Further studies are needed to explore the role of drug-induced hypovolemia in the association between the use of diuretics and LLE. The hypovolemia hypothesis could provide an explanation for the increased risk of LEA observed with SGLT2 inhibitors.

Disclosure: **L. Potier:** Grants; NOVO NORDISK, SANOFI, ELI LILLY. Honorarium; NOVO NORDISK, ELI LILLY, SANOFI, SERVIER. Non-financial support; SANOFI, ELI LILLY.

OP 03 Unravelling nephropathy

13

Linagliptin reduced renal injury and proteinuria in a rat model of crescentic nephritis

A.-L. Mayer[1], K. Amann[1], T. Klein[2], C. Daniel[1];
[1]Department of Nephropathology, University of Erlangen-Nürnberg, Erlangen, [2]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

Background and aims: Dipeptidyl peptidase 4 (DPP-4) inhibitors are a class of oral glucose lowering drugs, used in the treatment of type 2 diabetes. In human kidney biopsies we observed high DPP-4 expression in early crescent formation. This glomerular lesion occurs in various kidney diseases and is a pathogenic hallmark of renal dysfunction. Therefore, we investigated the potential involvement of DPP-4 in the pathogenesis of nephritis induced by anti-GBM (glomerular basement membrane antibody) in Wistar rats.

Materials and methods: Linagliptin (3 mg/kg/bw, $n = 11$) and vehicle ($n = 11$) were used to treat anti-GBM nephritis in 8-week regimens: either preventive or therapeutic (treatment started 4 weeks after model induction). Kidney function, morphologic changes, inflammation and fibrosis were monitored.

Results: Disease prevention with linagliptin in anti-GBM nephritic rats significantly ($p < 0.01$) reduced the number of crescents (51 ± 3% vs 65 ± 3%), glomerulosclerosis (score 1.2 ± 0.07 vs 1.6 ± 0.1), tubule-interstitial injury (score 1.2 ± 0.1 vs 1.8 ± 0.2), renal fibrosis (score 1.3 ± 0.13 vs 1.9 ± 0.14) and proteinuria (265 ± 29 vs 363 ± 22 mg/24h) compared with untreated nephritic rats. Furthermore, the preventive linagliptin regimen significantly reduced the number of $Pax8^+$ cells on the glomerular tuft by 17 ± 5% at day 14 ($p < 0.05$) and 60 ± 5% at week 8 ($p < 0.001$), indicating accelerated resolution of the cellular crescents. Therapeutic intervention with linagliptin resulted in weaker amelioration of renal disease at week 8, but significantly ($p < 0.05$) reduced renal fibrosis (score 1.4 ± 0.13 vs. 1.9 ± 0.14), crescent formation (52 ± 4% vs. 65 ± 3%) and $Pax8^+$ cells on glomerular tuft (65 ± 5.2% reduction) compared with vehicle. Proteinuria was also reduced, but this result did not reach significance.

Conclusion: DPP-4 inhibition with linagliptin ameliorates renal injury in a severe rat model with anti-GBM induced nephritis as shown by reduced crescents, proteinuria and fibrosis, and resolution of crescents. Therapeutic intervention with linagliptin showed weaker effects compared with preventive intervention.

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **A. Mayer:** Non-financial support; Boehringer Ingelheim.

14

Non-esterified free fatty acids (NEFA) can enhance the inflammatory response in renal tubules by inducing ATP release

H. Sun[1], Z. Sun[2], X. Ruan[3];
[1]Department of Endocrinology and Metabolism, the First Affiliated Hospital of Soochow University, Suzhou, China, [2]Department of Endocrinology and Metabolism, Zhongda Hospital, Nanjing, China, [3]John Moorhead Research Laboratory, Centre for Nephrology, University College London (UCL) Medical School, London, UK.

Background and aims: Diabetes mellitus is the leading cause of chronic kidney disease (CKD) in both developed and developing countries. The global widespread of CKD is rapidly becoming a worldwide health problem. The severity of tubulointerstitial inflammation has long been considered as a crucial determinant of progressive CKD. Although the pathogenesis of tubulointerstitial inflammation is poorly understood, one common association and likely pathogenic factor is proteinuria. However, excessive plasma non-esterified free fatty acids (NEFA) load in proteinuria can leak across the damaged glomeruli to be reabsorbed by

renal proximal tubular cells, and cause inflammatory tubular cells damage by as yet unknown mechanisms. The present study was designed to investigate these mechanisms induced by NEFA overload.

Materials and methods: HK-2 cells were incubated with palmitic acid (PA). THP-1 cells were differentiated into macrophages by phorbol-12-myristate-13-acetate (PMA). Mitochondrial reactive oxygen species (mtROS) production was determined using dihydroethidium, and extracellular ATP (eATP) was detected by an ATP Assay Kit. Caspase-3/7 activity was tested by Caspase-3/7 Green ReadyProbes Reagent, and YoPro-1 fluorescence has been used to quantify pannexin-1 (Panx1) activation. Transwell filter migration assay were used for the chemotaxis assay, and the monocyte chemoattractant protein-1 (MCP-1) in the supernatants was detected by MCP-1 ELISA kit. Expression of mRNA and protein were examined by quantitative RT-PCR and western blot, respectively.

Results: 1. NEFA induces mtROS-dependent caspase-3/7 activation in renal tubular cells, and then, the activated caspase-3/7 opens the Panx1 channel, leading to pathophysiological ATP release. 2. Both eATP and NEFA increase the secretion of MCP-1 from renal tubular cells, which induce monocyte infiltration. 3. NEFA stimulates interleukin-1β (IL-1β) release from both macrophages renal tubular cells. 4. eATP stimulates IL-1β release from both macrophages renal tubular cells via the P2X7R-mTOR-FOXO1-TXNIP/NLRP3 inflammasome pathway.

Conclusion: NEFA increase mtROS production and inflammatory stress, causing 'the first hit.' The first hit stimulates ATP release from Panx1 channel on renal tubules by activation of caspase-3/7. Then, playing as 'the second hit', eATP aggravates the tubular inflammatory response by increasing monocyte infiltration and stimulating inflammatory cytokine release from both macrophage and renal tubular cells via the P2X7R-mTOR-FOXO1-TXNIP/NLRP3 inflammasome pathway. This may cause a severe renal inflammatory response and renal dysfunction. Therefore, inhibition of the ATP release may be a potential point to alleviate renal inflammation and improve renal function.

Supported by: NSFC

Disclosure: **H. Sun:** None.

15

Overexpression of CD38 (NADase) in diabetic kidney disease results in renal mitochondrial oxidative stress and pathologies via NAD^+-dependent Sirt3 inactivation

Y. Ogura, M. Kitada, I. Monno, D. Koya;
Kanazawa Medical University, Kahokugun, Japan.

Background and aims: Diabetic kidney disease (DKD) is a leading cause of end-stage renal disease (ESRD) worldwide. Although the detailed pathogenesis of DKD is not elucidated yet, aging is recognized as one of the risk factors for the progression of ESRD. Therefore, aging-related mechanism by which DKD could aggravate would be a novel therapeutic target for DKD. Nicotinamide adenine dinucleotide (NAD) levels decrease during aging, and are involved in age-related metabolic unhealth. Previous report demonstrated that expression and activity of the CD38 (NADase) increased with aging, and that CD38 is required for the age-related NAD decline and mitochondrial dysfunction via a pathway mediated at least in part by regulation of Sirt3 activity. However, the role of CD38 in the pathogenesis for DKD has not elucidated yet.

Materials and methods: In this study, we evaluated the role of CD38 in modulation of mitochondrial oxidative stress which is related to altered Sirt3 activity, in the kidney of Zucker Diabetic Fatty rats (ZDFRs) and in cultured human renal tubular epithelial cells (HK2 cells) exposed high-glucose condition.

Results: At 28 weeks of age, the ZDFRs exhibited elevated HbA1c levels, heavier kidney weight, increase in urinary albumin, urinary liver type fatty acid binding protein (L-FABP) and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) excretion, histological tubulo-interstitial fibrosis and glomerulomegaly, and inflammation, compared to non-diabetic Zucker Lean rats. Additionally, in renal mitochondria, the NAD^+/NADH ratio was reduced, and acetylation levels of mitochondrial antioxidant enzymes, isocitrate dehydrogenase 2 (IDH2) and superoxide dismutase (SOD2), which are regulated by Sirt3, were increased in ZDFRs. Similarly, in cultured HK2 cells exposed to high-glucose condition, CD38 expression was increased, compared to cells under low-glucose condition, resulting in the reduction of NAD^+/NADH ratio and Sirt3 activity, which increased in acetylation levels of IDH2 and SOD2. Administration of the CD38 inhibitor, apigenin, to ZDFRs and HK2 cells, restored the NAD^+/NADH ratio, decreased the levels of IDH2 and SOD2 acetylation and renal mitochondrial oxidative stress.

Conclusion: Therefore, restoring Sirt3 activation by suppression of CD38 could be a novel therapeutic target for DKD.

Disclosure: **Y. Ogura:** None.

16

Soluble Nogo-B overexpression inhibits diabetes-mediated endothelial cell proliferation in a murine model of early diabetic glomerulopathy

I.P. Hernandez-Diaz[1], G. Fouli[1], C. Ricciardi[1], A. Hayward[1], D.E. Long[2], L. Gnudi[1];
[1]School of Cardiovascular Medicine & Sciences, King's College London, London, [2]Developmental Biology and Cancer, University College London, London, UK.

Background and aims: Impaired angiogenesis, as seen in early diabetic glomerulopathy (DG), is characterised by thin wall capillaries and increased vascular permeability and is paralleled by an increase in endothelial cells (ECs) number, mainly driven by cell proliferation. Nogo-B is expressed in the glomerular endothelium and podocytes, and is mainly localised in the endoplasmic reticulum; previous works has implicated Nogo-B in vascular remodelling where it promotes vascular integrity. Nogo-B expression is downregulated in the diabetic glomeruli and soluble Nogo-B (sNogo-B, a circulating 200AA N-terminus fragment of Nogo-B) has been shown to correct diabetes-mediated Nogo-B downregulation and albuminuria. In this work, we have studied the effects and putative cellular mechanisms of sNogo-B overexpression on ECs proliferation/impaired glomerular angiogenesis in a murine model of diabetes.

Materials and methods: 5–8 weeks old DBA2J male mice were injected i.p. with streptozotocin (40 mg/kg/day) for 5 days, sNogo-B overexpression in the circulation was induced by tail injection of adeno-associated vector (AAV) driving the expression sNogo-B (AAV driving the expression of green fluorescent protein [GFP] was utilised as control). Animals with glycaemia >22 mM were considered diabetic. Animals were killed after 12-weeks of diabetes and the right kidney was frozen in Optimal Cutting Temperature (OCT) compound for sectioning and histology with immunofluorescence with the ECs marker (CD31) and the proliferation marker (KI67). In parallel renal cortex cell lysate was utilised for the study of VEGFA levels with ELISA, and AKT, eNOS and GSK3β total and phosphorylated levels with western immunoblotting.

Results: Diabetes (D) resulted in a significant increase in glomerular ECs proliferation when compared to non-diabetic (ND) status (ND-GFP *vs* D-GFP, $p = 0.0001$); diabetes-mediated glomerular ECs proliferation was partially prevented by sNogo-B overexpression (D-GFP *vs* D-sNogo-B, $p = 0.005$). In ND mice, sNogo-B overexpression had no effect on ECs proliferation. VEGFA, AKT^{ser473} and $eNOS^{ser1177}$ phosphorylation (molecules/pathways involved in ECs proliferation) were upregulated in D mice (ND-GFP *vs* D-GFP, $p < 0.04$); sNogo-B overexpression partially inhibited diabetes-mediated VEGFA expression, and AKT^{ser473} and $eNOS^{ser1177}$ phosphorylation (D-GFP *vs* D-sNogo-B, $p < 0.03$). $GSK3\beta^{ser9}$ phosphorylation was unchanged in D-GFP animals when compared to ND-GFP ones; sNogo-B overexpression was paralleled by a significant upregulation of $GSK3\beta^{ser9}$ phosphorylation in ND mice (ND-GFP *vs* ND-sNogo-B, $p = 0.001$), while, in D mice, sNogo-B

overexpression was paralleled by a significant downregulation of GSK3β^{ser9} phosphorylation (D-GFP *vs* D-sNogo-B, $p = 0.03$).
Conclusion: sNogo-B overexpression prevents diabetes-mediated glomerular ECs proliferation, an event that seems to be related to sNogo-B-mediated inhibition of the pro-angiogenesis and permeability VEGFA/AKT^{ser473}/eNOS^{ser1177} phosphorylation pathway, and by GSK3β activation (reduced GSK3β^{ser9} phosphorylation) known to favor capillary formation/stability over ECs proliferation/angiogenesis. sNogo-B appears to ameliorate endothelial dysfunction in early DG and studies are ongoing to better dissect its role in the pathophysiology of DG.
Supported by: BHF
Disclosure: **I.P. Hernandez-Diaz:** None.

17

FoxO1 inhibits autophagosome-lysosome fusion leading to endothelial autophagic-apoptosis in diabetes

X. Wu[1], H. Zhang[1], K. He[2], X. Zhao[1], Y. Wu[1], S. Ge[3], Y. Shao[2];
[1]Endocrinology, the First Affiliated Hospital with Nanjing Medical University, Nanjing, [2]Thoracic surgery, the First Affiliated Hospital with Nanjing Medical University, Nanjing, [3]Neurology, the First Affiliated Hospital with Nanjing Medical University, Nanjing, China.

Background and aims: Endothelial dysfunction in response to various insults such as hyperglycemia, dyslipidemia and altered homeostasis is critical in the genesis of diabetic angiopathy. Recent data found that inadequate autophagy contributed to endothelial dysfunction in patients with diabetes. However, whether inadequate autophagy leads to endothelial cells (ECs) apoptosis remains unknown. The aim of this study is to investigate the relationship between inadequate autophagy and ECs apoptosis in diabetes and its underlying mechanism.
Materials and methods: Aortic vascular ECs were freshly isolated from the discarded vascular tissue of diabetic patients undergoing artery vascular replacement surgery. Cultured human aortic vascular ECs (HAECs) were stimulated with AGEs-bovine serum albumin or BSA. The expression levels of LC3-II, P-62, Rab7, Atg14, STX17, LAMP2, cleaved-caspase-3, Bcl-2, FoxO1, Ac-FoxO1, and p-FoxO1 were determined by western blotting. Autophagosomes were observed by electron microscopy. The apoptosis rate was evaluated by flow cytometry. The fusion of autophagosome and lysosomes was detected by immunofluorescence.
Results: Compared with non-diabetic subjects, the levels of LC3-II and p-62 were increased in ECs from diabetes. Western Blotting and immunofluorescence showed that the expressions of Atg14 and STX17 were decreased, and the co-localization of autophagosomes marker (LC3-II) with lysosomes marker (LAMP2), and Atg14 with STX17 were declined, suggesting inadequate autophagy with impaired autophagosome-lysosomal fusion in ECs from diabetic patients. AGEs induced HAECs autophagy in a time-dependent manner. For 24h, the expressions of LC3-II, Atg14, STX17 and Rab7 and the number of autophagosomes were gradually increased with no change of P-62, LAMP2 expression and apoptosis rates. For 48h, AGEs markedly upregulated LC3-II and p62 expression and the number of autophagosomes with decreased level of Atg14, STX17, Rab7 and co-localization of LC3-II with LAMP2, and Atg14 with STX17 which indicated the reduced autophagic flux with impaired autophagosome-lysosomal fusion. The apoptosis rates were significantly increased with elevated cleaved-caspase-3 level and declined Bcl-2 expression. Inhibition of autophagy with 3-MA could reduce AGEs-induced HAECs apoptosis, suggesting that activated autophagy contributes to ECs apoptosis. Higher levels of FoxO1, Ac-FoxO1 and Ac-FoxO1 binding to Atg7 were detected in AGEs-treated HAECs. Knockout FoxO1 by siFoxO1 reduced AGEs-induced autophagy, and increased the expression of Atg14, suggesting that FoxO1 regulates the expression of Atg14. Immunofluorescent staining showed that FoxO1 knockdown promoted the co-localization of LC3-II with LAMP2, and Atg14 with STX17 in HAECs exposed to AGEs, indicating that FoxO1 is crucial signaling molecular mediating ECs autophagy in diabetes.
Conclusion: Inadequate autophagy with impaired autophagosome-lysosomal fusion exists in ECs from diabetic patients. FoxO1 mediates AGEs-induced ECs autophagic apoptosis through impairing autophagosome-lysosomes fusion by inhibiting Atg14 expression, which may be a target for therapy of diabetic vascular complications.
Supported by: BRA2015389, LGY2016004
Disclosure: **X. Wu:** None.

18

Empagliflozin suppresses atherosclerotic lesion formation in apolipoprotein E deficient mice by inhibiting macrophage activation

T. Matsumura, S.M. Nishida, T. Senokuchi, N. Ishii, S. Nishida, T. Kondo, H. Motoshima, E. Araki;
Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan.

Background and aims: Cardiovascular disease (CVD) is one of the major causes of death in patients with type 2 diabetes (T2D). Recent studies suggest that SGLT2 inhibitors, which are novel class of glucose-lowering agents, reduce cardiovascular events in T2D patients with high risk of CVD. However, it remains uncertain whether the cardiovascular benefits merely depend on glucose-lowering effects or some other mechanism(s). Therefore, we examined whether empagliflozin suppressed the progression of atherosclerosis in diabetic and non-diabetic Apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice, and whether it had direct anti-atherogenic effects in macrophages.
Materials and methods: *Apoe*$^{-/-}$ mice (12 weeks age) were fed normal chow (NC) or a high fat diet (HFD), or treated with streptozotocin (120 mg/kg), and further treated with a placebo or empagliflozin (5 mg/kg/day) for 8 weeks. Mouse peritoneal macrophages from C57BL/6 mice were used for in vitro experiments. Atherosclerotic lesion size of aortic sinus and en face of aorta were estimated by oil-reo-O staining. Expression of 4-HNE, F4/80, Ki67 and Iba1 were visualized by fluorescence immunohistochemistry. Expression of SGLT2, MCP-1 and TNF-α were performed by Real-time RT-PCR and/or Western blot analysis. Intracellular ROS generation was measured by H_2DCF-DA. Cell proliferation was estimated by a CCK-8 assay kit and direct counting of the live cell number. Membrane currents of macrophages were performed by patch clump measurements.
Results: In all mouse models, there were no significant differences on dietary intake and lipid profile between placebo and empagliflozin-treated groups. However, body weight was lower in the empagliflozin group than the placebo group in NC-fed mice. Glucose levels during a food load test were lower in the empagliflozin group than the placebo group in all mouse models. Treatment with empagliflozin suppressed the progression of atherosclerotic lesions in the aortic sinus and en face of the whole aorta in all mouse models. The 4-HNE-positive area and number of proliferating macrophages in plaques, and mRNA expression of MCP-1 and TNF-α in the aorta were lower in empagliflozin groups than in control groups. Expression of SGLT2 mRNA and protein were confirmed in mouse peritoneal macrophages. Empagliflozin inhibited LPS-induced ROS generation, MCP-1 and TNF-α mRNA expression, and GM-CSF-induced cell proliferation, as well as suppressed glucose-sensitive inward current and glucose uptake in macrophages. Moreover, a pan-glucose transporter inhibitor or sodium channel blocker suppressed macrophage proliferation, suggesting the involvement of SGLT2 for macrophage activation.
Conclusion: We revealed that treatment with empagliflozin suppresses the progression of atherosclerosis in normoglycemic and hyperglycemic *Apoe*$^{-/-}$ mice. Moreover, we revealed for the first time that macrophages express functional SGLT2, and empagliflozin directly suppresses ROS generation, inflammatory responses, and cell growth of macrophages. These actions of empagliflozin may indicate that SGLT2 inhibitors may be beneficial for the treatment of diabetic macrovascular complications, and SGLT2 in macrophages may be a therapeutic target in normoglycemic patients with atherosclerotic diseases.

Supported by: Grants-in-Aid for Scientific Research from the Japan Society
Disclosure: **T. Matsumura:** Grants; This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 21591144 to T.M. and No. 20390259 to E.A.). Other; This project was financially supported by Boehringer Ingelheim.

OP 04 The adipose tissue: from biology to intervention studies

19

Insulin sensitising effects of vitamin D mediated through reduced adipose tissue inflammation and fibrosis

E. Lontchi-Yimagou[1], S. Kang[2], K. Zhang[1], A. Goyal[1], J. You[1], P. Kishore[1], E. Rosen[3], M. Hawkins[1];
[1]Albert Einstein College of Medicine, Bronx, [2]University of California, Berkeley, Berkeley, [3]Beth Israel Deaconess Medical Center, Boston, USA.

Background and aims: Despite epidemiologic evidence linking vitamin D deficiency with insulin resistance and type 2 diabetes, much controversy exists regarding whether vitamin D repletion has beneficial metabolic effects. Since vitamin D (25(OH)D) has anti-inflammatory and anti-fibrotic effects, expression of its receptor in adipocytes and macrophages suggests that 25(OH)D signaling could mediate paracrine effects within adipose tissue and improve insulin resistance. We designed parallel studies in humans and rodents to define the effects of vitamin D on adipose tissue inflammation and fibrosis, and on systemic insulin resistance.
Materials and methods: We performed a randomized, double-blinded placebo-controlled trial to examine the effects of repleting vitamin D levels to >30 ng/ml in 25(OH)D-deficient (<20 ng/ml), insulin resistant, overweight-to-obese humans ($n = 19$). A comprehensive study of whole-body insulin action was undertaken with stepped euglycemic (~90 mg/dL) hyperinsulinemic clamp studies, both before (1st visit) and after administration of vitamin D or placebo (2nd visit). Adipose tissue fibrosis and inflammation were quantified by 'real-time' rt-PCR and immunofluorescence in subcutaneous abdominal adipose tissue. To determine whether vitamin D's effects are mediated through adipocytes, we performed hyperinsulinemic clamp studies (4 mU/kg/min) and adipose tissue analysis in an adipocyte-specific vitamin D receptor knockout (VDR KO) mouse model (Adiponectin-Cre+VDR+/fl) following high fat diet feeding for 12 weeks.
Results: 25(OH)D repletion was associated with reductions in adipose tissue gene expression of inflammatory (0.6–0.7-fold decreased expression of *TNF-α, IL-6, iNOS and PAI-1*) and pro-fibrotic (0.4–0.8-fold decreased expression of *TGF-β1, HiF1α, Collagen I, V, VI and MMP7*) factors, decreased collagen VI immunofluorescence (19% reduction, $p = 0.02$) and improved hepatic insulin sensitivity in humans, with heightened suppression of endogenous glucose production (EGP) during hyperinsulinemic clamp studies (1.28 ± 0.20 *vs* 0.88 ± 0.18 mg/kg/min, $p = 0.03$). Despite no differences in body weight or adiposity, compared to wild type (WT), adipose-specific VDR KO mice exhibited increased adipose tissue expression of several pro-inflammatory (*Tnf-α*, *iNos*, *Pai-1*, *Mcp*-1 and *F4/80*; 4–10 fold) and pro-fibrotic genes (*Tgf-β1*, *Collagen VI*, and *Tsp1*; 2–4 fold), in concert with hepatic insulin resistance (EGP 10 ± 3 *vs* 3 ± 2 mg/kg/min in WT, $p = 0.021$). There were no changes in insulin-mediated glucose uptake in either humans or mice.
Conclusion: These complementary human and rodent studies establish a beneficial role of vitamin D to improve hepatic insulin resistance, likely by restraining adipose tissue inflammation and fibrosis. Thus, normalizing 25(OH)D levels could have metabolic benefits in targeted individuals.
Clinical Trial Registration Number: NCT01354964
Supported by: ADA
Disclosure: **E. Lontchi-Yimagou:** None.

20

FKBP51 ablation using CRISPR/Cas-9 impairs adipocyte differentiation

C. Castillejo-López[1], X.M. Abalo[2], C.O. Sidibeh[2], M.J. Pereira[2], P.G. Kamble[2], J.W. Eriksson[2];
[1]Immunology, Genetics and Pathology, Uppsala University, Uppsala, [2]Medical Sciences, Uppsala University, Uppsala, Sweden.

Background and aims: Prolonged exposure to high levels of endogenous or exogenous glucocorticoids (GC) has adverse effects on critical metabolic processes that can lead to insulin resistance, diabetes, central obesity and dyslipidemia. GC action depends on its binding to the cytosolic GC receptor, which act as a transcription factor tightly regulated by a chaperone protein complex that includes FKBP51 (FK506-binding protein 51). FKBP51 is implicated in stress-related psychiatric disorders. Recently, in a microarray study, we reported that the expression of the corresponing gene, *FKBP5*, was highly upregulated in human adipose tissue after 24 h treatment with a synthetic glucocorticoid, dexamethasone. Moreover, *FKBP5* SNPs were found to be associated with type 2 diabetes. In this study, we aimed to investigate the consequences of FKBP51 ablation in human adipocyte differentiation.
Materials and methods: We used CRISPR/Cas-9 methodology to generate two independent *FKBP5* knockouts in two different cell models: human preadipocytes isolated from stromal vascular cells obtained from human adipose tissue biopsies (non-diabetic healthy volunteers) and the preadipocyte SGBS cell line (Simpson-Golabi-Behmel Syndrome). The phenotype was analyzed in preadipocytes and in differentiated adipocytes by Western blot, RT-qPCR, and immunohistochemistry capacity of differentiated adipocytes.
Results: Gene editing in different cell cultures displayed a prevalence of at least 50% of null allelic mutations and on average of 75% protein reduction. Adipogenesis assays showed that *FKBP5*-KO preadipocytes had reduced ability to differentiate into mature adipocyte. The gene expression of adipogenic markers at different time points during differentiation was assessed, and *PPARG*, *FABP4*, *CD36,* and *ADIPOQ* expression was reduced by a ~60%, ~95%, ~95% and 90% ($p < 0.01$), respectively, compared to wild-type (WT) control cells. In addition, the degree of differentiation assessed by quantifying the amount of lipid accumulation on the 14th day of differentiation also showed a reduced accumulation of lipid in *FKBP5*-KO cells by about 30% ($p < 0.01$) compared to WT. Studies are ongoing to investigate the effect of dexamethasone on cell differentiation and glucose uptake capacity in differentiated *FKBP51*- KO and WT adipocytes.
Conclusion: We show proof-of-concept for CRISPR/Cas-9 gene editing and ablation in human adipose precursor cells of FKBP51, a chaperone protein modulating GC-receptor activity. The resulting phenotype includes a markedly impaired adipogenesis. This implies a critical role of FKBP51 in human adipose tissue that may influence insulin action and other metabolic functions.
Supported by: SRC, EXODIAB, EF, SDF, ALF
Disclosure: **C. Castillejo-López:** None.

21

Insulin regulates lipolysis and fat mass by upregulating growth/differentiation factor 3 in adipose tissue macrophages

T. Izumi, Y. Bu, K. Okunishi;
Gunma University, Maebeshi, Gunma, Japan.

Background and aims: Previous genetic studies in mice have shown that functional loss of activin receptor-like kinase 7 (ALK7), a type I transforming growth factor-β receptor, increases lipolysis to resist fat accumulation in adipocytes. Although growth/differentiation factor 3 (GDF3) has been suggested to function as a ligand of ALK7, it is unknown how GDF3 production is regulated under nutrient-excess conditions.
Materials and methods: We identified the cell source of GDF3 by biochemical and FACS fractionation of mouse white adipose tissue (WAT). We examined the effects of insulin on GDF3 expression in adipose tissue macrophages (ATMs) ex vivo and on body and WAT weights and serum nonesterified fatty acid levels in vivo in both ALK7-intact and ALK7-deficient obese mouse strains. To examine the involvement of ATMs and GDF3 in the insulin activity toward lipolysis and adiposity, we performed experiments of clodronate treatment and transplantation of bone marrow from GDF3-knockout mice to deplete macrophages and their GDF3, respectively.
Results: A physiologically low level of insulin converted $CD11c^-$ adipose tissue macrophages into GDF3-producing, $CD11c^+$ macrophages ex vivo, and directs ALK7-dependent accumulation of fat in vivo. Depletion of ATMs by clodronate upregulated adipose lipases and reduced fat mass in ALK7-intact obese mice, but not in their ALK7-deficient counterparts. Furthermore, depletion of ATMs or transplantation of GDF3-deficient bone marrow negated the in vivo effects of insulin on both lipolysis and fat accumulation in ALK7-intact obese mice.
Conclusion: Insulin efficiently inhibits lipolysis and accumulates fat primarily through the upregulation of GDF3 in ATMs, but not through its direct activities on adipocytes. The GDF3-ALK7 axis between ATMs and adipocytes represents a previously unrecognized mechanism by which insulin regulates both fat metabolism and mass.
Supported by: JSPS KAKENHI
Disclosure: **T. Izumi:** None.

22

The diabetes risk gene TCF7L2 regulates human adipose progenitor cell biology

M. Verma, N. Loh, M. Todorčević, K. Pinnick, A.V. Dam, M. Neville, F. Karpe, C. Christodoulides;
Oxford Centre for Diabetes Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

Background and aims: Dysfunctional adipose tissue *e.g.* as seen in obesity or lipodystrophy is associated with insulin resistance (IR) which predisposes to type 2 diabetes (T2D) and cardiovascular disease (CVD). Nonetheless, the risk of T2D and CVD is not uniform in similarly obese subjects. The adipose tissue (AT) response to chronic caloric overload (hypertrophy *vs.* hyperplasia) is a major determinant of susceptibility to IR. TCF7L2 is a key transcription factor involved in WNT signalling, a developmental pathway, which has a central role in AT biology. A common SNP in TCF7L2 (rs7903146) is the strongest genetic determinant of T2D risk in humans with the risk being higher in lean *vs.* obese subjects. We hypothesised that TCF7L2 modulates T2D risk partly *via* effects on AT biology.
Materials and methods: *In vitro* functional studies in primary and immortalised human adipose progenitor cells (APCs) and AT phenotyping in rs7903146 risk variant carriers.
Results: *Ex vivo TCF7L2* expression was higher in APCs compared to mature adipocytes (mADs) ($p < 0.001$, $n = 35–49$) and adipose endothelial cells ($p < 0.01$, $n = 5–6$) in both abdominal and gluteal depots. *Ex vivo TCF7L2* expression corelated positively with BMI in abdominal ($p = 0.001$, $R^2 = 0.28$, $n = 35–50$) and gluteal ($p = 0.04$, $R^2 = 0.12$, $n = 35–50$) APCs but not in mADs. Stable TCF7L2 knockdown (KD) with two independent shRNAs (low and high efficiency) in immortalised human abdominal APCs led to impaired proliferation ($p < 0.01$) and a dose-dependent increase in WNT signalling ($p < 0.001$) both basally and following WNT3a treatment. Notably, adipogenesis was enhanced ($p < 0.001$) with low efficiency TCF7L2 KD whilst being impaired ($p < 0.001$) with high efficiency TCF7L2 KD in both immortalized and primary human abdominal APCs. AT phenotyping showed reduced *ex vivo* TCF7L2 mRNA ($p = 0.015$, $n = 15–29$) and protein ($p = 0.04$, $n = 5–10$) levels *selectively* in abdominal APCs of T2D risk allele (T) carriers. Accordingly, *in vitro* reporter assays for *cis*-regulatory activity at rs7903146 revealed that the T2D risk allele (T) abrogates a weak enhancer in abdominal APCs (C *vs.* T, $p < 0.001$). Lastly, compared with homozygous carriers of the C allele, individuals homozygous for the T2D risk allele (T) displayed altered adipocyte size distribution in abdominal AT ($p < 0.001$, $n = 9–25$).
Conclusion: These results implicate TCF7L2 in human adipose progenitor biology, AT plasticity and susceptibility to T2D.
Supported by: Oxford-Novo Nordisk Postdoctoral Research Fellowship (MV)

Disclosure: **M. Verma:** Grants; This work was supported by Oxford-Novo Nordisk Postdoctoral Research Fellowship (MV). The funders had no role in study design, analysis or reporting of the current work.

23

Pros and cons of gastric bypass surgery in obese individuals with type 2 diabetes: nationwide, matched, observational cohort study

V. Liakopoulos, S. Franzén, A.-M. Svensson, M. Miftaraj, J. Ottosson, I. Näslund, S. Gudbjörnsdottir, B. Eliasson;
Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden.

Background and aims: Long-term effects of gastric bypass (GBP) surgery have been presented in observational and randomized studies, but there is still only limited data for obese persons with type 2 diabetes (T2DM), in particular regarding postoperative complications. We investigated postoperative outcomes after GBP in a nation-wide cohort.
Materials and methods: In this observational study, we merged data from the Scandinavian Obesity Surgery Registry, the National Diabetes Register and national databases. We matched persons with type 2 diabetes who had undergone GBP with persons not surgically treated for obesity, based on sex, age, BMI and propensity score. The risks of postoperative outcomes were assessed using Cox regression model adjusted for sex, age, BMI and socioeconomic status.
Results: 5321 T2DM patients who had undergone GBP and 5321 control persons were followed for up to 9 years. We confirm lower risks of all-cause mortality (49%) and cardiovascular disease (34%), found positive effects on severe kidney disease, but also demonstrate significantly increased risks (2 to 9-fold) of several short-term complications after GBP. There were higher rates of abdominal pain and gastrointestinal conditions frequently requiring additional surgical procedures, apart from reconstructive plastic surgery. Long-term, the risk of anemia was 92% higher, malnutrition appeared approximately 3-fold more often, while psychiatric diagnoses were 33% increased, and alcohol abuse was 3-fold higher than in the control group.
Conclusion: This nation-wide study confirms the benefits but also describes the panorama of adverse events after bariatric surgery in obese persons with T2DM. In order to maximize the benefit and minimize the risk of unfavorable results after bariatric surgery, a thorough and long-term follow-up and support of these patients seems paramount. Better selection of patients for such treatment could probably also improve results.
Supported by: SF, AMS, MM, JO, IN, SG, BE
Disclosure: **V. Liakopoulos:** None.

24

Differing gut hormone responses drive weight loss after Roux-en-Y gastric bypass and sleeve gastrectomy, with similar effects on glucose dynamics and insulin sensitivity

C. Liaskos[1,2], C. Koliaki[1], K. Alexiadou[1], I. Mourouzis[3], C. Pantos[3], G. Argyrakopoulou[1], A. Alexandrou[4], N. Katsilambros[1], T. Diamantis[4], N. Tentolouris[1], A. Kokkinos[1];
[1]First Department of Propaedeutic Medicine, School of Medicine, National and Kapodistrian University of Athens, Laiko General Hospital, Athens, Greece, [2]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine University, Düsseldorf, Germany, [3]Department of Pharmacology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [4]First Department of Surgery, School of Medicine, National and Kapodistrian University of Athens, Laiko General Hospital, Athens, Greece.

Background and aims: Bariatric surgery, the most effective method for the long-term treatment of class III obesity, exerts beneficial effects on glucose metabolism. Possible underlying mechanisms include massive loss of fat mass, restricted caloric intake and changes in gut hormones. Aim of our study was to compare the effects of Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) on glucose, insulin, ghrelin, PYY and GLP-1 levels.
Materials and methods: We recruited 28 obese patients, of which 11 underwent RYGB (age: 38.6 ± 8.2 years; BMI: 48 ± 6 kg/m^2) and 17 SG (age: 41.3.6 ± 8.1 years; BMI: 50.7 ± 7.3 kg/m^2, p = NS vs RYGB)]. They were examined preoperatively, as well as 3, 6, and 12 months after surgery. Blood samples were drawn before, and 30, 60, 90, 120, 150 and 180 min after consumption of a mixed meal for the measurement of glucose, insulin, ghrelin, PYY and GLP-1. Insulin resistance was estimated with the HOMA-IR index. Postprandial responses were expressed as area under the curve (AUC).
Results: There were no preoperative differences between groups in any of the parameters. Both experienced significant and comparable weight loss (BMI 12 months RYGB: 30.8 ± 5.2 vs SG: 34.4 ± 6.7 kg/m^2, p = NS). Glucose AUCs were reduced 6 ($p \leq 0.007$) and 12 months ($p \leq 0.002$) after both procedures, with no difference between groups (Glucose AUC 12 months RYGB: 15858.3 ± 1700 vs SG: 16750 ± 2660.3 mg min/dl, p = NS). Both operations led to significantly and comparably lower fasting insulin levels at all time points. HOMA-IR was profoundly decreased for both groups at all postoperative time points (HOMA-IR preop RYGB: 6.7 ± 5.6 vs SG: 8.2 ± 6.2, p = NS, and HOMA-IR 12 months RYGB: 1.6 ± 1.3 vs SG: 1.9 ± 0.9, p = NS). Fasting ghrelin decreased after SG (Ghrelin preop SG: 223.2 ± 77 vs 3 months: 129.3 ± 20.9, 6 months: 106.4 ± 15.8, 12 months: 128 ± 19.8 pg/ml, all $p \leq 0.02$ vs preop), while was increased at 12 months ($p = 0.04$ vs preop) after RYGB. Ghrelin AUC decreased at 3 months after SG ($p = 0.015$), with nonsignificant changes after RYGB. PYY AUC increased at 3, 6, and 12 months after RYGB (PYY AUC preop RYGB: 11406.7 ± 4593.9 vs 3 months: 19187.4 ± 5617.5, 6 months: 22405.8 ± 8259.2, 12 months: 24940.6 ± 9399.1 pg min/ml, all $p \leq 0.02$), and only at 3 months after SG ($p = 0.016$). GLP-1 AUC was significantly higher in the RYGB group compared to that after SG at 6 months (GLP-1 AUC 6 months RYGB: 9966 ± 2137.9 vs SG: 7507.7 ± 3011.1 pM min, $p = 0.046$).
Conclusion: RYGB and SG induce distinctly differing gut hormone responses, the first leading to a pronounced increase in PYY, and the second to a decrease in ghrelin. However, the satiety-inducing effects of both lead to comparable effects on weight loss, glucose dynamics and insulin sensitivity. It seems that weight loss *per se* is the primary driving force behind these metabolic improvements, while gut hormones play a secondary role.
Disclosure: **C. Liaskos:** None.

OP 05 Novel models for understanding complications

25

Finerenone improves the cardiovascular benefits after a return to a normal diet in the mouse model of high fat diet-induced obesity

M. Pieronne-Deperrois[1], L. Nicol[1], S. Messaoudi[2], A. Marouchtchak[1], V. Richard[1], P. Kolkhof[3], F. Jaisser[2], P. Mulder[1], A. Ouvrard-Pascaud[1];
[1]Inserm U1096, Medical school of Rouen-Normandy University, Rouen, France, [2]Inserm U1138, Cordeliers Institute, Paris 6 University, Paris, France, [3]Bayer Pharma AG, Wuppertal, Germany.

Background and aims: Patients with obesity exhibit high prevalence of left ventricular (LV) diastolic dysfunction. In a mouse model of high fat diet (HFD)-induced obesity, we assessed the benefit of a normalization of the diet in obese mice, and we hypothesized that the non-steroidal mineralocorticoid receptor (MR) antagonist Finerenone further improves heart function.

Materials and methods: Nine weeks old B6D2 male mice were fed a HFD (60% fat) or maintained on normal diet (CTL). After 16 weeks, obese mice were divided in 3 groups for 8 more weeks with either: *i)* HFD; *ii)* normal diet (HFD-STOP); *iii)* normal diet plus Finerenone 1 $mg.kg^{-1}.day^{-1}$mixed in the food (HFD-STOP+FINE).

Results: After 24 weeks of HFD in mice, blood pressure remained normal. However, compared to CTL after 16 weeks of HFD, mice showed 1) overweight and insulin resistance, 2) decreased Stroke Volume (SV) assessed by echocardiography, 3) reduced cardiac filling pressure (LV-End-Diastolic-Pressure, LVEDP: CTL 2.73 ± 0.1, HFD 4.73 ± 0.34 mmHg; $P < 0.001$) and impaired LV compliance (LV-End-Diastolic-Pressure-Volume-Relation, LVEDPVR: CTL 1.19 ± 0.26, HFD 4.77 ± 0.31 mmHg/RVU; $P < 0.001$) assessed by invasive hemodynamics, 4) reduced Coronary Reserve (CR: CTL 4.24 ± 0.71, HFD 1.27 ± 0.41 ml mg^{-1} min^{-1}; $P < 0.01$) assessed by MRI perfusion measurements and 5) reduced exercise ability in a stress-test on treadmill. After 24 weeks, HFD fed mice compared to CTL still had increased LV filling pressure, impaired LV compliance, reduced Coronary Reserve and limited exercise ability, plus worsened heart function including decreased LV fractional shortening and decreased cardiac output (CO). Switching HFD to normal diet in the HFD-STOP group from weeks 16 to 24, body weight decreased down to CTL values. In both HFD-STOP and HFD-STOP+FINE groups compared to HFD alone, diet normalization allowed improving insulin resistance, SV,CO and LV compliance, the latest being further improved by FINE (LVEDPVR: HFD-STOP 3.44 ± 0.39, HFD-STOP+FINE 2.28 ± 0.23 mmHg/RVU; $P < 0.05$). Interestingly, after diet normalization, there were raises of kidney weight and of albumin over creatinine urine ratio that were prevented by FINE (alb/creat/24hours: CTL 43.8 ± 4.5, HFD 43.5 ± 6.7, HFD-STOP 69.1 ± 7.3, HFD-STOP+FINE 44.3 ± 5.2; $P < 0.05$). Moreover, only the FINE treatment on top of diet normalization allowed improving LV filling pressure (LVEDP: CTL 2.73 ± 0.16, HFD 4.73 ± 0.34, HFD-STOP 4.53 ± 0.33, HFD-STOP+FINE 3.18 ± 0.26 mmHg; $P < 0.05$), Coronary Reserve (CR: CTL 3.76 ± 0.72, HFD 1.00 ± 0.33, HFD-STOP 1.19 ± 0.25, HFD-STOP+FINE 2.78 ± 0.67 ml mg^{-1} min^{-1}; $P < 0.05$) and total distance during the stress-test on treadmill.

Conclusion: When administered on top of diet normalization after HFD-induced obesity in mice, Finerenone improved albuminuria, led to further improvement of LV compliance and led to specific improvements of LV filling pressure and Coronary Reserve, likely contributing to improved performance in the running stress-test.

Supported by: Bayer-Pharma AG, Wuppertal, Germany and Cost-Admire BM1301

Disclosure: **M. Pieronne-Deperrois:** Grants; Bayer Pharma AG, Wuppertal, Germany and COST-Admire BM 1301.

26

Characterisation of an animal model of type 2 diabetes with potential application in the evaluation of new therapies for diabetic neuropathy

F. Ezquer[1], C. De Gregorio[1], D. Contador[1], D. Santapau[1], M. Campero[2], M. Ezquer[1];
[1]Center for Regenerative Medicine, School of Medicine, Universidad del Desarrollo, Santiago, [2]Department of Neurology and Neurosurgery, Hospital Clínico José Joaquín Aguirre, Universidad de Chile, Santiago, Chile.

Background and aims: Diabetic Neuropathy (DN) is one of the most common clinical complications of diabetes affecting up to 60% of diabetic patients. DN is characterized by progressive, distal-to-proximal degeneration of peripheral nerves, affecting both sensory and motor fibers. Evidences from diabetic patients suggest that reduced availability of neuroprotective factors in the nerves in combination with a chronic pro-inflammatory microenvironment contribute to the pathogenesis of DN. Nowadays, there is no effective clinical treatment for DN. Therefore; the generation of new therapeutic alternatives is highly desirable. Different animal models of Type 2 Diabetes Mellitus (T2DM) have been used to study the progression of nerve dysfunction in DN. However, a complete characterization of all functional and structural alterations present in these models and the kinetics of their appearance are lacking. The aim of this work was to characterize the main functional, structural and electrophysiological alterations present in one of the most commonly used animal model of T2DM

Materials and methods: Leptin receptor deficient mice (BKS.Cg^{m}+/+ $Lepr^{db}$/J) (BKS *db/db*) spontaneously develop severe obesity and chronic hyperglycemia at 4 weeks of age. Diabetic (*db/db*) and non-diabetic (*db/+*) mice were analyzed from 4 to 32 weeks of age to evaluate the progression of DN. To identify potential peripheral neurologic defects, we measured functional parameters including sciatic nerve conduction velocity and the responses to mechanical stimuli and noxious radiant heating in the hind paws. We correlated these functional measurements with previously described structural alterations in other DN models, including intraepidermal nerve fiber density (IENF) in the plantar surface of the hind paws, the presence of apoptotic Schwann cells in sciatic nerve and the presence of lymphocyte infiltration in sciatic nerve. The study was carried out along the principles of laboratory animal care

Results: Diabetic mice showed progressive impairments at functional level beginning at 8 weeks in withdrawal latency in plantar test and at 16 weeks in nociceptive threshold in Von Frey test. Furthermore, diabetic mice displayed decreased conduction velocity in sciatic nerve at 26 weeks of age. Based on the severity of these physiological parameters, we defined an early (18 weeks), mid (26 weeks) and late (32 weeks) phase of disease to analyze some structural parameters. Diabetic mice showed a significant reduction of IENF beginning at 18 weeks of age, an increased number of TUNEL positive Schwann cells in the sciatic nerves beginning at 26 weeks of age and a significant increase in the number of infiltrating T lymphocytes in sciatic nerves beginning at 26 weeks of age.

Conclusion: We produced a complete description of the behavioral, histological and electrophysiological parameters of an animal model of T2DM at an early, mid and late phase of the disease. We found a progressive correlation between physiological parameters with the structural defects. This animal model recapitulates many of the alterations associated to DN in humans, and these defects worsened as diabetic time increased. Therefore, it could be used at preclinical level to evaluate new therapeutic outcomes for DN patients.

Supported by: FONDECYT 1170712 to FE

Disclosure: **F. Ezquer:** None.

27

Cardiac metabolism of a lipodystrophic mouse model studied using hyperpolarised [1-^{13}C] pyruvate magnetic resonance spectroscopy

X. Teo, B. Bai, W. Chan, Y. Fu, W. Han, P. Lee;
Singapore Bioimaging Consortium, Singapore, Singapore.

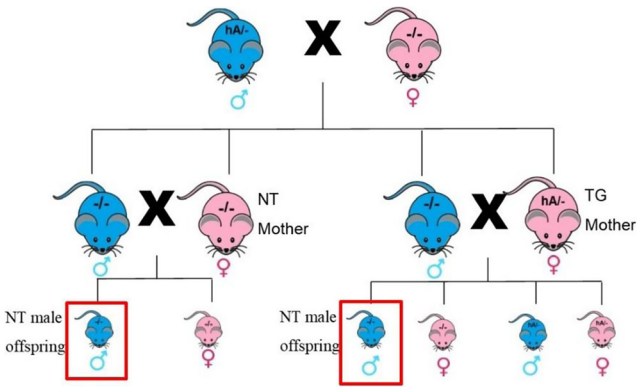
Background and aims: Berardinelli-Seip congenital lipodystrophy is a rare form of autosomal recessive disorder caused by loss-of-function mutation in BSCL2 gene that codes for *Seipin*. The phenotypes include severe insulin resistance, hypertriglyceridemia, and almost complete loss of adipose tissue. These overlap with the characteristics of type-2 diabetes. Hence, it is plausible that there would be underlying alteration in cardiac metabolism. *Seipin* knockout (SKO) mice, as a mouse model of lipodystrophy, have recently been shown to exhibit cardiac hypertrophy and dysfunction. Here, we investigated the *in vivo* cardiac pyruvate metabolism of SKO mice using hyperpolarized [1-^{13}C] pyruvate magnetic resonance spectroscopy (MRS).
Materials and methods: Twenty four weeks old SKO mice ($n = 5$) and their heterozygous or wildtype littermates as controls ($n = 5$) underwent ^{13}C MRS. The MRS experiments were performed on a 9.4 T MR scanner (Bruker Biospec), with the heart positioned on a dual ^{1}H/^{13}C a Butterfly 20 mm butterfly surface coil (Doty). [1-^{13}C] pyruvic acid was polarized in a preclinical hyperpolarizer (Hypersense, Oxford Instruments) and was neutralized with Tris/NaOH buffer for dissolution. The dissolution was injected (0.56 mmol/kg, *i.v.*) into the animals under isoflurane anaesthesia, followed by simultaneous acquisition of cardiac ^{13}C MR spectra for 2 minutes immediately after injection. A typical *in vivo* hyperpolarized cardiac ^{13}C MR spectra shows [1-^{13}C] pyruvate (170.8 ppm) and [1-^{13}C] pyruvate hydrate (179.1 ppm), and the downstream metabolites: lactate (183.0 ppm), [1-^{13}C] alanine (176.4 ppm), and [1-^{13}C] bicarbonate (160.8 ppm). To assess the diabetic status of the animals, a handheld glucose meter was used to measure blood glucose concentrations immediately after blood sampling. Blood insulin levels were measured from collected serum using ELISA. Data are means ± SEM.
Results: The SKO mice had higher blood glucose than their age-matched littermates at 24 weeks old (22.4 ± 3.0 mmol/L vs 9.4 ± 0.5 mmol/L, $p = 0.0038$).The SKO mice also exhibited higher insulin levels compared with the littermate controls (93.0 ± 41.7 ng/mL vs 1.7 ± 0.4 ng/mL, $p = 0.047$). Upon injection of hyperpolarized [1-^{13}C] pyruvate, the ^{13}C label incorporation from [1-^{13}C] pyruvate into [1-^{13}C] lactate was similar in SKO mice and controls ([1-^{13}C] lactate/total carbon: 0.105 ± 0.022 vs. 0.100 ± 0.010, $p = 0.90$). The ^{13}C label incorporation into [1-^{13}C] alanine was also not different between SKO mice and controls ([1-^{13}C] alanine/total carbon: 0.030 ± 0.008 vs. 0.032 ± 0.006, $p = 0.79$). However, the ^{13}C label incorporation into [1-^{13}C] bicarbonate was 2 times higher in SKO mice than in controls ([1-^{13}C] bicarbonate/total carbon: 0.0053 ± 0.0015 vs. 0.0024 ± 0.0005, $p = 0.008$), which reveals a higher PDH activity in the SKO mice.
Conclusion: The SKO mice exhibited increased cardiac PDH flux, which is a characteristic of heart failure development. Hyperpolarized ^{13}C MRS allows measurements of enzymatic flux *in vivo*, thus making a longitudinal study with lipodystrophic SKO mice possible.
*Supported by: A*STAR Biomedical Research Council*
Disclosure: **X. Teo:** None.

28

Role of amylin oligomer in transmission of type 2 diabetes between mother and offspring

C.-L. Chuang, S. Zhang, G. Amarsingh, G. Cooper;
University of Auckland, Auckland, New Zealand.

Background and aims: Pregnancy in diabetic women is associated with an increased risk of short- and long-term adverse consequences for the fetus and mother, the most significant of which is a predisposition to the development of metabolic syndrome and Type 2 diabetes mellitus (T2DM). There is substantive evidence for deposition of aggregated human amylin (hA) in organs of T2DM patients, including pancreas, heart, kidney and brain, consistent with haematogenous spread of aggregated hA from the islet. We aim to characterise a novel mechanism potentially responsible for the transmission of T2DM between the mother and offspring.
Materials and methods: hA over-expression transgenic (TG) and non-transgenic (NT) female mice as control were mated with NT male mice. NT male offspring from both TG and NT mother were subjected to more in-depth phenotypic characterization. Biochemical and physiological methods including measurements of litter-weights, litter-sizes, growth curves, blood glucose, intraperitoneal insulin tolerance test, intraperitoneal glucose tolerance test, and serum hormone (insulin, leptin, adiponectin) levels.
Results: NT male offspring from TG mother displayed several characteristics of T2DM. NT male offspring from TG mother weigh significantly more than NT male offspring from NT mother by 137 days of age. NT male offspring from TG mother also developed hyperinsulinaemia with insulin resistance, hyperleptinaemia with leptin resistance, and glucose intolerance between 120–240 days of age. More importantly, 70% of NT male offspring from TG mother developed diabetes, whereas none of NT male offspring from NT mother did. Moreover, they acquire this syndrome before their mothers become hyperglycaemic.
Conclusion: Non-diabetic hA over-expression TG mothers transmit T2DM to their NT offspring with high penetrance, whereas NT mothers do not, indicating amylin oligomer could potentially play a role in the transmission. The mechanism of inter-generational transmission of T2DM is vital to understand its familial clustering and increasing prevalence.



Supported by: MBIE
Disclosure: **C. Chuang:** None.

29

Pdx1-deficient zebrafish exhibit diabetes-induced altered function and structure of the pronephros and increased retinal sprouting angiogenesis

L.M. Wiggenhauser, S.J. Stoll, J. Kroll;
Department of Vascular Biology and Tumor Angiogenesis, European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

Background and aims: Diabetic microvascular complications, e.g. nephropathy, retinopathy and neuropathy, are a major cause of morbidity and quality of life decrease in the rising number of diabetes patients worldwide and therapy options are limited. Pdx1 is a transcription factor responsible for MODY 4 diabetes and necessary for pancreatic ß-cell maturation and insulin production. To enhance the available set of screening organisms for new interventions and uncover possible new mechanisms of mentioned complications, we established a new genetic animal

model in embryonic and adult zebrafish to study microvascular complications in a pathophysiological setting of type 1 diabetes mellitus by gene knockdown of *pdx1*.

Materials and methods: Pdx1-deficient zebrafish were generated using CRISPR/Cas9 mediated genome editing, targeting exon 1 of the zebrafish *pdx1* gene in the ABTL strain. Through selective breeding homozygous $pdx1^{-/-}$ mutants were generated and validated in different reporter lines via sequencing and western blot for Pdx1. To examine the effect of the *pdx1* gene knockdown transgenic fluorescent zebrafish embryos were studied by fluorescence and confocal laser scanning microscopy. The constructs Tg(hb9:GFP), Tg(fli1:EGFP) and Tg(wt1b:GFP) were utilised to investigate pancreatic, vascular and nephric changes respectively. To assess ultrafiltration fluorescence-labelled 70 kDa dextran was intracardially injected. The dextran was chosen to resemble the size of human albumin. Adult zebrafish were sacrificed for blood sugar measurements, histology and retinal preparations.

Results: $pdx1^{-/-}$ embryos show reduced pancreatic size and adult specimen have increased blood sugar values 2 hours after feeding ($P < 0.05$). Homozygous $pdx1^{-/-}$ knockdown in zebrafish leads to morphological changes of the developing pronephros and is accompanied by increased loss of dextran through the filtration barrier ($P < 0.05$). The embryonic trunk vasculature did not exhibit susceptibility to the genotypical influence. The adult retinal vasculature analysis uncovered increased sprouting angiogenesis in both heterozygous $pdx1^{+/-}$ and homozygous $pdx1^{-/-}$ zebrafish and changes in the vascular architecture ($P < 0.05$).

Conclusion: *pdx1* knockdown successfully impaired pancreas development and function in zebrafish and lead to changes in organs, which are vulnerable to microvascular complications. Increased loss of dextran in injected $pdx1^{-/-}$ zebrafish and increased retinal vascular sprouting are indicating of pathophysiological mechanisms similar to the human condition. These findings suggest that zebrafish are susceptible to *pdx1* knockdown-mediated diabetic complications in both kidney and retina and should be further evaluated as a potential research model.

Supported by: GRK1874

Disclosure: **L.M. Wiggenhauser:** None.

30

Downregulation of FKBPL influences metabolic and vascular function in experimental model of diabetes

A. Alqudah[1], R. McNally[1], N. Todd[1], K. Edgar[1], A. Short[1], D. Grieve[1], T. Robson[2], L. McClements[1];
[1]Queen's University Belfast, Belfast, UK, [2]Royal College of Surgons in Ireland, Dublin, Ireland.

Background and aims: There are currently over 400 million people living with diabetes in the world. Cardiovascular disease (CVD) is the leading cause of death globally and people with diabetes have a three-fold higher incidence of CVD. The underlying mechanisms implicated in the development of CVD in association with diabetes are linked to aberrant angiogenesis and endothelial dysfunction. FKBPL is a novel anti-angiogenic protein which has a critical role in physiological and pathological angiogenesis. While *Fkbpl* homozygous knockout mice resulted in embryonic lethality, $Fkbpl^{+/-}$ embryos were viable and developed normally but showed signs of early vascular dysfunction and leakiness. Based on these findings, we now investigate the effect of FKBPL downregulation on metabolic and vascular function in a streptozotocin (STZ)-induced diabetic mouse model.

Materials and methods: Both wild-type (WT, C57BL/6N) $Fkbpl^{+/+}$ and $Fkbpl^{+/-}$ mice were randomized between 10 and 12 weeks of age to either STZ treatment (5 consecutive STZ injections at 50 mg/kg/day) or vehicle control treatment with citrate buffer. Metabolic parameters were measured weekly. Insulin tolerance (ITT) and echocardiography tests were performed at 12 weeks of diabetes. Following 13 weeks of diabetes, organs were excised for immunofluorescent *ex-vivo* analysis. Comparisons were analyzed using one-way ANOVA.

Results: Blood glucose levels were higher in $Fkbpl^{+/-}$ diabetic mice compared to $Fkbpl^{+/+}$ diabetic controls during a period of 8 to 12 weeks of diabetes ($p < 0.05$, $n \geq 6$). Glycated haemoglobin (HbA1c) was higher in both non-diabetic and diabetic $Fkbpl^{+/-}$ mice compared to $Fkbpl^{+/+}$ controls (non-diabetic, 31 ± 0.9 mmol/mol vs. 27.25 ± 0.8 mmol/mol, $p < 0.05$, $n \geq 6$; diabetic, 88.7 ± 3.1 mmol/mol vs. 69.8 ± 3.5 mmol/mol, $p < 0.001$, $n \geq 6$). Notably, $Fkbpl^{+/-}$ non-diabetic mice gained more weight compared to $Fkbpl^{+/+}$ non-diabetic controls on a normal diet (10.92 ± 0.51 g vs. 7.05 ± 1.02 g, $p < 0.05$, $n \geq 6$). However, no differences in blood glucose levels were observed between these two groups of mice. The results of ITT at 12 weeks of diabetes showed a trend towards higher blood glucose levels in diabetic $Fkbpl^{+/-}$ mice compared to $Fkbpl^{+/+}$ diabetic controls (at 0 min, 33.3 ± 0 mmol/l vs. 29.12 ± 0.56 mmol/l, $p < 0.05$; at 120 min, 20.3 ± 3.02 mmol/l vs. 15.6 ± 1.61 mmol/l, $p = 0.09$, $n \geq 6$). This was associated with significant cardiac diastolic dysfunction, as indicated by reduced E/A ratio in both $Fkbpl^{+/+}$ and $Fkbpl^{+/-}$ diabetic mice compared to their respective controls ($p < 0.001$, $n \geq 6$), whilst E/A tended to be elevated in the non-diabetic $Fkbpl^{+/-}$ mice compared to $Fkbpl^{+/+}$ controls ($p = 0.08$, $n \geq 6$). Immunofluorescence staining of the hearts showed lower FKBPL protein expression in $Fkbpl^{+/+}$ diabetic mice compared to non-diabetic controls (mean fluorescence intensity: 0.12 ± 0.03 vs. 1 ± 0.17, $p < 0.01$, $n \geq 4$). Cardiac protein expression of intercellular adhesion molecule 1 (ICAM-1), a marker of endothelial dysfunction, was higher in diabetic animals as well as in $Fkbpl^{+/-}$ non-diabetic mice compared to $Fkbpl^{+/+}$ controls (mean fluorescence intensity: 1.37 ± 0.13 vs. 1 ± 0.04, $p < 0.01$, $n \geq 4$).

Conclusion: Our results suggest that FKBPL may play a key regulatory role in fat and glucose metabolism as well as irregular cardiac angiogenesis associated with diabetes. As such, FKBPL could be explored as a potential therapeutic target for prevention of cardiovascular complications of diabetes

Supported by: This project is funded by Hashemite University in Jordan

Disclosure: **A. Alqudah:** None.

OP 06 Beta cell connectivity and heterogeneity

31

Imaging Ins2 gene activity and single-cell RNA sequencing reveal heterogeneous beta cell states

H. Modi[1], H. Cen[1], S. Skovsø[1], X. Hu[1], N. Krentz[2], D. Dionne[1], S. Xuan[3], M. Huising[4], F. Lynn[1], J. Johnson[1];
[1]University of British Columbia, Vancouver, Canada, [2]University of Oxford, Oxford, UK, [3]University of Columbia, New York, USA, [4]University of California, Davis, USA.

Background and aims: Functional b-cell heterogeneity is well established and studied. Previous work from our group identified dynamic states marked by fluorescent proteins driven by the promoters of insulin and Pdx1. In order to study b-cell heterogeneity of insulin production with more accuracy, we turned to an *Ins2*^{GFP} knock-in/knockout mouse line, and other knock-in alleles. Here, our aims were to characterize heterogeneous b-cell states using mice and cells expressing the *Ins2*^{GFP} knock-in allele over time using live-cell imaging and single-cell RNA sequencing technology.
Materials and methods: Based on our preliminary data showing heterogeneity of GFP expression in the *Ins2*^{GFP} knock-in islets, we crossed this line with Ins1-mCherry transgenic mice that are known to show relatively stable mCherry expression. We conducted immunofluorescence of intact pancreatic sections and FACS analysis of dispersed islets to characterize GFP abundance. Dispersed islet cells from the resulting double-mutant *Ins2*$^{GFP/wt}$:Ins1-mCherry were studied over ~3 days using ImageXpress^{MICRO} live-cell imaging systems. Single-cell RNA sequencing, using the 10X Genomics platform, was performed on FACS purified GFP (+) and GFP- (-) b-cells from younger as well as older *Ins2*^{GFP} knock-in/knockout mice.
Results: Analysis of pancreatic tissue sections from *Ins2*^{GFP} knock-in mice showed that, at any given time, only about half of all b-cells were robustly GFP-positive, suggesting that not all b-cells have active transcription at the *Ins2* locus *in vivo*. FACS analysis confirmed *Ins2* mRNA and pre-mRNA were increased in GFP-positive cells compared to negative cells (861.9 ± 110.5 vs 97.2 ± 16.3, 2.06 ± 0.45 vs 1.01 ± 0.04 respectively). *In vitro* perifusion of islets isolated from *Ins2*$^{GFP/GFP}$ knock-in/knockout mice showed reduced insulin secretion at 20 mM glucose (AUC = 44.63 ± 4.7) compared to heterozygous *Ins2*$^{GFP/Wt}$ knock-in/knockout mice (AUC = 82.5 ± 6.3) and control *Ins2*$^{Wt/Wt}$ mice (AUC = 71.7 ± 10.2). Live-cell imaging of dispersed cells from *Ins1*^{mCherry}:*Ins2*$^{GFP/wt}$ mice revealed that GFP fluorescence flashed on and off in a sub-set of cells, suggesting bursts of transcription at the *Ins2* gene locus rather than stable heterogeneity. Using Cell Profiler software and custom R scripts, we tracked individual cell GFP activity and found that 153 out of 547 cells show flickering GFP activity. Principal component analysis identified 3 distinct clusters of *Ins2* gene activity cell behaviors. Single-cell RNA sequencing on FACS purified GFP-positive and GFP-negative population from islets isolated from young as well as old homozygous *Ins2*$^{GFP/GFP}$ and heterozygous *Ins2*$^{GFP/Wt}$ knock-in/knockout mice identified significantly up-regulated (Dapl1, Npy, Pgk1) and down-regulated (Xist, Nupr1, Rbp4) genes between low-GFP and high-GFP β-cells, which further gives insight about molecular features of this b-cell state.
Conclusion: Our results demonstrate the *Ins2*^{GFP} knock-in mice are a useful tool for studying b-cell heterogeneity, state transitions and plasticity. To the best of our knowledge, our observations are the first to find a previously uncharacterized form of b-cell plasticity and/or heterogeneity. Understanding the dynamics of insulin production has relevance for understanding the pathobiology of diabetes and for regenerative therapy research.
Supported by: JDRF
Disclosure: **H. Modi:** None.

32

Loss of beta cell heterogeneity disrupts normal islet function

D. Nasteska[1], G.A. Rutter[2], Q. Zhou[3], D.J. Hodson[1];
[1]University of Birmingham, Birmingham, UK, [2]Department of Medicine, Imperial College London, London, UK, [3]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, USA.

Background and aims: Immature β-cell subpopulations such as hubs give rise to heterogeneity in the islet, although it is still unclear how this may impact function *in situ*. To explore the contribution of heterogeneity to stimulus-secretion coupling and insulin release in the intact islet, we generated a loss of heterogeneity model using a viral strategy to upregulate β-cell maturity.
Materials and methods: Islets from wild-type mice (WT) were infected with an adenoviral vector carrying the polycistronic construct for *Pdx1, MafA, Ngn3* and *mCherry* (Ad3-NPM). Control islets (CT) were non-infected or treated with control virus (Ad-PATagRFP). Short hairpin RNA (shRNA) and corresponding scrambled control was used for knock-down of *Pdx1*. Gene expression was confirmed by qPCR and proteins detected by immunohistochemistry (IHC). High-speed spinning disk microscopy, coupled with biosensors/organic dye application was used for measuring Ca^{2+} fluxes, cAMP and ATP/ADP. HTRF assay was used to measure insulin secretion after stimulation with glucose and incretin mimetic.
Results: Forty eight hours post-infection (Ad3-NPM), *Pdx1* and *MafA* expression levels were increased (*Pdx1:* 9.9-fold *vs* CT, $p < 0.01$; *MafA:* 2.2-fold *vs* CT, $p < 0.01$), while *Ngn3* showed no change. *Pdx1* overexpression, as assessed by IHC, was largely restricted to Pdx1^{low} β-cells, inducing homogeneity across the β-cell population. The balance of the islet endocrine populations was unaffected, as concluded by normal α/β and δ/β ratios and normal α-specific gene expression (*Arx* and *Pax6*). Loss of heterogeneity impaired both glucose-stimulated (8.7 *vs* 5.2% content, CT *vs* Ad3-NPM; $p < 0.01$) and incretin-stimulated insulin secretion (56 *vs* 29% content, CT *vs* Ad3-NPM; $p < 0.01$). Ca^{2+} fluxes were blunted, both during high glucose ($\Delta F = 1.34$ *vs* 0.6 AU, CT *vs* Ad3-NPM; $p < 0.01$) and incretin stimulation ($\Delta F = 0.46$ *vs* 0.28 AU, CT *vs* Ad3-NPM; $p < 0.05$). cAMP levels but not ATP/ADP ratios were significantly decreased in Ad3-NPM islets (% forskolin max = 127 vs 71.5, CT *vs* Ad3-NPM; $p < 0.05$). The proportion of hubs (12 *vs* 8% hubs, CT *vs* Ad3-NPM; $p < 0.05$) and β-cell-β-cell connectivity (12.6 *vs* 6.0%; CT *vs* Ad3-NPM; $p < 0.05$) (i.e. coordination) were reduced, accompanied by decreased gene expression of *Gjd2*, encoding the gap junction protein connexin 36 (0.75-fold *vs* CT; $p < 0.05$). A reduction of expression was also seen in *Cacn1d* and *Cacnb2* subunits of the voltage-gated Ca^{2+} channels (0.75-fold and 0.67-fold *vs* CT; $p < 0.05$). Experiments in *Pdx1*-silenced islets (aiming to increase the Pdx1^{low} population) showed similarities to many of the above-listed results. *Pdx1* expression in the islet was reduced (0.7-fold vs CT), mainly due to a reduction in the Pdx1^{high} β-cell population, and this was associated with the absence of glucose-stimulated insulin secretion. Ca^{2+} amplitude in response to high glucose showed a significant drop ($\Delta F = 0.38$ *vs* 0.21 AU, CT *vs* shRNA treatment; $p < 0.01$), together with a decrease in hub proportion (12 *vs* 6% hubs, CT *vs* shRNA treatment; $p < 0.05$) and cell-cell connectivity (13.8 *vs* 8.9%; CT *vs*shRNA treatment; $p < 0.05$).
Conclusion: Loss of β-cell heterogeneity caused by changes in β-cell maturity prevents islets from mounting appropriate responses to stimuli. Cellular diversity within the beta complement thus appears to be an essential part of islet physiology.
Supported by: Diabetes UK, MRC, ERC
Disclosure: **D. Nasteska:** Grants; MRC, ERC, Diabetes UK.

33

Differential beta cell coupling patterns drive biphasic activity

M. Jaffredo[1], A. Pirog[2], É. Bertin[1], B. Catargi[3], S. Renaud[2], J. Lang[1], M. Raoux[1];

[1]CBMN, CNRS UMR 5248, Univ. Bordeaux, Bordeaux, [2]IMS, CNRS UMR 5218, Bordeaux INP, Univ. Bordeaux, Bordeaux, [3]CHU de Bordeaux, Hôpital Saint-André, Bordeaux, France.

Background and aims: After food intake, pancreatic islets secrete insulin with a biphasic pattern, which is impaired in type 2 diabetic patients. The mechanisms underlying this pattern have not been fully elucidated and the presence of distinct vesicle pools has been proposed as explanation. Electrical activity of islets consists of individual β cell activity (action potentials, APs) and the multicellular electrical response due to coupling between β cells (slow potentials, SPs). We addressed here the contribution of these two distinct activities to the 1st and the 2nd phase of β cell activity, and their modulation by physiological concentrations of GLP-1.
Materials and methods: Electrical activity (SPs and APs) of entire mice (C57Bl6/J, age 10–14 weeks) or human islets have been recorded on polymer-coated microelectrode arrays (MEA). These new electrodes allow simultaneous detection of APs (of very low amplitude) and SPs at a high time resolution (10'000 points/s x60 electrodes) for a prolonged period mimicking physiological digestion (2 h). Specific filters differentially detect SPs and APs and 3 parameters were analyzed at the same time: SP frequencies, SP amplitudes and AP frequencies. To investigate synchrony of SPs between different regions of the same islet, we used high density MEAs with an inter-electrode distance of 30 instead of 200 μm followed by analysis *via* Matlab.
Results: Islets were stimulated with glucose concentrations in the physiological range (5.5–8.2 mM). Electrical responses were biphasic for both SPs and APs. APs were mainly present during the 1st phase while the transition between the 1st and the 2nd phase is driven by SPs. In 2nd phase, the SP amplitude and synchronisation increased significantly (1st phase: 18.1 ± 2.3 μV; 2nd phase: 47.4 ± 5.5 μV, $p < 0.0001$), reflecting further electrical coupling and synchronisation of β cells. The intra-islet synchronisation was also further correlate using high density MEAs. The incretin GLP-1, at a physiological postprandial concentration (50 pM), did not change the individual activity of cells (APs) but increased specifically coupling (SPs) and only in the 2nd phase (37.7 ± 3.0 μV *vs* 47.0 ± 4.2 μV with GLP-1, $p < 0.0001$). Furthermore, when GLP-1 was applied in the presence of a subthreshold glucose concentration (5.5 mM), the hormone triggered only a 2nd phase. The biphasic electric profile was confirmed in human islets. Their exposure to a glucotoxic medium (20 mM glucose, 65 h) considerably increased basal activity and abolished the biphasic response as well as the discrimination between glucose concentrations. These glucotoxic effects were partially reversible.
Conclusion: Our data show that (i) electrical activity pattern shape the biphasic secretion and (ii) the transition period between the 1st and the 2nd phase results from increasing electrical synchronisation. Thus biphasic secretion is primarily dictated by changes in electrical activity rather than vesicle pools. The effects of GLP-1 on only coupling SP signals and only during the 2nd phase explain its clinical effects.
Supported by: ANR, Région Nouvelle Aquitaine - FEDER
Disclosure: **M. Jaffredo:** None.

34

Glucose regulates pancreatic islet beta cell calcium dynamics and intercellular connectivity in vivo

V. Salem[1], L. Delgadillo Silva[2], K. Suba[1], N. Akhtar[3], N. Mousavy[1], A. Martin-Alonso[1], E. Georgiadou[1], D.C.A. Gaboriau[4], S.M. Rothery[4], T. Stylianides[3], D.J. Hodson[5], P. Marchetti[6], L. Briant[7], N. Ninov[2], G.A. Rutter[1];
[1]Department of Medicine, Imperial College London, London, UK, [2]DFG-Center for Regenerative Therapies, Technische Universität Dresden, Dresden, Germany, [3]Loughborough University, Loughborough, UK, [4]Imperial College London, London, UK, [5]University of Birmingham, Birmingham, UK, [6]University of Pisa, Pisa, Italy, [7]Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

Background and aims: The coordinated function of pancreatic islet β cells is believed to be essential for the efficient release of insulin in response to elevated glucose concentrations. Whilst this behaviour has been demonstrated *in vitro* in isolated islets, its existence *in vivo*, where the islet is perfused and receives neural inputs, has not previously been examined. Here, we use the recombinant Ca^{2+} sensor GCaMP6 to explore connectivity in three living animal models: the zebrafish *D. rario*; in murine islets transplanted into the anterior chamber of the C57BL6 mouse eye (ACE), and in human islets engrafted in the ACE of immunodeficient *nu/nu* mice.
Materials and methods: *D. rario* bearing GCaMP6s and nuclear mCherry transgenes under the insulin promoter were immobilized and imaged on a Zeiss 780 laser-scanning confocal microscope (40x dipping objective, 488 nm illumination; 0.1 Hz data acquisition). Glucose manipulations were made through the bath or via direct intracardiac injection. Mouse (C57Bl6, Ins1Cre:GCaM6m$^{f/f}$) or human islets infected with adenovirus expressing GCaMP6m, were injected into the ACE then imaged 3–4 weeks later under isofluorane anaesthesia. Insulin or glucose were infused either via tail vein or IP to achieve "low glucose" concentrations (<6 mmol/l) or "high glucose" concentrations (>20 mmol/l). Data were collected on a modified Nikon Ti-E spinning disc confocal microscope (20x, 0.75 NA water immersion objective; 1–3 Hz). Corrections for movement, using nuclei as landmarks, were performed off-line using Fiji, and Ca^{2+} traces analyzed in manually-defined cytosolic regions with Image J and Igor.
Results: In each of the three systems, significant β cell Ca^{2+} dynamics existed under basal conditions. In zebrafish, these were rapidly abrogated upon glucose lowering via insulin injection or following the temporal inhibition of blood flow. The amplitude of the observed oscillations, and the degree of connectivity between individual β cells, were both increased in response to increases in circulating glucose concentrations. Examined in larger (>200 μm) mouse islets, Ca^{2+} waves often began at sites remote from capillaries, consistent with the existence of hub/pacemaker cells which serve as initiators of the waves. Increasing glucose concentrations augmented the proportion of connected β cells in zebrafish islets from <10 to 75 ± 9% ($p < 0.001$) of all cells, and correlation strength (R) from 0.15 ± 0.03 to 0.74 ± 0.1 ($p < 0.001$; $n = 6$ animals). Equivalent values for mouse islets were 65 to 86% ($n = 5$; $p = 0.02$) and R-values 0.34 ± 0.07 to 0.46 ± 0.08 ($n = 5$; $p = 0.05$). For human islets, elevated blood glucose increased connectivity from 58.3% to 63.9% ($n =$ 1 female, age 54, BMI 24.5).
Conclusion: These studies demonstrate glucose-regulated intercellular connectivity between β cells *in vivo*, and provide evidence for important hierarchical β cell behavior involving "hubs" and "followers" in initiating and sustaining Ca^{2+} dynamics and insulin secretion.
Supported by: Diabetes UK, Wellcome Trust, Medical Research Council
Disclosure: **V. Salem:** None.

35

Beta-screen: non-invasive, easy to use MEA-based parallelised screening system for intact islets of Langerhans

S. Schönecker[1], K.-H. Boven[1], U. Kraushaar[2];
[1]Multi Channel Systems, Reutlingen, [2]Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany.

Background and aims: Increase in blood glucose concentration leads to glucose-induced electrical activity of pancreatic beta cells resulting in insulin secretion. This electrical activity manifests in glucose concentration-dependent oscillations which can easily be recorded by microelectrode arrays (MEA). To increase the throughput of the measurements to be suitable for drug development we engineered a MEA-based parallelized recording system for primary rodent, human or stem cell derived islets of Langerhans called Beta-Screen.
Materials and methods: The recording chip consisting of five electrodes allows to record electrical oscillations from intact islets of Langerhans.

Eight chips can be connected to the device which enables recording from 40 islets simultaneously. Positioning is realized by suction and 8 chambers can be perfused independently. Intact murine Islets of Langerhans or islets isolated from human biopsies were used for recording. Electrical activity was recorded at 37°C as field potentials and quantified as fraction of plateau Phase (FOPP) or as number of single spike activity (spikes/5min).

Results: The glucose concentration response curve recorded with murine islets plotted as FOPP revealed a half-maximal activity of 12.26 ± 2 mM (n = 12) which is in line with traditional methods e.g. intracellular electrodes. The application of 150 μM diazoxide inhibited (n = 13) while 300 μM tolbutamide restored electrical activity (n = 13). Human islets of Langerhans displayed typical glucose-induced activity (10 mM, n = 10). Validation studies with tolbutamide and diazoxide showed the existence of functional K_{ATP} channels. Diazoxide reduced the mean spike activity from 833 ± 339 Spikes/5 min (10 mM glucose) to 8 ± 4 spikes/5 min. The additional application of tolbutamide restored spike activity up to 876 ± 310 spikes/5 min. TTX reduced spike activity from 1047 ± 314 to 199 ± 90 spikes/5 min after the application of 300 nM TTX (n = 11). The washout led to an increase of spike activity to 779 ± 301 spikes/min (n = 11). The glucose concentration response curve of the spike activity revealed an EC50 value of 8.66 ± 3.02 mM glucose.The glucose responsiveness in human islets recorded with the Beta-Screen is in line with other reports showing that the EC50 value of human beta-cells (~6 mM) is lower than in mouse beta-cells.

Conclusion: This study shows the successful development of a parallelized MEA chip, which allows to simultaneously record electrical oscillations from up to 40 intact islets of Langerhans. Previous attempts to use electrophysiological features as readout for a higher throughput screening failed due to technical limitations. The Beta-Screen device enables for the first time acute electrophysiological medium throughput recordings of intact islets. The validation of the Beta-Screen device revealed properties of electrical activity which are comparable to literature as well as to the acute recordings obtained with the classical MEA chips. This improvement of throughput will facilitate future studies with murine, human or stem cell derived islets of Langerhans. Moreover, it will facilitate basic research, e.g. in combination with knockout mice. Importantly, the capability to also record human islets opens numerous new possibilities for this approach, e.g. the system could be used as a quality control system prior to transplantation of human islets into patients with type 1 diabetes.

Supported by: BMBF-program KMUinnovativ: BiotechnologieBioChance, 0316162A, #0316162B

Disclosure: **S. Schönecker:** None.

36

Role of the very long chain fatty acid elongase 2 (Elovl2) in the control of beta cell Ca^{2+} dynamics and connectivity

E. Georgiadou;
Medicine, Section of Cell Biology and Functional Genomics, London, UK.

Background and aims: ELOVL2 is an enzyme that synthesizes ω3-polyunsaturated fatty acids (PUFAs) including DHA (docosahexaenoic acid). Recent comparisons of multiple mouse strains have implicated *Elovl2* in the control of insulin secretion. Correspondingly, mice deleted for *Elovl2*selectively in the β-cell display impaired glucose homeostasis. In order to understand the molecular mechanisms involved, we have explored the role of a) altered Ca^{2+} dynamics and b) β-cell communication in these changes.

Materials and methods: C57Bl/6N male mice bearing floxed *Elovl2* alleles were bred to Ins1Cre animals to achieve highly selective deletion in β-cells. Animals were fed with regular chow (RC) or high fat diet (HFD, 36% fat) for 3 months. Ca^{2+} imaging of whole isolated islets was performed after loading with Fluo-8 (Stratech;10 μM), and perifusion in modified Krebs-Ringer buffer (mM: 130 NaCl, 3.6 KCl, 0.5 NaH_2PO_4, 24 $NaHCO_3$, 24 $NaHCO_3$, 1.5 $CaCl_2$, 0.5 $MgSO_4$, 10 HEPES) containing 3 mM glucose previously equilibrated with 95% O_2 and 5% CO_2 at 34–36°C. Images were captured at 0.5 Hz on a Zeiss Axiovert microscope equipped with a 10X 0.3–0.5 NA oil immersion objective, coupled to a Nipkow spinning-disk head (Yokogawa CSU-10) and illuminated at 491 nm. Connected cell numbers were determined using in house software (Igor) and the strength of correlation (R) with a Matlab (Mathworks) script.

Results: An elevation in glucose concentration from 3 to 17 mM prompted biphasic, oscillatory increases in intracellular Ca^{2+} dynamics in islets isolated from wild type (WT) animals fed on a RC diet. Conversely, both phases were reduced in islets deleted for *Elovl2*. Ca^{2+} dynamics were also reduced in islets from WT mice maintained on HFD, and loss of *Elovl2* further suppressed the response to high glucose (p < 0.01, n = 15–17 islets RC vs HFD p < 0.05, n = 24–27 islets). Responses to depolarisation with 20 mM KCl were also reduced in islets from *Elovl2* KO mice vs WT controls maintained on either diet. The number of connected cell pairs was reduced by deletion of *Elovl2* by 15% (p > 0.05, n = 27 islets) when examined at 3 mM, but not in 17mM (p > 0.05, n = 27 islets) glucose in islets isolated from mice fed on HFD. The increase in R between cell pairs at 17mM vs 3mM Glucose was reduced by *Elovl2* deletion (20% on RC and 10% on HFD).

Conclusion: These data demonstrate that *Elovl2* is required for glucose and depolarisation induced islet-wide Ca^{2+} dynamics and intercellular connectivity. This may suggest that very long chain fatty acids, including DHA, influence β-cell membrane potential or Ca^{2+} channel activity. Our observations also demonstrate that maintenance of mice on HFD reduces Ca^{2+} dynamics and connectivity and therefore, that altered ratios of unsaturated:saturated fatty acids may be an important determinant of glucose-induced insulin secretion.

Disclosure: **E. Georgiadou:** None.

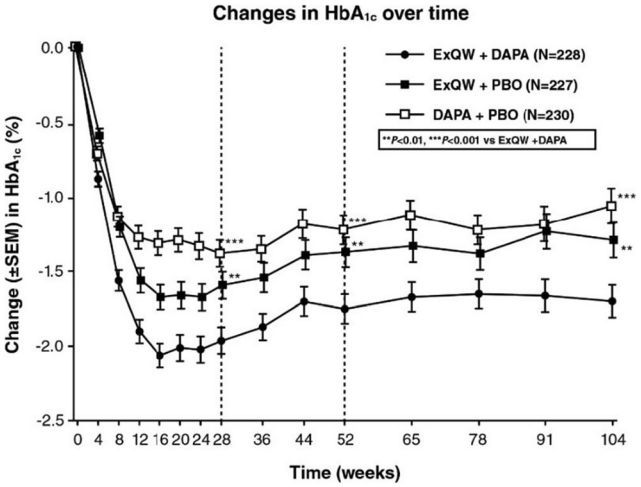
OP 07 New insights from clinical trials with incretin-based therapies

37

DURATION-8 randomised controlled trial 104-week results: efficacy and safety of once-weekly exenatide (ExQW) plus once-daily dapagliflozin (DAPA) vs ExQW or DAPA alone

E. Hardy[1], S.A. Jabbour[2], C. Guja[3], S. Bhattacharya[1], P.K. Öhman[1], J.P. Frías[4];
[1]AstraZeneca, Gaithersburg, USA, [2]Thomas Jefferson University, Philadelphia, USA, [3]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [4]National Research Institute, Los Angeles, USA.

Background and aims: In patients with type 2 diabetes mellitus (T2DM) uncontrolled on metformin alone, exenatide once weekly (ExQW) + dapagliflozin (DAPA) significantly reduced glycaemia, body weight and systolic blood pressure compared to ExQW + placebo (PBO) or DAPA + PBO at 28 weeks (DURATION-8 trial). Here, we examined the efficacy and safety of this combination after 104 weeks of double-blind therapy.
Materials and methods: In DURATION-8, adults with T2DM and inadequate glycaemic control despite stable metformin monotherapy (≥1500 mg/day) were randomly assigned to receive ExQW (2 mg s.c. injection) + DAPA (10 mg oral tablet), ExQW + PBO or DAPA + PBO for 28 weeks. Patients entered 52- and 104-week controlled extension periods where they continued to receive active treatment. HbA_{1c}, body weight and systolic blood pressure along with safety were evaluated after 104 weeks.
Results: Of 695 patients randomised, 431 (62%) completed 104 weeks; 4.3% patients withdrew due to adverse events (AEs). Absolute reductions and between-group differences in HbA_{1c} were achieved at week 28 and maintained over weeks -52 and -104 (Figure). ExQW + DAPA significantly reduced fasting plasma glucose (FPG; mg/dL) at week 28 compared with ExQW alone (least-square [LS] mean [SEM]; −20.1 [4.0]; $P \leq 0.001$) or DAPA alone (LS mean [SEM]; −16.6 [3.9]; $P \leq 0.001$), with clinically relevant results observed at week-52 and -104 (change in FPG from baseline for ExQW + DAPA vs ExQW alone and DAPA alone at weeks 52 and 104 [LS mean {SEM}]: −17.6 [4.1], −19.2 [5.9] and − 23.3 [4.0], −27.1 [6.0]; $P \leq 0.001$, respectively). Clinically relevant changes versus baseline in other efficacy endpoints (postprandial glucose, body weight and systolic blood pressure) were also observed for the ExQW + DAPA group at week 104. All evaluations at 104 weeks were exploratory. AEs and serious AEs (SAEs) were balanced across treatment groups. SAEs were reported in 7.4%, 7.8% and 7.7% of patients in the ExQW + DAPA, ExQW + PBO and DAPA + PBO groups, respectively. Hypoglycaemia incidence was low. None of the patients experienced major hypoglycaemia. Minor hypoglycaemia and other hypoglycaemic events were more frequent with ExQW + DAPA vs ExQW + PBO and DAPA + PBO (1.7% and 6.9% vs 0.0% and 3.5% vs 0.4% and 3.4%, respectively).
Conclusion: ExQW + DAPA maintained efficacy over 104 weeks with no unexpected safety concerns.



Clinical Trial Registration Number: NCT02229396
Supported by: Funding for this analysis was supported by AstraZeneca Pharmaceuticals LP.
Disclosure: **E. Hardy:** Employment/Consultancy; AstraZeneca.

38

Effect and safety of oral semaglutide monotherapy in type 2 diabetes: PIONEER 1 trial

M. Haluzik[1], J. Rosenstock[2], Y. Terauchi[3], O. Jeppesen[4], E. Christiansen[4], C.L. Hertz[4], V.R. Aroda[5,6];
[1]Institute for Clinical and Experimental Medicine, Prague, Czech Republic, [2]Dallas Diabetes Research Center at Medical City, Dallas, USA, [3]Yokohama City University, Yokohama, Japan, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Brigham and Women's Hospital, Boston, USA, [6]MedStar Health Research Institute Hyattsville, Hyattsville, USA.

Background and aims: Oral semaglutide, the first glucagon-like peptide-1 (GLP-1) receptor agonist in a tablet formulation, is in late-stage development for the treatment of type 2 diabetes (T2D).
Materials and methods: The effect and safety of oral semaglutide (3, 7, or 14 mg once daily) was assessed in this randomised, double-blind, placebo-controlled phase 3a trial in drug-naïve patients with T2D uncontrolled on diet and exercise ($n = 703$). The primary endpoint was change from baseline in HbA_{1c} at week 26. The primary estimand (treatment policy) evaluated the effectiveness regardless of trial product discontinuation or rescue medication use. A secondary estimand (hypothetical) evaluated the efficacy of trial product while on treatment without rescue medication using a mixed model for repeated measures (MMRM), and is the conventional statistical method used in many previous T2D studies.
Results: Baseline characteristics were balanced between treatment groups: approximately 49% of patients were female, mean age was 55 years and mean duration of diabetes was 3.5 years. Oral semaglutide resulted in clinically meaningful reductions in both HbA_{1c} (all doses)

and body weight (higher doses) at week 26 (Table). Adverse events (AEs) occurred in 58%, 53% and 57% for 3, 7, and 14 mg oral semaglutide, respectively, and 56% with placebo. The most common AE with oral semaglutide was transient mild or moderate nausea. Nausea occurred in 5–16% of patients with oral semaglutide vs 6% with placebo.

Conclusion: This trial represents the first phase 3 demonstration of the effect and safety of an orally administered GLP-1 receptor agonist. In conclusion, oral semaglutide demonstrated superiority vs placebo in reducing HbA_{1c} (all dose levels) and body weight (14 mg) and, consistent with the GLP-1 receptor agonist class, it was well tolerated in T2D uncontrolled on diet and exercise.

	Oral semaglutide 3 mg (n=175)		Oral semaglutide 7 mg (n=175)		Oral semaglutide 14 mg (n=175)		Placebo (n=178)	
Baseline HbA_{1c}, %-points	7.9		8.0		8.0		7.9	
Baseline body weight, kg	86.9		89.0		88.1		88.6	
ENDPOINTS AT WEEK 26 BY PRIMARY AND SECONDARY ESTIMANDS								
	PRIMARY	SECONDARY	PRIMARY	SECONDARY	PRIMARY	SECONDARY	PRIMARY	SECONDARY
Change from baseline in HbA_{1c}, %-points ± SE (primary endpoint)	-0.9 ± 0.1	-0.8 ± 0.1	-1.2 ± 0.1	-1.3 ± 0.1	-1.4 ± 0.1	-1.5 ± 0.1	-0.3 ± 0.1	-0.1 ± 0.1
HbA_{1c} (%-points) treatment difference vs placebo [95%CI]	-0.6[a] [-0.8; -0.4]	-0.7[a] [-0.9; -0.5]	-0.9[a] [-1.1; -0.6]	-1.2[a] [-1.5; -1.0]	-1.1[a] [-1.3; -0.9]	-1.4[a] [-1.7; -1.2]	–	–
Change from baseline in body weight, kg ± SE (secondary confirmatory endpoint)	-1.5 ± 0.3	-1.7 ± 0.3	-2.3 ± 0.4	-2.5 ± 0.3	-3.7 ± 0.3	-4.1 ± 0.3	-1.4 ± 0.3	-1.5 ± 0.3
Weight (kg) treatment difference vs placebo [95%CI]	-0.1 [-0.9; 0.8]	-0.2 [-1.0; 0.6]	-0.9 [-1.9; 0.1]	-1.0* [-1.8; -0.2]	-2.3[a] [-3.1; -1.5]	-2.6[a] [-3.4; -1.8]	–	–
Proportion of subjects with HbA_{1c} <7%, %	55.1[a]	59.1[a]	68.8[a]	71.9[a]	76.9[a]	80.3[a]	31.0	33.8
Proportion of subjects with weight loss ≥5%, %	19.6	21.3	26.9*	28.7*	41.3[a]	44.3[a]	14.9	15.7

n, number of randomised subjects in the full analysis set; SE, standard error; *p<0.05; [a]p<0.001 vs placebo. Baseline data are means. Changes and treatment differences are LSmeans; proportions are observed. The primary and confirmatory secondary endpoints were controlled for multiplicity for the primary estimand. The primary estimand (treatment policy) was evaluated by a pattern-mixture model using multiple imputation to handle missing data. The secondary estimand (hypothetical) was evaluated by a MMRM.

Clinical Trial Registration Number: NCT02906930
Supported by: Novo Nordisk A/S
Disclosure: **M. Haluzik:** Employment/Consultancy; Novo Nordisk, Eli Lilly, Sanofi Aventis, AstraZeneca, Novatin. Grants; Eli Lilly, BMS, AstraZeneca. Lecture/other fees; Novo Nordisk, Eli Lilly, Novartis, Sanofi, Aventis, Johnson & Johnson, AstraZeneca, Novatin, Medtronic, Mundipharma.

39

Common variants in the GLP-1 receptor are associated with glycaemic response to GLP-1 receptor agonists in observational and large RCT data: an IMI-DIRECT study

A.Y. Dawed[1], A. Mari[2], T.J. McDonald[3], N.R. Robertson[4], A. Mahajan[5], M. Walker[6], S. Gough[7], K. Zhou[1], I. Forgie[1], H. Ruetten[8], I. Pavo[9], S.G. Pillai[9], A.G. Jones[3], E.R. Pearson[1], for the DIRECT consortium;
[1]Molecular and Clinical Medicine, University of Dundee, Dundee, UK, [2]CNR Institute of Neuroscience, Padua, Italy, [3]NIHR Exeter Clinical Research Facility, University of Exeter and Royal Devon and Exeter Hospital, Exeter, UK, [4]Molecular and Clinical Medicine, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, [5]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, [6]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK, [7]Oxford Centre for Diabetes Endocrinology and Metabolism, NIHR Oxford Biomedical Research Centre, Oxford, UK, [8]Sanofi-Aventis Deutschland GmbH, TMED, Frankfurt, Germany, [9]Eli Lilly Research Laboratories, Indianapolis, USA.

Background and aims: Glycaemic response to GLP-1 Receptor Agonist (GLP-1RA) treatment varies markedly among patients with Type 2 Diabetes (T2D) yet the mechanism for this variation is uncertain. Common missense variants in the *GLP-1R* have previously been reported to alter GLP-1 mediated insulin secretion. We aimed to investigate how variants in the *GLP-1R* alter glycaemic response to the GLP-1RA in a locus wide meta-analysis.

Materials and methods: We performed a meta-analysis using data from four observational cohorts (DIRECT, PRIBA, GoDARTS and PROMASTER) in 1,238 subjects on liraglutide or exenatide and two randomized clinical trial cohorts from the AWARD (1,554 subjects from 5 trials on dulaglutide, liraglutide or exenatide) and the HARMONY (1,771 subjects from 7 trials on albiglutide or liraglutide) studies. In total, 4,563 T2D subjects were followed-up for 6 months after initiation of GLP-1RA. The association of variants in the *GLP-1R* region with reduction in glycated haemoglobin (HbA1c) after treatment were assessed using multiple linear regression assuming additive mode of inheritance.

Results: Gly168Ser (rs6923761) and rs2268640 were independently associated with reduction of GLP-1RA to lower HbA1c (Gly168Ser β (HbA1c change per allele) = −0.09%, $p = 3.59e^{-05}$, rs2268640 β per G allele = −0.10%, $p = 2.52e^{-07}$). The allele frequency for Gly168Ser and rs2268640 in a Caucasian population was 0.33 and 0.34, respectively. We then derived a genetic risk score, summing up these two variants from the HARMONY trials. The 43% of the population of HARMONY who carry no risk allele in either of the variants had a mean (SEM) HbA1c reduction of 0.99% (0.03) in response to GLP-1RA. In contrast, 53% of the population who carry 3 or more risk alleles had a mean (SEM) HbA1c reduction of 0.84% (0.03), a difference of 0.15% ($p < 0.001$). There was no significant impact of these SNPs on weight change in response to GLP-1RA. The Gly168Ser variant was previously shown to be associated with lower β-cell surface expression of *GLP-1R* and reduced intracellular calcium mobilization. The rs2268640 variant is a *cis*-eQTL, with carriers of the G allele having reduced expression of *GLP-1R* in the pancreas.

Conclusion: We observed significant associations between common variants in *GLP-1R* gene and GLP-1RA-induced HbA1c reduction with a large multi-ethnic cohort-collection. In HARMONY, the genetically determined effect on glycaemic response explains about $1/5^{th}$ of the overall response to GLP-1RA. This suggests that genetic variants in *GLP-1R* might explain part of the variability observed in the therapeutic response to GLP-1RA.

Supported by: IMI Joint undertaking under grant agreement no 115317,
Disclosure: **A.Y. Dawed:** None.

40

Potential impact of differential drop-in of open-label diabetes medications in EXSCEL

J.B. Buse[1], M.A. Bethel[2], R.A. Patel[2], S.R. Stevens[3], S.M. Gustavson[4], Y. Lokhnygina[3], A.F. Hernandez[3], R.R. Holman[2], for the EXSCEL Study Group;
[1]UNC School of Medicine, Chapel Hill, USA, [2]Diabetes Trials Unit, Oxford, UK, [3]Duke Clinical Research Institute, Durham, USA, [4]AstraZeneca, Gaithersburg, USA.

Background and aims: Greater drop-in of open label diabetes (DM) medications occurred during the EXenatide Study of Cardiovascular Event Lowering (EXSCEL) with placebo (P) than exenatide (E). As some DM medication classes reduced cardiovascular (CV) events in other outcome trials, we evaluated whether imbalanced use of concomitant DM medications during follow up may have impacted time to event analyses for major adverse CV events (MACE3; CV death, nonfatal myocardial infarction, or nonfatal stroke) or all-cause mortality (ACM).

Materials and methods: DM medication use was recorded by drug class at each study visit. Once initiated, new medications were assumed to continue for the study duration. For medication classes where drop-in occurred in >5% of participants and for open label glucagon-like peptide-1 receptor agonists [GLP-1 RA; 3% overall), Cox hazard models were performed by randomized treatment with right censoring at the drop-in visit. Cox hazard models for MACE3 were also recalculated by modelling the impact of drop-in medication by applying effect sizes derived from published trials: HR 1.02 for insulin, 0.99 for dipeptidyl peptidase-4 inhibitors (DPP-4i), 0.88 for GLP-1 RA, and 0.85 for sodium glucose transporter 2 inhibitors (SGLT2i). E vs P HRs for MACE3 and ACM were also recalculated using inverse probability weighting (IPW), preferentially weighting accumulated outcome data for participants who did not experience drop-in of DM medications.

Results: Concomitant DM medication use did not differ between groups at baseline, but during follow-up, drop-in use was more frequent in P for

biguanide (6.1 vs 4.8%), sulfonylurea (SU; 8.8 vs 6.9%), DPP-4i (10.6 vs 7.5%), insulin (13.8 vs 9.4%), SGLT2i (5.4 vs 3.7%), and GLP-1 RA (3.6 vs 2.5%). Using censoring analyses, E vs P HRs for MACE3 were minimally altered with drop-in medication (Table) but became nominally statistically significant with SU, SGLT2i or any DM medication. For ACM, neither the HR (95% CI) nor the *p* value were altered meaningfully. Modelled E vs P HRs for MACE3 dependent on published effect sizes were 0.92 (0.84, 1.01) for DPP-4i, 0.92 (0.84, 1.01) for GLP-1 RA, 0.92 (0.84, 1.01) for SGLT2i, and 0.92 (0.84, 1.01) for insulin. After IPW, E v. P HRs were 0.89 (0.78, 1.02), $p = 0.10$ for MACE3 and 0.82 (0.64, 1.04), $p = 0.104$ for ACM.

Conclusion: Observed MACE3 and ACM E vs P effect sizes in EXSCEL were robust to several methods of adjusting for the greater drop-in of open-label DM medications in P. Lower *p* values ($p < 0.05$) were observed for MACE3 after censoring for SU, SGLT2i, or any medication, suggesting drop-in medications can influence study outcomes. *P* values were consistently <0.05 for ACM. In summary, greater drop-in of cardioprotective medications with placebo can blunt signal detection and should be considered in the design and analysis of future trials.

		Censored open-label drop-in diabetes medication						
	None	Biguanide	SU	DPP-4i	Insulin	SGLT-2I	GLP-1RA	Any
Median (IQR) follow up (yrs)	3.3 (2.3, 4.4)	2.7 (2.0 4.0)	2.5 (1.7, 3.9)	2.5 (1.6, 3.7)	2.5 (1.9, 3.8)	2.5 (1.6, 3.9)	2.5 (1.6, 3.9)	2.0 (1.3, 3.4)
MACE-3								
HR (95% CI)	0.91 (0.83, 1.00) P=0.061	0.91 (0.83, 1.00) P=0.056	0.89 (0.81, 0.99) P=0.024	0.92 (0.83, 1.02) P=0.103	0.91 (0.82, 1.00) P=0.060	0.90 (0.82, 1.00) P=0.042	0.92 (0.83, 1.01) P=0.080	0.88 (0.79, 0.98) P=0.021
Events (E/P)	839/905	792/852	756/819	763/800	770/803	766/832	766/819	648/659
ACM								
HR (95% CI)	0.86 (0.77, 0.97) P=0.016	0.86 (0.76, 0.97) P=0.017	0.85 (0.75, 0.96) P=0.012	0.87 (0.77, 1.00) P=0.043	0.87 (0.76, 0.98) P=0.029	0.86 (0.76, 0.98) P=0.023	0.87 (0.77, 0.99) P=0.034	0.84 (0.72, 0.97) P=0.016
Events (E/P)	507/584	467/533	445/509	439/482	441/481	444/504	445/498	363/378
	E/P exenatide/placebo; CI confidence interval; DPP-4i dipeptidyl peptidase inhibitor; SGLT-2i sodium-glucose co-transporter 2 inhibitor; GLP-1 RA glucagon-like peptide-1 receptor agonist							

Clinical Trial Registration Number: NCT01144338
Supported by: AstraZeneca (Gaithersburg, MD)
Disclosure: **J.B. Buse:** Employment/Consultancy; Adocia, AstraZeneca, Dance Biopharm, Dexcom, Elcelyx Therapeutics, Eli Lilly, Fractyl, GI Dynamics, Intarcia Therapeutics, Lexicon, Merck, Metavention, NovaTarg, Novo Nordisk, Orexigen, PhaseBio, Sanofi, Shenzhen HighTide, Takeda, vTv Therapeutics. Grants; AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, GI Dynamics, GlaxoSmithKline, Intarcia Therapeutics, Johnson & Johnson, Lexicon, Medtronic, Merck, Novo Nordisk, Orexigen, Sanofi, Scion NeuroStim,, Takeda, Theracos and vTv Therapeutics, NIH. Stock/Shareholding; Insulin Algorithms and PhaseBio. Other; serves on the board of the AstraZeneca HealthCare Foundation.

41

Near-normoglycaemia, with meaningful discontinuations of prandial insulin, by adding weekly albiglutide to uncontrolled basal/bolus insulin-treated type 2 diabetes

J. Rosenstock[1], A. Nino[2], J. Soffer[2], J. Mallory[2], L. Erskine[2], A. Acusta[2], J. Dole[2], M.C. Carr[2], P. Home[3];
[1]Dallas Diabetes Research Center at Medical City, Dallas, USA, [2]GlaxoSmithKline, Collegeville, USA, [3]Newcastle University, Newcastle upon Tyrne, UK.

Background and aims: The glycemic efficacy of a weekly GLP-1 RA, albiglutide (Albi) 50 mg to replace prandial insulin lispro (Lis) was evaluated in type-2 diabetes mellitus (T2DM) inadequately controlled on a multiple daily insulin regimen (≥3 injections/day).
Materials and methods: Basal/bolus insulin was optimized during a 4-week run-in phase before randomization to: 1) Albi + optimized insulin glargine (Gla), with prandial Lis subsequently discontinued by week 4 ($n = 402$) or 2) optimized Lis + optimized Gla ($n = 412$).
Results: At week 26, the LS mean ± SE change from baseline in HbA1c was −34.9 ± 0.04 mmol/mol (−1.04% ± 0.04%) vs −35.5 ± 0.04 mmol/mol (−1.10% ± 0.04%) (treatment difference 0.7 [95% confidence interval (CI), −0.5, 1.9] mmol/mol or 0.06% [95% CI, −0.05, 0.17]%; non-inferiority $p < 0.0001$) (Table). In the Albi + Gla group, 218 subjects (54%) replaced all prandial insulin without reintroducing Lis through to week 26, resulting in a total daily insulin dose reduction of 61 U. Mean number of injections was reduced from 29 to 13 (mean change ± SD: −16 ± 8) per week. GI adverse events were higher in the Albi group (26% vs 13%). Albi + Gla was favorable for severe or documented symptomatic hypoglycemia (*n*: 230 [57%] vs 309 [75%]) and weight change (LS mean ± SE: −2.0 ± 0.2 vs +2.4 ± 0.2 kg; $p < 0.0001$) vs Lis + Gla.
Conclusion: Albi meaningfully improved glucose control; prandial insulin was stopped in 54% of participants, allowing substantial reductions in insulin dose and number of injections, less hypoglycemia, and body weight loss.

Table. Summary of population and results

	Albi + Gla (n=402)	Lis + Gla (n=412)
Age (years), mean ± SD	58 ± 9.4	58 ± 9.5
BMI (kg/m^2), mean ± SD	32.1 ± 4.5	32.5 ± 4.7
HbA1c, mmol/mol (%), mean ± SD		
Baseline	61 ± 7 (7.8 ± 0.6)	60 ± 7 (7.7 ± 0.6)
Week 26	56 ± 12 (6.7 ± 0.8)	65 ± 11 (6.6 ± 0.8)
LS mean difference (95% CI) mmol/mol (%)	0.7 (−0.5, 1.9) (0.06 [−0.05, 0.17])*	
FPG mmol/L (mg/dL), mean ± SD		
Baseline	8.0 ± 2.6 /144 ± 47	7.7 ± 2.6 / 139 ± 47
Week 26 FPG[a]	5.8 ± 2.0 /104 ± 47	6.3 ± 2.3 / 113 ± 41
LS mean difference (95% CI)	−0.6 (−0.9, −0.3) / −10 (−16, −5)†	
Total daily insulin dose (U), mean ± SD		
Baseline	80 ± 29	83 ± 32
Week 26	69 ± 33	130 ± 61
LS mean difference (95% CI)	−60.83 (−66.57, −55.10)‡	
Total number of weekly injections, mean ± SD		
Baseline	29 ± 2	28 ± 0
Week 26	13 ± 8	28 ± 0
Change from baseline	−16 ± 8	0
Body weight (kg), mean ± SD		
Baseline	87.7 ± 17.3	89.6 ± 18.1
Week 26	85.7 ± 17.5	92.0 ± 18.6
Difference, mean (95% CI)	−4.4 (−4.9, −3.8)‡	
Hypoglycemia (severe or documented symptomatic)		
Participants, n (%)	230 (57)	309 (75)
Odds ratio (95% CI)	0.43 (0.31, 0.60)§	
Adverse Events (AEs), n (%)		
Serious AEs	28 (7)	34 (8)
AEs leading to discontinuation	14 (4)	9 (2)
Gastrointestinal AEs	102 (26)	53 (13)

*Non-inferiority p <0.0001; †superiority p = 0.0004; ‡superiority p <0.0001; §p <0.0001 with the nonparametric Cochran-Mantel-Haenszel test. [a]Fasting plasma glucose (FPG) at week 26 was missing for all subjects and was imputed with fasting serum glucose at week 26.

Clinical Trial Registration Number: NCT02229227
Supported by: Funding for this abstract was provided by GSK.
Disclosure: **J. Rosenstock:** Employment/Consultancy; Eli Lily and Company, Novo Nordisk Inc., Sanofi, Janssen Pharmaceuticals, Inc., Boehringer Ingelheim Pharmaceuticals, Inc., Intarcia Therapeutics, Inc.. Lecture/other fees; Eli Lily and Company, Sanofi, Janssen Pharmaceuticals, Inc., Boehringer Ingelheim Pharmaceuticals, Inc., Intarcia Therapeutics, Inc.. Other; Novo Nordisk, Inc., Merck & Co. Inc., Pfizer Inc., Sanofi, Novo Nordisk, Inc., Bristol-Meyers Squibb Company, Eli Lily and Company, Intarcia Therapeutics, Inc., Genentech, Inc..

42

Diabetes prevention with lifestyle, linagliptin and metformin in patients with prediabetes: the PRELLIM project

R. Guardado-Mendoza[1,2], L. Jimenez-Ceja[1], D. Farfan[1], M. Alvarez-Canales[1], S. Salazar-Lopez[1], M. Montes de Oca[1], F. Angulo-Romero[1], M. Reyes-Escogido[1], E. Durán-Pérez[2], A. Aguilar-García[2];
[1]Metabolic Research Laboratory, University of Guanajuato, Leon, Guanajuato, [2]Hospital Regional de Alta Especialidad del Bajío, Leon, Guanajuato, Mexico.

Background and aims: Patients with impaired fasting glucose (IFG) + impaired glucose tolerance (IGT) have a high risk to develop T2DM. Lifestyle modifications and metformin are therapeutic options in these patients. The goal of this work was to evaluate the effect of linagliptin + metformin plus a lifestyle program on glucose metabolism, insulin secretion and beta cell function in patients with newly diagnosed IFG + IGT during 12 months.
Materials and methods: Patients had a basal metabolic evaluation including oral glucose tolerance test (OGTT) with insulin measurements, body composition, lipid profile and HbA1c. Patients with IFG+IGT, age 18–65 y were randomly assigned to: *i) lifestyle program plus linagliptin 2.5 mg/metformin 850 mg twice daily (**LM group**, n = 62)*, or *ii) lifestyle program plus metformin 850 mg twice daily* (***M group***, *n* = 58), with monthly follow-up and a 6 and 12 month metabolic evaluation. Insulin sensitivity, insulin secretion and beta cell function were calculated from the OGTT. The protocol was approved by the Ethical Committee. Intergroup differences were analyzed with a T test.
Results: There were not basal differences in age (48 vs 47 y), body composition (Weight 83 vs 83 kg, visceral fat 11.8 vs 11.6 AU), glucose metabolism (fasting glucose 108 vs 105 mg/dl, 30 minutes glucose 177 vs 170 mg/dl, 60 minutes glucose 200 vs 190 mg/dl, 90 minutes glucose 185 vs 181 mg/dl, and 120 minutes glucose 170 vs 165 mg/dl), insulin sensitivity and insulin secretion between LM and M group, respectively. At 12 months both groups had an improvement in weight (−5.4 vs −3.5 kg) visceral fat (−0.87 vs −0.44 AU) and insulin sensitivity (1.1 vs 1.18), all *p* = NS; however, LM group showed a better improvement in glucose at 0′ (−16 vs −6 mg/dl, *p* 0.001), 30′(−30 vs −11 mg/dl, p 0.007), 60′(−41 vs −14 mg/dl, *p* 0.008), 90′(−36 vs −18 mg/dl, *p* 0.061) and 120 minutes (−33 vs −19 mg/dl, *p* 0.118) during the OGTT (AUCglucose_OGTT −4008 vs −1639 mg/dl/120 min, *p* 0.005), insulin secretion (AUCins/AUCgluc_OGTT 0.14 vs −0.01, *p* 0.013), acute insulin response (0.50 vs −0.01, *p* 0.003), disposition index (1.1 vs 0.43, *p* 0.007), oral disposition index (0.10 vs 0.007, *p* 0.023), and HbA1c (−0.14 vs 0.23, *p* 0.007). Adherence to medications was 92 and 91% in the LM and M group, respectively.
Conclusion: Combination of linagliptin + metformin together with a lifestyle program improved better glucose metabolism, insulin secretion and beta cell function after 12 months in patients with IFG+IGT. This could be a useful preventive strategy in patients with prediabetes and a high risk of T2DM
Clinical Trial Registration Number: NCT03004612
Supported by: Hospital Regional de Alta Especialidad del Bajío
Disclosure: **R. Guardado-Mendoza:** None.

OP 08 Diabetes gazing into the crystal ball

43

Sex hormone binding globulin and development of insulin resistance: a longitudinal study

K. Ottarsdottir[1], A.G. Nilsson[2], M. Hellgren[1], U. Lindblad[1], B. Daka[1]; [1]Department of Public Health and Community Medicine, University of Gothenburg, Gothenburg, [2]Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Gothenburg, Sweden.

Background and aims: Previous cross-sectional studies and genetic studies using Mendelian randomization principle, have shown that low sex hormone binding globulin (SHBG) concentrations are associated to the risk of developing type 2 diabetes mellitus in both men and women. However, there is a lack of prospective studies investigating the association between SHBG and insulin resistance, and therefore we aim to investigate this association in a longitudinal study in men, premenopausal women, and postmenopausal women in a Swedish cohort.
Materials and methods: In this longitudinal observational study, a sample of 2816 subjects (*M* = 1400) were randomly selected from a Swedish population between 2002 and 2005 for a cohort study with the goal to detect risk factors for cardiovascular disease at an early stage. The cohort was followed up in 2012–2014. The mean follow-up time was 9.7 ± 1.4 years and the protocol was completed in a subset of 1327 (*M* = 657) individuals.Fasting blood samples were collected at both visits. Immunoassay technique was used for measurements of SHBG. The homeostatic model assessment of insulin resistance (HOMA-Ir) was used to define insulin resistance, and the variable was log-transformed in all statistical analyses due to distribution skewness. Analyses were stratified for sex and menopausal state as reported by the participants in a questionnaire at follow-up. As there was no self-report regarding menopause at baseline, 50 years of age was used as time of menopause at baseline and stratified analyses for age ≤50 or >50 were computed. Linear regressions were computed to investigate the association between SHBG and insulin resistance both in cross-sectional and longitudinal analyses.
Results: At baseline, concentrations of SHBG were significantly inversely associated with log transformed HOMA-Ir in men ($N = 1299$, $\beta = -0.213$, $p < 0.001$), premenopausal women ($N = 852$, $\beta = -0.087$ $p = 0.003$) and postmenopausal women ($N = 427$, $\beta = -0.246$ $p < 0.001$) in a model adjusting for age, lifestyle habits, hypertension, diabetes and waist-hip ratio. Similar results were found at follow-up; in men ($N = 546$, $\beta = -0.197$ $p < 0.001$), premenopausal women ($N = 152$, $\beta = -0.292$, $p < 0.001$) and in postmenopausal women ($N = 317$, $\beta = -0.237$ $p < 0.001$). In the longitudinal analysis in men, SHBG concentration at baseline was significantly inversely associated with log transformed HOMA-Ir at the follow-up in a multivariate model including age, lifestyle habits, hypertension, diabetes and waist-hip ratio, and log transformed HOMA-Ir at baseline ($N = 585$, $\beta = -0.087$ $p = 0.028$). Furthermore, there was a significant inverse association between SHBG levels at baseline and log transformed HOMA-Ir at follow-up in premenopausal ($N = 360$, $\beta = -0.092$ $p = 0.039$) and in postmenopausal women ($N = 215$, $\beta = -0.131$ $p = 0.012$) in the fully adjusted model.
Conclusion: In this study, SHBG levels could predict the deterioration of insulin resistance in both men and women, regardless of menopausal state. This might explain the previously shown association between SHBG level and type 2 diabetes.
Supported by: The Local Research and Development Council Göteborg och Södra Bohuslän
Disclosure: **K. Ottarsdottir:** None.

44

Broad changes in body mass index between age 10 and adulthood are associated with type 2 diabetes risk independently of adult body mass index

J. Tyrrell, H. Yaghootkar, S.E. Jones, R. Beaumont, A.R. Wood, M.A. Tuke, K.S. Ruth, R.C. Andrews, T.M. Frayling;
University of Exeter, Exeter, UK.

Background and aims: Obesity is a strong risk factor for type 2 diabetes, but the condition occurs across the body mass index (BMI) range. Age, sex, ethnic differences, varying body fat distribution and genetic factors all contribute to differences in type 2 diabetes risk independently of BMI. Here, we used the UK Biobank to test the hypothesis that an additional factor, approximate change in BMI between childhood and adulthood, would contribute to type 2 diabetes risk.
Materials and methods: We used data from 371,903 individuals of European ancestry in the UK Biobank, with a measure of adult BMI, self-reported perceived relative body size at age 10 and genetic data available. First, we validated the perceived body size at age 10 by investigating the association with a BMI genetic risk score. We then stratified individuals based on their adulthood BMI into overweight and obese. Logistic regression models were used to calculate the odds of type 2 diabetes for individuals who were thin at age 10 and were either now overweight or obese in comparison to overweight or obese individuals who perceived themselves to be average or plump at age 10.
Results: Individuals in the overweight BMI range (25–30 kg/m^2) but who reported being thin, average and plump at age 10 had an average BMI of 27.2, 27.3 and 27.5 kg/m^2 respectively. Despite these very similar current BMIs, individuals who on average had moved up these broad BMI centiles were at 1.53 [95%CI: 1.44, 1.62] higher odds of diabetes than someone who had remained in an average BMI centile. Obese individuals (≥30 kg/m^2) who reported being thin, average and plump at age 10 had an average BMI of 33.6, 33.5 and 34.9 kg/m^2 respectively. Despite slightly lower current BMI, the prevalence of type 2 diabetes was highest in those people, who on average had moved up the broad BMI centiles, with individuals who were thin at age 10, average at age 10 and plump at age 10 having a type 2 diabetes prevalence of 14.6%, 11.0% and 12.3% respectively. This equated to an odds ratio of type 2 diabetes of 1.07 [95%CI: 1.01, 1.13] for the group of people moving up these broad BMI centiles compared to those staying at the same broad centile. These findings were independent of an individuals birthweight and current BMI.
Conclusion: These findings suggest that individuals who remain in higher BMI centiles throughout life may adapt to excess weight in ways that lower the risk of type 2 diabetes in comparison to individuals of similar adult BMI that have moved up the BMI centiles since childhood.
Supported by: This study was supported by the Diabetes Research and Wellness Foundation
Disclosure: **J. Tyrrell:** None.

45

GWAS study on susceptibility to bacterial infections in patients with diabetes

J.R. Simonsen[1,2], A. Käräjämäki[3], I. Toppila[1,2], E. Ahlqvist[4], V. Harjutsalo[1,2], C. Forsblom[1,2], D.M. Aly[4], T. Tuomi[1], M. Lehto[1,2], L. Groop[4], P.-H. Groop[1,2], N. Sandholm[1,2];
[1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, University of Helsinki, Finland, Helsinki, Finland, [2]Abdominal Center of Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, Helsinki, Finland, [3]Department of Primary Health Care, Vaasa Central Hospital, Vaasa, Finland, Vaasa, Finland, [4]Department of Clinical Sciences, Lund University Diabetes Centre, Lund University, Skåne University Hospital, Malmö, Sweden, Lund, Sweden.

Background and aims: Diabetes increases the risk of infectious diseases. In addition to classical autoimmune disorders, genetic variations in the human leukocyte antigen (HLA) region have been associated with increased susceptibility to specific bacterial and viral infections e.g. malaria, HIV, tuberculosis, and hepatitis. Finland - being one of the best-documented genetic isolates - is an excellent ground for genetic studies of infection related diseases. The aim of the present genome-wide association study (GWAS) is to identify genetic risk factors, which increase susceptibility to bacterial infections in patients with diabetes.
Materials and methods: For patients with type 1 diabetes (the FinnDiane Study; $n = 5{,}092$) and type 2 diabetes (Diabetes Registry Vaasa Direva; $n = 3{,}499$), nationwide register data on antibiotic drug prescription purchases (The Social Insurance Institution of Finland-KELA; www.kela.fi) and bacterial infections treated at hospital (Hospital Discharge Register; www.stakes.fi) were collected between 1995 and 2014. Each antibiotic purchase and hospitalization was counted as one infection event. The total number of infection events was counted over the follow-up period for each patient. Follow-up years prior to the onset of diabetes as well as years after the diagnosis of end-stage renal disease were excluded. DNA samples were genotyped using HumanCoreExome BeadChips in both the FinnDiane and Direva cohorts. Genotype imputation with 1000 Genomes reference panel resulted in $8.4\text{x}10^6$ and $8.6\text{x}10^6$ SNPs in FinnDiane and Direva, respectively. GWAS analyses were performed with RvTests software using score test, adjusted for mean HbA1c, duration of diabetes, and relatedness matrix, and the results from the two cohorts were combined with fixed effects meta-analysis with METAL software.
Results: Based on the FinnDiane cohort, the narrow sense heritability of this infection risk related phenotype (annual infection rate adjusted for long-term glycemic control) was estimated to be ~26%. Meta-analysis revealed a low-frequency variant (minor allele frequency 1%) associated significantly genome-wide with infection frequency ($p = 2.97 \times 10^{-9}$) at chromosome 5 near the genes *SGCD* and *TIMD4*, with altogether eight SNPs with p value $<10^{-6}$ in the locus. Furthermore, 48 SNPs from 12 loci reached a suggestive p value $<1 \times 10^{-5}$.
Conclusion: Genetic variants at chromosome 5 are associated with an increased risk of bacterial infections in patients with diabetes.
Supported by: Folkhälsan Research Foundation, Novo Nordisk Foundation
Disclosure: **J.R. Simonsen:** Grants; Novo Nordisk Foundation (#NNF14SA0003), Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation. Honorarium; P-HG has received lecture honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi.. Other; P-HG is an advisory board member of AbbVie, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi.

46

Trial data show the proposed 5 diabetes subgroups from cluster analysis do predict drug response and diabetes progression but simple clinical measures are stronger predictors

J.M. Dennis, B.M. Shields, W.E. Henley, A.G. Jones, A.T. Hattersley;
University of Exeter Medical School, Exeter, UK.

Background and aims: A recent cluster analysis of clinical and biochemical data close to diagnosis in Scandinavian patients (Ahlqvist et al) proposed 5 novel diabetes subgroups (1 autoimmune and 4 non-autoimmune). If these novel subgroups have clinical utility they should help predict the disease progression and drug response of an individual patient. We aimed to use individual data close to diagnosis from a large randomised trial to see if we could replicate the subgroups derived from the cluster analysis. We then went on to evaluate whether the cluster subgroups outperformed simple clinical characteristics in predicting drug response, disease progression and complications in the protocol driven conditions of the clinical trial.
Materials and methods: We evaluated 4,351 participants aged 30–75 with newly diagnosed type 2 diabetes in the ADOPT drug efficacy trial who were randomised to metformin, sulfonylureas or thiazolidinediones

for up to 5 years. We replicated the K-means clustering analysis of Ahlqvist et al to derive 5 patient clusters based on baseline measures of age at diagnosis, HbA1c, BMI, HOMA-IR, HOMA-B and GAD autoantibody status. We compared cluster prevalence and characteristics obtained with those previously reported by Ahlqvist et al. We then tested the predictive ability of the clusters for key patient outcomes (HbA1c response over 1 year, HbA1c progression from year 1 to 5, and 5 year risk of chronic kidney disease stage 3 (CKD, defined as 2 consecutive GFR <60 measures)), and compared results with regression models using 3 continuous routine clinical measures (age of diagnosis, baseline HbA1c and BMI).

Results: The 5 cluster derived subgroups in ADOPT replicated closely those previously reported by Ahlqvist et al as the subgroups were similar in size and had similar baseline characteristics. HbA1c response up to 1 year did vary by cluster subgroups for each drug, however the model incorporating continuous measures of age at diagnosis, baseline HbA1c and BMI had far greater predictive ability (Metformin R^2 0.24 for clusters versus 0.41 for continuous measures ($p < 0.001$), sulfonylureas R^2 0.27 vs. 0.41 ($p < 0.001$), thiazolidinediones R^2 0.17 vs 0.35 ($p < 0.001$)). There was some evidence of differential HbA1c progression by cluster ($R^2 = 0.08$) but continuous measures predicted progression similarly ($R^2 = 0.09$), with older participants progressing more slowly ($p < 0.001$). CKD occurred in 5% of participants and there were differences in risk by cluster ($p < 0.001$), but we found much better CKD risk prediction using continuous measures (C-statistic (equivalent to the area under a ROC curve) 0.63 for clusters versus 0.79 for continuous measures ($p < 0.001$)).

Conclusion: We used cluster analysis of individual data from the ADOPT trial to replicate in both prevalence and clinical characteristics the 5 diabetes subgroups reported by Ahlqvist et al. We show that in a trial setting these cluster derived subgroups do predict drug-specific response, glycaemic progression and complications, to a modest degree. However, the simple clinical measures of age at diagnosis, baseline HbA1c and BMI had much greater predictive ability. These results suggest the best guide to defining a patient's progression and drug response will be to use simple and easily obtained clinical measures and not cluster derived subgroups.

Supported by: MR/N00633X/1

Disclosure: **J.M. Dennis:** None.

47

Excess mortality and cardiovascular disease in type 1 diabetes in relation to age at disease onset: a study of 27,195 patients with diabetes

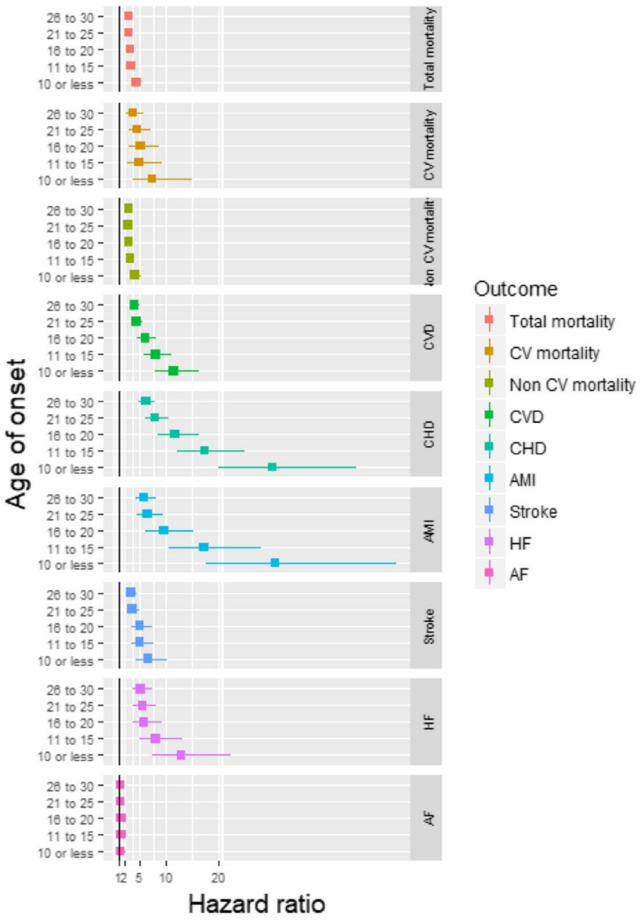
A. Rawshani[1], N. Sattar[2], S. Franzén[3], B. Eliasson[1], A.-M. Svensson[3], S. Gudbjörnsdottir[3];
[1]Institute of Medicine, Gothenburg, Sweden, [2]Institute of Cardiovascular and Medical Sciences, Glasgow, UK, [3]Swedish National Diabetes Register, Gothenburg, Sweden.

Background and aims: Age at diagnosis has emerged as an important risk marker in diabetes. This simple variable may carry valuable information on clinically important factors, pathophysiological mechanisms, age-related variations in clinical care, age-dependent differences in ability to cope with the disease *etc*. Recent studies have demonstrated that age at diagnosis can contribute to identifying subtypes of diabetes in adults, as well as predict risk factor trajectories. No study has examined how age at diagnosis relates to excess risk of death and cardiovascular (CV) outcomes, while accounting for duration of diabetes. To answer this question, we compared individuals with type 1 diabetes (T1D) to matched controls from the general population.

Materials and methods: We estimated the excess risk of all-cause mortality, CV mortality, non-CV mortality, acute myocardial infarction (AMI), stroke, CVD (composite of AMI and stroke), coronary heart disease (CHD), heart failure (HF) and atrial fibrillation (AF). Individuals with diabetes were categorized into five groups, according to age at diagnosis: 0 to 9 years, 10 to 14 years, 15 to 19 years, 20 to 24 years and 25 to 30 years. Analyses were performed using Cox regression, with adjustment for socioeconomic, demographic variables, comorbidities and duration of diabetes.

Results: A total of 27,195 persons with T1D and 135,178 matched controls were included. Median follow-up was 5.1 years; 924 patients with T1D and 1,405 controls died. We observed a remarkable association between age at diabetes onset and excess risk of death and all cardiovascular outcomes. For patients who developed type 1 diabetes before 10 years of age hazard ratio (95% CI) was 4.11 (3.24–5.22) for death, 7.38 (3.65–14.94) for CV death, 11.44 (7.95–16.44) for CVD, 30.50 (19.98–46.57) for CHD, 30.95 (17.59–54.45) for AMI, 6.45 (4.04–10.31) for stroke, 12.90 (7.30–22.51) for HF and 1.17 (0.62–2.20) for AF. Risks of these outcomes declined gradually with increasing age at onset of T1D. With the exception of AF, no hazard ratio fell below 2.0. Risk of non-CV mortality was also greatest among those with early onset of type 1 diabetes. Considering risk factor control, increasing age at diagnosis was associated with better glycemic control, higher blood pressure, higher prevalence of smoking, more physical activity, and higher socioeconomic status. Refer to Figure 1.

Conclusion: Age at onset of type 1 diabetes is a fundamental predictor of survival, as well as all cardiovascular outcomes with the exception of atrial fibrillation. Early onset type 1 diabetes is associated with up to 30 times increased risk of serious cardiovascular outcomes..



Supported by: Swedish Heart and Lung Foundation

Disclosure: **A. Rawshani:** None.

48

Determinants of progression of type 2 diabetes, a cross sectional analysis of UK BioBank

X. Wang[1,2], M. Lonergan[1], L. Donnelly[1], K. Zhou[1], E.R. Pearson[1];
[1]Molecular and Clinical Medicine, Dundee University, Dundee, UK, [2]Science for Life Laboratory, Medical Cell Biology, Uppsala University, Uppsala, Sweden.

Background and aims: The progression after a diagnosis of Type 2 Diabetes (T2D) is highly variable between individuals. We aimed to identify the factors which are associated with the progression of T2D using UK BioBank data.

Materials and methods: 25290 patients in UK Biobank have prevalent diabetes. Diabetes diagnosis type is self-reported in UK biobank so to ensure that we excluded Type 1 Diabetes (T1D) all of the participants had a T1D genetic risk score <0.2132, a robust cut off value to allow only 1% 'contamination' with T1D. We studied 6215 white European patients with T2D with duration <10 years. From these we identified 2 groups, each of 429 patients, matched for duration of diabetes. For each fast progressor (on insulin within 10 years) we identified an individual with the same duration of diabetes but who was diet treated (slow progressor). We investigated the association of clinical and biochemical factors with the risk of progression of T2D in these two groups using multiple logistic regression including enter covariates used here.

Results: When comparing the fast and slow progression groups, the fast progression group was associated with younger age at diagnosis, higher BMI, higher waist to hip ratio, more common usage of statin and fibrate. Interestingly, when we compared the family history of diabetes between the fast and slow progression groups, we observed that a maternal family history of diabetes was associated with greater odds of fast progression (OR = 1.47 [95% CI 1.01–2.12 $P = 0.033$]). There was no association with sibling or paternal family history of diabetes with progression.

Conclusion: Younger age at diagnosis, higher BMI, higher waist to hip ratio, and greater usage of statin and fibrate are associated with increased rate of progression of T2D. The higher prevalence of maternal history of diabetes in the fast progression group suggests an impact of maternal intrauterine environment on offspring diabetes progression which warrants further investigation.

Supported by: Swedish Research Council, O.E och Edla Johansson Foundation, Wellcome Trust

Disclosure: **X. Wang:** None.

OP 09 Pregnancy and gestational diabetes

49

Increasing prevalence of gestational diabetes according to the results of a population-based screening programme in Hungary between 2009–2017

A. Kun[1], E. Szabó[2,3], J. Tornoczky[4], Z. Kerenyi[5], Á.G. Tabák[3,6];
[1]Department Obstetrics and Gynecology, Tolna County Balassa Janos Hospital, Szekszard, Hungary, [2]Szent Imre Teaching Hospital, Budapest, Hungary, [3]1st Department of Medicine, Semmelweis University Faculty of Medicine, Budapest, Hungary, [4]Diabetes Care Outpatient Unit, Tolna County Balassa Janos Hospital, Szekszard, Hungary, [5]Diabetes Outpatient Clinic, Tóth Ilona Health Service, Budapest, Hungary, [6]Department of Epidemiology and Public Health, University College London, London, UK.

Background and aims: Increasing prevalence of gestational diabetes (GDM) was reported from several countries in the last decade believed to be related to the increasing age and level of obesity of pregnant women. Using data from a population-based screening from a Western county in Hungary, we investigated changes (unadjusted and adjusted for known GDM risk factors) in fasting, 1-hour and 2-hour glucose and the risk of gestational diabetes based on the WHO-2013 diagnostic criteria between 2009 and 2017.

Materials and methods: During a universal screening program in a Western Hungarian region 9,469 of 10,076 pregnant women (age 29.5 ± 5.6 years; mean ± SD) had a 75 g OGTT with the determination of fasting, 1-hour and 2-hour glucose between 16/JAN/2009 and 02/OCT/2017. Based on these OGTTs $n = 1{,}505$ (14.9%) women were diagnosed with GDM. Maternal pre-pregnancy (lifestyle, socioeconomic status, obstetrical, medical and family history) and early pregnancy risk factors (anthropometrics, blood pressure) were collected according to standardized protocols. Time trends in OGTT glucose values were investigated by multiple linear regression, GDM risk by logistic regression with adjustments for calendar time and known risk factors.

Results: Prevalence of GDM increased from 11.6% in 2009 to 15.1% in 2017. Among pre-pregnancy risk factors, maternal age, maternal smoking, positive family history of diabetes, living in a relationship and living in the county capital; among early pregnancy risk factors, body weight and systolic blood pressure were related to both the risk of gestational diabetes and showed an association with the date of the OGTT during the observation period according to univariate analysis (all $p < 0.05$) and thus were deemed potentially explanatory of the time trend in GDM prevalence. Fasting glucose showed a yearly increase of 0.019 (SE 0.002) mmol/l per year in a model adjusted for GDM risk factors without a time trend (model 1), while the addition of all potential explanatory variables except BMI (model 2) decreased this trend to 0.015 (SE 0.002), and further adjustment for BMI (model 3) to 0.008 (SE 0.002) mmol/l. The yearly increase in 1-hour and 2-hour glucose was larger (0.11 SE 0.01 and 0.053 SE 0.006 mmol/l, respectively - model 1) but the attenuation of the coefficients was much smaller in further adjustment (0.08 SE 0.01 and 0.036 SE 0.006 mmol/l, respectively - model 3) although body weight remained the most important explanatory variable. The risk of GDM increased by 9% per year (OR 1.09 95%CI 1.06–1.12 -model 1) which was attenuated to 4.8% (OR 1.048 95%CI 1.02–1.08) in model 3 with body weight being the most important explanatory variable.

Conclusion: The prevalence of GDM significantly increased in the last decade in Hungary reaching epidemic proportions based on WHO-2013 diagnostic criteria. Almost half of this increase was explained by adverse time trends in GDM risk factors (such as body weight, age, family history) however increasing levels of obesity seemed to be most important underlying factor.

Disclosure: **A. Kun:** None.

50

Early-onset gestational diabetes compared to late gestational diabetes: maternal characteristics and obstetrical outcomes in a French cohort of 2948 patients

A. Vambergue[1], A. Raynaud[2], A. Caron[1], S. Thinat[2], M. Cazaubiel[2], W. Karrouz[2], G. Ficheur[1], P. Deruelle[1];
[1]University of Lille, Lille, [2]Department of Diabetology, Lille, France.

Background and aims: Based on the IADPSG recommendations, the French guidelines suggested screening with fasting plasma glucose (FPG) test at the first prenatal visit and a 75-g OGTT between 24 and 28 weeks' gestation for gestational diabetes (GDM) diagnosis. The aim of this study was to compare the clinical characteristics and pregnancy outcomes of women with GDM who were diagnosed and treated early in pregnancy (<20 weeks of gestation) with women who were diagnosed and treated late in the pregnancy (24–28 weeks of gestation).
Materials and methods: This study was carried out in Lille between February 2011 and December 2016. Women with risk factors were screened for GDM with a FPG at the first prenatal visit and between 24–28 weeks with a 75-g OGTT using the IADPSG criteria. During this period, we diagnosed 3460 women with GDM. We analyzed the data in a cohort of 2948 women: 1445 women were diagnosed and treated early in pregnancy (<20 weeks of gestation) and 1503 women late in the pregnancy (24–28 weeks of gestation). All women were treated according to the French guidelines. We analyzed the association between time of diagnosis (early versus late) and large for gestational age (LGA) after adjustment on confounding factors as age, parity, pregestational BMI. LGA was defined above the 90th percentile of gestational age adjusted for parity, fetal sex and maternal biometrics.
Results: 41.7% of women were classified as early-onset gestational diabetes. There was no significant difference for age and parity in the two groups. Women with early-onset GDM had a higher BMI ($p < 0.001$) and were more often treated by insulin (41.2% versus 22.4% $p < 0.001$) compared to late GDM. The mean of HbA1c was significantly lower in the early-onset GDM compared to late GDM group ($p < 0.001$). The rate of LGA was not significantly different between the groups. The rate of obstetric were not significantly different between the 2 groups except a lower rate of shoulder dystocia in the early-onset GDM ($p < 0.05$). In multivariate analysis, there was not significant relationship between time of diagnosis and LGA (RR: 1.07 CI 95% 0.88–1.30).
Conclusion: In this large cohort, a high proportion of women with GDM have been diagnosed early in pregnancy. However, the time of diagnosis has no influence on the rate of LGA. There is an urgent need for randomized controlled trials that investigate any benefits and possible harms of treatment of early-onset GDM
Disclosure: **A. Vambergue:** None.

51

Pregnancy and neonatal diabetes outcomes in remote Australia: the PANDORA study

L. Maple-Brown[1], A. Brown[2], I.-L. Lee[1], F. Barzi[1], C. Connors[1], J.A. Boyle[3], E. Moore[4], C. Whitbread[1], M. Kirkwood[1], D. Longmore[1], K. O'Dea[1], J. Oats[5], H.D. McIntyre[6], P. Zimmet[3], J.E. Shaw[7];
[1]Menzies School of Health Research, Darwin, [2]South Australian Health and Medical Research Institute, Adelaide, [3]Monash University, Melbourne, [4]Aboriginal Medical Services Alliance, NT, Darwin, [5]Melbourne University, Melbourne, [6]Mater Medical Research Institute, Brisbane, [7]Baker Heart and Diabetes Institute, Melbourne, Australia.

Background and aims: In Australia's Northern Territory, 33% of babies are born to Indigenous mothers, and type 2 diabetes in pregnancy prevalence is 10 times higher than in non-Indigenous mothers. The PANDORA study is a longitudinal birth cohort recruited from a hyperglycaemia in pregnancy register. Here we assess relationships between metabolic markers in cord blood and perinatal outcomes.
Materials and methods: Data include antenatal and birth clinical information, cord blood, neonatal anthropometry. Of 1135 women (48% Indigenous, 32% Europid, 7% Indian subcontinent), 900 had diabetes: 175 type 2 diabetes (T2D), 86 newly diagnosed diabetes in pregnancy (DIP), 639 GDM. Women without hyperglycaemia in pregnancy were also recruited (NGT, $n = 235$). Glucose, lipids, c-peptide, c-reactive protein (CRP) were measured in cord blood for 723 babies (NGT = 168, GDM/DIP = 442, T2D = 113). Data were analysed using t-tests, chi-squared tests, multivariate logistic regression and mediation analysis for outcomes of birth weight z-score, large for gestational age (LGA), neonatal fat (calculated from anthropometry) and small for gestational age (SGA). C-peptide, triglycerides were log-transformed.
Results: Among those with cord blood samples, diabetes type differed for Indigenous ($n = 374$) and non-Indigenous ($n = 349$) women (T2DM, 27% vs 3%; GDM/DIP, 50% vs 73%, $p < 0.001$), with similar proportions with NGT (23% vs 23%). Indigenous women were younger and had a higher BMI. Trends were evident across diabetes types (NGT to GDM/DIP to T2D) for: increasing c-peptide [mean (95% CI), 0.35 nmol/L (0.31, 0.39), 0.39 nmol/L (0.37, 0.42), 0.66 nmol/L (0.57, 0.75), $p < 0.001$], decreasing glucose [4.4 mmol/L (4.2, 4.6), 4.1 mmol/L (3.9, 4.2), 4.1 mmol/L (3.9, 4.4), $p < 0.029$], decreasing HDL-cholesterol [0.7 mmol/L (0.66, 0.74), 0.66 (0.63, 0.68), 0.56 (0.52, 0.61), $p < 0.001$], decreasing triglycerides [0.43 mmol/L (0.40, 0.47), 0.37 (0.35, 0.69), 0.43 (0.39, 48), $p = 0.001$], increasing rates of CRP >0.3 mg/L (26%, 49%, 47%, $p < 0.001$). After adjustment for diabetes type, maternal age, ethnicity, BMI and parity; c-peptide and glucose (inverse) were independently associated with birthweight z-score, LGA and neonatal fat. Triglycerides were associated, independent of c-peptide and glucose, directly with SGA and inversely with birthweight z-score. Adjustment for medication did not change results. On pathway analysis c-peptide mediated 20% (95% CI 15%–38%) of the contribution of maternal BMI to LGA and 14% (10%–22%) for birth weight z-score.
Conclusion: In this observational birth cohort, high c-peptide was the metabolic marker that was independently associated with neonatal adiposity outcomes. The inverse relationship of cord blood triglycerides with birthweight (and direct with SGA) is consistent with previous European GDM studies, hypothesised to be related to enhanced lipoprotein lipase activity with increased adipose tissue. Longitudinal study of the cohort will inform the significance of these findings on the child's subsequent risk profile.
Supported by: Australian NHMRC #1032116, #107833
Disclosure: **L. Maple-Brown:** None.

52

Randomised controlled trial of very tight versus less tight glycaemic targets in women with gestational diabetes: preliminary results

P. Popova[1,2], A. Tkachuck[1], Y. Bolotko[1], A. Gerasimov[1], E. Pustozerov[1,3], E. Vasilyeva[1], O. Li[1], I. Zazerskaya[1], E. Grineva[1,2];
[1]Almazov National Medical Research Centre, Saint Petersburg, [2]Saint Petersburg Pavlov State Medical University, Saint Petersburg, [3]Saint Petersburg State Electrotechnical University, Saint Petersburg, Russian Federation.

Background and aims: There is wide variation in international guidelines concerning glycaemic treatment targets for women with gestational diabetes (GDM). We conducted a randomized clinical trial to assess the effect of different intensities of glycaemic control in pregnant women with GDM on maternal and infant health outcomes.
Materials and methods: We randomly assigned women who were in the 8th to 31st week of gestation and who met the World Health Organization (WHO 2013) criteria for GDM to 2 groups per target glycaemic levels: GDM1 (very tight glycaemic targets, fasting blood glucose (FBG) <5.1 mmol/L and <7.0 mmol/L postprandial) and GDM2 (less tight glycaemic targets, <5.3 mmol/L and <7.8 mmol/L, respectively). All participants were instructed on diet and lifestyle changes. In case of

exceeding the target blood glucose levels (in 2 or more measurements per week in group 1 and in more than 1/3 of measurements per week in group 2) insulin therapy was started. The primary study outcome was the incidence of large for gestational age (LGA) infants. Secondary outcomes were: for the baby - composite of neonatal death or severe morbidity (nerve palsy, bone fracture and shoulder dystocia), gestational age at birth, birthweight, macrosomia (birth weight >4000 g), small-for-gestational age (SGA) and hypoglycaemia; for the woman - pre-eclampsia, mode of birth, mean daily fasting and postprandial capillary glucose (PPG) concentration during treatment, proportion of glucose values within target, proportion of women requiring insulin therapy.
Results: The women from GDM1 ($N = 201$) and GDM2 ($N = 194$) groups did not differ in terms of age (31.9 ± 4.6 vs 31.8 ± 4.5 years, $p = 0.681$) and pre-pregnancy BMI (25.4 ± 5.4 vs 25.8 ± 6.6 kg/m^2, $p = 0.481$). We observed no significant difference between the groups in the frequency of LGA infants (15.9% and 16.5%, for GDM1 and GDM2, respectively, $p = 0.892$) using intention-to-treat analysis. There were no perinatal deaths. Between the two groups, there were no significant differences with regard to the frequency of the composite outcome (2.0% and 1.6%, $P = 1.0$), gestational age at birth (38.9 ± 1.3 vs 38.8 ± 1.5 weeks, $p = 0.223$), birthweight (3435 ± 489 vs 3396 ± 519 g, $p = 0.438$), macrosomia (13.4% and 11.9%, respectively, $p = 0.653$), SGA (9.0% and 9.3%, respectively, $p = 1.0$), hypoglycaemia (7.6% and 8.1%, $p = 1.0$), pre-eclampsia (16.8% and 16.3%, $p = 1.0$), cesarian section rate (24.5% and 30.6%, $p = 0.214$), and mean daily FBG (4.8 ± 0.4 mmol/L vs 4.9 ± 0.4 mmol/L, $p = 0.066$). GDM1 group achieved lower mean daily PPG values (6.2 ± 0.5 mmol/L vs 6.4 ± 0.6 mmol/L, $p < 0.001$). The proportion of women with glucose values within GDM1 targets was 29% and 16% in GDM1 and GDM2 groups, respectively ($p = 0.007$). The proportion of women with glucose values within GDM2 targets was 85.2% and 70.5% in GDM1 and GDM2 groups, respectively ($p = 0.002$). The proportion of women requiring insulin therapy was higher in GDM1 compared to GDM2 group (49% and 27%, $p < 0.001$).
Conclusion: Striving for very tight target glycaemic levels in women with GDM did not improve pregnancy outcomes but almost doubled the proportion of women requiring insulin therapy.
Clinical Trial Registration Number: AAAA-A16-116012210374-0
Supported by: This study was partly funded by the RSF (project no. 15-14-30012)
Disclosure: **P. Popova:** None.

53

Glyburide versus insulin for the prevention of perinatal complications of gestational diabetes: a pragmatic, non inferiority, randomised trial

F. Lorenzini[1], H. Affres[2], C. Rolland[3], A. Vambergue[4], M. Cazaubiel[4], H. Legardeur[5], P. Rosenberg[6], N. Bourcigaux[7], F. Maillot[8], A. Rod[9], I. Heron[10], V. Castera[11], S. Laboureau[12], F. Bretelle[13], M.-V. Senat[14,15];
[1]CHU, Toulouse, [2]APHP, Le Kremlin Bicêtre, [3]APHP, Clamart, [4]CHRU, Lille, [5]APHP, Colombes, [6]CH, Poissy, [7]APHP, Paris, [8]CHU, TOURS, [9]CHU Côte de Nacre, Caen, [10]CHU, Rouen Bois Guillaume, [11]Hopital Saint Joseph, Marseille, [12]CHU, Angers, [13]APHM, Marseille, [14]APHP, Le Kremlin-Bicêtre, [15]INSERM, Le Kremlin Bicêtre, France.

Background and aims: Use of oral hypoglycemic drugs in gestational diabètes (GDM) is unusual in european countries, because of results of metanalysis of 7 randomized clinical trials (RCT) comparing Glyburide and Insulin, showing an increase in macrosomia and neonatal hypoglycemia. However, primary outcome in those trials was maternal blood glucose control and not prevention of neonatal complications. Thus, we decided to compare oral glyburide and subcutaneaous insulin for prevention of perinatal complications in women with GDM requiring pharmacotherapy
Materials and methods: We conducted a non-inferiority RCT between 2012 and 2016 in France. Participants were women with singleton pregnancies and GDM diagnosed between 24 and 34 weeks. They were randomly assigned to receive Glyburide (2.5 to 20 mg/d) or Insulin after failure of 10-day well-conducted lifestyle measures.Primary outcome was a composite neonatal criterion including macrosomia, neonatal hypoglycemia or hyperbilirubinemia. Secondary outcomes were maternal glycemic control, maternal/neonatal complications, including maternal hypoglycemia, and maternal satisfaction. Non-inferiority was demonstrated if the difference in composite criteria between groups was <7%.
Results: 914 women were randomized; 18% switched from Glyburide to Insulin. 367 and 442 women and their neonates were analyzed in the Glyburide and the Insulin groups, respectively, in a per-protocol perspective. Frequency of the composite criterion was 23.4% in Insulin group and 27,6% in Glyburide group. Difference was 4.2% 95%CI [−2%; 10.5%]. RR: 1.2 [0.9; 1.5], mainly due to neonatal hypoglycemia, more frequent in Glyburide group. However, we found no difference in severity of hypoglycemia, neonatal transfer, or delivery complications. Maternal glycemic control during pregnancy was significantly better in Glyburide group, mainly for fasting glycaemia. Maternal hypoglycemia <0.4 g/l were more frequent in the Glyburide group, but decrease along the study. Women satisfaction was significantly better in the Glyburide group.
Conclusion: Non-inferiority of glyburide was not demonstrated since the non-inferiority limit was included in the confidence interval. Nevertheless, easy use of oral glyburide, small difference in frequency of the composite criterion, improved maternal glycemic control with low number of symptomatic hypoglycemia, and better maternal satisfaction , should encourage to consider glyburide as an option in treatment strategy of GDM. Prescription strategy of oral drug, including diet advice remains to be established to avoid maternal hypoglycemia.
Clinical Trial Registration Number: NCT01731431
Supported by: DRCAPHP
Disclosure: **F. Lorenzini:** None.

54

Which growth standards should be used to assess infant size in pregnancies affected by type 1 diabetes? An ancillary study from the CONCEPTt clinical trial

C.L. Meek[1], R. Corcoy[2], D.S. Feig[3], H.R. Murphy[4];
[1]Metabolic Research Laboratories, University of Cambridge, Cambridge, UK, [2]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [3]Mount Sinai Hospital, Toronto, Canada, [4]University of East Anglia, Norwich, UK.

Background and aims: Pregnant women with type 1 diabetes (T1D) are at risk of multiple complications during pregnancy and frequently give birth to infants who are large for gestational age (LGA: >90th percentile for gestational age). However, there is considerable controversy about the optimal method for assessing and comparing infant size at birth. Although neonatal growth references have been used in the past, growth standards are now in common use, which describe expected fetal growth and birthweight under optimal conditions. Examples include the INTERGROWTH and GROW data which incorporate various neonatal and/or maternal parameters to enhance the precision of the birthweight centile estimate. The CONCEPTt study identified high levels of LGA in T1D offspring. The aim of the current study was to assess the methods available for calculation of birthweight centile, and to compare which approach is most useful for infants of women with T1D.
Materials and methods: The CONCEPTt trial was a multicentre, open-label, randomised controlled trial which recruited women with T1D in pregnancy or planning pregnancy. Women monitored their blood glucose using home capillary glucose monitoring (HGM) and were randomized to receive additional continuous glucose monitoring (CGM). Participants were recruited at 31 hospitals in Europe, Canada and North America. 200 infants were born to mothers enrolled in CONCEPTt. Birthweight and anthropometry were measured by trained staff. Centiles were

calculated for each infant using GROW, INTERGROWTH and WHO standards. Rates of LGA were compared for each centile method.
Results: 200 infants were born during CONCEPTt, with mean gestational age 37.0 weeks (sd 1.7 wks) and a mean birthweight of 3564 g (sd 714 g). Using country specific data for GROW, which incorporates maternal height, weight, ethnicity and parity and infant sex and gestational age, the mean birthweight centile was 81.9, median 94.9, and 122 (61%) of infants were LGA. Using INTERGROWTH, which incorporates infant sex and estimated gestational age, the mean centile was 85.2, median 94.6, and 132 (66%) of infants were LGA. WHO standards identified only 29% of infants as LGA. Calculation of LGA using GROW standards identified 48/70 (69%) infants admitted to NICU for >24 hours and 32/43 (74%) infants with neonatal hypoglycaemia. Calculating LGA by INTERGROWTH standards, identified 51/70 (73%) infants admitted to NICU and 32/43 (74%) infants with neonatal hypoglycaemia. For SGA, GROW standards identified 3/70 infants with NICU admission and 1/43 infants with neonatal hypoglycaemia. For SGA, INTERGROWTH standards identified 2/70 infants with NICU admission and 0/43 infants with neonatal hypoglycaemia.
Conclusion: Rates of LGA varied depending upon the standards used. This is most likely due to their different approaches to preterm infants. INTERGROWTH and GROW centiles performed similarly but each has strengths and limitations when applied to the T1D population. INTERGROWTH and GROW were both able to identify similar proportions of infants at risk of complications. WHO standards do not make allowance for preterm infants which renders them unsuitable for use in T1D offspring.
Clinical Trial Registration Number: NCT01788527
Supported by: JDRF, NIHR, CCTN
Disclosure: **C.L. Meek:** None.

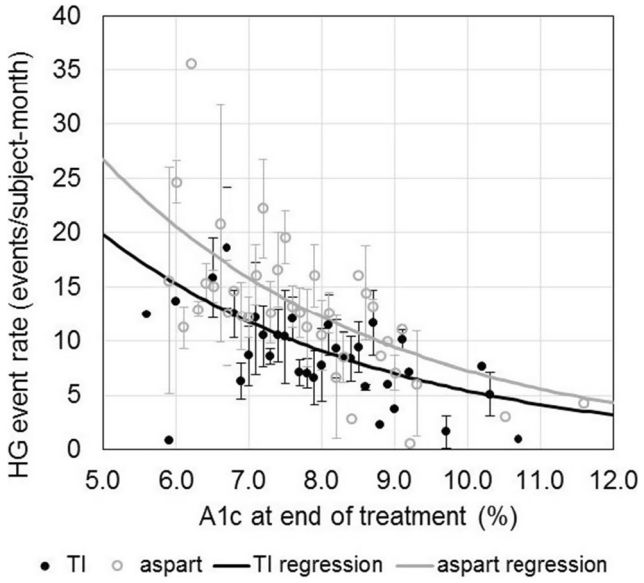
OP 10 Where, when and hows of rapid acting insulins

55

Total and severe hypoglycaemia is reduced with use of inhaled Technosphere Insulin (TI) relative to insulin aspart in type 1 diabetes

F. Pompilio[1], L. Blonde[2], S. Bruce[3], M. Grant[4], D.M. Kendall[1];
[1]MannKind Corporation, Westlake Village, [2]Ochsner Clinic, New Orleans, [3]MannKind Corporation (Clinical Development Consultant), Westlake Village, [4]MannKind Corporation, Danbury, USA.

Background and aims: Hypoglycemia (HG) and fear of HG limit effective insulin therapy and contribute to suboptimal glycemic control. Ultra-short acting insulins reduce HG risk by providing glucose-lowering effect early and reducing the risk of late post-prandial HG. AFFINITY-1, a treat-to-target study in T1D on multiple daily injection therapy, demonstrated one such ultra-short acting insulin, TI, was non-inferior to SC aspart in A1C reduction. Consistent with its action profile, a lower rate of HG was observed in TI users overall, particularly in the 2–5 h post-meal interval and in those achieving target A1C <7%. This analysis evaluates the rates of HG as a function of A1C achieved in subjects treated with TI compared to insulin aspart at the end of 24 weeks of treatment.
Materials and methods: In this post hoc analysis rates of HG were compared relative to end of treatment A1C levels in subjects treated with either TI or insulin aspart from the AFFINITY 1 study. A total of 18,706 HG events occurred in 129 subjects treated with TI and 150 subjects treated with insulin aspart. A negative binomial regression including treatment, region, type of basal insulin, and A1C at end of treatment was conducted to determine LS-mean HG rates for the two treatment groups.
Results: On average, subjects on TI experienced 30% fewer HG events than those on aspart: 6983 events in 129 subjects (54.1 events per subject) vs. 11723 events in 150 subjects (78.2 events per subject). The incidence of severe HG was similarly reduced (21.7% vs. 31.3%). Additionally, subjects on TI who reported severe HG experienced fewer events than those on aspart (59 events in 28 subjects or 2.1 severe HG events per subject during the trial) vs. 127 events in 47 subjects or 2.7 severe HG events per subject during the trial). Mean rates for all HG and severe HG obtained from combined SMBG and AE reporting were significantly lower with TI than with aspart. The negative binomial regression analysis yielded an LS-mean HG rate for patients on TI 26% lower than comparable patients on aspart across the entire A1C range (mean ratio: 0.74, 95%CI: 0.68–0.81).
Conclusion: TI's rapid onset and ultra-short action provide insulin when needed at meals and between meals. This profile improves overall and prandial glucose control and, as demonstrated in AFFINITY-1, has the potential to reduce the risk of late post-meal HG.



Clinical Trial Registration Number: NCT01445951
Disclosure: **F. Pompilio:** None.

56

Structured self-management education for insulin pump therapy (INPUT): results from a randomised controlled trial

D. Ehrmann[1], B. Kulzer[1], M. Schipfer[1], B. Lippmann-Grob[2], T. Haak[2], N. Hermanns[1];
[1]Research Institute of the Diabetes Academy Bad Mergentheim (FIDAM), Bad Mergentheim, [2]Diabetes Clinic Mergentheim, Bad Mergentheim, Germany.

Background and aims: Continuous subcutaneous insulin infusion (CSII) offers many technological features which allow users to individualise their therapy to optimise glycaemic control. However, patients need a vast amount of skills and training to effectively use these features. No structured education programme has been developed specifically for CSII-therapy that has been evaluated in a randomised controlled trial. We developed a structured self-management education program for CSII-therapy (INPUT) and evaluated its efficacy in a randomised controlled trial with a six-month follow-up.
Materials and methods: 254 patients with CSII-therapy were randomized to either receive the INPUT education programme or treatment-as-usual. All patients were already performing CSII-therapy for 8.7 ± 6.8 years, with a mean diabetes duration of 23.1 ± 12.6 years. Primary outcome was reduction in HbA1c from baseline to six months after the end of the intervention. Secondary outcomes were incidence of severe hypoglycaemic events requiring third party assistance, diabetes distress, depressive symptoms, and usage of pump features.
Results: At the six-month follow-up, the INPUT-group showed a significant reduction in HbA1c (8.33 ± 0.8 vs. 8.04 ± 0.9; $p < 0.0001$), but HbA1c in the control group remained unchanged (8.33 ± 1.0 vs. 8.27 ± 1.0; $p = 0.11$). The between-group difference in HbA1c reduction was significant, favouring INPUT (Δ −0.22%, 95% CI −0.38 to −0.06; $p = 0.003$). Furthermore, the chance to achieve optimal glycaemic control at follow-up (HbA1c <7.5%) was 1.98-times higher in the INPUT-group than in the control group (95% CI 1.04 to 3.78; $p = 0.03$). Incidence rate ratio of severe hypoglycaemia was 3.55-times higher for participants in the control group than for those in the INPUT-group (95% CI 1.50 to 8.43; $p = 0.004$). Diabetes distress (Δ −5.80; 95% CI −8.87 to −2.73; $p = 0.0003$) and depressive symptoms (Δ −2.08; 95% CI −3.89 to −0.29; $p = 0.011$) were significantly more reduced in the INPUT-group. After the end of the intervention, participants in the INPUT-group also started to use temporary basal rates ($p = 0.014$) as well as bolus options ($p = 0.01$) more often than participants in the control group.
Conclusion: In this study, participants already performed CSII-therapy without achieving optimal glycaemic control. The beneficial effects of participating in INPUT were not only seen in medical outcomes such as improvement in glycaemic control and reduction of severe hypoglycaemic events, but also in psychosocial outcomes and behavioural changes. Therefore, the efficacy of the INPUT education programme could be demonstrated. Considering the higher costs of CSII-therapy, these beneficial effects of a relatively inexpensive group education programme have health-economic implications.
Clinical Trial Registration Number: NCT02868931
Supported by: This study was supported by an unrestricted grant by Berlin-Chemie AG
Disclosure: **D. Ehrmann:** Grants; Berlin-Chemie AG.

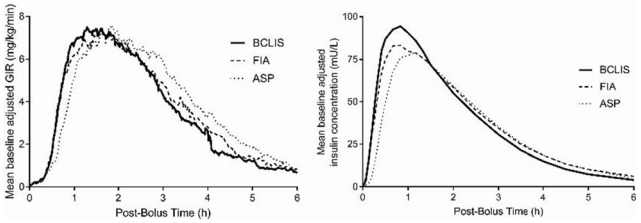
57

The ultra-rapid insulin BioChaperone Lispro shows favourable pharmacodynamics and pharmacokinetics compared to faster insulin aspart and insulin aspart in insulin pumps

G. Meiffren[1], O. Klein[2], C. Seroussi[1], A. Ranson[1], J. Arrubla[2], J. Correia[1], M. Gaudier[1], O. Soula[1], R. Soula[1], B. Alluis[1], S. Glezer[1], T. Heise[2], B. Bode[3];
[1]Adocia, Lyon, France, [2]Profil, Neuss, Germany, [3]Atlanta Diabetes Associates, Atlanta, USA.

Background and aims: BioChaperone Lispro is an ultra-rapid insulin lispro formulation designed to better mimic the physiological timing of prandial insulin action than conventional insulin analog formulations and to achieve a more stable blood glucose control. This clinical trial is the first to investigate the pharmacodynamic (PD) and pharmacokinetic (PK) properties of the two ultra-rapid insulins BioChaperone Lispro (BCLIS) and faster insulin aspart (FIA) and of the conventional insulin analog aspart (ASP).
Materials and methods: Forty-three otherwise healthy participants with type 1 diabetes were enrolled in this phase 1 single center, double blind, randomised, three period cross-over clinical trial. Each insulin formulation was administered with an insulin pump under automated euglycaemic clamp conditions (blood glucose target 5.5 mmol/L). A priming dose was injected around 5 hours before a bolus dose of 0.15 U/kg given at time = 0 on top of a 0.01 U/kg/h basal infusion maintained from pump priming to the end of the clamp procedure, 10 hours after the bolus administration. PK was assessed using a validated assay for insulin aspart and insulin lispro.
Results: BCLIS was associated with significantly higher early insulin exposure ($AUC_{INS\ 0\text{-}1h}$ BCLIS 68 ± 27; ASP 43 ± 22; FIA 59 ± 21 h.mU/L, $p < 0.001$ for BCLIS vs ASP; $p = 0.028$ for BCLIS vs FIA), lower late exposure ($AUC_{INS\ 2\text{-}6h}$ 81 ± 43 vs. 95 ± 41 vs. 93 ± 45 h mU/L, $p < 0.001$; $p = 0.002$) and earlier time to late half-maximum exposure ($t_{late0.5INSmax}$ 147 ± 48 vs 183 ± 68 vs 165 ± 59 min, $p < 0.001$; $p = 0.003$) than FIA and even higher differences to ASP (figure). Compared to ASP, BCLIS had significantly faster-on and faster-off activity with higher area under the glucose infusion rate curves in the first two hours ($AUC_{GIR\ 0\text{-}2h}$, mean ± SD 592 ± 275 vs. 500 ± 244 mg/kg, $p < 0.0038$), lower $AUC_{GIR\ 2\text{-}6h}$ (784 ± 402 vs 980 ± 453 mg/kg, $p = 0.0015$) and earlier times to early and late half-maximum GIR ($t_{early0.5GIRmax}$ 44 ± 22 vs 58 ± 19 min, $p < 0.0001$; $t_{late0.5GIRmax}$ 210 ± 68 vs 232 ± 52 min, $p = 0.0020$). Compared to FIA, BCLIS showed similar early glucose-lowering effects and a significantly lower late glucose-lowering effect with reaching $t_{late0.5GIRmax}$ earlier (147 ± 48 vs 165 ± 59 min, $p = 0.0017$). All three formulations were safe and well tolerated.
Conclusion: Administered with an insulin pump, BCLIS exhibits ultra-rapid PK and PD properties compared to ASP, and favorable profiles compared to the ultra-rapid FIA formulation.

Figure: Mean baseline adjusted glucose infusion rate (GIR) (left) & PK (right) profiles after a subcutaneous bolus dose of BCLIS, FIA or ASP administered with an insulin pump on top of a basal infusion.



Clinical Trial Registration Number: NCT03179332
Supported by: Adocia
Disclosure: **G. Meiffren:** Employment/Consultancy; Adocia. Stock/Shareholding; Adocia.

58

Long-term safety and efficacy of intraperitoneal insulin infusion from implanted pumps in a large series of patients with type 1 diabetes and initial high glucose variability

E. Renard[1,2], B. Guerci[3,4], N. Jeandidier[5,6], EVADIAC;
[1]Department of Endocrinology, Diabetes, Nutrition, CHU Montpellier, Montpellier, [2]Institute of Functional Genomics, University of Montpellier, INSERM, CNRS, Montpellier, [3]Department of Endocrinology, Diabetes and Nutrition, CHU Nancy, Vandoeuvre les Nancy, [4]University of Lorraine, Nancy, [5]Department of Endocrinology, Diabetes and Nutrition, CHU Strasbourg, Strasbourg, [6]University Louis Pasteur, Strasbourg, France.

Background and aims: Intra-peritoneal (IP) delivery allows an alternative route for insulin therapy in patients with type 1 diabetes (T1D) who present high glucose variability under subcutaneous insulin treatment. We assessed from the data of a post authorization safety study the long-term safety and efficacy on glucose control of IP insulin therapy.
Materials and methods: Two hundred and fifty-three patients followed in 12 university hospitals have been enrolled in a multinational, multicenter, observational, prospective cohort study for patients with T1D who are treated with Insuman Implantable 400 IU/mL in Medtronic MiniMed implantable pumps. Visits occurred according to routine clinical practice for the use of an implantable pump, which is at refill visits (every 40–45 days) and at ad hoc visits related to complications of the insulin treatment regimen or pump. The primary objective of the study was to better characterize identified risks of severe hypoglycemia, hyperglycemia (caused by insulin underdelivery due to pump jamming, pump dysfunction or catheter occlusion), pump pocket infection, abnormal healing (at the surgical incision site after device implantation), and skin erosion. Data after a follow-up of 0.9 ± 0.3 year (>1 year in 97.6%) has been analyzed, representing a cumulated experience of 343.5 patient-years (PY).
Results: The cohort includes 241 T1D patients who had been using MIP for 15.1 ± 7.7 years at inclusion and 12 new patients. The patient characteristics at inclusion were: 149F/104M, age: 56.6 ± 10.9, BMI: 25.7 ± 4.3, T1D duration: 35.4 ± 12.1 years, HbA1c: 7.6 ± 1.0%. IP insulin was motivated by brittle diabetes in 68% cases and frequent severe hypoglycemia in 26.9%. Comorbities included: cardiovascular diseases in 26%, retinopathy in 51.4%, nephropathy in 23.3%, neuropathy in 31.5%. Premature discontinuation occurred in 4 cases: 2 by patient decision and 2 deaths of cardiovascular origin. The incidences of severe hypoglycemia, hyperglycemia due to insulin underdelivery, pump pocket infection and skin erosion were 7.3, 18, 1.2 and 0.3 per 100 PY, respectively. No ketoacidosis was reported. Surgical outcomes included 7 temporary and 1 definitive explantations, and 9 catheter replacements (incidence: 2.6/100 PY). Longer duration of IP experience was significantly associated with lower risk of hyperglycemic events.
Conclusion: Our study shows sustained efficacy of IP insulin on glucose control with a low incidence of severe hypoglycemia in these patients with multiple comorbidities and initial high glucose variability. Hyperglycemic episodes related to underdelivery events were limited and solved in most cases with no surgical intervention. This data supports the utility of IP insulin delivery from implanted pumps in T1D patients with major glucose control issues.
Supported by: SANOFI
Disclosure: **E. Renard:** Honorarium; Medtronic, Sanofi.

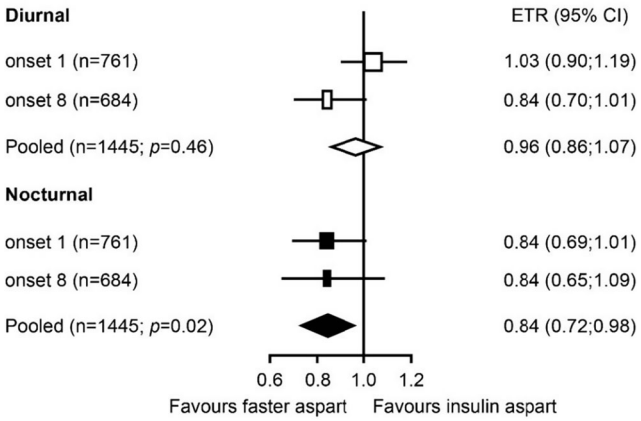
59

Hypoglycaemia with mealtime fast-acting insulin aspart versus insulin aspart across two large type 1 diabetes trials

C. De Block[1], A. Carlson[2], L. Rose[3], T. Gondolf[4], A. Gorst-Rasmussen[4], T.W. Boesgaard[4], W. Lane[5];
[1]Department of Endocrinology-Diabetology-Metabolism, Antwerp University Hospital, Antwerp, Belgium, [2]International Diabetes Center, Minneapolis, USA, [3]Institute of Diabetes Research, Münster, Germany, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Mountain Diabetes and Endocrine Center, Asheville, USA.

Background and aims: Hypoglycaemia is a ubiquitous challenge with insulin treatment in type 1 diabetes (T1D), with nocturnal episodes of particular concern. The aim of this analysis was to investigate the safety of mealtime fast-acting insulin aspart (faster aspart) across two large double-blind, treat-to-target, randomised T1D trials comparing faster aspart and insulin aspart (IAsp).
Materials and methods: Analysis of severe (as defined by the American Diabetes Association) or blood glucose-confirmed (3.1 mmol/L [<56 mg/dL]) hypoglycaemia was performed across two trials evaluating the efficacy and safety of faster aspart vs IAsp by multiple daily injections in adults with T1D: a 52-week trial in combination with insulin detemir (onset 1; $n = 761$), and a 26-week trial in combination with insulin degludec (onset 8; $n = 684$).
Results: Faster aspart was confirmed to be non-inferior to IAsp regarding change from baseline in HbA_{1c} in both trials, with a statistically significantly greater HbA_{1c} reduction with faster aspart in onset 1. Importantly, nocturnal hypoglycaemia rates were consistently lower with faster aspart vs IAsp in both trials (pooled estimated treatment rate ratio [ETR] 0.84 [95% CI: 0.72;0.98]; $p = 0.02$) (Figure), while no significant difference was observed for overall (pooled ETR 0.94 [95% CI: 0.85;1.05]) and diurnal hypoglycaemia rates (pooled ETR 0.96 [95% CI: 0.86;1.07]) with some heterogeneity across trials.
Conclusion: Analysis across two large trials supports the safety of mealtime faster aspart, with lower rates of nocturnal hypoglycaemia with faster aspart vs IAsp.

Figure: Diurnal and nocturnal severe or blood glucose-confirmed hypoglycaemic events[a]



[a]An episode that is severe (requiring assistance of another person to actively administer carbohydrate or glucagon, or take other corrective actions) or blood glucose-confirmed by a plasma glucose value 3.1 mmol/L (<56 mg/dL) with or without symptoms consistent with hypoglycaemia.
Pooled ETR and CI is obtained from a fixed-effects meta-analysis. Study duration of onset 1 was 52 weeks and onset 8 was 26 weeks. For onset 1, weighted contribution is 63.5% for diurnal hypoglycaemia and 65.2% for nocturnal hypoglycaemia. For onset 8, weighted contribution is 36.5% for diurnal hypoglycaemia and 34.9% for nocturnal hypoglycaemia.
CI, confidence interval; ETR, estimated treatment rate ratio; faster aspart, fast-acting insulin aspart; N, total number of subjects in faster aspart/insulin aspart arm.

Clinical Trial Registration Number: NCT01831765; NCT02500706
Supported by: Novo Nordisk
Disclosure: **C. De Block:** Lecture/other fees; Abbott, AstraZeneca, A. Menarini Diagnostics, Lilly, MSD, Novartis, Novo Nordisk, Sanofi, Johnson & Johnson.

60

Ultra rapid lispro (URLi) reduces postprandial glucose excursions vs lispro in patients with type 1 diabetes at multiple meal-to-dose timing intervals

L. Plum-Morschel[1], J.K. Leohr[2], R. Liu[2], S.R. Reddy[2], M.A. Dellva[2], S.T. Lim[3], M.T. Loh[3], M.P. Knadler[2], T.A. Hardy[2], C. Kazda[4];
[1]Profil Mainz GmbH & Co. KG, Mainz, Germany, [2]Eli Lilly and Company, Indianapolis, USA, [3]Lilly-NUS Centre Clin Pharm Pte Ltd, Singapore, Singapore, [4]Lilly France, Neuilly-sur-Seine, France.

Background and aims: Ultra Rapid Lispro (URLi; LY900014), a novel mealtime insulin in Phase 3 development, is shown to reduce postprandial glucose after subcutaneous injection.
Materials and methods: This 2-part, randomised, double-blind, Phase 1b study evaluated the differences in PK and PD between URLi and lispro (Humalog®) in 30 patients with T1D. Part A used a 6-period crossover design to evaluate safety and compare PK and postprandial glucose response to solid mixed meal tolerance tests (MMTT) with the same, individualised doses of URLi or lispro at different injection-to-mealtime intervals (−15, 0, and +15 min). Part B evaluated the safety, PK, and PD during 2 wks of multiple daily dosing (immediately before a meal) in a parallel design. Patients were stabilised overnight to a fasting blood glucose level of 7 mmol/L before the MMTT procedure.
Results: In Part A, URLi reduced glucose excursions (assessed as change in area under the concentration curve vs. time [ΔAUC]) vs. lispro during the first 2 hrs (ΔAUC0-2h) and entire 5 hrs (ΔAUC0-5h) of the MMTT regardless of dose timing (Figure). URLi reduced ΔAUC0-2h by 103% ($p = 0.008$), 39% ($p = 0.031$), and 16% ($p = 0.096$), and ΔAUC0-5h by 40% (p = NS), 44% ($p = 0.097$), and 42% ($p = 0.026$) vs. lispro at −15, 0, and +15 min (significance level = 0.1). The PK and PD profiles for URLi and lispro were sustained after 2 wks of outpatient dosing (Part B). Similar numbers of hypoglycaemic events occurred between treatments during MMTTs. During 2 wks of outpatient dosing, the number of events was numerically lower for URLi vs. lispro. Local tolerability was similar between treatments.
Conclusion: These results provide preliminary evidence that URLi may improve postprandial glucose control in T1D.

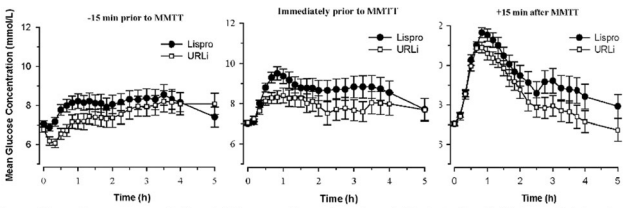


Figure: Mean glucose concentration (±SE) versus time when dosed 15 min before (left), immediately prior (middle), and 15 min post-test meal (right) by treatment following a single dose (Part A).

Clinical Trial Registration Number: NCT02703350
Disclosure: **L. Plum-Morschel:** None.

OP 11 Pain is in the brain

61

Painful diabetic neuropathy is characterised by impaired sensory cortex and thalamic haemodynamic response to exogenous pain

M. Greig[1], I.D. Wilkinson[2], R. Gandhi[1], D. Selvarajah[2], S. Tesfaye[1];
[1]Royal Hallamshire Hospital, Sheffield, [2]University of Sheffield, Sheffield, UK.

Background and aims: Diabetic peripheral neuropathic pain (DPNP) negatively impacts quality of life of affected individuals and exacts an enormous socio-economic cost. Currently treatments provide inadequate management of pain in many patients. Our understanding of the risk factors that underlie the development of chronic neuropathic pain is limited. Recent studies have suggested an important contribution of dysfunction in descending pain modulatory circuits to pain 'chronification'. The aim of this study was to measure cerebral perfusion of the pain processing areas of the brain using MR-Dynamic Susceptibility Contrast (MR-DSC) imaging at rest and under experimental pain condition.
Materials and methods: 74 subjects [55 with T1DM (20 DPNP, 23 painless-DPN, 13 no-DPN) and 19 healthy non diabetic volunteers] underwent detailed clinical and neurophysiological assessment (NISLL+7 tests of nerve function; DN4 pain questionnaire). MR images were obtained at 3T using a MR-DSC, T2*-weighted technique (TR/TE = 12]0/35 ms; 72 dynamics) to assess the passage of a bolus of intravenous gadolinium-chelate through cerebral vascular bed. Subjects were scanned at baseline and during 90s of heat-pain applied to the right lateral thigh (non-neuropathic area). The time-to-peak (TTP) concentrations of gadolinium in the right and left thalami (Rt-T and Lt-T), and right and left sensory cortices (Rt-SC and Lt-SC), were measured.
Results: At baseline, the mean TTP concentrations (s) in the regions of interest (ROIs) were shorter in the DPNP group [*e.g.* Rt-T: Mean (SD): 9.22(1.13) *vs* HV 9.83 (0.99), no-DPN 9.59 (0.90), painless-DPN 9.94 (0.97)] although these were not statistically significant (e.g. Rt-T, $p = 0.058$). However, the change in TTP in response to thermal pain was significantly prolonged in the DPNP group in the ROIs: Lt-T ($p = 0.021$), RT ($p = 0.003$), Lt-SC ($p = 0.009$), Rt-SC ($p = 0.008$). Whilst healthy volunteers respond to thermal pain by shortening the TTP in ROIs, the DPNP group do the reverse ($p < 0.05$).
Conclusion: Subjects with painful-DPN have a paradoxical delay in TTP in response to exogenous thermal pain. This suggests that chronic neuropathic pain state may result in a failure to mount a hemodynamic response to external pain indicating abnormal pain processing and impaired descending inhibition. This novel finding may serve as an objective marker of chronic DPNP, and a potential target for the development of novel treatments.
Supported by: EFSD
Disclosure: **M. Greig:** None.

62

Pain network functional connectivity in painful diabetic neuropathy: Resting State Functional MRI study

D. Selvarajah, I.D. Wilkinson, M. Awadh, K. Teh, S. Pallai, S. Tesfaye; University of Sheffield, Sheffield, UK.

Background and aims: Painful neuropathy (Painful-DPN) affects up to a fifth of patients with diabetes and can lead to progressive disability and poor quality of life. There are no objective biomarkers and current treatments are less than optimal. We examined the resting functional connectivity of the cortical pain network in painful DPN as a possible objective biomarker for neuropathic pain.
Materials and methods: 54 patients with diabetes (No DPN, $n = 16$; Painful DPN, $n = 23$ Painless DPN, $n = 15$) and 16 healthy volunteers underwent detailed clinical and neurophysiological assessments (NIS[LL]+7tests). Resting state fMRI data were acquired at 3T

(Achieva, Philips Healthcare) and data analysis was performed using the Conn Functional Connectivity Toolbox in SPM. Seed region of interest analysis was performed in the somatosensory cortex and insula cortex to represent the sensory discriminatory and affective components of pain processing respectively.
Results: There was increased functional connectivity in the somatosensory cortex (−42, −22, 56; TFCE, corrected $p < 0.05$) and reduced functional connectivity in the insular cortex (34, 62, 60; TFCE, corrected $p < 0.05$) in patients with painful DPN compared to other study cohorts. Somatosensory functional connectivity significantly correlated overall neuropathy severity score ($r = 0.57$; $p = 0.03$). There were no significant correlations between quantitative pain assessments with somatosensory functional connectivity (HADS-A $r = -0.35$, $p = 0.20$), Short Form 36, $r = -0.43$; $p = 0.11$ and Chronic Pain Acceptance Questionnaire $r = -0.16$, $p = 0.57$). Conversely, insula cortex functional connectivity was significantly correlated with affective measures of the chronic pain condition (HADS-A $r = -0.48$, $p = 0.02$; SF-36 $r = -0.51$, $p = 0.01$; CPAQ $r = -0.65$, $p = 0.001$) but not with neuropathy composite score ($r = -0.09$, $p = 0.70$).
Conclusion: This is the first study to examine resting state pain network functional connectivity in DPN. We have demonstrated that abnormal pain network functional connectivity reflects closely the roles of each brain region. Alterations in functional connectivity of the insula cortex, which is involved with interoceptive awareness and the emotional experience, correlated with subjective measures of pain and behaviour unique to the chronic pain condition. Whereas, the somatosensory cortex which is involved with nociceptive/sensory discrimination was more closely related to objective measures of neuropathy severity based on neurophysiological assessments. This novel, quick (five minute) MRI scan captures the multi-dimensional aspects of chronic pain and has a great potential to be an objective assessment tool in both clinical trials and practice.
Supported by: European Foundation for the Study of Diabetes R/145328
Disclosure: **D. Selvarajah:** None.

63

Pericyte mediated reduction in spinal cord blood flow in diabetic neuropathic pain

R.P. Hulse[1], M.E. Da Vitoria Lobo[2], D.O. Bates[2];
[1]Nottingham Trent University, Nottingham, [2]University of Nottingham, Nottingham, UK.

Background and aims: The role that the neurovascular network within the spinal cord plays in regulating nociception has not been investigated; especially in neuropathic pain. We have recently identified that blood vessels in the spinal cord of diabetic animals are narrower than in non-diabetic animals, and that this was accompanied by development of pain. We hypothesise that this reduction in vessel diameter could be a result of vasoconstriction, related to changes in the cells surrounding these vessels (pericytes) due to alterations in the hormone angiotensin II, and activation of its receptors.
Materials and methods: All Experiments were designed in accordance with UK Home Office legislation, Animals (Scientific Procedures) Act 1986 and ARRIVE guidelines. A rodent model of type 1 diabetes was induced in Female Sprague dawley rats (~200 g) ($n = 6$/group). Streptozotocin (intraperitoneal 50 mg/kg) was administered and animals were insulin supplemented. All studies were carried out with age matched controls. Animals body weight was monitored and levels of blood glucose determined (hyperglycaemia >15 mmol/l). 8 weeks following streptozotocin administration, animals were administered with hypoxyprobe (60 mg/kg) intraperitoneal 30 minutes prior to being terminally anaesthetised (intraperitoneal 60 mg/kg Sodium Pentobarbital) and cardiac perfused with 4% paraformaldehyde. Lumbar spinal cords were extracted and processed (40 μM thick sections) for confocal microscopy to identify the endothelium (CD31), pericytes (NG2, PDGFRβ) and AT1R.
Results: In diabetic animals that displayed neuropathic pain there was a significant reduction in vessel diameter in the spinal cord versus age matched controls ($p < 0.0001$). This was associated with increased levels of hypoxia indicated through increased hypoxyprobe staining in the dorsal horn of the spinal cord of diabetic animals ($p < 0.05$, $p < 0.0001$). Furthermore, this vasconstriction in diabetic animals was significantly prevalent when in close proximity to pericytes (AT1R positive, $p < 0.05$).
Conclusion: This demonstrates that pericyte function has a role in modulating the neurovascular network and pain. This highlights a novel mechanism by which diabetic neuropathic pain may manifest.
Supported by: EFSD microvascular research programme and Rosetrees Trust
Disclosure: **R.P. Hulse:** None.

64

Regional brain volume reduction in diabetic peripheral neuropathy: a magnetic resonance imaging volumetry study

S. Tesfaye, S. Pallai, F. Heiberg-Gibbons, K. Teh, I.D. Wilkinson, D. Selvarajah;
University of Sheffield, Sheffield, UK.

Background and aims: Diabetic peripheral neuropathy (DPN) is a common and serious complication, which was hitherto considered a disease of the peripheral nervous system. The is increasing evidence of significant central nervous system involvement in DPN. We have conducted a series of magnetic resonance imaging (MRI) experiments to examine structural brain alterations in DPN.
Materials and methods: 102 patients with type 1 and 2 diabetes (34 No DN, 34 Painless DN & 34 Painful DN) and 34 healthy volunteers underwent detailed clinical and neurophysiological assessments. All subjects underwent 3-dimensional T1-weighted brain MRI (3.0T, Philips). Brain volume analysis was performed using SIENAX (www.fmrib.ox.ac.uk/fsl) and Freesurfer (http://surfer.nmr.mgh.harvard.edu/). Segmented brain volumes (total brain, peripheral and total grey, white matter and CSF) and regional cortical thickness (postcentral gyrus, precentral gyrus and insula cortex) were measured.
Results: Groups were matched for age and gender ($p > 0.05$). Total brain volume was significantly lower in both neuropathy groups (painful DN [1401.7 (10.7)ml], painless DN [1393.5 (69.6)ml]) compared to the HV [1457.2(79.2)ml] and No DN [1437.2(60.9)ml]; ANOVA $p \leq 0.01$). Total grey matter volume was significantly lower in painful DN [713.9(67.1)ml] and painless DN [717.2(42.4)ml] compared to controls (HV [758.4(46.5)] ; $p \leq 0.01$; No DN [747.3(41.1)]; $p = 0.015$). There were no significant differences in white matter (ANOVA $p = 0.18$) and CSF (ANOVA $p = 0.23$) volumes. Painful DN subjects had significantly lower cortical thickness in the right postcentral gyrus [1.83(0.14)mm vs HV 1.91(0.13)mm]; ($p = 0.02$); left precentral gyrus [2.31(0.16)mm vs HV [2.39(0.12)mm]; ($p = 0.02$) and no DN [2.38(0.14)mm]; ($p = 0.04$); and left insula [2.81(0.15)mm] vs HV [2.97(0.14)mm];($p \leq 0.01$).
Conclusion: This is the largest cohort study of brain volume changes in subjects with DN examined to date. We have demonstrated significant reduction in grey matter volume in painful and painless DN subjects. In painful DN this is localised within the somatomotor cortex and insula. These findings highlight significant CNS involvement in DN that provides clues to the pathogenesis of this condition.
Supported by: European Foundation for the Study of Diabetes
Disclosure: **S. Tesfaye:** None.

65

Efficacy of platelet rich plasma injection in diabetic neuropathy: double blinded randomised controlled trial

W.A.M. Khalifa[1], M.M. Hassanien[2], A.-R. Al-awamy[3], H.M. Abdelhafez[4], Y.S. Hussien[2];
[1]Internal Medicine, Assiut University, Assiut, [2]Rheumatology and Rehabilitation department, Faculty of Medicine, Assiut University,

Assiut, [3]Anesthesia department, Assuit University, Assiut, [4]Clinical Pathology department, Assiut University, Assiut, Egypt.

Background and aims: Till now there is no available effective therapy for the treatment of diabetic peripheral neuropathy (DPN). Autologous platelet rich plasma (PRP) is an easy and cost effective method as it provides necessary growth factors for axon regeneration. Aim: To evaluate the clinical efficacy of PRP perineural injection in the treatment of DPN compared to traditional medical treament
Materials and methods: Double blinded randomized controlled trial was conducted. All included patients had type2DM, DPN of at least 5 years. Neuropathy was assessed by the modified Toronto Clinical Neuropathy score (mTCNs). Baseline pain and nerve conduction studies were done.Regardless of age and gender participants were divided into two groups, both the control and experimental groups received primary treatment and strictly control blood glucose. Group I underwent PRP perineural injection under ultrasound guidance. Group II received medical treatment only. Patients were followed for 6 months. Independent student's test was used for comparisons between groups.
Results: The study included 60 patients, 33(55%) were female with a mean age of 35.27 ± 12.86 years with duration of DPN 7.42 ± 1.51 years. 40 patients underwent PRP peri-neural injection. Significant symptomatic improvement in group I versus group II (P value ≤0.001). both mean motor nerve conduction velocity and Sural conduction velocity were accelerated in group I after 6 months of PRP application (P value <0.05, ≤0.001 respectivly), also mTCNS had improved in group I post PRP injection (P value ≤0.001).
Conclusion: Application of PRP perineural injection is an effective adjunct therapy in diabetic peripheral neuropathy, also it significantly improves neuropathic symptoms
Clinical Trial Registration Number: NCT03250403
Disclosure: **W.A.M. Khalifa:** None.

66

Improvement in painful diabetic neuropathy after 3 months from administration of a supplement containing SOD, ALA, B_{12} and Carnitine

T. Didangelos, E. Karlafti, K. Tziomalos, Z. Kontoninas, C. Margaritidis, N. Krikis, A. Hatzitolios;
Diabetes Center, 1st Prop. Department of Internal Medicine, "AHEPA" Hospital, Aristotele University, Thessaloniki, Greece.

Background and aims: To investigate the effect of a new combination of four elements [Superoxide Dismutase (SOD), Alpha Lipoic Acid (ALA), Acetyl L-Carnitine (AC), Vit. B12] contained in one pill in Painful Diabetic Neuropathy (PDN). It is a combination of two antioxidants plus Vit B12 and Carnitine.
Materials and methods: In current prospective, double-blind, placebo controlled, age matched study, 65 patients with Diabetes Mellitus Type 2 (DMT2), 31 women, with mean age 63 ± 11 years, mean duration of DM 15 years randomized in two groups: group A: $n = 32$ received placebo and group B: $n = 33$ received the pill with the combination of the four elements (SOD, ALA, B12, ACL). All patients were on treatment either with a combination of antidiabetic drugs or with a combination with insulin and drugs. Treatment of diabetes did not change during the three months of follow up. The following methods for detecting Diabetic Peripheral and Autonomic Neuropathy (DPN, DAN) used: Michigan Neuropathy Screening Instrument Questionnaire and Examination (MNSIQ and MNSIE), measurement of vibration perception threshold with biothesiometer (BIO) and Cardiovascular Reflex Tests (CRT): R-R variation during deep breathing [assessed by mean circular resultant (MCR)], Valsalva maneuver (Vals), 30:15 ratio and blood pressure response to standing (OH). We used a pain (PS) and a quality of life (QL) questionnaire, also.
Results: All indices of measurements between the two groups including HbA1c (group A 6.8 ± 1.2 vs group B 7.2 ± 1.2 $p = 0.660$) did not differ at baseline. The following indices increased significantly in group B (baseline vs final): BIO 35 ± 13 vs 28 ± 15 ($p < 0.001$), MNSIQ 4.3 ± 3.0 vs 4.2 ± 2.99 ($p = 0.009$), QL 39.0 ± 11.4 vs 37.2 ± 10.9 ($p < 0.001$) and PAIN 20.5 ± 7.1 vs 18.6 ± 6.7 ($p < 0.001$). Indices of CARTS and MNSIE did not differ significantly in group B (baseline vs final). We did not observe a significant change in all indices: in group A (placebo group).
Conclusion: In current study after three months from the administration of the combination with four elements in one pill, we observed an improvement in vibration perception threshold as measured by biothesiometer, in Pain, in Quality of Life and in MNSI Questionnaire. The pill contains two anti-oxidants (SOD, ALA), Vit B12 and Acetyl L-Carnitine and those could be helpful in the management of painful symptoms in patients with PDN or could be a good starting point for a valid adjuvant for the treatment of pain symptoms.
Disclosure: **T. Didangelos:** None.

OP 12 Beta cell identity, degeneration and type 2 diabetes

67

$tRNA^{Gln}$ fragmentation in patient iPSC-derived beta-like cells mediates apoptosis in TRMT10A diabetes

C. Cosentino[1], S. Toivonen[1], E. Diaz Villamil[1], S. Demine[1], A. Schiavo[1], N. Pachera[1], D. Balboa[2], T. Otonkoski[2], P. Marchetti[3], D.L. Eizirik[1], M. Cnop[1,4], M. Igoillo-Esteve[1];
[1]ULB Center for Diabetes Research, Univertsité Libre de Bruxelles, Bruxelles, Belgium, [2]Research Programs Unit, Molecular Neurology and Biomedicum Stem Cell Centre, University of Helsinki, Helsinki, Finland, [3]Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy, [4]Division of Endocrinology, Erasmus Hospital, Université Libre de Bruxelles, Bruxelles, Belgium.

Background and aims: Loss-of-function mutations in *TRMT10A*, a transfer RNA (tRNA) methyltransferase, cause early onset diabetes and microcephaly. tRNAs play a crucial role in cellular homeostasis and post-transcriptional modifications, such as methylation, modulate tRNA function and fragmentation. tRNA-derived halves (tiRNAs, 29–50 nt) and fragments (tRFs, 14–30 nt) are a new class of functional small noncoding RNAs, involved in cellular stress responses. Here we investigated the molecular mechanisms underlying β-cell demise in TRMT10A deficiency.
Materials and methods: Induced pluripotent stem cell (iPSC) lines were derived from a TRMT10A-deficient patient and healthy controls. TRMT10A expression was silenced in EndoC-βH1 cells by siRNA. Reactive oxygen species (ROS) were measured using HPF fluorescent probe. qRT-PCR was used to detect guanine-9 methylation (m_1G^9) in tRNAs. tRNA fragmentation was assessed by Northern blot and qRT-PCR. Synthetic tRNA fragments and tRF inhibitors were transfected by lipofection. Apoptosis was examined by nuclear dyes, Western blot and immunocytochemistry.
Results: iPSCs from controls and TRMT10A diabetic patients were successfully differentiated into β-like cells using a 7-stage protocol. Stage-7 cells expressed insulin and glucagon-like peptide-1 receptor mRNA at levels comparable to EndoC-βH1 cells and human islets ($n = 9–12$). In iPSC-β-like cells and TRMT10A-depleted EndoC-βH1 cells (≥70% knockdown, $p < 0.001$) m_1G^9 methylation was reduced in a subset of cytosolic tRNAs, including $tRNA^{Gln}$ ($p < 0.05$, $n = 6–12$). Hypomethylation of $tRNA^{Gln}$ resulted in fragmentation and increased 5′-$tiRNA^{Gln}$ and 5′-tRF^{Gln} in patient-derived cells (1.5 ± 0.5 fold increase vs controls, $p < 0.05$ $n = 3–6$). Transfection of TRMT10A-competent EndoC-βH1 cells with synthetic 5′-$tiRNA^{Gln}$ and 5′-tRF^{Gln} induced apoptosis. Conversely, transfection of antisense oligonucleotides targeting 5′-$tiRNA^{Gln}$ and 5′-tRF^{Gln} protected TRMT10A-deficient β-cells from apoptosis ($23 \pm 2\%$ apoptosis in TRMT10A-silenced cells vs $17 \pm 2\%$ following antisense transfection, $p < 0.05$). TRMT10A deficiency induced oxidative stress ($p < 0.05$, $n = 5$) and triggered the intrinsic pathway of apoptosis. The ROS scavengers Tiron (25 μM) and NAC (1 mM) protected TRMT10A-deficient β-cells from apoptosis ($20 \pm 2\%$ apoptosis without NAC vs $14 \pm 2\%$ with NAC, $p < 0.05$, $n = 4$).
Conclusion: Using patient iPSC-derived β-like cells and RNA interference we show that TRMT10A deficiency induces oxidative stress and activation of the intrinsic pathway of apoptosis. TRMT10A deficiency leads to hypomethylation and fragmentation of tRNAs. We demonstrate that 5′-$tRNA^{Gln}$ fragments are key mediators of β-cell death. Our study provides unequivocal evidence for the importance of tRNA modifications in human pancreatic β-cells and identifies tRNA hypomethylation and fragmentation as a novel mechanism of β-cell demise in diabetes.
Supported by: T2DSystems European Union's Horizon 2020, FNRS, ARC, SFD-Novartis, EFSD
Disclosure: **C. Cosentino:** None.

68

Role for a lncRNA at the Pax6 locus in controlling beta cell identity and function

R.M. Callingham[1], S. Itzkovitz[2], L. Farack[2], T.J. Pullen[1], G.A. Rutter[1];
[1]Cell Biology and Functional Genomics, Imperial College Lonodn, London, UK, [2]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

Background and aims: Long non-coding RNAs (lncRNA) regulate expression of several β-cell transcription factors (TFs) (*Pdx1, Nkx2.2*). *Pax6* is an essential TF for endocrine development of both β- and α-cells, and is preferentially expressed in the latter. Here, we examine the role of a lncRNA (*Pax6os1*) expressed from the *Pax6* locus in β-cell function.
Materials and methods: *Pax6os1* expression was silenced in MIN6 β-cells using small interfering RNAs (siRNA). RNA sequencing was performed on an Illumina platform (HiSeq 2000) and differentially expressed genes identified with DESeq2. Cell proliferation was assessed using a Cyquant kit (Thermo Fisher). Subcellular location was determined with fractionation and differential centrifugation as well as single molecule fluorescent *in situ* hybridization (smFISH). The MIT CRISPR design tool was used to development a two gRNA system to delete a ~733 bp region spanning *Pax6os1*exon 1 and its upstream promoter in MIN6 cells and C57BI6/J mice.
Results: Silencing of *Pax6os1* led to a $25 \pm 0.07\%$ ($n = 9$ independent experiments, $p = 0.0015$) increase in *Pax6* expression. RNA-seq revealed differential expression of genes involved in β-cell identity, including 'disallowed' β-cell genes. Upregulation of essential β-cell genes (*Ins1, Gck, Slc2a2, Pdx1*) with a coordinated downregulation of genes involved in alternative β-cell fates (*Slc16a1, Ldha, Pdk1*) were observed. Cellular proliferation was significantly reduced ($p < 0.05$) 72 h post siRNA transfection. Assessment of subcellular localisation in MIN6 cells revealed that *Pax6os1* was enriched 2.5-fold ($p = 0.0001$, $n = 3$) within the cytoplasm, while localisation of *Pax6* mRNA also tended to be preferentially (1.6-fold) enriched in the nuclear fraction. mRNA encoding other islet TFs (*Pdx1, Nkx6.1*) showed no preferential retention in the nucleus. Cytoplasmic localisation of *Pax6os1*and *Pax6* were enhanced by incubation for 6 h at elevated (25 vs 5 mmol/l) glucose concentrations. Prolonged glucose incubation (15 h) further exacerbated *Pax6os1* and *Pax6* cytoplasmic localisation (ns). Single molecule FISH on fixed pancreatic tissue of C57BI6/J mice confirmed the enriched nuclear location of *Pax6* mRNA ($p = 0.0015$) while *Pax6os1* was too weakly expressed for reliable quantification. Removal of exon 1 plus ~600 bp upstream of *Pax6os1* was achieved in MIN6 cells using to two gRNAs. The same system was used to generate a *Pax6os1* null mouse line which will be subject to phenotypic characterisation with the addition of diabetic challenge.
Conclusion: Our findings indicate that *Pax6os1* is a negative regulator of *Pax6* expression. Interestingly, *Pax6* mRNA sequestration in the nucleus, and *Pax6os1* action, thus appear to attenuate *Pax6* expression and function in the β-cell. Enhanced *Pax6* function during hyperglycaemia may facilitate a drift in β-cell identity towards an α-cell fate.
Supported by: DUK
Disclosure: **R.M. Callingham:** None.

69

Islet microRNA miR-183-5p is a regulator of beta cell apoptosis and dedifferentiation in NOD mouse pancreatic islet

F. Mancarella[1], G. Ventriglia[1], L. Nigi[1], G.E. Grieco[1], N. Brusco[1], C. Gysemans[2], D. Cook[2], C. Mathieu[2], G. Sebastiani[1], F. Dotta[1];
[1]Diabetes Unit Department of Medical Science, Surgery and Neuroscience, University of Siena, Italy; Umberto Di Mario Foundation ONLUS, Toscana Life Sciences, Siena, Italy, [2]Laboratory of Experimental Medicine, University of Leuven, Leuven, Belgium.

Background and aims: MicroRNAs (miRNAs) are a class of small non coding RNAs that negatively regulate gene expression. Several studies

demonstrated that miRNAs could be involved in cell dedifferentiation and apoptosis, as well as in pancreas development and β cell function, and that their alteration may contribute to the development of type 1 diabetes. Aim of this study was to analyze the expression profile of miRNAs in pancreatic islets of diabetic NOD mice, in order to gain insight into their possible role in β cell damage.

Materials and methods: Expression profile of 384 miRNAs was analyzed using Taqman Array Microfluidic cards in islet endocrine tissue collected using Laser Capture Microdissection (LCM) from NOD SCID, NOD Normoglycemic and recent diabetic NOD mice (3 mice per group). RT-PCR single assay validation on differentially expressed microRNAs was also performed from the same mice as well as from another cohort of $n = 5$ NOD SCID, $n = 8$ NOD Normoglycemic and $n = 8$ recent diabetic NOD mice. Additionally, NOD mouse pancreatic islets were separately captured based on the degree of islet infiltration (insulitis score) and analyzed for expression of miRNA and genes of interest (respectively miR-183-5p, ALdh3a1 and Foxo1). Finally, differentially expressed miRNAs were modulated in MIN6 murine cell line for 48h and treated or not with a cytokine mix (IFNγ, IL-1β, TNFα) for 24h in order to evaluate apoptosis (picnotic nuclei count) and miRNA target genes expression.

Results: MiRNA expression profiling revealed a significant downregulation ($p < 0.05$) of miR-183-5p in LCM-captured islets from NOD recent onset diabetic vs normoglycemic and NOD SCID mice; this was confirmed by single assay analysis. Of note, miR-183-5p resulted downregulated ($p < 0.05$) in heavily infiltrated islets vs low infiltrated islets, highlighting that miR-183-5p reduced expression correlates to the degree of islet inflammation. Interestingly, in highly inflamed islets the reduction of miR-183-5p was paralleled by a higher expression of dedifferentiation marker Aldh3a1 as well as by a reduction of the differentiation marker FOXO1, thus potentially linking miR-183-5p downregulation to an ongoing dedifferentiation process. In murine β-cell line MIN6, miR-183-5p inhibition significantly reduced cytokines-induced apoptosis ($p < 0.001$), indicating that miR-183-5p could be able to modulate apoptotic mechanisms under cytokine stress. Indeed, bioinformatic analysis of miR-183-5p target genes revealed the anti-apoptotic transcription factor Bach2, whose expression was significantly higher ($p < 0.05$) in MIN6 transfected with miR-183-5p inhibitor. Of note, among miR-183-5p target genes, we identified the epithelial-mesenchymal transition modulator Quaking, thus supporting the hypothesis that β-cells undergo dedifferentiation during inflammation as a potential protective mechanism.

Conclusion: In conclusion, miR-183-5p reduction in NOD mouse pancreatic islets may contribute to beta-cell dedifferentiation and to protection from apoptosis, through the modulation of anti-apoptotic factor Bach2 and, potentially, Quaking.

Disclosure: **F. Mancarella:** None.

70

Metabolic insufficiency caused by cellular stresses is implicated to beta cell dedifferentiation in a mouse model of Wolfram syndrome

K. Shinoki-Amo[1], K. Tanabe[1], M. Hatanaka[1], H. Masutani[2], Y. Tanizawa[1];
[1]Department of Endocrinology, Metabolism, Hematological Sciences and Therapeutics, Yamaguchi University Graduate School of Medicine, Ube, [2]Tenri Health Care University, Tenri, Japan.

Background and aims: β cell failure is central in the pathogenesis of both type 1 and type 2 diabetes. It results from reduced β cell mass and function. Wolfram syndrome, caused by the *WFS1* gene mutations, is characterized by insulin dependent diabetes mellitus and optic atrophy. Genetically determined pancreatic β cell loss results from augmented ER and oxidative stresses. We investigated the contribution of these two processes to β cell failure using mice lacking *Wfs1*.

Materials and methods: In vivo metabolic profile was assessed by standard methods. We used genetic lineage tracing to examine beta cell fate. Pancreatic tissues obtained from various age of mice were stained by the antibodies to Insulin, Glucagon, Somatostatin, MafA, Pdx1, Neurogenin3 and so on. For islet studies, we assessed energy metabolisms and mitochondria function by metabolomics analysis and a use of extracellular metabolic flux analyzer.

Results: In the $Wfs1^{-/-}$ mice, β cells become dedifferentiated and revert to endocrine progenitor-like cells expressing Neurogenin3. Lineage-tracing experiments demonstrated that loss of β cells was mainly due to β cell de-differentiation and that a subset of β cells takes α cell fate. Such β cell plasticity appears after nursing, independently of hyperglycemia, and becomes more apparent along with diabetes progression accompanied with no significant increase in apoptosis. We have found that genetic inhibition of Txnip, which is a stress response molecule involving in various cellular processes, preserved β cell mass and maintained glucose homeostasis in the $Wfs1^{-/-}$ mice. This suggests its roles in the regulation of β cell plasticity in the setting of *Wfs1* deficiency. One clue to the mechanisms underlying β cell dedifferentiation was the reduction of acetyl-CoA, citrate and ATP content in the $Wfs1^{-/-}$ islets. Although glycolysis assessed by measuring extracellular acidification rate after glucose loading was apparently decreased, metabolomics analysis revealed that pyruvate and intermediate glycolytic products were apparently accumulated. These abnormalities in glucose catabolic process were correlated with an increase in phosphorylated pyruvate dehydrogenase (p-PDH). Importantly, Txnip directly interacts with the both PDH kinase as well as PDH, indicating its involvement in the regulation of PDH activity. Indeed, islets of $Wfs1^{-/-}$ mice lacking *Txnip*demonstrated a robust reduction of phosphorylated PDH and a restoration of capabilities of ATP production in response to glucose. Consistently, severely decreased glycolysis and oxygen consumption rate after glucose loading in $Wfs1^{-/-}$ islets were significantly reversed by *Txnip* deficiency.

Conclusion: These finding illustrate energy insufficiency associated with impaired glucose catabolism in β cells under the chronic stress conditions and suggest that β cells may possibly become dedifferentiated to adapt to metabolic insufficiency caused by unresolvable stresses. This provides new insights into molecular mechanisms underlying β cell loss in diabetes related to cellular stresses, such as Wolfram syndrome.

Supported by: Grant-in-Aid for Scientific Research, Takeda Science Foundation

Disclosure: **K. Shinoki-Amo:** Grants; a Grant-in-Aid for Scientific Research (grant number 15K21198), a Junior Scientist Development Grant supported by Novo Nordisk Pharma Ltd., Grants for young researchers from the Japan Association for Diabetes Education and Care and Banyu Life Science Foundation International.

71

An islet gene expression module containing AldoB is correlated with progression of hyperglycaemia and type 2 diabetes in humans

M. Ibberson[1], F. Burdet[1], K.-P. Knoch[2], M. Barovic[2], A. Dahl[2], P. Marchetti[3], A. Schulte[4], M. Solimena[2];
[1]Vital-IT, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland, [2]TU Dresden, Dresden, Germany, [3]University of Pisa, Pisa, Italy, [4]Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany.

Background and aims: Surgical specimens of metabolically phenotyped pancreatectomized patients (PPP) represent a novel source for studies on pancreatic islets in health and type 2 diabetes (T2D). In this study we sought to identify islet gene co-expression modules correlated with progression of hyperglycemia and T2D.

Materials and methods: Based on their medical history and preoperative values for fasting glucose, HbA1c and OGTT 138 PPP were classified as non-diabetic (ND, $n = 18$), impaired glucose tolerant (IGT, $n = 44$), having <1 year-long diabetes secondary to the pancreatic disease (T3cD, $n = 37$) or long standing (mean duration: 11 yrs) type 2 diabetes (T2D, $n = 39$), according to the ADA guidelines. Islets were retrieved from snap-frozen surgical specimens by laser capture microdissection (LCM) and

profiled by RNAseq. Weighted gene co-expression network analysis (WGCNA) was performed on normalised count data to identify islet gene co-expression modules, which were then correlated to available clinical traits.

Results: A total of 40 gene co-expression modules were identified with a mean size of 500 genes. Correlation of these modules to 25 clinical traits led to the identification of a module of 78 genes that was the most strongly correlated to HbA1c, fasting glucose, 1H glucose during OGTT and diabetes status. *ALDOB*, encoding Fructose-1,6-bisphosphate aldolase, was the highest-ranking gene in this module in terms of intramodular connectivity and HbA1c correlation and was the top "Hub" gene for this trait. Other top-ranking "Hub" genes for HbA1c were involved with extracellular matrix remodelling, lipid transport and metabolism.

Conclusion: We present an unsupervised analysis of islet gene expression data from the largest biobank of LCM surgical pancreas specimens to date. Exploration of the islet gene co-expression module uncovers mechanisms of islet response to chronic elevated glucose in patients and/or islet dysfunction in type 2 diabetes.

Supported by: EU-IMI IMIDIA, EU-IMI RHAPSODY, DZD e.V

Disclosure: **M. Ibberson:** None.

72

Beta Cell and Diabetes Platform (BCDP): a sustainable solution for IMI project data

I. Xenarios[1], R. Liechti[1], M. Gossel[2], M. Solimena[3], P. Marchetti[4], A.M. Schulte[2], C. Magnan[5], C. Cruciani-Guglielmacci[5], R. Scharfmann[6], O. Bernard-Poenaru[7], D. Madsen[8], B. Thorens[9], P. Hecht[7], W. Kramer[10], M. Ibberson[1];

[1]Vital-IT, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland, [2]Sanofi Deutschland, Frankfurt, Germany, [3]TU Dresden, Dresden, Germany, [4]University of Pisa, Pisa, Italy, [5]Paris Diderot University, Paris, France, [6]INSERM U1016, Paris, France, [7]Servier, Suresnes, France, [8]Novo Nordisk, Copenhagen, Denmark, [9]University of Lausanne, Lausanne, Switzerland, [10]Biomedical and Scientific Consulting, Mainz, Germany.

Background and aims: A wealth of data and results are collected by projects funded by the Innovative Medicines Initiative and other EU projects. Often though, once the project reaches completion the only way to share knowledge is through publication, which only represent a fraction of the total data and results generated. We aim to provide a framework for sustainable access to IMI project data in the diabetes area.

Materials and methods: Data and results from the IMI1 project IMIDIA are hosted at the Swiss Institute of Bioinformatics (SIB). Integrative analysis was performed during IMIDIA and made available through various web tools for data exploration. Data from the project were converted into a standardized format, Resource Description Framework (RDF) to enable future interoperability with other resources. Clinical data were aligned using CDISC standards. Web tools provide continued access to data and results to BCDP consortium partners.

Results: BCDP so far contains islet gene expression, lipidomics and clinical/phenotypic data derived from human organ donors and pancreatectomized patients ($N = 498$) and six metabolically challenged mouse strains (C57BL/6, DBA2/J, 129S2, BALB/cJ, AKR and A/J) followed over time. Patient parameters available include standard measures such as age, gender, BMI and multiple functional parameters such as HbA1c, GSIS and OGTT measurements. Available mouse phenotypes are GSIS, OGTT, ITT and beta/alpha cell mass estimations. Web-based tools are available to (i) query the human biobank for detailed patient/sample information, (ii) analyse gene expression profiles across datasets and (iii) analyse gene co-expression networks and their correlations to the different clinical and functional parameters. For mouse, web-based tools are available to (i) analyse how physiological measurements differ across strains and diets over time, (ii) analyse how plasma and islet lipids changes across strains and diets over time, (iii) analyse gene expression changes induced by HF diet across strains and time-points, and (iv) analyse gene co-expression networks and their correlations to physiological measurements. Functionality also exists for cross-querying between human and mouse: for example, genes selected through analysis of human data can be used to directly query the mouse data or *vice versa* using orthology relationships (NCBI Homologene).

Conclusion: The BCDP is the first fully funded sustainable platform for an IMI diabetes resource. IMIDIA represents the cornerstone for the BCDP. SIB is currently acting as data coordinator for several IMI projects in the diabetes area and the plan is to include further interoperable datasets in the future. The BCDP platform is already in use for the IMI2 project RHAPSODY. This therefore represents an opportunity to provide sustainable access to multiple IMI projects whilst ensuring different access restrictions on these data due to ethical/legal constraints.

Supported by: EU-IMI IMIDIA, BCDP Consortium

Disclosure: **I. Xenarios:** None.

OP 13 GLP1 receptor agonists, SGLT2 inhibitors and the kidney: new lessons from large clinical trials

73

Cardiovascular safety and efficacy of exenatide once-weekly in patients with moderate renal dysfunction in the EXenatide Study of Cardiovascular Event Lowering (EXSCEL)

A.F. Hernandez[1], M.A. Bethel[2], G.L. Bakris[3], P. Merrill[1], S.M. Gustavson[4], B.G. Katona[4], P. Ohman[4], Y. Lokhnygina[1], R.J. Mentz[1], R.R. Holman[2], for the EXSCEL Study Group;
[1]Duke Clinical Research Institute, Durham, USA, [2]Diabetes Trials Unit, Oxford, UK, [3]University of Chicago Medicine, Chicago, USA, [4]AstraZeneca, Gaithersburg, USA.

Background and aims: EXSCEL, a multinational, randomized, placebo-controlled cardiovascular (CV) outcome trial of 2 mg once-weekly exenatide added to usual care, demonstrated CV safety in patients with type 2 diabetes (T2D) with or without previous CV disease. We report the impact of exenatide on confirmed CV outcomes, all-cause mortality, and key CV safety parameters according to baseline renal function (moderate dysfunction [<60 mL/min/1.73 m^2] and within Stage 3 [3a: eGFR 45–59 or 3b: 30–44 mL/min/1.73 m^2] chronic kidney disease).
Materials and methods: For the subgroups by baseline renal function, Cox proportional hazards models were fit to the time to first event of the three-component major adverse CV event (MACE-3) composite outcome (death from CV causes, nonfatal myocardial infarction, or nonfatal stroke). Secondary outcomes were time to all-cause mortality, death from CV cause, nonfatal or fatal myocardial infarction, nonfatal or fatal stroke, hospitalization for heart failure, and hospitalization for acute coronary syndrome.
Results: Of 14,752 patients in the ITT population, 3191 (22%) had eGFR <60, 2288 (16%) had eGFR 45–59 and 889 (6%) had eGFR 30–44 mL/min/1.73 m^2. Participants with moderate renal dysfunction had a higher mean age (67 vs 61 years) and longer duration of T2D (median [IQR] 14 [9, 21] vs 11 [6, 17] years). In univariate subgroup analyses, there was no significant interaction between randomized treatment and renal function, either based on eGFR thresholds (±60 mL/min/1.73 m^2; p for interaction = 0.12) or on CKD stages (p for interaction = 0.51). In those with eGFR <60 mL/min/1.73 m^2, first MACE-3 events occurred in 283 (18.1%) participants in the exenatide group and 284 (17.5%) in the placebo group (hazard ratio [HR] 1.01, 95% CI 0.86–1.19). HR and 95% CI for other important CV outcomes are shown in the Table.
Conclusion: In patients with moderate renal dysfunction, 2 mg once-weekly exenatide had a neutral impact on CV outcomes. In univariate analyses unadjusted for multiplicity, modest risk reductions were seen with exenatide in those with baseline eGFR ≥60 mL/min/1.73 m^2 for MACE-3, all-cause mortality, CV death and fatal or non-fatal stroke.

Table. Hazard ratio (HR) and 95% confidence interval (CI) for CV outcomes for allocation to 2mg once-weekly exenatide compared with placebo, according to baseline renal function

eGFR mL/min/1.73m²	MACE-3 HR (95%CI)	CV Death HR (95%CI)	F/tF MI HR (95%CI)	F/NF stroke HR (95%CI)	ACM HR (95%CI)	HF HR (95%CI)	ACS HR (95%CI)
≥60 (n=11,514)	0.86 (0.77, 0.97)	0.77 (0.64, 0.93)	0.97 (0.83, 1.14)	0.74 (0.58, 0.93)	0.78 (0.67, 0.91)	0.84 (0.66, 1.07)	1.07 (0.93, 1.23)
<60 (n=3191)	1.01 (0.86, 1.19)	1.10 (0.86, 1.40)	0.95 (0.77, 1.18)	1.17 (0.82, 1.67)	1.01 (0.83, 1.23)	1.08 (0.81, 1.44)	1.00 (0.82, 1.22)
Stage 3a: 45-59 (n=2288)	0.97 (0.79, 1.20)	1.05 (0.77, 1.43)	0.95 (0.73, 1.25)	1.23 (0.79, 1.90)	1.01 (0.79, 1.30)	0.80 (0.55, 1.16)	1.02 (0.80, 1.31)
Stage 3b: 30-44 (n=889)	1.11 (0.84, 1.47)	1.18 (0.79, 1.75)	0.99 (0.69, 1.41)	1.07 (0.57, 2.01)	1.01 (0.73, 1.40)	1.80 (1.12, 2.90)	1.00 (0.71, 1.41)

MACE-3: composite of death from cardiovascular causes (CV death), nonfatal myocardial infarction (MI), nonfatal stroke.
F: fatal. NF: nonfatal. ACM: all- cause mortality. HF: hospitalization for heart failure. ACS: hospitalization for acute coronary syndrome

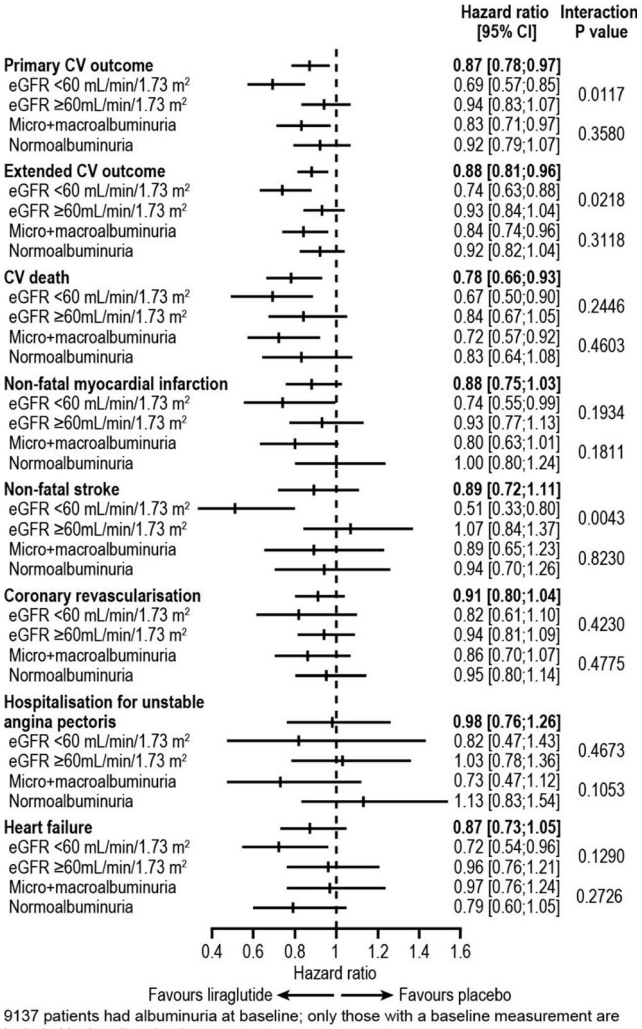
Clinical Trial Registration Number: NCT01144338
Supported by: AstraZeneca (Gaithersburg, MD)
Disclosure: **A.F. Hernandez:** Grants; AstraZeneca, GlaxoSmithKline, Merck, Novartis. Honorarium; AstraZeneca, Bayer, Boehringer Ingelheim, Boston Scientific, Merck, Novartis, Pfizer.

74

Liraglutide reduces major cardiovascular events in patients with chronic kidney disease: results from the LEADER trial

N. Poulter[1], J. Mann[2], V. Fonseca[3], O. Mosenzon[4], I. Raz[4], H. Frimer-Larsen[5], B. von Scholten[5], T. Idorn[5], on behalf of the LEADER Trial Steering Committee and Investigators;
[1]Imperial College London, London, UK, [2]KfH Kidney Center, Munich, Germany, [3]Tulane University Health Sciences Center, School of Medicine, New Orleans, USA, [4]Hadassah Hebrew University Hospital, Jerusalem, Israel, [5]Novo Nordisk A/S, Søborg, Denmark.

Background and aims: People with type 2 diabetes (T2D) and chronic kidney disease (CKD) are at high risk of cardiovascular (CV) events. We analysed the effect of liraglutide vs placebo (PBO) on CV outcomes in patients with T2D and CKD in the LEADER trial.
Materials and methods: LEADER was a randomised, double-blind, multicentre, CV outcome trial with liraglutide 1.8 mg/day vs PBO, both in addition to standard of care for 3.5–5 years, in 9340 patients with T2D and high CV risk. The primary composite outcome was defined as first occurrence of death from CV causes, non-fatal myocardial infarction, or non-fatal stroke. The expanded composite CV outcome additionally included coronary revascularisation, and hospitalisation for unstable angina pectoris or hospitalisation for heart failure (HF). In this analysis, CV outcomes were assessed in patients with CKD based on estimated GFR (eGFR) (<60 and ≥60 mL/min/1.73 m^2) and on albuminuria (≥30 mg/g: micro/macroalbuminuria and <30 mg/g: normoalbuminuria).
Results: The mean eGFR in patients with baseline eGFR <60 (n = 2158) and ≥60 mL/min/1.73 m^2 (n = 7182) was 45.7 ± 10.9 and 90.8 ± 21.6 mL/min/1.73 m^2, respectively. Versus PBO, liraglutide was associated with reductions in the risk of the primary composite outcome: HR 0.69 (CI 0.57; 0.85) for the eGFR <60 subgroup and HR 0.94 (CI 0.83; 1.07) in the eGFR ≥60 subgroup. Equivalent reductions in the expanded composite CV outcome were observed (Figure). In the eGFR <60 mL/min/1.73 m^2 subgroup, liraglutide significantly reduced the risk of CV death, non-fatal stroke and hospitalisation for HF vs PBO (Figure). Liraglutide also reduced the risk of the primary composite outcome, the expanded composite CV outcome and CV death in the micro/macroalbuminuria subgroup vs PBO (Figure).
Conclusion: In LEADER, there was a significant reduction of major CV events in patients with CKD.



9137 patients had albuminuria at baseline; only those with a baseline measurement are included in the albuminuria group.

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **N. Poulter:** Other; Support: Novo Nordisk A/S.

75

Canagliflozin and cardiovascular outcomes in patients with chronic kidney disease

V. Perkovic[1], B.L. Neuen[1], T. Ohkuma[1], B. Neal[1], D.R. Matthews[2], D. de Zeeuw[3], K.W. Mahaffey[4], G. Fulcher[5], M. Desai[6], Q. Li[1], H. Deng[6], N. Rosenthal[6], M. Jardine[1], G. Bakris[7];
[1]The George Institute for Global Health, Sydney, Australia, [2]Oxford Centre for Diabetes, Endocrinology and Metabolism and Harris Manchester College, University of Oxford, Oxford, UK, [3]University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [4]Stanford Center for Clinical Research, Department of Medicine, Stanford University School of Medicine, Stanford, USA, [5]Royal North Shore Hospital, Sydney, Australia, [6]Janssen Research & Development, LLC, Raritan, USA, [7]University of Chicago Medicine, Chicago, USA.

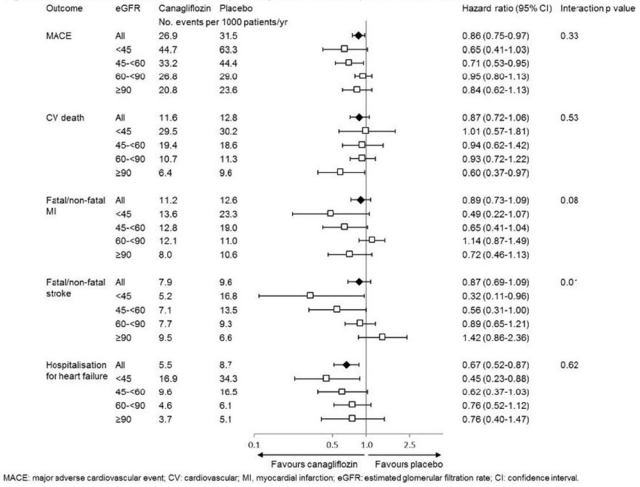
Background and aims: SGLT2 inhibitors are approved for glucose lowering in type 2 diabetes (T2DM) and have proven cardiovascular (CV) benefits, but are not approved for people with significantly reduced kidney function as glycaemic efficacy is dependent on glomerular filtration. This analysis of the CANagliflozin cardioVascular Assessment Study (CANVAS) Program assessed effects of canagliflozin on CV outcomes in patients with T2DM and established CV disease or ≥2 CV risk factors according to baseline kidney function.

Materials and methods: The primary outcome of the CANVAS Program was the composite of CV death, nonfatal myocardial infarction, and nonfatal stroke (major adverse cardiovascular events [MACE]). The primary outcome and other CV outcomes were assessed in participants by baseline estimated glomerular filtration rate (eGFR; <45, 45–<60, 60–<90, and ≥90 ml/min/1.73 m^2). Incidence rates, hazard ratios (HRs), and 95% confidence intervals (CIs) were calculated for each outcome.

Results: The CANVAS Program included 2039 patients (20.1%) with baseline eGFR <60 ml/min/1.73 m^2 (mean age 68 y, BP 138/76 mmHg, HbA1c 8.3%, eGFR 49 ml/min/1.73 m^2, median urinary albumin:creatinine ratio 22 mg/g). Effects of canagliflozin on HbA1c and body weight were smaller in patients with eGFR <60 vs ≥60 ml/min/1.73 m^2 (−0.43 vs −0.64%, *P*-heterogeneity <0.0001, and −1.16 vs −1.43 kg, *P*-heterogeneity = 0.0002), but BP effects were similar (−3.89 vs −4.11 mmHg, *P*-heterogeneity = 0.21). Relative effects on the primary and most other CV outcomes were similar across four eGFR subgroups, with possible heterogeneity suggested only for the exploratory outcome of stroke (Figure). Results for almost all safety outcomes were also consistent across eGFR subgroups.

Conclusion: Despite smaller glycaemic effects in people with reduced eGFR, the cardioprotective benefits of canagliflozin are similar across different levels of kidney function.

Figure. Cardiovascular outcomes in participants by baseline eGFR (eGFR <45, 45-<60, 60-<90, and ≥90 ml/min/1.73 m²).



MACE: major adverse cardiovascular event; CV: cardiovascular; MI, myocardial infarction; eGFR: estimated glomerular filtration rate; CI: confidence interval.

Clinical Trial Registration Number: NCT01032629, NCT01989754
Supported by: Janssen Global Services, LLC
Disclosure: **V. Perkovic:** Grants; Australian National Health and Medical Research Council (Senior Research Fellowship and Program Grant). Lecture/other fees; AbbVie, Astellas, AstraZeneca, Bayer, Baxter, Bristol-Myers Squibb, Boehringer Ingelheim, Durect, Eli Lilly, Gilead, GlaxoSmithKline, Janssen, Merck, Novartis, Novo Nordisk, Pfizer, Pharmalink, Relypsa, Roche, Sanofi, Servier. Other; AbbVie (steering committee), Boehringer Ingelheim (steering committee), GlaxoSmithKline (steering committee), Janssen (steering committee), Pfizer (steering committee).

76

Lesser eGFR decline with dulaglutide regardless of weight changes in people with type 2 diabetes and moderate to severe chronic kidney disease (AWARD-7)

K.R. Tuttle[1], M.C. Lakshmanan[2], B. Rayner[3], R.S. Busch[4], A.G. Zimmermann[2], D.B. Woodward[2], F.T. Botros[2];

[1]Providence Health Care, Spokane, USA, [2]Eli Lilly and Company, Indianapolis, USA, [3]Division of Nephrology and Hypertension, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa, [4]Division of Community Endocrinology, Albany Medical Center, Albany, USA.

Background and aims: Body weight (BW) changes may affect muscle mass and thus creatinine (Cr) levels. Estimating glomerular filtration rate (eGFR) by Cr-based equations may not accurately reflect changes in kidney function when BW changes. While muscle mass changes affect serum Cr levels, cystatin C is not affected by muscle mass changes. Dulaglutide (DU) treatment was associated with BW loss and lesser eGFR (Cr-CKD-EPI) decline in people with type 2 diabetes (T2D) and moderate to severe chronic kidney disease (CKD) compared to insulin glargine (IG) (Table). The aim was to evaluate if the lesser eGFR decline observed with DU is related to BW loss.
Materials and methods: Changes in eGFR were evaluated at the end of the treatment period (52 weeks) with Cr-based equations (MDRD, CKD-EPI) and cystatin C-CKD-EPI equation. Creatinine clearance (CrCL) was evaluated with the Cockcroft-Gault equation. Pearson correlation analysis was used to determine the relationship between change in body weight and changes in serum Cr, cystatin C, or eGFR.
Results: Baseline characteristics were similar between treatments ([mean ± SD] eGFR (Cr-CKD-EPI): 38.3 ± 12.8 mL/min/1.73 m^2, HbA_{1c}: 8.6 ± 1.0%, age: 64.6 ± 8.6 y, T2D duration: 18.1 ± 8.7 y). All equations consistently show that eGFR remained stable with DU, but significantly decreased with IG regardless of BW loss in DU or gain in IG (Table). Since BW is a factor in CrCL calculations, compared to eGFR equations, BW loss in DU led to bias toward greater reductions in CrCL. This bias disappeared when using lean BW (Table). Overall, there were no correlation between changes in body weight and changes in serum creatinine ($r = -0.006$, $n = 473$, $p = 0.904$), serum cystatin C ($r = -0.056$, $n = 470$, $p = 0.224$), or eGFR (Cr-CKD-EPI [$r = -0.074$, $n = 473$, $p = 0.106$], cystatin C-CKD-EPI [$r = -0.041$, $n = 471$, $p = 0.379$]).
Conclusion: In conclusion, compared to IG, DU was associated with lesser eGFR decline in people with T2D and moderate to severe CKD regardless of BW changes.

Table. Effects of dulaglutide and glargine on body weight, eGFR, and creatinine clearance in the study participants at 52 weeks

Treatment Group	Body weight (kg)	eGFR (mL/min/1.73 m^2)			Creatinine clearance (CrCL) (mL/min)	
		CKD-EPI cystatin-C	CKD-EPI creatinine	MDRD creatinine	Total body weight (kg)	Lean body weight (kg)
DU 1.5 mg	-2.7**## (-3.58,-1.75)	-0.7# (-2.5,1.0)	-1.1 (-2.4,0.2)	-0.4# (-1.8,1.0)	-2.0* (-3.7, -0.2)	-0.8# (-1.9,0.3)
DU 0.75 mg	-1.7**## (-2.59,-0.83)	-0.7# (-2.4,1.1)	-1.5* (-2.8,-0.2)	-1.3 (-2.7,0.1)	-2.9* (-4.6, -1.1)	-1.6* (-2.7,-0.5)
IG	1.6** (0.73,2.41)	-3.3** (-5.1,-1.6)	-2.9** (-4.2,-1.6)	-2.5** (-3.9,-1.1)	-2.9* (-4.6,-1.1)	-2.4** (-3.5,-1.4)

Data presented as change from baseline LSM (95% CI); safety population. *2-sided p < 0.05 and **2-sided p < 0.001 change from baseline, #2-sided p < 0.05 and ##2-sided p < 0.001 versus insulin glargine.
Abbreviations: CI=confidence interval; CKD=chronic kidney disease; Cr=creatinine; DU=dulaglutide; eGFR=estimated glomerular filtration rate; IG=insulin glargine; LSM=least squares mean

Clinical Trial Registration Number: NCT01621178
Supported by: Eli Lilly and company
Disclosure: **K.R. Tuttle:** Employment/Consultancy; Eli Lilly and Company, Boehringer Ingelheim, Astra Zeneca, Gilead.

77

Lixisenatide and renal outcomes in patients with type 2 diabetes: a post-hoc analysis of the ELIXA trial

M.H.A. Muskiet[1], H.J.L. Heerspink[2], L. Tonneijck[1], Y. Huang[3], M. Liu[4], A. Saremi[3], D.H. van Raalte[1];
[1]Diabetes Center, Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands, [2]Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, Netherlands, [3]Sanofi, Bridgewater, NJ, USA, [4]BDM Consulting, Somerset, USA.

Background and aims: Limited data exist on the long-term effects of glucagon-like peptide-1 (GLP-1) receptor agonists on kidney function and albuminuria in type 2 diabetes (T2D).
Materials and methods: This was a post hoc analysis of ELIXA, which was a study of cardiovascular safety of lixisenatide (Lixi) vs placebo over a median follow-up of 25 months in 6068 patients with T2D and an acute coronary event ≤180 days before screening. A mixed-effect model with repeated measures for comparisons between treatment groups of changes in urine albumin-to-creatinine ratio (UACR) was performed. The estimated glomerular filtration rate (eGFR, per the 4-variable modification of diet in renal disease formula) and UACR by baseline albuminuria status were assessed. A Cox proportional hazards model adjusted for baseline and on-trial HbA_{1c} was used to estimate the incidence of macroalbuminuria in patients without macroalbuminuria at baseline.
Results: Slower progression of UACR was seen with Lixi vs placebo. In the mixed-effect model, the interaction between treatment and baseline UACR categories was significant ($p < 0.01$). UACR percent change from baseline was lower for Lixi vs placebo in patients with micro- or macroalbuminuria (Table). In the Cox proportional hazards model, Lixi was associated with a 23% lower risk for first macroalbuminuria event in patients without baseline macroalbuminuria (HR 0.77; 95% CI: 0.62, 0.96; $p = 0.0174$). eGFR was not significantly different for Lixi vs placebo overall or by baseline albuminuria status.
Conclusion: In patients with T2D and a recent acute coronary event, the renal benefit of Lixi was beyond glycaemic control. Lixi reduced UACR progression in patients with baseline micro- or macroalbuminuria and was associated with lower incidence of macroalbuminuria.

Table. Change in UACR from baseline to Week 108 in the ELIXA trial according to baseline albuminuria status (ITT population)

		Placebo		Lixi	
		BL	Week 108	BL	Week 108
Normoalbuminuria (BL UACR <30 mg/g)	UACR ± SD, mg/g	8.1 ± 4.7 (n=2191)	11.3 ± 11.8 (n=1101)	8.2 ± 4.8 (n=2250)	11.5 ± 11.6 (n=1110)
	Percent change from BL ± SE, %*	46.0 ± 4.0		44.3 ± 3.9	
	Difference ± SE (95% CI), %†	–1.7 ± 5.2 (–11.9, 8.5); p=0.7424			
Microalbuminuria (BL UACR ≥30 mg/g to <300 mg/g)	UACR ± SD, mg/g	76.5 ± 46.6 (n=596)	85.6 ± 117.4 (n=280)	73.6 ± 47.2 (n=552)	62.6 ± 94.2 (n=239)
	Percent change from BL ± SE, %*	14.8 ± 8.6		–6.4 ± 7.6	
	Difference ± SE (95% CI), %†	–21.1 ± 10.8 (–42.3, 0.0); p=0.0505			
Macroalbuminuria (BL UACR ≥300 mg/g)	UACR ± SD, mg/g	930.3 ± 809.5 (n=207)	960.8 ± 1312.5 (n=87)	959.2 ± 858.3 (n=182)	577.8 ± 955.2 (n=76)
	Percent change from BL ± SE, %*	11.0 ± 14.5		–31.6 ± 9.7	
	Difference ± SE (95% CI), %†	–42.6 ± 16.6 (–75.0, –10.2); p=0.0084			

All data are geometric means unless stated otherwise
*Percent change from BL and between treatment group differences were calculated with log-transformed UACR values using the MMRM with treatment (Lixi, placebo), visit (Weeks 24, 76, and 108), region, intake of ACE inhibitors at BL (yes or no) and intake of ARB at BL (yes or no) as fixed effects, and both treatment and BL UACR by visit interaction. Results in the log scale were back-transformed to provide the estimates of the geometric means
†Lixi vs placebo; calculated based on estimates from the MMRM
ARB=angiotensin-receptor blocker; BL=baseline; ITT=intent-to-treat; MMRM=mixed-effect model with repeated measures; SE=standard error

Clinical Trial Registration Number: NCT01147250
Supported by: Sanofi
Disclosure: **M.H.A. Muskiet:** Employment/Consultancy; Eli Lilly and Co, Novo Nordisk A/S.

78

Semaglutide treatment and renal function in the SUSTAIN 6 trial

R. Silver[1], J. Gumprecht[2], T. Vilsbøll[3,4], T. Hansen[5], J. Pettersson[5], H. Vrazic[5], J. Wilding[6];
[1]Southern New Hampshire Medical Center, Nashua, USA, [2]Medical University of Silesia, Katowice, Poland, [3]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [4]University of Copenhagen, Hellerup,

Denmark, [5]Novo Nordisk A/S, Søborg, Denmark, [6]University of Liverpool, Liverpool, UK.

Background and aims: Semaglutide is a glucagon-like peptide-1 analogue for the once-weekly treatment of type 2 diabetes (T2D). Semaglutide demonstrated superior glycaemic control and body weight loss vs placebo and active comparators across the SUSTAIN phase 3a clinical trial programme. SUSTAIN 6 was a 2-year cardiovascular (CV) outcomes trial conducted in 3,297 subjects with T2D at high risk for CV events that compared subcutaneous semaglutide 0.5 mg or 1.0 mg once weekly vs placebo. The results showed that semaglutide-treated subjects had a significant 26% lower risk of major adverse CV events (MACE: a primary composite outcome of non-fatal myocardial infarction, non-fatal stroke or CV death) vs those receiving placebo over 2 years (hazard ratio [HR], 0.74; 95% confidence interval [CI], 0.58;0.95). This *post hoc* analysis assessed the effect of semaglutide on renal function and renal adverse events (AEs) by baseline estimated glomerular filtration rate (eGFR [Modification of Diet in Renal Disease model; MDRD]) in SUSTAIN 6.

Materials and methods: Changes in renal function, urine albumin-to-creatinine ratio (UACR) and acute renal AEs were assessed in subjects categorised by baseline eGFR (mL/min/1.73 m^2: normal [≥90], mild impairment [<90], moderate impairment [<60] and severe impairment [<30]).

Results: Overall, mean eGFR decreased from baseline to week 104 across all treatment groups and subgroups. The largest decreases were in subjects with normal renal function: −9.6 vs −7.4 mL/min/1.73 m^2 with semaglutide 0.5 mg vs placebo and −8.6 vs −6.5 mL/min/1.73 m^2 with semaglutide 1.0 mg vs placebo, respectively. The corresponding changes in eGFR from baseline were −4.8 vs −4.2 mL/min/1.73 m^2 and −3.2 vs −5.6 mL/min/1.73 m^2 for subjects with mild renal impairment; −2.1 vs −4.8 mL/min/1.73 m^2 and −2.4 vs −4.2 mL/min/1.73 m^2 for subjects with moderate renal impairment; and −4.1 vs −1.8 mL/min/1.73 m^2 and −0.5 vs −2.6 mL/min/1.73 m^2 for subjects with severe renal impairment. UACR decreased with increasing renal impairment with semaglutide 1.0 mg, but not with other treatment groups (**Table**). The number of AEs related to acute renal failure was generally higher in subjects with greater renal impairment at baseline, except with semaglutide 0.5 mg. The proportion of subjects experiencing new or worsening nephropathy was lower with both semaglutide doses vs placebo: 36/826 (4.4%) vs 54/824 (6.6%) with semaglutide 0.5 mg, and 23/822 (2.8%) vs 45/825 (5.5%) with semaglutide 1.0 mg. The majority of this was due to reductions in persistent macroalbuminuria: 22 (2.7%) vs 42 (5.1%) and 19 (2.3%) vs 38 (4.6%), respectively.

Conclusion: No renal-related safety issues were observed with semaglutide regardless of baseline renal function in SUSTAIN 6.

Table. UACR (change from baseline to week 104) and renal-related safety endpoints (week 104) stratified by baseline renal function in the SUSTAIN 6 trial

	Semaglutide 0.5 mg		Placebo 0.5 mg		Semaglutide 1.0 mg		Placebo 1.0 mg	
	Value	N	Value	N	Value	N	Value	N
UACR, mg/mmol [SD]								
Normal	−5.8 [61.6]	191	4.7 [59.1]	196	−0.8 [15.9]	206	5.1 [52.2]	200
Mild	−3.2 [40.5]	267	−1.0 [43.2]	261	−4.7 [36.8]	287	3.2 [36.4]	272
Moderate	−9.2 [73.4]	195	12.3 [114.1]	171	−4.9 [64.2]	168	5.4 [73.5]	173
Severe	−50.5 [132.8]	20	−43.3 [157.0]	28	−56.0 [239.2]	25	−16.0 [192.3]	31
AEs related to acute renal failure, n (%) [R]								
Normal	2 (0.8) [0.4]	247	10 (4.1) [2.6]	242	5 (2.0) [1.1]	245	7 (2.8) [1.4]	253
Mild	26 (8.0) [4.9]	327	23 (6.9) [4.5]	335	16 (4.5) [3.4]	357	25 (7.2) [4.6]	347
Moderate	37 (16.2) [11.5]	228	25 (11.7) [7.6]	214	16 (8.3) [5.8]	192	24 (12.5) [7.7]	192
Severe	1 (4.8) [3.1]	21	6 (21.4) [13.1]	28	3 (12.0) [7.2]	25	7 (21.2) [14.0]	33

Renal function categories based on MDRD eGFR: Normal, ≥90 mL/min/1.73 m^2; Mild, <90 mL/min/1.73 m^2; Moderate, <60 mL/min/1.73 m^2; Severe, <30 mL/min/1.73 m^2. Missing UACR values were derived from mixed model for repeated measurements. 'N' numbers for UACR vary from 'N' numbers for AEs because the baseline and end-of-treatment data for UACR were not available for all subjects. Subjects are adults with T2D at high risk of CV AEs. AE, adverse event; CI, confidence interval; CV, cardiovascular; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; R, event rate per 100 patient-years of exposure (calculated as the duration of the on-treatment period); SD, standard deviation; T2D, type 2 diabetes; UACR, urine albumin-to-creatinine ratio.

Clinical Trial Registration Number: NCT01720446
Supported by: Novo Nordisk A/S research support
Disclosure: **R. Silver:** Employment/Consultancy; Novo Nordisk, Foundation Medical Partners. Lecture/other fees; Novo Nordisk, AstraZeneca, Lilly.

OP 14 The bottom line: What's the best basal insulin?

79

Superior efficacy of insulin degludec/liraglutide vs insulin glargine as add-on to sodium-glucose co-transporter-2 inhibitor in patients with type 2 diabetes: DUAL IX trial

A. Philis-Tsimikas[1], L.K. Billings[2,3], R. Busch[4], C. Morales Portillo[5], R. Sahay[6], N. Halladin[7], R. Gronskyte[7], S. Harris[8];
[1]Scripps Whittier Diabetes Institute, San Diego, USA, [2]NorthShore University HealthSystem, Evanston, USA, [3]University of Chicago Pritzker School of Medicine, Chicago, USA, [4]Albany Medical Centre, Albany Medical Centre, USA, [5]Hospital Virgen Macarena, Seville, Spain, [6]Osmania Medical College, Hyderabad, India, [7]Novo Nordisk, Søborg, Denmark, [8]Western University, London, Canada.

Background and aims: The DUAL IX study investigated the safety and efficacy of insulin degludec/liraglutide (IDegLira) versus insulin glargine 100 units/mL (IGlar U100) as an add-on to sodium-glucose co-transporter-2 inhibitor (SGLT2i) ± other oral antidiabetic drug therapy (OAD) in patients with type 2 diabetes (T2D).

Materials and methods: In this 26-week, phase 3b, open-label trial, 420 patients with T2D uncontrolled on SGLT2i ± other OADs were randomised 1:1 to receive add-on therapy of IDegLira or IGlar U100 (100 units [U]/mL). Starting doses were 10 U in both treatment arms. Doses were titrated twice-weekly to a fasting glucose target of 4.0–5.0 mmol/L; only IDegLira had a maximum dose (50 dose steps). Analysis values for HbA_{1c}, body weight and insulin dose based on analysis of covariance model with treatment, pre-trial OAD and region as factors and corresponding baseline value as covariate; missing data are imputed using unconditional reference-based multiple imputation including data obtained after premature treatment discontinuation. Number of hypoglycaemic episodes were analysed by negative binomial regression model with a log link and the logarithm of the exposure time +7 days as offset; the model includes treatment and pre-trial OAD as fixed factors and missing data are imputed using multiple imputation. Hypoglycaemia defined as severe (requiring the assistance of another person) or blood glucose-confirmed (<3.1 mmol/L) symptomatic hypoglycaemic episodes.

Results: Mean HbA_{1c} decreased from 8.2% at baseline to 6.3% at week 26 for IDegLira and from 8.4 to 6.7% for IGlar U100; IDegLira superiority confirmed ($p < 0.0001$). IDegLira treatment resulted in unchanged mean body weight versus 2.0 kg weight gain with IGlar U100 ($p < 0.0001$). The rate of treatment-emergent hypoglycaemic episodes was 58% lower ($p = 0.0035$) with IDegLira (0.37 events/patient-year of exposure [PYE]) versus IGlar U100 (0.90 events/PYE). Total daily insulin dose after 26 weeks was 36 U for IDegLira versus 54 U for IGlar U100 ($p < 0.0001$). Adverse event rates were low in both treatment arms with no unexpected safety issues.

Conclusion: Superiority of IDegLira versus IGlar U100 as an add-on to SGLT2i was confirmed for glycaemic control, body weight, hypoglycaemia rate and total daily insulin dose.

DUAL IX trial
Change in HbA_{1c} and mean body weight, hypoglycaemic episodes and total daily insulin dose at end of trial

		IDegLira (n=210)	IGlar U100 (n=210)	Treatment Contrast [95% CI]	*p*-value
HbA_{1c}, % (SD)	Baseline	8.2 (0.9)	8.4 (1.1)	–	–
	EOT	6.3 (0.8)	6.7 (0.8)	–	–
	Δ from baseline	–1.9 (1.0)	–1.7 (1.1)	ETD –0.36 [–0.50; –0.21]	<0.0001
Mean body weight, kg (SD)	Baseline	89.3 (17.6)	87.2 (17.2)	–	–
	EOT	89.4 (17.8)	89.5 (17.8)	–	–
	Δ from baseline	–0.0 (3.8)	2.0 (3.9)	ETD –1.92 [–2.64; –1.19]	<0.0001
Hypoglycaemic episodes, episode/PYE		0.37	0.90	ERR 0.42 [0.23; 0.75]	0.0035
Total daily insulin dose at EOT, U/day (SD)		36.2 (13.4)	53.5 (26.1)	ETD –15.37 [–19.60; –11.13]	<0.0001

Δ, change; ETD, estimated treatment difference; EOT, end of trial; ERR, estimated rate ratio; HbA_{1c}, glycated haemoglobin; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; PYE, patient-year of exposure; U, units

Clinical Trial Registration Number: NCT02773368
Supported by: Novo Nordisk A/S
Disclosure: **A. Philis-Tsimikas:** Grants; Research support: Dexcom, Inc.; Novo Nordisk A/S; Sanofi; Mylan. Honorarium; Advisory Panel: Dexcom, Inc.; Eli Lilly and Company; Merck & Co., Inc.; Novo Nordisk A/S; Sanofi; AstraZeneca.

80

Similar glycaemic control and less or comparable hypoglycaemia with insulin glargine 300 U/ml vs degludec 100 U/ml in insulin naive type 2 diabetes: the BRIGHT randomised study

A. Cheng[1], J. Rosenstock[2], R. Ritzel[3], Z. Bosnyak[4], C. Devisme[5], P. Stella[6], A.M.G. Cali[7], X. Wang[8], J. Frias[9], R. Roussel[10], G.B. Bolli[11];
[1]Division of Endocrinology and Metabolism, University of Toronto, Toronto, Canada, [2]Dallas Diabetes Research Center at Medical City, Dallas, USA, [3]Klinikum Schwabing and Klinikum Bogenhausen, Städtisches Klinikum München GmbH, Munich, Germany, [4]Sanofi, Paris, France, [5]AIXIAL, Boulogne-Billancourt, France, [6]Sanofi, Budapest, Hungary, [7]Sanofi, Tokyo, Japan, [8]Sanofi, Beijing, China, [9]National Research Institute, Los Angeles, USA, [10]Assistance Publique Hôpitaux de Paris, Bichat Hospital, Paris, France, [11]Perugia University Medical School, Perugia, Italy.

Background and aims: BRIGHT is the first head-to-head clinical trial investigating the efficacy and safety of insulin glargine 300 U/ml (Gla-300) vs insulin degludec 100 U/ml (IDeg-100).
Materials and methods: In this 24-week, multinational, open-label, parallel-group, treat-to-target trial, 929 insulin-naïve adults with type 2 diabetes (T2DM) inadequately controlled with oral antihyperglycemic drugs ± glucagon-like peptide-1 receptor agonists, were randomised 1:1 to once-daily Gla-300 or IDeg-100. Primary endpoint: HbA_{1c} change from baseline to week 24. Secondary endpoints included hypoglycaemia, blood glucose levels, and adverse events.
Results: The included individuals had mean HbA_{1c} of 8.6%, diabetes duration of 10.6 years and BMI of 31.5 kg/m^2. Non-inferiority of Gla-300 vs IDeg-100 was demonstrated for the primary endpoint (**Table**). Gla-300 had similar fasting self-measured plasma glucose reduction to IDeg-100 (**Table**), with final daily insulin doses of 0.54 and 0.43 U/kg from starting evening doses, per label, of 0.2 U/kg and 10 U/day (0.12 U/kg), respectively. Over the 24-week period, incidence of confirmed (≤3.9 mmol/l) or severe hypoglycaemia was comparable, but event rates were lower with Gla-300 vs IDeg-100, by 14% at any time of day (24 h) and 19% at night (00:00–05:59 h) (**Table**).
Conclusion: BRIGHT showed that Gla-300 provides similar glycaemic control to IDeg-100, with less or comparable hypoglycaemia, in previously inadequately controlled, insulin-naïve adults with T2DM.

Table. Key efficacy and safety results for Gla-300 vs IDeg-100, over the 24-week on-treatment period

		Gla-300 N=462	IDeg-100 N=462
Efficacy parameters (ITT population)			
HbA_{1c}, %	Baseline	8.72 ± 0.83	8.57 ± 0.80
	Week 24	7.03 ± 0.79	7.03 ± 0.77
	LS mean change (baseline to week 24) ± SE	−1.64 ± 0.04	−1.59 ± 0.04
	LS mean difference (95% CI)	−0.05 (−0.15 to 0.05)*	
*Fasting SMPG**, mmol/l*	Baseline	9.9 ± 2.3	9.5 ± 2.1
	Week 24	6.4 ± 1.3	6.3 ± 1.2
	LS mean change (baseline to week 24) ± SE	−3.2 ± 0.1	−3.3 ± 0.1
	LS mean difference (95% CI)	0.06 (−0.11 to 0.23)	
Safety parameters (safety population)		N=462	N=462
Confirmed (≤3.9 mmol/l) or severe hypoglycaemia at any time of day (24 h)	Incidence, n (%)	307 (66.5)	319 (69.0)
	OR (95% CI)	0.88 (0.66 to 1.17)	
	Rate, events (events/participant-year)	1969 (9.34)	2258 (10.83)
	RR (95% CI)	0.86 (0.81 to 0.92)	
Nocturnal (00:00–05:59 h) confirmed (≤3.9 mmol/l) or severe hypoglycaemia	Incidence, n (%)	132 (28.6)	133 (28.8)
	OR (95% CI)	0.99 (0.74 to 1.32)	
	Rate, events (events/participant-year)	385 (1.83)	472 (2.26)
	RR (95% CI)	0.81 (0.71 to 0.92)	
Treatment-emergent AE/ serious treatment-emergent AE, %		43.7/4.5	47.8/4.3

*Primary endpoint; p<0.0001 for non-inferiority (non-inferiority margin 0.3 %); **Fasting SMPG target was 4.4–5.6 mmol/l; baseline and week 24 values are mean ± standard deviation.
AE, adverse event; CI, confidence interval; ITT, intention-to-treat; LS, least-squares; OR, odds ratio; RR, rate ratio; SE, standard error; SMPG, self-measured plasma glucose

Clinical Trial Registration Number: NCT02738151
Supported by: Sanofi
Disclosure: **A. Cheng:** Employment/Consultancy; Abbott, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, Novo Nordisk, Sanofi, Servier, Takeda. Honorarium; Abbott, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, Novo Nordisk, Sanofi. Lecture/other fees; Abbott, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, Novo Nordisk, Sanofi.

81

Reducing insulin degludec around regular exercise improves time spent in euglycaemia in people with type 1 diabetes: a randomised cross-over trial

O. Moser[1], M.L. Eckstein[1], A. Mueller[2], P. Birnbaumer[2], F. Aberer[3], G. Koehler[3], C. Sourij[3], H. Kojzar[3], P. Holler[4], H. Simi[4], P. Pferschy[3], P. Dietz[2], R.M. Bracken[1], P. Hofmann[2], H. Sourij[3];
[1]Swansea University, Swansea, UK, [2]University of Graz, Graz, Austria, [3]Medical University of Graz, Graz, Austria, [4]FH JOANNEUM, Bad Gleichenberg, Austria.

Background and aims: Insulin degludec (IDeg) is associated with a similar risk of exercise-induced hypoglycaemia compared to insulin glargine. Though adjustment of bolus insulin is commonly recommended around exercise with an unaltered background of IDeg, no research has explored the impact of intermittent IDeg dose reduction in patients regularly exercising on a few consecutive days. Therefore, the aim of this study was to compare the time spent in euglycaemia in people with type 1 diabetes (T1D) during 5 consecutive days of continuous moderate-intensity exercise, on either 100% or 75% of their usual IDeg dose.
Materials and methods: 9 participants with T1D (4 females, age 32.1 ± 9.0 years, BMI 25.5 ± 3.8 kg/m^2, HbA_{1c} 7.2 ± 2.8% (55 ± 7 mmol/mol)) performed a cardio-pulmonary exercise test on a cycle ergometer to determine maximum oxygen uptake (VO_{2max}) as well as the first (LTP_1) and the second lactate turn points (LTP_2). Afterwards, a flash glucose monitoring sensor was inserted, and participants were switched to IDeg if not running on that insulin before. 3 days before the first exercise phase participants were randomised to either 100% or 75% of their usual IDeg dose. Then participants exercised on a cycle ergometer for 55 min at a moderate intensity (midpoint between LTP_1 and LTP_2 (~65% VO_{2max})) for 5 consecutive days in the evening at the clinical research facility. After a washout period of 4 weeks, participants performed the second exercise phase

for 5 consecutive days with the remaining allocation. Time spent in eu- (3.9–10 mmol/l), hypo- (<3.9 mmol/l) and hyperglycaemia (>10 mmol/l), AUC for these glycaemic ranges, numbers of hypoglycaemic events, glycaemic CV and insulin as well as carbohydrate intake were compared for the entire 5 days. Data were compared between groups by paired t-test and Wilcoxon matched-pairs signed rank test, $p < 0.05$.

Results: A 25% reduction in IDeg dose around regular exercise achieved a longer time spent in euglycaemia ($p = 0.04$) with no effect on numbers of hypoglycaemic events ($p = 0.91$) or time spent in hypo- ($p = 0.07$) or hyperglycaemia ($p = 0.38$) (table 1). The amount of carbohydrates and dose of bolus insulin injections were similar between the two dosing regimens ($p > 0.05$).

Conclusion: This is the first study demonstrating that people with T1D should be encouraged to reduce IDeg dose by 25% when performing regular exercise on consecutive days.

Table 1 Comparison of groups over the 5-day period (mean ± SD)

	75% IDeg dose	100% IDeg dose	p-value
Time $_{euglycaemia}$ (min (%))	4008 ± 938 (62 ± 15)	3566 ± 856 (57 ± 14)	0.04
Time $_{hypoglycaemia}$	270 ± 165 (4 ± 3)	240 ± 112 (4 ± 2)	0.07
Time $_{hyperglycaemia}$	2187 ± 1046 (34 ± 16)	2440 ± 1094 (39 ± 18)	0.38
AUC $_{euglycaemia}$ (min × mmol/l)	28372 ± 6684	25187 ± 6384	0.03
AUC $_{hypoglycaemia}$	1347 ± 1474	1032 ± 1017	0.05
AUC $_{hyperglycaemia}$	29062 ± 15274	31749 ± 15269	0.49
Hypoglycaemic events (n)	4.8 ± 3.4	4.7 ± 2.9	0.91
CV $_{glycaemia}$ (%)	40 ± 7	39 ± 7	0.57
Prandial insulin used (IU)	72 ± 32	73 ± 40	0.89
Correction insulin used (IU)	20 ± 10	17 ± 10	0.31
Prandial carbohydrates (g)	739 ± 237	648 ± 115	0.10
Correction carbohydrates (g)	219 ± 112	259 ± 114	0.17

Clinical Trial Registration Number: DRKS00013477
Supported by: Novo Nordisk: Unrestricted grant to Medical University of Graz
Disclosure: **O. Moser:** Grants; Novo Nordisk A/S: Unrestricted grant to Medical University of Graz.

82

Efficacy, safety and patient-reported outcomes (PROs) of patient- vs physician-led titration of Gla-300 in uncontrolled type 2 diabetes: the pan-European TAKE CONTROL study

E. Delgado[1,2], D. Russell-Jones[3], A. Dauchy[4], G. Dimitriadis[5], H.A. Frandsen[6], L. Popescu[7], A. Roborel de Climens[8], B. Schultes[9], K. Strojek[10], M. Bonnemaire[4];
[1]Department of Medicine, University of Oviedo, Oviedo, Spain, [2]Endocrinology and Nutrition Service, Hospital Universitario Central de Asturias, Oviedo, Spain, [3]Department of Diabetes and Endocrinology, Royal Surrey County Hospital, Guildford, UK, [4]Global Diabetes Division, Sanofi, Paris, France, [5]National and Kapodistrian University of Athens Medical School, Attikon University Hospital, Athens, Greece, [6]Department of Internal Medicine, Amager Hospital, Copenhagen, Denmark, [7]Global R&D Operations, Sanofi, Bucharest, Romania, [8]Global Medical Evidence Generation, Sanofi, Lyon, France, [9]eSwiss Medical & Surgical Center, Department of Internal Medicine, Endocrinology, Diabetes & Metabolism, St. Gallen, Switzerland, [10]Department of Internal Diseases, Diabetology and Cardiometabolic Diseases SMDZ, Zabrze, Silesian Medical University, Zabrze, Poland.

Background and aims: People with type 2 diabetes (T2DM) require effective insulin titration to achieve HbA_{1c} targets. However, most fail to achieve HbA_{1c} goals in clinical practice, owing to patient- and physician-related barriers. This study evaluated whether the second-generation basal insulin, insulin glargine 300 U/ml (Gla-300) empowered patients to self-titrate effectively.

Materials and methods: This 24-week, multicentre, randomised, open-label, parallel-group study compared the efficacy and safety of a simple Gla-300 titration algorithm (fasting self-monitored blood glucose [SMBG] >7.2 mmol/l, +3 U; <4.4 mmol/l, −3 U), when managed by patients vs physicians, in people with uncontrolled T2DM. Participants ($N = 631$) from 10 European countries were randomised 1:1 to each group.

Results: Baseline characteristics were similar in both groups. The least squares (LS) mean difference for patient- vs physician-led groups in HbA_{1c} change from baseline was −0.13% [95% CI: −0.2619 to −0.0004], demonstrating superiority for self-titration ($p = 0.0247$). The proportion of participants achieving a fasting SMBG of 4.4–7.2 mmol/l without confirmed (<3.0 mmol/l) or severe hypoglycaemia was 67% and 58% in the patient- and physician-led groups ($p = 0.0187$), and 31.2% and 23.7% of participants, respectively, achieved HbA_{1c} <7% ($p = 0.0269$). Severe hypoglycaemia was reported in 0.6% and 0.3% of the patient- and physician-led groups, respectively. Similar decreases in LS mean Diabetes Distress Scale total score were observed in both groups from baseline to week 24: −0.24 (95% CI: −0.32 to −0.15) in the patient- and −0.16 (−0.25 to −0.08) in the physician-led group (difference: −0.07 [−0.19 to 0.04]). More patients with high distress (mean total score ≥3) were observed in the physician-led titration group at week 24 (12.2% vs. 8.5% in the patient-led group). LS mean Diabetes Empowerment Scale scores similarly improved from baseline to week 24 in the patient- (0.19 [0.14 to 0.24] and physician-led (0.12 [0.07 to 0.17]) groups (difference: 0.07 [0.00 to 0.14]).

Conclusion: Self-titration of Gla-300 in T2DM provides more effective glycaemic control without increased hypoglycaemia, with as-confident and a trend towards less-distressed patients, versus physician-led titration.

Clinical Trial Registration Number: EudraCT: 2015-001626-42
Supported by: Sanofi
Disclosure: **E. Delgado:** Employment/Consultancy; AstraZeneca, Novo Nordisk, Lilly, Sanofi, GlaxoSmithKline, Pfizer, Almirall, Novartis, Abbott Laboratories, Esteve, and Merck Sharp & Dohme. Grants; AstraZeneca, Novo Nordisk, Sanofi, Pfizer, and Roche. Honorarium; AstraZeneca, Novo Nordisk, Lilly, Sanofi, GlaxoSmithKline, Pfizer, Almirall, Novartis, Abbott Laboratories, Esteve, and Merck Sharp & Dohme. Lecture/other fees; AstraZeneca, Novo Nordisk, Lilly, Sanofi, GlaxoSmithKline, Pfizer, Almirall, Novartis, Abbott Laboratories, Esteve, and Merck Sharp & Dohme.

83

The clinical benefits of IDegLira in DUAL VII were achieved while using a simple regimen with fewer injections and dose adjustments compared with basal-bolus therapy

E.M. Miller[1], E. Jodar[2], K. Khunti[3], D. Sugimoto[4], P. Orsy[5], M.F. Ranthe[5], A. Viljoen[6];
[1]Diabetes Nation, Bend, USA, [2]University Hospital Quiron Salud, Madrid, Spain, [3]Leicester General Hospital, Leicester, UK, [4]Cedar Crosse Research Center, Chicago, USA, [5]Novo Nordisk A/S, Søborg, Denmark, [6]Lister Hospital, Stevenage, UK.

Background and aims: Complex treatment regimens, such as basal-bolus insulin therapy (BB), are associated with lower compliance, greater treatment burden and poor patient satisfaction. Complex regimens are also a major concern for physicians since they require more resources and clinical decisions, both of which become more problematic as type 2 diabetes (T2D) progresses. In DUAL VII, insulin degludec/liraglutide (IDegLira) resulted in non-inferior HbA_{1c} reductions (as per the trial design), weight loss (−0.9 vs. 2.6 kg), and an 89% reduction in rates of hypoglycaemia compared with BB in patients with T2D. This *post hoc* analysis evaluated the treatment complexity of IDegLira vs. BB in terms of number of injections and dose adjustments.

Materials and methods: In a 26-week, open-label trial, patients with T2D uncontrolled on metformin and 20–50 U insulin glargine 100 U/mL (IGlar U100) were randomised 1:1 to IDegLira ($N = 252$) or BB (IGlar U100 + insulin aspart ≤4 times/day; $N = 254$). IDegLira was initiated at 16 dose steps/units (U) (16 U insulin degludec +0.58 mg liraglutide); initial IGlar U100 dose was the pre-trial dose (mean: 33 U). Both were titrated twice-weekly, based on the mean of three pre-breakfast self-monitored plasma glucose (SMPG) readings, to a target of 4–5 mmol/L. Insulin aspart was initiated at 4 U/main meal and titrated twice-weekly to a pre-prandial and bedtime SMPG target of 4–6 mmol/L. This analysis reports the observed mean number of insulin injections and dose adjustments during 26 weeks.

Results: Despite the lower starting basal insulin component dose with IDegLira vs. BB, the number of basal insulin dose adjustments were similar during treatment (Table). The mean number of bolus insulin adjustments increased steadily during the trial to 200 per patient (median [min; max]: 218 [1; 569]). 66.5% of patients in the BB group were receiving ≥3 bolus injections/day at Week 26 in addition to their basal insulin and SMPG measurements in connection with each injection.

Conclusion: Burdensome regimens impact on patients' quality of life, treatment adherence and ability to achieve good glycaemic control. Compared with BB, the clinical benefits of IDegLira (comparable HbA_{1c} reduction, lower hypoglycaemia rates and weight loss) are achieved using a more convenient regimen in the DUAL VII study. In addition to the clinical benefits, this simple regimen has the advantages of fewer daily injections, SMPG readings and dose adjustments, requiring fewer clinical decisions.

Regimen complexity at 26 weeks

	IDegLira	Basal–bolus
Mean number (SD) of basal insulin dose adjustments	16.6 (6.8)	17.1 (10.2)
Mean number (SD) of bolus insulin dose adjustments	–	200.1 (118.6)
Number (%) of patients receiving the following bolus injections/day		
0	–	3 (1.3)
1	–	18 (7.8)
2	–	56 (24.3)
≥3	–	153 (66.5)

IDegLira, insulin degludec/liraglutide

Clinical Trial Registration Number: NCT02420262
Supported by: Novo Nordisk
Disclosure: **E.M. Miller:** Honorarium; Eli Lilly, Boehringer Ingelheim, Novo Nordisk, Astra Zeneca, Janssen, Intarsia, BD, Abbott.

84

CONFIRM: a comparative effectiveness study of insulin degludec and insulin glargine 300 units/ml (glargine U300) in insulin-naive patients with type 2 diabetes

J. Tibaldi[1], S. Haldrup[2], V. Sandberg[2], M.L. Wolden[2], H.W. Rodbard[3]; [1]Fresh Meadows Diabetes and Endocrinology, New York, USA, [2]Novo Nordisk A/S, Søborg, Denmark, [3]Clinical Research, Rockville, USA.

Background and aims: The Clinical Outcome Assessment of the Effectiveness of Insulin Degludec in Real-life Medical Practice (CONFIRM) study compared the real-world effectiveness of insulin degludec (degludec) and insulin glargine 300 units/mL (glargine U300) in insulin-naïve patients with type 2 diabetes.

Materials and methods: This retrospective, non-interventional, comparative effectiveness study used electronic health records of US-based patients from Explorys, with propensity-score matching to balance baseline characteristics between cohorts. The primary endpoint, Δ HbA_{1c} from baseline to 6 months' follow-up, was estimated using a repeated-measure analysis with subject as random effect. Rate of hypoglycaemic episodes (defined using International Classification of Diseases codes 9/10) and proportion of patients with hypoglycaemia were estimated using negative binomial and logistic regression, respectively. Time-to-discontinuation of basal insulin was analysed using a Cox Proportional Hazard model. This study included adults with type 2 diabetes treated with oral antidiabetic drugs, intensified with either degludec or glargine U300.

Results: Data from 4056 patients were analysed. After matching, baseline characteristics of the groups were comparable ($n = 2028$ in each group). At follow-up, Δ HbA_{1c} was significantly lower with degludec (−1.5%) versus glargine U300 (−1.2% [treatment difference, −0.3%, $p = 0.029$]). Rates of hypoglycaemia were significantly lower with degludec versus glargine U300 (rate ratio: 0.70, $p = 0.045$). Similarly, the proportion of patients experiencing hypoglycaemia was significantly lower with degludec (odds ratio: 0.64; $p < 0.01$). Patients treated with glargine U300 had a 37% higher risk of treatment discontinuation versus degludec (hazard ratio: 1.37, $p < 0.01$).

Conclusion: Data from the largest real-world comparative effectiveness study of degludec and glargine U300 to date demonstrated improved glycaemic control, lower rates of hypoglycaemia and lower risk of discontinuation with degludec versus glargine U300.

Supported by: Novo Nordisk
Disclosure: **J. Tibaldi:** Employment/Consultancy; Novo Nordisk. Lecture/other fees; Novo Nordisk.

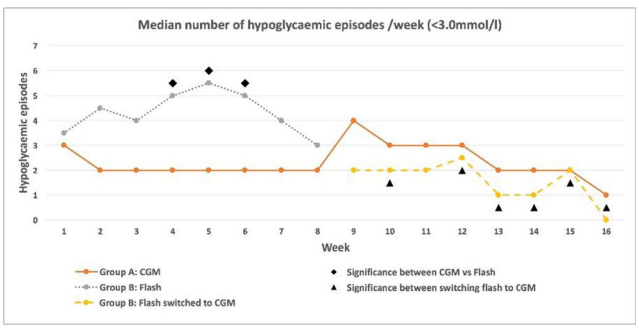
OP 15 Technological advances in the treatment of diabetes

85

THE I-HART CGM study: hypoglycaemic episodes reduced with continuous glucose monitoring compared to Flash in adults with type 1 diabetes

P. Avari[1], V. Moscardo[2], N. Jugnee[1], M. Reddy[1], N. Oliver[1];
[1]Imperial College London, London, UK, [2]Universitat Politècnica de Valencia, Valencia, Spain.

Background and aims: The I-HART CGM Study is the first head-to-head glucose monitoring study designed to assess impact of flash and continuous glucose monitoring (CGM) in highest risk adults with type 1 diabetes mellitus (T1DM). We have previously shown CGM was associated with reduced hypoglycaemia exposure compared to flash. In this analysis, we assess the number of hypoglycaemic episodes in each group.
Materials and methods: Forty participants with highest risk diabetes (Gold Score ≥4 or recent severe hypoglycaemia using insulin injections) were recruited to this randomized, parallel group trial. Following two weeks of blinded CGM, participants were randomized to CGM (DexcomG5; $n = 20$) or flash (Freestyle Libre; $n = 20$) for 8-weeks. An open extension phase enabled participants on CGM to continue for a further 8 weeks, and those on flash to switch to CGM over this period. A non-parametric analysis was performed. Significance was calculated between flash and CGM throughout the study period, and between flash switching to CGM with the corresponding weekly interval. The number of hypoglycaemic episodes were analyzed for each week. Each episode of hypoglycaemia was defined with a duration of 20 minutes and a separation time of 15 minutes.
Results: Over the first 8 weeks, a reduced number of serious hypoglycaemic episodes (<3.0 mmol/L) was observed with CGM compared to flash during each consecutive week. Statistical significance between groups was observed at weeks 4–6 ($p < 0.05$). Less serious hypoglycaemic episodes (<3.9 mmol/L) showed similar reduction in events at weeks 5 and 6 ($p < 0.05$), however, the difference between the two groups was less marked. Between 8–16 weeks, no significant change was observed in the group continuing on CGM. A significant reduction in hypoglycaemic events was observed when switching flash to CGM ($p < 0.05$) for serious hypoglycaemia <3.0 mmol/L at weeks 10 and 12–16 ($p < 0.05$). For hypoglycaemia <3.9 mmol/L, reduced events were noted at weeks 13, 14 and 16.
Conclusion: Real-time CGM shows greater beneficial impact on reducing hypoglycaemic episodes compared with flash in adults with T1DM at highest risk of hypoglycaemia. In particular, the benefits of CGM was observed with more serious, clinically important hypoglycaemia (<3.0 mmol/l). However, the significant effect on reducing hypoglycaemic episodes with CGM was not sustained throughout the 16 weeks, and may reflect the small cohort numbers or user fatigue with alarms. Switching flash to CGM significantly reduced hypoglycaemic episodes. These findings are important when selecting monitoring techniques to minimize the clinical and cost, impact of hypoglycaemia.



Clinical Trial Registration Number: NCT03028220
Supported by: Investigator-initiated study funded by Dexcom
Disclosure: **P. Avari:** Other; Investigator-initiated study funded by Dexcom.

86

First assessment of the performance of an implantable continuous glucose monitoring (CGM) system through 180 days in a primarily adolescent population with type 1 diabetes

A. Abitbol[1], R. Aronson[2], R. Rastogi[3], C. Mdingi[3], X. Chen[3], K.S. Tweden[3];
[1]LMC Diabetes & Endocrinology, Oakville, Canada, [2]LMC Diabetes & Endocrinology, Toronto, Canada, [3]Senseonics, Incorporated, Germantown, USA.

Background and aims: An implantable continuous glucose monitoring (CGM) system (Eversense® XL, Senseonics, Maryland USA) recently received CE Mark for 180-day duration in adults. The current study is the first investigation of the performance of the Eversense XL through 180 days in a primarily adolescent population with type I diabetes (T1D).
Materials and methods: This study was a prospective, single-center, single-arm, 180-day study that evaluated the effectiveness and safety of the implantable CGM system among Canadian adolescent and adult participants with T1D. Effectiveness measures included mean absolute relative difference (MARD), system agreement with Yellow Springs Instrument (YSI) glucose values, and Clarke Error Grid analysis using paired CGM and reference YSI glucose analyzer values. Adult participants were inserted with two sensors and adolescent participants were inserted with one sensor in the upper arm. CGM system accuracy studies were performed every 30 days. The safety assessment included the incidence of insertion/removal-procedure and device-related serious adverse events (SAEs) through 180 days post-insertion.
Results: Thirty-Six participants (30 adolescent/6 adult, 13 female/23 male, mean age 17 ± 9.2 years, mean BMI 22 ± 4 kg/m^2) received the CGM system. One subject withdrew at Day 1 due to intravenous access issues. CGM system agreement with YSI glucose within 15 mg/dL or 15% of YSI glucose values ($N = 7163$) through 60, 120 and 180 days was 82.9%, 83.6% and 83.4% (95% CI: 79.7%–85.5%), respectively. Overall MARD was 9.4% (95% CI: 8.6%–10.5%). Clarke Error Grid analysis showed 99% of paired values in clinically acceptable error zones A and B. No insertion/removal or device-related SAEs were reported.
Conclusion: The Eversense XL CGM system is safe and accurate through 180 days of Sensor wear in a primarily adolescent population.
Supported by: Senseonics, Incorporated
Disclosure: **A. Abitbol:** Grants; Senseonics.

87

Development of a computerised, guideline based continuous glucose monitoring (CGM) directed therapy algorithm to assist physicians in the management of patients with type 2 diabetes

R.A. Vigersky, S. Guan, C. McMahon, B. Huhta, P. Agrawal, R. Buechler, P. Chung, O. Cohen, G. Hamer, J. Smith, S. Lee, R. Morawiec, L. Resurreccion, K. Saad, F. Kaufman;
Medtronic Diabetes, Northridge, USA.

Background and aims: To address the clinical inertia that often occurs in T2D treatment, facilitate interpretation of CGM-derived data, and promote adherence to professional organizations' guidelines, a computerized decision support system (CDSS) for physicians has been developed.
Materials and methods: The CDSS is based on retrospective CGM data and linked to the medication guidance of the American Diabetes Association and the American Association of Clinical Endocrinology. While CDSS's are used in diabetes management, none have included CGM-derived data with pattern analysis to help address the wide range

of pharmacotherapeutic possibilities in patients with T2D. Inputs to the system include: 1) A1C target; 2) current medication dose and frequency; and 3) retrospective CGM data. The CDSS algorithm includes all classes of diabetes medications except dopamine agonists and bile acid sequestrants and considers a wide range of baseline medication states from therapy-naïve to triple therapy combinations. Major patterns are identified by a pattern-recognition algorithm. The CDSS provides 0–6 therapy considerations (by class) matching the clinically most important pattern with the predominant action of a drug. These considerations are based on mean sensor glucose, current medication(s) and dose(s), and presence/absence of hyperglycemic symptoms. Therapy considerations include making no change in the baseline therapy, increasing or reducing the dose of a current medication(s), or adding/substituting/stopping a medication including the initiation of insulin therapy, where appropriate. Since large scale head-to-head trials of comparative effectiveness are not available for most drugs, the therapy considerations are arranged alphabetically. Importantly, physicians must apply their professional judgement in assessing these options and consider important clinical factors such as past medication use/tolerance, allergies, renal and hepatic function before making a therapy decision.

Results: The CDSS output from over 300 cases was presented to over 75 physicians in the U.S., Europe, and Asia. Examples: In a patient taking metformin and glimepiride with a primary pattern of low sensor glucose overnight, therapy considerations would be: "reduce or stop sulfonylurea" and "consider replacing sulfonylurea with a non-hypoglycemia inducing medication"; in a patient on glipizide-XR, metformin and basal insulin the morning with a primary pattern of high sensor glucose from 1600–2400 hours, a therapy consideration would be: "increase basal insulin". There was a high degree of concordance with the pattern identification and therapy considerations. The CDSS may be customized to reflect the guidance from other professional organizations or governmental bodies.

Conclusion: We anticipate that CDSS's such as this one will become important tools in assisting physicians to make appropriate medication changes in a complex therapy environment.

Disclosure: **R.A. Vigersky:** None.

88

Continuous glucose monitoring in healthy non-diabetic participants: a multicentre prospective study

A. Peters[1], Z. Li[2], S. DuBose[2], R. Beck[2], V. Shah[3], R.S. Weinstock[4], M. Tansey[5], D. Sparling[6], S. Woerner[7], F. Vendrame[8], R. Bergenstal[9], J. Sherr[10], J. Shine Dyer[11], S.E. Watson[12], B. Tamborlane[10];
[1]USC, Los Angeles, [2]JCHR, Tampa, [3]BDC, Aurora, [4]SUNY Upstate, Syracuse, [5]Univ Iowa, Iowa City, [6]Univ Oklahoma, Oklahoma City, [7]Indiana Univ, Indianapolis, [8]Univ Miami, Miami, [9]IDC Park Nicollet, St Louis Park, [10]Yale, New Haven, [11]COPEDS, Columbus, [12]Wendy Novak, Louisville, USA.

Background and aims: Much effort has been placed on standardization of CGM-based outcomes that are increasingly being used in clinical research related to newer therapeutics and devices. This study was undertaken to determine the distribution of sensor glucose levels in healthy, non-diabetic participants using the recently approved DexCom G6 system.

Materials and methods: In this multicenter study, healthy, non-diabetic children and adults (age 7 to 80 years, BMI <25 kg/m^2 or between 5th and 85th percentile, and HbA1c <5.7%) were included. Each subject wore a blinded DexCom G6 for ~10 days and kept a daily log of exercise, meals, and sleep. Only participants with no positive islet antibodies and at least 72 hours of CGM data were analyzed. Participants were divided into 5 age cohorts for analysis.

Results: A total of 201 healthy non-diabetic participants were screened and 151 enrolled and analyzed. The cohort was 67% female, 84% non-Hispanic White, and had mean HbA1c of 5.1%. Participants ≥18 years old had mean BMI of 24 kg/m^2 and those <18 years had mean BMI percentile of 51%. Overall mean 24-hour sensor glucose level was 100 ± 7 mg/dL, a finding that was consistent except for those participants 60+ years who had slightly higher glucose levels (104 ± 9 mg/dL). Peak postprandial glucose was 129 mg/dL with no major differences across age groups. Overall, meal related increases in sensor glucose resulted in daytime glucose levels 2 mg/dL higher than nighttime values. Sensor glucose levels above 120 and below 70 mg/dL were not uncommon across all age groups but sensor values above 180 mg/dL were rarely observed except in participants 60+ years old (Table). In all age groups, sensor levels <54 mg/dL were rarely seen. Overnight mean and nadir sensor glucose levels following exercise days were slightly lower compared with sedentary days, but the differences were not statistically significant (data not shown).

Conclusion: As greater emphasis is placed on glycemic metrics beyond HbA1c levels, the current study provides a normative set of sensor glucose levels that can be used for comparison for clinical trials. It is noteworthy that sensor glucose levels >180 and <54 mg/dL were very uncommon in our healthy non-diabetic participants, which support these levels as the thresholds for clinically important hyper- and hypoglycemia in diabetes. With improvements in both pharmacologic agents and mechanical solutions the ultimate goal may be to attain tighter glycemic control in those living with diabetes by altering the hyperglycemic threshold to 160 mg/dL.

Table: Sensor glucose levels overall and by age in healthy non-diabetic subjects (n=151)

		Age (Years)				
	All (n=151)	7 to 11 (n=26)	12 to 17 (n=30)	18 to 24 (n=28)	25 to 59 (n=41)	60+ (n=26)
Overall Glucose Control						
Mean glucose (mg/dL) – mean ± SD	100±7	99±7	98±6	99±6	99±6	104±9
Glucose coefficient of variation (%) - mean ± SD	16%±3%	16%±2%	15%±2%	18%±3%	16%±3%	17%±4%
% Time in range 70 to 120 mg/dL – median (IQR)	88% (81%, 91%)	89% (83%, 91%)	91% (85%, 92%)	85% (80%, 90%)	88% (85%, 91%)	79% (69%, 85%)
Hyperglycemia						
% Time above 120 mg/dL - median (IQR)	9.8% (5.5%, 15.5%)	8.4% (4.8%, 14.9%)	7.0% (5.0%, 12.1%)	10.3% (5.8%, 15.1%)	9.8% (5.7%, 11.4%)	16.3% (10.5%, 26.4%)
% Time above 140 mg/dL – median (IQR)	2.0% (0.9%, 4.1%)	1.7% (0.8%, 2.9%)	1.2% (0.3%, 2.0%)	2.4% (1.2%, 4.5%)	2.1% (1.1%, 3.1%)	4.1% (1.3%, 8.6%)
% Time above 160 mg/dL – median (IQR)	0.3% (0.1%, 0.9%)	0.2% (0.1%, 0.8%)	0.2% (0.0%, 0.2%)	0.4% (0.2%, 1.1%)	0.4% (0.2%, 0.9%)	0.6% (0.1%, 2.9%)
% Time above 180 mg/dL – median (IQR)	0.0% (0.0%, 0.2%)	0.0% (0.0%, 0.1%)	0.0% (0.0%, 0.0%)	0.0% (0.0%, 0.4%)	0.0% (0.0%, 0.2%)	0.1% (0.0%, 0.5%)
Hypoglycemia						
% Time below 70 mg/dL – median (IQR)	1.1% (0.3%, 2.9%)	1.0% (0.3%, 1.7%)	1.7% (0.6%, 2.6%)	1.0% (0.4%, 3.4%)	1.0% (0.3%, 2.3%)	1.0% (0.2%, 3.4%)
% Time below 60 mg/dL – median (IQR)	0.2% (0.0%, 0.5%)	0.1% (0.0%, 0.3%)	0.2% (0.0%, 0.5%)	0.1% (0.0%, 0.6%)	0.2% (0.0%, 0.4%)	0.2% (0.0%, 0.6%)
% Time below 54 mg/dL – median (IQR)	0.0% (0.0%, 0.2%)	0.0% (0.0%, 0.1%)	0.0% (0.0%, 0.2%)	0.0% (0.0%, 0.2%)	0.0% (0.0%, 0.1%)	0.1% (0.0%, 0.1%)

Supported by: Leona M. and Harry B. Helmsley Charitable Trust
Disclosure: **A. Peters:** None.

89

Decreased time <70 mg/dl for patients previously using pumps, multiple daily injections, CGM or no CGM before using a predictive low glucose suspend system: the PROLOG study

B.A. Buckingham[1], Z. Li[2], J.E. Pinsker[3], G.P. Forlenza[4], E. Cengiz[5], L. Ekhlaspour[1], M. Church[3], P. Wadwa[4], S.A. Weinzimer[5], W. Woodall[2], B.B. Dokken[6], V. Swanson[6], J. Lum[2], C. Kollman[2], R. Beck[2];
[1]Department of Pediatric Endocrinology, Stanford University, Palo Alto, [2]Jaeb Center for Health Research, Tampa, [3]Sansum Diabetes Research Institute, Santa Barbara, [4]Barbara Davis Center, Aurora, [5]Department of Pediatric Endocrinology and Diabetes, Yale University, New Haven, [6]Department of Clinical Affairs, Tandem Diabetes Care, San Diego, USA.

Background and aims: Hypoglycemia is a major concern for patients with type 1 diabetes. The predictive low glucose suspend (PLGS) feature on the t:slim X2 insulin pump with Basal-IQ Technology allows for automatic basal rate suspension when the sensor glucose is predicted to reach 80 mg/dL within 30 min, and resumes immediately when glucose begins to rise. Previous studies have found increased hyperglycemia associated with some PLGS systems.

Materials and methods: A randomized crossover trial was conducted at 4 sites in the US. Participants had type 1 diabetes (age ≥6 years, n = 103) and were previously treated with MDI (n = 17) or pump therapy (n = 86), either with (n = 87) or without CGM (n = 16). Subjects used the t:slim X2

with Basal-IQ (PLGS) during one 3-week period and sensor augmented pump (SAP) during another 3-week period. The order of treatment was randomized. The primary outcome was the percentage of CGM sensor glucose values <70 mg/dL compared between treatment arms using a repeated measures regression model. Pump suspension or resumption of insulin did not generate alarms.

Results: Sensor time <70 mg/dL decreased by 31% relative to SAP in the PLGS arm (4.5% ± 3.9% SAP vs. 3.1% ± 2.8% PLGS, mean values; $P < 0.001$) with no change in mean glucose between groups (159 ± 27 mg/dL SAP vs. 159 ± 25 mg/dL PLGS). Time in range (70 mg/dL–180 mg/dL) modestly but significantly increased by 3% in the PLGS arm relative to the SAP arm (63% ± 15% SAP vs. 65% ± 15% PLGS, mean values, $P < 0.001$). The mean duration of pump suspensions was 18 minutes, and only 3% lasted for >1 hr. The mean number of suspensions each day was 5.7 ± 4.3 which was associated with a significant reduction in basal insulin delivery from a mean of 21.5 units/d to 20.3 units/d ($P < 0.001$), while bolus delivery was unchanged. There was a 30% decrease in mean percent time <70 mg/dL for subjects previously using pumps and a 34% decrease for subjects previously using MDI. For subjects previously using CGM there was a 33% decrease in time <70 mg/dL, and for non-CGM users there was a 20% decrease. Participants found the system easy to use, documented with a high System Usability Score of 88.8 out of 100.

Conclusion: The t:slim X2 with Basal-IQ was safe and associated with a significant reduction of hypoglycemia and increased time in range compared to SAP. Subjects experienced with or naïve to pump and sensor technologies had similar reductions in hypoglycemia and user satisfaction ratings.

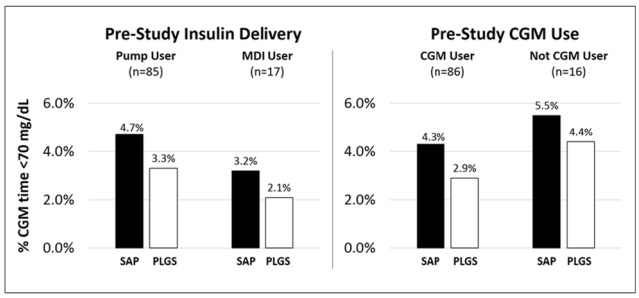


Figure 1: Mean percent CGM time <70 mg/dL in insulin delivery and CGM use subgroups during SAP and PLGS study arms

Clinical Trial Registration Number: NCT03195140
Supported by: Tandem Diabetes Care
Disclosure: **B.A. Buckingham:** Grants; Research funding.

90

Closed-loop insulin delivery in suboptimally controlled type 1 diabetes: a multicentre, 12-week, randomised trial

M. Tauschmann[1], H. Thabit[1,2], J.M. Allen[1], J. Sibayan[3], C. Kollman[3], P. Cheng[3], M.L. Evans[1,4], D.B. Dunger[1], D. Elleri[5], R.M. Bergenstal[6], F. Campbell[7], V.N. Shah[8], A. Criego[6], L. Leelarathna[2], R. Hovorka[1];
[1]University of Cambridge, Cambridge, UK, [2]Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK, [3]Jaeb Center for Health Research, Tampa, USA, [4]Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK, [5]Royal Hospital for Sick Children, Edinburgh, UK, [6]International Diabetes Center, Minneapolis, USA, [7]Leeds Children's Hospital, Leeds, UK, [8]University of Colorado, Denver, USA.

Background and aims: We assessed the safety and effectiveness of day-and-night hybrid closed-loop insulin delivery compared with sensor augmented pump therapy in youths and adults with suboptimally controlled type 1 diabetes.

Materials and methods: In an open-label, multi-centre, multi-national (UK and USA), single-period, parallel study, we randomly assigned subjects with type 1 diabetes aged 6 years and older treated with insulin pump therapy and suboptimal glycaemic control (HbA1c between 7.5% and 10%) to receive either closed-loop insulin delivery with Cambridge control algorithm ($n = 46$) or sensor-augmented pump therapy ($n = 40$; control) over 12 weeks of unrestricted living. Training on study pump and continuous glucose monitor took place over a 4-week run-in period.

Results: In an intention to treat analysis and relative to run-in period, closed-loop increased time that glucose was in target range by 13 ± 8 percentage points compared with a 2 ± 6 percentage point increase in control group (primary endpoint; $p < 0.001$; closed-loop vs control). In closed-loop group, HbA1c was reduced from screening value of 8.3 ± 0.6% to 8.0 ± 0.6% post run-in and 7.4 ± 0.6% post intervention. In control group these values were 8.2 ± 0.5%, 7.8 ± 0.6% and 7.7 ± 0.5%; reductions in A1c levels were significantly greater in closed-loop group compared to control group (mean difference in change 0.4%; 95% CI, 0.2% to 0.6%; $p < 0.001$). Mean sensor glucose was lower in closed-loop group ($p < 0.001$) as was the time spent with sensor glucose levels below 3.9 mmol/L ($p = 0.008$) and above 10.0 mmol/L ($p < 0.001$) (table). Time spent with glucose levels in significant hypoglycaemia (<2.8 mmol/L) was not different between interventions ($p = 0.11$). Similarly, total daily insulin dose was not different ($p = 0.09$). No severe hypoglycaemia occurred. One diabetic ketoacidosis presented in closed-loop group due to infusion set failure and none in control group.

Conclusion: Hybrid closed-loop is safe and improves glucose control and HbA1c while reducing the risk of hypoglycaemia across a wide age range in suboptimally controlled type 1 diabetes supporting adoption of closed-loop in clinical practice.

Table. Study endpoints by treatment group

	Closed-loop (N=46)	Control (N=40)	p-value*
Time spent at glucose level (%)			
3.9-10.0 mmol/L**	**65 ± 8**	**54 ± 9**	**<0.001**
<3.9 mmol/L	2.6 (1.9-3.6)	3.9 (1.7-5.3)	0.008
<2.8 mmol/L	0.3 (0.2-0.6)	0.5 (0.2-0.9)	0.11
>10.0 mmol/L	32 ± 8	42 ± 10	<0.001
>16.7 mmol/L	3.5 (1.9-4.6)	4.4 (2.9-6.5)	<0.001
Mean glucose (mmol/L)	8.9 ± 0.7	9.7 ± 1.0	<0.001
Glucose SD (mmol/L)	3.5 ± 0.5	3.8 ± 0.5	<0.001
Glucose CV (%)	40 ± 4	40 ± 4	0.50
Total daily dose (U/kg/day)	0.81 ± 0.25	0.71 ± 0.19	0.09

Randomised population: 23 children, 19 adolescents/young adults, and 44 adults
Data presented as mean±SD or median (interquartile range)
* Closed-loop vs. control, adjusted for run-in ** Primary endpoint

Clinical Trial Registration Number: NCT02523131
Supported by: JDRF
Disclosure: **M. Tauschmann:** None.

OP 16 Diabetes and mortality

91

Impaired mitochondrial function of human ventricular myocardium in insulin resistance and type 2 diabetes

E. Zweck[1,2], D. Scheiber[1,2], T. Jelenik[1], P. Horn[2], S. Albermann[1,2], U. Boeken[3], D. Saeed[3], M. Kelm[2,4], M. Roden[1,5], R. Westenfeld[2], J. Szendroedi[1,5];
[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research, Heinrich Heine University and German Center for Diabetes Research, Düsseldorf, [2]Division of Cardiology, Pulmonology and Vascular Medicine, Medical Faculty, Heinrich Heine University, Düsseldorf, [3]Clinic for Cardiovascular Surgery, Medical Faculty, Heinrich Heine University, Düsseldorf, [4]Cardiovascular Research Institute Düsseldorf, Medical Faculty, Heinrich Heine University, Düsseldorf, [5]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany.

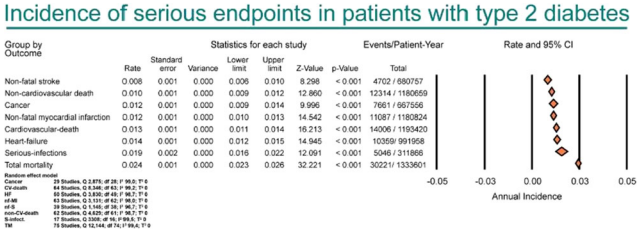
Background and aims: Type 2 Diabetes Mellitus (T2DM) is associated with increased risk of heart failure independent of other risk factors like coronary artery disease and arterial hypertension. While underlying mechanisms are only partially understood, recent studies detected mitochondrial alterations in atrial tissue of patients with T2DM suffering from ischemic or valvular heart disease and requiring open-heart surgery. These studies yielded controversial results concerning a direct link between insulin resistance and mitochondrial dysfunction. We hypothesized that mitochondrial capacity and coupling efficiency are reduced in ventricular tissue of insulin resistant and humans with T2DM and normal heart function.
Materials and methods: High resolution respirometry was performed ex-vivo in transcatheter ventricle biopsies of 29 healthy heart transplant recipients with normal left ventricular function (left ventricular ejection fraction: 64 ± 8%) and without allograft rejection. We assessed three-hour oral glucose insulin sensitivity (OGIS), fasting insulin resistance via homeostasis model assessment (HOMA-IR) and redox potential (ORP) reflecting systemic oxidative stress.
Results: Glucose tolerant humans (CON; $n = 16$) and T2DM ($n = 13$) had comparable age (54 ± 14 vs. 58 ± 12 years), sex (81% vs. 85% male) and time since transplantation (26 ± 24 vs. 25 ± 26 months), while body mass index was higher in T2DM (25.0 ± 2.9 vs. 28.1 ± 4.9 kg/m^2; $p < 0.05$). Myocardial state 3 respiration was 20% lower in T2DM compared to CON at saturating levels of octanoyl carnitine (101 ± 25 vs. 81 ± 20 pmol/(s*mg); $p < 0.05$), 23% lower at additional glutamate (123 ± 35 vs. 95 ± 27 pmol/(s*mg); $p < 0.05$), but not different at additional succinate (178 ± 60 vs. 143 ± 51 pmol/(s*mg)). Lipid-linked respiration related negatively to Hb_{A1c} ($r = -0.45$; $p < 0.05$) and fasting blood glucose levels ($r = -0.48$; $p < 0.05$), but positively to OGIS ($n = 18$; $r = 0.57$; $p < 0.05$). Respiration with additional substrates of complex I and II of the respiratory chain specifically correlated with OGIS ($r = 0.51$; $p < 0.05$). Respiratory control ratios (RCR) on lipids were 21% lower in T2DM (1.6 ± 0.4 vs. 1.2 ± 0.3; $p < 0.05$) and correlated negatively with Hb_{A1c} ($r = -0.44$; $p < 0.05$) and HOMA-IR ($r = -0.488$; $p < 0.05$). Additional substrates of complex I and II resulted in 22% lower RCR in T2DM (2.7 ± 0.8 vs. 2.1 ± 0.5; $p < 0.05$). ORP was 24% higher in T2DM (127 ± 23 vs. 157 ± 25 mV; $p < 0.01$) and related to Hb_{A1c} ($r = 0.45$; $p = 0.02$).
Conclusion: This study demonstrates reduced mitochondrial respiration and coupling efficiency in ventricular myocardium of humans with T2DM, which associates whole-body insulin resistance, impaired glycemic control and oxidative stress. These findings point to cardiomyocyte energy metabolism as a novel target for T2DM-related heart failure.
Clinical Trial Registration Number: NCT03386864
Supported by: German Research Council (SFB1116) and Research commission of HHU (JS)
Disclosure: **E. Zweck:** None.

92

Total mortality: the key-feature of type 2 diabetes

J. vor dem Esche;
Codiplan GmbH, Herrsching am Ammersee, Germany.

Background and aims: Until today, most clinical trials performed in patients with type 2 diabetes have focussed on cardiovascular outcomes only, taking not into consideration non-cardiovascular causes of death and other major health-threats such as serious infections. Therefore, lethal and non-lethal, but potentially life-threatening events are meta-analysed.
Materials and methods: The data gathered from randomized controlled clinical trials conducted in patients with type 2 diabetes are evaluated by rates per person-years to define a disease appropriate ranking of serious outcomes.
Results: In the seventy-two selected publications, total mortality with an annual rate of 2.4% is by far the dominant outcome (R: 0.0244; 95% CI: 0.0229–0.0258, $p < 0.0001$), independent from the baseline risks. These figures are based on the analysis of all trials and all endpoints of interest (1,342,482 patient-years with 30,221 lethal events). Approximately 44% of the mortality events are non-cardiovascular death events. The results serious infections (R: 0.015; 95% CI: 0.012–0.019), cancer (R: 0.012; 95% CI: 0.008–0.016), heart failure (R: 0.012; 95% CI: 0.008–0.015), non-fatal myocardial infarction (R: 0.011; 95% CI: 0.008–0.015), and non-fatal stroke (R: 0.007; 95% CI: 0.004–0.011) are ranked from two to six.
Conclusion: Total mortality is the key-feature of type 2 diabetes. Apart from non-fatal myocardial infarction and non-fatal stroke, serious infections, cancer, and heart-failure should be included in a primary safety endpoint. By focussing on cardiovascular morbidity and mortality only, the current practice to evaluate efficacy and safety of antiglycaemic treatment strategies appears not to be fully appropriate for the real threats associated with type 2 diabetes.



Disclosure: **J. vor dem Esche:** None.

93

Screening for diabetes and early treatment reduces mortality in peripheral arterial disease over seven years

C. Hoebaus[1], C.T. Herz[2], G. Pesau[1], B. Zierfuss[1], R. Koppensteiner[1], G.-H. Schernthaner[1];
[1]Medicine II - Angiology, Medical University of Vienna, Vienna, [2]Medicine III - Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria.

Background and aims: Diabetes mellitus type 2 (T2D) is a well-known risk factor for atherosclerosis development. The combination of T2D and peripheral arterial disease (PAD) is known to reduce survival. We investigated if intensified screening and early treatment for T2D is able to enhance survival over seven years in elderly PAD patients.
Materials and methods: Repetitive screening for T2D was performed in a PAD patient cohort ($N = 367$, 123 women, Fontaine stage I-II) three times every six month. Procedures included a 75 g oGTT and HbA1c measurement. T2D was defined using the current ADA guidelines or by active use of an anti-diabetic agent. Newly detected T2D was primarily

treated with metformin. Patients were stratified according to HbA1c (cut-off 53 mmol/mol) in adequate glucose control (AGC) or inadequate glucose control (IGC) at baseline and mean HbA1c was calculated over the first year. Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation. Data is presented as median (25th–75th percentile). Mann-Whitney-*U* and log-rank test were used as appropriate. Survival curves were calculated by the Kaplan-Meier method.

Results: This PAD cohort included 229 patients with presumed absence of glucose disturbance and 138 patients with known T2D. Initial intensified screening revealed 26 new T2D patients; 13 additional patients were diagnosed by screening over one year. T2D patients with AGC ($N = 100$) vs. IGC ($N = 64$) at baseline exhibited similar patient age (78 (69–83) vs. 76 (68–82) years, $p = 0.521$), LDL-cholesterol (2.36 (1.94–2.94) vs. 2.56 (2.08–3.01) mmol/L, $p = 0.746$), and eGFR (63.7 (52.2–81.4) vs. 64.8 (52.5–77.2) ml/min/1.73 m², $p = 0.683$), but presented a higher BMI (27.7 (25.1–30.8) vs. 28.2 (26.3–31.8) kg/m², $p = 0.05$). 94 PAD patients deceased during the study period. Survival of PAD patients decreased between patients without T2D ($N = 202$, 78.8%) to patients with AGC ($N = 100$, 73%), and patients with IGC ($N = 64$, 62.5%) over seven years ($p = 0.019$). Similar survival rates after the one-year screening were seen in patients categorized according to mean HbA1c for patients without T2D (81.7%), AGC (75%), and IGC (58.9%) ($p = 0.02$). The mortality difference between patients without T2D (18.3%) and AGC (25%) was not statistically significant ($p = 0.164$). During the first year of observation patients with newly diagnosed T2D exhibited similar survival rates to those with AGC (71.8 vs. 75%, $p = 0.786$; figure 1).

Conclusion: This study highlights that adequate glucose control in elderly PAD patients is able to reduce long-term mortality rates. Furthermore, this trial underlines the importance to actively screen for diabetes in PAD patients to treat T2D early and ameliorate diabetes complications and patient survival.

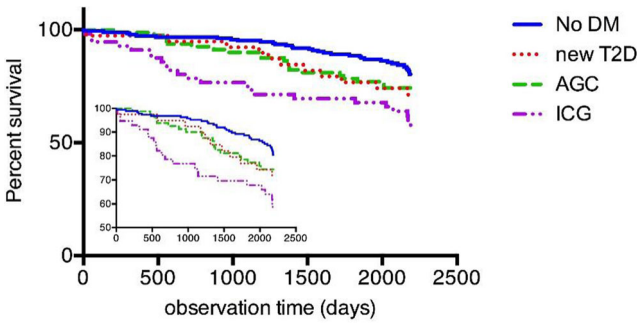


Figure 1. All-cause survival is depicted according to mean Hba1c over the first study year

Disclosure: **C. Hoebaus:** None.

94

Effects of treatment-achieved HbA_{1c} on incidence of micro-/macrovascular complications in patients with diabetes

M. Harada[1], K. Fujihara[1], T. Osawa[1], M. Yamamoto[1], M. Kaneko[1], M. Ishizawa[1], Y. Matsubayasi[1], T. Yamada[1], N. Yamanaka[2], S. Hiroyasu[2], S. Kodama[1], H. Sone[1];

[1]Diabetes and Endocrinology, Niigata University, Niigata, [2]Japan Medical Data Center Co, Tokyo, Japan.

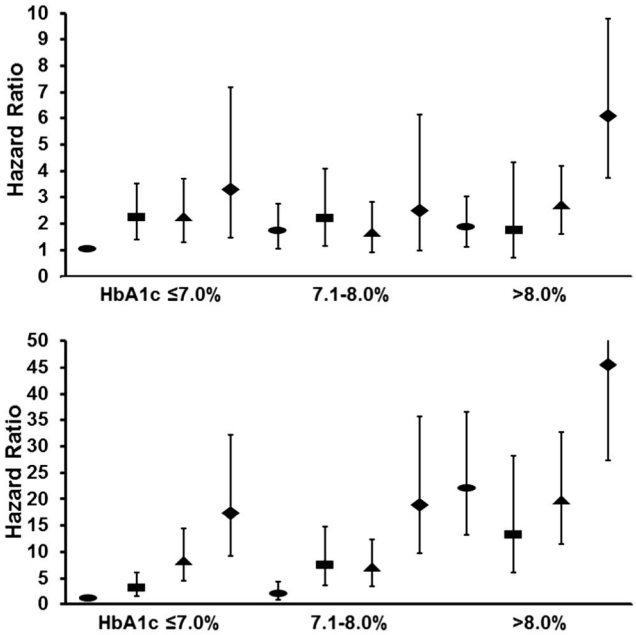
Background and aims: Although a so-called "J"- or "U"-shaped relationship between treatment-achieved HbA1c and risk of coronary artery disease (CAD) has been repeatedly reported, it is not yet clarified whether the association is maintained across various treatment modalities including diet only for both macro- and microvascular complications of diabetes. Thus, we investigated the effects of treatment-achieved HbA1c on the incidence of CAD and treatment-required diabetic eye disease (TRDED) in four treatment groups, i.e. diet only, insulin (INS), sulfonylurea (SU) and antihyperglycemic agents other than glinides, SU, or INS.

Materials and methods: We analyzed data using a nationwide claim-based database that included 296,504 people who belong to a health insurance provider for company employees and their dependents in Japan. Participants aged 18–72 years between 1 April 2008 and 31 March 2013 were included, with the final follow up ending 31 August 2016. Of the 295,570 individuals with available data, data were analyzed on 14,633 without CAD at baseline and with health examination data that included blood tests. Treatment modalities were classified into four groups: diet only, INS, SU, and antihyperglycemic agents other than glinides, SU or INS. Treatment-achieved HbA1c was categorized as follows: ≤7.0, 7.1–8.0, and >8.0. Cox regression model identified variables related to the incidence of CAD and TRDED according to treatment modalities and HbA1c category.

Results: A significant linear trend in the association between HbA1c and CAD events was only seen in the diet only group. Significantly higher risks for CAD were observed in the INS and SU groups whose HbA1c was ≤7.0% or >8.0% compared to diet only group patients with HbA1c ≤7.0%. Conversely, risk for TRDED was strongly dependent on achieved HbA1c regardless of treatment modalities. However, risks of TRDED did not differ significantly between categories of ≤7.0% and 7.1–8.0% among SU and INS groups.

Conclusion: These results implied the necessity of setting different target HbA1c goals according to treatment modalities for prevention of micro-/macrovascular complications.

Figure



Adjusted hazard ratios (95%CI) for (upper) coronary artery disease and (bottom) treatment required diabetic eye disease by 4 treatment modalities (diet only, circles; antihyperglycemic agents other than glinides, sulfonylurea or insulin, squares; sufonylurea, triangles; insulin, rhombus) in three HbA1c categories. Covariates include sex, age, smoking status, BMI, systolic blood pressure, HDL cholesterol, LDL cholesterol

Disclosure: **M. Harada:** None.

95

Effect of preoperative metformin use on lactate levels in patients with type 2 diabetes undergoing coronary artery bypass graft surgery

S.K. Mishra[1], T. Bano[1], M.S. Kuchay[1], Y. Mehta[2], A. Mithal[1];
[1]Division of Endocrinology & Diabetes, Medanta The Medicity Hospital, Gurugram- Delhi NCR, [2]Institute of Critical Care and Anesthesiology, Medanta The Medicity Hospital, Gurugram- Delhi NCR, India.

Background and aims: Lactic acidosis is one of the rare but serious complications associated with metformin use. Relative hypoxemia and hypovolemia during major surgery are risk factors for lactic acidosis in patients with diabetes. Guidelines regarding perioperative use of metformin are debatable. According to some guidelines, metformin should be withdrawn 48 hours before major surgery. Therefore, current study was undertaken to examine whether perioperative use of metformin is associated with increased risk of lactate levels.
Materials and methods: In this prospective observational study, 1,800 consecutive subjects who underwent CABG surgery were enrolled from November 2015 to October 2017. A total of 860 subjects with elective CABG surgeries were included for final analysis; out of which 426 (49.5%) patients with type 2 diabetes received metformin (group 1), 263 (30.5%) subjects with type 2 diabetes were non metformin users (group 2) and 171 (19.8%) patients were having no diabetes (group 3). Subjects with eGFR <30 ml/min/1.73^2 (MDRD formula), severe left ventricular ejection fraction (<30%), overt thyroid dysfunction and hemodynamic instability were excluded. Metformin was continued till night before surgery in eligible subjects. Lactate levels were monitored using arterial blood gas (ABG) machine RADIOMETER ABL 800 BASIC. ABG was done in preoperative period, immediate postoperative period, and then every 6 hourly for 24 hrs. Other parameters recorded included pH, PCO2 and bicarbonate. Student t test was used for comparison of individual quantitative parameters. One-way- analysis of variance ANOVA was used to test the difference between the means of different groups.
Results: Baseline characteristics were similar between groups for age, gender and body mass index. There was increased prevalence of hypertension, triple vessel coronary artery disease, chronic kidney disease and low ejection fraction amongst patients with type 2 diabetes as compared to patients with no diabetes. Intra-operatively there was no difference between groups with respect to type of CABG surgery (on pump or off pump) and use of venous or radial grafts. In all groups there was increase in lactate levels and decrease in pH and bicarbonate levels postoperatively. Mean preoperative pH in groups 1, 2 and 3 were 7.43, 7.44 and 7.40 respectively. Mean postoperative pH in groups 1, 2 and 3 were 7.40, 7.40 and 7.40 respectively. There was no significant difference between groups in postoperative pH. Mean preoperative lactate levels (mmol/l) in groups 1, 2 and 3 were 1.66, 1.64 and 1.48 respectively. Mean postoperative lactate levels in groups 1, 2 and 3 were 1.94, 2.06 and 2.04 respectively. There was no statistical significance for lactate levels among metformin users (group 1) and non-diabetes patients (group 1 vs. 3, $p = 3 = 0.138$). Similarly, there was no difference for lactate levels between group 2 (diabetes without metformin) vs. group 3 (no diabetes) ($p = 0.740$). Although there was significant change in lactate levels between group 1 vs. group 2 ($p = 0.032$), but the change in lactate levels was well below the cut-off for lactic acidosis.
Conclusion: Current study suggests that continuation of metformin in preoperative period is not associated with raised lactate levels in subjects undergoing CABG surgery.
Disclosure: **S.K. Mishra:** None.

96

Intermittent fasting delays the progression of cardiomyopathy in a pre-diabetic obese rat model

D. Abdurrachim, X.-Q. Teo, C.-C. Woo, J. Lalic, P.T.H. Lee;
Singapore Bioimaging Consortium (SBIC), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.

Background and aims: Diabetes increases the risk for heart failure by two to three times. Alterations in cardiac substrate metabolism in diabetes have been thought to contribute to the development of diabetic cardiomyopathy. Caloric restriction has been proposed to be beneficial for cardiometabolic health. In this study, we investigated the effects of caloric restriction via intermittent fasting (IF) on cardiac function and metabolism in pre-diabetic obese spontaneously hypertensive heart failure-prone (SHHF) rats.
Materials and methods: Pre-diabetic obese SHHF rats (10 weeks of age) were randomly divided into non-fasting group ($N = 8$) and IF group ($N = 7$). IF group was fasted for 24 hours on alternate days for a period of six months. At 5 weeks and 6 months of IF, magnetic resonance imaging (MRI) was performed to measure left ventricular mass (LVM) and ejection fraction (EF). In addition, at 6 months of IF, cardiac ^{13}C magnetic resonance spectroscopy (MRS) was performed to measure cardiac pyruvate utilization in vivo, by quantifying the production of downstream metabolites (i.e. [1-^{13}C] lactate, [1-^{13}C] alanine, and [1-^{13}C] bicarbonate) within 2 minutes after hyperpolarized [1-^{13}C] pyruvate injection. Blood glucose, FFA, TG, and insulin were determined at 6 months of IF. Statistical significance was determined using Student's t-test for data with a single time point, and using a repeated measures ANOVA with Bonferroni post hoc test for data with more than one time points.
Results: After 6 months of IF, body weight gain was 46% less in IF rats than in non-fasting rats ($P < 0.001$). In IF rats, fed blood glucose levels tended to be 30% lower than in non-fasting rats ($P = 0.06$), while fed serum FFA, TG, and insulin levels were 61%, 40%, and 85% lower compared with non-fasting rats ($P = 0.014$, $P < 0.001$, $P = 0.021$, respectively), which suggests an improvement of insulin sensitivity in the IF rats. Non-fasting rats exhibited progressive LV hypertrophy as indicated by an increase in LVM/tibia length (LVM/TL) by 44% and 115% at 5 weeks ($P < 0.001$) and 6 months of study period ($P < 0.001$) compared with baseline, respectively. The increase in LVM/TL in non-fasting rats was accompanied by a 6% decrease in EF at 6 months of study period ($P = 0.032$ vs. baseline). In contrast, the increase in LVM/TL was lower in IF rats (i.e. 25% and 76% at 5 weeks ($P = 0.011$) and 6 months of IF ($P < 0.001$) compared with baseline, respectively), while EF was maintained ($P > 0.99$ at 6 months of IF vs. baseline). At 6 months of IF, the production of [1-^{13}C] bicarbonate upon injection of [1-^{13}C] pyruvate was 33% higher in IF rats than in non-fasting rats ($P = 0.042$), which indicates higher cardiac pyruvate dehydrogenase (PDH) flux in the IF rats. The production of [1-^{13}C] alanine was 48% higher in IF rats compared with non-fasting rats ($P < 0.001$), while the production of [1-^{13}C] lactate was not altered in the IF rats ($P = 0.109$ vs. non-fasting rats).
Conclusion: Six months of IF delays the progression of LV hypertrophy and prevents cardiac dysfunction in pre-diabetic obese SHHF rats, which may be associated an improvement in insulin sensitivity and an increase in cardiac pyruvate utilization. Our results suggest that IF may improve cardiac-metabolic health by modulating cardiac substrate metabolism.
*Supported by: A*STAR Biomedical Research Council*
Disclosure: **D. Abdurrachim:** None.

OP 17 Exercise: running back and forth from the gym to the culture dish

97

Effects of different types of physical activity on metabolic control in type 1 diabetic patients

I. Stotl[1], T. Kambic[2], A. Zdolsek[2];
[1]University Medical Centre Ljubljana, Ljubljana, [2]Faculty of Sport, University of Ljubljana, Ljubljana, Slovenia.

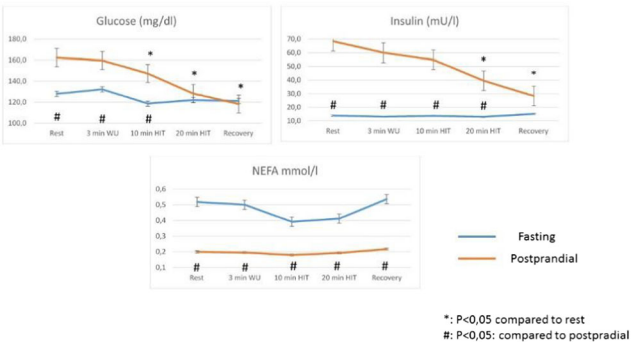
Background and aims: Physical activity (PA) has an important role in treatment of patients with type 1 diabetes. Research shows that PA doesn't usually affect HbA1c, but it can increase the frequency of hypoglycaemia in type 1 diabetic patients. Non-exercise activity thermogenesis (NEAT) represents the additional energy expenditure besides active sports-like exercise and resistance training in daily life. To date the effect of NEAT in patients with type 1 diabetes was not investigated; therefore the aim of this study was to determine the effects of PA and NEAT on metabolic control in this group of patients.
Materials and methods: A total of 109 subjects with type 1 diabetes (55 women and 54 men) - average age of 38 ± 10 years, average weight of 77.33 ± 15.70 kg, HbA1c 7.03 ± 0.89 were included in the study. Participants were asked to complete multiple PA questionnaires, including NEAT questionnaire, WHO physical activity questionnaire and hypoglycaemia questionnaire. Clinical data such as frequency of hypoglycaemia, insulin therapy, HbA1c, blood pressure and serum lipid profiles were also obtained to investigate the relationship with PA and NEAT score.
Results: Regression model predicting levels of HbA1c from total amount of PA (WHO, combined moderate and vigorous PA) revealed positive multiple correlation between total PA and HbA1c levels ($r = 0.20$), while the regression model was not significant. After total amount of PA was separated into moderate and vigorous PA only the latter was significantly correlated with HbA1c ($r = 0.20$; $P < 0.05$). There was a significant regression model predicting HbA1c from NEAT score ($p < 0.05$) with positive correlation between NEAT score and HbA1c ($r = 0.23$; $P < 0.05$). Moreover, a regression model predicting frequency of hypoglycaemia from NEAT score was borderline significant ($P = 0.051$), whereas correlation between NEAT score and frequency of hypoglycaemia was negatively significant ($r = -0.198$; $P < 0.05$). A significant regression model ($P < 0.01$) was obtained when predicting systolic blood pressure from moderate, vigorous PA and NEAT score. Beta coefficient showed significant effect of vigorous PA ($\beta = 0.36$; $P < 0.01$) and NEAT score ($\beta = -0.31$; $P < 0.01$) on systolic blood pressure levels. Similar effects and correlations were not obtained when the predictions of diastolic blood pressure were made. There was a significant negative correlation between vigorous PA ($r = -0.31$; $P < 0.05$) and total serum cholesterol ($r = -0.301$; $P < 0.05$), while NEAT score and moderate PA were not significantly correlated with total cholesterol levels. Furthermore, a significant regression model ($P < 0.05$) was obtained when serum value of low-density lipoprotein (LDL-C) was predicted from moderate, vigorous PA and NEAT score. Vigorous PA ($r = -0.324$; $P < 0.05$) and NEAT score ($r = -0.229$; $P < 0.05$) were significantly correlated with (LDL-C) levels. Additionally, when predicting serum levels of high-density lipoprotein (HDL-C) and triglycerides from combined PA and NEAT score, no significant effects or correlation were observed.
Conclusion: Our data suggest that higher amount of NEAT is associated with lower frequency of hypoglycaemia, lower systolic blood pressure, lower LDL-C and higher HbA1c in type 1 diabetic patients. We have also demonstrated a positive metabolic effect of vigorous PA levels on total serum cholesterol and LDL-C concentration.
Disclosure: **I. Stotl:** None.

98

Combined effects of timing of exercise and high intensity exercise on plasma NEFA, glucose and insulin concentrations in type 2 diabetic and prediabetic men

P. Calders[1], B. Lapauw[2], S. Shadid[3], B. Celie[1];
[1]Rehabilitation Sciences and Physiotherapy, University of Ghent, Ghent, [2]Endocrinology, University Hospital of Ghent, Ghent, [3]Endocrinology, University Hospital of Ghent, Ghent, Belgium.

Background and aims: Exercise is beneficial in type 2 diabetes (T2DM), but both the preferred exercise type (continuous or interval) and timing of exercise (fasted versus fed) is still on debate. Postprandial continuous exercise has been shown to acutely blunt glycaemia in both healthy persons and patients with T2DM, while exercising in the fasted state did not affect glycaemia. There are no available data about the effects of fasted state continuous exercise in T2DM. In a healthy population this exercise type acutely increases free fatty acid concentration in both fasted and postprandial state. Considering this lack of knowledge and because there are no data available on the effects of an acute high intensity exercise bout (HIT) in T2DM patients, the aim of this study was to evaluate the effect of HIT on glucose, insulin and NEFA concentration in fasted versus postprandial state in patients with pre(diabetes and T2DM.
Materials and methods: We have studied 11 adult overweight or obese males with prediabetes or T2DM, defined as an HbA1c ≥6.0%, using metformine monotherapy ($n = 9$) or diet/exercise ($n = 2$). Patients were tested three times. First, they performed an incremental exercise test until exhaustion on a cycle ergometer to evaluate maximal oxygen consumption (VO2max). The second and third test (cycling), consisted of 3 minutes warming up at 50W, followed by 20 minutes HIT (ten times: 60 seconds at 80% of VO2max and 60 seconds at 50 Watt), finishing with 40 minutes recovery (including 3 minutes cooling down). Exercise tests were carried out in fasted or postprandial state (90 minutes after a standard meal (carbohydrates: 56%; fat: 22%; proteins: 22%)). Nutritional status was randomized. Glucose, insulin and non-esterified fatty acids (NEFA) were measured before and after warming up, after 10, 20 minutes of HIT and 40 minutes recovery in a venous blood sample. Data are expressed as mean (SD). A Repeated measures ANOVA was executed with post hoc Sidak to evaluate interaction effects (time * condition) and time effects. Significance level was set at $P < 0.05$.
Results: Patients had a mean age of 42 (5.6) years; a mean BMI of 31.9 (5.11) kg/m^2, an HbA1c of 6.6 (0.49) % and a relative maximal oxygen uptake of 21.4 (5.33) ml/kg min. *Glucose, insulin and NEFA concentration in fasting and postprandial condition (fig 1):* Glucose and insulin concentration decreased significantly in postprandial state during HIT ($P < 0.05$ versus pre-HIT), but remained stable in the fasting state. Resting NEFA concentrations were increased in the fasting versus the postprandial condition ($P < 0.05$) and remained stable during HIT ($P < 0.05$).
Conclusion: Postprandial interval exercise possesses the ability to blunt postprandial glycaemia in patients with prediabetes and T2DM while NEFA concentrations are higher in the fasted state but remain stable during HIT.



Disclosure: **P. Calders:** None.

99

Interleukin-6 blockade ameliorates the effect of exercise on cardiac fat in abdominally obese individuals

R. Christensen[1], L. Lang Lehrskov[1], A.-S. Wedell-Neergaard[1], R. Krogh-Madsen[1], K. Karstoft[1], H. Ellingsgaard[1], J. Rosenmeier[2], B.K. Pedersen[1];
[1]The Capital Hospital of Denmark, Copenhagen, [2]Bispebjerg Hospital, Copenhagen, Denmark.

Background and aims: Excessive cardiac adipose tissue has been associated with the incidence and severity of type 2 diabetes and cardiovascular disease. Exercise training reduces cardiac adipose tissue and may therefore be a strategy to prevent type 2 diabetes and coronary heart disease. The underlying mechanisms for exercise-mediated adaptations of cardiac fat are unclear, but may involve actions of interleukin-6 (IL-6). IL-6 is a myokine which is released in response to exercise and it has been shown to increase lipolysis in adipose tissue. Whether this mechanism is operative in cardiac adipose is unknown. Therefore, the aim of this study was to investigate whether blocking of IL-6 can ameliorate the effects of exercise on cardiac fat volume.
Materials and methods: This was a 12-week, double-blinded, randomised, placebo-controlled exercise and drug intervention trial. 52 abdominally obese participants were enrolled to endurance exercise (3 x sessions per week of interval-based high-intensity training of 70–85% of VO_2 max) or no exercise groups combined with IL-6 blockade (Tocilizumab, 8 mg/kg, toci) or placebo (saline). Cardiac fat volume was assessed by MRI at baseline and post intervention. Data is expressed as mean ± SD. A 2-way ANCOVA was used to assess whether IL-6 blockade influenced the effect of exercise on cardiac fat.
Results: 13 (25%) were men, with an age average of 44 ± 13 years. Baseline mean cardiac fat was 203 ± 111 ml. There were no differences in baseline cardiac fat levels between participants ($p = 0.07$). Cardiac fat was reduced by 16% (95% CI −31; −1, $p = 0.041$) after 12 weeks of endurance exercise compared to no exercise (Figure 1). The reduction in cardiac fat was ameliorated in the group that exercised and received IL-6 blockade ($p = 0.021$). IL-6 blockade alone did not lead to any significant changes on cardiac fat in groups that did not exercise ($p = 0.083$) (Figure 1).
Conclusion: In a randomised placebo-controlled exercise and drug intervention we found that IL-6 blockade can ameliorate the effects of exercise on cardiac fat in abdominally obese individuals. These data suggest IL-6 is required to mediate the adipose reducing effects of exercise specifically on cardiac adipose tissue.

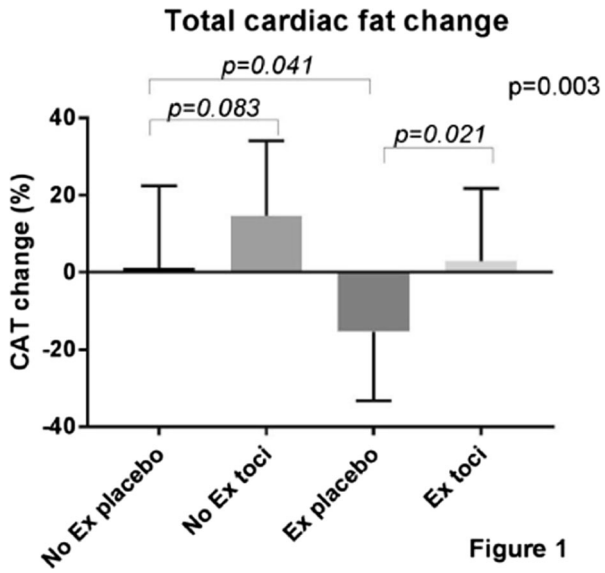


Figure 1

Clinical Trial Registration Number: NCT02901496
Supported by: Danish Heart Association
Disclosure: **R. Christensen:** None.

100

Contraction-induced changes in mitochondrial function and insulin sensitivity of myocytes rely on the functional Ndufb6 subunit of the electron transport system complex I

T. Jelenik[1], S.W. Görgens[1], N. Krako Jakovljevic[1], I. Rokitta[1], N.M. Lalic[2], J. Eckel[1], M. Roden[1];
[1]German Diabetes Center, Duesseldorf, Germany, [2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

Background and aims: Impaired mitochondrial function associates with insulin resistance in skeletal muscle, yet causal relationships and underlying mechanisms are unclear. In humans, we have previously shown that the G/G-single nucleotide polymorphism (SNP) in the Ndufb6 subunit of the mitochondrial complex I relates to impaired mitochondrial plasticity after exercise and insulin resistance. Here we hypothesize that reduced Ndufb6 activity impairs oxidative capacity and inhibit insulin signaling in contracted myotubes.
Materials and methods: C2C12 myotubes were treated with Ndufb6 siRNA to induce its knockdown (siNdufb6) or negative control siRNA (NT). The myotubes underwent studies under basal, palmitate-treated, and/or electrical pulse-stimulated (EPS) conditions, the latter simulating muscle cell contraction ($n = 4$–6). Mitochondrial oxidative capacity was assessed in digitonin-permeabilized cells using high-resolution respirometry. Reactive oxygen species (ROS) were detected by DCF fluorimetry. Insulin signaling at the level of Akt phosphorylation was assessed at baseline and in insulin-treated cells by Western blots.
Results: After 24 h of siRNA treatment, Ndufb6 mRNA and protein levels were silenced by 70% (siNdufb6: 0.24 ± 0.06 vs. NT: 0.82 ± 0.15 AU; $p < 0.05$) and by 40% (0.61 ± 0.07 vs. 0.99 ± 0.11; $p < 0.05$), respectively. Complex I-linked state u respiration was 36% lower in siNdufb6 than in NT (211 ± 15 vs. 327 ± 19 pmol/s/10^6cells; $p < 0.05$). While there were no differences in respiration with octanoyl-carnitine, EPS-stimulated complex I-linked respiration was markedly decreased in siNdufb6 myotubes (271 ± 10 vs. 435 ± 34 pmol/s/10^6cells; $p < 0.001$). However, ROS production was 19% higher in siNdufb6 ($p < 0.01$) and not further stimulated by palmitate. In contrast to NT, EPS did not rescue the palmitate-induced reduction in pAkt(Ser473), which was decreased by 35% in siNdufb6 (0.72 ± 0.15 vs. 1.10 ± 0.05 AU; $p < 0.01$).
Conclusion: Reduced Ndufb6 activity redirects electrons from oxidative phosphorylation towards electron leakage. Lower oxidative capacity and higher ROS production could contribute to the impaired insulin sensitivity and lower exercise responsiveness, as observed in humans with the G/G-SNP in the Ndufb6 gene.
Supported by: MIWF NRW, BMG, BMBF, DZD e.V., DDG, Dr. Eickelberg Stiftung
Disclosure: **T. Jelenik:** None.

101

Impaired exercise performance and glucose disposal in Tbc1d4-deficient mice is rescued by regular exercise training

A. Chadt[1,2], C.A. Springer[1,2], L. Toska[1], A. Grieß[1], S. Karpinski[1], C. de Wendt[1], M. Dille[1], H. Backes[3], A. Cremer[3], J.C. Brüning[3], H. Al-Hasani[1,2];
[1]German Diabetes Center, Duesseldorf, [2]German Center for Diabetes Research (DZD), Düsseldorf, [3]Max Planck Institute for Metabolism Research, Cologne, Germany.

Background and aims: The Rab-GTPase-activating protein TBC1D4 (=AS160) represents a key regulator of insulin-mediated glucose transport into skeletal muscle and adipocytes. Moreover, as a direct target of

AMPK, TBC1D4 plays a crucial role in contraction-dependent skeletal muscle metabolism. In mice, *Tbc1d4*-deficiency leads to substantially reduced levels of the insulin-responsive glucose transporter GLUT4 in skeletal muscle and adipocytes. Recently, a muscle-specific loss-of-function variant in the *TBC1D4* gene was identified in the Greenlandic population which defines a specific subtype of non-autoimmune diabetes characterized by elevated post-prandial glucose levels. Our aim was to elucidate whether regular exercise training can overcome the initial defect in glucose disposal using *Tbc1d4*-deficient mice.

Materials and methods: Wildtype (WT) and *Tbc1d4*-deficient (D4KO) mice were fed a high-fat diet (HFD) with 60% fat from calories from week 8 on, and subjected to forced exercise training on treadmills for 4 weeks starting from week 12. Glucose sensitivity and physical condition were determined and compared with sedentary controls. *Ex vivo* analyses of skeletal muscle and adipose tissue and *in vivo* PET (Positron-emission tomography) imaging were conducted to determine insulin responsiveness of peripheral tissues. Statistical analysis was performed using two-way ANOVA.

Results: Sedentary D4KO mice showed impaired physical activity during an acute exercise exhaustion test that was rescued after 4 weeks of treadmill training (trained vs sedentary D4KO; 8.2 ± 0.3 vs 9.5 ± 0.4 min, $n = 8$, $p < 0.05$). In addition, glucose and insulin tolerance were significantly improved in D4KO mice following the exercise intervention (AUC trained vs sedentary D4KO; glucose tolerance: 464.2 ± 29.4 vs 333.7 ± 8.1 a.u., $n = 8$–9, $p < 0.05$; insulin tolerance: 580.1 ± 15.6 vs 472.0 ± 25.5 a.u., $n = 8$, $p < 0.001$). Interestingly, no compensation for the initial reduction in GLUT4 protein abundance nor for the impaired insulin-stimulated glucose uptake in skeletal muscle was achieved due to the exercise training. In contrast, total GLUT4 protein content and insulin-stimulated glucose transport were substantially increased in white adipose tissue from D4KO animals (trained vs sedentary D4KO; GLUT4 protein: 56.9 ± 6.6 vs 91.1 ± 9.6 a.u., $n = 12$–15, $p < 0.05$; insulin-stimulated glucose uptake: 52.2 ± 8.1 vs 148.7 ± 36.8 CPM/mg lipid, $n = 12$; $p < 0.01$), completely ameliorating the initial impairment caused by the *Tbc1d4*-deficiency. Moreover, gene expression of a set of browning factors such as *Ucp-1* and *Cidea* were significantly elevated in white adipose tissue from trained D4KO mice, indicating enhanced mitochondrial activity.

Conclusion: In summary, our results demonstrate that deletion of the RabGAP TBC1D4 leads to impaired physical activity, presumably due to reduced glucose uptake into skeletal muscle and adipose tissue. After treadmill training, D4KO mice were able to normalise their exercise performance, glucose and insulin tolerance, respectively, to a degree comparable to WT controls. We show that the adipose tissue is responsible for these beneficial effects, potentially by a combination of pathways leading to increased GLUT4 content and enhanced mitochondrial activity.

Disclosure: **A. Chadt:** None.

102

Roles of neutrophils and IL-1 in intramuscular immunometabolic niche for priming GLUT4 translocation during exercise

M. Kanzaki[1], S. Sekiai[1], H. Hatakeyama[1], C. Chaweewannakorn[2], K. Sasaki[2], Y. Hagiwara[3], E. Itoi[3], M. Tsuchiya[4];
[1]Biomedical Engineering, Tohoku University, Sendai, [2]Graduate School of Dentistry, Tohoku University, Sendai, [3]Orthopaedic Surgery, Tohoku University, Sendai, [4]Tohoku Fukushi University, Sendai, Japan.

Background and aims: Immunomodulation of metabolism involving pro-inflammatory interleukin-1 (IL-1) has been intensively investigated under unfavorable conditions of excess nutrition such as obesity, and obesity-induced low-grade chronic inflammation involving IL-1 has been widely implicated as a detrimental factor in insulin resistance. Recently, however, employing a masticatory behavior (Restrained/Gnawing) model, we demonstrated that mice deficient in both IL-1α and IL-1β (IL-1-KO mice) exhibited apparent dysregulation of muscle glucose uptake with accompanying ease of fatigability during masseter muscle activity (PLoS ONE, Chiba et al 2015). Our previous study also revealed that neutrophils producing IL-1β were markedly recruited within working masseter muscles. To further explore our findings, the present studies were designed to clarify the favorable IL-1 actions and its underlying mechanism on the working skeletal muscles by using the running-wheel-based exercise model and electric pulse stimulation (EPS)-evoked in situ muscle contraction.

Materials and methods: We examined IL-1-KO mice and neutrophil-depleted mice, focusing on muscle GLUT4 translocation, by utilizing the running-wheel-based exercise model and EPS-evoked in situ muscle contraction model.

Results: Upon 2h of walking exercise, IL-1-KO mice displayed rapid exhausted behavior attributable to the dysregulation of exercise-stimulated GLUT4 translocation and glucose uptake along with the depletion of intramuscular glycogen. We also found in WT mice that neutrophils producing IL-1β were markedly recruited within the working skeletal muscle tissues such as quadriceps femoris muscles (QFMs). To directly investigate significance of the neutrophils, we utilized the neutrophil-depleted mice and found that neutrophil depletion resulted in essentially the same phenotypes with IL-1 KO mice in terms of the undermined walking performance in accordance with the impaired exercise-dependent glucose uptake. Intravital-imaging analysis using the skeletal muscle-specific GLUT4-EGFP-expressing transgenic mice demonstrated that EPS-evoked in situ contraction promptly induced GLUT4 translocation to sarcolemma and T-tubules, which was remarkably blunted in the neutrophil-depleted mice. Biochemical analysis of exercise-related intracellular signals demonstrated that both IL-1-KO mice and neutrophil-depleted mice displayed obvious derangements in the Rac1 signaling cascades, while no obvious defects in the AMPK signaling cascade, including Tbc1d1 (Ser237) phosphorylation, were observed.

Conclusion: Taken together, these findings shed new light on the roles of IL-1 and neutrophils, which emerges to be positively involved in exerting exercise-dependent responses especially on muscle glucose homeostasis via GLUT4 translocation. Importantly, these metabolic benefits involving both IL-1 and neutrophils directly engaged in fatigue alleviation of the working skeletal muscles.

Supported by: KAKENHI

Disclosure: **M. Kanzaki:** None.

OP 18 From stem cells to human pancreas development

103

A new dual reporter embryonic stem cell line for the purification of SOX9-positive pancreatic progenitors

O. Naujok, C. Davenport, J. Kresse, U. Diekmann;
Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany.

Background and aims: The high mobility group box transcription factor SOX9 is involved in the maintenance of embryonic and adult ducts cells, which are believed to serve as a pool for NGN3-positive endocrine progenitors committed to islet progeny. In order to analyze the role of SOX9 during pancreatic differentiation of human embryonic stem cells (ES cells) we sought to establish a conservative dual reporter cell line by means of CRISPR/Cas9-mediated homology directed repair. This reporter line shall give insights into developmental processes during human pancreatic organogenesis and pave the way for the purification of pure pancreatic cells by magnetic or fluorescence-activated cell sorting.
Materials and methods: Three sgRNAs targeting ±15 bp next to the stop codon of the *SOX9* locus were cloned and validated by the T7EI-assay. Then HES3 ES cells were nucleofected with the functional CRISPR/Cas9 and a targeting vector comprising a P2A-H-2KK-F2A-GFP2 gene cassette flanked by 500 bp 5′ and 3′ homology arms. A floxed hygromycin gene was used for clonal selection of targeted cells. Cell clones were then screened by PCR and DNA sequencing and then the functionality of the knock-in was tested by differentiation into pancreatic endoderm using an adopted differentiation protocol.
Results: Out of three designed CRISPR/Cas9 the sgRNA T5 effectively introduced DSBs into the *SOX9* locus of the HEK293 model cell line. The nucleofection of HES-3 embryonic stem cells with T5 and the HDR vector yielded after selection in 48 cell clones from which 32 showed a homozygous integration, 7 a heterozygous integration, and 9 clones that were only resistant to hygromycin without homology directed repair. All tested clones activated GFP2 upon differentiation into pancreatic endoderm. The HES3 SC30 clone was then used for further characterization. GFP2-positive cells appeared after PDX1-positive duodenal cells at d8 with a peak of 50–60% positive cells at day 12–13 of differentiation as measured by flow cytometry. FACS-sorted GFP2-positive cells expressed SOX9, PDX1, HNF6, and NKX6.1 but not NGN3 whereas GFP2-negative cells remained largely negative for these markers. GFP2-positive cells also expressed the surface antigen H-2KK which allowed simple MACS-assisted cell purification. Further differentiation of SOX9/H-2KK/GFP2-positive cells showed that these cells are highly capable of endocrine differentiation into insulin/C-peptide-positive and glucagon-positive cells.
Conclusion: In summary, this study reports the derivation of a new ES dual reporter cell line which comprises the knock-in of the fluorescence reporter GFP2 and the surface antigen H-2KK into the *SOX9* locus. Due to the design of the targeting vector, the *SOX9* locus remains unharmed so that GFP2 and H-2KK are expressed along with SOX9 under the control of the endogenous gene promoter. The analysis of GFP2-expressing cells showed that they co-express SOX9, PDX1, HNF6, and NKX6.1, all markers of the pancreatic endoderm. The duality of the reporter gene knock-in allows the purification by magnetic or fluorescence-activated cell sorting. Thus, we conclude that this cell line is a powerful tool to study the mechanisms of duct-to-islet conversion during pancreatic development. Furthermore, this cell line can be used to purify pancreatic endodermal cells for cell replacement therapy of diabetes.
Supported by: DFG project # 329435715
Disclosure: **O. Naujok:** None.

104

PKC activation promotes resolution of polyhormonality toward alpha cell fate in human pluripotent stem cell (HPSC) derived alpha cells

B. Tyrberg[1], Q.P. Peterson[2,3], A. Veres[2], J. Kenty-Ryu[2], M.Q. Slama[3], L. Chen[1], Q. Zhou[2], M.R. Brown[3], A. Matveyenko[3], M. Sörhede-Winzell[1], D.A. Melton[2];
[1]Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Mölndal, Sweden, [2]Stem Cell and Regenerative Biology, Harvard University, Cambridge, USA, [3]Physiology and Biomedical Engineering, Mayo Clinic, Rochester, USA.

Background and aims: HPSCs holds great promise as a source of specialized cells for drug discovery and mechanistic interrogation of cell signaling. In diabetes research pancreatic endocrine cells are critical when studying hormone secretion and cell proliferation/survival, but primary cell supply is scarce. Currently, only insulin-producing beta-cell lines and beta-cell HPSC protocols are available. Therefore, we aimed to develop a differentiation protocol specifically generating alpha-cells.
Materials and methods: We have developed a protocol to differentiate glucagon-producing alpha-cells from HPSCs based on our previously published beta-cell differentiation protocol. We substantially modified stage 4–5 of the protocol driving alpha-cell differentiation only by BMP and ALK5 inhibition (LDN193189, SJN2511). Briefly, for the phenotypic screen of HPSCs in differentiation stage 6, we plated cells in 384-well plates and treated with compounds for 4 days. We examined the differentiated alpha-cells *in vitro* for glucagon secretion and *in vivo* following transplantation into immunodeficient mice.
Results: Like primary cells, these alpha-cells secrete glucagon in response to low glucose (low glucose: 0.51 ± 0.2 pM per 1000 cells vs. high glucose: 0.28 ± 0.1 pM per 1000 cells, $P < 0.05$), and their ultrastructure resembles primary human alpha-cells. Following transplantation, mice demonstrate elevated fasting blood glucose (7.5 ± 0.5 mM vs. 5.6 ± 0.4 mM, $P < 0.05$) and transplanted cells prevent hypoglycemia in response to insulin. Surprisingly, the cells are bihormonal *in vitro* and also express insulin, although not secreting insulin (alpha cells: 0.08 ± 0.04 μIU/ml per 1000 cells vs. human islets: 10.6 ± 0.3 μIU/ml per 1000 cells, $P < 0.05$). We hypothesized that the cells are not fully mature and that interfering with the appropriate signaling pathway would convert the cells to monohormonal alpha-cells. In a phenotypic screen with the bihormonal alpha-cells we found that the PKC activator PdbU increase glucagon expression and diminish insulin expression. By modifying stage 6 of the differentiation protocol with PdbU we now achieve 46 ± 4% monohormonal alpha-cells instead of 23 ± 3% without PdbU treatment.
Conclusion: We have developed the first HPSC differentiation protocol that generates monohormonal alpha-cells in large quantities. This complements HPSC derived beta-cells and makes possible recreation of human islet mini-organs with multiple endocrine cell types and intact paracrine signaling. The cells are consequently suitable for drug discovery and interrogation of human alpha-cell biology that has hitherto not been possible.
Supported by: AstraZeneca, NIDDK
Disclosure: **B. Tyrberg:** Employment/Consultancy; AstraZeneca. Grants; AstraZeneca.

105

Endocrine cell specification and beta cell maturation require the transcriptional co-activator MED15

A.Z. Kadhim[1], E.E. Xu[2], T. Speckmann[2], C. Nian[2], R. Cullen[1], R. Shi[2], D.S. Luciani[2], S. Taubert[1], F.C. Lynn[2];
[1]Medical Genetics, The University of British Columbia, Vancouver, [2]Surgery, The University of British Columbia, Vancouver, Canada.

Background and aims: The Mediator complex, a co-regulator required for RNA polymerase II activity, interacts with specific transcription

factors through distinct subunits such as MED15. These interactions promote the expression of defined gene sets both during development and for tissue homeostasis. As MED15 is highly expressed starting in nascent immature beta cells, we generated several developmental stage specific knockout mice to determine a role for MED15 during pancreatic islet differentiation. The results obtained from these lines will allow us to dissect when and where MED15 is required for beta cell differentiation, maturation and function.

Materials and methods: We crossed *Pdx1-Cre*, *Neurog3-Cre*, and *Ins1-Cre* transgenic mice with *Med15*$^{flox/flox}$ mice to delete *Med15* in pancreatic progenitors, endocrine progenitors, and beta cells, respectively. RNA-Sequencing, Taqman, and immunofluorescence analyses were performed to determine changes in expression in these knockout models. Glucose uptake assays were performed using fluorescent glucose 2-NBDG, and insulin secretion along with mitochondrial activity were assessed via perifusion and Seahorse XF extracellular flux analyzer, respectively. Co-immunoprecipitation (CoIP) in mouse insulinoma (MIN6) cells was used to identify MED15 interacting partners. Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) in MIN6 cells and primary islets was used to determine MED15 genomic occupancy.

Results: In the *Pdx1-Cre* model, we observed a reduction of Neurog3$^+$ cells, demonstrating that MED15 is required for endocrine progenitor specification. As expected, 8-week old mice showed reduced viability, were glucose intolerant, and had fasting hyperglycemia. The *Neurog3-Cre*; *Med15*KO mice showed a 50% reduction in beta cell mass (student's t test, $p < 0.05$). Transcriptome profiling demonstrated that MED15 is required for expression of the beta cell maturation markers UCN3, IAPP, MAFA, and GLUT2 in the *Ins1-Cre* model. In agreement with reduced GLUT2 expression, *Ins1-Cre*; *Med15* KO cells had impaired glucose uptake, reduced glucose-stimulated oxygen consumption, and reduced first-phase insulin secretion. Using native CoIP experiments in MIN6 cells, we found that MED15 interacts with NKX6-1. Furthermore, MED15 was found to bind NKX6-1 bound enhancer loci, both in MIN6 and mouse islets. As such, we conclude that MED15 drives beta cell maturation by interacting with the transcription factor NKX6-1 at genomic enhancer regions.

Conclusion: Taken together, these results demonstrate a critical role for MED15 in endocrine lineage specification, differentiation, and beta cell maturation. As the Mediator complex has not previously been studied in the pancreas, we provide the first evidence of its importance in this tissue. A greater understanding of how Mediator and MED15 regulate beta cell maturation could help refine the generation of cell-based therapies for diabetes.

Supported by: CDA

Disclosure: **A.Z. Kadhim:** Grants; Canadian Diabetes Association operating grant 2015-2018.

106

GPR56 is highly expressed by pancreatic progenitor cells and it regulates beta cell development and function

O.E. Olaniru, K. Toczyska, A.-M. Cujba, P. Atanes, R. Sancho, P.M. Jones, S.J. Persaud;
Department of Diabetes, King's College London, London, UK.

Background and aims: GPR56, the most abundant islet-expressed G protein coupled receptor, is known to regulate proliferation, apoptosis and organ development. We have previously reported that GPR56 activation by its endogenous ligand, collagen III, led to potentiation of glucose-stimulated insulin secretion from islets. Here, we investigated the expression and function of GPR56 in developing mouse and human pancreases and determined the effect of a small peptide agonist of GPR56, TYFAVLM, on β-cell function.

Materials and methods: Using IHC and RNAscope in-situ hybridisation (ISH), the expression of GPR56 and its co-localisation with PDX1$^+$, NGN3$^+$ and SOX9$^+$ progenitor cells was investigated in human fetal pancreas (CS13, 10, 12, 14 and 17 post conception weeks) and mouse pancreas at embryonic days E11, E13, E15, E18 and at P9. GPR56 mRNA expression in pancreatic progenitors derived from human induced pluripotent stem cells (hiPSCs) was determined by qPCR. Pancreas sections from WT and GPR56 KO mice were immunoprobed for Ki67, BrdU, insulin, glucagon, and images were quantified by Image J. Effects of 100 μM TYFAVLM on insulin secretion from human islets were investigated by radioimmunoassay, while its effects on β-cell apoptosis and intracellular calcium [(Ca^{2+})i] were investigated by measuring caspase 3/7 activities and calcium microfluorimetry respectively, in native β-cells and in β-cells in which GPR56 had been knocked down by CRISPR-Cas9.

Results: ISH and IHC revealed that GPR56 was strongly expressed by endocrine progenitor cells in developing mouse and human pancreas, with high expression early in pancreas development and lower expression as the cells differentiated (pancreas, % area GPR56$^+$ cells; E11: 0.87 ± 0.04, E13: 0.85 ± 0.06, E15: 0.36 ± 0.08, E16: 0.15 ± 0.05); (hiPSCs, GPR56 mRNA relative to GAPDH; undifferentiated hiPSCs: 0.11 ± 0.01, End stage (ES)1: 0.26 ± 0.01, ES2: 0.36 ± 0.01, ES3: 0.21 ± 0.03, ES4: 0.001 ± 0.003). GPR56 was then upregulated in pancreas at the stage of β-cell replication (% area GPR56$^+$ cells; E18: 0.15 ± 0.05, P9: 0.47 ± 0.07, $n = 10$). The number of cells proliferating and remaining in the cell cycle was significantly lower in GPR56KO islets at P9 (BrdU$^+$Ki67$^+$ cells/μm^2; WT: 115.9 ± 18.2, KO: 50.9 ± 6.3, $n = 3$, $p < 0.05$), leading to less β-cells (% β-cells/islet; WT: 68.5 ± 0.8, KO: 54.8 ± 3.0, $n = 3$, $p < 0.05$), but higher numbers of α-cells in GPR56KO islets (% α-cells/islet; WT: 17.7 ± 0.9, KO: 33.7 ± 2.8, $n = 3$, $p < 0.01$). TYFAVLM increased [(Ca^{2+})i] in WT β-cells but not in GPR56KO β-cells (basal to peak ratio; WT, 2mM glucose: 0.02 ± 0.01, +TYFAVLM: 0.13 ± 0.01, $n = 3$, $p < 0.01$; KO, 2 mM glucose: 0.01 ± 0.003, +TYFAVLM: 0.02 ± 0.001, $p > 0.2$) and it potentiated glucose-induced insulin secretion from human islets (Insulin pg/islet/min; 2 mM glucose: 3.21 ± 0.3, 20 mM glucose: 5.25 ± 0.72, 20 mM glucose+TYFAVLM: 12.2 ± 1.85, $n = 3$, $p < 0.05$). TYFAVLM protected WT β-cells from cytokine-induced apoptosis, but had no effect in GPR56KO β-cells (luminescence values; WT, control: 306,910 ± 11,301, +TYFAVLM: 268,499 ± 11,315, +10% FBS: 158,668 ± 8,151, $n = 8$, $p < 0.0001$; KO, control: 177,575 ± 9208, +TYFAVLM: 187,823 ± 10,620, +10% FBS: 104,482 ± 10,932, $n = 8$, $p > 0.1$).

Conclusion: Our data suggest that GPR56 plays an important role in islet development, and it is required for an appropriate α-/β-cell ratio in islets. Moreover, GPR56 is activated by TYFAVLM to stimulate insulin secretion and protect β-cells from apoptosis.

Supported by: Diabetes UK, Society for Endocrinology, Human Developmental Biology Resource

Disclosure: **O.E. Olaniru:** None.

107

Modelling congenital hyperinsulinism in patient stem cell derived beta like cells

V. Lithovius, J. Saarimäki-Vire, D. Balboa, J. Ustinov, S. Eurola, H. Grym, T. Otonkoski;
Biomedicum Stem Cell Center, University of Helsinki, Helsinki, Finland.

Background and aims: Mutations in the genes encoding the K_{ATP}-channel of the pancreatic beta cell are the most common cause of congenital hyperinsulinism (CHI). In Finland, the most common single cause is the *ABCC8* mutation V187D, which leads to a trafficking defect of the SUR1 protein, causing a drug resistant, severe form of the disease, characterized by continuous insulin secretion regardless of blood glucose concentration. Our objective was to recapitulate this CHI phenotype with patient induced pluripotent stem cell (iPSC) -derived beta-like cells *in vitro* and in a humanized mouse model. This model can be used to investigate the effect of the K_{ATP}-channel defect on the development, function, proliferation and apoptosis rates of human beta cells *in vitro* and *in vivo*. The

model also enables future studies in testing novel pharmaceuticals and PET-tracers for improved management and diagnostics of CHI.

Materials and methods: iPSCs were derived from a patient with severe, diazoxide unresponsive diffuse CHI caused by homozygous *ABCC8*-V187D -mutation. iPSCs from a healthy donor and the patient iPSCs corrected with CRISPR/Cas9 technology were used as controls. The cells were differentiated towards beta-cell fate, using a 7-stage, 35-day protocol which yielded islet-like clusters containing 20–40% insulin-positive monohormonal cells (beta-like cells; BLCs). These were studied *in vitro* with static sequential exposures to glucose and pharmaceuticals acting on K_{ATP}-channels and other targets. The BLCs were transplanted to immunocompromised NOD scid gamma mice and subjected to an insulin tolerance test 4 months after transplantation. The explanted grafts were stained and quantitative analyses on the endocrine cell populations and proliferation and apoptosis rates were conducted.

Results: Mutant BLCs failed to shut down secretion when exposed to diazoxide as compared to healthy control cells (fold change to basal 1.00 ± 0.19 vs. 0.44 ± 0.11 $p < 0.001$) and did not increase secretion in response to tolbutamide (fold change 0.91 ± 0.25 $n = 5$ vs. 1.56 ± 0.31, $p < 0.001$, $n = 5$). The mutant BLCs could inhibit their secretion in response to clonidine and calcium chelation similarly to control cells. The CHI-mice had lower fasting blood glucose (4.3 ± 1.6 vs. 7.6 ± 2.3 mmol/l, $p < 0.05$) and higher human C-peptide (816 ± 298 vs. 152 ± 58 pmol/l, $p < 0.01$). Most importantly, human C-peptide secretion was not inhibited by insulin-induced hypoglycemia in the CHI-mice (reduction at 40 min after insulin administration 13.5 ± 26.7% $n = 5$ vs. 73.8 ± 11.6% $n = 8$, $p < 0.01$). The CHI-explants had more insulin+ cells (47.7 ± 9.7% vs. 19.2 ± 13.3%, $p < 0.01$) and fewer somatostatin+ cells (2.7 ± 1.0% vs. 7.7 ± 2.8%, $p < 0.05$), there was no difference in the proliferation rate of the explanted insulin+ cells. (All Mean ± 95%CI)

Conclusion: We have successfully recapitulated the CHI phenotype in beta-like cells derived from patient iPSC and created a humanized mouse model for CHI.

Supported by: Finnish Diabetes Association

Disclosure: **V. Lithovius:** None.

108

New insights into beta cell development: a specific CNOT1 mutation impairs early pancreatic and neurological development in both humans and mice

A.T. Hattersley[1], R. Watson[2], S.E. Flanagan[1], S. Ellard[1], H. Heimberg[3], A. Caliebe[4], A. Deeb[5], A. Green[2], W.J. Weninger[6], E. De Franco[1], I. Barroso[2];

[1]University of Exeter Medical School, Exeter, UK, [2]Wellcome Sanger Institute, Hinxton, UK, [3]Vrije Universiteit, Brussels, Belgium, [4]Institut fuer Humangenetik, Keil, Germany, [5]Mafraq Hospital, Abu Dhabi, United Arab Emirates, [6]Medical University of Vienna, Vienna, Austria.

Background and aims: Discovering the genes causing pancreatic agenesis in humans is crucial to identify factors needed for human pancreatic and hence beta-cell development. To date we and others have identified 6 causes of pancreatic agenesis, many of which were not initially suspected from mouse studies. We aimed to identify the genetic cause in patients where the genetic aetiology of pancreatic agenesis is not known as this has the potential to give key insights into development of the human pancreas.

Materials and methods: We investigated 9 patients without an identified aetiological mutation from an international cohort of 106 patients with pancreatic agenesis. We performed exome sequencing on the DNA from the cases and all available parents to allow detection of *de novo* heterozygous mutations that represent the commonest causes of both pancreatic agenesis and neonatal diabetes in patients born to unrelated parents. To allow more detailed study of the mechanisms whereby the aetiological gene caused pancreatic agenesis, we created a mouse line harbouring the *Cnot1* mutation with CRISPR. The mouse model was phenotyped in detail including volumetric measurement of the fetal pancreas.

Results: The same novel missense variant in *CNOT1*, p.(Arg535Cys), was identified in 3 patients. This mutation was predicted to affect the protein's function in silico and was proven to have arisen *de novo* in the 2 families where both unaffected parents were available. The three patients not only had pancreatic agenesis requiring both insulin treatment and exocrine replacement therapy but also had holoprosencephaly (a failure of forebrain fusion) in 2 cases and probable holoprosencephaly in the 3rd (brain imaging was declined by the parents). We hypothesised a mutation-specific mechanism and generated a mouse line harbouring the same C*not1* mutation by CRISPR engineering. Gross morphological assessment suggested no phenotype in heterozygous mice while homozygosity for the mutation was embryonically lethal. We therefore investigated mouse embryos at E14.5. Homozygous embryos had markedly abnormal brain development (exencephaly $p = 3.2 \times 10^{-9}$), eye defects (coloboma $p = 5.5 \times 10^{-8}$) and significantly reduced dorsal pancreas size.

Conclusion: Our results identify a specific mutation in *CNOT1* as the genetic cause of a rare syndrome of pancreatic agenesis and holoprosencephaly. The phenotype in homozygous mice carrying the mutation recapitulates the human disease, confirming causality of the specific mutation. CNOT1 has been proposed to have a role in maintaining stem cells in a pluripotent state. We therefore propose a new mechanism resulting in pancreatic agenesis resulting from stem cells being maintained in their pluripotent state and prevented from differentiating towards the pancreatic line. This study establishes *CNOT1* as a key gene in very early pancreatic and neurological development in man and mouse, consistent with the proposed role in stem cells.

Supported by: Wellcome Trust

Disclosure: **A.T. Hattersley:** None.

OP 19 SGLT2 inhibitors: new mechanisms and clinical evidence

109

Bioenergetics of myeloid angiogenic cells: its role in the damage induced by stearic acid and in the protective action of empagliflozin

F. Fantuzzi[1], V. Spigoni[1], G. Cinquegrani[1], R. Aldigeri[1], E. Derlindati[1], A. Dei Cas[2], R.C. Bonadonna[2];
[1]University of Parma, Parma, [2]University of Parma and AOU of Parma, Parma, Italy.

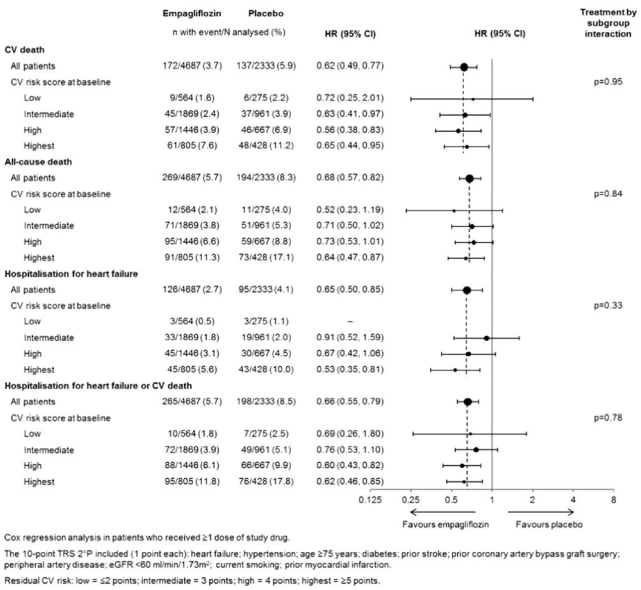
Background and aims: In muscle biopsies of patients with type 2 diabetes, dapagliflozin reduced glucose oxidation and ATP synthesis - as a consequence of decreased tricarboxylic acid (TCA) cycle flux. In our lab, empagliflozin and dapagliflozin curbed inflammation and oxidant stress induced by stearic acid (SA) in human myeloid angiogenic cells (MAC; elsewhere named endothelial progenitor cells). We asked the question whether cell bioenergetics may be involved in the effects exerted by SA and/or SGLT2-inhibitors in MAC, which are thought to play major roles in atherosclerosis and cardiovascular risk. Aim of the study was to assess in human MAC whether: 1. SA-induced increases in inflammation and oxidant stress are accompanied by bioenergetic alterations; 2. empagliflozin anti-lipotoxic action is concomitant with coherent changes in bioenergetic metabolism.
Materials and methods: MAC were isolated from peripheral blood of healthy volunteers and incubated in the presence/absence of SA (100 μM) for 3 hours with/without empagliflozin (EMPA 100 μM). Respiration (O_2 consumption rate: VO_2) and glycolysis (GLYC; measured as extracellular acidification rate) were recorded in real-time by Seahorse technology (XFp Extracellular Flux Analyzer, Agilent). Basal and maximal VO_2, ATP-linked and non-mitochondrial respiration and spare respiratory capacity were quantified by serially adding oligomycin (ATP synthase inhibitor), FCCP (protonophore) and rotenone/antimycin A (inhibitors of complex I and III of electron transport chain) into the culture medium, according to a well established protocol. All parameters were adjusted for the number of viable cells.
Results: SA, at the concentration (100 μM) causing inflammation and increased oxidant stress, extensively altered cell bioenergetics of human MAC, with overall reductions both in basal/maximal VO_2, ATP production and spare respiratory capacity (all $p < 0.05$ or less *vs* control), all pointing to mitochondrial dysfunction, and in GLYC ($p < 0.05$), indicating no induction of Warburg effect. EMPA, at the concentration counteracting SA-induced lipotoxicity, both alone and in the presence of SA, caused alterations in cell bioenergetics quite similar to those induced by SA alone ($p < 0.05$ or less *vs* control).
Conclusion: In human MAC: 1. SA induces extensive alterations in cell bioenergetics, concomitantly with increase in inflammation and oxidant stress; 2. EMPA may inhibit TCA cycle/mitochondrial respiration, extending a previous observation made with dapagliflozin in muscle biopsies and suggesting a potential class effect; 3. the protective effect of EMPA against SA-induced lipotoxicity is unlikely to be mediated through bioenergetic metabolism.
Supported by: Fondazione Diabete e Ricerca in collaboration with Eli Lilly Italia
Disclosure: **F. Fantuzzi:** Grants; Fondazione Diabete e Ricerca in collaboration with Eli Lilly Italia.

110

Empagliflozin reduces mortality and hospitalisation for heart failure irrespective of cardiovascular risk score at baseline

D. Fitchett[1], S.E. Inzucchi[2], C.P. Cannon[3], D.K. McGuire[4], O.E. Johansen[5], S. Sambevski[6], U. Hehnke[6], J.T. George[6], B. Zinman[7];
[1]St Michael's Hospital, Division of Cardiology, University of Toronto, Toronto, Canada, [2]Section of Endocrinology, Yale University School of Medicine, New Haven, USA, [3]Cardiovascular Division, Brigham and Women's Hospital, Boston, USA, [4]University of Texas Southwestern Medical Center, Dallas, USA, [5]Boehringer Ingelheim Norway KS, Asker, Norway, [6]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [7]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Canada.

Background and aims: In the EMPA-REG OUTCOME trial in patients with type 2 diabetes and established cardiovascular (CV) disease, empagliflozin added to standard of care reduced CV death vs placebo by 38% (HR 0.62 [95% CI 0.49, 0.77]), all-cause death by 32% (HR 0.68 [95% CI 0.57, 0.82]) and hospitalisation for heart failure (HHF) by 35% (HR 0.65 [95% CI 0.50, 0.85]). We investigated whether residual CV risk at baseline influenced the effect of empagliflozin on these outcomes.
Materials and methods: We investigated CV death, all-cause death, HHF and the composite of HHF or CV death with empagliflozin vs placebo in subgroups by degree of CV risk at baseline based on the 10-point TIMI Risk Score for Secondary Prevention (TRS 2°P). *P* values for treatment-by-subgroup interaction were obtained from tests of homogeneity of treatment group differences among subgroups with no adjustment for multiple testing.
Results: Based on the TRS 2°P risk score, of 7020 patients who received study drug in the EMPA-REG OUTCOME trial, 12%, 40%, 30% and 18% were at low, intermediate, high and highest residual CV risk, respectively, at baseline. In the placebo group, from low to highest predicted risk, the proportion of patients with CV death increased from 2.2% to 11.2% and the proportion of patients with HHF increased from 1.1% to 10.0%. Effects of empagliflozin on CV death, all-cause death, HHF and HHF or CV death were consistent across subgroups by baseline CV risk score (Figure).
Conclusion: The benefits of empagliflozin on key clinical outcomes in the EMPA-REG OUTCOME trial occurred irrespective of residual CV risk at baseline.



Cox regression analysis in patients who received ≥1 dose of study drug.
The 10-point TRS 2°P included (1 point each): heart failure; hypertension; age ≥75 years; diabetes; prior stroke; prior coronary artery bypass graft surgery; peripheral artery disease; eGFR <60 ml/min/1.73m²; current smoking; prior myocardial infarction.
Residual CV risk: low = ≤2 points; intermediate = 3 points; high = 4 points; highest = ≥5 points.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **D. Fitchett:** Honorarium; Sanofi, Merck & Co., Amgen, AstraZeneca, Eli Lilly and Company and Boehringer Ingelheim.

111

Canagliflozin versus other antihyperglycaemic agents on the risk of below-knee amputation for patients with type 2 diabetes: a real world analysis of >700,000 US patients

P. Ryan[1], J.B. Buse[2], M. Schuemie[1], F. DeFalco[3], Z. Yuan[1], P. Stang[1], J.A. Berlin[4], N. Rosenthal[3];
[1]Janssen Research & Development, LLC, Titusville, [2]University of North Carolina School of Medicine, Chapel Hill, [3]Janssen Research & Development, LLC, Raritan, [4]Johnson & Johnson, Titusville, USA.

Background and aims: Sodium glucose co-transporter 2 inhibitors (SGLT2i) are indicated for treatment of type 2 diabetes mellitus (T2DM); some SGLT2i have reported a cardiovascular (CV) benefit and some have reported a risk of below-knee amputation (BKA).
Materials and methods: US claims databases were analysed using a prespecified protocol to examine canagliflozin (CANA)-associated effects on BKA and hospitalisation for heart failure (HHF) versus other SGLT2i and non-SGLT2i antihyperglycaemic agents (AHAs). Analyses used a propensity score adjusted new user design with numerous sensitivity analyses. The 4 databases included 142,000 new users of CANA, 110,000 of other SGLT2i, and 460,000 of non-SGLT2i AHAs. Meta-analysis results are reported when heterogeneity across databases was not substantial ($I^2 < 0.4$).
Results: There was no evidence of increased risk of BKA with CANA versus non-SGLT2i AHAs or other SGLT2i in on-treatment or intention-to-treat (ITT) analyses (Table). HHF benefits were demonstrated in these analyses, consistent with clinical trials. Similar BKA and HHF results were seen in a subgroup with established CV disease.
Conclusion: In this large comprehensive analysis, neither CANA nor other SGLT2i showed an increased risk of amputation versus non-SGLT2i AHAs. Because on-treatment median exposure was <6 months, future observational studies with longer duration are needed. This study helps to further characterise the potential benefits and harms of SGLT2i as observed in routine clinical practice to complement the evidence from clinical trials and prior observational studies.

Table. Risk of BKA and HHF in the overall population.*

				On-treatment†			Intent-to-treat (ITT)‡		
		Exposure (n/PY)		Outcomes, n			Outcomes, n		
Outcome	Comparison	CANA	Comp	CANA	Comp	HR (95% CI) p value Cal p value§	CANA	Comp	HR (95% CI) p value Cal p value§
BKA	CANA vs non-SGLT2i	111,332/ 53,125	445,367/ 256,646	60	481	0.75 (0.40-1.41) p = 0.25 Cal p = 0.30	295	1,308	1.01 (0.93-1.10) p = 0.71 Cal p = 0.51
	CANA vs other SGLT2i	69,554/ 31,369	98,169/ 41,666	40	53	1.14 (0.67-1.93) p = 0.48 Cal p = 0.53	171	209	1.13 (0.99-1.29) p = 0.06 Cal p = 0.06
HHF	CANA vs non-SGLT2i	111,332/ 53,116	445,367/ 255,504	124	2,979	0.39 (0.26-0.60) p = 0.01 Cal p = 0.00	810	7,081	**
	CANA vs other SGLT2i	69,554/ 31,363	98,169/ 41,667	56	73	0.90 (0.71-1.13) p = 0.22 Cal p = 0.28	352	381	1.07 (0.95-1.20) p = 0.16 Cal p = 0.32

PY, patient-years; Comp, comparator; Cal, calibrated; HR, hazard ratio; CI, confidence interval.
*Time-to-first-post-index-event analysis using variable-ratio propensity score matching.
† 'On-treatment' period was defined as the time from 1 day after exposure to all days through the period of persistent exposure allowing for 30 day gap between successive exposures until the final exposure record. The time will be censored if the patient starts a non-metformin AHA other than the cohort defining drug(s).
‡ 'Intent-to-treat' (ITT) period was defined as the time from 1 day after exposure to the last day of observation available in the database.
§ A set of negative control outcomes were used to calibrate p values to control for any potential systematic errors after propensity score adjustment.
**Meta-analytic estimate was not imputed due to observed heterogeneity ($I^2 = 0.59$). HR (95% CI) in the 4 databases were: 0.63 (0.52-0.75), 0.94 (0.70-1.25), 0.83 (0.69-1.00), and 0.73 (0.63-0.83).

Supported by: Janssen Global Services, LLC
Disclosure: **P. Ryan:** Employment/Consultancy; Janssen Research & Development, LLC.

112

The inTandem 2 study: 52-week efficacy and safety of sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, as adjunct therapy to insulin in adults with type 1 diabetes

B. Cariou[1], T. Danne[2], P. Banks[3], M. Brandle[4], H. Brath[5], E. Franek[6], J.A. Kushner[7], P. Lapuerta[3], D.K. McGuire[8], A. Peters[9], S. Sangeeta[10], P. Strumph[3];
[1]l'institut du thorax, Department of Endocrinology, CHU de Nantes, Nantes, France, [2]Department of Diabetes, Endocrinology and Clinical Research, Hannover Medical School, Hannover, Germany, [3]Lexicon Pharmaceuticals, Inc., The Woodlands, USA, [4]Department of Internal Medicine, Kantonsspital St Gallen, St Gallen, Switzerland, [5]Diabetes Outpatient Clinic, Health Center South, Vienna, Austria, [6]Polish Academy of Sciences, Warsaw, Poland, [7]Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, USA, [8]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, USA, [9]Keck School of Medicine of the University of Southern California, Los Angeles, USA, [10]Lexicon Pharmaceuticals, Inc, The Woodlands, USA.

Background and aims: Sotagliflozin (SOTA), a dual SGLT1 and SGLT2 inhibitor, is currently in development as an oral adjunct to insulin in type 1 diabetes (T1D).
Materials and methods: In this double-blind, 52-week European study, 782 adults with Type 1 diabetes (T1D) treated with multiple daily insulin injections or pump therapy were randomized 1:1:1 to placebo ($n = 258$), sotagliflozin (SOTA) 200 mg ($n = 261$) or SOTA 400 mg ($n = 263$) once daily after 6 weeks of insulin optimization. Primary endpoint was change from baseline in A1C at Week 24. Other endpoints were A1C, documented hypoglycemia (DH), weight and fasting plasma glucose (FPG) change at Week 52, patient (pt) reported outcomes (PROs) and net clinical benefit (NCB) - the proportion of pts with A1C <7.0% without severe hypoglycemia (SH) or diabetic ketoacidosis (DKA).
Results: Baseline characteristics were similar between groups. Compared with placebo, treatment with SOTA 200 or 400 mg improved A1C and pt satisfaction at Week 24 and reduced A1C, DH rate, weight, FPG and pt distress at Week 52 (Table). More pts achieved NCB in the SOTA arms vs placebo (Table). Pts receiving SOTA 400 mg had the least SH events, but more genital mycotic infections, DKA, and diarrhea than placebo.
Conclusion: SOTA 200 and 400 mg provided statistically significant A1C reductions that were sustained ($P < 0.05$) at Week 52, as well as improvement in DH and PROs. There was more DKA, but less SH, with SOTA 400 mg relative to placebo at Week 52.

Efficacy (mITT population) and Safety (safety population) Results

	Placebo n=258	SOTA 200 mg n=261	SOTA 400 mg n=263
Mean A1C at Baseline, after 6-week insulin optimization, %	7.79	7.74	7.71
Outcomes at Week 24			
A1C LSM diff from placebo, % ± SE (P-value)	-	–0.37±0.06 (P<0.001)	–0.35±0.06 (P<0.001)
Outcomes at Week 52			
A1C LSM diff from placebo, % ± SE (P-value)	-	–0.21±0.07 (P=0.003)	–0.32±0.07 (P<0.001)
FPG LSM diff from placebo, mmol/L ± SE	-	–0.27±0.33 (P=0.41)	–0.87±0.33 (P=0.008)
Daily insulin LSM diff from placebo, IU ± SE (P-value)	-	–2.81±1.14 (P=0.014)	–3.37±1.14 (P=0.003)
DH rate[a], LSM diff from placebo ± SE (P-value)	-	–0.03±0.01 (P=0.017)	–0.03±0.01 (P=0.006)
Body weight LSM diff from placebo, kg ± SE (P-value)	-	–2.18±0.36 (P<0.001)	–2.92±0.36 (P<0.001)
Mean daily bolus insulin dose at Baseline, IU	32.1	31.1	31.9
Bolus insulin dose mean change from Baseline, % ± SE	4.21±3.25	–3.48±3.24	–7.94±3.23
Bolus insulin LSM diff from placebo, % ± SE (P-value)	-	–7.70±4.41 (P=0.08)	–12.15±4.40 (P=0.006)
Net clinical benefit at Week 52			
A1C <7.0% without SH and without DKA, n (%)	37 (14.3)	67 (25.7)	70 (26.6)
Safety outcomes over 52 weeks			
Any TEAE, n (%)	158 (61.2)	178 (68.2)	181 (68.8)
TEAEs leading to study discontinuation, n (%)	9 (3.5)	10 (3.8)	18 (6.8)
Treatment-emergent serious adverse events, n (%)	17 (6.6)	26 (10.0)	21 (8.0)
Death, n (%)	2 (0.8)	0	0
DKA[b], n (%)	0	6 (2.3)	9 (3.4)
Severe hypoglycemia, n (%)	13 (5.0)	13 (5.0)	6 (2.3)
Diarrhea, n (%)	9 (3.5)	12 (4.6)	19 (7.2)
Genital mycotic infection, n (%)	6 (2.3)	24 (9.2)	29 (11.0)
Patient-reported outcomes			
DTSQ score LSM diff from placebo at Week 24 ± SE (P-value)		2.0±0.4 (P<0.001)	1.7±0.4 (P<0.001)
DDS2 score LSM diff from placebo at Week 52 ± SE (P-value)		–0.2±0.2 (P=0.23)	–0.3±0.2 (P=0.046)

DDS2, two-item Diabetes Distress Screening Scale; DTSQ, diabetes treatment satisfaction questionnaire; LSM diff, least squares mean difference; mITT, modified intent-to-treat; SE, standard error; TEAE, treatment emergent adverse events. [a]Events per patient per day ≤3.0 mmol/L (≤55 mg/dL); [b]Positively-adjudicated events discontinuation of drug due to DKA was: 0% placebo, 0% SOTA 200 mg, and 1.9% for SOTA 400 mg.

Clinical Trial Registration Number: NCT02421510
Supported by: Lexicon Pharmaceuticals, Inc/Sanofi
Disclosure: **B. Cariou:** Grants; Sanofi, Regeneron Pharmaceuticals, Pfizer. Honorarium; Amgen, AstraZeneca, Genfit, Pierre Fabre, Eli Lilly and Company, MSD Merck & Co., Novo Nordisk, Sanofi. Other; Sanofi, Regeneron Pharmaceuticals.

113

SGLT2i vs bolus insulin as add-on to stable basal insulin treatment in type 2 diabetes and risk of cardiovascular disease and mortality: a nationwide observational study

J.W. Eriksson[1], J. Bodegard[2], A. Norhammar[3], M. Thuresson[4], D. Nathanson[5], T. Nyström[5];

[1]Uppsala University, Uppsala, Sweden, [2]AstraZeneca Nordic-Baltic, Oslo, Norway, [3]Karolinska Institutet, Stockholm, Sweden, [4]Statisticon AB, Uppsala, Sweden, [5]Södersjukhuset, Stockholm, Sweden.

Background and aims: Cardiovascular outcome trials (CVOTs) have recently shown improved prevention of cardiovascular (CV) disease with sodium-glucose cotransporter-2 inhibitors (SGLT2i). Large observational studies have indicated similar effects in broader T2D populations. A significant proportion of the CVOT-patients were on concomitant insulin treatment. The aim of this observational study was to examine the risk of cardiovascular disease and mortality in new users of SGLT2i versus bolus insulin, when added to stable basal insulin treatment in T2D. These patients have advanced T2D with a need for treatment intensification.
Materials and methods: All T2D patients on basal insulin treatment (intermediate-, long-acting- or premixed insulin for >1 year) were identified in mandatory, nationwide health care registries during 2013–2016. New users of SGLT2i and bolus (short-acting) insulin as add-on to basal insulin treatment were matched 1:1 by propensity scores for the likelihood of receiving SGLT2i (calculated by using >90 relevant patient characteristic variables). The primary combined endpoint was hospitalization for heart failure (HHF; in-/outpatient) or CV mortality. In addition, major cardiovascular adverse events (MACE; myocardial infarction, stroke or CV mortality) and severe hypoglycaemia (hospital admission) were assessed. Patients were followed until death, end of study period or treatment discontinuation. Unadjusted Cox survival models estimated hazard ratios.
Results: A total of 2 988 patients were identified after matching, mean age 64 years, 32% had established CV disease with no imbalances at baseline. Follow-up time was 2 736 patient years (mean 0.92 years), in the SGLT-2i group dapagliflozin contributed with 87% and empagliflozin with 13%. Add-on of SGLT-2i versus bolus insulin to stable insulin treatment was significantly associated with lower risk of the combined outcome (HR 95% CI; 0.51:0.32–0.82), and also HHF and CV mortality separately; MACE (0.54:0.34–0.87) and severe hypoglycemia (0.61:0.40–0.93), which was the most common event. No significant differences for myocardial and stroke were found.
Conclusion: In patients with ongoing basal insulin treatment, add-on of SGLT2i versus bolus insulin carried significantly less risk of CV mortality, HHF and MACE. Notably, the event rate of severe hypoglycaemia in this population was lower with SGLT2i treatment.

	SGLT-2i No. Events (Event rate per 100 patient-years)	Bolus insulin No. Events (Event rate per 100 patient-years)	Hazard ratio	CI 95%	p-value
HHF or CV mortality	28 (1.93)	50 (3.90)	0.51	(0.32-0.82)	0.005
HHF	21 (1.45)	35 (2.73)	0.54	(0.32-0.93)	0.027
CV mortality	8 (0.55)	19 (1.46)	0.40	(0.17-0.91)	0.030
MACE (includes CV mortality)	27 (1.86)	46 (3.57)	0.54	(0.34-0.87)	0.011
Myocardial infarction	13 (0.89)	16 (1.24)	0.73	(0.35-1.52)	0.400
Stroke	9 (0.62)	14 (1.08)	0.59	(0.25-1.36)	0.214
Severe hypoglycemia	35 (2.44)	53 (4.20)	0.61	(0.40-0.93)	0.022
All-cause mortality	18 (1.23)	56 (4.31)	0.30	(0.18-0.51)	<0.001

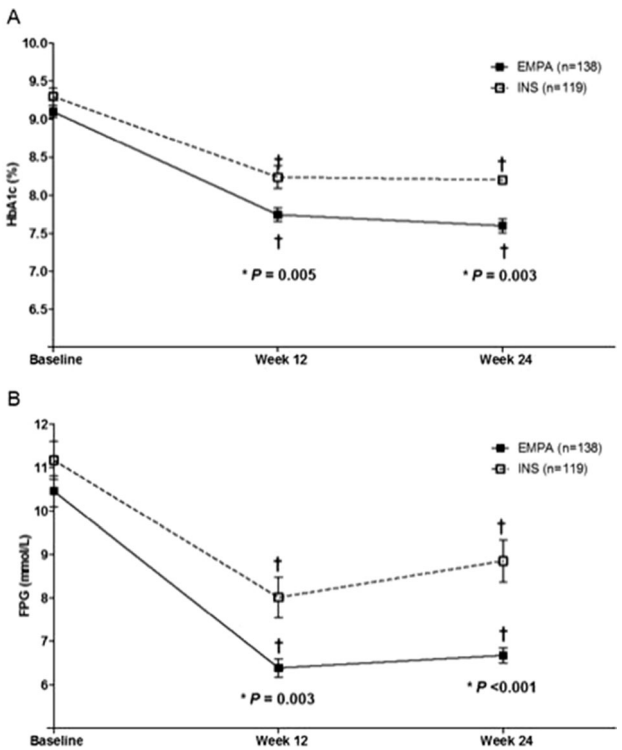
Supported by: AstraZeneca
Disclosure: **J.W. Eriksson:** Grants; AstraZeneca, Bristol-Myers-Squibb. Honorarium; AstraZeneca, NovoNordisk. Lecture/other fees; Sanofi, AstraZeneca.

114

Efficacy and safety of empagliflozin-based quadruple therapy compared to insulin glargine-based therapy in poorly controlled type 2 diabetes

E. Ku, H. Jeon, T. Oh;
Internal medicine, Chungbuk National University Hospital, Cheongju-si, Republic of Korea.

Background and aims: Empagliflozin is a potent and selective sodium-glucose cotransporter 2 (SGLT2) inhibitor. Empagliflozin showed the glucose lowering effects in the mono- or dual combination therapy with metformin, sulfonylurea (SU), dipeptidyl peptidase 4 inhibitors (DPP4i) and insulin. However, there was no study with quadruple combination with empagliflozin. The aim of this study was to examine the efficacy and safety of the empagliflozin as add-on therapy compared to basal insulin-based oral antidiabetic agents in patients with type 2 diabetes mellitus (T2DM) inadequately controlled on triple oral antidiabetic agents (OADs) in a real clinical practice.
Materials and methods: A single center, 24-week, open-label, prospective study in 257 patients comparing empagliflozin (25 mg/day, EMPA, n = 138) and insulin glargine (INS, n = 119) added to metformin (2000 mg/day) plus SU (glimepiride 8 mg/day) plus DPP4i (maximal dose). The inclusion criteria were defined as follow; HbA_{1c}: 7.5–12%, with stable OADs dose before 12 weeks. Efficacy outcomes included the change in HbA1c, fasting plasma glucose (FPG), bodyweight, systolic blood pressure (SBP) from baseline to week 24. Safety outcomes included adverse events (AEs), hypoglycemia, genitourinary tract infections (GUTIs) and laboratory tests.
Results: At week 24, reductions from baseline in mean HbA_{1c} were significantly greater in the EMPA group (−1.47 ± 1.19%) compared with the INS group (−1.17 ± 1.44%) (P = 0.003). Significant changes were also observed in FPG (−69.3 ± 57.2 vs −40.1 ± 65.9 mg/dL, EMPA and INS, respectively, P < 0.001). Reductions from baseline in bodyweight were significantly greater with the EMPA group (−1.5 ± 8.1 vs +1.2 ± 3.0 kg, P < 0.001). In terms of the differences in the SBP; changes from baseline at week 24 were −3.0 ± 14.6 mmHg with the EMPA group and +5.6 ± 21.6 mmHg with the INS group (P = 0.008). Confirmed hypoglycemic AEs were in 10.9% and 26.1%, of EMPA and INS group, respectively (P = 0.002). Events consistent with GUTIs were reported in 5.1% of subjects on EMPA group and 0.8% of subjects on INS group, respectively (P = 0.072).
Conclusion: The quadruple OADs combination of empagliflozin resulted in a significant reduction in HbA_{1c} and FPG compared with the insulin glargine-based OADs combination therapy at week 24 in patients with inadequately controlled T2DM.



Disclosure: **E. Ku:** None.

OP 20 Overriding mechanisms of NAFLD

115

Role of the receptor RANK and its ligand RANKL in the development of NAFLD

F. Phan[1,2], M. Hu[1], I. Hainault[1], V. Ratziu[1,3], O. Bourron[1,2], F. Foufelle[1];
[1]INSERM UMRS1138 Cordeliers Research Centre, Paris, [2]Assistance Publique-Hôpitaux de Paris, Department of Diabetology, Paris, [3]Assistance Publique-Hôpitaux de Paris, Department of Hepatology, Paris, France.

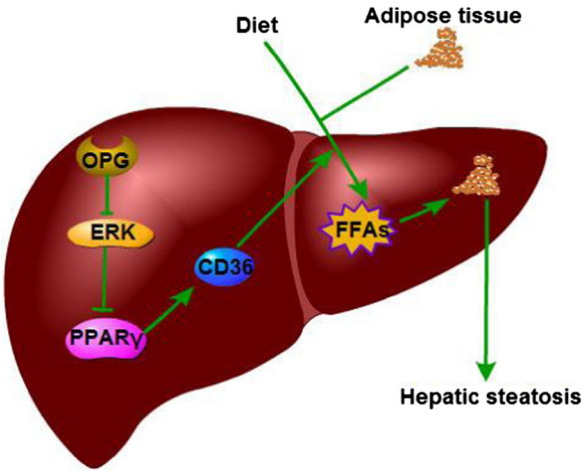
Background and aims: Non Alcoholic Fatty Liver Disease (NAFLD) is highly associated with type 2 diabetes. Hepatic steatosis represents the first stage of NAFLD and is characterized by lipid accumulation and insulin resistance. Lipotoxicity promotes ballooning and inflammation leading to steatohepatitis (NASH). Thus, insulin resistance and inflammation play a major role in the progression of the disease. Nuclear factor-kB (NF-kB) plays a central role in inflammation. Its hepatic activation leads to insulin resistance, steatosis and inflammation. Here, we are interested in the role of the TNF receptor related member RANK and its ligand RANKL, known to induce NF-κB activity, in the onset and progression of NAFLD.
Materials and methods: Mice overexpressing RANK in the myeloid lineage (TgRANK) are fed under normal chow (NC) or high fat diet (HFD). The expression of RANK and those of lipogenic and gluconeogenic enzymes are measured by RT-qPCR from the livers of NAFLD-related animal models, from biopsies of NAFLD patients and from the liver of TgRANK. Hepatic insulin signalling is analysed by Western blot.
Results: RANK expression is markedly induced in the liver of NASH-related mice models. Its expression is the highest in hepatic macrophages and proportional to the inflammatory state. In human biopsies, RANK expression is increased in NASH patients compared to NAFL and control patients. Interestingly, in our human cohort, RANK expression is negatively correlated with fasting glycaemia. Mice overexpressing RANK in the myeloid cells are generated and analysed. These mice display a lower basal glycemia and an improved insulin sensitivity during glucose tolerance test and insulin sensitivity test. In the liver of TgRANK, the phosphorylation of key proteins of the insulin signaling pathway is enhanced compared to control littermates. As a consequence, the expression of lipogenic enzyme is increased and those of gluconeogenic enzyme decreased. We next try to understand how RANK overexpression in macrophages, can induce this metabolic phenotype. We observe that TgRANK macrophages secrete more IL10 than macrophages of control mice. In the presence of insulin, treatment of primary hepatocytes with IL10 enhances insulin signaling. Conditional medium isolated from TgRANK macrophages trigger insulin signalling when added to cultured hepatocytes. We finally challenge RANK-overexpressing mice with 8 weeks of HFD and show that these animals are protected from hepatic steatosis and insulin resistance.
Conclusion: In conclusion, our results reveal that RANK expression is increased in macrophages under NAFLD conditions. We believe this could be a compensatory mechanism against insulin resistance and inflammation seen in NAFLD.
Supported by: ANR
Disclosure: **F. Phan:** None.

116

Osteoprotegerin promotes hepatic steatosis through ERK-PPARγ-CD36 pathway

Y. Lin[1], L. Li[1], G. Yang[2];
[1]Key Laboratory of Diagnostic Medicine (Ministry of Education) and Department of Clinical Biochemistry, Chongqing Medical University, Chongqing, [2]Department of Endocrinology, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterized dysregulated lipid homeostasis and an aberrant accumulation of triglycerides in the liver, but underlying mechanisms are unknown. It has been reported that circulating Osteoprotegerin (OPG) is associated with metabolic diseases in humans, including NAFLD. However, the studies of mechanism are lacking. The purpose of the present study is to investigate the roles of OPG in the progression of obesity-associated fatty liver and possible mechanisms.
Materials and methods: Expression of hepatic OPG was investigated in mouse and human livers with hepatic steatosis. In vitro, adenovirus expressing OPG (Adv-OPG) and small hairpin RNA (Adv-shOPG) were used in L02 cells and Hepal1-6 cells and OPG-Fc was used in mouse primary hepatocytes (MPHs) to investigate the role of OPG on hepatic steatosis. In vivo, hepatic triglyceride metabolism and related genes were analyzed in male WT and OPG knockout ($OPG^{-/-}$) mice fed with standard diet (SD) or high fat diet (HFD). Then, MPHs from CD36 knockout ($CD36^{-/-}$) mice were treated with OPG-Fc to further verify if OPG regulation of lipid accumulation in the liver relies on CD36. GW9662, a PPARγ inhibitor, and SCH772984, an ERK inhibitor, were respectively used to verify the association of OPG with CD36, PPARγ and ERK in cultured cells.
Results: OPG expression was down-regulated in obese mice than in SD-fed C57BL/6 mice, and down-regulated in NAFLD patients than in healthy individuals. In vitro, up-regulation of OPG in cell lines and MPHs increased TG contents and lipid droplets. In contrast, Ad-shOPG treatment decreased lipid accumulation. In vivo, under SD and HFD feeding, hepatic steatosis in $OPG^{-/-}$ mice were significantly decreased compared with WT mice. In parallel, decreased steatosis were found in MPHs from $OPG^{-/-}$ mice compare with MPHs from WT mice. Importantly, the deficiency of OPG reduced expressions of CD36 and PPARγ in the liver of $OPG^{-/-}$ mice and cultured cells. In contrast to, overexpression of OPG increased CD36 and PPARγ expressions in cultured cells. The effect of OPG on lipid accumulation was blocked by the deficiency of CD36 in MPHs from $CD36^{-/-}$ mice. With a series of truncated promoters and site-directed mutated promoter of CD36 gene, luciferase assay showed that PPREs binding site of CD36 promoter was indispensable for OPG activation of CD36 promoter activity. Treatment with GW9662, a PPARγ inhibitor, could completely eliminate OPG-induced CD36 protein expression. Furthermore, the overexpression or deficiency of OPG led to decreasing or increasing ERK phosphorylation in the liver of $OPG^{-/-}$ mice and cultured cells. And SCH772984, an ERK inhibitor, eliminated the decrease of CD36 and PPARγ expression induced by knockdown of OPG.
Conclusion: These data indicate that OPG play an important role in NAFLD through targeting ERK-PPARγ-CD36 pathway.



Supported by: National Natural Science Foundation of China (81670755, 81570752)
Disclosure: **Y. Lin:** None.

117

NREP bridges TGF-beta signalling and lipid metabolism in the epigenetic programming of NAFLD

D.F. De Jesus[1,2], K. Orime[1], E. Dirice[1], D. Kaminska[3], C.-H. Wang[1], J. Hu[1], V. Mannisto[3,4], A.M. Silva[5], Y.-H. Tseng[1], J. Pihlajamaki[3,4], R.N. Kulkarni[1];
[1]Joslin Diabetes Center and Harvard Medical School, Boston, USA, [2]University of Porto, Porto, Portugal, [3]University of Eastern Finland, Kuopio, Finland, [4]Kuopio University Hospital, Kuopio, Finland, [5]University of Tras-os-Montes and Alto Douro, Vila Real, Portugal, [6]University of Eastern Finland, Kuopio, Finland.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) prevalence is increasing worldwide and few studies have associated maternal diabetes and altered birth weights with increased risk for NAFLD. We aimed to determine the genetic and epigenetic effects of paternal versus maternal genetic insulin resistance on the developmental programming in the offspring of the liver-specific insulin receptor knockout (LIRKO) mice.
Materials and methods: We used a unique non-dietary model manifesting hyperglycemia and hyperinsulinemia - two hallmarks of gestational and type 2 diabetes. Male control F1 offspring from father LIRKO (FL), mother LIRKO (ML) or control mothers and fathers (C) were weaned on a chow or high-fat-diet (HFD) and followed for 3 months. We further validated a set of candidate genes by using an *in-vitro* model of hepatic steatosis in HepG2 cells and human primary hepatocytes. Finally we validated the clinical translation of these findings by evaluating our candidate genes expression in samples from patients with steatosis.
Results: FL and ML showed impaired growth and body weight composition. FL and ML developed hepatic steatosis compared to C when challenged with HFD and exhibited increased hepatic expression of lipogenesis-associated genes. Hepatic transcriptomic and genome-wide DNA methylation analyses of FL and ML on chow diet presented enriched-pathways associated with TGF-β signaling and lipid synthesis. FL and ML hepatic NREP mRNA levels were decreased 50% ($p < 0.05$) on HFD compared to C. *In-vivo* and *in-vitro* modeling of hepatic steatosis in HepG2 and human primary hepatocytes decreased *NREP* mRNA and protein levels. Knock-down experiments performed in HepG2 cells revealed that *NREP* acts by regulating triglyceride and cholesterol synthesis transcriptional network via regulation of ATP-citrate lyase (ACL) in a phospho-AKT dependent manner. To investigate the translational relevance of these findings, we investigated NREP levels in patients with steatosis. Consistently, *NREP* mRNA levels were decreased by 40% ($p < 0.05$) in patients with steatosis as compared to controls and looking into public available datasets we were able to identify a strong negative correlation between *NREP* and *ACL* expression. Next, to examine whether plasma NREP levels mimic the changes seen in patients with NAFLD, we analyzed NREP plasma levels in controls ($n = 106$), simple steatosis ($n = 36$) and NASH ($n = 28$) patients in a comprehensive obese liver biopsy-proven cohort (the Kuopio cohort). Indeed, several clinical parameters negatively correlated with NREP levels, including steatosis grade ($p < 0.0002$) and NAFL activity score ($p < 0.0001$).
Conclusion: These findings and recent preclinical trials implicating ACL in NAFLD highlight the translation relevance of our findings. Together these data suggest that prenatal insulin resistance epigenetically regulates a novel protein, NREP that has detrimental effects on metabolic adaptation and transcriptional regulation of hepatic metabolism in the development of NAFLD.
Supported by: SFRH/BD/51699/2011, R01 DK67536, R01 DK103215, R01 DK055523,
Disclosure: **D.F. De Jesus:** None.

118

Role of PKD1 in the control of liver endoplasmic reticulum stress responses during non-alcoholic fatty liver disease progression

P. Rada[1,2], A. Mosquera[1,2], C. García-Monzón[3], T. Iglesias[1,4], Á.M. Valverde[1,2];
[1]Instituto de Investigaciones Biomédicas Alberto Sols (UAM-CSIC), Madrid, [2]Centro de Investigación Biomédica en Red sobre Diabetes y enfermedades metabólicas asociadas (Ciberdem), Madrid, [3]Liver Research Unit, Instituto de Investigación Sanitaria Princesa, University Hospital Santa Cristina, CIBERehd, Madrid, [4]Centro de Investigación Biomédica en Red sobre enfermedades neurodegenerativas (CIBERNED), Madrid, Spain.

Background and aims: Protein kinase D1 (PKD1) is a ubiquitous serine/threonine kinase belonging to the CAMK family. It is increasingly implicated in the regulation of fundamental biological processes such as apoptosis, cell proliferation, trafficking and oxidative stress. It has been previously reported that PKD1 plays a role in different tissues including skin, immune cells, cardiac myocytes and pancreas. However, its role in liver metabolism remains unclear.
Materials and methods: A mouse model that lacks PKD1 in hepatocytes was generated by using the Cre-loxP system ($PKD1^{\Delta Hep}$ and $PKD1^{fl/fl}$ as control mice). Primary hepatocytes from $PKD1^{fl/fl}$ and $PKD1^{\Delta Hep}$ mice were isolated by two-step collagenase perfusion. As an *in vivo* model of non-alcoholic fatty liver disease (NAFLD) linked to obesity, 8 week-old male $PKD1^{\Delta Hep}$ and $PKD1^{fl/fl}$ mice were fed Chow (CHD) or High Fat Diet (HFD) for 20 weeks. Parameters that assess glucose homeostasis, whole body and hepatic insulin sensitivity as well as NAFLD progression were analyzed.
Results: Primary hepatocytes from $PKD1^{\Delta Hep}$ mice presented higher levels of endoplasmic reticulum (ER) stress markers under basal conditions (pPERK $p < 0.01$ vs $PKD1^{fl/fl}$; pIRE1α $p < 0.05$ vs $PKD1^{fl/fl}$; CHOP $p < 0.05$ vs $PKD1^{fl/fl}$) and after palmitic acid (PA) stimulation. In addition, under conditions of chow diet, liver sections of $PKD1^{\Delta Hep}$ mice showed a more pronounced dilation of the ER lumen compared to $PKD1^{fl/fl}$ mice, suggesting that PKD1 might contribute to the maintenance of ER homeostasis in the liver. Since obesity induce ER stress response and insulin resistance, male mice from both strains were fed HFD for 20 weeks. After this period, $PKD1^{\Delta Hep}$ mice exhibited a higher increase in body weight when compared to $PKD1^{fl/fl}$ mice ($p < 0.05$ vs HFD-fed $PKD1^{fl/fl}$: $PKD1^{fl/fl}$ 47.4 ± 2.4 g, $PKD1^{\Delta Hep}$ 53.9 ± 1.2 g). Moreover, glucose (GTT), insulin (ITT) and pyruvate (PTT) tolerance tests revealed that HFD-fed $PKD1^{\Delta Hep}$ mice presented a moderate alteration in glucose homeostasis and a significant decrease in hepatic insulin sensitivity than control mice (PTT $p < 0.05$ vs HFD-fed $PKD1^{fl/fl}$ mice). These results were also confirmed by analysis of phosphorylation levels of insulin receptor and AKT ($p < 0.001$ vs HFD-fed $PKD1^{fl/fl}$ mice). Interestingly, histological analysis revealed that steatosis, inflammation and ballooning (NAS score) were slightly but not significantly higher in $PKD1^{\Delta Hep}$ mice than in $PKD1^{fl/fl}$ mice under CHD, but more importantly, when both mice were fed HFD, these differences were statistically significant between both genotypes (steatosis $p < 0.001$ vs HFD-fed $PKD1^{fl/fl}$; inflammation $p < 0.05$ vs HFD-fed $PKD1^{fl/fl}$; ballooning $p < 0.001$ vs HFD-fed $PKD1^{fl/fl}$).
Conclusion: Taken together, our results strongly suggest a possible protective role of PKD1 to maintain the liver ER homeostasis that could delay the progression of liver pathologies associated to ER stress such as NAFLD.
Supported by: IJCI-2014-19381 (MINECO), SAF2015-65267-R (MINECO/FEDER), CIBERdem (ISCiii)
Disclosure: **P. Rada:** None.

119

Therapeutic effect of a novel long-acting GLP-1/GIP/Glucagon triple agonist (HM15211) in NASH and fibrosis animal models

J. Kim, I. Choi, J. Lee, E. Park, Y. Kim, S. Jung, S. Kim;
Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea.

Background and aims: Nonalcoholic steatohepatitis (NASH), a progressive form of nonalcoholic fatty liver disease (NAFLD), may lead to end stage liver disease including cirrhosis and hepatocellular carcinoma. Despite its severity and prevalence, NASH currently lacks effective therapies. In this respect, we developed a novel long-acting, GLP-1/GIP/Glucagon triple agonist, HM15211. HM15211, with a unique activity profile, provides synergistic benefits on body weight loss and lipid profile improvement while avoiding hyperglycemic risk. Previously, we showed that HM15211 exerts potent reductions in body weight and hepatic triglycerides (TG) in DIO mice. In addition, HM15211 shows liver preferential distribution, suggesting a potential treatment for NASH. Here, we evaluated the effect of HM15211 in NASH and fibrosis in Methionine choline-deficient (MCD) diet mice model with various disease induction periods utilizing up-to-date imaging techniques and histopathological analysis. In addition, efficacy of HM15211 was further evaluated in obese and NASH monkeys.
Materials and methods: MCD-diet mice model was induced for 6 and 10 weeks for moderate and severe fibrosis induction, respectively. Comparators including liraglutide 50 nmol/kg, twice-daily (BID) (for 6 week induction) or selonsertib and OCA 30 mg/kg once-daily (QD) (for 10 week induction) were used along with HM15211 0.72 nmol/kg, once every 2 days (Q2D). During the treatment, MRI/MRS was utilized for real-time liver fat analysis. At the end of treatment, the animals were sacrificed for liver TG, TBARS (oxidative stress assay), hydroxyproline assay (surrogate for hepatic collagen fiber), marker gene expression analysis, and NAFLD activity score (NAS). Lastly, obese and NASH cynomolgus monkeys were treated with HM15211 (starting with 3 weeks titration period followed by 9 weeks single dose treatment) to further evaluate its therapeutic efficacy in non-human primates.
Results: HM15211 treated MCD-diet mice (6 weeks induction) showed significant reduction in hepatic TG (−82.6% *vs.* vehicle) and TBARS (−60.7% *vs.* vehicle), which coincided with significant reduction in blood liver functional markers such as ALT and bilirubin. Time course MRI/MRS imaging further confirmed the progressive steatosis resolution by HM15211 while liraglutide showed minimal effect. Furthermore, histopathological analysis indicated that HM15211 significantly reduced hepatic inflammatory gene expression and NAS (1.3 for HM15211, 3.4 for liraglutide, and 2.7 for vehicle). In MCD-diet mice with overt liver fibrosis (10 weeks induction), NASH improvement in HM15211 treated groups was confirmed with improved blood liver functional markers and related gene expression along with reduced hydroxyproline contents. Moreover, HM15211 treated groups showed complete reversal in NAS while selonsertib and OCA had marginal effect (0.0 for HM15211, 1.2 for selonsertib, 0.9 for OCA, and 2.1 for vehicle). Finally, HM15211 effectively treated obese and NASH cynomolgus monkeys as indicated by potent BWL, improved liver functional markers and histopathological analysis.
Conclusion: According to its efficacy in various NASH animal models, HM15211 may offer therapeutic potential in NASH and fibrosis as well as obesity.
Disclosure: **J. Kim:** Employment/Consultancy; Employee of Hanmi Pharm. Co., Ltd.

120

Patients with an impaired fructolysis are characterised by an increased intrahepatic triglyceride content

N. Simons[1,2], F.-G. Debray[3], N.C. Schaper[1,4], M.E. Kooi[2,5], E.J.M. Feskens[6], C.E.M. Hollak[7], L. Lindeboom[8,9], J.A.P. Bons[10], D.J. Lefeber[11,12], C.G. Schalkwijk[1,2], C.D.A. Stehouwer[1,2], D. Cassiman[13], M.C.G. Brouwers[1,2];
[1]Department of Internal Medicine, MUMC, Maastricht, Netherlands, [2]CARIM, Maastricht, Netherlands, [3]Department of Medical Genetics, Metabolic Unit, University Hospital Liège, Liège, Belgium, [4]CAPHRI, Maastricht, Netherlands, [5]Department of Radiology and Nuclear Medicine, MUMC, Maastricht, Netherlands, [6]Division of Human Nutrition, Wageningen University, Wageningen, Netherlands, [7]Department of Internal Medicine, AMC, Amsterdam, Netherlands, [8]Department of Radiology and Nuclear Medicine, Department of Human Biology/Human Movement Sciences, MUMC, Maastricht, Netherlands, [9]NUTRIM, Maastricht, Netherlands, [10]Central Diagnostic Laboratory, MUMC, Maastricht, Netherlands, [11]Translational Metabolic Laboratory, RadboudUMC, Nijmegen, Netherlands, [12]Department of Neurology, RadboudUMC, Nijmegen, Netherlands, [13]Department of Gastroenterology-Hepatology and Metabolic Center, University Hospital Leuven, Leuven, Belgium.

Background and aims: Dietary fructose has been suggested to play an important role in the pathogenesis of nonalcoholic fatty liver disease (NALFD). However, the exact underlying mechanism remains unclear. Fructose could contribute to NAFLD as either a substrate for *de novo* lipogenesis (direct pathway) or as a signal molecule that induces transcription factors such as carbohydrate-responsive element-binding protein (ChREBP) that stimulate *de novo* lipogenesis (indirect pathway). The study of inborn errors of fructose metabolism may aid to make a distinction between these two pathways. Since aldolase B is involved in the final step of fructolysis, patients with a deficiency in this enzyme serve as an excellent human model to study the contribution of the indirect pathway to the pathogenesis of fructose-induced NAFLD (independent of the direct pathway).
Materials and methods: In this case-control study, intrahepatic triglyceride (IHTG) content was measured using proton magnetic resonance spectroscopy (^{1}H-MRS) in 15 confirmed aldolase B deficient (aldoB$^{-/-}$) patients and 15 age-, sex-, and BMI-matched control subjects. All individuals filled out a three-day food diary to determine their daily fructose intake and underwent an oral glucose tolerance test (OGTT). Plasma transferrin glycosylation patterns, used as a surrogate for hepatic fructose-1-phosphate concentrations, were measured with high-resolution mass spectrometry.
Results: IHTG content was higher in aldoB$^{-/-}$ patients when compared to healthy controls (median IHTG content: 2.5% and 0.6% respectively, $p = 0.001$). Plasma glucose excursions during the OGTT were higher in aldoB$^{-/-}$ patients, resulting in a significantly different area under the curve ($p = 0.043$). The most fructose-intolerant patients -indicated by the lowest dietary fructose intake- had the highest IHTG content (Spearman's rho = −0.77, $p = 0.001$). Hypoglycosylated transferrin was more abundant in aldoB$^{-/-}$ patients when compared to controls ($p < 0.001$) and tended to be higher in aldoB$^{-/-}$ patients with relevant IHTG content (i.e. >3%, $n = 5$) compared to those without ($n = 10$) ($p = 0.09$).
Conclusion: This study demonstrates that the direct pathway is not necessary for the pathogenesis of fructose-induced NAFLD in humans. The increased IHTG content in aldoB$^{-/-}$ suggests that intermediates of fructolysis, i.e. fructose-1-phosphate, play a prominent role through indirect pathways.
Supported by: Netherlands Heart Foundation (grant 2015T042) and Stofwisselkracht
Disclosure: **N. Simons:** Grants; Netherlands Heart Foundation, Stofwisselkracht.

OP 21 Predicting complications

121

Reduce the risk for repeat percutaneous coronary intervention: the importance of HbA_{1c} control in prediabetes

H. Yu[1], Y. Zhang[2], X. Liu[3], J. Li[1];
[1]Tongji University School of Medicine, Shanghai, [2]Shanghai Tenth People's Hospital affiliated to Tongji University School of Medicine, Shanghai, [3]Shanghai Tongji Hospital affiliated to Tongji University School of Medicine, Shanghai, China.

Background and aims: According to the current guidelines, patients with percutaneous coronary intervention (PCI) were recommended to have strict lipid profile and plasma glucose control. However, many patients with well-controlled risk factors also experienced repeat PCI. The risk factors for repeat PCI were still unclear.
Materials and methods: Consecutive 5545 inpatients with CHD were enrolled from the Shanghai Eastern Hospital Affiliated to Tongji University from November 2011 to March 2015. Anthropologic measurements and medical records of these patients were collected.
Results: 5545 participants, including 3559 acute coronary syndrome (ACS) patients, received PCI procedure, of whom the incidence of repeat PCI was 10.80% in total population and 10.65% in ACS patients, respectively. In multivariate logistic regression, prediabetes (HbA1c 39–46 mmol/mol, 5.7–6.4%) and diabetes (HbA1c ≥48 mmol/mol, 6.5%) were significant and independent risk factors for repeat PCI with OR of 1.61 (1.21–2.13) and 2.97 (2.13–4.12), respectively. Similar results were observed in ACS patients, with OR of 1.87 (1.30–2.68) and 3.32 (2.16–5.10), respectively. Moreover, even in patients with LDL-C <1.80 mmol/L, poorly glycemic-control (HbA1c ≥39 mmol/mol, 5.7%) was also significant and independent risk factor for repeat PCI with OR of 2.16 (1.18–3.95).
Conclusion: Prediabetes and diabetes are crucial risk factors for the repeat PCI in post-PCI patients, even in those with well-controlled lipid profile.

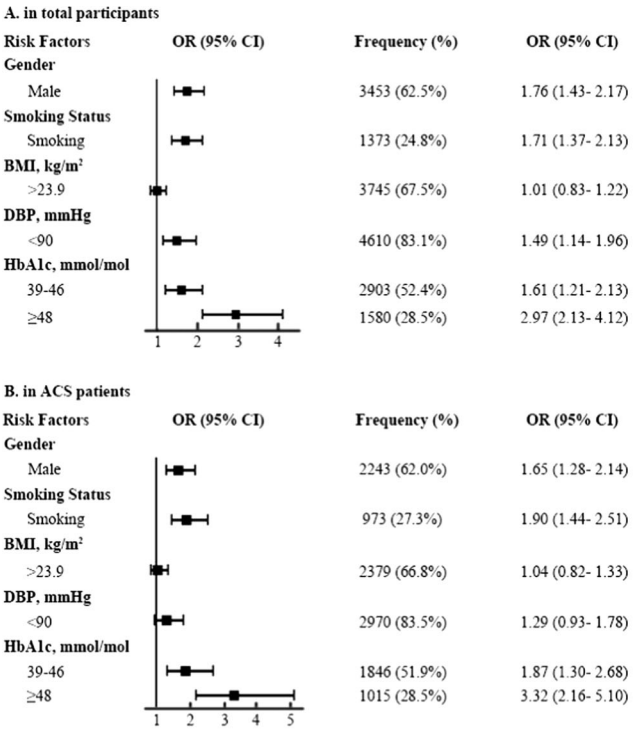


Figure 1. Association of risk factors with repeat PCI after adjustment for lesion number, left main disease and medication treatments [a]

Variables were graded according to current guidelines and their clinical significance respectively, see details in Methods.
ACS, acute coronary syndrome; DBP, diastolic blood pressure; PCI, percutaneous coronary intervention.
[a] including statin, dual anti-platelet, antihypertensive and antidiabetic therapy.

Supported by: National Nature Science Foundation of China, Shanghai municipal government
Disclosure: **H. Yu:** None.

122

The updated mean HbA_{1c} level is more strongly related to mortality compared to a single HbA_{1c} measurement: The Hoorn Diabetes Care System Cohort study

G. Nijpels[1], P. Elders[1], J. Beulens[2], F. Rutters[2], A. van der Heijden[1];
[1]General Practice and Elderly Care, VU University Medical Center, Amsterdam, [2]Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, Netherlands.

Background and aims: When the association between glycemic control and mortality risk for people with type 2 diabetes (T2D) is studied, most often the last glycemic measurement available is used. However, T2D is a progressive disease and treatment can lower HbA1c. Furthermore, previously intensive glycemic control was associated with increased mortality rates. To date, the relative importance of average HbA1c levels over time, as proxy for longer term glycemic control in T2D in the association with mortality remains uncertain. Therefore, we compared last measured HbA1c and the updated mean of HbA1c and their association with relative mortality risk in people with T2D
Materials and methods: A prospective observational study of 14.420 people with T2D included in the Hoorn Diabetes Care System (DCS) cohort was performed. The DCS includes all people with T2D in the region West-Friesland in the Netherlands. From 1997 till 2017, data were collected every year on among others, blood pressure, HbA1c, lipids, smoking status. All-cause mortality was extracted from national registries. 4,960 people with T2D included in the DCS within two years after the diagnosis of T2D with at least five HbA1c follow-up measurements were included. The last measured HbA1c and the updated mean of the

HbA1c level ($T = 1$ to $T = 5$) were associated to all-cause mortality with two Cox regression models, adjusted for sex, age, smoking status, diabetes duration, total cholesterol and systolic blood pressure. Because a diabetes diagnosis at a younger age may result in a higher complication risk, effect modification by age of diabetes onset (<55 years, 55–65 years, and ≥65 years) was tested by adding cross product terms with HbA1c to the model. Hazard ratios (HR's and 95% confidence intervals (CIs) are reported.

Results: During a mean follow-up period of 6.7 years (range: 0–16 years), 770 people (15.5%) died. Statistically significant effect modification was found between age of onset of diabetes and both measures of HbA1c ($p < 0.1$). Consequently, all results were stratified for age of diabetes onset. The fully adjusted models are shown in the table, with updated mean of HbA1c showed stronger associations with mortality in all categories of age of onset of diabetes, compared to assessing only the last HbA1c measurement. For both measures of HbA1c, the association with mortality (per 1% increase) was stronger in in people with an age of onset <55 years (HR: 1.50 (95% CI: 1.24 to 1.81), compared to onset of diabetes ≥65 years (HR: 1.14 (95%CI: 1.00 to 1.29)) (Table).

Conclusion: In this prospective cohort of people with T2D, the updated mean of HbA1c is more strongly associated with all-cause mortality, compared to last measured HbA1c, even more pronounced in people with a diagnosis at early age. The predictive role of the course of HbA1c levels for mortality should be further investigated.

Table. Hazards ratio for mortality risk by HbA1c (%) according to category of age of onset of diabetes.

Single measurement of HbA1c	< 55 years	55-65 years	≥ 65 years
Model 1	1.37 (1.17 to 1.61)	1.11 (0.97 to 1.28)	1.08 (0.96 to 1.21)
Model 2	1.38 (1.17 to 1.63)	1.11 (0.97 to 1.27)	1.08 (0.97 to 1.21)
Updated mean of HbA1c			
Model 1	1.50 (1.25 to 1.81)	1.21 (1.03 to 1.42)	1.13 (0.99 to 1.28)
Model 2	1.50 (1.24 to 1.81)	1.20 (1.03 to 1.40)	1.14 (1.00 to 1.29)

Model 1: adjusted for age and sex
Model 2: further adjusted for smoking status, diabetes duration, total cholesterol, and systolic blood pressure.

Supported by: DFN
Disclosure: **G. Nijpels:** None.

123

Trends in prevalence, incidence and mortality of type 1 and type 2 diabetes in Denmark 1996–2016

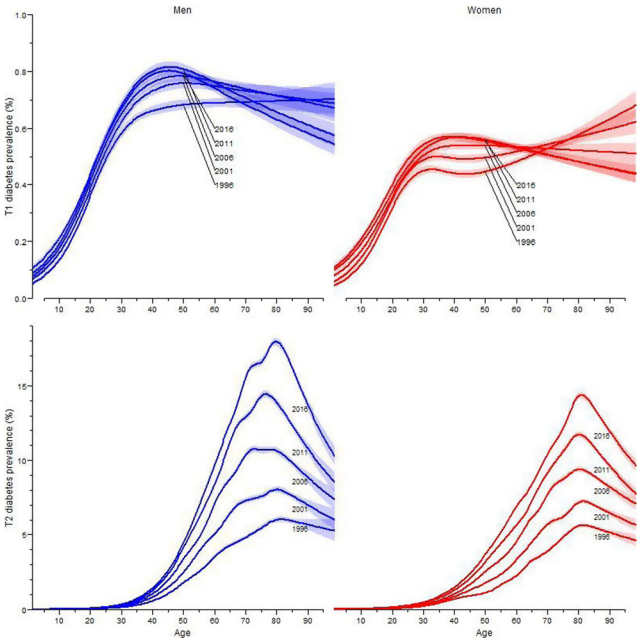
B. Carstensen, P.F. Rønn, M.E. Jørgensen;
Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: Registers provide a unique opportunity to assess diabetes demography; however, many diabetes registers cannot distinguish between type 1 and type 2 diabetes. Thus, the current national prevalence and incidence rates of diabetes are either done for the total diabetes population or limited by imprecise classification of type 1 and type 2 diabetes. We aimed to provide more precise estimates of the prevalence, incidence and mortality separately for type 1 and type 2 diabetes during a 20-year period in Denmark.

Materials and methods: We constructed a Danish national diabetes register based on existing health care registers in Denmark. We included persons as diabetes patients based on first diabetes diagnosis in the National Patient Register or Danish Adult Diabetes Database (DADD); purchase of any anti-diabetic medication (Prescription Register); use of podiatry or eye examination for diabetic complications (Health Services Registers). Classification of type was based on the clinical reports in the DADD. Analyses were conducted separately for diabetes type and sex. For dates 1 January 1996 to 2016 we modeled prevalence by age using a binomial model with log-link. Incidence and mortality rates were analysed using Poisson regression. Incidence rates were modeled by current age, calendar time and birth cohort using natural splines. Mortality rates were additionally modeled by diabetes duration and age at diagnosis, also using natural splines.

Results: At the start of 1996, a total of 23,652 persons were alive with type 1 diabetes and 69,975 with type 2 diabetes. In 2016 the numbers were 30,244 and 245,879 respectively. The overall prevalence of type 1 diabetes increased from 0.42% to 0.52% (0.6% per year) and from 1.3% to 4.3% (5.1% per year) for type 2 diabetes. The fraction of type 1 diabetes of all diabetes patients was 50% at 40 years of age and about 10% for ages 60+. The age-specific prevalences from 1996–2016 are shown in figure 1 for men and women. We observed an average annual decrease in incidence of type 1 diabetes of 0.7% per year, and increase in incidence rates of type 2 diabetes of 3.5% per year before 2012 and a decrease of 10% per year from 2012 to 2016. Mortality rates showed a slight decrease of 1.5% per year for type 1 patients and 3.5% per year for type 2 patients. The overall mortality rate ratio between type 1 and type 2 patients was 1.29 (controlling for age and calendar time).

Conclusion: In the period 2012–2016 we saw a decrease in incidence of type 2 diabetes which was not seen for type 1, which makes the decrease unlikely to be a registration artifact. A reduction in mortality was observed for both type 1 and 2 but most for type 2, probably due to improved treatment and/or earlier diagnosis. The mortality RR between type1 and type 2 may to some extent be explained by differing duration of disease, however it is not meaningful to control for disease duration in the comparison because the overlap of age and duration between type 1 and type 2 diabetes is limited.



Disclosure: **B. Carstensen:** None.

124

Cardiovascular disease risk in OGTT-diagnosed diabetes patients with and without confirmation by HbA_{1c} values: the Whitehall II study

A.G. Tabák[1,2], E.J. Brunner[2], M.J. Shipley[2], M. Kivimaki[2,3];
[1]Semmelweis University, Budapest, Hungary, [2]University College London, London, UK, [3]University of Helsinki, Helsinki, Finland.

Background and aims: Several diabetes associations currently recommend non-fasting test (HbA1c- A1C) for diagnosing diabetes leading to declining use of fasting glucose and oral glucose tolerance test (OGTT) in clinical practice. It is well known that the overlap between people

diagnosed by an OGTT or an A1C is limited. We investigated (1) the natural history of OGTT-based diabetes in terms of confirmation by A1C and (2) the risk of cardiovascular disease (CVD) in people with A1C-confirmed and unconfirmed OGTT-diagnosed diabetes compared to the diabetes-free background population.

Materials and methods: During clinical phases 7 (2002–2004) and 9 (2007–2009) of the Whitehall II cohort study, both A1C and OGTT tests were performed ($N = 9149$). We determined how many of the 384 incident diabetes cases diagnosed only by OGTT were confirmed by AIC-based diagnosis during the next 14 years and examined which factors predicted confirmation. We followed participants for CVD up to 2015 to investigate differences in CVD risk between participants with health-care diagnosed diabetes at phase 7 ($n = 632$), incident diabetes cases at phases 7 and 9, and those without diabetes throughout the study ($n = 8133$).

Results: During a mean follow-up of 12 years, 223 (58.1%) of the 384 OGTT cases were confirmed by HbA1c. The OGTT-diagnosis was more frequently confirmed in people with higher BMI ($HR_{per\ 1kg/m2} = 1.07$, 95%CI 1.05–1.10), higher fasting glucose ($HR_{per\ 1mmol/l} = 1.20$, 95%CI 1.12–1.29), and if both the fasting and 2-hour glucose were diagnostic (HR = 1.99, 95%CI 1.27–3.10). After adjustment for age, sex, ethnicity and social status, participants with health-care diagnosed diabetes and A1C-confirmed OGTT-based diabetes had an increased CVD risk (HR = 1.87, 95%CI 1.50–2.30 and HR = 1.63, 95%CI 1.11–2.41, respectively), while OGTT-cases not confirmed by AIC had risk (HR = 1.13, 95%CI 0.75–1.69) similar to the diabetes-free population. Further adjustment for classical CVD-risk factors similarly attenuated the excess risk associated with health-care diagnosed diabetes and A1C-confirmed diabetes although the latter no longer reached statistical significance at conventional levels.

Conclusion: OGTT and A1C methods identify different populations with diabetes and only a subsample of OGTT cases will be confirmed by A1C during an extended follow-up. The OGTT-cases not confirmed by A1C have a CVD risk similar to diabetes-free population, while A1C-confirmed OGTT cases have an increased CVD risk. These findings suggest that losing OGTT cases that cannot be confirmed by A1C is not harmful in term of CVD prevention.

Supported by: US NIA, BHF, UK MRC

Disclosure: **A.G. Tabák:** None.

125

Multiplex proteomics for prediction of major cardiovascular events in type 2 diabetes

C. Nowak[1], A.C. Carlsson[1], C.J. Östgren[2], F.H. Nyström[2], M. Alam[3], T.R. Feldreich[3], J. Sundström[4], J.J. Carrero Roig[5], J. Leppert[4], P.O. Hedberg[4], A.C. Cordeiro[6], L. Lind[4], E. Ingelsson[7], T. Fall[4], J. Ärnlöv[1,3]; [1]Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Huddinge, Sweden, [2]Linköping University, Linköping, Sweden, [3]Dalarna University, Falun, Sweden, [4]Uppsala University, Uppsala, Sweden, [5]Karolinska Institutet, Solna, Sweden, [6]Dante Pazzanese Institute of Cardiology, Sao Paolo, Brazil, [7]Stanford University School of Medicine, Stanford, USA.

Background and aims: Multiplex proteomics could improve understanding and risk prediction of major adverse cardiovascular events (MACE) in type 2 diabetes. This study assessed 80 cardiovascular and inflammatory proteins for biomarker discovery and prediction of MACE in type 2 diabetes.

Materials and methods: We combined six prospective studies of 30-to-77 year-olds with type 2 diabetes in whom 80 circulating proteins were measured by proximity extension assay. Multivariable-adjusted Cox regression was used in a discovery/replication design to identify biomarkers for incident MACE. We used gradient boosted machine learning in a random 75% training subsample to assess whether adding proteins to an established risk model based on the Swedish National Diabetes Register improved prediction of MACE in the separate 25% validation subsample.

Results: In 1,211 adults with type 2 diabetes (32% women), 211 experienced a MACE over 6.4 ± 2.3 years. We replicated associations (<5% false discover rate) between eight proteins and risk of MACE: matrix metalloproteinase-12, interleukin-27 subunit alpha, T-cell immunoglobulin/mucin domain-1, fibroblast growth factor-23, protein S100-A12, tumor necrosis factor receptor (TNFR)-1, TNFR-2 and TNF-related apoptosis-inducing ligand receptor-2. Addition of the 80-protein assay to the established risk model improved discrimination in the separate validation sample from 68.6% (95%-CI, 68.2%–68.9%) to 74.8% (95%-CI, 74.6%–75.1%).

Conclusion: We identified eight protein biomarkers, four of which are novel, for risk of MACE in community residents with type 2 diabetes, and found improved risk prediction by combining multiplex proteomics with an established risk model. Multi-protein arrays may improve the selection of persons with diabetes for more aggressive cardiovascular prevention.

Supported by: Vetenskapsrådet, EU Horizon 2020, HLF, Landstinget Dalarna

Disclosure: **C. Nowak:** None.

126

Reduced eGFR and/or increased urinary albumin excretion rate are powerful determinants of survival among insulin treated patients with type 2 diabetes in routine practice

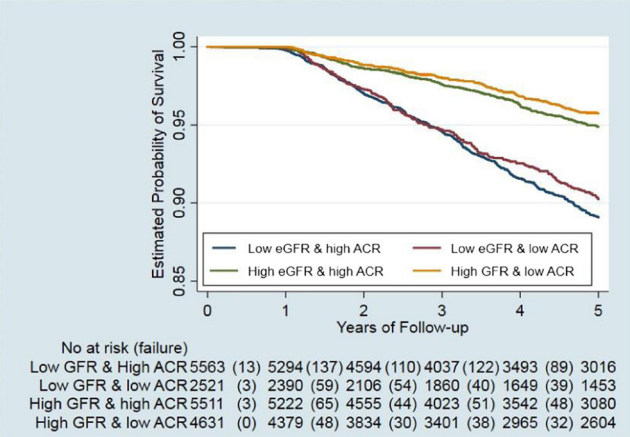
U. Anyanwagu, R. Donnelly, I. Idris;
Royal Derby Hospital Centre, University of Nottingham, Derby, UK.

Background and aims: Low estimated glomerular filtration rate (eGFR) and increased urinary albumin-to-creatinine ratio (ACR) are well recognised diagnostic and prognostic markers of chronic kidney disease (CKD) and cardiovascular (CV) risk, but their precise relationship with CV disease and total mortality among insulin-treated Type 2 Diabetes (T2D) patients in routine clinical care is unclear.

Materials and methods: We analysed data for insulin users with T2D from UK general practices between 2007 and 2014 and examined the association between mortality rates and CKD [categorised by low eGFR (<60 mL/min/1.73 m^2); high eGFR (≥60 mL/min/1.73 m^2); low ACR (<300 mg/g); and high ACR (≥300 mg/g) at insulin initiation] after a 5-year follow-up period using Cox proportional hazard models.

Results: A total of 18,227 patients with T2DM on insulin therapy were identified (mean age: 61.5 ± 13.8 yrs, mean HbA1c: 8.6 ± 1.8%; 53% male). After adjusting for confounders, mortality curves for each CKD category are shown below (log-rank p value <0.001). When compared to adults on insulin therapy with an eGFR <60 and an ACR ≥300 (low eGFR + high ACR) after a follow up period of 5 years, patients with an eGFR <60 and an ACR <300 (low eGFR + low ACR) had a 6% lower mortality rate (aHR: 0.94; 95%CI: 0.79 to 1.12); those with an eGFR >60 and an ACR ≥300 (high eGFR + high ACR) had a 20% lower mortality rate (aHR: 0.80; 95%CI: 0.68 to 0.96); and those with an eGFR >60 and an ACR <300 (high eGFR + low ACR) had the lowest death rate (28% less; aHR: 0.72; 95%CI: 0.59 to 0.87) after adjusting for confounders.

Conclusion: This study shows that among a big cohort of insulin-treated T2DM patients in routine practice, the combination of reduced eGFR with increased ACR was associated with the greatest risk of premature death, followed closely by those with reduced eGFR and normal ACR levels. Adoption of aggressive CV risk management strategies to reduce mortality in patients with a low eGFR and albuminuria is essential in these high risk patients with T2D.



Disclosure: **U. Anyanwagu:** None.

OP 22 Sweetening the endothelium

127

High glucose exposure reduces DNA demethylation of cAMP response element (CRE) region in eNOS promoter during pro-angiogenic CD34$^+$ stem cell differentiation

M. Vinci, V. Vigorelli, J. Resta, S. Genovese, G. Pompilio;
Centro Cardiologico Monzino - IRCCS, Milan, Italy.

Background and aims: Diabetic patients have reduced number and activity of circulating CD34$^+$ stem cells. This clinical observation has been associated with serious endothelial dysfunction and elevated risk of adverse cardiovascular events, strengthening the concept that the functional impairment of CD34$^+$ stem cells allows further progression of vascular disease. The expression of endothelial nitric oxide synthase (eNOS) in CD34$^+$-derived endothelial progenitor cells (EPCs) is a marker of proangiogenic differentiation and its product, the nitric oxide (NO), plays a key role in the mechanisms of bone marrow mobilization, migration, and homing. NO bioavailability along with proangiogenic properties of CD34$^+$-derived EPCs are impaired in diabetic patients. We aimed at investigating whether reduced proangiogenic capacities of CD34$^+$-derived EPCs and altered NO bioavailability induced by hyperglycemia were associated with a defective epigenetic eNOS promoter activation and gene expression.

Materials and methods: CD34$^+$ cells were purified from cord blood of healthy donors and expanded in normal-glucose (NG; with 30 mM mannitol for osmotic control) or high-glucose (HG; 30 mM) serum-free medium plus cytokines (FLT3, SCF, IL3 and IL6) for up to 20 days. NG and HG-CD34$^+$ cells were then cultured in proangiogenic EGM2 medium. After 15 days NG and HG-CD34$^+$-derived EPC phenotype was evaluated by DiLDL-UEA-1 double-positive cell count. Their proangiogenic properties were assessed by the ability to form colony forming units-endothelial cell (CFU-EC) and by incorporation into endothelial tube-like structure. NO production and eNOS mRNA expression were detected by flow cytometry with DAF-2DA and qPCR, respectively. The DNA methylation of eNOS promoter was assessed by two-step qPCR method followed by bisulfite cloning Sanger sequencing. Data were then analyzed by QUMA software (http://quma.cdb.riken.jp/).

Results: After 15 days of culture in proangiogenic medium NG and HG-CD34$^+$-derived EPCs were evaluated for their proangiogenic phenotype. HG-CD34$^+$-derived EPCs displayed a significantly lower number of double-positive stained cells (DiLDL-UEA-1) than their NG counterpart ($n = 3$; 0.59 ± 0.03 vs 0.44 ± 0.03; $p \leq 0.05$). The reduction of EPC differentiation marker associated with an impaired ability of HG-CD34$^+$-derived EPCs to form CFU-EC ($n = 4$; 6.74 ± 1.0 vs 3.38 ± 1.2; $p \leq 0.01$) and to incorporate into endothelial tube-like structure ($n = 9$; 92.7 ± 11.8 vs 27.7 ± 7.8; $p \leq 0.01$). In line with the impairment of proangiogenic functionality, HG cells also showed reduced eNOS mRNA expression ($n = 3$; FC 1 ± 0.2 vs 0.5 ± 0.1; $p \leq 0.05$) and NO production ($n = 3$; $69\% \pm 10$ vs $28\% \pm 5$ $p \leq 0.05$). We further evaluated DNA demethylation events occurring on eNOS promoter of CD34$^+$stem cells during proangiogenic differentiation. Interestingly, we found that eNOS mRNA transcription in CD34$^+$-derived EPCs occurred after CRE region DNA demethylation of eNOS promoter. Consistent with gene expression data, eNOS promoter of HG cells displayed higher DNA CRE region methylation as assessed by two-step qPCR method ($n = 5$; FC 1 ± 0.3 *vs* 4.9 ± 1.6; $p \leq 0.05$) and bisulfite sequencing.

Conclusion: CRE region DNA demethylation of eNOS promoter is involved in eNOS mRNA expression and fosters proangiogenic differentiation of CD34$^+$ stem cells. This activating epigenetic modification is impaired by high glucose concentration.

Supported by: Ricerca Finalizzata, Ministero della Salute [PE-2011-02348537]

Disclosure: **M. Vinci:** None.

128

Apabetalone (RVX-208) an epigenetic modifier lowers risk of MACE in diabetes patients with CVD by affecting monocyte adhesion to endothelial cells

N.C.W. Wong[1], L. Tsujikawa[1], E. Kulikowski[1], C. Calosing[1], S. Wasiak[1], D. Gilham[1], C. Halliday[1], J.O. Johansson[2], M. Sweeney[2];
[1]Resverlogix Corp., Calgary, AB, Canada, [2]Resverlogix Inc., San Francisco, USA.

Background and aims: Apabetalone (RVX-208, 200 mg/d) when given orally to patients with type 2 diabetes mellitus (T2DM) and CVD for 6 months leads to a 57% relative risk reduction in major adverse cardiovascular events (MACE). RVX-208 is a selective inhibitor (BETi) of bromodomain extra-terminal (BET) proteins that are epigenetic readers of acetylated lysines in the tail of histones. The benefits on CVD in T2DM patients is explored here by examining effects of RVX-208 to disrupt a key pathologic step of monocyte adhesion to endothelial cells and the genes underlying this process in CVD. Cellular adhesion and genes believed to be part of this process are examined in response to T2DM defects, high glucose (HG, 25.6 mM) and increased dietary metabolite trimethyl-amine oxide (TMAO).
Materials and methods: Cultured THP-1 monocytes, HUVEC endothelial cells and primary human hepatocytes (PHH) exposed to HG or varying levels TMAO (10–100 uM).
Results: In HG or TMAO, adhesion of THP-1 to HUVEC cells was enhanced almost 2.4-fold but 20 uM RVX-208 blocked this process by 30–70%. In addition, HG induced Very Late Antigen-4 (VLA-4) mRNA, a gene mediating THP-1 adhesion by 1.3-fold and RVX-208 suppressed it >50%. Similarly, BETi blocked TMAO induction of VLA-4 mRNA by >50% in THP-1. In HUVECs RVX-208 abrogated HG induction of E-selectin and MYD88 mRNA by 2- and 1.3-fold, respectively and lowered TMAO induction of these mRNAs by >50%. Microbiome processing of dietary phospholipids followed by hepatic flavin mono-oxygenase-3 (FMO3) metabolism yields TMAO. In PHH exposed for 24 hrs to RVX-208, FMO3 mRNA was lowered by 40% but it also suppressed an important regulator of FMO3 gene transcription, farnesoid X receptor (FXR). BETi suppressed both FXR mRNA and protein within 6 hrs by >80% suggesting a direct effect of BETi on the FXR gene. Additionally, ChiP data showed that BRD4, a BET protein, dissociated rapidly from FXR DNA upon exposure to RVX-208. Since BRD4 guides a complex containing RNA pol II along actively transcribed genes containing highly acetylated histones, dissociation of BRD4 from FXR DNA halts transcription of the gene.
Conclusion: Apabetalone inhibits HG and TMAO enhanced adhesion of THP-1 to HUVECs, a process that is believed to trigger the vascular inflammation component of CVD. RVX-208 suppresses genes that underlie cellular adhesion; VLA-4 in THP-1 and both E-selectin plus MYD88 in HUVECs. BETi blocks not only activity of TMAO but also its production by inhibiting FXR expression, a regulator of FMO3 gene transcription. The rapid actions of BETi in dissociating BRD4 along with pol II from FXR DNA suggests a direct effect of RVX-208 on FXR gene transcription. Studies here examine potential roles of monocytes, endothelial cells, hepatocytes and microbiome in CVD that contribute to enhanced CVD risks in T2DM. Apabetalone a BETi affects activity of these cells by altering function in such a way as to limit their contribution to CVD. This action of BETi across cell types suggests that RVX-208 affects many pathways underlying CVD and provides potential explanation for why apabetalone in targeting BRD4, leads to many benefits for lowering CVD risks in T2DM patients.
Disclosure: **N.C.W. Wong:** Employment/Consultancy; Resverlogix Corp. Stock/Shareholding; Resverlogix Corp.

129

Liraglutide improves vascular dysfunction via regulating cAMP-independent PKA-SIRT1/AMPK-PGC1α pathway in perivascular adipose tissue in obese mice

X. Sun, F. Han, N. Hou;
Department of Endocrinology, Affiliated Hospital of Weifang Medical University, Weifang, China.

Background and aims: Perivascular adipose tissue (PVAT), with characteristics of both white and brown adipose tissues (BAT), loses its anti-contractile effect in endothelial-dependent pathway and exacerbates endothelial dysfunction in obese subjects. Glucagon-like peptide-1 receptor (GLP-1R) agonist possessed cardiovascular protective effects including restoring endothelial dysfunction in obesity. However, these studies were performed with the conditions of PVAT removed. Therefore, the aim of this study was to determine whether liraglutide, GLP-1R agonist, could improve vascular dysfunction (both anti-contractile effect of PVAT and endothelial function) via regulating PVAT-related signaling pathways in obesity.
Materials and methods: C57BL/6 mice were fed with a normal-chow diet or a high-fat diet (HFD) with or without liraglutide treatment. Vascular contraction and endothelial-dependent vasodilation (EDV) of the thoracic aorta with or without PVAT (PVAT+ or PVAT−) were determined by the response to norepinephrine or acetylcholine, respectively. Protein levels of PKA-SIRT1/AMPK-PGC1α signaling pathways in PVAT were determined by western blot. BAT-related gene, pro-inflammatory and antioxidant genes expression in PVAT were analyzed by PCR.
Results: Treatment of HFD mice with liraglutide improved metabolic profiles, glucose tolerance and insulin sensitivity (body weight, 29.2 ± 0.6 g vs. 38.5 ± 0.4 g; glucose, 102.22 ± 3.66 mg/dl vs. 114.54 ± 3.64 mg/dl; NEFA 0.86 ± 0.16 mmol/L vs. 1.21 ± 0.25 mmol/L; AUC glucose 21899 ± 483 vs. 29121 ± 828; AUC insulin 9404 ± 130 vs. 11587 ± 413; $P < 0.05$). PVAT from control mice had a significant anti-contractile effect on the aortic rings, which were attenuated in HFD mice (maximal attenuation rate: 3.56% vs. 15.01%; $P < 0.05$). However, it was improved when treatment of HFD mice with liraglutide or pre-incubation of PVAT with liraglutide *ex vivo*, with the maximal attenuation rates increasing from 3.56% to 12.40% and from 4.23% to 14.48%, respectively ($P < 0.05$). For EDV, there was a significant impairment in EDV in aortic rings (both PVAT+ and PVAT−) from HFD mice and this impairment was significantly exacerbated by PVAT (34.8% vs. 47.4%, $P < 0.05$). However, liraglutide treatment alleviated the impairment with no significant difference in maximal vasodilation between PVAT+ ring and PVAT− ring (68.9% vs. 70.9%, $P < 0.05$). Pre-incubation of aortic ring (PVAT+) from HFD mice with liraglutide *ex vivo* also restored this impairment of EDV. However, Blockade or knockdown PKA, SIRT1 or AMPK but not cAMP, attenuated or abolished these beneficial effects of liraglutide on the anti-contractile capability and endothelial function. Liraglutide treatment induced browning-related genes expression (Cidea and UCP-1), activated AMPK-eNOS signaling pathway. Additionally, we observed liraglutide effectively enhanced heme oxygenase-1 gene expression and reduced TNF-α expression, indicative of antioxidant and anti-inflammatory abilities. These beneficial effects were due to activation of PKA-SIRT1/AMPK-PGC1α signaling pathway by cAMP-independent way, as demonstrated by western blot and PCR.
Conclusion: Our study indicates that liraglutide improves vascular dysfunction via regulating cAMP-independent PKA-SIRT1/AMPK-PGC1α pathway in PVAT from obese mice. These findings provide a novel mechanism for cardiovascular protection of liraglutide via regulating PVAT function in obesity.
Supported by: National Natural Science Foundation of China (81600663,81400829)
Disclosure: **X. Sun:** None.

130

Methylglyoxal driven endothelial dysfunction in hyperglycaemia targets protein folding, protein synthesis, glycolysis and gluconeogenesis pathways

N. Rabbani[1], A. Ashour[2], Z. Irshad[1], J. Larkin[3], M. Xue[1], P.J. Thornalley[1];
[1]University of Warwick, Coventry, UK, [2]Taif University, Taif, Saudi Arabia, [3]University of Oxford, Oxford, UK.

Background and aims: Endothelial cell (EC) dysfunction in diabetes is linked to development of diabetic vascular complications. It is characterized by increased inflammatory signalling, expression of adhesion molecules and secretion of inflammatory cytokines**,** apoptosis and processes supporting atherosclerosis. ECs accumulate methylglyoxal (MG) and MG-derived advanced glycation endproduct (AGE)-modified proteins in hyperglycemia - suppression of which prevents EC dysfunction. Our aim is to identify proteins modified by MG that may be mediators of EC dysfunction in hyperglycemia.
Materials and methods: Human aortal endothelial cells (HAECs) were incubated in primary culture with 5 mM (model normoglycemia) or 20 mM glucose (model hyperglycemia) for 6 days and cytosolic extracts prepared. A cytosolic extract of human endothelial HMEC-1 cells was incubated with exogenous MG to increase the modification by MG-derived AGE, MG-H1, by 10-fold - typical of the upper limit of increased MG in diabetes. Cytosolic protein extracts were reduced, alkylated and digested with trypsin and lys-C and tryptic peptides analysed by nanoflow liquid chromatography-Orbitrap Fusion™ (Thermo) high resolution mass spectrometry. Proteins were identified by detection of at least 2 unique peptides using Progenesis QI 2.0 software (Nonlinear Dynamics, Newcastle, UK). REACTOME, INTERPRO and receptor binding domain (RBD) analysis was used for pathway, protein domain enrichment and functional domain analysis, respectively.
Results: In high glucose concentration cultures of HAECs, only two proteins were detected with MG modification: rho GDP-dissociation inhibitor 2 (RhoGDI2) and far upstream element-binding protein 2 (FUBP2); others were < limit of detection. In MG-modified HMEC1 cytosolic extract, 1262 proteins were detected of which 220 (17%) had MG modification. MG-H1 modification was on 411 sites with highest modification found on pyruvate kinase-M, β-actin, α-enolase and heat shock protein 90-beta. Pathways analysis showed that MG-modified proteins were enriched in: protein folding, protein synthesis, glycolysis and gluconeogenesis. Enriched protein domain targets of MG modification were: TCP-1 and GroEL chaperonins, phosphoserine and phosphothreonine binding sites of 14-3-3 proteins, proteasome alpha/beta subunits and conserved sites of aminoacyl-tRNA synthases. All have conserved functional arginine residues. To assess if MG modification was likely associated with functional impairment, we deduced the proportion of the MG modifications found in sites of protein functional interaction by RBD analysis. There was a relatively high proportion of MG modification in functional sites, 36%, with at least one modification on 47% of proteins.
Conclusion: MG modification of FUBP2 - checkpoint for inflammatory cytokines - may increase inflammatory signaling, and modification of RhoGDI2 may activate Rac2 and endothelial NADPH oxidase, driving oxidative stress in ECs in hyperglycemia. MG glycation is damaging because its produces loss of arginine residue charge and arginine targets are enriched in protein functional domains. MG modification may mediate impaired chaperone activity, serine/threonine kinase signalling, proteasomal proteolysis and protein synthesis associated with vascular complications of diabetes.
Disclosure: **N. Rabbani:** None.

131

Increased hexokinase-2-catalysed entry of glucose into glycolysis: key driver of metabolic dysfunction in endothelial cells in hyperglycaemia

P. Thornalley[1], Z. Irshad[1], M. Xue[1], A. Ashour[2], N. Rabbani[1];
[1]University of Warwick, Coventry, UK, [2]Taif University, Taif, Saudi Arabia.

Background and aims: Metabolic dysfunction of endothelial cells in hyperglycemia contributes to the development of vascular complications of diabetes. Multiple pathways of metabolic dysfunction are involved, including mitochondrial dysfunction, hexosamine, protein kinase C and advanced glycation endproduct pathways. This requires increased entry of glucose into glycolysis catalysed by hexokinase-1 and -2 (HK-1 and HK-2). These enzymes are saturated with glucose substrate even under normoglycemic conditions. We investigated the mechanism of increased entry of glucose into glycolysis in endothelial cells in model hyperglycemia *in vitro*.
Materials and methods: Human aortal endothelial cells (HAECs) were incubated in primary culture with 5 mM glucose (model normoglycemia) or 20 mM glucose (model hyperglycemia) for 6 days. Glucose consumption and net flux of formation of L-lactate were measured by enzymatic assays. Fructosyl-lysine (FL) content of cell protein was determined by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. HK-1 and HK-2 mRNA was quantified by RT-PCR and HK-1 and HK-2 protein by label-free quantitative proteomics in cytosolic protein extracts by nanoflow liquid chromatography-Orbitrap Fusion™ mass spectrometry (Thermo), corroborated by Western blotting.
Results: In high glucose cultures, FL content of cellular protein was increased, compared to low glucose control (5.71 ± 1.05 versus 2.92 ± 1.60 mmol/mol lys, $n = 3$; +96%, $P < 0.01$), indicating persistent cytosolic hyperglycemia linked to GLUT1-dependent glucose uptake. The flux of glucose consumption in high glucose concentration was increased, compared to low glucose concentration control (2079 ± 246 versus 1175 ± 87 nmol/10^6 cells/day, $n = 8$; +77%, $P < 0.01$). The net flux of formation of L-lactate was also increased. Proteomics analysis showed the abundance of HK-1 was unchanged in high glucose concentration cultures whereas HK-2 was increased 40% ($P < 0.05$). This was confirmed by Western blotting. There was no increase of HK-1 and HK-2 mRNA, suggesting that HK-2 protein is selectively stabilised from proteolysis in high glucose concentration. HK-2 is degraded by chaperone-mediated autophagy where heat shock cognate 71 kDa protein binds to motif $_{712}$QRFEK$_{716}$. This motif is directly involved in the binding of glucose at the active site in the C-terminal domain of HK-2 but not in HK-1. In the presence of cytosolic hyperglycemia, increased binding of glucose masks the degradation motif and HK-2 protein is stabilised to proteolysis. The turnover number of HK-2 is ca. 5-times that of HK-1 and hence glucose metabolism is highly sensitive to increased HK-2 protein. Increased cellular glucose-6-phosphate in high glucose concentration cultures displaces HK-2 from mitochondria, driving mitochondrial dysfunction in hyperglycemia. Decrease of HK-2 to normal levels by treatment with *trans*-resveratrol-hesperetin combination corrected increased HK-2, glucose consumption and metabolic dysfunction.
Conclusion: Increased HK-2-catalysed entry of glucose into glycolysis is a key driver of metabolic dysfunction in endothelial cells in hyperglycemia. Combination of GLUT1-dependent glucose uptake and HK-2 expression are likely key criteria for damaging effects which may explain the sensitivity of the vasculature, kidney, retina and peripheral nerve to damage in hyperglycemia.
Disclosure: **P. Thornalley:** None.

132

Comparative study in various model organisms regarding the effect of the loss of glyoxalase 1

B. Fuchs[1], J. Morgenstern[1], J. Tyedmers[1], E. Lodd[2], J. Kroll[2], D. Schuhmacher[3], M. Freichel[3], P. Nawroth[1,4], T. Fleming[1,4];
[1]Department of Internal Medicine I and Clinical Chemistry, University Hospital Heidelberg, Heidelberg, [2]Medical Faculty Mannheim, Center for Biomedicine and Medical Technology Mannheim, Mannheim,

[3]Institute of Pharmacology, University Heidelberg, Heidelberg, [4]German Center for Diabetes Research (DZD), Neuherberg, Germany.

Background and aims: The generation of methylglyoxal-derived advanced glycation end products has an important role in the development of diabetes and late diabetic complications. Detoxification by glyoxalase 1 (GLO1) might therefore play an important role in the context of dicarbonyl-induced damage in patients suffering from diabetes. This assumption was based on findings in less developed organisms. Recent findings in murine cell lines indicate that GLO1 is less significant in higher organisms. It was shown that loss of GLO1 is compensated by increased Aldo-Keto-Reductase and Aldehyde Dehydrogenase activity and improves the resistance against various toxins and radiation. Therefore, the major aim of this study was to address potential differences in complex organisms regarding their dependency on GLO1.
Materials and methods: The total knock-out of GLO1 ($GLO1^{-/-}$) was achieved in several murine cell lines and in a mouse model using the CRISPR-Cas9 technique. $GLO1^{-/-}$ of yeast, C. elegans and zebrafish were provided by collaboration partners. To determine the effect of $GLO1^{-/-}$, the survival rate after toxin exposure was determined by counting the survival/living organisms. MG-H1 expression was quantified via Western blotting. Intracellular levels of MG and MG-H1 were measured by LC-MS/MS.
Results: $GLO1^{-/-}$ models of less evolved organisms such as yeast and C. elegans were more sensitive to hydrogen peroxide and formaldehyde. In those organisms MG-H1 was accumulated. The $GLO1^{-/-}$ zebrafish embryos seem to be slightly resistant against toxins compared to wild-type animals. Furthermore, a mouse model showed an increased natural survival rate when GLO1 is missing.
Conclusion: Complex organisms are less dependent on GLO1 and less prone to damage despite the loss of GLO1 than expected. In $GLO1^{-/-}$ mice and isolated murine cells, $GLO1^{-/-}$ phenotype even showed a protective character. Therefore, we claim that the loss of GLO1 has less severe effects on higher organisms than expected and might even lead to an advantage on survival. The clinical relevance of GLO1 and possible compensatory pathways on the development of late diabetic complications in different organs has to be addressed in future studies.
Disclosure: **B. Fuchs:** None.

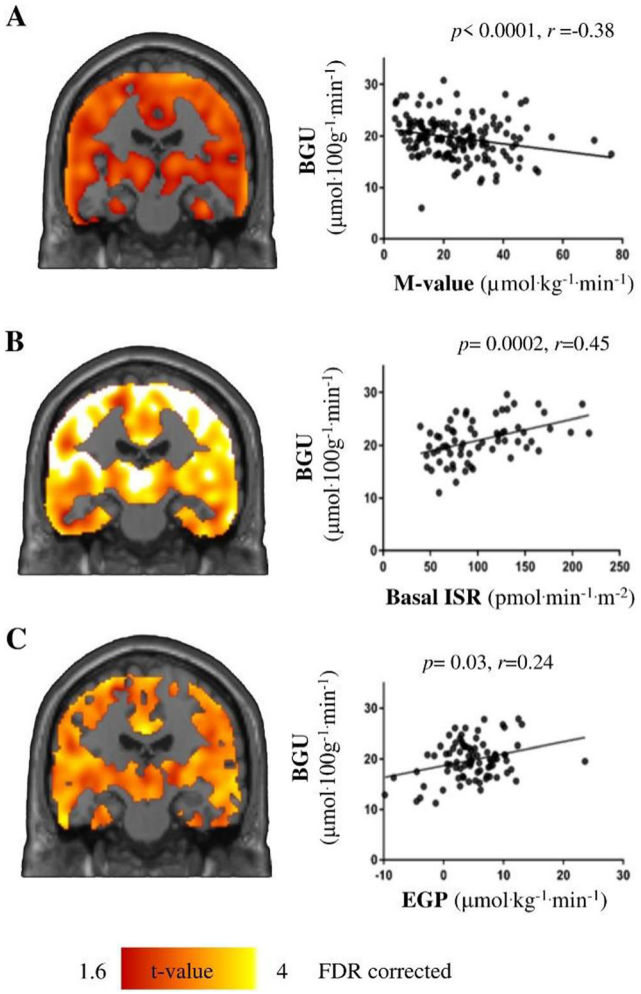
OP 23 How our brain impacts on diabetes

133

The influence of brain metabolism in insulin secretion and action assessed with FDG-PET in humans

E. Rebelos[1], M. Bucci[1], M.-J. Honka[1], A. Latva-Rasku[1], M. Laakso[2], K.A. Virtanen[1], J.C. Hannukainen[1], L. Nummenmaa[1], A. Mari[3], P. Iozzo[4], P. Nuutila[1];
[1]Turku PET Centre, Turku, Finland, [2]Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland, [3]Institute of Neuroscience, National Research Council, Padua, Italy, [4]Institute of Clinical and Experimental Physiology, Pisa, Italy.

Background and aims: Recent intervention studies have shown that brain glucose uptake (BGU) measured during euglycemic hyperinsulinemia and [^{18}F]FDG-PET imaging is increased in subjects with impaired glucose tolerance and morbidly obese subjects compared to normal glucose tolerant and lean subjects. The aim of this study was to evaluate insulin stimulated BGU and endogenous glucose production (EGP) and beta cell function in a larger cohort of subjects studied in our center.
Materials and methods: Data from 151 subjects were pooled together. Brain FDG-PET images were similarly pre-processed and BGU parametric images were calculated. Statistical analysis was performed with Statistical parametric mapping (SPM). Endogenous glucose production ($n = 132$) was calculated with FDG data. Beta cell function ($n = 67$) was estimated by C-peptide modelling during OGTT.
Results: In the whole dataset ($n = 151$) insulin-stimulated BGU was negatively associated with age ($p = 0.0006$, $r = -0.28$), M-value ($p < 0.0001$, $r = -0.38$) (**Figure** 1A) and positively associated with BMI ($p < 0.0001$). In a multivariate analysis including age, BMI, M-value, gender only the association between insulin-stimulated BGU and age, and insulin-stimulated BGU and M-value remained significant. Across all subjects ($n = 132$) there was no association between insulin-stimulated BGU and insulin-suppressed EGP. When dividing the subjects in tertiles of BMI, a positive association between BGU and EGP was found in the higher 2 tertiles ($p = 0.03$, $r = 0.24$) (**Figure** 1C), but not in the lean's group. We found a positive association between insulin-stimulated BGU and basal insulin secretion ($p = 0.0002$, $r = 0.45$, $n = 67$) (**Figure** 1B) and total insulin secretion ($p = 0.002$, $r = 0.37$, $n = 67$). After correcting for the M-value the association between BGU and basal insulin secretion remained significant ($p < 0.001$), but not for total insulin secretion.
Conclusion: In this large dataset including subjects across a wide spectrum of age, BMI, and insulin sensitivity, BGU was associated positively with EGP in the overweight and obese subjects. BGU also associated positively with basal insulin secretion and negatively with insulin sensitivity. These data suggest that central metabolism in humans, assessed with insulin-stimulated BGU, is involved in the regulation of both insulin secretion and peripheral insulin action.



Clinical Trial Registration Number: NCT01931540, NCT00793143
Supported by: Academy of Finland
Disclosure: **E. Rebelos:** None.

134

Hypothalamic orexin system prevents the development of non-alcoholic fatty liver disease in diet-induced obese mice

H. Tsuneki, K. Kon, S. Takata, T. Maeda, K. Otsuka, T. Wada, T. Sasaoka;
Department of Clinical Pharmacology, University of Toyama, Toyama, Japan.

Background and aims: Whole-body energy balance is maintained by inter-organ networks. In this mechanism, hypothalamic orexin system plays a central role by synchronizing the daily rhythms of sleep-wake, feeding-fasting, and glucose/energy metabolism. However, it remains unclear whether orexin prevents metabolic disorders under the conditions of obesity. Therefore, we investigated the functional significance of orexin in preventing non-alcoholic fatty liver disease (NAFLD) in obese mice.
Materials and methods: Male orexin knockout (OXKO) mice, female OXKO mice with or without ovariectomy (OVX), and their controls were fed a high fat diet (HFD) for 16–24 weeks. Also, OVX-OXKO mice and their controls were fed a high fat and high fructose diet (HFFD) for 20 weeks. Orexin A was intracerebroventricularly (ICV) injected to type 2 diabetic db/db mice. The expression profiles of mRNAs and miRNAs in peripheral tissues were examined by RT-qPCR and GeneChip microarray analyses, respectively. Hepatic fibrosis was determined by Sirius-red staining.
Results: HFD-fed male OXKO mice showed severer obesity and impairment of glucose tolerance than wild-type (WT) controls. In the liver and white adipose tissue (WAT), the tissue weight, the levels of chronic inflammation markers (e.g., Mcp-1 mRNAs), and hepatic triglyceride content were more rapidly increased in OXKO than WT mice on HFD. Also, in females, these metabolic parameters were markedly increased in the liver and WAT of OXKO mice when compared to WT mice on HFD, and the levels were further increased by ovariectomy (i.e., in OVX-OXKO mice). Pathway analysis of hepatic miRNA profiles demonstrated that orexin deficiency promoted development of NAFLD. Importantly, hepatic fibrosis developed in OXKO but not WT mice on HFD. Moreover, when fed HFFD, obvious fibrosis was observed in the liver sections of OXKO but not WT mice, despite similar hepatic triglyceride accumulation. The fibrosis was exacerbated in the following order: HFD/HFFD-fed controls << HFD-fed OXKO ≤ HFD-fed OVX-OXKO < HFFD-fed OVX-OXKO. Repetitive ICV injection of orexin A reduced the levels of proinflammatory markers in the WAT of db/db mice.
Conclusion: The present results provide the first evidence that endogenous orexin contributes to prevent obesity-related disorders, including NAFLD, in both sexes of mice. Since the orexin expression is down-regulated by diabetic hyperglycemia, enhancement of the orexin action is considered to be a novel therapeutic approach to prevent obesity/type 2 diabetes-induced NAFLD.
Supported by: JSPS KAKENHI Grant Number JP15K09380, JP15K15599
Disclosure: **H. Tsuneki:** None.

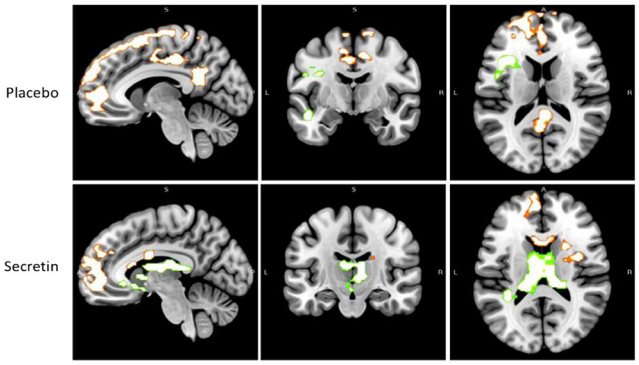
135

Secretin activates brown fat and induces satiation in humans

S. Laurila[1,2], M. Lahesmaa[1,2], L. Sun[2], K. Braun[3,4], K. Virtanen[2], K. Laitinen[5], M. Klingenspor[3,4], L. Nummenmaa[2], P. Nuutila[1,2];
[1]Department of Endocrinology, Turku University Hospital, Turku, Finland, [2]Turku PET Centre, University of Turku, Turku, Finland, [3]ZIEL - Institute for Food and Health, Technical University Munich, Freising, Germany, [4]Chair for Molecular Nutritional Medicine, EKFZ - Else Kröner-Fresenius Center, Technical University Munich, Freising, Germany, [5]Department of Biomedicine, University of Turku, Turku, Finland.

Background and aims: Cold-induced brown adipose tissue (BAT) activation in humans is by now a well known phenomenon. Recent preclinical studies suggest that secretin induces satiation in mice through activation of an endocrine gut - BAT - brain axis. Our aim was to investigate whether secretin administration effects BAT activation and satiation in humans as well.
Materials and methods: Fifteen healthy, normal weight males (age 41.6 ± 12.1 years, BMI 24.0 ± 1.9) were recruited. The study consisted of two phases: the assessment of BAT metabolic activity using PET/CT (n = 15) and brain functional magnetic resonance imaging (fMRI) to investigate satiation (n = 10). In both parts, study subjects were randomized and blinded to receive placebo (saline) and secretin (2 IU/kg secretin pentahydrochloride) prior to scans on separate days. For PET studies, tissue glucose uptake was measured with [^{18}F]-FDG and whole body energy expenditure monitored by indirect calorimetry. For fMRI studies, neural activity intensity response was assessed while subjects viewed appetizing and bland food images. Subjects filled in a subjective visual analogue scale questionnaire to measure composite satiety score.
Results: Secretin induced an increase in glucose uptake in BAT (1.1 ± 0.7 vs 0.7 ± 0.3 μmol/100 g/min, p = 0.02) and skeletal muscle (1.2 ± 0.5 vs 0.8 ± 0.2 μmol/100 g/min, p = 0.002). Secretin administration also induced an increase in whole body energy expenditure (1680 ± 150 vs 1640 ± 130 kilocalories/day, p = 0.01). The composite satiety score at fasting was increased after secretin administration compared to placebo (46.4 ± 10.3 vs 41.1 ± 12.03 millimeters p = 0.01). Secretin also

downregulated the brain reward circuitry during the fMRI food-reward experiment (statistical significance was thresholded at $p < 0.05$).
Conclusion: In the post-absorptive state, secretin not only increases whole body energy expenditure by activating brown adipose tissue and muscle, but also induces satiation and attenuates reward. These results suggest that secretin has a role in regulating appetite and food intake in humans, possibly via the activation of BAT.



Contrasts are made between appetizing and bland foods.
Orange signifies increased and green signifies reduced activity

Clinical Trial Registration Number: NCT03290846
Supported by: Academy of Finland
Disclosure: **S. Laurila:** None.

136

Impaired brain plasticity in obesity: effects of bariatric surgery and gut hormones

G. Daniele[1], A. Dardano[1], C. Lunghi[2], L. Giusti[1], A. Ciccarone[1], F. Santini[1], G. Ceccarini[1], R. Bellini[3], C. Moretto[3], R. Miccoli[1], G. Penno[1], M. Morrone[2], S. Del Prato[1];
[1]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, [2]Department of Translation Research on New Technologies in Medicine and Surgery, University of Pisa, Pisa, [3]Bariatric and Metabolic Surgery Unit, University of Pisa, Pisa, Italy.

Background and aims: Obesity and diabetes are associated with reduced plasticity in the hippocampus and impairment of memory and learning. It is still unclear whether obesity can alter plasticity in the sensory cortex. Gut hormones play a crucial role in neuroplasticity (NP) but to which extent it can mediate obesity's effects on NP and cognition is still poorly evaluated. The aims of the study were to evaluate: i) the effect of obesity and bariatric surgery (RYGB) on NP ii) the relationship between NP and gut hormones (GLP-1, GIP and VIP) changes 6 months (6m)after RYGB iii) the relationship between NP, BDNF, Leptin and cognitive performance
Materials and methods: NP was assessed testing binocular rivalry between orthogonal gratings (size: 2°, contrast: 50%, SF: 2cpd) before and after 2 h of monocular deprivation (index of brain plasticity in the visual cortex). NP evaluation has been performed on 20 healthy volunteers (NS) (age 26 ± 10 years, BMI 21.7 ± 2.6 kg/m^2) and 31 obese subjects (OB) (age: 40 ± 11 years; BMI: 41.6 ± 6.7 kg/m^2) in fasting condition. A subgroup of OB ($n = 13$; BMI 45.8 ± 4.9 kg/m^2; age 43.7 ± 9.5 years; HbA1c 41.5 ± 5.4 mmol/mol) underwent a 75 g OGTT before and 6m after RYGB. NP was performed at baseline, 1, 3 and 6 m after RYGB. Gut hormones, BDNF, leptin and cognitive performance were assessed at baseline and 6 m after RYGB
Results: In the whole population NP was lower in OB as compared to NS (0.12 ± 0.05 vs. 0.04 ± 0.08, $p < 0.0001$) and NP was inversely correlated with BMI ($r = -0.55$; $p < 0.001$). In the OB subgroup 6 m after RYGB a significant BMI reduction (45.8 ± 4.9 to 34.3 ± 1.6 kg/m^2; $p < 0.001$) was associated withimproved glucose metabolism (HOMA-IR: 4.7 ± 0.9 to 1.6 ± 0.4; $P = 0.006$; Disposition Index: 0.07 ± 0.02 to 0.81 ± 0.30 mUI × ml^{-1}/mg × dl^{-1} × 1/mUI × ml^{-1}; $P < 0.05$). NP was progressively restored (ANOVA: F(3.24) = 5.7, $p = 0.002$) with a 10 fold increase of NP 6 m after RYGB (0.01 ± 0.03 to 0.11 ± 0.04; $p = 0.008$). Post-OGTT GLP-1 increased (5336 ± 2263 to 11132 ± 3412 pmol/l × 120 min; $p < 0.05$) as well as GIP (4140 ± 3659 to 5791 ± 4537 pg/ml × 120 min; $p = 0.01$). The NP increase was correlated to active GLP-1 and negatively with GIP increase($p < 0.05$). VIP levels did not change 6 m after RYGB with no correlation with NP. Fasting plasma leptin decreased (73.8 ± 45.5 to 14.3 ± 5.9 pmol/l; $p < 0.008$) and it was inversely correlated with NP increase ($p < 0.05$). Baseline BDNF was inversely correlated with fasting insulin ($r = -0.76$; $p = 0.007$) and it did not change after 6m RYGB. Post-RYGB BDNF inversely correlated with NP ($p < 0.05$) but positively with both total and active GLP1 ($p < 0.05$). NP correlated with cognitive performance ($p < 0.05$). In a multiple linear regression analysis, addition of post-RYGB gut hormones, BDNF and Leptin to BMI and fasting glucose improved the r^2 associated to post-RYGB NP (r^2 change: 0.881; F change: 10732.19; $p = 0.007$)
Conclusion: Obesity is associated with abnormal NP in visual cortex that can be reversed by weight-loss following bariatric surgery, supporting a strong effect of peripheral metabolism on early sensory plasticity and function. The relationship between NP increase, circulating gut hormones, BDNF and Leptin suggest a potential role of these hormones in the NP restoration and cognitive function in humans
Clinical Trial Registration Number: NCT03414333
Disclosure: **G. Daniele:** None.

137

Genetic disruption of Adipose Triglycerides Lipase (ATGL) in mediobasal hypothalamic neurons induces overweight and metabolic disturbances

R. Manceau[1], K. Bouyakdan[1], A. Fisette[1], D. Rodaros[1], G. Mitchell[2], S. Fulton[1], T. Alquier[1];
[1]Cardiometabolic, CRCHUM, Montreal, [2]CRCHU Ste Justine, Montreal, Canada.

Background and aims: Adipose Triglyceride Lipase (ATGL) acts as the first lipase in the hydrolysis of triglycerides (TG). Recent studies show that ATGL in peripheral tissues plays major roles on energy homeostasis. We found that ATGL is expressed in the mediobasal hypothalamus (MBH) and in hypothalamic neuronal cell lines, in line with our recent study suggesting that neurons accumulate TG. ATGL expression is increased in the MBH of high fat-fed mice that maintain a healthy body weight compared to mice that become obese. In addition, ATGL expression in the MBH is increased in response to fasting. This suggests that increased ATGL may play a role in maintaining a healthy metabolic profile. We propose that hypothalamic ATGL regulates lipid metabolism in the brain that in turn contributes to energy balance.
Materials and methods: To test this hypothesis, synapsin-Cre or -GFP expressing AAV are stereotaxically injected in the arcuate nucleus (ARC) of male ATGL *flox* mice to KO ATGL specifically in neurons (ARC-ΔATGL).
Results: First, we validated that ATGL expression is reduced by 50% in ARC-ΔATGL mice compared to ARC-WT. We found that ARC-ΔATGL have increased weight gain on a chow diet compared to control animals that is associated with reduced energy expenditure and increased food intake and fat mass. In addition, chow-fed ARC-ΔATGL mice have an increased fasting glycaemia and mild glucose intolerance. Finally, pharmacological inhibition of ATGL in hypothalamic neurons in vitro increases intracellular TG content.
Conclusion: Together, our findings suggest that the ATGL pathway in MBH neurons beneficially regulates glucose and energy homeostasis by

mechanisms that may involve regulation of TG and lipid droplets metabolism.
Supported by: NSERC
Disclosure: **R. Manceau:** Grants; NSERC.

138

Brain insulin action stimulates pancreatic insulin secretion: results from hyperglycaemic clamps

M. Heni[1,2], R. Wagner[1,2], C. Willmann[1,2], B.A. Jaghutriz[1,2], A. Vosseler[1,2], C. Kübler[1], A. Peter[1,2], H.-U. Häring[1,2], H. Preissl[1,2], S. Kullmann[2,3], A. Fritsche[1,2];
[1]Division of Endocrinology, Diabetology, Nephrology, Vascular Disease and Clinical Chemistry, University Hospital Tübingen, Tübingen, [2]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tübingen, Tübingen, [3]German Center for Diabetes Research (DZD), Neuherberg, Germany.

Background and aims: Animal studies and initial correlative analyzes in humans indicate that insulin action in the brain may affect pancreatic insulin secretion. An important brain region for this process appears to be the hypothalamus. Like other human brain areas, the hypothalamus can also develop insulin resistance. We now investigated whether induction of brain insulin action by intranasal insulin influences pancreatic insulin secretion.
Materials and methods: 15 young, healthy men (27 ± 2.3 years) with a large BMI spectrum (21–28 kg/m²) underwent two hyperglycemic clamps (target blood glucose: 10 mmol/l). In this double-blind study, subjects were randomized to receive 160 units of insulin as a nasal spray on one day and placebo on the other. On another day, the insulin sensitivity of the hypothalamus was determined by functional magnetic resonance imaging.
Results: Glucose levels were comparable on both study days. In the whole group, C-peptide levels were not significantly different between conditions. Though, there was a significant interaction between insulin sensitivity of the hypothalamus x nasal spray x time on C-peptide levels ($p = 4 \times 10^{-05}$). The group was therefore divided according to median hypothalamic insulin sensitivity. In the group with high insulin sensitivity of the brain, C-peptide levels were higher after insulin nasal spray than after placebo spray ($p_{(\text{nasal spray x time})} = 0.004$). This effect was especially noticeable after minute 10 of the hyperglycemic clamp. In the group with low brain insulin sensitivity, the nasal spray did not affect C-peptide levels ($p_{(\text{nasal spray x time})} = 0.4$).
Conclusion: In participants with high hypothalamic insulin sensitivity, insulin action in the brain enhanced insulin secretion from pancreatic beta cells. This reaction could, for example, contribute to a sufficient suppression of hepatic glucose production by portal venous insulin in the postprandial state.
Clinical Trial Registration Number: NCT02870361
Supported by: EFSD, DZD
Disclosure: **M. Heni:** None.

OP 24 Beta cell signal transduction: new concepts

139

Extended synaptotagmin-1 controls insulin secretion through diacylglycerol transport at ER-PM contact sites

B. Xie, O. Idevall-Hagren;
Medical Cell Biology, Uppsala Univerisity, Uppsala, Sweden.

Background and aims: The endoplasmic reticulum (ER) is essential for protein and lipid synthesis and Ca^{2+} homeostasis. Dysfunctional ER is associated with β-cell failure and death in diabetes. The ER form contacts with other cellular compartments, including the plasma membrane (PM), and these sites are important reactions centres where ion and lipid exchange occurs. The Extended Synaptotagmins (E-Syts) are ER-localized proteins that bind the PM and transport lipids in a Ca^{2+}-dependent manner. Recent studies have shown that the E-Syts participate in diacylglycerol (DAG) clearance from the PM, but the biological relevance of this transport mechanism is still unclear. The aim of our study was to investigate if E-Syt-mediated signalling at ER-PM contacts plays a role in insulin secretion.
Materials and methods: TIRF microscopy was used to study the subcellular distribution kinetics of fluorescently tagged E-Syts and changes in Ca^{2+} (R-GECO) and DAG (mCh/GFP-C1aC1b) levels. An optogenetic tool to generate artificial ER-PM contacts was developed. MIN6 cells were used for all experiments. Cell lines stably expressing non-targeting or E-Syt1-targeting shRNA were also generated. Secretion from single cells was measured using a pH-sensitive fluorescent reporter (VAMP2-pHl) and from batches of cells using the AlphaLISA technique (Perkin-Elmer).
Results: Immunoblotting revealed high expression of E-Syt1 in both MIN6 cells and mouse islets. Fluorescence-tagged E-Syt1 was recruited to the PM by both K^+- and glucose induced Ca^{2+}-increases, and this resulted in expansion of ER-PM contacts that co-localized with L-type voltage-dependent Ca^{2+} channels (55 ± 7% enrichment, $P < 0.001$). Insulin secretion stimulates transient and repetitive DAG formation (spiking) in the PM by autocrine activation of PLC. DAG formation correlated spatio-temporally with E-Syt1 PM-binding, and the overexpression of E-Syt1 suppressed DAG spiking frequency (52%, $P < 0.001$) and amplitude (49%, $P < 0.001$) whereas E-Syt1 knockdown increased spike amplitude (50%, $P < 0.001$) and duration (20%, $p < 0.05$). Together, these observations indicate a role of E-Syt1 in DAG clearance. E-Syt1 knockdown was associated with 76% reduction ($n = 3$, $p < 0.05$) in glucose-stimulated insulin secretion. Optogenetic ER-PM contacts were constructed by replacing the C-terminus of E-Syt1 with CRY2 and anchoring CIBN in the PM by a transmembrane domain. Blue light illumination stimulated CRY2-CIBN binding, resulting in the generation of ER-PM contacts. These contacts did not affect K^+- or glucose-induced Ca^{2+} influx, but suppressed the frequency of secretagogue-induced DAG spikes by 61% ($P < 0.05$). The light-induced expansion of ER-PM contacts resulted in amplification of K^+-induced insulin granule exocytosis (50%, $P < 0.001$).
Conclusion: Plasma membrane DAG levels are regulated by E-Syt1 at ER-PM contact sites in a Ca^{2+} dependent manner. The E-Syt1-mediated removal of DAG positively regulates insulin secretion by an unknown mechanism, and the findings more broadly identify ER-PM contacts as important reaction centres for the regulation of insulin secretion.
Disclosure: **B. Xie:** None.

140

Regulation of pancreatic beta cell insulin secretion by RGS2

T. Speckmann, P.V. Sabatini, C. Nian, D.S. Luciani, F.C. Lynn;
Dept. of Surgery, University of British Columbia, Vancouver, Canada.

Background and aims: Insulin-secreting β-cells in the pancreatic islets of Langerhans are important regulators of glucose homeostasis. During

type 2 diabetes (T2D), which is associated with hyperglycemia and obesity, excess nutrients such as high glucose or the fatty acid palmitate can cause β-cell stress and exhaustion. We previously found that the calcium-dependent transcription factor, *Neuronal PAS domain protein 4* (NPAS4), can alleviate β-cell stress by reducing both insulin production and secretion, though it remains unknown which target genes mediate this effect. Because regulator of G protein signalling (RGS) protein family members are known to regulate β-cell function and survival, we *hypothesized* that the direct NPAS4 target gene *Regulator of G protein signalling 2* (RGS2) mediates these β-cell cytoprotective effects.

Materials and methods: To test our hypothesis, *Rgs2* was adenovirally overexpressed (Ad-*Rgs2*) and compared to control overexpression (Ad-βGal) in primary mouse islets and MIN6 cells. Furthermore, *Rgs2* loss of function was studied by using CRISPR-Cas9 to derive clonal *Rgs2* knockout MIN6 cell lines.

Results: Compared to control, *Rgs2* overexpression blunted glucose-stimulated insulin secretion by 30% or 55–70% in mouse islets or MIN6 cells, respectively, whereas KCl-induced insulin release and total insulin content remained unchanged ($p < 0.05$, Student's t test). When examining what caused the decrease in insulin secretion, we discovered that glucose-induced intracellular calcium (Ca^{2+}_i) amplitudes were diminished by 17% in Ad-*Rgs2* overexpressing MIN6 cells compared to control ($p < 0.05$, Student's t test). Moreover, Ad-*Rgs2* islets exhibited 30% reduced oxygen consumption rate (OCR) at high glucose ($p < 0.05$, Student's t test). In order to further elucidate the mechanism by which RGS2 represses glucose-stimulated insulin secretion, clonal *Rgs2* knockout cell lines were derived and loss of RGS2 expression confirmed by western blot. Initial results indicate that insulin secretion is elevated in *Rgs2* knockout cells.

Conclusion: To date, our results suggest that activity-dependent *Rgs2* expression tempers glucose metabolism leading to reduced depolarization, reduced Ca^{2+}_i, and reduced insulin secretion. Of note, *RGS2* expression levels were found significantly reduced in islets from T2D donors, indicating that a population of T2D patients may benefit from restoration of *RGS2*. Additionally, these studies provide a new model for future study of how *Rgs2* expression impacts β-cell function.

Supported by: CIHR MOP-142222

Disclosure: **T. Speckmann:** None.

141

Evaluation of the acute metabolic effects and specificity of GPR55 agonists (Abn-CBD and AM251) on islet and enteroendocrine cell function

A.G. McCloskey, M.G. Miskelly, P.R. Flatt, A.M. McKillop;
Diabetes Research Group, Ulster University, Coleraine, UK.

Background and aims: Type-2-diabetic therapies which enhance beta cell regeneration and function are needed and interest has focused on G-protein coupled receptors (GPCRs). G-protein coupled receptor 55 (GPR55), a novel endocannabinoid receptor has been identified as a potential anti-diabetic target, through the regulation of islet and enteroendocrine cell function. GPR55 function was evaluated by identifying and utilising selective GPR55 agonists and assessing their potential as novel therapeutic agents.

Materials and methods: CRISPR/Cas9 gene editing was employed to develop a clonal pancreatic GPR55 knockout BRIN-BD11 cell line. Specificity and insulinotropic activity of GPR55 agonists were assessed in wild type and GPR55 knockout BRIN-BD11 cells, with potency confirmed in human 1.1B4 cells. GPR55 expression and distribution were assessed by qPCR and western blotting in BRIN-BD11 cells and by immunohistochemistry in high fat fed (HFF) induced diabetic mouse pancreas and small intestine. Acute metabolic effects of agonist monotherapy (0.1 μM/kg BW) and combinational therapy (DPP-IV inhibitor [50 mg/kg BW]) *in-vivo* were investigated in HFF-induced diabetic NIH-Swiss mice. Animals were subjected to an oral glucose tolerance test and received either glucose alone (18mmol/kg BW) or glucose in combination with GPR55 agonist monotherapy and/or combinational therapy.

Results: Targeted CRISPR/Cas9 gene editing diminished GPR55 mRNA ($p < 0.001$) and protein expression ($p < 0.001$) in pancreatic BRIN-BD11 cells, with sanger sequencing confirming bi-allelic deletion of the GPR55 start codon. Synthetic agonists Abn-CBD (10^{-9}–10^{-4}M) and AM251 (10^{-8}–10^{-4} M) augmented insulin secretion from BRIN-BD11 and 1.1B4 cells at 5.6 mM ($p < 0.05$–$p < 0.001$) and 16.7 mM ($p < 0.05$–$p < 0.001$) glucose, with no cytotoxic effects. The insulinotropic response of Abn-CBD and AM251 was attenuated ($p < 0.05$) when assessed using the GPR55 knockout BRIN-BD11 cell line. Upon agonist treatment, insulin ($p < 0.01$) mRNA expression was upregulated in BRIN-BD11 cells, with no significant change in GPR55 mRNA expression observed. Confirmatory GPR55 protein expression was demonstrated by western blotting and levels of insulin content by radioimmunoassay. Immunohistochemistry demonstrated regions of co-localisation between GPR55 and insulin in the pancreatic islet and incretin hormones in the small intestine. Orally administered Abn-CBD and AM251 (0.1 μmol/kg BW) improved glucose excursion ($p < 0.001$), increased plasma insulin ($p < 0.001$), gastric inhibitory polypeptide ($p < 0.05$), glucagon-like peptide 1 ($p < 0.05$) and improved satiety ($p < 0.001$) in HFF-induced diabetic mice. Abn-CBD and AM251 agonist combinational therapy (Sitagliptin) diminished DPP-IV activity ($p < 0.001$), whilst improving glucose excursion ($p < 0.05$) through enhanced insulin ($p < 0.05$) and incretin ($p < 0.05$) hormone secretion from islet and enteroendocrine cells respectively.

Conclusion: Abn-CBD and AM251 activate GPR55 and potentiate insulin secretion from BRIN-BD11 cells and HFF-induced diabetic mice. *In-vivo* findings present GPR55 agonist monotherapy and combinational therapy as a novel approach for the treatment of type-2-diabetes.

Supported by: DUK PhD studentship

Disclosure: **A.G. McCloskey:** Grants; Diabetes UK PhD studentship.

142

Activation of PKD1 by autocrine ATP signalling in pancreatic beta cells

S. Khan;
Pharmacology, University of Alberta, Edmonton, Canada.

Background and aims: β-cells co-secrete the neurotransmitter ATP along with insulin. ATP acts as a positive autocrine signal in β cells by activating P2Y1 receptors and resulting in activation of Phospholipase C and production of DAG. However, the downstream signaling that couples P2Y1 activation to insulin secretion remains to be fully elucidated. Since DAG has been shown to activate Protein Kinase D1 (PKD1) to potentiate glucose stimulated insulin secretion, we hypothesize that autocrine ATP signaling activates downstream PKD1 to regulate insulin secretion.

Materials and methods: Western blotting was performed to study agonist-induced, depolarization-induced and antagonist-inhibited activation of PKD1 in response to KCl in INS 832/13 insulinoma cells and in mouse islets. Insulin secretion was measured from intact PKD1 knockout islets. Capacitance measurements of exocytosis were employed in single mouse β cells. Expression of PKD1 mRNA was analysed by RT-PCR in human islets. Correlation between the insulinotropic capacity of PKD1 activation and donor characteristics was examined in human islets.

Results: The P2Y1 receptor agonists, MRS2365 and ATP, induce PKD1 phosphorylation at S916 in mouse islets. Similarly, direct depolarization with KCl causes activation of PKD1. A reduction in PKD1 activation was observed upon application of P2Y1 antagonist, MRS 2500. Insulin secretion was measured from PKD1 KO mouse islets, where potentiation of insulin secretion elicited by P2Y1 activation was lost. Activation of P2Y1 increased the exocytotic response of mouse β cells in a PKD1-dependent manner. Finally, RT-PCR analysis confirmed expression of PKD1 in human islets and the study of donor characteristics revealed a correlation

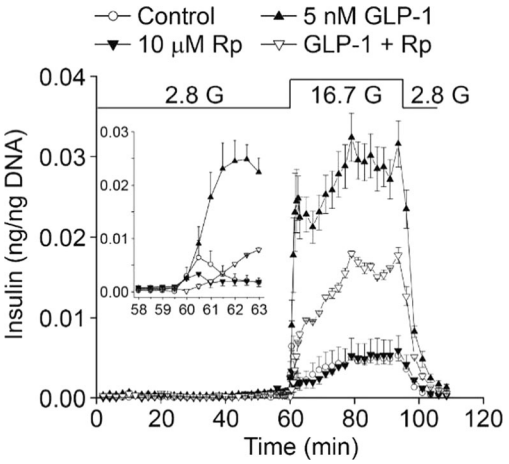
between the activation of PKD1 and stimulation index - ability of the islets to produce insulin when stimulated by high glucose.
Conclusion: A P2Y1 receptor-dependent activation of PKD1 by ATP increases insulin secretion in mouse islets. In human islets, PKD1 may be involved with potentiation of glucose induced insulin secretion.
Disclosure: **S. Khan:** None.

143

cAMP-dependent and -independent actions of GLP-1 to potentiate 1st and 2nd phase GSIS as revealed by Rp-8-Br-cAMPS-pAB - a dual antagonist of PKA and Epac activation

O. Cabrera[1], J. Ficorilli[1], K.R. Jones[2], O.G. Chepurny[3], C.A. Leech[3], F. Schwede[4], G.G. Holz[3];
[1]Islet Biology and Diabetes Drug Hunting Team, Eli Lilly and Company, Indianapolis, USA, [2]Advanced Testing Lab, Blue Ash, USA, [3]Medicine, SUNY Upstate Medical University, Syracuse, USA, [4]BIOLOG Life Sci. Inst., Bremen, Germany.

Background and aims: The novel cAMP antagonist prodrug Rp-8-Br-cAMPS-pAB (Rp-pAB) blocks PKA and Epac activation, and it was reported to act in human and rat islets to abolish 1st -phase glucose-stimulated insulin secretion (GSIS) while instead having a minimal inhibitory action on 2nd -phase GSIS. The aim of the present study was to determine if and how Rp-pAB also alters the ability of incretin hormone GLP-1 to potentiate 1st and 2nd phase GSIS. Such an analysis was expected to reveal cAMP -dependent and -independent actions of GLP-1 that might be selective for 1st or 2nd phase GSIS.
Materials and methods: Perifusion studies of SD rat islets were performed in which GSIS was initiated and terminated by step-wise changes of the glucose concentration (G) from 2.8 to 16.7 mM to 2.8 mM. Rp-pAB and GLP-1 were administered during the initial perifusion in 2.8G, and then also during perifusion with 16.7G. Perifusate fractions were assayed for insulin content, and insulin release was quantified relative to whole-islet DNA content. Static incubation assays using mouse islets were also performed to investigate if very low concentrations of GLP-1 (1 or 10 pM) potentiate GSIS at steady-state 5.6G or 11.1G.
Results: Rat islets exhibited biphasic insulin secretion when challenged with 16.7G (see Figure). GLP-1 (5 nM) potentiated 1st and 2nd -phase GSIS by 4.2-fold and 6.3-fold during assay intervals 60–65 and 65–95 min, respectively. For islets treated with Rp-pAB in the absence of GLP-1, 1st-phase GSIS was nearly abolished, whereas 2nd-phase GSIS was not altered. GLP-1 failed to potentiate 1st-phase GSIS from islets treated with Rp-pAB, whereas it retained a smaller but significant 3.75-fold ability to potentiate 2nd-phase GSIS. These actions of GLP-1 were significant ($n = 3$; $p < 0.05$ for 5 nM GLP-1) and dose-dependent at 100 pM–30 nM. FRET assays using AKAR3 and H188 confirmed that Rp-pAB blocked PKA and Epac activation by GLP-1. Very low concentrations of GLP-1 (1 or 10 pM) failed to potentiate GSIS from mouse islets at either 5.6G or 11.1G, whereas 10 nM GLP-1 was effective.
Conclusion: GLP-1 exerts a cAMP-dependent action to potentiate 1st-phase GSIS from rat islets, an effect that is abrogated by Rp-pAB. Since Rp-pAB reduces but fails to fully block the action of GLP-1 to potentiate 2nd-phase GSIS, there exist cAMP- dependent and -independent actions of GLP-1 to potentiate 2nd-phase GSIS. We propose that GLP-1 enables cAMP-dependent insulin exocytosis to occur during 2nd-phase GSIS, while also enhancing cAMP-independent exocytosis that normally predominates during 2nd-phase GSIS in the absence of GLP-1. Potentially, a cAMP-dependent mechanism of insulin exocytosis explains 1st-phase GSIS, and it is recruited by GLP-1 so that it becomes operational during 2nd-phase GSIS.



Supported by: R01-DK069575
Disclosure: **O. Cabrera:** None.

144

GLP-1 analogues protect beta cells in models of Wolfram syndrome

P. Salpea[1], S. Toivonen[1], C. Cosentino[1], A. Musuaya[1], N. Pachera[1], P. Marchetti[2], C. Brown[3], F. Urano[3], D.L. Eizirik[1], M. Cnop[1,4], M. Igoillo-Esteve[1];
[1]ULB Center for Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium, [2]Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy, [3]Department of Medicine, Washington University School of Medicine, Washington, USA, [4]Division of Endocrinology, Erasmus Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Background and aims: Wolfram syndrome is a rare autosomal recessive orphan disease. The clinical manifestations are young onset diabetes, optic nerve atrophy and deafness. Most Wolfram patients carry mutations in *WFS1*. WFS1 deficiency results in endoplasmic reticulum (ER) stress, leading to neurodegeneration and pancreatic β-cell dysfunction and death. Glucagon-like peptide-1 (GLP-1) analogs and the cAMP inducer forskolin have been shown to protect β-cells from ER stress. Our aim is to test whether GLP-1 analogs confer protection in *in vitro* and *in vivo* Wolfram syndrome models
Materials and methods: WFS1 was silenced in human EndoC-βH1 β-cells and human islets by RNA interference. Wolfram syndrome patients' induced pluripotent stem cells (iPSCs) were differentiated into β-like cells. Synthetic ER stress was induced using tunicamycin (5 μg/ml). β-cell apoptosis was evaluated by Hoechst 33342/propidium iodide staining. Expression of ER stress markers was examined by qPCR. Whole body wfs1 knockout (KO) mice (homozygous exon 8 deletion on 129S background) were treated for 12 weeks with exendin-4 (10 μg/kg/day) or vehicle using miniosmotic pumps. Glucose tolerance was evaluated before, during and at the end of treatment by intraperitoneal glucose tolerance tests.
Results: WFS1 silencing (>70% knockdown, $n = 6$, $p < 0.001$) sensitized EndoC-βH1 cells to tunicamycin-induced apoptosis (29 ± 3% apoptosis in WFS1-deficient cells vs 12 ± 1% apoptosis in control cells, $n = 5$, $p < 0.01$) and increased mRNA expression of the ER stress marker CHOP ($p < 0.001$). Exendin and forskolin protected WFS1-deficient EndoC-βH1 cells from ER stress (29 ± 3% apoptosis with tunicamycin alone vs 22 ± 1% with tunicamycin + exendin or 10 ± 0.3% with tunicamycin + forskolin, $n = 5$, $p < 0.01$). iPSCs from 4 Wolfram syndrome patients were successfully differentiated *in vitro* into β-like cells using a 7-stage protocol. Forskolin protected Wolfram iPSC-β-like cells from tunicamycin-induced apoptosis ($n = 4$, $p < 0.001$) and increased expression of the ER chaperone BiP. wfs1 KO mice had impaired glucose

tolerance compared to wild type littermates as early as 6 weeks of age ($n = 17$, $p < 0.01$). 12 weeks of exendin administration improved glucose tolerance of wfs1-deficient mice when compared to vehicle-treated KO animals ($n = 8$–9 per group, $p < 0.05$).

Conclusion: cAMP induction by exendin and forskolin protects WFS1-deficient β-cells from ER stress-induced apoptosis. *In vivo*, exendin treatment improves glucose tolerance of wfs1 KO mice. These findings provide further evidence for the protective properties of GLP-1 analogs in the context of β-cell ER stress, and suggest that GLP-1 analogs hold preventive and therapeutic potential for Wolfram syndrome-related diabetes.

Supported by: Eye Hope Foundation

Disclosure: **P. Salpea:** Grants; Projet réalisé avec le soutien du Fons Eye Hope, géré par la Fondation Roi Baudoin.

OP 25 Hypoglycaemia: consequences and prevention

145

Severe hypoglycaemia and cardiovascular or all-cause mortality in the Korean population

S.-A. Cha[1], J.-S. Yun[1], K. Han[2], S.-H. Ko[1], Y.-B. Ahn[1];
[1]Internal medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Suwon, [2]Biostatistics, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.

Background and aims: Previous studies have associated hypoglycemia with an increase in cardiovascular disease and mortality. We investigated the association between the number of severe hypoglycemia (SH) and the risk of cardiovascular disease (myocardial infarction, stroke, congestive heart failure) and all-cause mortality in patients with type 2 diabetes using the National Health Insurance Service database which covers the entire Korean population.

Materials and methods: Baseline and follow-up data ($n = 1{,}583{,}149$) from the patients with T2DM for the period 2006–2016 were retrieved from the National Health Insurance System database. Type 2 diabetes, SH, and major comorbidities were identified using the International Classification of Diseases 10 codes and medication information. We counted the number of SH episodes according to ICD-10 codes during the three years (2006–2009) prior to the index date within the year of 2009–2010. The primary outcome was a new development of 1) myocardial infarction, 2) stroke, 3) congestive heart failure and 4) all-cause mortality.

Results: 20,064 (1.2%) developed at least one SH event during the first three years prior to the index date. The mean follow-up duration was 7.2 years. After adjustment for multiple confounding factors, including age, sex, socioeconomic status, hypertension, dyslipidemia, use of insulin and sulfonylurea, number of metabolic syndrome factors, the presence of major comorbidities, the hazard ratio (HR) of cardiovascular diseases or all-cause mortality significantly increased sequentially. [the group who experienced zero SH episode vs. one SH episode, HR 1.96 95% CI (1.91–2.02); vs. two SH episodes, HR 2.36 (2.22–2.50); vs. three SH episodes, HR 3.14 (2.90–3.41); *P* for trends <0.001]. Similar findings were noted the relationship for the number of SH episodes with myocardial infarction, stroke, and congestive heart failure. The sensitivity analysis which analyzed the 804,503 subjects who had received national health examination did not change the significance of the main findings.

Conclusion: We demonstrated that, in the entire Korean population, the number of SH episode is associated with an increased risk for all cardiovascular outcomes and all-cause mortality. The patients who experienced recurrent SH episode may have a greater risk of cardiovascular events and mortality.

Disclosure: **S. Cha:** None.

146

Preserved glucose response to low-dose glucagon after exercise in insulin pump-treated individuals with type 1 diabetes: a randomised crossover study

I.I.K. Steineck[1,2], A. Ranjan[1,2], S. Schmidt[1,2], T.R. Clausen[3], J.J. Holst[4], K. Nørgaard[5];
[1]Department of Endocrinology, research unit (afs. 159), Hvidovre University Hospital, University of Copenhagen, Hvidovre, [2]Danish Diabetes Academy, Odense University Hospital, Odense, [3]Obesity Research, Novo Nordisk A/S, Måløv, [4]Department of Biomedical Sciences and NNF Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, [5]Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: To compare the increase in plasma glucose after a subcutaneous injection of 200 μg glucagon given after 45 minutes of

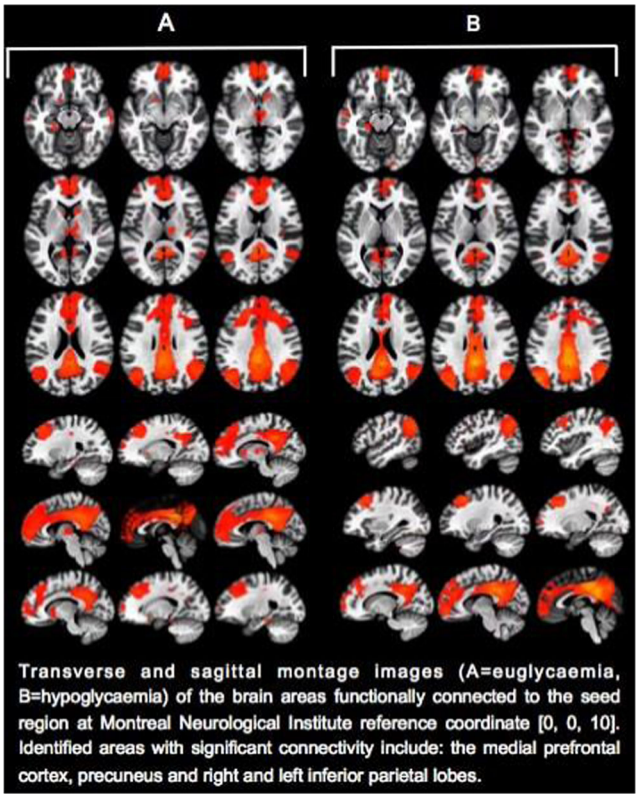
cycling versus resting and to investigate the effects of glucagon when injected before compared with after 45 minutes of cycling.
Materials and methods: Fourteen insulin pump-treated individuals with type 1 diabetes completed three visits in a randomized, placebo-controlled, single-blinded crossover study. Baseline (mean and range) HbA1c 54 (43–65) mmol/mol or 7.1 (6.1–8.1) %, age 45 (23–66) years, BMI 26 (21–30) kg/m^2, diabetes duration 26 (8–51) years. On each visit, participants consumed a standardized breakfast two hours prior to 45 minutes of cycling or resting. A subcutaneous injection of 200 μg glucagon was either given after cycling, after resting or before cycling and frequent blood sampling occurred until two hours after exercise.
Results: The glucose response to glucagon was higher after cycling compared with after resting (mean ± SD incremental peak: 2.6 ± 1.7 versus 1.8 ± 2.0 mmol/l, $P = 0.02$). As expected, plasma glucose decreased during cycling (−3.1 ± 2.8 mmol/l) but less so when glucagon was given before cycling (−0.9 ± 2.8 mmol/l, $P = 0.002$). The number of subjects reaching hypoglycemia (glucose values ≤3.9 mmol/l) was the same on the three days.
Conclusion: Moderate cycling for 45 minutes did not impair the glucose response to glucagon compared to the glucose response after resting. The glucose fall during cycling was diminished by a pre-exercise injection of 200 μg glucagon. Thus a small glucagon dose can potentially be used to treat exercise-induced mild hypoglycemia and diminish the glucose fall during exercise.
Clinical Trial Registration Number: NCT02882737
Supported by: Danish Diabetes Academy, Zealand pharma
Disclosure: **I.I.K. Steineck:** Grants; Dansih Diabetes Academy sponsored by Novo Nordisk Foundation, Zealand Pharma. Lecture/other fees; Speaker grants from Roche Diabetes Care., Speaker grants from Rubin Medical.

147

The impact of hypoglycaemic stress on the connectivity of the default mode network in healthy controls

C.E.D. Osborne, O. O'Daly, M. Nwokolo, F.O. Zelaya, P. Choudhary; King's College London, London, UK.

Background and aims: As a physiological stressor, hypoglycaemia is known to stimulate the brain's physiological stress pathway. The brain plays a key role in recognising this stress and generating protective symptomatic and hormonal responses that serve to maintain normoglycaemia. In patients with type 1 diabetes, allostatic mechanisms can cause these responses to become blunted, increasing the risk of dangerous hypoglycaemia. Areas of interest in recognizing this physiological stress are the resting state networks (RSNs), which are networks of brain regions that show similar spontaneous low frequency oscillations while at rest. The default mode network (DMN) is a RSN, which demonstrates reduced connectivity during task-based activity or changes to the system. We investigated whether hypoglycaemic stress disrupts the connectivity of the DMN.
Materials and methods: Fourteen healthy participants underwent a hyperinsulinaemic, two-step glucose clamp procedure during which two blood oxygen-level dependent (BOLD) RSN functional magnetic resonance images (fMRI) were obtained. At euglycaemia [5mmol/L] and hypoglycaemia [2.6 mmol/L] we collected symptom scores using a visual analogue scale and counter-regulatory hormone responses through blood sampling. Seed-to-voxel analysis using statistical parametric mapping was performed using the CONN toolbox in SPM12. The posterior cingulate cortex (PCC) was defined *a priori* as a seed region for the default mode network.
Results: We identified connectivity in the DMN in euglycaemia as well as hypoglycemia. A DMN mask was designed on WFU Pickatlas and applied to the data and formal statistical comparison was performed using a Student's paired t-test. Only results with a family wise error corrected (FWE-corr) cluster size of $p < 0.05$, at a cluster forming threshold of $p < 0.001$ were considered significant. A significant weakening of PCC to right angular gyrus connectivity was identified during hypoglycaemia: $p_{FWE\text{-}corr} = 0.03$; $z = 4.47$; cluster size (k) = 43; Montreal Neurological institute (MNI) coordinate [x, y, z] = [38, −58, 36]. The degree of connectivity alteration was not related to the magnitude of change in serum adrenaline or symptom score. Whole brain exploratory analysis revealed no additional significant regional effects.
Conclusion: During hypoglycemia, we found a reduced functional connectivity primarily in the right angular gyrus, an area involved in converging and processing multisensory information in order to interpret and respond to events. The impact of hypoglycaemia on other RSNs is being explored. Disruption of these RSNs may be key in the process of internal recognition of hypoglycaemia and triggering of the stress response.



Transverse and sagittal montage images (A=euglycaemia, B=hypoglycaemia) of the brain areas functionally connected to the seed region at Montreal Neurological Institute reference coordinate [0, 0, 10]. Identified areas with significant connectivity include: the medial prefrontal cortex, precuneus and right and left inferior parietal lobes.

Supported by: Diabetes UK; NIHR/Wellcome Trust CRF
Disclosure: **C.E.D. Osborne:** Grants; Diabetes UK, NIHR/Wellcome Trust CRF.

148

Individualised nursing support reduces mortality in patients with type 2 diabetes following severe hypoglycaemia requiring ambulance attendance

K. Kulavarasalingam[1], B. Whittam[1], S. Cassidy[1], C. James[2], P. Baxter[3], S. Pearson[1,4], R.A. Ajjan[1,4];
[1]St James's University Hospital, Leeds, [2]Yorkshire Ambulance Service, Wakefield, [3]Division of Biostatistics, Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), University of Leeds, Leeds, [4]Division of Cardiovascular and Diabetes Research, LICAMM, University of Leeds, Leeds, UK.

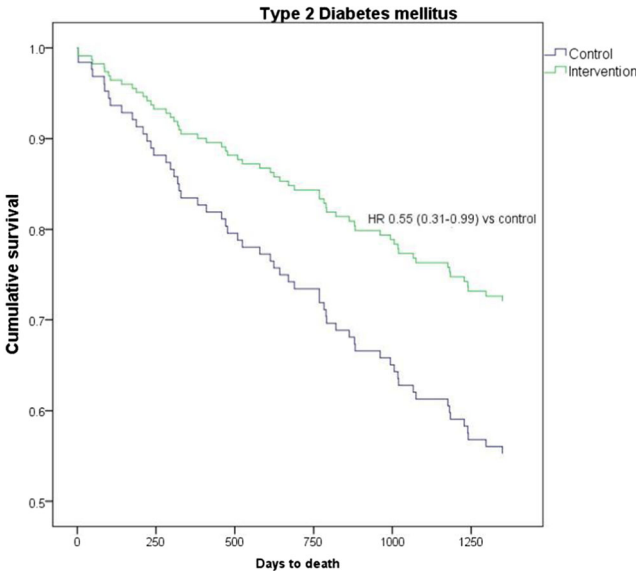
Background and aims: Mortality in patients with diabetes following emergency services call out for severe hypoglycaemia is high and it is unclear whether this can be modified using structured intervention. The aim of this work was to assess the impact of nurse-led intervention on

mortality of patients with diabetes following ambulance call out for hypoglycaemia.

Materials and methods: Patients with diabetes requiring ambulance services intervention for hypoglycaemia, in an area covering 5 million people, were recruited into the study after informed consent. Patients were randomised 1:1 to either receiving intensive nurse-led support (intervention arm) or managed using existing pathways (control arm). A third group of patients did not wish to participate in the study but agreed to have their data collected and were managed as per the control arm. Those assigned to the intensive arm received structured intervention that involved individualised nurse follow up with regular contact in order to alter therapy and provide support for a total period of 3 months. The primary outcome was all cause mortality comparing two study arm during the follow up period.

Results: A total of 323 individuals were recruited into the study between Feb-2013 and Dec-2017. Study withdrawal and lost contact occurred in 24 individuals (7.4%), while the remaining 299 patients were followed up for a median of 915 (IQR: 463–1358) days. Of these patients, 137 (45.8%) had type 1 diabetes mellitus (T1DM) and 150 (50.2%) had type 2 diabetes mellitus (T2DM).In patients with T1DM, there was no difference in mortality in the intervention compared with the control arm [10.4% vs 9.0%, respectively; $p = 0.79$; HR 1.20 (0.39–3.68)]. In contrast, patients with T2DM showed a significant reduction in mortality in the intervention compared with the control arm [27.8% vs 44.8%, respectively; $p = 0.04$, HR 0.55 (0.31–0.99)]. Cox regression analysis suggested that the relatively short period of intervention of 3 months continued to have an effect on mortality for over 3 years (Figure 1).

Conclusion: Our data suggest that in patients with T2DM and severe hypoglycaemic requiring ambulance call-out, close nurse-led individualised intervention reduces mortality compared with standard care. Large scale multicentre studies are warranted to investigate the role of structured nurse intervention on reducing mortality in T2DM patients with a history of severe hypoglycaemia. Figure 1



Supported by: LifeScan, part of Johnson & Johnson
Disclosure: **K. Kulavarasalingam:** None.

149

Reduction in severe hypoglycaemia in paediatric type 1 diabetes during the first year of continuous glucose monitoring: real-world data from the DPV registry

J. Hermann[1], R. Holl[1], B. Rami-Merhar[2], C. Freiberg[3], M. Papsch[4], A. Thon[5], B. Heidtmann[6], K. Placzeck[7], D. Agena[8], T. Kapellen[9], B. Schenk[10], J. Wolf[11], T. Danne[12], DPV initiative;

[1]Institute for Epidemiology, Ulm, Germany, [2]University of Vienna, Vienna, Austria, [3]University of Goettingen, Goettingen, Germany, [4]Childrens Hospital, Gelsenkirchen, Germany, [5]MHH, Hannover, Germany, [6]Catholic Children Hospital, Hamburg, Germany, [7]University Childrens Hospital, Halle, Germany, [8]Pediatric Practice, Hildesheim, Germany, [9]University Children Hospital, Leipzig, Germany, [10]Pediatric Clinic, Schwerin, Germany, [11]Childrens Hospital, Paderborn, Germany, [12]Childrens Hospital, Hannover, Germany.

Background and aims: Controlled studies indicated improved metabolic control in type-1 patients on continuous glucose monitoring. Patients in clinical studies are often biased towards patients with higher education level, higher adherence and better self-management. We therefore used real-world data from the German-Austrian-Luxemburg diabetes patient follow-up (DPV) registry, which includes >80% of pediatric patients in the participating countries, to longitudinally follow metabolic control (HbA1c) and acute complications (severe hypoglycemia, diabetic ketoacidosis (DKA)) in pediatric subjects during the first year after the initiation of continuous glucose monitoring (CGM or FGM).

Materials and methods: Anonymized patient records from the DPV registry were analyzed, using SQL for data integration and SAS 9.4 for statistical analysis. Patients with type-1 diabetes (T1-DM), less than 18 years of age, more than 1 year of diabetes duration and both baseline (6 months prior to CGM start) and at least 1 year of follow-up after initiation of continuous glucose monitoring were selected. Documented sensor use for at least 50% of the observation time was required. Severe hypoglycemia was defined by events requiring external help, or leading to coma or convulsion. DKA was defined by a pH <7.3. Non-parametric paired statistics (McNemar) and Poisson-regression models for repeated measurements were used.

Results: 3171 pediatric patients (median age 11.8 [Q1–Q3: 8.8–14.4] years, median DM-duration 3.9 [2.2–6.5] years, 51.7% males) fulfilled the inclusion criteria. 60.9% of subjects were treated with insulin pumps, 19.1% reported migration background. Metabolic control (median baseline HbA1c: 7.46% [6.86–8.11]/58.0 [51.4–65.1] mmol/mol) did not change within 6 or 12 months after initiation of CGM use. Rate of severe hypoglycemia was 10.7 events per 100 patient-years during the 6 months prior to CGM use, and decreased to 7.6 events during the first 6 months and to 5.9 events 6–12 months after CGM onset ($p < 0.002$). 3.9% of patients experienced a severe hypo event during the baseline period, compared to 2.1% of patients during sensor use ($p < 0.003$). Rate of DKA did not change significantly (baseline: 1.6 events/100 pat.-years, 6–12 months follow-up: 1.2 events per 100 pat.-years).

Conclusion: In this on average well-controlled pediatric group of type-1 patients, initiation and continuous use (>50% of days) of CGM was associated with rapid and persistent reduction of reported severe hypoglycemic events. Reduction of severe hypoglycemia was not accompanied by deterioration of metabolic control. Longer follow-up and additional end-points (e.g. hospitalization) together with subgroup-analysis on baseline metabolic control, diabetes treatment and type of glucose monitoring will allow additional insights into outcome of CGM.

Supported by: German Diabetes Society (DDG), German Center for Diabetes Research (DZD), A
Disclosure: **J. Hermann:** None.

150

Nasal glucagon: a viable alternative to treat insulin-induced hypoglycaemia in adults with type 1 diabetes

J. Suico[1], U. Hövelmann[2], S. Zhang[1], T. Shen[1], B. Bergman[1], J. Sherr[3], E. Zijlstra[2], B. Frier[4], L. Plum-Moerschel[5];

[1]Eli Lilly & Company, Indianapolis, USA, [2]Profil Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany, [3]Yale University, New Haven, USA, [4]University of Edinburgh, Edinburgh, UK, [5]Profil Profil Institut für Stoffwechselforschung GmbH, Mainz, Germany.

Background and aims: Any insulin-treated individual with diabetes is at risk of severe hypoglycaemia (SH). Glucagon is available as a rescue medication in these instances. Currently available commercial glucagon products require reconstitution and injection, which are cumbersome during an emergency situation. Nasal glucagon (NG) is a nasally administered, drug-device combination product that consists of a dry powder spray formulation with 3-mg synthetic glucagon contained within a single-use device. This study in adults with type 1 diabetes (T1D) aimed to demonstrate non-inferiority between intramuscular glucagon (IMG) and NG as treatment for insulin-induced hypoglycaemia.
Materials and methods: This randomised, two-period, crossover trial was conducted at two clinical sites and used a NG drug product manufactured at commercial scale. The comparator was glucagon [rDNA origin] injection. Hypoglycaemia (plasma glucose [PG] <3.3 mmol/L) was induced by an intravenous insulin infusion. Five minutes after stopping insulin, either 3-mg NG or 1-mg IMG was administered followed by multiple PG measurements up to 90 min. Treatment success was defined as an increase in PG to ≥3.9 mmol/L or an increase of ≥1.1 mmol/L from the PG nadir within 30 min of receiving glucagon. Non-inferiority of NG was declared if the upper limit of the two-sided 95% CI of the difference in percentage of patients achieving treatment success (IMG-NG) was <10%. Besides spontaneously reported adverse events (AEs), a Nasal and Non-Nasal Symptom Questionnaire (NNSQ) assessed local tolerability of NG.
Results: Of the 66 participants included in the primary efficacy analysis who received both NG and IMG, 100% achieved treatment success. The study demonstrated non-inferiority of NG to IMG. All participants achieved treatment success by 25 min with the mean time to treatment success of 11.4 min (NG) and 9.8 min (IMG). As shown in Figure 1, similar glucose responses were observed with NG and IMG within 40 min post glucagon administration. No deaths or other serious AEs occurred. Forty-eight AEs occurred after NG and 51 after IMG. Most AEs were mild and transient, and the frequency was similar between IMG and NG. Treatment-emergent AEs with an incidence ≥5% were nausea (31% NG; 42% IMG), vomiting (14% NG; 17% IMG), and headache (16% NG; 10% IMG). After NG, very common (≥10%) symptoms from the NNSQ included watery eyes, nasal itching, nasal congestion, runny nose, sneezing, redness of eyes, itchy eyes, and itching of throat.
Conclusion: Nasal glucagon was as efficacious and safe as intramuscular glucagon for the treatment of insulin-induced hypoglycaemia in adults, thus supporting the use of nasal glucagon as a rescue treatment for severe hypoglycaemia.

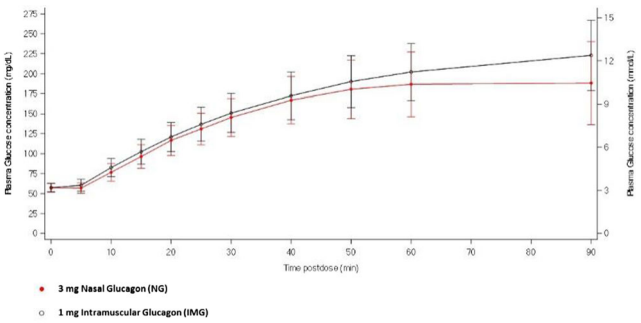


Figure 1. Arithmetic mean (±SD) profile of plasma glucose concentration after a single dose of 3 mg NG or 1 mg IMG

Clinical Trial Registration Number: NCT03339453
Disclosure: **J. Suico:** Stock/Shareholding; Eli Lilly Shareholder.

OP 26 Diabetes: eat and heart beat

151

Effects of increased fiber and reduced red meat intake, combined with caloric restriction, on cardiometabolic risk: a randomised and controlled dietary intervention study

C. Willmann[1,2], M. Heni[1,2], K. Linder[1,2], R. Wagner[1,2], N. Stefan[1,2], J. Machann[2,3], H.-U. Häring[1,2], A. Fritsche[1,2];
[1]Department of Internal Medicine IV, University Hospital of Tübingen, Tübingen, [2]Institute for Diabetes Research and Metabolic Diseases (IDM) of the Helmholtz Center Munich at the University of Tübingen, Tübingen, [3]Section on Experimental Radiology, Department of Diagnostic and Interventional Radiology, University Hospital Tübingen, Tübingen, Germany.

Background and aims: Epidemiological studies suggest that increased intake of red meat associates with a higher, while increased intake of fibers associates with a lower risk of type 2 diabetes. We, thus, conducted a randomized intervention study to investigate the effects of these nutritional factors on glucose and lipid metabolism, body fat distribution and liver fat content, in subjects at increased risk of type 2 diabetes.
Materials and methods: This prospective, randomized and controlled dietary intervention study was performed over six months. In the control group ($N = 40$) the participants decreased their daily caloric intake by 400 Kcal. In addition to this caloric restriction, the "no red meat" group ($N = 48$) lowered the intake of red meat and the "fiber" group ($N = 44$) increased intake of fibers to 40 gr/d. Before and after the intervention, anthropometric parameters and a frequently-sampled oral glucose tolerance test were performed. Body fat mass and distribution and liver fat content were assessed by magnetic resonance imaging and ^{1}H-MR spectroscopy.
Results: Glucose tolerance and insulin sensitivity improved during the intervention in all groups (all $p < 0.03$). Body fat mass, as well as visceral fat mass decreased in all groups (all $p < 0.03$). Multivariate analysis revealed that these changes did not differ between the groups. Liver fat content decreased significantly in the "no red meat" and "fiber" groups, but not in the control group.
Conclusion: Our data suggest that reduced intake of red meat or increased intake of fibers may have favourable effects on liver fat content. However, in combination with caloric restriction, there seems to be no additional beneficial impact on the improvement of other cardiometabolic risk parameters.
Clinical Trial Registration Number: NCT 03231839
Supported by: BMBF, DZD
Disclosure: **C. Willmann:** None.

152

Eldecalcitol, a vitamin D analogue, for diabetes prevention in impaired glucose tolerance: DPVD study

T. Kawahara[1], G. Suzuki[2], T. Inazu[3], S. Mizuno[4], F. Kasagi[4], Y. Okada[1], Y. Tanaka[1], DPVD clinical study group;
[1]University of Occupational and Environmental Health, Kitakyushu, [2]International University of Health and Welfare Clinic, Ohtawara, Japan, [3]Ritsumeikan University, Kusatsu, [4]Radiation Effects Association, Tokyo, Japan.

Background and aims: In observational studies, it was clear that vitamin D deficiency is associated with insulin resistance and risk of future diabetes. However, the efficacy of vitamin D supplementation in randomized controlled trials for improving glucose tolerance or prevention of type 2 diabetes is still controversial.
Materials and methods: We conducted the *Diabetes Prevention on Vitamin D* (DPVD) study which was a large, randomized, double-blind, placebo-controlled study to examine whether eldecalcitol, an active form of vitamin D analog, can reduce the risk of type 2 diabetes in patients with

impaired glucose tolerance. Participants were randomly assigned to receive eldecalcitol or placebo. The primary endpoint was the incidence of type 2 diabetes and the secondary endpoint was the conversion to normoglycemia. The study duration was 3 years.

Results: The mean follow up was 2.6 years. A total of 1256 participants were enrolled in the study. Fifty-seven of 630 (9.0%) participants in the eldecalcitol group and 64 of 626 (10.2%) in the placebo group developed type 2 diabetes (hazard ratio, 0.87; 95% confidence interval, 0.68 to 1.09; $p = 0.37$). In the subgroup participants with vitamin D deficiency (serum 25-hydroxyvitamin D <20 ng/ml), the difference of the incidence of type 2 diabetes between the two groups was greater; however, there was no statistical significance. Two hundred ninety-five of 630 (46.8%) participants in the eldecalcitol group and 267 of 626 (42.7%) in the placebo group achieved normoglycemia (hazard ratio, 1.10; 95% confidence interval, 0.93 to 1.31; $p = 0.45$). At the end of the study, the mean fasting plasma glucose level was significantly lower in the eldecalcitol group (110.5 mg/dl) than the placebo group (112.8 mg/dl, $p = 0.046$), though plasma glucose levels 2 hours after an oral glucose load were not significantly lower in the eldecalcitol group (165.0 mg/dl vs. 163.1 mg/dl, $p = 0.071$). No serious adverse events related to the intervention were recorded.

Conclusion: Our study showed that treatment with eldecalcitol was not associated with a reduction in the incidence of type 2 diabetes or an increase in the conversion to normal glucose tolerance among patients with impaired glucose tolerance.

Clinical Trial Registration Number: UMIN000010758

Supported by: DPVD clinical study programme

Disclosure: **T. Kawahara:** None.

153

Sweetened beverage consumption is associated with autoimmune diabetes in adults only among low risk HLA genotype carriers

J.E. Löfvenborg[1], E. Ahlqvist[2], L. Alfredsson[1], T. Andersson[1,3], M. Dorkhan[2], L. Groop[2,4], A. Rosengren[2], T. Tuomi[5,6], A. Wolk[1], S. Carlsson[1];

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of Clinical Sciences, Lund University, Malmö, Sweden, [3]Center for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden, [4]Finnish Institute of Molecular Medicine, Helsinki University, Helsinki, Finland, [5]Div. of Endocrinology, Abdominal Centre, Helsinki University Hospital, Helsinki, Finland, [6]Folkhälsan Research Center, Helsinki, Finland.

Background and aims: Sweetened beverage consumption is associated with increased risk of type 2 diabetes (T2D) as well as autoimmune diabetes. Findings for type 1 diabetes in children suggest that HLA genotype may modify the association. We aimed to investigate whether the association between sweetened beverage intake and latent autoimmune diabetes in adults (LADA) and T2D is modified by HLA DR-DQ genotype.

Materials and methods: Swedish case-control data including incident cases of LADA ($n = 384$) and T2D ($n = 1240$) with matched population-based controls ($n = 879$) was used. Cases were classified based on onset age (≥35 years), GAD autoantibodies (GADA) and C-peptide. Information on diet and potential confounding factors was obtained through an extensive health and lifestyle questionnaire. HLA genotyping was based on SNP data and grouped as high/low risk. Logistic regression models adjusted for age, sex, education, physical activity, smoking, and alcohol intake were used to estimate OR of diabetes (95% CI) per 1 daily 200 mL serving. Dietary factors had little impact on the estimates and are thus not included. BMI was considered as a mediator separately. The association with GADA, insulin resistance (HOMA-IR) and beta cell function (HOMA-B) was explored through linear regression.

Results: Consumption of sweetened beverages was associated with increased risk of LADA and T2D; each daily 200 mL serving conferred 15% increased risk of LADA (OR 1.15, 95% CI 1.01–1.30) and 20% increased risk of T2D (OR 1.20, 95% CI 1.08–1.33). The increased risk may partly be mediated through BMI (Table 1). In HLA-stratified analysis, the association between sweetened beverages and LADA was present also after adjustment for BMI but only for those having low risk HLA genotypes (OR 1.25, 95% CI 1.00–1.56). Similar tendencies were seen for T2D. Sweetened beverage intake was positively associated with HOMA-IR in individuals with low risk HLA genotypes but not in those with high risk HLA variants. These associations were similar in LADA and T2D. Sweetened beverage intake was not associated with HOMA-B or GADA (Table 1).

Conclusion: Our findings suggest that the increased risk of LADA and T2D conferred by consumption of sweetened beverages only pertains to individuals with low risk HLA genotypes, mainly through mechanisms related to overweight and insulin resistance. This concurs with contemporary literature indicating that environmental factors are more important in the development of autoimmune diabetes in low risk HLA genotypes.

Table 1. OR and 95% CI of LADA and type 2 diabetes, and change in GADA, HOMA-IR, and HOMA-B per one daily 200 mL serving of sweetened beverages.

Per 1 daily serving of sweetened beverages	LADA			Type 2 diabetes		
	Overall	High risk HLA[a]	Low risk HLA[b]	Overall	High risk HLA[a]	Low risk HLA[b]
Cases/controls	384/879	235/278	149/601	1240/879	389/278	851/601
OR[c] (95% CI)	1.15 (1.01-1.30)	1.04 (0.87-1.25)	1.32 (1.06-1.64)	1.20 (1.08-1.33)	1.08 (0.92-1.27)	1.27 (1.11-1.45)
OR[d] (95% CI)	1.11 (0.97-1.26)	1.01 (0.84-1.21)	1.25 (1.00-1.56)	1.06 (0.95-1.18)	0.92 (0.76-1.11)	1.13 (0.97-1.31)
% change in GADA[c]	6.4% (p=0.4888)	11.4% (p=0.3606)	-2.4% (p=0.8609)	---	---	---
% change in GADA[d]	9.6% (p=0.2906)	12.8% (p=0.2898)	5.3% (p=0.7092)	---	---	---
% change in HOMA-IR[c]	5.2% (p=0.0900)	0.8% (p=0.8165)	17.5% (p=0.0047)	3.5% (p=0.0008)	3.8% (p=0.1260)	3.5% (p=0.0018)
% change in HOMA-IR[d]	4.4% (p=0.1421)	0.5% (p=0.8958)	13.0% (p=0.0293)	3.3% (p=0.0015)	2.1% (p=0.3881)	3.7% (p=0.0012)
% change in HOMA-B[c]	1.5% (p=0.6900)	1.3% (p=0.7601)	2.6% (p=0.7562)	-1,5% (p=0.1776)	-1.0% (p=0.6930)	-1.7% (p=0.1860)
% change in HOMA-B[d]	-0.6% (p=0.8679)	-0.1% (p=0.9819)	-5.0% (p=0.5108)	-1.7% (p=0.1362)	-2.6%(p= 0.3025)	-1.5% (p=0.2162)

[a] High risk HLA: DR4/4, DR3/4, DR3/3, DR4-DQ8. [b] Low risk HLA: DR4/x, DR3/x, DRx/x, DR4-DQ7), where x=neither DR4 nor DR3.
[c] Model 1 adjusted for age, sex, education, physical activity, smoking, alcohol intake. [d] Model 2 adjusted for same as model 1 + BMI.

Supported by: VR, Forte, Swedish Diabetes Association, NovoNordisk, SNF

Disclosure: **J.E. Löfvenborg:** None.

154

Plant versus animal based diets and insulin resistance, prediabetes and type 2 diabetes: the Rotterdam Study

T. Voortman, M. Zuurmond, O. Franco, Z. Chen;
Erasmus University Medical Center, Rotterdam, Netherlands.

Background and aims: Vegan or vegetarian diets have been suggested to reduce type 2 diabetes (T2D) risk. However, not much is known on whether variation in the degree of having a plant-based versus animal-based diet may be beneficial for prevention of T2D. Therefore, we aimed to investigate whether level of adherence to a diet high in plant-based foods and low in animal-based foods was associated with insulin resistance, prediabetes, and T2D.

Materials and methods: Our analysis included 6798 participants (62.7 ± 7.8 years) from the Rotterdam Study (RS), a prospective population-based cohort in the Netherlands. Dietary intake data were collected with food-frequency questionnaires at baseline of three sub-cohorts of RS (RS-I-1: 1989–93, RS-II-1: 2000–01, RS-III-1: 2006–08). We constructed a continuous plant-based dietary index (range 0–92) assessing adherence to a plant-based versus animal-based diet. Higher score on the plant-based dietary index reflected more plant-based foods intake and less animal-based foods intake. Insulin resistance at baseline and follow-up was assessed using homeostasis model assessment of insulin resistance (HOMA-IR). Prediabetes and T2D were collected from general practitioners' records, pharmacies' databases, and follow-up examinations in our research center until 2012. We used linear mixed models to examine associations of score on the plant-based dietary index with longitudinal HOMA-IR, and used cox proportional-hazards regression models to examine associations of score on the plant-based dietary index with risk of prediabetes and T2D.

Results: During median 5.7 years, and 7.3 years of follow-up, we documented 928 prediabetes cases and 642 T2D cases. After adjusting for

sociodemographic and lifestyle factors, a higher score on the overall plant-based dietary index was associated with lower insulin resistance (per 10 points higher score on the index per day: β = −0.09; 95% CI: −0.10; −0.08), lower prediabetes risk (HR = 0.89; 95% CI: 0.81; 0.98), and lower T2D risk (HR = 0.82; 95% CI: 0.73; 0.92)). After additional adjustment for BMI, associations attenuated and remained statistically significant for longitudinal insulin resistance (−0.05 (−0.06; −0.04)) and T2D risk (0.87 (0.79; 0.98)), but no longer for prediabetes risk.
Conclusion: A more plant-based and less animal-based diet may lower risk of insulin resistance, prediabetes and T2D. These findings strengthen recent dietary recommendations to adopt a more plant-based diet.
Clinical Trial Registration Number: NTR6831
Disclosure: **T. Voortman:** None.

155

Beneficial effects of three months exercise on plasma adipokines levels and inflammation-related gene expression in subcutaneous adipose tissue in men with prediabetes

H.L. Gulseth[1,2], S. Lee[1,2], T.M. Langleite[2], F. Norheim[2], K.I. Birkeland[1,2], C.A. Drevon[2];
[1]Oslo University Hospital, Oslo, [2]University of Oslo, Oslo, Norway.

Background and aims: Obesity and insulin resistance promote several changes in adipose tissue including production and secretion of adipokines. Physical exercise improves insulin sensitivity perhaps through effects on adipokines. Our aim was to examine the effect of long-term exercise on large-scale adipose tissue gene expression and plasma adipokines concentrations, and their relationships with insulin sensitivity in men with or without prediabetes (PD).
Materials and methods: In the MyoGlu clinical study of 26 sedentary men (13 prediabetes (PD) and 13 controls) aged 40–65 years, a 12-weeks intensive combined strength and endurance exercise intervention increased insulin sensitivity by 30%. Before and after 12 w of intervention insulin sensitivity was measured by hyperinsulinemic-euglycemic-clamp, transcriptomics by global RNA-sequencing and RT-PCR of adipose tissue and skeletal muscle biopsies, and plasma adipokines by ELISA. .
Results: Intersected results from three approaches to RNA-sequencing analysis of adipose tissue revealed 90 genes in PD and seven genes in controls responding to 12 w exercise. mRNA-sequencing and RT-PCR results were highly coherent. Evidence for encoding secreted proteins existed for 62/90 and 5/7 genes in PD and controls respectively. The 90 genes that responded for exercise in PD were mostly related to the immune system and inflammatory processes. They displayed elevated expression levels in PD at baseline, but were partly normalized after 12 w exercise, as compared to controls. Baseline expressions of these genes were negatively correlated with insulin sensitivity both at baseline ($r = -0.49$, $p = 0.016$) and with changes in response to 12 w exercise ($r = -0.46$, $p = 0.025$) across all men. Adipose tissue, but not skeletal muscle expression levels of *LEP, ADIPOQ, IL6, SFRP4* and *OPG* (but not *THBS4*) correlated with corresponding plasma protein concentrations. Plasma SFRP4 and OPG concentrations were elevated in PD vs. controls at baseline, and were lowered after 12 w exercise in PD, attenuating the group difference.
Conclusion: We discovered dysregulated inflammation-related genes in PD, which were negatively associated with insulin sensitivity, and partly normalized after 12 w of physical exercise as compared to controls. These gene expression patterns were reflected in plasma adipokines concentrations, and may provide important links to glucose metabolism.
Clinical Trial Registration Number: NCT01803568
Disclosure: **H.L. Gulseth:** None.

156

Gut microbiome, insulin resistance, and type 2 diabetes: a large population-based study

Z. Chen[1], D. Radjabzadeh[2], O. Franco[1], M. Ikram[1], A. Uitterlinden[2], R. Kraaij[2], T. Voortman[1];
[1]Department of Epidemiology, Erasmus Medical Center, Rotterdam, [2]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Background and aims: Few data on gut microbiome linked to development of type 2 diabetes (T2D) are available. Therefore, we aimed to examine associations of gut microbiome with insulin resistance and T2D in a large Dutch middle-aged and elderly population.
Materials and methods: Our current cross-sectional study was embedded within the Rotterdam study (RS), a population-based cohort study including people aged ≥45 years living in the Ommoord District of Rotterdam. The study has been approved by the Medical Ethics Committee of Erasmus University Medical Center and all participants gave written informed consent. We included 1146 participants (median age: 57 years, 25%–75% range: 51–61 years) from the second examination cycle of the third sub-cohort of the Rotterdam study (RS-III-2: 2012–2014). For the 1146 participants, we collected stool in 2012–13. We detected gut microbiome via stool using sequencing of the 16S rRNA gene. Fasting blood was drawn in 2012–13 to measure glucose and insulin, we calculated the homeostatic model assessment of glucose (HOMA-IR) for insulin resistance. We identified T2D cases using information from general practitioners, pharmacies' databases, and follow-up examinations in our research center until 2012. Alpha diversity and beta diversity of gut microbiome were quantified by Shannon index and Bray-Curtis distance, respectively. We used linear regression models to examine association between Shannon index and insulin resistance, and used logistical regression models to examine association between Shannon index with T2D. Adonis permutation *P* value calculation was used to examine whether Bray-Curtis distance differed by insulin resistance and T2D. We used Multivariate Association with Linear Models (MaAsLin) to examine gut microbial communities in relation to insulin resistance and T2D at multiple taxonomical levels from phylum to genus. We confined the analyses to 11 phyla, 19 classes, 25 orders, 44 families, and 184 genera.
Results: Of 1146 participants, 90 participants had T2D. Of 1056 participants without T2D, 1022 participants had data on insulin resistance (median: 2.1, 25%–75% range: 1.6–2.7). After multivariate adjustment for technical covariates (run batch, time-in -email of stool), age, sex, total energy intake, diet quality score, education, smoking, physical activity, and BMI, higher Shannon index was associated with lower HOMA-IR ($\beta = -0.13$ (95%CI: −0.28, −0.09)), and lower odds of T2D (OR = −0.44 (−0.85, −0.03)). Bray-Curtis distance of beta-diversity was also linked to insulin resistance (genus level, $R^2 = 0.005$, $p = 0.001$), and T2D (genus level, $R^2 = 0.003$, $p = 0.001$). In MaAsLin analyses, higher relative abundance of two genera: *Acetitomaculum* ($\beta = -0.001$, $p = 3*10^{-9}$, $q = 0.004$) and *RuminococcaceaeUCG010* ($\beta = -0.006$, $p = 2*10^{-4}$, $q = 0.02$) were associated lower HOMA-IR, and participants with T2D had lower relative abundance of two genera: *Clostridiumsensustricto1* ($\beta = -0.04$, $p = 4*10^{-7}$, $q = 0.0001$) and *RuminococcaceaeUCG010* ($\beta = -0.03$, $p = 6*10^{-7}$, $q = 0.0001$).
Conclusion: Our findings indicate that increased diversity of gut microbiome may be beneficial for prevention of T2D. Especially, genera: *Acetitomaculum, RuminococcaceaeUCG010, and Clostridiumsensustricto1* may play an important role in the development of T2D.
Clinical Trial Registration Number: NTR6831
Disclosure: **Z. Chen:** None.

OP 27 Liver at large

157

Insulin regulates the hypothalamic mitochondrial chaperone complex Hsp60/10 and impacts the mitochondrial stress response

K. Wardelmann[1,2], S. Blümel[1,2], M. Rath[1,2], K. Warnke[1,2], E. Alfine[1,2], B.-F. Belgardt[3,2], A. Kleinridders[1,2];
[1]German Institute of Human Nutrition (DifE), Potsdam-Rehbrücke, [2]German Center for Diabetes Research (DZD), Neuherberge, Germany, [3]German Diabetes Center (DDZ), Düsseldorf, Germany.

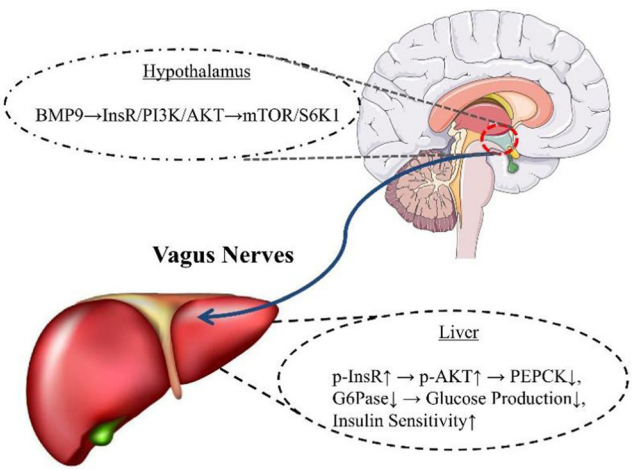
Background and aims: Type 2 diabetic (T2D) mice exhibit brain insulin resistance, mitochondrial dysfunction and a decrease in the heat shock protein (Hsp)60. Hsp60 with its co-chaperone Hsp10 are crucial for mitochondrial matrix protein folding and represent the key protein-complex of the mitochondrial unfolded protein response (UPRmt) which is induced by the accumulation of misfolded/unfolded proteins. UPRmt is crucial for neuronal health and brain function and knockdown (KD) of UPRmt genes causes metabolic alterations and neurodegeneration. Thus understanding the metabolic regulation of these chaperones and identifying positive regulators for this pathway is vital to ensure proper brain function. Here, we investigate the effect of insulin action on UPRmt regulation and mitochondrial function *in vitro* using hypothalamic neurons and *in vivo* by characterizing the regulation of UPRmt in insulin-resistant brains.
Materials and methods: To this end, we analyzed the expression of key regulators of the UPRmt in insulin deficient and resistant brains using db/db mice, streptozotocin-induced diabetic mice along with C57BL/6N mice on a high fat diet. To address the effect of insulin or insulin resistance on gene and protein expression, we either treated the hypothalamic cell line CLU183 with 100 nM of insulin or mimic insulin-resistance by pretreating cells with 250 μM palmitate. Following we analyzed mitochondrial chaperones expression levels and mitochondrial respiration using the Seahorse XF Analyzer. To further emphasize the direct effect of insulin, we investigated insulin-induced regulation of key proteins of the UPRmt using qPCR and western blot technique.
Results: We observed that T2D mice suffer from brain insulin resistance together with mitochondrial dysfunction which are linked to reduced levels of Hsp60 and 10. Interestingly, mouse models deficient for insulin signaling or exhibiting reduced insulin sensitivity showed a reduction in brain specific Hsp60 and Hsp10 expression by ~50% along with a decrease of their transcription factor CHOP, a key mediator of UPRmt and ER stress. Fitting to these data, we could also demonstrate that the gene expression of Hsp60 and Hsp10 in serum-starved hypothalamic cells over the course of 24 hours is decreased by 15–30%. Palmitate-induced insulin resistance also causes decreased Hsp60 protein expression with a concomitant reduction in basal mitochondrial respiration. Conversely, both chaperones are upregulated by 25–60% after 16h of 100 nM insulin stimulation in hypothalamic neurons on mRNA and protein expression level, as well as a four-fold increased gene expression of CHOP, demonstrating that hypothalamic UPRmt is controlled by insulin action.
Conclusion: In conclusion, we were able to demonstrate for the first time that the hypothalamic UPRmt genes Hsp60 and Hsp10 are insulin regulated genes. Along with it, our results show clearly the importance of functional insulin signaling for the regulation of UPRmt and with this, the ill-fate of mitochondrial function in an insulin-resistant brain.
Supported by: DFG
Disclosure: **K. Wardelmann:** None.

158

BMP9 in the hypothalamus regulates hepatic glucose production and hepatic insulin sensitivity through the central PI3K/Akt/mTOR pathway

Q. Li[1], L. Li[1], G. Yang[2];
[1]Key Laboratory of Diagnostic Medicine (Ministry of Education) and Department of Clinical Biochemistry, Chongqing Medical University, Chongqing, [2]Department of Endocrinology, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China.

Background and aims: Recent studies have shown that bone morphogenetic protein 9 (BMP9) whoes ligands are found to exist in hypothalamus is associated with glucose metabolism and insulin resistance (IR). However, the precise mechanism for how the central BMP9 signaling regulate hepatic glucose production (HGP) and IR remains unclear. The present study was designed to investigate the effects of BMP9 activity in hypothalamus on glucose metabolism and insulin sensitivity and explored the possible mechanism.
Materials and methods: We first performed intracerebroventricular (ICV) injections of adenovirus expressing BMP9 (Ad-BMP9) or adenovirus encoding enhanced green fluorescence protein (Ad-GFP) and evaluated activation of potential signaling candidates. Moreover we examined the expression of hypothalamic BMP9 in db/db, normal chow diet (NCD) feeding or high fat diet(HFD)-fed WT, Adipoq-/- mice and ICV Ad-BMP9 mice. Subsequently, energy expenditure was measured in mice treated with ICV Ad-BMP9 or Ad-GFP. We then examined the effects of overexpression of hypothalamic BMP9 and the hyperinsulinemic-euglycemic clamp (HEC) in NCD- or HFD-fed mice. Furthermore the mRNA and protein levels of PEPCK and G-6-Pase were examined to investigate the effects of ICV Ad-BMP9 on improving hepatic IR. We further examined the effects of ICV Ad-BMP9 on insulin's ability to promote the immunostaining of phosphatidylinositol 3, 4, 5-trisphosphate (PIP3) formation in the hypothalamic neurons.
Results: We found that BMP9 expression in the hypothalamus was downregulated in obese or IR mice. The overexpression of BMP9 in the hypothalamus decreased body weight, food intake and blood glucose, and elevated energy expenditure in HFD feeding mice. Importantly, central BMP9 ameliorated hepatic IR and suppressed HGP in HFD-fed mice. Central BMP9 induced hepatic insulin action and the related metabolic effects were abolished by ICV rapamycin, an inhibitor of the mTOR signaling. Furthermore, central BMP9 increased insulin's ability to promote insulin receptor (InsR) and Akt phosphorylation and to lead phosphatidylinositol 3, 4, 5-trisphosphate formation in hypothalamic neurons. Thereby, the current study provided the first evidence suggesting that activating BMP9 in hypothalamus ameliorates central IR by promoting insulin's ability to activate the mTOR/PI3K/Akt pathways and revealed that the central nervous system (CNS) may be an important target for the metabolic action of BMP9.
Conclusion: These findings reveal a novel role of BMP9 in CNS for the regulation of glucose metabolism and hepatic insulin sensitivity through the central PI3K/Akt/mTOR pathway in vivo.



Supported by: National Natural Science Foundation of China (NO:81570752, 81670755)
Disclosure: **Q. Li:** None.

159

Hepatocyte p110α controls insulin signalling but is dispensable for free fatty acid and glucose sensing

M. Regnier[1], A. Polizzi[1], C. Lukowicz[1], S. Smati[1], A. Fougerat[1], S. Ellero-Simatos[1], K. Anderson[2], F. Lasserre[1], Y. Lippi[1], J. Bertrand-Michel[3], L. Stephens[2], N. Loiseau[1], C. Postic[4], A. Montagner[3], H. Guillou[1];
[1]Toxalim, UMR 1331, Toulouse, France, [2]Babraham Institute, Cambridge, UK, [3]I2MC, Toulouse, France, [4]Institut Cochin, Paris, France.

Background and aims: Class IA phosphoinositide 3-kinase (PI3K) is involved in insulin signaling through the production of the second messenger phosphatidylinositol 3,4,5 tri-phosphate (PIP3). Mouse with hepatocyte-specific deletion of p110α ("p110α hep-/-"), the catalytic subunit of PI3Kα are glucose intolerant but protected from hepatic steatosis. We used a system biology approach to identify pathways regulated by p110α *in vivo*.
Materials and methods: In this study, p110α hep+/+ and p110α hep-/- were used in different nutritional states: fed, fasted and refed. We combined transcriptomic, lipidomic, proteomic, metabolomic and histological approaches. *In vivo* studies were conducted under the EU guidelines for the use and care of laboratory animals, and they were approved by an independent Ethics Committee.
Results: As previously reported, we confirm that p110α deletion leads to glucose intolerance without steatosis in response to aging and to high fat-induced obesity. We also provide evidence that "p110α hep-/-" mice have normal circadian control of liver transcriptome. Then, we investigated the influence of hepatocyte p110α-dependent signaling on liver transcriptome and proteome in fed and in fasted mice. In line with the role of PI3Kα in insulin signaling, we observed that major transcriptional targets of insulin are disrupted when p110α is lacking in fed mice. This is associated with decreased phosphorylation of insulin-activated proteins. Interestingly, we show that this depends in a 50% reduction in PI(3,4,5)P3 production in response to insulin *in vivo*. However, we found that p110α is dispensable for Chrebp-mediated glucose sensing in hepatocytes. More surprisingly, in fasted mice, p110α deficiency is also very influential on liver transcriptome and lipidome. Gene ontology analysis revealed a major effect on PPARα signaling. Given the well-established role of PPARα in fasting, we further analysed the expression of PPARα target genes in "p110α hep-/-" mice. These genes, including the hepatokine FGF21, which is produced by hepatocytes in a PPARα-dependant response to adipose lipolysis, were highly increased in "p110α hep-/-" fasted mice. Conversely, p110α is dispensable for the inhibition of PPARα target during refeeding. This led us to postulate that, in "p110α hep-/-" mice, the fasting-induced changes in PPARα activity leading to an increase in FGF21 expression and secretion depends on adipose tissue fatty acid remodeling. Consistent with this hypothesis, we found that liver and adipose tissue fatty acid profile is modified in "p110α hep-/-" mice in response to fasting.
Conclusion: Altogether, our data evidence that liver p110α dependent effect on AKT is dispensable for glucose and free fatty acid signaling by CHREBP and PPARα respectively. Moreover, we highlight lipolysis as the dominant signal for hepatocyte PPARα activity.
Disclosure: **M. Regnier:** None.

160

Longitudinal study of the pathogenesis of hepatic insulin resistance in diet-induced obese mice

C. Geißler[1], C. Krause[1], M. Kähler[2], I. Cascorbi[2], H. Kirchner[1];
[1]Medical Department I, University of Lübeck, Lübeck, [2]Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Kiel, Germany.

Background and aims: Excess caloric intake leads to obesity and insulin resistance, which is also associated with hepatic fat accumulation. The transition from glucose tolerance to insulin resistance is characterized by altered expression of metabolically relevant genes. As longitudinal studies are scarce, it often remains unclear when this shift happens and whether these changes are cause or consequence of insulin resistance. Therefore we designed a longitudinal experiment in mice to study alterations in gene expression during the development of insulin resistance and to identify the timepoint of the metabolic switch.
Materials and methods: Our longitudinal study based on the frequently used diet-induced obesity model of C57BL/6N mice that were fed high fat diet (HFD) containing 60% fat for up to 12 weeks. Control mice were fed with standard chow. After 1, 2, 4, 8 and 12 weeks 8 mice of each group underwent an i.p. glucose tolerance test and the day after mice were sacrificed for tissue collection. RNA and DNA was extracted from liver. To identify differentially regulated metabolic pathways and genes in early and/or late stages of insulin resistance in liver a transcriptome profiling using microarrays was performed. Differentially expressed genes were validated by qRT-PCR. Hepatic triglyceride (TG) levels were determined by a calorimetric assay. For statistical analysis a 2-Way ANOVA with Holm-Bonferroni correction was used.
Results: The glucose tolerance test revealed that 8 weeks of HFD are sufficient to induce glucose intolerance ($p = 0.0005$). Short term feeding with HFD for 1 and 2 weeks led to slightly elevated TG-levels ($p < 0.01$ and $p < 0.0001$, respectively). HFD-feeding for 8 and 12 weeks led to excessive TG accumulation in the liver ($p < 0.0001$ for both). Pathway analysis of the differentially expressed genes revealed an involvement of the fatty acid metabolism and peroxisome proliferator-activated receptor (PPAR) signaling. 1 week of HFD-feeding resulted mainly in decreased expression of genes activated by PPAR signaling, for example *Fasn* (qRT-PCR data, fold change 0.32, $p < 0.0001$) and *Scd1* (qRT-PCR data, fold change 0.08, $p < 0.0001$), whereas 12 weeks of HFD-feeding induced higher mRNA level of genes activated by PPAR signaling, for instance *Cd36* (qRT-PCR data, fold change 7.52, $p < 0.0001$).
Conclusion: These results indicate that feeding a diet rich in fat causes glucose intolerance after already 8 weeks. However, even before manifestation of the insulin resistance gene expression of metabolically important genes is altered. The transcriptome profiling shows a distinct expression pattern of genes at early and late timepoints in liver. The results indicate a metabolic switch between week 4 and week 8 of HFD-feeding. In the future we will analyze if epigenetic mechanisms are responsible for this switch in hepatic gene expression and diabetes etiology.
Supported by: Emmy-Noether-Program from the DFG
Disclosure: **C. Geißler:** None.

161

Leptin therapy suppresses alanine utilisation in type 1 diabetic mice independent of glutamic pyruvic transaminase

M.M. Kwon[1], S. Chen[2], R.K. Baker[1], P.R. Cullis[2], T.J. Kieffer[1];
[1]Cellular and Physiological Sciences, University of British Columbia, Vancouver, [2]Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.

Background and aims: Leptin lowers blood glucose levels in diabetic rodents and decreased glucose production in the liver has been suggested to be the mechanism. Since the amino acid alanine is a substrate for glucose production, we sought to determine whether alanine utilization is suppressed by leptin and assess the role of glutamic pyruvic transaminase (*Gpt*), the gene responsible for alanine catabolism, in leptin action.
Materials and methods: We administered leptin by pumps to streptozotocin (STZ)-diabetic mice and performed alanine tolerance tests

and pyruvate tolerance tests as a comparison on day 5 post pump implant. Alanine tolerance tests pointed towards decreased alanine utilization, and the gene responsible for alanine catabolism, *Gpt*, was downregulated in leptin treated STZ-mice. To determine whether this downregulation of *Gpt* is required and sufficient for the anti-diabetic actions of leptin, we performed 2 complementary studies. We overexpressed *Gpt* in the liver of STZ-diabetic mice by hydrodynamic gene delivery then tested the efficacy of leptin therapy 4 days after plasmid injection. In another study, we used siRNA encapsulated in nanoparticles to knockdown *Gpt* in the liver of STZ-diabetic mice then tracked blood glucose levels.

Results: Upon observing normalization of blood glucose levels in leptin treated STZ-mice (22.2 ± 0.4 vs 15.2 ± 5.7 mM day -1 and day 3), STZ-leptin and non-diabetic controls were fasted to the point of mild hypoglycemia and injected with alanine or pyruvate on day 5. In non-diabetic controls, alanine injection increased blood glucose levels (4.7 ± 0.1 vs 6.9 ± 0.4 mM at 0 and 30 minutes), and injection of pyruvate, the breakdown product of alanine, also increased blood glucose levels (4.7 ± 0.2 vs 11.2 ± 0.3 mM at 0 and 30 minutes). In contrast, alanine did not increase blood glucose levels in leptin treated STZ-mice (5.4 ± 0.5 vs 4.5 ± 0.5 mM at 0 and 30 minutes) but injection of pyruvate increased blood glucose levels in leptin treated STZ-mice (5.8 ± 0.6 vs 9.3 ± 0.9 mM at 0 and 30 minutes). These data suggest that alanine breakdown may be blocked by leptin; thus, we measured *Gpt* transcript levels in the liver. *Gpt* transcript levels were downregulated by ~2 fold in leptin treated mice compared to STZ-diabetic controls on day 4 ($p < 0.0001$). Administration of plasmid encoding *Gpt* to leptin treated STZ-mice led to overexpression of *Gpt* in the liver by ~3 fold compared to leptin treated mice receiving empty plasmids as controls ($p = 0.02$). Leptin similarly lowered blood glucose levels in STZ-mice with overexpression of *Gpt* (9.7 ± 0.6 mM) and controls (8.8 ± 0.8 mM) by day 7 post leptin therapy. In a separate study, *Gpt* siRNA led to knockdown of *Gpt* in the liver by ~5 fold and ~10 fold for low and high doses of siRNA, respectively, compared to negative controls receiving *F7* siRNA ($p < 0.01$ for both low and high). STZ-mice with knockdown of *Gpt* remained hyperglycemic on day 10 post siRNA delivery (23.2 ± 0.9 and 23.4 ± 0.9 mM for low and high) comparable to *F7* siRNA controls (24.7 ± 0.7 and 24.6 ± 0.4 mM for low and high doses).

Conclusion: Leptin treated STZ-mice cannot utilize alanine to produce glucose but their ability to utilize pyruvate, a product of alanine breakdown, remains intact. *Gpt*, the gene responsible for alanine breakdown is downregulated by leptin but this is neither required nor sufficient for the anti-diabetic actions of leptin.

Supported by: CIHR

Disclosure: **M.M. Kwon:** None.

162

Circular dorsal ruffles and IR internalisation

M. Araujo-Correia[1,2], R. Machado de Oliveira[1], D.C. Barral[1], M. Macedo[1];

[1]CEDOC - Centro de Estudos de Doenças Crónicas, Lisboa, [2]ProRegeM - Doctoral Programme in Mechanisms of Disease and Regenerative Medicine, Lisboa, Portugal.

Background and aims: In the postprandial state, insulin is released from the pancreas into the bloodstream, targeting insulin-sensitive organs such as liver, promoting glycolysis and lipogenesis, and skeletal muscle allowing for glucose uptake. Insulin binds to its receptor (IR) at the surface, and the newly-formed complex is rapidly internalized into the cell. Insulin-IR uncoupling leads to signal termination, and to either receptor degradation in lysosomes or recycling to the plasma membrane. The internalization and endocytic process can be mediated by clathrin and caveolin vesicles, or be independent of these proteins. Circular dorsal ruffles (CDRs) are ring-shaped actin-rich structures that form exclusively at the dorsal surface of cells between 5 and 30 min after growth factor stimulation. These are dynamic and transient structures, responsible for tyrosine kinase receptor internalization and fast recycling. IR internalization and trafficking are crucial for peripheral insulin bioavailability, through the balance between insulin secretion and clearance, and to maintain glucose homeostasis in its target organs. Here, we propose that **CDRs are a non-canonical pathway involved in the internalization and fast recycling of the IR both in liver and skeletal muscle, and that this pathway can be disrupted by NO-induced inflammation.**

Materials and methods: Hepa 1-6 mouse hepatocytes, primary mouse hepatocytes and L6 rat muscle cells were used to characterize IR internalization. Cells were insulin stimulated for different timepoints, and processed for immunofluorescence, using phalloidin and cortactin (actin cytoskeleton), as well as antibodies against the IR and nitric oxide sintase (NOS). We also impaired CDR formation by silencing WAVE1, to understand the impact of the absence of these structures in the insulin signaling pathway. Finally, to mimic an inflammatory environment, we overexpressed iNOS and further assessed CDR formation.

Results: Our results show that, upon insulin stimulation, Hepa 1-6 cells and primary mouse hepatocytes form CDRs. We detect CDRs as early as 1 min after stimulation and observe that IR localizes to these structures, suggesting that CDRs mediate IR internalization. Moreover, CDRs are also present in stimulated L6 rat muscle cells, suggesting an important role for these structures in IR internalization in insulin-sensitive tissues. The number of cells with CDRs also increases with increasing insulin concentrations. Preliminary results suggest that disruption of CDR formation by WAVE1 silencing leads to impaired insulin signaling, as inferred by a decrease in Akt phosphorylation. Finally, iNOS overexpression leads to a decrease in CDR formation when compared to sham-transfected cells, suggesting that an inflammatory environment might disrupt this pathway, therefore IR internalization.

Conclusion: Herein, we observed insulin-induced CDR formation in hepatocytes and skeletal muscle cells. Moreover, CDR disruption impairs the insulin signaling pathway and that inflammation impairs CDR formation. This IR internalization route might be a major contributor for the receptor's availability to activate insulin signaling pathways and promote glucose uptake, but also in the insulin internalization itself, allowing for the fast recycling of the receptor and for insulin to fulfill its functions and be metabolized in the liver.

Supported by: FCT - PTDC/BIM-MET/2115/3014;PD/BD/52427/2013

Disclosure: **M. Araujo-Correia:** None.

OP 28 Novel drug therapies: moving beyond GLP1

163

Effects of the novel dual GLP-1R/GCGR agonist SAR425899 on postprandial glucose metabolism in overweight/obese subjects with type 2 diabetes

B. Goebel[1], M. Schiavon[2], R. Visentin[2], M. Riz[1], C. Dalla Man[2], C. Cobelli[2], T. Klabunde[1];
[1]Sanofi, Frankfurt, Germany, [2]University of Padova, Padova, Italy.

Background and aims: SAR425899 is a novel dual glucagon-like peptide-1 receptor/glucagon receptor (GLP-1R/GCGR) agonist. A randomized, double-blind, phase I multiple-ascending-dose study in 27 overweight/obese subjects with type 2 diabetes receiving SAR425899 demonstrated decreased body weight with a safety profile comparable with GLP-1R agonists. Here, we report additional analyses of the effect of SAR425899 on key processes in glucose metabolism.
Materials and methods: Subjects were randomized to receive daily subcutaneous administrations of low-dose SAR425899 (30, 60, 90 μg) or high-dose SAR425899 (60, 120, 180 μg) for 28 days; dose escalation occurred after Days 7 and 14. Mixed meal tests were conducted before treatment (Day −1) and on Days 1 and 28. Oral glucose and C-peptide minimal models were used to quantify metabolic indices of glucose absorption, insulin sensitivity, and β-cell responsiveness.
Results: High-dose SAR425899 had positive effects on glucose control from Day 1. Percent change in area under the curve for rate of meal glucose appearance between 0 and 120 min from Day −1 to Day 28 was −14% and −19% with low- and high-dose SAR425899, respectively. Change in insulin sensitivity was 104% and 262%, respectively. Change in β-cell function was 127% and 145%, respectively (Table).
Conclusion: After 28 days of treatment, SAR425899 improved postprandial glucose control by significantly reducing glucose absorption rate, increasing insulin sensitivity, and enhancing β-cell function.

Table. Effect of SAR425899 on key processes in glucose metabolism

Treatment	Day	$AUCRa_{0-120}$ (mg/kg)	SI (10^{-4} dL/kg/min/[μU/mL])	Φ (10^{-9} min^{-1})	DI (10^{-14} dL/kg/min^2/[pmol/L])
Low-dose SAR (30, 60, 90 μg)	−1	390 [337–483]	2.42 [1.59–3.48]	17.3 [11.3–23.3]	107 [24–150]
	1	412 [393–458]	1.46 [1.08–2.39]	22.9 [14.5–28.7]	76 [32–110]
	28	364 [272–454]	**4.68*** [3.01–5.86]	**40.8*†** [24.6–69.5]	**224*†** [166–679]
High-dose SAR (60, 120, 180 μg)	−1	482 [430–584]	1.25 [0.70–2.51]	20.9 [14.9–30.8]	55 [23–82]
	1	**382*** [310–519]	2.79 [1.97–5.26]	**26.5*** [22.6–50.4]	**183*** [99–230]
	28	**407*** [373–456]	**8.00*** [3.68–17.27]	**49.1*†** [33.2–62.5]	**579*** [313–2085]

Values are reported as median [interquartile range]
*p<0.05 from paired Wilcoxon signed rank test against Day −1
†p<0.05 from paired Wilcoxon signed rank test against Day 1
$AUCRa_{0-120}$=area under the rate of meal glucose appearance curve between 0 and 120 min; DI=total disposition index; SI=insulin sensitivity promoting glucose utilization and inhibiting glucose production; Φ=total β-cell responsivity index

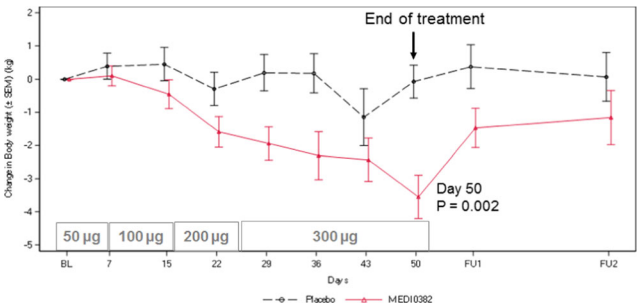
Clinical Trial Registration Number: NCT02411825
Supported by: Sanofi
Disclosure: **B. Goebel:** Employment/Consultancy; Sanofi.

164

MEDI0382, a dual GLP-1 glucagon receptor agonist, promotes rapid glucose control and significant weight loss in patients with type 2 diabetes

V. Parker[1], P. Ambery[1], D. Robertson[1], M. Posch[2], L. Plum-Moerschel[3], T. Wang[4], M. Petrone[1], T. Heise[5], J. Meier[6], B. Hirshberg[4];
[1]MedImmune, Cambridge, UK, [2]Charité Research Organisation GmbH, Berlin, Germany, [3]Profil, Mainz, Germany, [4]MedImmune, Gaithersburg, USA, [5]Profil, Neuss, Germany, [6]Ruhr-University, Bochum, Germany.

Background and aims: MEDI0382 is a GLP-1/glucagon receptor dual agonist under development for the treatment of type 2 diabetes mellitus and nonalcoholic steatohepatitis. Balanced GLP-1 and glucagon receptor agonism is predicted to achieve improved glycemic control with clinically significant weight loss via increased energy expenditure and central effects on appetite. The primary objective was to evaluate glucose AUC reduction during a mixed-meal test and body weight change in cohort 1.
Materials and methods: A randomized double-blind placebo-controlled phase 2a study was undertaken to evaluate the efficacy of MEDI0382 and tolerability in different titration schedules. Subjects recruited ($n = 65$) had type 2 diabetes mellitus and were on metformin monotherapy with an HbA1c of 6.5–8.5% and body mass index of 27–40 kg/m^2. Subjects received once-daily subcutaneous MEDI0382 uptitrated from 50 μg to 300 μg in either one or two weekly titration steps (cohorts 1 and 2, respectively) or placebo.
Results: After 49 days of dosing, significant weight loss of 3.4% (3.3 kg) vs placebo, ($P = 0.002$; Figure 1) was observed and 11/26 (42.3%) achieved weight loss of ≥5% ($P = 0.040$). Both postprandial and fasting glucose were significantly decreased; −27.8% for glucose AUC vs placebo ($P < 0.001$) and −1.8 mmol/L for fasting glucose vs placebo ($P < 0.001$). This equated to a reduction in HbA1c of 0.6% vs placebo ($P < 0.001$). Remarkably, this improvement in glycemic parameters was evident after just 7 days of dosing of 50 μg of MEDI0382 (glucose AUC, −27.4% vs placebo, $P < 0.001$ and fasting glucose −1.85 mmol/L vs placebo, $P < 0.001$). Treatment-related adverse events occurred more often with MEDI0382 (31/46 = 67.4%), the most frequent being decreased appetite in 13/46 (28.3%). Nausea and vomiting were recorded in 5/26 (19.2%) and 3/26 (11.5%) after weekly titration and 7/20 (35%) and 4/20 (20%) after two-weekly titration. A significant increase in heart rate of 7.8 bpm was observed after 49 days of dosing, but there were no significant changes in systolic or diastolic blood pressure.
Conclusion: MEDI0382 administered for up to 49 days promoted significant weight loss and led to rapid reduction in both fasting and postprandial glucose levels. The tolerability profile in cohort 1 was comparable to that of marketed GLP-1 analogs; lengthening the titration interval did not improve gastrointestinal tolerability.
Figure 1. Weight loss in kilograms in cohort 1.



Clinical Trial Registration Number: NCT03244800
Disclosure: **V. Parker:** Employment/Consultancy; MedImmune. Stock/Shareholding; AstraZeneca.

165

Neuroprotective effects of HM15211, a novel long-acting GLP-1/GIP/glucagon triple agonist in the neurodegenerative disease models

J. Kim, S. Lee, S.-H. Lee, S. Jung, Y. Kim, I. Choi, S. Kim;
Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea.

Background and aims: HM15211 is a novel long-acting GLP-1/glucagon/GIP triple agonist that is being developed for the treatment of obesity and non-alcoholic fatty liver disease (NASH). Accumulating evidences have shown that obesity, type 2 diabetes, and NASH increase the risk of

developing progressive neurodegenerative disease such as Parkinson's disease (PD) and Alzheimer's disease (AD). In addition to peripheral contributions, each of incretins consisting HM15211 have neuroprotective effects in several brain diseases like AD, PD, and ischemia. Previously, we demonstrated that HM15211 exerted neuroprotective effects in MPTP induced subacute Parkinson's disease mice model. Here, we evaluated 1) the neuroprotective effects of HM15211 in chronic MPTP/probenecid Parkinson's disease model, and 2) the protection of Alzheimer's disease progression in db/db mice.

Materials and methods: Chronic Parkinson's disease mice model was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in combination with probenecid intraperitoneal injection, twice a week for 5 weeks and HM15211 was subcutaneously administered once a week for 6 weeks. A db/db mice are well-established diabetic model and reported that db/db mice develop hyperphosphorylation of tau as they grew older. Thus we chose db/db mice to elucidate the prophylactic effect of HM15211 on Alzheimer's disease. Six weeks old db/db mice were subcutaneously treated with HM15211, once every two days for 12 weeks. For motor function evaluation of chronic PD model, the traction test, pole test and rotarod test were conducted before sacrifice. To assess the histological changes, hemi brain of all mice were sectioned and stained. And for the molecular changes, striata of chronic PD model and cortex were dissected from the other hemi brain and lysed with RIPA buffer and assayed with ELISAs.

Results: Dopaminergic neuronal death in MPTP/probenecid induced chronic PD model was confirmed by immunohistochemistry against tyrosine hydroxylase. The dopaminergic neuronal death was protected by HM15211, which was derived from anti-inflammatory and anti-oxidative stress effect by HM15211. Also HM15211 decreased alpha synuclein in striatum of chronic mice PD model. Together with these efficacies, HM15211 significantly improved the MPTP/probenecid induced motor impairments in behavior tests (rotarod, pole test, and traction test). In db/db mice, after 12 weeks of treatment, HM15211 reversed inflammatory cytokines and oxidative stress marker, which were increased in db/db mice. Also, increased phosphorylated tau in db/db mice was decreased by HM15211.

Conclusion: Based on these observations, HM15211 might be a potential therapeutic option for the neurodegenerative disease.

Disclosure: **J. Kim:** None.

166

MEDI4166, a novel antibody-peptide fusion molecule: multiple-ascending-dose study in patients with type 2 diabetes

G. Carlson[1], W. Cook[2], L. Morrow[3], M. Petrone[4], N. White[4], T. Wang[2], P. Ambery[4], B. Hirshberg[2], M. Jain[4];
[1]AstraZeneca, Gaithersburg, USA, [2]MedImmune, Gaithersburg, USA, [3]ProSciento, Chula Vista, USA, [4]MedImmune, Cambridge, UK.

Background and aims: Glucagon-like peptide-1 (GLP-1) agonists are widely utilized for the treatment of type 2 diabetes mellitus (T2DM) with proven effects on glycemic control and, in some cases, beneficial effects on cardiovascular (CV) outcomes. High-intensity statin therapy is recommended in T2DM patients with established CV disease and those at increased CV risk. However, some T2DM patients at high CV risk may require additional lowering of their low-density lipoprotein (LDL)-cholesterol (LDL-c) levels or may be intolerant to high-intensity statin therapy. Blockade of antiproprotein convertase subtilisin/kexin type 9 (PCSK9) is an effective method to lower LDL-c and decrease CV risk by decreasing degradation of the LDL receptor. MEDI4166 is a novel antibody-peptide genetic fusion molecule comprising a PCSK9 antibody and a GLP-1 agonist. Its dual mechanisms of action are designed to lower glucose and LDL-c in patients with T2DM, with the potential to reduce CV risk.

Materials and methods: In this multicenter, double-blind, placebo-controlled, multiple-ascending-dose study, adult subjects with T2DM receiving metformin monotherapy and LDL-c ≥70 mg/dL were randomized to receive subcutaneous MEDI4166 once weekly for 5 weeks at doses of 50 mg [(n = 9), 200 mg (n = 18), or 400 mg (n = 21), or placebo (n = 15) in 3 separate cohorts. The co-primary endpoints were change from baseline to day 36 in LDL-c and area under the plasma glucose concentration-time curve ($AUC_{0\text{-}4h}$) after a mixed-meal tolerance test (MMTT).

Results: Overall, 63 subjects were randomized, of which 55 (87%) completed the study. After multiple doses of MEDI4166, LDL-c levels were significantly decreased vs placebo (Table); no statistically or clinically significant reductions in glucose $AUC_{0\text{-}4h}$ after MMTT were observed. The pharmacokinetic profile supported weekly dosing. Adverse events (AEs) occurred in 79% (38/48) of MEDI4166 treated subjects and 87% (13/15) placebo treated subjects. Gastrointestinal symptoms and injection site reactions were the most common AEs in both groups. No serious AEs were observed. Two subjects discontinued from the study due to AEs; 1 subject in MEDI4166 200-mg dose group due to hyperglycemia considered unrelated to the investigational product, and 1 subject in the placebo group due to dyspepsia. One subject in the 50-mg and 5 subjects in the 400-mg groups discontinued for other reasons. No significant laboratory, vital sign, or ECG abnormalities were identified. Of 48 subjects receiving MEDI4166, 11 (22.9%) showed a treatment-induced antidrug antibody response.

Conclusion: After multiple weekly dosing with MEDI4166 across the dose range of 50–400 mg, significant dose-dependent reductions in LDL-c were observed. There was no improvement in postprandial glucose control versus placebo. Overall, MEDI4166 was well tolerated.

	Change from baseline					
	LDL-c (mg/dL)			Glucose $AUC_{0\text{-}4}$ (mg·h/dL)		
Dose	LS mean	95% CI	*P*	LS mean	95% CI	*P*
Placebo (n = 15)	−1.3	−13.6, 11.0	—	−87.1	−179.3, 5.0	—
MEDI4166						
50 mg (n = 9)	−48.4	−64.3, −32.5	<0.0001	−195.6	−318.7, −72.5	0.1616
200 mg (n = 18)	−76.1	−87.4, −64.9	<0.0001	−76.2	−157.4, 5.0	0.8591
400 mg (n = 21)	−75.9	−86.3, −65.5	<0.0001	−46.7	−128.6, 35.2	0.5145

Clinical Trial Registration Number: NCT02524782

Disclosure: **G. Carlson:** Employment/Consultancy; AstraZeneca. Stock/Shareholding; AstraZeneca.

167

Impact of praliciguat, a soluble guanylate cyclase stimulator, on blood pressure and metabolic parameters in patients with diabetes and hypertension

J.P. Seferovic[1], P. Wilson[1], K.E. Carlson[1], J. Jung[1], J.D. Wakefield[1], P. Miller[1], J.G. Chickering[1], L. Morrow[2], M.G. Currie[1], G.T. Milne[1], A.T. Profy[1], J.P. Hanrahan[1];
[1]Ironwood Pharmaceuticals Inc., Cambridge, [2]ProSciento, Inc., Chula Vista, USA.

Background and aims: Praliciguat (IW-1973), a soluble guanylate cyclase stimulator, increased nitric oxide (NO)-mediated signaling and reduced fasting plasma glucose and proteinuria in an animal model of diabetic nephropathy. In healthy subjects, repeated oral doses of praliciguat (15–40 mg) were well tolerated and lowered blood pressure (BP). We evaluated 2 dosing regimens of praliciguat in a phase 2a trial in patients with type 2 diabetes mellitus (T2DM) and hypertension (HTN).

Materials and methods: In a double-blind, randomized, placebo (PBO)-controlled trial, the effects of oral praliciguat were assessed in 26 patients with T2DM and HTN on stable antihyperglycemic and BP-lowering therapies. Two praliciguat regimens were evaluated: 1) 40 mg once daily (QD) for days 1–14 (N = 10), and 2) 20 mg twice daily for days 1–7 then 40 mg QD for days 8–14 (N = 10). Fasting plasma glucose, serum lipids,

apolipoprotein B, and BP (by 24-h ambulatory BP monitoring; ABPM) results are presented. Least squares mean differences from PBO and associated 95% confidence intervals (LSM [95% CI]) from analysis of covariance models with treatment as a fixed effect and baseline as a covariate are shown. Post-hoc subgroup analyses by concomitant medication use and baseline BP levels were also performed.

Results: Results were similar for both regimens after 14 days of treatment and were therefore combined. Relative to PBO, praliciguat-treated patients overall had decreases in fasting plasma glucose (−13 mg/dL [−32, 7]) and in the subgroup of 16 patients who were using only oral antihyperglycemic agents without insulin (−19 mg/dL [−37, −2]). A similar trend was observed in this subgroup of patients in homeostatic model assessment of insulin resistance-HOMA-IR (−23% [−56, 9]). Total and low-density lipoprotein cholesterol decreased in praliciguat-treated vs. PBO patients overall (−26 mg/dL [−44, −7]) and −20 mg/dL [−37, −3], respectively) and in the subgroup of 18 patients on concomitant statin therapy (−17 mg/dL [−44, 10] and −16 mg/dL [−41, 9], respectively). Lowering of apolipoprotein B levels was also suggested to be more pronounced after praliciguat compared to PBO treatment overall (−119 μg/mL [−295, 57]) and in those on statin therapy (−61 μg/mL [−281, 160]). There were decreases in 24-h ABPM mean arterial pressure (MAP) in praliciguat- vs. PBO-treated patients, overall (−5 mmHg [−10, 1]) and in subgroup of 10 patients with baseline MAP >92 mmHg (overall median at baseline; −14 mmHg [−23, −5]). In contrast, these decreases were not observed in the subgroup with baseline MAP ≤92 mmHg (2 mmHg [−4, 8]). A similar pattern was seen for both systolic and diastolic BP. Tolerability of praliciguat was acceptable in this trial.

Conclusion: In this small study, praliciguat lowered fasting plasma glucose and lipid levels in T2DM patients with HTN on standard therapies, including oral antihyperglycemic agents and statins. These results suggest that praliciguat may lower BP in these T2DM patients, especially those with higher baseline BPs. The metabolic and hemodynamic effects of praliciguat are being further evaluated in ongoing studies of diabetic nephropathy and heart failure with preserved ejection fraction.

Clinical Trial Registration Number: NCT03091920

Disclosure: **J.P. Seferovic:** Employment/Consultancy; J.P.Seferovic is an employee of Ironwood Pharmaceuticals Inc.

168

Effect of leptin replacement therapy (LRT) on survival and disease progression in generalised and partial lipodystrophy (GL, PL)

K. Cook[1], O. Ali[1], D. Gupta[1], D. Holmqvist[1], D. Lee[1], C. Ng[1], P. Bradt[2], R. Brown[3];
[1]Analysis Group, Menlo Park, [2]Aegerion Pharmaceuticals, A Novelion Therapeutics Company, Cambridge, [3]National Institutes of Health, Bethesda, USA.

Background and aims: Lipodystrophy (LD) is an ultra-rare disease associated with significant morbidity and mortality. Effects of LRT on metabolic disease in GL and PL have been studied; but effects on mortality are unknown. We investigated these effects using data from GL and PL patients treated with LRT at the NIH ($N = 114$) and cared for but not treated with LRT at 3 centers in the US and Turkey ($N = 178$).

Materials and methods: Four abnormalities (liver, kidney, heart, and HbA1c ≥6.5%) were considered. LRT patients had a mean of 2.8 abnormalities prior to treatment, while the mean for untreated patients was 0.7 at a similar age. We used a matching approach to create comparable samples of treated and untreated patients. Each treated patient was matched (using Mahalanobis distance) to an untreated patient to balance across age, gender, type of LD, and number of abnormalities. LRT treatment effect was examined via Cox proportional hazards models of 1) mortality and 2) development of subsequent abnormalities. Additionally, the relationship between abnormalities and mortality was studied in the sample of untreated patients.

Results: A Cox proportional hazards model relating treatment to mortality yielded a hazard ratio for LRT of 0.34 ($p = 0.047$), meaning that LRT was associated with a 66% decrease in mortality risk. Adjusting for covariates including gender, type of LD, and type of organ abnormality results in a larger decrease in mortality risk (HR 0.21, $p < 0.01$). One possible mechanism for the effect of LRT on mortality is its role in mitigating or resolving organ abnormalities. A time-varying Cox proportional hazards model relating number of abnormalities present (0 to 4) to mortality among untreated patients found a positive relationship between additional abnormalities and mortality (HR 3.2, $p < 0.01$). Separately, we found that LRT reduced the likelihood of developing a third (HR 0.47, $p < 0.01$) or fourth abnormality (HR 0.46, $p < 0.05$).

Conclusion: These are the first data suggesting that LRT reduces mortality in LD.

Clinical Trial Registration Number: NCT00025883

Supported by: Aegerion Pharmaceuticals, A Noevlion Therapeutics Company

Disclosure: **K. Cook:** Employment/Consultancy; Analysis Group, Aegerion Pharmaceuticals, A Novelion Therapeutics Company.

OP 29 Epigenetics: beyond the genes

169

Placental microRNA expression patterns in pregestational diabetes and identification of specific potential biomarkers

A. Ibarra[1], B. Vega[2,3], M. Armas[2,3], D. González[2,3], S. Perera[4], R. Horres[5], R. Valls[4], J.C. Wiebe[3], A.M. Wägner[1,3];
[1]Endocrinology, Complejo Hospitalario Universitario Insular Materno Infantil de Canarias, Las Palmas de Gran Canaria, Spain, [2]Gynecology, Complejo Hospitalario Universitario Insular Materno Infantil de Canarias, Las Palmas de Gran Canaria, Spain, [3]Instituto Universitario de Investigaciones Biomédicas y Sanitarias. Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [4]Anaxomics SL, Barcelona, Spain, [5]GenXPro GmbH, Frankfurt, Germany.

Background and aims: Offspring of mothers with type 1 diabetes (T1D) are at increased risk of developing T1D, but have a lower risk than offspring of fathers with T1D. This could be explained by intrauterine, epigenetic effects, detectable at birth. Our aim was to assess the effect of maternal T1D on placental expression of miRNAs.

Materials and methods: Samples of the maternal and foetal sides of the placenta were obtained from women with T1D ($N = 38$, 3rd trimester HbA_{1c} 6.4 (0.9)%) and type 2 diabetes (T2D) ($N = 32$, HbA_{1c} 6.1 (0.7)%), women whose partner had T1D ($N = 15$) and controls matched for age and gestational age ($N = 59$). In ten "pools" of 8–10 samples, massive sequencing of mRNA and miRNAs was performed. Selecting miRNAs with a difference in expression between groups ($p < 0.1$) and an inverse difference in the target mRNAs (fold-change <−1/>1), 5 new and 8 known miRNAs were identified. The following were selected for validation via qPCR (EXIQON miRCURY Universal RT Kit; cel-miR-39-3p as internal control) in the individual samples in each pool: miR-372-3p2, miR-127-3p, miR-145-5p, miR-373-3p2, miR-125b-5p, miR-19a-5p, miR-20a-5p and novel Chr11-134. Quantile normalisation was performed and ΔCT values (with miR-16-5p as reference due to its stable expression across groups) were compared (pair-wise, cluster- and principal component analysis (PCA). Data mining techniques were also used to identify potential biomarkers (single miRNAs or combinations of 2–3) specific for T1D and T2D (foetal and maternal sides of the placenta). Feature normalization, selection, extraction and transformation were followed by the application of base classifiers (binomial GLM and naive Bayes). Validation was performed using the "leave one out" method. Classifiers with a cross validated $p < 0.05$ and a cross-validated balanced accuracy >70% were considered.

Results: Analysis of the first 96 samples revealed that the miRNAs that best discriminate the T1D group from the control groups are 19a-5p, 125b-5p, 20a-5p and Chr11-134. The table shows the most relevant classifiers for T1D and T2D. Replication in 188 additional samples is ongoing.

Conclusion: MiRNA expression patterns can distinguish T1D placentas from controls. No common classifiers for T1D and T2D were identified, although two miRNAs were included as classifiers for both T1D and T2D on the maternal side of the placenta and another two on the foetal side of the placenta. Thus, these miRNAs could be related to intrauterine hyperglycemia, and are therefore interesting candidate classifiers for further study.

	Foetal side			Maternal side			
	crosVal-Balanced ACC	crosVal-AUC	CrosVal-pValue	crosVal-Balanced ACC	crosVal-AUC	CrosVal-pValue	
miR-19a-5p miR-20a-5p	0.76	0.77	1.15E-03	-	-	-	T1D
miR-19a-5p miR-125b-5p	0.73	0.79	1.43E-03	-	-	-	T1D
miR-125b-5p miR-127-3p miR-373-3p2	0.76	0.76	1.95E-03	-	-	-	T1D
miR-125b-5p Chr11-134	-	-	-	0.73	0.72	1.43E-03	T1D
miR-125b-5p miR-145-5p Chr11-134	-	-	-	0.77	0.75	2.95E-04	T1D
miR-125b-5p miR-373-3p2 Chr11-134	-	-	-	0.75	0.73	2.41E-04	T1D
miR-20a-5p miR-125b-5p	0.75	0.78	2.86E-02	-	-	-	T2D
miR-19a-5p miR-20a-5p miR-125b-5p	0.80	0.82	9.88E-03	-	-	-	T2D
miR-19a-5p miR-20a-5p miR-373-3p2	0.75	0.73	3.49E-02	-	-	-	T2D
miR-20a-5p miR-125b-5p miR-145-5p	-	-	-	0.75	0.6	2.86E-02	T2D

Most promising classifiers obtained from placenta to discern pregnant women with T1D or T2D.

Disclosure: **A. Ibarra:** None.

170

Inhibition of lncRNA Lincpint expression affects insulin secretion and apoptosis in mouse pancreatic beta cells

Y. Li[1], Y. Zhu[2], Q. Yuan[1];
[1]Department of Endocrinology, the first Affiliated Hospital of Nanjing Medical University, Nanjing, [2]Jiangsu Medical Magazine, Nanjing, China.

Background and aims: Insulin, which is produced and secreted from pancreas islet, is essential for maintaining glucose homeostasis. The decrease of insulin biosynthesis and islet mass may induce to β cell dysfunction, ultimately to various diabetes mellitus. Lots of transcription factors and microRNAs are found to modulate islet function, while a newly identified set of regulatory factors, the long noncoding RNAs (lncRNAs), are rarely known. LncRNAs are recognized as a new class of transcripts longer than 200nt that unable to translate into proteins. They regulate gene expression at transcription, epigenetic and translation levels. Moreover, lncRNAs participate in a variety of biological processes, such as cellular development, differentiation, and cell apoptosis. Recent evidence indicates that lncRNAs are involved in maintaining islet function and diabetes occurrence. Lincpint is a long intergenic noncoding RNA, and is necessary for development and cell function. Here, we investigated its biological functions in mouse pancreatic β cells both in vivo and vitro.

Materials and methods: Lincpint expression levels were detected in BALB/c mice tissues (including heart, liver, spleen, muscle, kidney, pancreas) and pancreatic islets from C57BL/KsJ-$lepr^{db}$/$lepr^{db}$ (db/db) mice and age-matched nondiabetic littermates by quantitative real time PCR (qRT-PCR). RNA interference was used to knockdown Lincpint expression in MIN6 cells and BALB/c mice. The effect of Lincpint on islets β cells proliferation, apoptosis and insulin secretion were assessed by MTT, flow cytometry and glucose stimulated insulin secretion (GSIS). Intraperitoneal glucose tolerance test (IPGTT), ELISA and immunohistochemistry were performed to evaluate the effect of Lincpint in vivo.

Results: Lincpint was highly expressed in pancreas compared to other tissues ($P < 0.05$) and rich in islets rather than exocrine glands from BALB/c mice ($P < 0.05$), but downregulated in db/db mice islets compared to littermates' ($P < 0.05$). Lincpint could be regulated by different concentrations of glucose in MIN6 cells ($P < 0.05$). Moreover, silencing Lincpint expression in vitro suppressed insulin synthesis and secretion ($P < 0.05$), and increased cell apoptosis ($P < 0.05$). In vivo, blood glucose, serum insulin and positive islet area were decreased after knockdown Lincpint expression ($P < 0.05$).

Conclusion: In our study, we demonstrated that Lincpint was highly expressed in nondiabetic mice pancreas and islets, but decreased in db/

db mice islet. As well as, Lincpint could affects insulin secretion and apoptosis in mouse pancreatic β cells. The present findings suggest that Lincpint may contribute to maintain β cells function, and is worthy for further investigation due to its potential for diabetes treatment.
Disclosure: **Y. Li:** None.

171

Impact of type 2 diabetes-associated variants at the STARD10 locus on chromatin conformation and human beta cell function

M. Hu[1], P.J. Gadue[2], G.A. Rutter[3];
[1]Imperial College London, London, UK, [2]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, USA, [3]Department of Medicine, Imperial College London, London, UK.

Background and aims: Genome-wide association studies (GWAS) have identified more than 100 *loci* in the human genome associated with type 2 diabetes. The majority of these are located in intergenic or intragenic regions suggesting that the implicated variants may alter chromatin conformation. This, in turn, is likely to influence the expression of nearby or more remotely located genes to influence beta cell function. At present, however, detailed molecular and functional analyses are still lacking for most of these variants. We recently analysed one of these loci, within the *STARD10* gene, and mapped five causal variants in an islet-specific enhancer cluster. Here, we aimed to understand how these variants affect enhancer activity and beta cell function.
Materials and methods: Human foetal pancreas-derived EndoC-βH1 cells were used throughout. Promoter-luciferase reporter, chromatin conformation capture, chromatin immunoprecipitation-qPCR, and glucose-stimulated insulin secretion (GSIS) assays were deployed to identify and assess the function of individual enhancers. CRISPR/Cas9 genome editing was used to create the required mutations or deletions.
Results: Using published ATAC-seq data, we sub-cloned 10 open chromatin regions (0.2–2 kb) at the *STARD10* locus and analysed their transcriptional activity using promoter-luciferase assays. Of these, one, termed HS1b, displayed a six-fold increase of luciferase activity [luciferase/renilla ratio: Control, 1.25 ± 0.06 *vs.* HS1b, 7.82 ± 0.17; Student's t-test, $p < 0.001$; $n = 3$]. Deletion of HS1b using CRISPR/Cas9 led to a significant reduction of *STARD10* expression (fold; Control 1 *vs.* HS1b-del, 0.486 ± 0.04; $p < 0.001$, $n = 3$) and a concomitant lowering of glucose-stimulated insulin secretion (GSIS) (15/0.5 mM glucose, fold change: control, 4.23 ± 0.41 *vs.* HS1b-del, 2.21 ± 0.06; $p < 0.05$; $n = 3$). Deletion of a 4.0 kb DNA region containing the five causal variants also reduced GSIS (15/0.5 mM glucose, fold change: Control, 1.70 ± 0.077 *vs.* SNPs-del, 1.36 ± 0.078; $p < 0.05$; $n = 3$) and lowered *STARD10* expression (fold: Control 1 *vs.* SNPs-del, 0.528 ± 0.042; $p < 0.001$, $n = 3$). Chromosome conformation capture assays (4C and 3C) identified two enhancer regions, HS1 and HS5, as interacting with the causal variants, including the indel rs140130268 with highest causal probability. Immunoprecipitation-qPCR assay using a CCCTC binding factor (CTCF) antibody confirmed the existence of multiple CTCF binding sites in both HS regions. To test the hypothesis that CTCF binding sites may create a chromatin loop defining an enhancer complex, we mutated four of the CTCF binding sites one by one. This resulted in a significant ($p < 0.05$; $n = 3$) impairment in GSIS assays [Control, 4.05 ± 0.46 *vs.* HS5-1-Mu, 2.75 ± 0.07; HS5-2-Mu, 2.66 ± 0.13; HS1-1-Mu, 3.49 ± 0.02 and HS1-2-Mu, 3.47 ± 0.57] and ~18–25% reduction in *STARD10* expression [Control, 1.0 *vs.* HS5-1-Mu, 0.82 ± 0.045; HS5-2-Mu, 0.74 ± 0.050; HS1-1-Mu, 0.82 ± 0.03; and HS1-2-Mu, 0.81 ± 0.043; $p < 0.05$; $n = 3$].
Conclusion: These data demonstrate that modification of enhancer elements at the *STARD10* locus affects beta cell function. The causal variants, which are physically associated with enhancer regions, are likely to exert their effects through the formation and activity of an enhancer-cluster complex which alters the expression of *STARD10* and possibly other genes.
Supported by: Wellcome Trust
Disclosure: **M. Hu:** None.

172

Histone acetylation and transcriptome mapping reveals distinct glucose-regulated genomic regions mediated by histone acetyltransferase p300 in pancreatic beta cells

P. Bompada[1], P. volkov[1], R. Andersson[1], M. Bysani[1], S. Atanur[2], C. Luan[1], J. Omella[1], E. Renström[1], Q. Zhang[3], M. Ridderstråle[1], J. Ferrer[2], L. Groop[1], Y. De Marinis[1];
[1]Lund University, Malmö, Sweden, [2]Imperial College London, London, UK, [3]University of Oxford, Oxford, UK.

Background and aims: Histone acetylation is an essential part of gene regulation controlled by histone acetyltransferase (HAT). Previous studies showed that hyperglycemia induces histone 3 lysine 9 acetylation (H3K9ac) at gene promoters, which leads to gene expression changes. However, it is not known which genomic regions genome-wide are directly targeted by HATs at hyperglycemia, or how this may change histone acetylation and subsequently gene expression. In this study, we mapped global H3K9ac enrichment and gene expression changes induced by high glucose mediated by HAT p300 in pancreatic beta cell line INS 823/12.
Materials and methods: HAT p300 was silenced by CRISPR/Cas9 in INS1 832/13 cells. Wild-type (WT) and *Ep300* knock-out (KO) cells were treated in 5 (LG) or 25 mM (HG) glucose for 24 hours. For ChIP seq, cells were cross-linked by formaldehyde and chromatin was sonicated. DNA-protein complex was precipitated using H3K9ac antibody. Library was constructed on de-crosslinked DNA fragments and sequenced on Ilumina NextSeq 500. For RNA sequencing, mRNA was isolated by RNeasy and library was constructed using TruSeq Stranded Total RNA Library Prep Kit and sequenced on Illumina NextSeq 500.
Results: In WT cells, high glucose changed expression in 10453 genes (5042 upregulated and 5429 downregulated). When p300 KO was compared to WT at high glucose, 8943 genes were differentially expressed (4342 increased; 4600 decreased), among which 3040 genes overlapped with high glucose-sensitive genes in WT cells. The remaining 5902 genes were identified as glucose-sensitive p300-dependent genes. To understand if the gene expression changes caused by p300 KO were directly linked to histone acetylation enrichment facilitated by p300, we performed ChIP seq using H3K9ac antibody. Glucose induced changes in H3K9ac enrichment were observed in 240 genomic regions, 49% of which are in the promoter regions. Two were identified at transcription termination sites (TTS) in the Ins1 and Ins2 genes, and 32 in other intron/exon regions. When compared to WT-HG, p300 KO cells at high glucose had differential H3K9ac enrichment in 113 genomic regions, including 33% at the promoter regions. All differentially enriched H3K9ac are distributed among 21 chromosomes. Around 30% of H3K9ac changes in p300 KO (HG) were gene promoter acetylation decreases, which led to dramatic changes in respective gene expression detected by RNA seq. In the Ccnd2 (Cyclin D2) gene, H3K9ac decreased by ~5 fold at Ccnd2 promoter in p300 KO, which was associated with 600-fold decrease in the Ccnd2 gene expression. In nine genes, p300 KO led to increased H3K9ac enrichment and increased gene expression. The top gene is Stx16 (Syntaxin 16), p300 KO led to a 3-fold increase in H3K9ac at promoter, which was associated with 122-fold increase in Stx16 gene expression. Gcg (Glucagon) had 700-fold decrease in gene expression in p300 KO, but no significant H3K9ac changes were detected. This may imply that decreased Gcg gene expression may be mediated by other histone acetylation marks facilitated by p300, or this is a downstream effect via another gene expression changes.
Conclusion: Our study has revealed distinct genomic regions that are regulated by high glucose-induced H3K9ac enrichment changes mediated by HAT p300.
Supported by: Swedish research council, Hjelt foundation, IMI BEAT-DKD
Disclosure: **P. Bompada:** None.

173

Role of microRNAs in the adipocyte morphology in relation to the family history of type 2 diabetes

P. Mirra[1], A. Desiderio[1], C. Nigro[1], M. Longo[1], L. Parrillo[1], R. Spinelli[1], F. Zatterale[1], F. Fiory[1], G.A. Raciti[1], U. Smith[2], F. Beguinot[1];
[1]URT GDD of the IEOS-CNR & University of Naples "Federico II", Napoli, Italy, [2]The Lundberg Laboratory for Diabetes Research at the University of Gothenburg, Gothenburg, Sweden.

Background and aims: Individuals with a family history (first-degree relatives, FDRs) of type 2 diabetes (T2D) are more likely to develop the disease, but the precise factors accounting for this increase in risk are poorly understood. However, emerging evidence suggests that epigenetic mechanisms, including microRNAs (miRNAs), may bridge genes and shared environmental components amongst family members. In euglycemic non-obese T2D-FDRs, metabolic defects have been reported and an impaired insulin sensitivity has been associated with signs of adipose tissue dysfunction and an enlarged cell size of subcutaneous adipocytes. To gain further insight into the pathogenesis of T2D, the present study aims at exploring the relationship between miRNAs and molecular mechanisms underlying the altered adipocyte morphology in euglycemic non-obese T2D-FDRs, focusing on targets that may be involved in the dysregulation of adipocyte number or size.
Materials and methods: Using the Illumina HiSeq 2000 platform, miRNA and mRNA expression profiles were examined in cultured adipose precursors from subcutaneous adipose tissue (scAT)-derived stromal vascular fractions (SVFs) of euglycemic non-obese T2D-FDRs ($n = 9$) and matched control subjects without any T2D familiarity ($n = 11$). Advanced analyses were applied to identify miRNAs and mRNAs differentially expressed between the two groups, in addition to miRNA-target interactions. Luciferase reporter assays were performed to test the proposed miRNA-target interactions.
Results: miRNA expression analysis in cultured adipose precursors from scAT-SVFs revealed 34 miRNAs differentially expressed between T2D-FDRs and controls, of which 23 are down- and 11 up-regulated. Subsequently, we confirmed that *miR-23a-5p*, *miR-193a-5p* and *miR-193b-5p* are significantly down-regulated in T2D-FDRs compared to controls. Interestingly, we observed a significant inverse correlation between the levels of each of these three miRNAs and the adipocyte cell size. Furthermore, using computational algorithms to predict miRNA targets, we realized that pathways related to adipocyte commitment/differentiation and function are enriched for these miRNAs. In addition, we integrated the mRNA expression data with the miRNA target predictions to support our proposal of new miRNA-target interactions and verified them using 3′UTR-reporter constructs and miRNA specific mimics in HEK 293 cells. Therefore, we demonstrated that the identified miRNAs may contribute to the up-regulation of IGF2 (insulin-like growth factor 2) and MXRA5 (Matrix Remodeling Associated 5), whose levels are positively and significantly correlated with the adipocyte cell size in euglycemic non-obese T2D-FDRs.
Conclusion: The decreased expression of *miR-23a-5p*, *miR-193a-5p* and *miR-193b-5p* observed in adipose precursors is a common feature of T2D-FDRs. Furthermore, our findings suggest an effective role of these miRNAs in adipocyte differentiation and/or function. Thus, they may be proposed as new biomarkers and/or targets for therapeutic interventions.
Disclosure: **P. Mirra:** None.

174

CRTC1 variants and DNA methylation in eating behaviour and adipose tissue biology in humans

L. la Cour Poulsen[1], K. Rohde[1,2], M. Keller[2], M. Stumvoll[2,3], A. Tönjes[3], P. Kovacs[2], A. Horstmann[4], A. Villringer[4,5], A. Dietrich[6], M. Blüher[2,3], Y. Böttcher[1,2];
[1]University of Oslo/Ahus, Lørenskog, Norway, [2]IFB Adiposity Diseases, Leipzig, Germany, [3]Dept. of Medicine, University of Leipzig, Leipzig, Germany, [4]Max-Planck-Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, [5]Clinic of Cognitive Neurology, University of Leipzig, Leipzig, Germany, [6]Dept. of Surgery, University of Leipzig, Leipzig, Germany.

Background and aims: CREB-regulated transcription coactivator 1 (CRTC1) is involved in various biological processes including energy metabolism. Genetic variants within *CRTC1* (*e.g.* rs757318) have been related to body fat mass (BFM) and BMI in humans, while *CRTC1* knockout mice display clear changes in eating behaviour. We aimed at identifying genetic variants within *CRTC1* that are associated with eating behaviour in humans.
Materials and methods: 1,036 individuals from the Sorbs population were selected for initial analysis from which 540 completed the German 3-factor eating questionnaire. Using data obtained previously from a SNP array (blood), a total of 12 SNPs were extracted, and rs7256986 was newly genotyped. All variants were analysed for their association with eating behaviour, while only non-diabetics were included for analysis of the relation with metabolic and anthropometric variables. Analyses were done in additive, dominant and recessive modes of inheritance. Because rs7256986 introduces a CpG site, pyroseq was applied in a subcohort of $N = 42$ to test for differential methylation at this site, and its relationship to eating behaviour and to metabolic and anthropometric traits was tested. To validate our findings, a second independent German cohort with eating behaviour data was used ($N = 314$). A third cohort ($N = 77$) was used to analyse the impact of rs7256986 on *CRTC1* DNA methylation and gene expression in human adipose tissue from paired visceral (OVAT) and subcutaneous (SAT) depots. Calculations were completed using SPSS 24 and METAL. A P value over 0.05 was assumed to show correlation.
Results: Five SNP variants associate with eating behavior factors including restraint, disinhibition, and/or hunger while four relate further to alcohol or coffee intake ($P < 0.05$). Eight SNPs relate to alcohol intake only. Pyroseq at rs7256986 ($N = 42$) revealed that G-allele carriers have a 19.5 (genotype GG) or 10.7 fold (genotype GA) increase in DNA methylation as compared to homozygote A-allele carriers ($P < 0.0001$). Further, a neighboring CpG site was found to be co-methylated with higher methylation among GG carriers ($P < 0.01$). Methylation at both sites correlates with increased restraint ($P = 0.00025$). A meta-analysis on rs7256986 association with eating behavior using data from Sorbs and the second independent cohort shows the same trends for disinhibition and restraint. In the third independent cohort, G-allele carriers show increased *CRTC1* gene expression level ($P = 0.006$) and lower waist circumference ($P = 0.018$) as compared to AA-carriers. Further, *CRTC1* gene expression correlates positively with DNA methylation in OVAT ($P = 0.035$), while the latter is negatively associated with BFM ($P = 0.045$). Moreover, a negative correlation of DNA methylation ($P = 0.016$) and gene expression ($P = 0.023$) with BMI was observed.
Conclusion: Our data suggest an effect of *CRTC1* SNPs on eating behavior and DNA methylation. *CRTC1* gene expression relates to rs7256986 and DNA methylation in OVAT and, further, to BMI in an independent cohort, suggesting a potential role for *CRTC1* in adipose tissue biology.
Supported by: ADI-K6e-96 (Y.B.); EU-Scientia Fellowship from the University of Oslo (KR)
Disclosure: **L. la Cour Poulsen:** None.

OP 30 Retinopathy: a different look at the eyes

175

Perinatal starvation increases risk for diabetic retinopathy in adulthood

O. Fedotkina[1], R.B. Prasad[2], R. Jain[2], L. Cherviakova[3], N. Khalimon[4], T. Svietleisha[4], T. Buldenko[5], I. Artner[2], V. Kravchenko[6], A.M. Zhydenko[3], A. Vaiserman[7], M. Khalangot[6], P.M. Nilsson[2], V. Lyssenko[1,8];
[1]Department of Clinical Science, University of Bergen, Bergen, Norway, [2]Diabetes and Endocrinology, Department of Clinical Sciences, Lund University Diabetes Center, Lund, Sweden, [3]Chernihiv Regional Hospital, Chernihiv, Ukraine, [4]Chernihiv City Hospital, Chernihiv, Ukraine, [5]Public Health Authority of Chernihiv regional public administration, Chernihiv, Ukraine, [6]Komisarenko Instutute of Endocrinology and Metabolism, Kyiv, Ukraine, [7]Chebotarev Instutute of Gerontology, Kyiv, Ukraine, [8]Lund University Diabetes Center, Lund, Sweden.

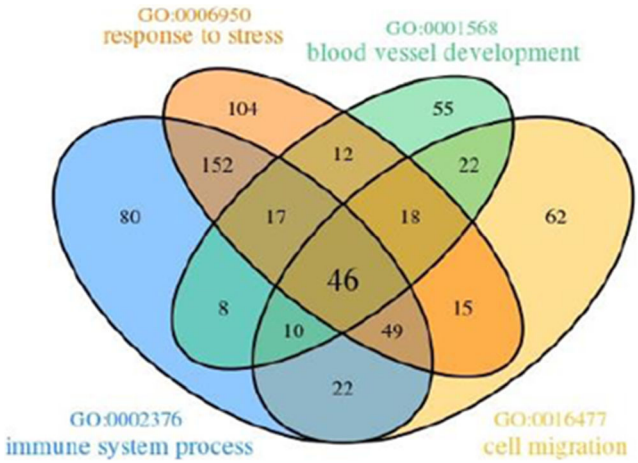
Background and aims: Perinatal exposure to stressful conditions has been associated with a number of adverse consequences later in life. A recent study has demonstrated that the Ukrainian Holodomor famine in 1930'ies as associated with 1.47-fold increased risk for type 2 diabetes in adulthood. In the present study, we investigated prevalence of diabetic retinopathy as a late consequence of perinatal exposure to famine. We also studied the extent to which genetic variants associated with metabolic risk factors might influence the risk of diabetic retinopathy in a population from northern Ukraine with historical famine exposure.
Materials and methods: We obtained records on diabetes retinopathy from 101,095 patients with type 2 diabetes from Ukraine national diabetes registry, of whom 3,061 had proliferative retinopathy. We analyzed a panel of 169 SNPs in 3,634 patients with type 2 diabetes from the Chernihiv region of northern Ukraine with a history of Holodomor famine exposure to identify genetic determinants of proliferative retinopathy. Logistic regression adjusted for sex, age at visit, diabetes duration, and corrected for family relationships was used to study association of SNPs with diabetic retinopathy. We calculated crossover odds ratio for interaction between additive model of DNA variants and year of birth adjusted for sex, age, and diabetes duration using logistic regression model.
Results: There were 53,321 (34% men) patients with type 2 diabetes included from the exposed to Holodomor famine regions in Chernihiv and Kiev, and 47,774 (37% men) from unexposed regions in Volyn and Rivne, extracted from the Ukraine national diabetes registry. The odds ratio for diabetes retinopathy was increased for subjects born during Holodomor, World War II and post-war famine periods 1932–1947 ($p < 0.001$). A strong interaction between DNA variants and perinatal exposure to famine on the risk of diabetic retinopathy was observed for 11 genes (*ADRA2A*, *DCD*, *PCSK9*, *CNDP2*, *CYP2C19*2*, *MADD*, *ADCY5*, *VEGFA*, *CDKN2B*, *PIK3CG*, and *PROX1*) (odds-ratio heterogeneity test I^2 value >75, $p < 0.05$).
Conclusion: These results demonstrate an association between intrauterine exposure to famine with the risk of diabetic retinopathy in adulthood. Underlying genomic factors seem to predominantly involve mechanisms regulating neuronal functions.
Supported by: BFS, VR, UiB, NNF
Disclosure: **O. Fedotkina:** None.

176

Starvation induced changes in transcriptome of retinal cultures

T. Özgümüs[1], A. Fedotkina[1], M. Keindl[1], I. Artner[2], R. Jain[2], V. Lyssenko[1,2];
[1]Department of Clinical Science, University of Bergen, Bergen, Norway, [2]Department of Clinical Science, Lund University, Lund, Sweden.

Background and aims: Retinopathy is a severe complication in patients with diabetes leading to visual impairment and blindness. The prevalence of diabetes and associated complications are particularly increasing in the developing countries with histories of malnutrition and poor living conditions. Starvation is a strong exposure, which may introduce irreversible changes in the cellular physiology, even after the exposure has been eliminated. In particular, there is a limited understanding of the starvation effects on the cells in the developmental stages. The molecular understanding of the processes affected by embryonic starvation in the retina may reveal fundamental insights into novel and effective treatment strategies.
Materials and methods: In this study, the long-term effects of starvation on the RNA expression of mice embryonic retinal cultures were examined using RNA sequencing ($n = 5–6$). Cells were starved for 6 hours and subsequently cultured in a normal glucose media for 6 days before sampling. The control cells were cultured in a normal culture environment. The RNA extracts were sequenced using TruSeq Stranded Total RNA with RiboZero (Illumina). The alignment of the transcripts and differential expression analysis were done with using Kallisto 0.43.1 and edgeR 3.20.9, respectively.
Results: The retinal cell culture deconvolution analyses demonstrated downregulated expression of cell markers, such as *Glul* and *Slc1a3* (*Glast*) for Müller cells, *Tubb3* for all types of neurons, *Prkca* for bipolar neurons, *Rbfox3* for retinal ganglion cells, *Opn1sw* for photoreceptors, and *Pax6* for progenitor cells, while vasculature cell marker *Flt1* (*Vegfr1*) was upregulated in the starved samples ($p < 0.01$, FDR <0.05). No differences were observed for *Nes*, *Pecam1*, *Calb1* and *Calb2*, and *Opn3* genes. Gene ontology enrichment analyses suggested upregulation of the genes for GO terms such as response to stimulus ($p = 4.3E-23$), signalling receptor activity ($p = 4.8E-22$), blood vessel development ($p = 3.1E-18$), cell migration ($p = 2.1E-16$), immune system process ($p = 1.2E-12$) and response to stress ($p = 5.8E-8$) in the starved cells as compared to the control cultures (Figure 1). We are presently looking for the association of genetic variants in these genes with retinopathy in patients with type 2 diabetes.
Conclusion: These data demonstrate that embryonic starvation exhibits detrimental and long-term effects on the transcriptome of the neurovascular unit in the retina, and thereby could increase susceptibility for the development of retinopathy.



Supported by: BFS, UiB, SRC
Disclosure: **T. Özgümüs:** None.

177

NSE, a novel biomarker, is elevated as an indicator of diabetic retinopathy including macular oedema

J. Li, M. Yan, Y. Zhang, M. Xie;
The First Affiliated Hospital of Nanjing Medical Univers, Nanjing, China.

Background and aims: Diabetic retinopathy (DR) has been a leading cause of legal blindness in working adults. In addition, diabetic macular edema (DME) is the most common cause of visual loss in both proliferative and non-proliferative retinopathy. Diabetic retinopathy is generally viewed as a neurovascular disease. Early DR includes a neurodegenerative component. In this regard, neuron-specific enolase (NSE), catalyzing the conversion of 2-phosphoglycerate into phosphoenolpyruvate in neurons, a new emerging biomarker of nerve deficits may be involved in retinopathy. Following nerve injury as a result of chronic exposure to ischemia or hypoxia, NSE is readily released into blood and detected in sera. We aimed to investigate the relationship between serum NSE and diabetic retinopathy including macular edema.
Materials and methods: Participants included type 1or 2 diabetes mellitus and healthy control subjects ($n = 392$). Patients with peripheral neuropathy were excluded from the study using the methods as we previously reported, i.e. through a combined assessment of clinical manifestations and functional tests of nerve deficits. Other diseases associated with possible sources of elevated NSE were also excluded. In this cross-sectional study, diabetic retinopathy status was assessed by fundus photographs. Based on International Classification of Diabetic Retinopathy or Early Treatment Diabetic Retinopathy Study (ETDRS), retinopathy and the macular edema were defined and sub-categorized, respectively. Serum neuron specific enolase (NSE) was measured using electrochemiluminescence immunoassay. Co-variables for diabetic retinopathy and NSE were obtained from fasting blood samples and interviewer- questionnaire.
Results: NSE was slightly elevated in diabetic subjects in contrast to healthy subjects and obviously increased in diabetic subjects with retinopathy compared without (8.3 (2.0) vs. 7.6 (1.5), $p = 0.037$ and 8.3 (2.0) vs. 9.5 (2.7), $p = 0.004$, respectively). In addition, NSE levels increased with and were closely correlated to the stages of retinopathy without macular edema ($r = 0.60$ (0.50–0.71), $p = 0.002$) and stages of macular edema with comparable retinopathy ($r = 0.58$ (0.46–0.69), $p = 0.006$). The association of NSE with diabetic retinopathy was independent (OR: 1.31 (1.12–1.65), $p = 0.017$), after the diabetic state and other potential confounders affecting NSE levels were considered (e.g., HbA1c, duration, age, gender, renal status, and medicines). The optimal cut-off point for serum NSE levels for differentiating patients with diabetic retinopathy including macular edema from without was 9.3 μg/l with a sensitivity of 67.5% and a specificity of 69.8%.
Conclusion: Our study demonstrated for the first time that serum neuron specific enolase levels were elevated in diabetes and independently related to diabetic retinopathy including macular edema. Our findings suggest that NSE may be a potential biomarker of diabetic retinopathy. Future prospective studies are warranted to clarify the relationship.
Disclosure: **J. Li:** None.

178

Improving diabetic retinopathy screening using deep learning

J.C. Mellor[1], A. Ochs[2], H.M. Colhoun[2], P. McKeigue[1];
[1]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, [2]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK.

Background and aims: The Scottish Diabetic Retinopathy Screening programme uses manual scoring of fundus images to grade retinas on an ordinal scale from 0 to 4 and maculas on a scale from 0 to 2. The current protocol is to rescreen every 12 months, except those with R2 or M1 findings who are rescreened at 6 months. A previous study has shown that by using clinical covariates including past retinal scores it is possible to stratify patients by their risk of referable disease and thus to reduce the workload of the programme while maximizing the early detection of referable disease. The manual grading does not capture all useful information from these images relevant to a risk model for diabetic retinopathy. Deep learning, a class of machine learning algorithms based on neural networks, has produced state-of-the-art performance in learning to extract features from and classify images. The objective of this study was to establish whether using deep learning on the retinal images can improve the prediction of referable retinopathy at future examinations beyond that obtained with clinical data and manual scores alone.
Materials and methods: We used 30,604 manually graded retinal images from 3,290 people in the Scottish Type 1 Diabetes Biorecource linked to clinical data for the years 2007 to 2016. We used data from the Kaggle 2015 Diabetic Retinopathy Detection Competition to train an Inception-V4 convolutional neural network (CNN) to predict the manual grading of diabetic retinopathy. The 384 features derived from this CNN were calculated on the images obtained on the Scottish cohort. A generalised linear model (with complementary log-log link) was used to model the time to referable retinopathy. A model including only clinical covariates and manual scores was compared with models that included these variables plus the deep learning features. Two alternative approaches to limiting the effective number of deep learning variables were compared: forward-selection, and a Bayesian approach that used a "horseshoe" prior to learn the distribution of effect sizes. Predictive performance was evaluated on a test dataset not used to learn the model.
Results: The model with only clinical covariates and manual scores achieved an AUROC of 0.81 for prediction of referable retinopathy at the next examination. With deep learning features added by forward selection, the AUROC was improved to 0.88 (gain in test log-likelihood 153.5 natural log units). With deep learning features included with a horseshoe prior on the distribution of effect sizes, the AUROC was improved further to 0.91 (gain in test log-likelihood 256.1 nat log units compared with baseline model).
Conclusion: These results show that there is predictive information in the fundus images beyond the manual grading, and that this information can be extracted using deep learning. In principle this can be used in screening programmes to personalize screening schedules so as to reduce the time for which patients referable disease remain undiagnosed while also reducing the overall workload of the programme.
Supported by: DUK
Disclosure: **J.C. Mellor:** None.

179

Association between diabetic retinopathy and Parkinson's disease: the Korean national health insurance service database

E. Koh, S. Lee, J. Jang, C. Woo, K.-U. Lee;
Asan Medical Center, Seoul, Republic of Korea.

Background and aims: Studies have shown an association between diabetes and Parkinson's disease (PD). The retina is a part of the central nervous system (CNS); it was proposed that diabetic retinopathy (DR) and PD share common pathophysiology of dopamine deficiency. However, no epidemiologic studies have investigated the relationship between these two diseases. The aim of this study was to evaluate the association between DR and incident PD using a population-based database.
Materials and methods: 14,912,368 participants who underwent regular health check-up from 2005 to 2008 in Korean National Health Insurance Service database were included. Subjects were classified into non-diabetes (non-DM), DM without DR, and DM with DR groups at baseline. These patients were followed-up until the date of the incident PD, death, or December 31, 2013. Cox proportional hazards regression analysis was used to evaluate the association between DR and incident PD.
Results: During the period, 34,834 subjects were newly diagnosed with PD. The incidence of PD was 2.74, 8.39 and 15.51 per 10,000 person-years for the non-DM, DM without and with DR groups, respectively. In multivariate Cox proportional hazard models, DR groups were associated with significantly higher risk of PD than non-DM or DM without DR groups even after adjusting for age, gender, fasting plasma glucose level, insulin usage, and other possible risk factors.

Conclusion: Concurrent DR was associated with an increased risk of incident PD. Future studies are necessary to ascertain whether increased risk of PD in DR is due to a long-lasting poor glycemic control in subjects with DR, or a dopamine deficiency in the CNS.
Disclosure: **E. Koh:** None.

180

Proteomic analysis of retinas from patients with type 2 diabetes reveals mediators of neurodegenerative diseases

O. Simo-Servat[1], C. Hernández[1], J. Sundstrom[2], S. Weber[2], Y. Zhao[2], M. Dunklebarger[2], N. Tiberti[3], T. Laremore[4], M. Garcia-Ramirez[1], A. Barber[2], T. Gardner[5], R. Simó[1];
[1]Vall d'Hebron Research Institute, Barcelona, Spain, [2]Penn State Hershey Eye Center, Hershey, USA, [3]University of Technology, Sidney, Australia, [4]Proteomics and Mass Spectometry Core Facility, Penn State University, University Park, USA, [5]Kellog Eye Center, University of Michigan Medical School, Ann Arbor, USA.

Background and aims: Type 2 diabetic patients have a significantly higher risk of developing neurodegenerative diseases and, in particular, Alzheimer's disease than non-diabetic subjects. In addition, current evidence suggests that retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy. Since the retina is ontogenically brain-derived tissue it could be postulated that in patients developing neurodegeneration in the brain there is a co-occurring neurodegenerative process in the retina. The main goal of the present study was to examine whether in the diabetic human retina there are common proteins and pathways shared with brain neurodegenerative diseases.
Materials and methods: A proteomic analysis was performed on three groups of postmortem retinas matched by age: non-diabetic control retinas ($n = 5$), diabetic retinas without glial activation ($n = 5$) and diabetic retinas with glial activation ($n = 5$). Retinal lysates from each group were pooled and run on an SDS-PAGE gel. Bands were analyzed sequentially by LC/MS using an Orbitrap Mass Spectrometer. Pathway enrichment analysis was performed using Ingenuity Pathway Analysis (IPA) bioinformatics platform. To identify the unique pathways related to neurodegenerative processes of each group, the Ontology and Function algorithm within IPA was used to restrict the enriched pathway dataset to those to "Neurotransmitters and Other Nervous System Signaling".
Results: A total of 2190 proteins were identified across all groups. Pathway analysis to "Neurotransmitters and Other Nervous System Signaling" revealed that 35 pathways are specifically represented in a single group (non-diabetic donors, diabetic donors without GA and diabetic donors with GA). The most relevant of these pathways were the following: "Neuroprotective Role of THOP1 in Alzheimer's Disease" and "Unfolded protein response" pathways were uniquely enriched in control retinas. By contrast, "Dopamine degradation" and "Parkinson's signaling" were enriched only in diabetic retinas with glial activation. The "Neuroregulin signaling", "Synaptic long term potentiation", and "Amyloid processing" pathways were enriched in diabetic retinas with no glial activation.
Conclusion: Proteomic analysis of diabetic retinas in early stages of diabetic retinopathy reveals mediators of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Our findings not only open up new therapeutic strategies against diabetes-induced retinal neurodegeneration but could be useful to further understand the neurodegenerative processes that occur in the brain of persons with diabetes.
Disclosure: **O. Simo-Servat:** None.

OP 31 Nephropathy: bedside and back to bench

181

Trajectories of estimated GFR in patients with and without albuminuria

M.E. Jørgensen[1,2], D. Vistisen[1], G.S. Andersen[1], A. Hulman[3,4], F. Persson[1], P. Rossing[1,5];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]National Institute of Public Health, Southern Denmark University, Copenhagen, [3]Aarhus University, Aarhus, [4]Danish Diabetes Academy, Odense, [5]Copenhagen University, Copenhagen, Denmark.

Background and aims: It is a common assumption that in patients with diabetes, a decline in kidney function is preceded by albuminuria. However, around one third of patients with an estimated GFR (eGFR) below 60 mL/min/1.73 m^2 have normoalbuminuria. The aim of this study was to assess the difference in the development over time in kidney function for patients with and without albuminuria from their first recording of a low eGFR.
Materials and methods: 930 patients with type 1 diabetes and 1,974 with type 2 diabetes treated in an outpatient clinic in Denmark during the period 2001–2017 were followed from their first recorded low eGFR (<60 mL/min/1.73 m^2) and onwards. Median (interquartile range) follow-up time was 4.2 years (1.5–7.6). Each patient had at least two clinical measurements with a median (interquartile range) of 7 (4–13) measurements per patient. A total of 28,288 clinical measurements were used in the analysis. GFR was estimated using the CKD-EPI formula. Albuminuria status was classified as normal (urinary albumin creatinine ratio <30 mg/g), micro (30–299 mg/g) or macro (≥300 mg/g). Trajectories of eGFR development following the first recorded low eGFR over time were estimated by mixed-effects models adjusting for sex, age at diabetes diagnosis, diabetes duration, calendar time and use of renin-angiotensin system (RAS) blockers. Interactions between time and albuminuria status and time and use of RAS blockers were included and tested. Albuminuria status and RAS blockade were included as time-varying covariates and eGFR was log-transformed prior to analysis. The analysis was stratified by diabetes type.
Results: Trajectories of eGFR development were overall similar in type 1 and type 2 diabetes (*Figure 1*). Albuminuria status significantly modified the development in eGFR ($P < 0.001$), with the steepest decline in kidney function among patients with macroalbuminuria: in patients with no RAS blockade, the annual decline was 7.0% and 6.7% in type 1 and type 2 patients respectively. RAS blockade had little effect on the trajectories. Both type 1 and type 2 diabetes patients with normoalbuminuria and who were not in RAS blockade had an estimated annual decline in eGFR of 4.2% from the adjusted model. For patients with a first recorded low eGFR of 59 mL/min/1.73 m^2 this amounted to a decline of 20 mL/min/1.73 m^2 over a 10-year period. In comparison, the average 10-year decline in eGFR in the background population age 40+ and without diabetes is around 10 mL/min/1.73 m^2.
Conclusion: Patients with type 1 or type 2 diabetes with an estimated GFR below 60 mL/min/1.73 m^2 are on average on a declining trajectory of kidney function that is increased by the presence of albuminuria.

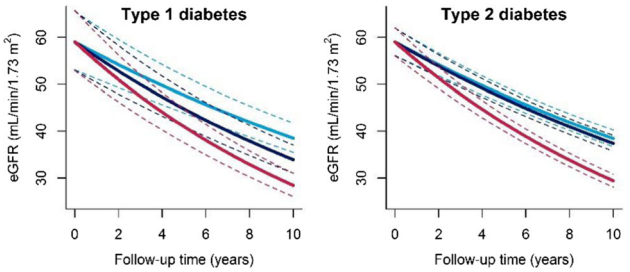


Figure 1 Estimated eGFR trajectories by diabetes type for patients with no RAS blockade and with a first recorded low eGFR of 59 mL/min/1.73/m^2. Time 0 is the first clinical visit with a recorded low eGFR. Curves are shown for patients with normoalbuminuria (light blue) microalbuminuria (dark blue) and macroalbuminuria (red)

Disclosure: **M.E. Jørgensen:** None.

182

Chronic kidney disease and risk of mortality, cardiovascular events and severe hypoglycaemia in type 2 diabetes: DEVOTE results

A. Amod[1], S.S. Emerson[2], S.P. Marso[3], D.K. McGuire[4], T.R. Pieber[5], R. Pop-Busui[6], R.E. Pratley[7], B. Zinman[8], M.V. Hansen[9], T. Jia[9], T. Mark[9], N.R. Poulter[10], on behalf of the DEVOTE Study Group;
[1]University of KwaZulu-Natal, Durban, South Africa, [2]University of Washington, Seattle, USA, [3]HCA Midwest, Kansas City, USA, [4]University of Texas Southwestern Medical Center, Dallas, USA, [5]Medical University of Graz, Graz, Austria, [6]University of Michigan, Ann Arbor, USA, [7]Florida Hospital, Orlando, USA, [8]Mount Sinai Hospital, University of Toronto, Toronto, Canada, [9]Novo Nordisk A/S, Søborg, Denmark, [10]Imperial College London, London, UK.

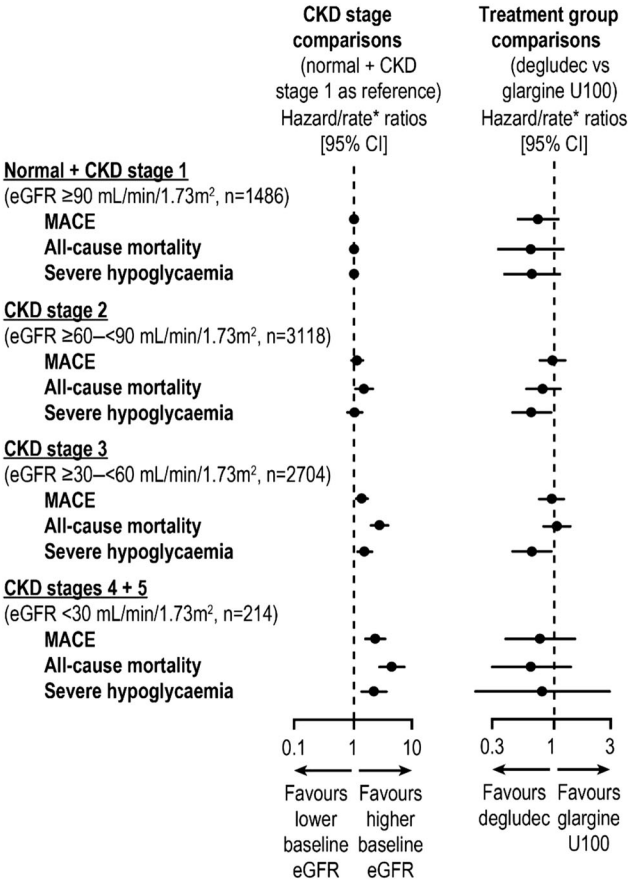
Background and aims: Type 2 diabetes (T2D) is associated with an increased risk of cardiovascular disease (CVD) and chronic kidney disease (CKD). CKD is a known risk factor for major adverse cardiovascular events (MACE), all-cause mortality and hypoglycaemia. This secondary, pooled analysis from DEVOTE examined whether baseline CKD stages were associated with an increased risk of MACE, all-cause mortality or severe hypoglycaemia in patients with T2D.

Materials and methods: DEVOTE was a treat-to-target, randomised, double-blind trial in 7637 patients with T2D at high cardiovascular (CV) risk, treated once daily with insulin degludec or insulin glargine 100 units/mL. Based on eGFR levels (mL/min/1.73 m^2), patients were divided into four CKD groups: normal + CKD stage 1 ($n = 1486$), CKD stage 2 ($n = 3118$), CKD stage 3 ($n = 2704$) and CKD stage 4 + 5 ($n = 214$). Severe hypoglycaemia was defined as an episode requiring the assistance of another person to actively administer carbohydrate or glucagon, or to take other corrective actions.

Results: According to baseline CKD stages (CKD stages 2–5), more patients had a history of CVD (CKD stages 3–5), were older and had lower HbA_{1c} versus those with normal kidney function (normal + CKD stage 1). The risk of MACE and all-cause mortality was significantly higher ($p < 0.05$) in those with a higher baseline CKD stage (Figure). There was a significantly higher rate of severe hypoglycaemia for CKD stages 3 and 4 + 5 versus CKD stage 2 or normal + stage 1. There were no significant interactions between treatment and CKD stages. Comparisons between treatment groups by CKD stage mirrored those from the primary analyses.

Conclusion: Increasing severity of baseline CKD stages was associated with a higher risk of MACE, all-cause mortality and rates of severe hypoglycaemia in patients with T2D at high CV risk.

Figure. Comparisons of effect of baseline CKD stages and basal insulin treatment (degludec vs glargine U100) on time to first MACE, all-cause mortality and severe hypoglycaemia in patients with T2D at high CV risk.



*Rate ratios only apply to the endpoint of severe hypoglycaemia, hazard ratios apply to the endpoints MACE and all-cause mortality. All endpoints reported here were externally adjudicated by the Event Adjudication Committee and pre-specified. The MACE endpoint was composed of cardiovascular mortality, non-fatal myocardial infarction, or non-fatal stroke. CI, confidence interval; CKD, chronic kidney disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; glargine U100, insulin glargine 100 units/mL; MACE, major adverse cardiovascular event; T2D, type 2 diabetes.

Clinical Trial Registration Number: NCT01959529
Supported by: Novo Nordisk
Disclosure: **A. Amod:** Employment/Consultancy; Servier, Medscheme, Discovery Health. Honorarium; Novo Nordisk, Astra Zeneca, MSD, Merck-Serono, Boeringher Ingelheim, Aspen Pharmacare, sanofi-aventis, Novartis, Ascendis Health, Cipla Medpro.

183

AVP gene variants, plasma copeptin and nephropathy in type 2 diabetes

G. Velho[1], L. Potier[1,2], K. Mohammedi[3], F. Fumeron[1,4], S. Hadjadj[5], M. Marre[2,4], R. Roussel[2,4];
[1]INSERM U1138, Centre de Recherche des Cordeliers, Paris, [2]Hôpital Bichat, AP-HP, Service de Diabétologie, Paris, [3]Université de Bordeaux, Faculté de Médecine Paul Broca, Bordeaux, [4]Université Paris Diderot, Sorbonne Paris Cité, UFR de Médecine, Paris, [5]CHU de Poitiers, Service d'Endocrinologie, Poitiers, France.

Background and aims: Experimental evidence supports a causal role for vasopressin (or antidiuretic hormone) in the development of chronic kidney disease and diabetic nephropathy through V2 receptor activation. Plasma copeptin, the COOH-terminal portion of pre-provasopressin and

a surrogate marker of vasopressin, was shown to be positively associated with the development and progression of diabetic nephropathy, and with end stage renal disease (ESRD) in type 1 and type 2 diabetes. To address the causality of this association, we assessed the association of *AVP* gene variants with plasma copeptin and with the risk of renal events during follow-up in a prospective cohort of people with type 2 diabetes.

Materials and methods: We studied 3077 French participants from the DIABHYCAR study (men 73%; micro- and macroalbuminuria in 76% and 24% of participants at baseline, respectively), a multicentre clinical trial on ramipril and renal and cardiovascular complications. A renal event during follow-up (median 5 years) was defined as the doubling of serum creatinine or the occurrence of ESRD (dialysis or transplantation requirement). Plasma copeptin was measured in baseline samples by an immunoluminometric assay. Six SNPs were chosen in the haplotype block containing the *AVP*gene (chr20p13): rs6084265, rs6084264, rs3761249, rs2282018, rs2770381 and rs1410713.

Results: All SNPs were significantly associated with baseline plasma copeptin. rs6084265: 8.0 ± 0.3 (CC), 8.6 ± 0.2 (TC) and 8.8 ± 0.3 pmol/L (TT; $p = 0.04$). rs6084264: 8.9 ± 0.3 (CC), 8.2 ± 0.2 (TC) and 7.7 ± 0.4 pmol/L (TT; $p = 0.005$). rs3761249: 8.4 ± 0.2 (TT), 8.6 ± 0.3 (TG) and 6.5 ± 1.0 pmol/L (GG; $p < 0.05$). rs2282018: 8.8 ± 0.3 (TT), 8.2 ± 0.2 (TC) and 7.8 ± 0.4 pmol/L (CC; $p = 0.01$). rs2770381: 7.8 ± 0.3 (AA), 8.5 ± 0.2 (AC) and 9.3 ± 0.3 pmol/L (CC; $p = 0.0002$). rs1410713: 8.7 ± 0.2 (CC), 8.5 ± 0.2 (CA) and 7.6 ± 0.4 pmol/L (AA; $p = 0.04$; ANCOVA, adjusted for sex, age, duration of diabetes, HbA1c, urinary albumin excretion and estimated glomerular filtration rate at baseline). The cumulative incidence of renal events during follow-up was 2.4% ($n = 75$). Baseline plasma copeptin was significantly associated with renal events in a Cox analysis. Hazard ratio (HR) for 1 unit of log[copeptin]: 3.12, 95% C.I. 2.18–4.42, $p < 0.0001$. Five SNPs were associated with renal events. HR (and 95% C.I.) were 2.23 (1.13–4.39), $p = 0.02$, for the T-allele of rs6084265 in a codominant model; 1.95 (1.32–2.97), $p = 0.0007$, for the C-allele of rs6084264 in a codominant model; 1.57 (1.10–2.29), $p = 0.01$, for the T-allele of rs2282018 in a codominant model; 1.80 (1.02–3.03), $p = 0.04$, for the CC-genotype (vs AX) of rs2770381; 1.77 (1.10–2.89), $p = 0.02$, for the CC-genotype (vs XA) of rs1410713 (all Cox analyses with same adjustments as above plus study treatment randomisation ramipril vs placebo).

Conclusion: We observed an association of plasma copeptin with severe renal events, and of allelic variations in the *AVP* locus with plasma copeptin and with the renal events in a cohort of people with type 2 diabetes. This pattern of mendelian randomisation supports the causality of the association of plasma copeptin (and vasopressin) with diabetic nephropathy.

Disclosure: **G. Velho:** None.

184

Cytosine methylation sequencing predicts the development of diabetic complications

I. Khurana[1], M. Ziemann[1], A. Kaspi[1], A.M. Syreeni[2], C. Forsblom[2], R. Ma[3], J.C.N. Chan[3], M.E. Cooper[1], P.-H. Groop[2], A. El-Osta[1];
[1]Monash University, Melbourne, Australia, [2]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, Helsinki, Finland, [3]Hong Kong Institute of Diabetes and Obesity, Hong Kong, Hong Kong.

Background and aims: While genome-wide methylation studies are typically performed using BeadChip array technology, this does not provide sufficient coverage to construct an integrated epigenetic regulatory network (ERN), important because the development of diabetic complications is considered a complex polygenic-multifactorial disorder. To address knowledge gap, we examined DNA methylation to define the ERN in the Finnish Diabetic Nephropathy (FinnDiane) cohort. Participants from FinnDiane study were selected based on the presence or absence of diabetes and diabetic kidney disease (DKD).

Materials and methods: Participants from FinnDiane study were selected based on the presence or absence of diabetes and diabetic kidney disease (DKD). Using methyl-CpG capture followed by massive parallel sequencing (methyl-seq) in leukocytes derived from 39 individuals, we detected differentially methylated regions (DMRs) associated with DKD

Results: Gene body-related regions made up >60% of the methylation differences, with <10% localised to exons. Integrative methylation analyses reveal 494 differentially methylated genes that intersect CTCF binding sites (181 genes with increased and 313 genes with reduced methylation). To determine the significance of DNA methylation changes we assessed 95 individuals using a FinnDiane validation cohort. We also assessed DKD-associated DMRs in a replication cohort from the Hong Kong Diabetes Registry. We show CTCF binding sites are sensitive to loss-of-methylation with gain-of-function in diabetes implicated in DKD involving insulin signalling, PI3K cascade, integrin cell interactions and lipid metabolism. These clinical findings were tested ex vivo using primary human diabetic vascular cells and renal derived podocytes. We confirm the transition from normal to high glucose conditions regulates insulin signalling genes mTOR, RPTOR, IRS2, FGF1 and GAB1, mediated by DNA methylation. We provide proof of concept that methylation regulates the activity of genes functionally important in signalling of insulin.

Conclusion: Not only does the epigenetic regulatory network presented here strengthen the evidence base against methylation changes merely being an epiphenomenon but the identification of core pathways and gene targets will be a useful resource and better understanding of regulatory mechanisms that contribute to the pathogenesis of diabetic kidney disease.

Supported by: NHMRC

Disclosure: **I. Khurana:** None.

185

Whole genome sequencing of individuals with type 1 diabetes reveals novel susceptibility loci for diabetic nephropathy

J. Haukka[1,2], N. Sandholm[1,2], E. Valo[1,2], M. Parkkonen[1,2], V. Harjutsalo[3], C. Forsblom[1,2], P.-H. Groop[1,2];
[1]Folkhälsan Institute of Gemetics, Folkhälsan Research center, Helsinki, [2]Abdominal Center Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, [3]The Chronic Disease Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland.

Background and aims: In recent years genome-wide association studies have yielded a few genome-wide significant common variants for diabetic nephropathy, most of them associated with end-stage renal disease. Compared to microarray chip based study designs, whole genome sequencing offers unrivalled capability to study low-frequency and rare susceptibility variants with larger effect on disease risk. Here, we performed a whole genome sequencing study in FinnDiane patients with type 1 diabetes and extreme phenotype regarding diabetic nephropathy.

Materials and methods: Altogether 599 subjects were recruited from the Finnish Diabetic Nephropathy Study (FinnDiane) cohort. The individuals represent extreme phenotypes for diabetic nephropathy: 299 cases had developed severe diabetic nephropathy (macroalbuminuria or ESRD), whereas 300 controls had retained normal AER for at least 35 years. Groups have similar age of diabetes onset (median cases: 11.7y, controls: 12.3y) but the cases had slightly higher HbA_{1c} (median cases: 9.0%, controls: 8.0%).The sequencing was conducted using Illumina HiSeq X platform by Macrogen Inc with at least 30x coverage. The short read data was processed and variants were called individually for each sample using Isaac aligner and variant caller according to Macrogen's standard pipeline. Of the 599 samples, 584 samples passed the QC and were included in the analyses.

Results: On average the participants had 3.7 million variants deviating from the reference, and in total the cohort included 22 million variants. Four variants on chromosome 22q11, in strong LD with each other,

reached the genome-wide significant p value threshold (5×10^{-8}), with the lowest p value 2.56 × 10-10. One SNP on chromosome 2p11.2 and one insertion on chromosome 5q21.1 reached suggestive p values of 1.53×10^{-7} and 8.88×10^{-7}, respectively. The intergenic chromosome 22 SNPs were close to PRDOH and several non-protein coding genes. Interestingly the same region resulted in the highest linkage peak in a previous linkage study in FinnDiane sibling pairs. However, none of the genes within 50 kb of the variants have previously been associated with any kidney diseases. Of note, these variants localize to a region of the genome with low mapping quality and require further validation.

Conclusion: This whole genome sequencing study reveals novel risk loci for diabetic nephropathy. However, the chromosome 22 findings need to be validated with more robust read processing and variant calling. Joint variant calling using Broad Institute's best practices guidelines with Genome Analysis Toolkit is in progress.

Supported by: Folkhälsan Research F, WE Stockmann F, Novo Nordisk F, Academy of Finland

Disclosure: **J. Haukka:** None.

186

Cooperative regulation of insulin signalling genes by DNA methylation in human podocytes

A. Jørgensen[1,2], I. Khurana[1], A. El-Osta[1];
[1]Department of Diabetes, Monash University, Melbourne, Australia, [2]Metropolitan University College, Copenhagen, Denmark.

Background and aims: Diabetic kidney disease (DKD) is the most common cause of end stage renal disease (ESRD). DKD is associated with abnormal changes in tubular and glomerular cells in the kidney. Podocyte injury, dysfunction, and loss are central to the development and progression of DKD, however, the precise mechanism remains poorly understood. While the pathogenesis of DKD is complex we now appreciate that seemingly disconnected pathways mediated by hyperglycemia are integrated by genetic and environmental determinants. Nowhere is this more evident than in type 1 diabetes (T1D). For instance, while some patients with T1D develop DKD after many years of disease, the majority do not, despite similar levels of hyperglycemia. We postulate glucose-induced DNA methylation changes activate insulin signaling pathways in the human podocyte.

Materials and methods: We cultured human podocytes in normo-glucose (NG) and high-glucose (HG) for 15 days. DNA methylation patterns were assessed using methyl-binding-domain capture (MBDCap) followed by qPCR. Transcription factor binding was monitored by chromatin immunoprecipitation (ChIP) assay. Quantitative real-time PCR (qPCR) was used to assay gene expression and high throughput protein arrays to detect changes in insulin signaling.

Results: We found changes in DNA methylation patterns and gene activity of mechanistic target of rapamycin (mTOR), regulatory-associated protein of mTOR (RPTOR), insulin receptor substrate 1 (IRS-2), GRB2 associated binding protein 1 (GAB1) and fibroblast growth factor 1 (FGF1) were associated with insulin signaling in hyperglycemic podocytes. For example, mTOR DNA methylation was significantly reduced (~60% for HG) and inversely correlated with CTCF transcription factor binding. Furthermore, increased gene expression levels are consistent with mTOR protein activity in human podocytes under hyperglycemic conditions.

Conclusion: Hyperglycemic podocytes undergo dramatic changes in DNA methylation that regulate the expression of genes such as mTOR implicated in the regulation of insulin signaling pathways. The regulatory capacity of CTCF that we have identified suggests other methylation sites could also be prime candidates for gene control in human podocytes.

Disclosure: **A. Jørgensen:** None.

OP 32 Beta cells stick together to fight insulin resistance

187

Determinants and pathological role of insulin hypersecretion in non-diabetic adults and adolescents

D. Tricò[1], A. Natali[1], S. Arslanian[2], A. Mari[3], E. Ferrannini[4];
[1]University of Pisa, Pisa, Italy, [2]University of Pittsburgh Medical Center, Pittsburgh, USA, [3]National Research Council, Padua, Italy, [4]National Research Council, Pisa, Italy.

Background and aims: The prevailing view of the natural history of type 2 diabetes (T2D) is that an early loss of insulin sensitivity precedes and causes a progressive increase of β cell insulin secretion, which would initially compensate for the insulin resistance to maintain glucose homeostasis. However, mechanisms underlying insulin hypersecretion are not fully understood, and growing evidence suggests that an inappropriate increase of insulin secretion may be the first event leading to T2D. Our aim was to identify insulin hypersecretion, independent of insulin resistance, and its role in the early derangements of glucose homeostasis.

Materials and methods: In 1,287 non-diabetic adults followed up for 3 years, insulin secretion rate (ISR) and β cell function parameters were estimated by C-peptide modelling during an OGTT. Insulin sensitivity (M/I) was measured by a hyperinsulinaemic-euglycaemic clamp. After regressing ISR on M/I, subjects in the upper tertile of the distribution of residuals were defined as hypersecretors (HyperS), while the other subjects as normosecretors (NormoS). This analysis was repeated in a multiethnic cohort of 182 obese adolescents.

Results: HyperS were more likely to be females ($p = 0.001$) and to have family history of T2D ($p = 0.02$) than NormoS. Furthermore, HyperS showed older age ($p = 0.0003$), higher fasting ($p = 0.005$) and post-OGTT glucose levels ($p < 0.0001$), BMI ($p = 0.03$), percent fat mass ($p < 0.0001$), LDL cholesterol ($p = 0.001$), fasting triglycerides ($p < 0.0001$), insulin-suppressed NEFA ($p < 0.0001$), γ-glutamyl-transferase ($p = 0.001$), as well as lower physical activity ($p = 0.005$) and HDL cholesterol ($p = 0.002$), compared to NormoS, despite similar M/I ($p = 0.10$). Among β cell function parameters, HyperS had higher β cell glucose sensitivity ($p < 0.0001$) and ISR at 5 mmol/L glucose ($p < 0.0001$), after adjustments for age, sex, and BMI. In multiple logistic analysis, significant predictors of HyperS were female sex (OR 3.62[0.13–0.57], $p = 0.0004$), post-OGTT glucose (OR 1.81[1.45–2.26], $p < 0.0001$), triglycerides (OR 2.03[1.28–3.20], $p = 0.002$), physical activity (OR 0.82[0.67–0.99], $p = 0.04$), and HDL cholesterol (OR 0.52[0.30–0.93], $p = 0.02$). Similarly defined HyperS adolescents showed higher fasting glucose ($p = 0.01$), post-OGTT glucose ($p < 0.0001$), LDL cholesterol ($p = 0.04$) and fasting triglycerides ($p = 0.01$) compared to NormoS. Furthermore, HyperS adolescents were more likely to be African Americans than American Whites ($p = 0.003$). At follow up, HyperS had increased odds of developing altered glucose tolerance or T2D (OR 1.49 [1.01–2.21], $p = 0.046$), after adjusting for age, sex, BMI, and fasting glucose.

Conclusion: We identified the phenotype of HyperS, where sex, race, post-OGTT glucose levels, triglycerides, HDL cholesterol, and physical activity were main determinants of insulin secretion fully independently of insulin sensitivity in both non-diabetic adults and adolescents. Furthermore, we found that inappropriately elevated insulin secretion relative to insulin sensitivity predicts deteriorations of glucose control over time. This evidence challenges the traditional view of insulin secretion as a mere adaptive mechanism to insulin resistance, and warrants further studies to identify the mechanisms of primary insulin hypersecretion and potential approaches to its prevention and treatment.

Supported by: EU grant QLG1-CT-2001-01252, AstraZeneca (Sweden)

Disclosure: **D. Tricò:** None.

188

Quantifying beta cell mass non-invasively with PET probe: ^{18}Fluorine-labelled Exendin4

N. Fujita[1], H. Fujimoto[1], K. Hamamatsu[1], T. Murakami[1], H. Kimura[2], K. Toyoda[1], H. Saji[3], N. Inagaki[1];
[1]Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, [2]Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Kyoto, [3]Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

Background and aims: β-cell function and β-cell mass (BCM) play important roles in insulin secretion. Cross-sectional studies on harvested pancreas have reported that BCM decreases in patients with type 2 diabetes. Thus, a method to quantify BCM non-invasively and repeatedly will enable us to improve our understanding on the pathophysiology of diabetes. However, the method has not been established yet. Therefore, we aim to examine whether BCM can be quantified non-invasively using a glucagon-like polypeptide-1 (GLP-1) receptor targeting probe, ^{18}Fluorine-labeled Exendin4 (^{18}F-Ex), with positron emission tomography (PET).
Materials and methods: The specificity of ^{18}F-Ex to GLP-1 receptors was assessed by a binding assay with a membrane-prepared recombinant human GLP-1. Specific accumulation to β-cells was evaluated by autoradiography with pancreatic sections of transgenic MIP-GFP mice after intravenously injecting ^{18}F-Ex. A blocking study was also performed with a pre-injection of excess non-radiolabeled Exendin(9-39). Organ specificity was investigated by a bio-distribution study with 6-week-old male BL6 mice. PET images of 12-week-old female NOD mice (4 hyperglycemic and 6 euglycemic) were taken at 30 min after injecting ^{18}F-Ex intravenously. Pancreata of the 10 NOD mice were harvested after obtaining PET images. The percentage of ^{18}F-Ex uptake in the pancreas was calculated by measuring the radioactivity of injected ^{18}F-Ex and harvested pancreas using a gamma counter. Pancreatic sections were stained with an anti-insulin antibody. BCM was calculated by multiplying the relative insulin-positive area by pancreatic weight. The percentage of ^{18}F-Ex uptake in the pancreas was compared between hyperglycemic and euglycemic NOD mice. The correlation between BCM and the percentage of ^{18}F-Ex uptake in the pancreas was also examined.
Results: A similar IC_{50} of ^{18}F-Ex and GLP-1(7-36) amide was confirmed in the binding assay analysis (0.070 nM and 0.045 nM, respectively). Radioactive signals in autoradiography were co-localized with fluorescent signals that are detected in insulin-producing β-cells on the pancreatic sections of MIP-GFP mice. The radioactive signals were blocked with the pre-injection of excess non-radiolabeled Exendin(9-39). In the bio-distribution study, the pancreatic uptake of ^{18}F-Ex was the highest at 30 min after injection (23.8%ID/g) and was retained for 2 h (17.5%ID/g). On the PET image, the pancreas was able to be identified and was distinguishable from other surrounding organs. *In vivo* radioactive signals from the pancreas were apparently weaker in the hyperglycemic NOD mice than in the euglycemic NOD mice. A significant difference was found between the hyperglycemic and euglycemic NOD mice in the percentage of ^{18}F-Ex uptake in the harvested pancreas (10.8%ID/g vs 18.4%ID/g, $p = 0.02$). The percentage of ^{18}F-Ex uptake in the harvested pancreas significantly correlated to BCM ($R = 0.72$).
Conclusion: ^{18}F-Ex was proven to specifically accumulate to β-cells. BCM significantly correlated with ^{18}F-Ex uptake in the harvested pancreas that can be non-invasively quantified using PET. Therefore, BCM could be non-invasively estimated by PET with ^{18}F-Ex *in vivo*.
Disclosure: **N. Fujita:** None.

189

Glucose homeostasis, insulin secretion and beta cell transcriptomics of mice with beta cell specific insulin resistance

S. Skovsø[1], D.A. Dionne[1], E. Panzhinskiy[1], H. Modi[1], J. Kolic[1], P. Overby[1], D. Hutchinson[1], S. Marcil[1], X. Hu[1], H.L. Noh[2], S. Suk[2], S. Flibotte[3], L.C. Alonso[4], J.K. Kim[4,2], J.D. Johnson[1];
[1]Diabetes Research Group, Life Sciences Institute, Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada, [2]Program in Molecular Medicine, Department of Medicine, University of Massachusetts Medical School, Worcester, USA, [3]Department of Zoology, University of British Columbia, Vancouver, Canada, [4]Division of Diabetes, Department of Medicine, University of Massachusetts Medical School, Worcester, USA.

Background and aims: The potential role of β-cell insulin resistance in diabetes remains somewhat enigmatic. Previously, autocrine insulin action has alternately been proposed to decrease or increase its own secretion, and the consequences of β-cell insulin resistance would differ in each scenario. The *Ins1*^{Cre} mouse model has become available and permits gene deletion specifically in the β-cells, but not in the brain. Our aim was to examine insulin release and gene expression on β-cells from mice lacking one or both alleles of the insulin receptor (*Insr*) gene.
Materials and methods: Male and female knockout *Insr*$^{f/f}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ mice, heterozygous *Insr*$^{f/wt}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ mice, and *Insr*$^{wt/wt}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ mice littermate controls were fed a low fat diet (LFD) or a high fat diet (HFD). Glucose tolerance, insulin tolerance, and insulin secretion (all *i.p.*) were assessed at multiple ages. Insulin secretion was also examined using the hyperglycemic clamp technique. RNAseq was conducted on FACS-purified, recombined β-cells, with Reactome analysis of differentially expressed pathways.
Results: Glucose homeostasis was significantly ($p < 0.05$) improved in LFD female *Insr*$^{f/f}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ and *Insr*$^{f/wt}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ mice when compared to controls at 9, 21 and 39 weeks. There were no significant differences between groups of male mice, or between groups of HFD-fed mice, suggesting the possibility that global insulin resistance may obscure these effects and prompting us to focus on female LFD-fed mice to understand the mechanisms of improved glucose tolerance. In these mice, no difference in insulin tolerance was observed at 10, 22 or 40 weeks. However, plasma insulin levels were higher following *i.p.* glucose challenge ($p = 0.055$), explaining their improved glucose tolerance. Similarly, insulin levels were exaggerated while plasma glucose levels were maintained at ~350 mg/dl in hyperglycemic clamp experiments. The glucose infusion rates required to maintain hyperglycemia during clamp trended higher in *Insr*$^{f/f}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ versus littermate controls likely due to a higher insulin secretion occurring during β-cell specific insulin resistance. Together, these data support the hypothesis that glucose stimulated insulin release inhibits its own secretion during hyperglycemic conditions. Reactome analysis of RNAseq data pointed to significant differences in genes regulating β-cell function in *Insr*$^{f/f}$:*Ins1*$^{cre/wt}$:nTnG$^{+/-}$ versus controls.
Conclusion: Loss of β-cell *Insr* increases insulin levels during glucose challenge thereby improving glucose homeostasis in young mice, consistent with the concept that glucose stimulated insulin release normally inhibits its own secretion during hyperglycemic conditions. By contributing to hyperinsulinemia, β-cell specific insulin resistance may play an early role in the initiation of type 2 diabetes pathogenesis.
Supported by: CIHR
Disclosure: **S. Skovsø:** None.

190

FGF family members may represent novel drivers of beta cell dedifferentiation in type 2 diabetes

S. Knight[1], N. Mullooly[1], B.J. Isherwood[1], L. Holmberg-Schiavone[2], C. Wennberg.Huldt[3], G. O'Mahony[3], S. Gopel[3];
[1]Discovery Sciences, IMED Biotech Unit, AstraZeneca, Cambridge, UK, [2]Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden, [3]Cardiovascular, Renal and Metabolic Disease, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.

Background and aims: Recent evidence suggests β-cell dedifferentiation as a key driver for the loss of pancreatic β-cell function. We adapted a previously established model of dedifferentiation using the human β-cell line, EndoC-βH1 to (i) enable high-throughput systematic investigation of the pathways involved in dedifferentiation in Type 2 diabetes (T2D) and (ii) identify small molecules capable of reversing the dedifferentiated phenotype.
Materials and methods: We performed a phenotypic screen using the EndoC-βH1 model of dedifferentiation to monitor the ability of 916 proteins of the secretome to alter the differentiation status of the human pancreatic β-cell line. Dedifferentiation was monitored by measuring changes in the subcellular location and expression of the β-cell marker MAFA (key regulator of glucose stimulated-insulin secretion) and the pancreatic progenitor marker SOX9. We further profiled small molecules in an FGF2-driven model of EndoC-βH1 dedifferentiation to assess the potential to reverse the effect.
Results: FGF9, FGF18 and FGF4 were identified as novel biological modulators of the dedifferentiation phenotype, along with previously established FGF1 and FGF2. FGF9 induced the strongest phenotypic effect with a 2 fold increase in SOX9 nuclear intensity and a 1.5 fold reduction in MAFA nuclear/cytoplasmic localisation compared to FGF2 control. The unique ability of the FGF family members from 916 secreted proteins to alter the dedifferentiation phenotype suggests that FGF signalling may be an important player in dedifferentiation. 13 of the 18 secreted FGFs were constituents of the tested library, however many members including FGF7 and FGF10 from the FGF7 subfamily and FGF23, FGF21 and FGF19 from the endocrine FGF subfamily did not induce dedifferentiation indicating that specific FGF-FGFR or FGF+co-factor-FGFR interactions are likely to govern the dedifferentiation phenotype. Indeed further studies indicate that a FGFR1c antibody is capable of partially inhibiting FGF2 induced changes in MAFA and SOX9 in both EndoC-βH1 and human primary islets. Using single cell RNA sequencing, FGFR1, FGF2 and FGF9 were found to have increased expression in pancreatic cells from T2D, with FGF9 and FGFR1 showing increased expression in β-cells. The FGF2-driven dedifferentiation of the EndoC-βH1 cell line was found to be reversible through treatment with small molecule inhibitors of MAPK/ERK and TGFβ signalling. For example, a MEK1 inhibitor (pEC50 = 5.5, pIC50 = 6.9), and a TGFβR1/TGFβR2 inhibitor (pEC50 = 5, pIC50 = 5.2) restored MAFA and reduced SOX9 protein expression to dedifferentiated cells to similar levels as that observed for undifferentiated cells.
Conclusion: We identified members of FGF family that may represent novel drivers of dedifferentiation in T2D. We further show increased transcriptional expression of FGF9 in pancreatic β-cells from T2D patients. We demonstrated reversibility of the phenotype through pharmacological intervention, supporting the pharmacological targeting of redifferentiation for therapeutic intervention in T2D. To our knowledge this is the first example of small molecule driven reversibility of a dedifferentiated phenotype in a human-based pancreatic β-cell model.
Disclosure: **S. Knight:** None.

191

Hyperglycaemia adversely affects mitochondrial function in pancreatic islets

E. Haythorne[1], A.I. Tarasov[2], F.M. Ashcroft[1];
[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, [2]OCDEM, University of Oxford, Oxford, UK.

Background and aims: Glucose metabolism is essential for glucose-stimulated insulin secretion from pancreatic β-cells. Metabolically generated ATP causes ATP-sensitive potassium (K_{ATP}) channel closure, membrane depolarisation and calcium influx, which stimulates exocytosis. There is accumulating evidence to suggest that β-cell mitochondrial metabolism is impaired in type 2 diabetes mellitus (T2DM), and contributes to reduced insulin secretion. As hyperglycaemia is common to all forms of diabetes, we aimed to determine if hyperglycaemia adversely affects mitochondrial function and thereby ATP production in pancreatic islets.
Materials and methods: In human patients and animal models of T2DM, hyperglycaemia often occurs in conjunction with elevated levels of circulating lipids, making it difficult to distinguish between the deleterious effects of glucose and lipids. We therefore used the βV59M mouse model in which hyperglycaemia is not accompanied by dyslipidaemia. In these mice, tamoxifen-inducible expression of a constitutively open K_{ATP} channel specifically in pancreatic β-cells inhibits insulin secretion and rapidly elevates blood glucose (>20 mmol/l). Diabetes was induced at 12 weeks of age and islets isolated 2 weeks later. Non-diabetic littermates were used as controls. Cellular Oxygen Consumption Rate (OCR) was monitored in real-time using the XF-24 extracellular flux analyser (Seahorse Bioscience, Inc.). Imaging the kinetics of ATP in β-cells was performed on a zoom microscope AxioZoom.V16 (Carl Zeiss) and utilised fluorescent sensor for Mg^{2+} (Mg-Green, ThermoFisher) as a surrogate for ATP.
Results: In comparison to islets from control mice, islets from diabetic mice showed a significant reduction in the % increase in OCR when ambient glucose was raised from 2 to 20 mmol/l (diabetic = 48.44 ± 8.98 ± vs. control = 91.29 ± 7.26% increase in OCR, $p < 0.005$; $n = 9$–12, 6 animals/genotype). Sequential addition of the ATP-synthase inhibitor oligomycin produced significantly less inhibition of OCR in islets from diabetic mice compared to control, indicating hyperglycaemia reduces the activity of ATP-synthase (diabetic = 88.72 ± 6.43 vs control = 123.05 ± 5.59% decrease in OCR, $p < 0.05$; $n = 5$–7, 3 animals/genotype). Subsequent addition of rotenone and antimycin A, which inhibit complex 1 and 3 of the electron transport chain respectively, supressed OCR to the same degree in both diabetic and control islets, indicating no difference in the level of mitochondrial leak. ATP increase in response to 20 mmol/l glucose was reduced in hyperglycaemic islets by 56 ± 6% (control = 1147 islets, diabetic = 423 islets).
Conclusion: Our results demonstrate that hyperglycaemia, independent of changes in circulating lipids, leads to impaired mitochondrial respiration and ATP production in pancreatic islets. Mitochondrial metabolism is essential for the stimulation of insulin secretion by glucose and therefore hyperglycaemia-induced mitochondrial dysfunction is likely to contribute to β-cell failure in T2DM. It remains to be determined if the deleterious effects of hyperglycaemia are limited to β-cells or if they occur in all islet cell types.
Supported by: ERC
Disclosure: **E. Haythorne:** None.

192

The liver-alpha-cell axis during weight loss in type 2 diabetes

J. Otten, A. Stomby, M. Waling, E. Chorell, M. Ryberg, M. Svensson, J.J. Holst, T. Olsson;
Umeå University, Umeå, Sweden.

Background and aims: The concept of a *liver-alpha-cell axis* has recently been described: increasing levels of amino acids stimulate glucagon secretion which, in turn enhances amino acid turnover in the liver by increasing ureagenesis. Thus, alpha cell function is essential for maintaining normal amino acid levels. Alanine and glutamine in particular are reported to stimulate glucagon secretion and alpha cell proliferation, respectively. We aimed to investigate the effect of weight loss by a Paleolithic diet with/without exercise on fasting amino acids, endogenous glucose production (EGP) and glucagon levels during a mixed meal to elucidate the *liver-alpha-cell* axis.
Materials and methods: Thirty-two overweight patients with type 2 diabetes were randomized to either a Paleolithic diet (PD) or a Paleolithic diet combined with supervised exercise (PD-EX). Subjects were served a solid mixed meal test at baseline and after 12 weeks. Glucose, insulin and glucagon were measured at 0, 30, 60, 120 and 180

min with calculation of the total area under the curve for the response. On another study day, fasting glutamine and alanine were measured with GC-MS and suppression of EGP was examined with the hyperinsulinemic euglycemic clamp technique with [6,6-$^2H_2$]glucose as a tracer and with an insulin infusion of 40 mU/m^2/min.

Results: Median weight loss was 7 kg in both study groups. Fasting glucagon tended to decrease in both study groups. Postprandial glucagon decreased by 22% in the PD-group ($P < 0.01$) and by 21% in the PD-EX group ($P = 0.13$). Fasting alanine decreased in the PD group ($P < 0.01$). Fasting glutamine increased non-significantly in both groups. Suppression of EGP increased by 26% in the PD group ($P < 0.05$) and by 10% in the PD-EX group ($P = 0.75$). The increased suppression of EGP during the intervention, was associated with a) reduction of postprandial glucagon levels ($r_S = -0.65$, $P < 0.01$), b) decreasing fasting glucagon levels ($r_S = -0.51$, $P < 0.05$) and c) the increasing fasting glutamine levels ($r_S = 0.51$, $P < 0.05$).

Conclusion: Weight loss decreases glucagon levels in concert with improved suppression of endogenous glucose production of the liver/kidney. The latter is associated with increasing glutamine levels, which may be involved in the regulation of the *liver-alpha-cell axis*.

Clinical Trial Registration Number: NCT01513798

Supported by: Swedish Diabetes Research Foundation, Heart and Lung Foundation,

Disclosure: **J. Otten:** None.

OP 33 Dietary patterns and metabolic regulation

193

Integration of half-day carbohydrate restriction into a hypocaloric Mediterranean-type diet in overweight and obese subjects: an open label, randomised, controlled trial

D. Tsilingiris, D. Dellis, I. Eleftheriadou, A. Tentolouris, M. Karanasiou, A. Meimari, G. Dellis, C. Dimosthenopoulos, S. Lazarou, A. Kokkinos, P.P. Sfikakis, N. Tentolouris;

1st Department of Propaedeutic Internal Medicine, Medical School, National and Kapodistrian University of Athens, Greece, Athens, Greece.

Background and aims: Among available modalities for weight loss, carbohydrate restriction diets achieve rapid weight decline with concomitant improvements in metabolic risk factors. Adherence to a strict carbohydrate restriction regimen is often hard to achieve. Aim of the present study was to investigate the effect of a half-day (morning) carbohydrate restriction diet (HCR-D) on weight loss and metabolic risk factors in overweight and obese subjects.

Materials and methods: A total of 70 overweight and obese individuals [41 (58.6%) females, 24 (34%) with type 2 diabetes, mean age 49.9 ± 10.7 years, mean body mass index-BMI 33.7 ± 3.9 kg/m^2)] were randomly allocated between two hypocaloric dietary regimens: HCR-D and a Mediterranean-type diet that served as control. Participants in the HCR-D group were permitted a minimum of 300 kcal of very low carbohydrate breakfast and a mid-morning snack, while a maximum of 300 kcal of typical Mediterranean early daily meals were allowed in the control group. Both diets were identical from midday thereafter. Participants were followed up over a period of 2 months. Somatometric and laboratory data were collected at screening and at follow-up completion.

Results: Baseline clinical and laboratory characteristics were comparable between the two groups. As compared to baseline, individuals in both groups achieved significant and clinically meaningful reductions in body weight, BMI, waist circumference and body fat mass. However, reductions were more pronounced in the HCR-D compared to the control group in weight, BMI, waist circumference and fat mass (mean differences between groups 3.45 kg, 1.52 kg/m^2, 3.21 cm, 1.45 kg, respectively, all $p < 0.001$). Furthermore, compared to the control group more participants in the HCR-D achieved loss of 5–10% of body weight by the end of the 1st month (77.1 vs 31.4%, $p < 0.001$), as well as 5–10% and >10% of weight by the end of the 2nd month (65.7 vs. 57.1% and 34.3 vs. 8.6% respectively, $p < 0.001$). All individuals in the HCR-D group achieved loss of ≥5% baseline weight by the end of the intervention as compared to 65.7% in the control group ($p < 0.001$). Participants in both groups achieved similar reductions in fasting serum glucose (FSG) and HbA_{1c} (−4.23 vs. −6.40 mg/dl, and 0.26% vs. −0.24%, respectively all $p > 0.05$) as well as improvements in the homeostatic model assessment index for insulin resistance (HOMA-IR) (−0.91 vs. −0.78, $p > 0.05$). Among individuals with type 2 diabetes, weight loss, BMI and weight circumference reductions from baseline were significantly greater for those allocated to the HCR-D than the control group (mean differences between groups 3.76 kg $p = 0.002$, 1.21 kg/m^2 $p = 0.005$, 3.49 cm $p = 0.002$ respectively). Loss of fat mass and improvements in FSG, HbA_{1c} and HOMA-IR were similar for participants with diabetes in both groups.

Conclusion: Application of morning carbohydrate restriction on a Mediterranean diet resulted in greater and more rapid weight loss while retaining metabolic benefits regarding glycemia-related parameters. Such strategies may combine the beneficial effects of carbohydrate restriction diets with improved adherence.

Disclosure: **D. Tsilingiris:** None.

194

Three meals diet with high energy breakfast is an effective strategy for weight loss, reduction of glucose variability and of total daily insulin dose in type 2 diabetes

D. Jakubowicz[1], O. Froy[2], S. Tsameret[2], J. Wainstein[1], I. Raz[3], M. Menaged[1], Y. Bar-Dayan[4], N. Mor[4], T. Ganz[4], Z. Landau[4];
[1]Wolfson Medical Center, Tel Aviv University, [2]Institute of Biochemistry, The Hebrew University of Jerusalem, [3]Diabetes Unit, Hadassah University Hospital, The Hebrew University of Jerusalem, [4]Wolfson Medical Center, Holon, Israel.

Background and aims: Obese patients with uncontrolled type 2 diabetes (T2D) and insulin resistance (IR), often require high total daily insulin dose (TDID), which leads to weight gain, worsening IR and hyperglycemia, requiring further increase of TDID, a vicious cycle ever-increasing TDID, weight gain, persistent hyperglycemia and high risk for diabetes complications. We have previously shown that a 3-meal diet (3Mdiet) with timing schedule, consisting of high energy breakfast (B) and Lunch (L) and low-energy dinner (D), can improve glycemic control, overall glycemia, reduce appetite and promote weight loss (WL) in obese and orally treated patients with T2D. Our aim was to compare the effects of 3Mdiet vs. 6-meals diet evenly distributed throughout the day (6Mdiet) on WL, overall glycemia, glucose variability (GV), appetite and TDID reduction in uncontrolled insulin treated T2D.
Materials and methods: Twenty-eight T2D (age 69 ± 7 yrs; BMI: 32.2 ± 5 kg/m^2; since 19.9 ± 8 yrs) were randomly assigned to12 weeks of isocaloric nutritional intervention with either 3Mdiet (1600 ± 200 kcal, B:L:D: 50:33:17%) or 6Mdiet (1600 ± 200 kcal, B:L:D: 20:25:25% + 3 snacks 10% each). Overall glycemia and GV including Daily-Risk-for Hyperglycemia (HBGI) and Average-Daily-Risk-Range (ADRR), were assessed for 14 days by continuous glucose monitoring (CGM) at baseline and at 12 weeks. Insulin dose was titrated biweekly.
Results: After 12 weeks improvement of the following parameters demonstrated in 3Mdiet compared to 6Mdiet, respectively: WL (−5.0 ± 0.9 kg vs. +0.26 ± 0.3 kg, $p < 0.05$). HbA1c decreased by −1.2 ± 0.3% (8.2 ± 1% to 7.0 ± 0.3%) vs −0.2 ± 0.4% (7.96 ± 1% to 7.7 ± 0.4%) ($p < 0.05$). Overall glycemia decreased in 3Mdiet by −40 ± 10 mg/dl (169 ± 23 to 129 ± 11 mg/dl) vs. −18 ± 16 mg/dl (174 ± 24 to 156 ± 20 mg/dl) in 6Mdiet ($p < 0.05$). There was no correlation between Δ body weight and Δ overall glycemia in neither of the groups ($R^2 = 0.0363$ for 3Mdiet, and $R^2 = 0.0135$ for 6Mdiet). GV indices were reduced in 3Mdiet by 62% for HBGI and by 55% for ADRR compared to 6Mdiet ($P < 0.05$). Overall VAS_{100} hunger scores were reduced by −18 ± 3 in 3Mdiet, but increased by 2 ± 1.7 in 6Mdiet ($p < 0.05$). Similarly, craving scores (especially for carbohydrates/starches) were augmented by 4 ± 5.1 with 6Mdiet, while in 3Mdiet were significantly reduced by −36 ± 7.7 ($p < 0.05$). At the end of the intervention, TDID increased by 4.9 ± 14 units/day (from 70.6 ± 17 to 75.5 ± 11 units/day) in 6Mdiet, whereas was significantly reduced by −27 ± 16 units/day (from 73.5 ± 16 to 33.8 ± 15.2 units/day) in 3Mdiet ($p < 0.05$).
Conclusion: In uncontrolled insulin treated T2D, the 3Mdiet with high energy breakfast was shown to be more effective than the traditional diet with six small meals evenly distributed throughout the day, for WL, overall glycemia, HbA1c, glucose variability, appetite and for the reduction of insulin requirements. Importantly, the reduction of overall glycemia and glucose variability, was independent of weight loss suggesting beneficial effect of meal timing schedule consisting on 3Mdiet. Therefore, meal timing with 3Mdiet with high-energy-breakfast should be strategy to improve diabetes control and outcome with less daily insulin dose.
Clinical Trial Registration Number: NCT02709915
Supported by: Israel Ministry of Health
Disclosure: **D. Jakubowicz:** None.

195

The incretin effect of essential amino acids (EAA) in youth and ageing

H. Abdulla[1], J.J. Bass[1], T. Stokes[2], S.H. Gorissen[2], S.M. Phillips[2], B.E. Phillips[1], K. Smith[1], I. Idris[1], P.J. Atherton[1];
[1]Molecular, Metabolic and Clinical Physiology Group, MRC-ARUK Centre for Musculoskeletal ageing research - School of Medicine - University of Nottingham, Derby, UK, [2]Department of Kinesiology, Mcmaster University, Hamilton, Canada.

Background and aims: The oral ingestion of glucose triggers greater insulin release than a comparable glucose challenge delivered intravenously. However, whether this phenomenon - known as the 'incretin effect' - applies to ingested volumes of dietary essential amino acids (EAA) during intake of meals is ill-defined. Furthermore, since EAA (and insulin) are important components of skeletal muscle protein anabolism and muscle loss is accelerated with ageing, exploration of how ageing impacts any incretin effect of EAA is warranted. We thus explored: i) whether EAA could induce an incretin effect, and ii) whether ageing impacted this incretin effect.
Materials and methods: A 15 g mixed EAA beverage was administered orally to two groups of younger ($N = 8$; mean age 25.6 ± 1.5 y) and older ($N = 8$; mean age 69.1 ± 1 y) healthy volunteers. Another group of younger volunteers ($N = 9$; mean age 21 ± 1 y) were given an equivalent intravenous (IV) beverage aiming to achieve matched plasma AA profiles. Oral EAA were given as a drink dissolved in an aqueous solution and consumed over 2 min. For IV delivery, 15 g of mixed EAA were infused at a rate of 133 mg.min^{-1} for 15 min followed by a rate of 289 mg.min^{-1} for a further 45 min. Plasma concentrations of AA, insulin, glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were quantified over a period of 120 min. Analytically, AA were derivatised as their N-acetyl-N-propyl esters and their concentrations determined against a standard curve of known concentrations using gas chromatography mass spectrometry (GC-MS). Milliplex Map, commercial ELISA kits, were used to determine insulin and gut hormone abundance. Statistical analyses was performed in Graphpad Prism 7 via ANOVA and t-tests as appropriate.
Results: EAA produced rapid insulinaemia and aminoacidaemia with total AA, sum EAA and sum branched-chain AA matched between oral and IV groups. Insulin levels peaked at 1353.37 pmol l^{-1} at 30 min following oral feeding compared to 782.4 pmol l^{-1} at 60 min following IV feeding. EAA peaked at 3395.39 μM at 45 min during IV infusion compared to 2891.73 μM following oral intake (*p*: NS). There was an approximate 45% incretin effect on calculating insulin response in the first 60 min. GIP increased following oral intake (452.28 pmol l^{-1} vs 232 pmol l^{-1} , $p < 0.05$) coinciding with insulin elevations, while no significant changes were observed with GLP-1. No differences were observed between younger and older groups in the profile of insulin or incretins following oral EAA intake (Figure. 1)
Conclusion: In response to oral vs. IV plasma essential aminoacidemia, oral EAA intake induced an incretin effect associated with augmented GIP, but not GLP-1. No ageing effects were observed on EAA stimulated incretin hormone release. Thus, postprandial levels of EAA induce a GIP-mediated incretin effect, unaffected by age, supporting the notion of EAA as *acute* nutritive therapeutics in Diabetes and ageing.
Clinical Trial Registration Number: NCT02370745
Disclosure: **H. Abdulla:** None.

196

Long-term effects of weight loss on muscle strength and bone mineral density in adults with overweight or obesity: a PREVIEW sub-study

R.V. Seimon[1], S. McClintock[1], J. Dodds[1], R. Muirhead[1], S. Brodie[1], J. Zibellini[1], A. Das[1], A.L. Wild-Taylor[1], J. Honeywood[1], M. Fogelholm[2], A. Raben[3], J. Brand-Miller[1], A. Sainsbury[1];

[1]The University of Sydney, Camperdown, Australia, [2]University of Helsinki, Helsinki, Finland, [3]University of Copenhagen, Copenhagen, Denmark.

Background and aims: Low energy diets, using meal replacement products, are an effective weight loss tool. However, little is known about changes in musculoskeletal integrity with these diets, particularly in people with overweight/obesity of different age or sex. Therefore, we aimed to determine the effect of a low energy (total meal replacement) diet on muscle strength and bone mineral density (BMD) in specific populations of adults.

Materials and methods: This sub-study is part of the PREVIEW randomised controlled trial (www.previewstudy.com). In total, 155 younger (27–45 years) and older (46–70 years) men and women with overweight/obesity (BMI >25 kg/m^2) and pre-diabetes were recruited and underwent a total meal replacement diet (~3400 kJ/day) for 2 months using Cambridge Weight Plan products, followed by a weight maintenance program until 36 months. At 0 (baseline), 2, 12 and 36 months, dominant handgrip strength were measured via hand dynamometry, and BMD was measured at the hip and spine using dual-energy X-ray absorptiometry. An intention-to-treat (ITT) analysis was conducted using a generalized linear mixed-model, with time and group as factors.

Results: Body weight was significantly reduced (by 5.8–12.4%) compared to baseline values in all groups at 2, 12 and 36 months, except in younger women, where body weight returned to values not significantly different from baseline by 36 months (Table 1). At 36 months, all groups showed significant reductions from baseline in dominant handgrip strength (Table 1). These reductions represented 6.8–11.8% of baseline handgrip strength, which is on par with the amount of muscle strength lost per decade (8–10%) in adults >40 years. In addition, at 12 months, older but not younger men and women exhibited significant reductions from baseline in dominant handgrip strength. Older women also showed decreases from baseline in hip BMD at 12 months (-0.02 ± 0.004 g/cm^3, $P < 0.001$), and decreases in spine BMD at 12 (-0.014 ± 0.006 g/cm^3) and 36 months (-0.035 ± 0.006 g/cm^3; $P < 0.05$ for both), with no significant change from baseline in any other group at 2, 12 or 36 months.

Conclusion: Strategies to monitor and protect against loss of muscle strength and BMD during use of low energy (total meal replacement) diets are called for, particularly in older women. However, given the known gravity of obesity-related health complications, concerns about potential effects of low energy diets on muscle strength and BMD should not deter clinicians from using these diets to manage overweight/obesity, with due care to monitor and protect musculoskeletal integrity before, during and after the diet.

Table 1: Changes in body weight and handgrip strength in younger and older men and women

Outcome	Time (months)	Younger men	Younger women	Older men	Older women
Body weight (kg)	0	108.2±4.8 (n=14)	102.3±3.4 (n=28)	102.9±3.2 (n=32)	96.3±2.0 (n=81)
	2	95.5±4.7* (n=14)	91.8±3.3* (n=25)	90.0±3.1* (n=32)	86.2±1.9* (n=81)
	12	97.6±5.1* (n=11)	92.7±3.6* (n=18)	92.5±3.4* (n=26)	86.5±2.1* (n=77)
	36	101.7±5.1* (n=10)	99.5±3.7 (n=14)	95.1±3.3* (n=25)	90.9±2.1* (n=61)
Dominant handgrip strength (kg)	0	43.9±2.9	38.0±2.0	39.1±1.9	36.3±1.2
	2	40.8±2.8	37.3±2.0	38.8±1.9	35.3±1.2
	12	42.6±3.1	37.6±2.2	36.6±2.0*	34.0±1.3*
	36	40.6±3.1*	35.3±2.1*	36.5±1.9*	32.4±1.2*

Data as means ± SEM. *P<0.05 versus baseline

Clinical Trial Registration Number: NCT02030249
Supported by: EU 7th FP, NHMRC
Disclosure: **R.V. Seimon:** None.

197

High dietary glycaemic load is associated with increased levels of plasma and urinary methylglyoxal hydroimidazolones (MG-H1) in type 2 diabetes: the CODAM study

K. Maasen[1,2], M.M. van Greevenbroek[1,2], C.J.H. van der Kallen[1,2], C.D.A. Stehouwer[1,2], C.G. Schalkwijk[1,2];
[1]CARIM School for Cardiovascular Diseases, Maastricht University Medical Centre, Maastricht, [2]Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, Netherlands.

Background and aims: Accumulation of advanced glycation endproducts (AGEs) and AGE-precursors (dicarbonyls) contributes to the development of diabetic complications. We previously found increased circulating levels of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) after an oral glucose tolerance test and mixed meal test. Glycaemic Index (GI) is a value assigned to foods based on how quickly they affect blood glucose and Glycaemic Load (GL) represents carbohydrate quality and quantity in a serving of that food. In this study, we examined associations of dietary GI and GL with dicarbonyls and AGEs.

Materials and methods: Cross-sectional analyses were performed in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM [$n = 574$, 25% Type 2 Diabetes (T2DM), 59 ± 7 years]). GI and GL were derived from a Food Frequency Questionnaire. Dicarbonyls and AGEs were measured in fasting state by UPLC-MS/MS. MGO, GO and 3-DG were measured in plasma and free forms of hydroimidazolone (MG-H1), N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL) were measured in both plasma and urine. Protein-bound CML, CEL and pentosidine were measured in plasma. Multiple linear regression was performed with log-transformed and standardized dicarbonyls and AGEs (dependent variables), and standardized dietary GI or GL content (main independent variables). Models were adjusted for age, sex, glucose metabolism status, BMI, smoking, physical activity, medication, kidney function, alcohol intake, and dietary fat and fibre.

Results: GI was not significantly associated with dicarbonyl or AGE levels ($-0.070 < \beta < 0.051$, $0.1 < p$ value < 0.8). GL was associated with plasma MG-H1 after adjustment for age and sex ($\beta = 0.137$, 95%CI [0.048; 0.227], $p = 0.003$). Statistical significance was just lost in the fully adjusted model ($\beta = 0.133$, 95%CI [−0.018; 0.284], $p = 0.084$). A similar positive association was observed between GL and urinary MG-H1 ($\beta = 0.168$, 95%CI [−0.001; 0.338], $p = 0.052$). Significant interaction with glucose metabolism status was found for the association between GL and plasma MG-H1, urinary MG-H1 and plasma CEL (all $p < 0.1$). After stratification by glucose metabolism status, GL was most strongly associated with circulating and urinary MG-H1 in T2DM individuals ($\beta = 0.369$, 95%CI [0.038; 0.700], $p = 0.029$ and $\beta = 0.367$, 95%CI [0.014; 0.721], $p = 0.042$ respectively). In addition, GL was in the total cohort inversely associated with GO ($\beta = -0.174$ 95%CI [−0.327; −0.021], $p = 0.026$) and protein-bound pentosidine ($\beta = -0.184$, 95%CI [−0.335; −0.033], $p = 0.017$) in the fully adjusted model.

Conclusion: Circulating and urinary levels of MG-H1 were higher in individuals who had a higher glycaemic load in their habitual diet. These associations remained statistically significant after adjustments for potential confounders in T2DM but not in the total cohort. The lack of association with GI suggests that dietary carbohydrate quantity rather than quality is important for the effect of diet on circulating AGEs. The fact that the most consistent and positive association was observed for MG-H1 suggests that dietary GL induces MG-H1 via transient increases in MGO.

Supported by: NVWA
Disclosure: **K. Maasen:** None.

198

Effects of the bitter taste receptor agonist, denatonium benzoate, on postprandial glycaemia, gastric emptying and energy intake in type 2 diabetes

C. Xie, X. Wang, M.J. Bound, J. Grivell, R.L. Young, K.L. Jones, M. Horowitz, C.K. Rayner, T. Wu;
Adelaide Medical School, University of Adelaide, Adelaide, Australia.

Background and aims: The gastrointestinal tract, like the tongue, has the capacity to "taste" nutrients through activation of G protein coupled-receptors (GPCRs). Emerging evidence from preclinical models and healthy humans has linked activation of gastrointestinal bitter taste receptors (BTRs) to stimulation of gastrointestinal hormones and altered gut motility in association with suppression of energy intake and a reduction in postprandial glycaemic excursions. We have evaluated the effects of the BTR agonist, denatonium benzoate (DB), on gastric emptying, postprandial glycaemia and energy intake in type 2 diabetes (T2DM).
Materials and methods: 16 T2DM patients managed by diet ± metformin (7 male and 9 female; mean age 66.6 ± 1.0 years; BMI 31.3 ± 1.1 kg/m^2; HbA1c 6.6 ± 0.1%; duration of known diabetes 6.4 ± 1.2 years), were studied on 4 occasions (Parts A and B; 2 days each) in double-blind, randomised fashion. In Part A, subjects consumed a gelatin capsule containing either 30 mg DB (a dose shown to reduce energy intake in healthy subjects in our unpublished study) or sodium chloride (control) with 150 mL water and 30 min later consumed a standardised mashed potato meal (1541.8 kJ) labelled with 100 μL ^{13}C-octanoic acid to evaluate gastric emptying and postprandial glycaemia. In Part B, subjects consumed the capsule containing DB or control and were offered a standardised *ad libitum* buffet meal after 30 min, to evaluate energy intake. In part A, "Arterialised" venous blood was sampled every 30 min for measurement of plasma glucose and breath samples collected for the determination of the gastric half-emptying time (T50). Energy intake was quantified using Foodworks software. Data are means ± SEM. $P < 0.05$ was considered statistically significant.
Results: Subjects tolerated the studies well, without nausea. Compared with control, DB had no effect on gastric emptying (T50: DB 176 ± 10 min vs. control 178 ± 12 min) or plasma glucose after the standardised mashed potato meal, but reduced both energy intake (DB 3254 ± 377 kJ vs. control 3695 ± 467 kJ, $P = 0.046$) and the weight of food intake (DB 935 ± 78 g vs. control 1034 ± 93 g, $P = 0.027$) at the buffet meal.
Conclusion: In relatively well controlled T2DM, oral administration of the BTR agonist, DB, has no effect on gastric emptying or postprandial glycaemia, but suppresses energy intake. Stimulation of intestinal BTRs may represent a novel approach to the management of obesity in T2DM.
Clinical Trial Registration Number: HREC/16/RAH/498
Supported by: NHMRC
Disclosure: **C. Xie:** None.

OP 34 Novelty in adipose tissue biology and lipid metabolism

199

Chronic hyperadiponectinaemia ameliorates bone quality in aged mice by promoting osteogenesis rather than inhibiting bone resorption

S. Otabe[1], Y. Xiaohong[1], T. Ohki[1], T. Hashinaga[1], N. Wada[1], H. Nakayama[1], N. Tohgi[2], M. Nomura[1], K. Yamada[3];
[1]Department of Medicine, Kurume University School of Medicine, Fukuoka, [2]Department of Medicine, Yamada Memorial Hospital, Mihara, [3]Department of Medicine, Asakura Medical Association Hospital, Asakura, Japan.

Background and aims: Fragility bone fractures, caused by osteoporosis, are an important global concern, particularly among our aging population. Furthermore, alterations in bone quality are the underlying basis for fragility fractures in patients with diabetes. We previously demonstrated that adiponectin—a physiologically active substance secreted specifically by adipocytes—possesses anti-aging effects in mammals. Here, we used transgenic mice to investigate the effects of adiponectin at the third vertebral body and femoral bone, as two common sites of bone fracture in older persons.
Materials and methods: Transgenic mice with hyperadiponectinemia *derived from only hepatocytes* from birth and C57BL6 mice (control) were fed with normal chow ad libitum and were sacrificed at the age of 60 weeks ($n = 4$ per group, males and females). The serum levels of human and mouse adiponectin were measured using ELISA kits for human adiponectin (Otsuka, Japan) and mouse adiponectin (AdipoGen, Korea), respectively, without detectable cross-reactivity. Mice were injected with calcein and tetracycline labels before sacrifice, and samples were assayed for various measures of *bone histo*morphometry evaluated by the Bone Ito Histomorphometry Institute (Nigata, Japan).
Results: There was no significant difference in the bodyweight of C57BL/6N and transgenic mice in males (36.1 ± 4.8 g vs. 30.4 ± 1.5 g) or females (27.1 ± 4.7 g vs. 31.5 ± 3.3 g). The concentration of hepatocyte-derived exogenous human adiponectin in male and female transgenic mice was high: 686.3 ± 287.7 μg/mL and 596.3 ± 302.1 μg/ml, respectively. Adipocyte-secreted endogenous mouse adiponectin was significantly higher in the transgenic mice (203.1 ± 45.3 μg/mL and 221.7 ± 76.2 μg/mL) than in C57BL/6N mice (31.5 ± 11.2 μg/mL and 33.0 ± 11.7 μg/mL) in males and females, respectively. Cancellous bone volume at the third lumbar vertebra was not significantly different between C57BL/6N and transgenic mice, respectively, in females (16.86% ± 4.00% vs. 16.19% ± 0.45%), but was significantly different in males, with higher values in the transgenic mice (11.33% ± 0.54% vs. 18.63% ± 0.30%, respectively; $P < 0.001$). Mineral apposition rates at the third lumbar vertebra and the femoral shaft were significantly higher in transgenic mice than in C57BL6 mice in both males (2.15 ± 0.20 vs. 1.39 ± 0.11; $P < 0.001$, 1.47 ± 0.20 vs. 0.59 ± 0.16 μm/day; $P < 0.0005$) and females (2.86 ± 0.13 vs. 1.67 ± 0.37; $P < 0.0009$, 1.62 ± 0.06 vs. 0.96 ± 0.09 μm/day; $P < 0.0001$). Moreover, bone formation rates at the bone surface at both the third lumbar vertebra and femoral shaft were significantly higher in transgenic mice as compared with C57BL6 mice in males (0.198 ± 0.056 vs. 0.096 ± 0.022; $P < 0.02$, 0.157 ± 0.055 vs 0.029 ± 0.030 mm^3/mm^2/year; $P < 0.006$) and in females (0.357 ± 0.014 vs 0.188 ± 0.021; $P < 0.0001$, 0.417 ± 0.171 vs 0.126 ± 0.015 mm^3/mm^2/year; $P < 0.02$). There were no differences in eroded surface/bone surface (%), or osteoclast number/bone surface (N/mm) between C57BL/6N and transgenic mice in both sexes.
Conclusion: Chronic hyperadiponectinemia significantly promotes osteogenesis in aged mice. Maintaining high adiponectin serum levels or administering an analogue could prevent diabetes-associated bone fracture.
Disclosure: **S. Otabe:** None.

200

The dual PPARα/γ-agonist tesaglitazar robustly induces browning of white fat in vitro and in vivo

T. Kroon[1], M. Harms[1], D. Nilsson[2], A. Lindblom[1], P. Gennemark[1], G. O'Mahony[1], V. Osinski[3], C. MacNamara[3], J. Boucher[1,2];
[1]Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden, [2]Wallenberg Centre for Molecular and Translational Medicine, The Lundberg Laboratory for Diabetes Research, University of Gothenburg, Gothenburg, Sweden, [3]Department of Medicine, Cardiovascular Research Center, University of Virginia, Charlottesville, USA.

Background and aims: Peroxisome proliferator-activated receptors (PPARs) are key transcription factors that regulate both white and brown fat development and function, and the conversion of white into brown-like adipocytes. Here we investigated whether PPARα and PPARγ possess a synergistic ability to induce browning of white fat.
Materials and methods: *In vitro* experiments were performed in primary mouse white preadipocytes and *in vivo* studies were conducted in lean and diet-induced obese C57bl/6 mice, treated with various selective PPARα, selective PPARγ or dual PPARα/γ agonists. Cell and tissue gene expression was measured by qPCR. Body and tissue weight, food intake, energy expenditure, liver lipid content and plasma glucose, insulin and lipids were quantified.
Results: *In vitro* testing of structurally diverse dual PPARα/γ agonists in mouse preadipocytes identified tesaglitazar as the most robust inducer of uncoupling protein 1 (*Ucp1*), increasing *Ucp1* by several hundred fold after an 8-day treatment. Tesaglitazar increased expression of a broad range of brown fat-related genes, including the key transcriptional regulators *Pgc1α* and *Prdm16*, and was associated with increased mitochondrial content. Importantly, tesaglitazar also strongly induced browning *in vivo* in both lean and obese mice. Tesaglitazar increased *Ucp1* mRNA levels >200-fold in the inguinal fat of obese mice, largely exceeding the 5-fold increase observed with rosiglitazone treatment. Tesaglitazar-induced browning was associated with increased energy expenditure, enhanced insulin sensitivity, reduced liver steatosis and an overall improved metabolic profile compared to rosiglitazone and vehicle control groups. I*n vitro* however, rosiglitazone and tesaglitazar were found to be equally potent *Ucp1* inducers, and combining rosiglitazone with the selective PPARα agonist WY14643, did not result in any further increase in *Ucp1*. Fibroblast growth factor 21 (FGF21) is a hormone mostly produced by the liver, which has been shown to induce browning of white fat in rodent models and is under PPARα control. Following tesaglitazar treatment, FGF21 liver expression and circulating levels were increased 3- and 8-fold, respectively, but were not changed with rosiglitazone treatment. Moreover, linear regression revealed a strong correlation between circulating FGF21 levels and inguinal fat *Ucp1* mRNA expression ($r^2 = 0.75$, $P < 0.001$), suggesting that FGF21 plays a role in the browning effect of tesaglitazar.
Conclusion: PPARγ agonism alone is sufficient for the conversion of white into brown-like adipocytes *in vitro,* but dual PPARα/γ agonism is superior to PPARγ agonism alone at inducing white fat browning *in vivo*, through additional PPARα-mediated increase in FGF21. Together, these findings identify a novel opportunity to develop compounds with robust browning of white fat, through modulation of multiple PPARs.
Disclosure: **T. Kroon:** Employment/Consultancy; AstraZeneca employee.

201

AAV-mediated overexpression of BMP7 induces white adipose tissue adipogenesis and reverses insulin resistance

E. Casana, V. Jimenez, V. Sacristan, S. Muñoz, C. Jambrina, J. Rodo, S. Darriba, C. Mallol, M. Garcia, X. Leon, I. Grass, S. Franckhauser, F. Bosch;
Universitat Autonoma de Barcelona, Bellaterra, Spain.

Background and aims: Obesity and type 2 diabetes are strongly associated and a major health problem because of their alarmingly growing prevalence worldwide. The hypertrophic expansion of white adipose tissue (WAT) promotes ectopic fat accumulation and development of insulin resistance whereas WAT hyperplasia is associated with preservation of insulin sensitivity. Several members of the Bone Morphogenetic Protein (BMP) family have been shown to play a role in white and/or brown adipogenesis and energy homeostasis. Although BMP7 has extensively been reported to induce brown adipogenesis in vitro, its role on WAT expandability and its potential contribution to insulin sensitivity remains to be elucidated.
Materials and methods: Specific overexpression of BMP7 in WAT was obtained by means of intra-depot administration of adeno-associated viral (AAV) vectors encoding for a murine optimized BMP7 coding sequence under the control of the CAG promoter in conjunction with target sequences of the liver-specific microRNA122a and the heart-specific microRNA1. To achieve specific overexpression of BMP7 in the liver, intravenous administration of AAV vectors bearing a murine optimized BMP7 coding sequence and the liver-specific hAAT promoter was carried out.
Results: Local administration of AAV vectors encoding BMP7 in WAT resulted in hyperplasic expansion of WAT together with reduced liver steatosis and amelioration of insulin sensitivity in both HFD-fed and ob/ob obese mice. In contrast, the AAV-mediated overexpression of BMP7 specifically in the liver did not promote WAT hyperplasia although the circulating levels of BMP7 achieved were similar to those obtained after intra-WAT administration of AAV vectors. Nevertheless, when liver-derived BMP7 circulating levels were further increased, body weight and insulin sensitivity were normalised in HFD-fed as well as in ob/ob mice.
Conclusion: Altogether, the results of this study unravel a new role of BMP7 on white adipogenesis. In addition, this study highlights the therapeutic potential of AAV-mediated BMP7 gene therapy to ameliorate obesity and insulin resistance.
Supported by: SAF2014-54866R, 2014-SGR-1669, ICREA Academia award to F.B., EFSD/MSD
Disclosure: **E. Casana:** None.

202

PCSK9 deficiency results in altered insulin secretion and glucose intolerance: the role of the LDL receptor

G. Norata, L. Da Dalt, M. Ruscica, C. Perego, A. Catapano;
University of Milan, Milan, Italy.

Background and aims: *PCSK9* loss of function genetic variants are associated with lower LDL cholesterol but also with higher plasma glucose levels and increased risk of type 2 diabetes mellitus. Here we investigated the molecular mechanisms underlying this association.
Materials and methods: *Pcsk9* KO, WT, *Pcsk9/Ldlr* DKO, *Ldlr* KO, albumin *AlbCre+/Pcsk9*$^{LoxP/LoxP}$ (liver selective *Pcsk9* knock-out mice) and *AlbCre-/Pcsk9*$^{LoxP/LoxP}$ mice were used. GTT, ITT, insulin and C-peptide plasma levels, pancreas morphology and cholesterol accumulation in pancreatic islets were studied in the different animal models.
Results: Glucose clearance was significantly impaired in *Pcsk9* KO mice fed a standard or a high fat diet for 20 weeks compared to WT animals, insulin sensitivity however was not affected. A detailed analysis of pancreas morphology of *Pcsk9* KO mice vs controls revealed larger islets with increased accumulation of cholesteryl esters, paralleled by increased insulin intracellular levels and decreased plasma insulin and C-peptide levels. This phenotype was completely reverted in *Pcsk9/Ldlr* DKO mice implying the LDLR as the PCSK9 target responsible for the phenotype observed. Further studies in albumin *AlbCre+/Pcsk9*$^{LoxP/LoxP}$, which lack detectable circulating PCSK9, also showed a complete recovery of the phenotype, thus indicating that circulating, liver-derived PCSK9, the principal target of monoclonal antibodies, does not impact beta cells function and insulin secretion.

Conclusion: PCSK9 critically controls LDLR expression in pancreas perhaps contributing to the maintenance of a proper physiological balance to limit cholesterol overload in beta cells. This effect is independent of circulating PCSK9, and is probably related to locally produced PCSK9. These data suggest that anti-PCSK9 therapies, which target mainly circulating PCSK9, might have a limited impact on beta cell dysfunction and the incidence of diabetes in contrast to Mendelian randomization analysis where the effect of global PCSK9 deficiency was investigated.
Disclosure: **G. Norata:** Grants; Pfizer. Lecture/other fees; Amgen, Sanofi, Alnylam.

203

Newly discovered regulator of lipid metabolism: pyruvate dehydrogenase kinase 1

C.L.K. Leung, S. Karunakaran, B.X. Hu, J. Dong, L. Tang, S. Wei, Z. Rajwani, J.D. Johnson, S.M. Clee;
Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada.

Background and aims: Pyruvate dehydrogenase kinases (PDKs) are a family of enzymes with isoforms 1–4 that have been studied as a therapy for diabetes and cancers. PDKs are mitochondrial proteins that inactivate pyruvate dehydrogenase, which regulates the entry of pyruvate into the Kreb's Cycle. This generates acetyl-CoA that can either be oxidized or used for lipid synthesis. Previous studies have shown that PDK2 and 4 knockout mice have improved glucose tolerance and glucose oxidation. Downregulation of PDK2 and 4 has been shown to ameliorate diabetes. However, PDK1 is regulated differently and its role in whole body glucose and lipid metabolism has not been directly tested. We hypothesized that decreasing PDK1 would increase glucose tolerance and lipid metabolism.
Materials and methods: We studied PDK1 knockout (KO) and wildtype (WT) littermate mice fed a high fat diet (60% fat, with sucrose) from weaning. Body weights were measured weekly. At 10 weeks, we conducted insulin tolerance test (ITT) using 1 U/kg intraperitoneal insulin dose. At 6 and 11 weeks, we conducted glucose tolerance tests (GTT) using 1 g/kg intraperitoneal glucose. At 12 weeks, we collected 4 hour fasted cardiac bleeds. We used enzymatic colorimetric assays to measure triglyceride, cholesterol and glucose levels in plasma samples. Insulin from GTT and cardiac bleeds were measured using ELISAs. We conducted a chloroform methanol lipid extraction on 12 week old flash frozen heart and livers from PDK1 KO mice to determine cholesterol and triglyceride content. We isolated primary islets and conducted perifusion of KO and WT mice using 120 matched islets from each. Perifusion solutions were made of 3 mM glucose (basal), 20 mM glucose or basal glucose with 30 mM KCl in Krebs Ringer Bicarbonate buffer and were flowed through at a rate of 1 mL/minute for 20–30 minutes for each secretagogue with 30 minutes basal glucose in between each. Perifusion fractions (1.7 mL) were collected every 5 minutes and measured for insulin using radioimmunoassay.
Results: Mice with reduced PDK1 had body weights similar to WT littermates ($p > 0.05$). We found modestly improved glucose tolerance in mice lacking PDK1 ($p = 0.2$), but no difference in fasting glucose and insulin levels ($p > 0.05$). We also conducted insulin tolerance tests and were unable to detect a difference between WT and KO ($p > 0.05$). From perifusion of isolated primary islets, we were also unable to detect a difference in insulin secretion between WT and KO ($p > 0.05$). However, we found a larger role of PDK1 on lipid metabolism that was not previously shown in other PDKs. We did not observe effects on fasting cholesterol levels, but found mice with reduced PDK1 had a 68% reduction in triglyceride levels ($p = 0.02$). We have found no changes in cholesterol levels in liver and heart ($p > 0.05$). We also found no evidence for altered hepatic triglyceride contents. However, PDK1 KO hearts had a 43% increase in triglyceride levels ($p = 0.0003$).
Conclusion: Our data suggest reduced PDK1 is associated with a modest improvement in glucose tolerance, no changes in insulin sensitivity. Importantly, we found a significant reduction of plasma triglycerides, but increased triglyceride concentrations in hearts. Together these data suggest an important and previously unknown role of PDK1 in metabolism and of PDKs in lipid metabolism, and that reducing PDK1 may have significant effects on glucose and lipid metabolism.
Supported by: NSERC, HSF, CRC
Disclosure: **C.L.K. Leung:** None.

204

Evaluation of changes in serum lipid intermediate oxidation products in the progress of type 2 diabetes development

M. Ciborowski[1], J. Godzien[2], J. Siroka[3], F. Traldi[2], M. Gorska[1], C. Barbas[2], A. Kretowski[1];
[1]Medical University of Bialystok, Bialystok, Poland, [2]Universidad CEU San Pablo, Madrid, Spain, [3]Palacký University in Olomouc, Olomouc, Czech Republic.

Background and aims: Oxidative stress is considered as one of the factors underlying the development of insulin resistance and type 2 diabetes mellitus (T2DM). A major mechanism responsible for increased oxidative stress is hyperglycemia-induced production of reactive oxygen species (ROS). Formation of the final products of lipid peroxidation: 4-hydroxynonenal (HNE) and malondialdehyde (MDA) is proceeded by appearance of lipids with fatty acids oxidized to their hydroxyl -OH, dihydroxyl -$(OH)_2$, peroxyl-OOH, keto- and epoxy forms. In case of phosphatidylcholines (PCs), these intermediate oxidation products are important signalling molecules associated with platelet aggregation, proliferation and migration of vascular smooth muscle cells, inflammatory response, endoplasmic reticulum stress and apoptosis. In this study changes in serum oxidized fatty acids (oxFAs) and oxidized PCs (oxPCs) were evaluate during the progress of T2DM development.
Materials and methods: The study group consisted of 204 individuals divided into 4 age and sex-matched groups: healthy controls (52 ± 11 years, BMI = 28 ± 5), subjects with IR but without dysglycemia (47 ± 11 years, BMI = 30 ± 8), with prediabetes (PD) (53 ± 9 years, BMI = 30 ± 7), and type 2 diabetes (T2DM) (53 ± 10 years, BMI = 32 ± 7). Serum samples were fingerprinted using LC-QTOF-MS. The analytical standard mixture of oxPCs was used to evaluate MS/MS fragmentation pattern characteristic to different PCs oxidation products (hydroxyl -OH, dihydroxyl -(OH)2, peroxyl -OOH, keto =O and epoxy forms). Based on that information we searched for oxFAs and oxPCs in the fingerprinting data. Differences in the level of found oxidized lipids between control, IR, PD and T2DM individuals were evaluated by Welch's t-test.
Results: In the studied samples we have found hydroxyl-FAs and hydroxyl-PCs as well as -OOH, -$(OH)_2$ and =O modified PCs; e.g.: hydroxyl docosahexaenoic and hydroxyl arachidonic acids, PC (34:2-OH), PC (34:2-OOH), PC (36:6-$(OH)_2$) or PC (36:2=O). There was a characteristic pattern of changes in oxidized lipid species related to T2DM development. In comparison to healthy controls increase in IR (+44–78%, $p = 0.02$–0.05) and PD groups (+50–101%, $p = 0.05$) was observed. Surprisingly, in subjects with overt type 2 diabetes serum lipid intermediate oxidation products levels were decreased (−25–85%, $p = 0.001$–0.04) in comparison to controls.
Conclusion: To our knowledge this is the first study identifying serum lipid intermediate oxidation products and evaluating their change in the course of T2DM development. A decrease of oxidized lipids in T2DM patients is probably due to the formation of lipid peroxidation end-products (HNE and MDA).
Supported by: Leading National Research Centre in Poland (KNOW 2012–2017)
Disclosure: **M. Ciborowski:** None.

OP 35 Genetics of diabetes across the life course

205

Physical activity and the risk of LADA, results from a Swedish case-control study and the Norwegian HUNT Study

R. Hjort[1], E. Ahlqvist[2], L. Alfredsson[1], V. Grill[3,4], L. Groop[2], A. Rosengren[2], T. Tuomi[5,6], B.O. Åsvold[3,7], S. Carlsson[1];
[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of Clinical Sciences in Malmö, Clinical Research Centre, Lund University, Malmö, Sweden, [3]Department of Endocrinology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway, [4]Department of Clinical and Molecular Medicine, NTNU, Norwegian University of Science and Technology, Trondheim, Norway, [5]Abdominal Center, Endocrinology, Helsinki University Central Hospital, Research Program for Diabetes and Obesity, University of Helsinki, Helsinki, Finland, [6]Folkhälsan Research Center, Helsinki, Finland, [7]K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Trondheim, Norway.

Background and aims: Physical activity (PA) has been linked to a reduced risk of type 2 diabetes (T2D) by ways of improving insulin sensitivity in key metabolic organs. We set out to investigate if PA was associated with a reduced risk of latent autoimmune diabetes in adults (LADA), and whether the association was modified by HLA genotype.
Materials and methods: We used data from a Swedish population-based case-control study with incident cases of LADA ($n = 474$, GADA-positive) and T2D ($n = 1593$ GADA-negative) and matched controls ($n = 3032$), and prospective data from the Norwegian HUNT Study, including 1,012,957 person-years of follow-up (1986–2008) and incident cases of LADA ($n = 147$) and T2D ($n = 2002$). We estimated odds ratios (OR) and hazard ratios (OR) of diabetes in relation to self-reported leisure time PA in four (high, moderate, low vs. sedentary) and two categories (active vs. sedentary) and adjusted for age, sex, family history of diabetes, smoking and BMI (kg/m^2). Analyses of the Swedish data were stratified by HLA DR-DQ genotypes.
Results: High PA was associated with a reduced risk of LADA in both the Swedish (OR 0.66, 95% CI 0.45–0.97) and Norwegian (HR 0.45, 95% CI 0.23–0.90) data (Table 1). The association did not persist after adjustment for BMI. High PA was also associated with a reduced risk of T2D, which was attenuated but remained after adjustment for BMI. Stratification by HLA genotype indicated that PA was associated with LADA only among those with low-risk genotypes. Sedentary compared to active LADA patients had higher mean BMI (31.2 vs. 27.6, $p < 0.0001$), were more insulin resistant (HOMA 3.60 vs. 2.60, $p = 0.0032$), had better beta-cell function (HOMA 51.1 vs. 36.6, $p = 0.0113$) and higher proportion of HLA low-risk genotypes (31.9 vs. 20.4, $p = 0.0372$).
Conclusion: These findings indicate that physical activity is associated with a reduced risk of LADA, which is mediated by beneficial effects on body weight and likely involve insulin sensitivity. A protective effect does not seem to apply to individuals with high-risk HLA genotypes, in whom a beneficial effect from physical activity on body weight and insulin sensitivity may not be enough to prevent or postpone onset of LADA.

Table 1. Physical activity and the risk of LADA and Type 2 diabetes.

	LADA				Type 2 diabetes			
	ESTRID		HUNT		ESTRID		HUNT	
	OR (95% CI)*	OR (95% CI)**	HR (95% CI)*	HR (95% CI)**	OR (95% CI)*	OR (95% CI)**	HR (95% CI)*	HR (95% CI)**
Physical activity level								
Sedentary	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Low	0.76 (0.57-1.02)	1.06 (0.78-1.44)	0.67 (0.42-1.06)	0.76 (0.48-1.21)	0.53 (0.44-0.64)	0.88 (0.70-1.10)	0.71 (0.63-0.80)	0.86 (0.76-0.97)
Moderate	0.61 (0.44-0.85)	0.98 (0.69-1.40)	0.86 (0.59-1.27)	0.92 (0.62-1.35)	0.34 (0.27-0.42)	0.75 (0.58-0.97)	0.79 (0.79-0.88)	0.91 (0.82-1.01)
High	0.66 (0.45-0.97)	1.20 (0.80-1.82)	0.45 (0.23-0.90)	0.54 (0.27-1.08)	0.33 (0.25-0.44)	0.87 (0.63-1.21)	0.56 (0.47-0.66)	0.73 (0.62-0.87)
Active vs. Sedentary								
With high-risk HLA	0.69 (0.40-1.22)	1.03 (0.56-1.90)	-	-	0.49 (0.30-0.80)	1.05 (0.57-1.92)	-	-
With low-risk HLA	0.50 (0.29-0.85)	0.67 (0.38-1.18)	-	-	0.45 (0.32-0.62)	0.82 (0.56-1.22)	-	-

ESTRID - Epidemiological Study of Risk Factors for LADA and Type 2 diabetes (Swedish study). HUNT - Nord-Trøndelag Health Study (Norwegian study).
*Adjusted for age and sex. **Adjusted for age, sex, BMI, family history of diabetes and smoking.
High-risk (DR4-DQ8, DR4/3-DQ8, DR3/4, DR3/3, DR4/4, and DQA1*0501-DQB1*0201) and low-risk (DR3/X, DR4/X, DR4-DQ7 and DRXX) HLA genotypes.

Supported by: VR, Forte, Swedish Diabetes Association, NovoNordisk, GlaxoSmithKline
Disclosure: **R. Hjort:** Grants; VR, Forte, Swedish Diabetes Association, NovoNordisk, GlaxoSmithKline Norway.

206

Genetic discrimination between LADA and type 1 diabetes within the MHC

R. Mishra[1], J.P. Bradfield[2], D.L. Cousminer[1], A. Chesi[1], K.M. Hodge[1], H. Hakonarson[2], BMDCS, D. Mauricio[3], N.C. Schloot[4], K.B. Yderstræde[5], B.F. Voight[6], S. Schwartz[7], B.O. Boehm[8,9], R.D.G. Leslie[10], S.F.A. Grant[1];
[1]Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, USA, [2]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, USA, [3]Hospital Universitari Germans Trias i Pujol, Badalona, Spain, [4]German Diabetes Center, Düsseldorf, Germany, [5]Odense University Hospital, Odense, Denmark, [6]Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA, [7]Main Line Health System, Wynnewood, USA, [8]Department of Internal Medicine I, Ulm University Medical Centre, Ulm, Germany, [9]LKC School of Medicine, Nanyang Technological University, Singapore and Imperial College London, London, UK, [10]ActionLADA/Department of Immunobiology, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

Background and aims: Studies on type 1 diabetes (T1D) and type 2 diabetes have revealed significant insights into novel biological mechanisms underlying diabetes, yet the genetic etiology of latent autoimmune diabetes in adults (LADA) remains largely unknown; furthermore, improved biomarkers of LADA are required to optimize diagnosis. Our genome-wide association assessment shows that the major histocompatibility complex (MHC) harbors the strongest association with LADA; however, the association is clearly attenuated compared to observations in T1D cohorts. While T1D susceptibility has long been known to be principally harbored within the MHC Class II genes *HLA-DQB1* and *HLA-DRB1*, variation in the MHC class I genes *HLA-A* and *HLA-B* have been subsequently shown, through conditional analysis, to increase T1D risk further. Given that MHC Class I markers have also been shown to be associated with lower age-at-diagnosis in T1D, the adult-onset disease of LADA is well placed to shed further light on this association.
Materials and methods: To investigate potential genetic discriminators at the MHC between LADA and T1D, we attempted recapitulation of findings in Nejentsev et al using an imputation-based approach and performing forward stepwise conditional logistic regression of the MHC region in T1D cases ($n = 1990$) and controls ($n = 2856$) from the Wellcome Trust Case Control Consortium (WTCCC), and in a well-phenotyped LADA cohort ($n = 978$) using population-based controls ($n = 1057$).
Results: We confirmed the strongest T1D associations at *HLA-DRB1* and *HLA-DQB1* ($P = 6.10 \times 10^{-175}$ and $P = 2.90 \times 10^{-219}$, respectively), as well as the independent effect of *HLA-B* ($P = 1.67 \times 10^{-14}$) and *HLA-A* ($P = 5.25 \times 10^{-8}$) to T1D. We then performed the conditional analysis in the LADA and population-based controls cohort, observing significant association at *HLA-DRB1* and *HLA-DQB1* ($P = 5.93 \times 10^{-22}$ and $P = 4.68 \times 10^{-13}$, respectively), although of diminished effect size when compared to T1D. Notably, we did not observe significant independent effects in *HLA-B* or *HLA-A* for LADA. After sensitivity analyses through the systematic decreasing of the sample size of T1D and WTCCC controls in order to contrast with the LADA vs controls sample size, the independent effects of *HLA-B* and *HLA-A* consistently remained.
Conclusion: Despite significant association at *HLA-DQB1* and *HLA-DRB1* with LADA, we did not observe independent effects of *HLA-B* and *HLA-A*. These results highlight a potential use for MHC Class I markers in differentiating T1D from LADA.

Supported by: NIH (R01 DK085212)
Disclosure: **R. Mishra:** Employment/Consultancy; Nanette C. Schloot is currently employed by Lilly Germany.

207

Development and validation of a clinical prediction model to identify adult patients (aged 18–50) with type 1 diabetes requiring early insulin therapy

A.L. Grubb[1], K. Patel[1], R.A. Oram[1,2], A.V. Hill[1], C. Angwin[1], T.J. McDonald[1,2], M.N. Weedon[1], A.T. Hattersley[1,2], K.R. Owen[3,4], B.M. Shields[1], A.G. Jones[1,2];
[1]National Institute for Health Research Exeter Clinical Research Facility, Exeter, [2]Royal Devon and Exeter NHS Foundation Trust, Exeter, [3]Oxford Centre for Diabetes Endocrinology and Metabolism, Oxford, UK, [4]Oxford NIHR Biomedical Centre, Oxford, UK.

Background and aims: Correctly determining diabetes subtype at diagnosis is important to ensure optimal treatment and education, but is often difficult, particularly in young adults, where misclassification is common. We aimed to develop a clinical prediction model combining clinical features and GAD islet-autoantibodies to accurately identify patients with type 1 diabetes.
Materials and methods: We studied 1,352 participants in Exeter-based cross-sectional cohorts diagnosed with diabetes between the ages of 18 and 50. Our study outcome was type 1 diabetes, which was robustly defined as the presence of both severe endogenous insulin deficiency (C-peptide <200 pmol/L) and rapid insulin requirement (≤3 years). We examined the relationship between clinical features (age at diagnosis and BMI), GAD islet-autoantibodies and the presence of type 1 diabetes using logistic regression. We developed two prediction models based on; 1) clinical features 2) clinical features and GAD islet-autoantibodies. External validation of the models was performed using 701 participants taken from the Young Diabetes in Oxford study (UK).
Results: Type 1 diabetes was present in 13% of participants in the Exeter cohort ($n = 179/1352$). Participants with type 1 diabetes were younger at diagnosis (median 30 years versus 44 years, $p < 0.001$), had a lower BMI (median 26 kg/m^2 versus 34 kg/m^2, $p < 0.001$) and a higher percentage of them were GAD positive (62% versus 5%, $p < 0.001$). Age of diagnosis, BMI and GAD islet-autoantibodies were discriminative and independent predictors of type 1 diabetes ($p < 0.001$ for all). The model combining clinical features was highly discriminative (AUC ROC 0.90 [95% CI 0.88, 0.93]); adding GAD improved discrimination (AUC ROC 0.96 [0.95, 0.97] $p < 0.001$). Type 1 diabetes was present in 19% of the participants in the Young Diabetes in Oxford study ($n = 134/701$). In the external validation, both models still showed excellent discrimination (clinical features AUC ROC 0.86 [0.82, 0.89]; clinical features + GAD AUC ROC 0.92 [0.89, 0.95] $p < 0.001$).
Conclusion: This is the first study to show that a clinical prediction model combining age at diagnosis, BMI and GAD islet-autoantibodies can accurately identify type 1 diabetes in a group of patients where misclassification is most common. GAD islet-autoantibodies add additional discrimination over and above clinical features, and may be helpful where clinical features are inconclusive. Our model has excellent discrimination and routine use of this model in clinical practice is likely to reduce misclassification.
Supported by: Wellcome Trust, NIHR
Disclosure: **A.L. Grubb:** None.

208

Genetic determinants of type 1 diabetes

A. Spiliopoulou[1], S.J. McGurnaghan[2], C.N.A. Palmer[3], P.M. McKeigue[1], H.M. Colhoun[2,4], on behalf of the SDRNT1BIO Investigators;
[1]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, [2]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, [3]Ninewells Hospital and Medical School, University of Dundee, Dundee, [4]Department of Public Health, NHS Fife, Kirkcaldy, UK.

Background and aims: The Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO) study is a nationally representative cohort of 6127 patients with a clinical diagnosis of T1DM (time to insulin use within 12 months) without restriction on age of onset. We performed a case-control GWAS of T1DM using the SDRNT1BIO cohort and non-diabetic controls from the background population; the Generation Scotland Family Health Study (GS).
Materials and methods: The study included 5172 unrelated T1DM cases from SDRNT1BIO and 7497 unrelated controls from GS. Samples were genotyped using the Illumina Human CoreExome and the OmniExpressExome arrays, and imputed using the online service of the Sanger Institute. GWAS was performed with SNPTEST, adjusting for age, sex and three principal components. Replication was sought in UK Biobank (UKBB). The Immunobase platform was used to define known genetic regions associated with T1DM. The ENSEMBL-VEP and GENOSCORES platforms were used to annotate GWAS findings.
Results: From 54 autosomal regions reported as genome wide significant for T1DM in Immunobase, 50 were replicated at the Bonferroni corrected p value threshold ($p < 0.01/54$), with 20 regions reaching significance below 2×10^{-6}. We confirmed a previously suggested genetic association at the *TNFRS11B* locus (strongest association with intronic SNP rs4242592, $p = 6 \times 10^{-9}$) and identified a novel association for T1DM at the *NOTCH2/ADAM30* locus (strongest association with intronic SNP rs406767, $p = 6 \times 10^{-11}$). The latter was stronger in cases diagnosed after age 25 (OR = 1.34, 95% CI = 1.22–1.49) than in cases diagnosed before age 25 (OR = 1.19, 95% CI = 1.09–1.30). *NOTCH2* has been reported as a genetic locus for type 2 diabetes mellitus (T2DM) in a large GWAS meta-analysis but has not been previously detected as a T1DM locus. The association with rs406767 was replicated for T1DM in UKBB ($p = 4 \times 10^{-4}$). rs406767 is in linkage disequilibrium ($R = 0.89$) with a quantitative trait locus (QTL) of *NOTCH2* expression in whole blood ($p = 2 \times 10^{-9}$), and the allele associated with lower *NOTCH2* expression is associated with higher T1DM risk in SDRNT1BIO and UKBB and higher T2DM risk in UKBB ($p = 2 \times 10^{-6}$). The *TNFRSF11B* locus was reported as a suggestive association in a large T1DM GWAS meta-analysis. Our study confirms this association at genome-wide significance. The direction of effect on T1DM is consistent in UKBB, but the association does not reach replication significance. The allele associated with higher T1DM risk at the *TNFRSF11B* locus is also associated with lower methylation of *TNFRSF11B* in whole blood ($p = 3 \times 10^{-76}$) and with increased bone mineral density ($p = 2 \times 10^{-17}$). *TNFRSF11B* encodes osteoprotegerin, a decoy receptor for the receptor activator of nuclear factor kappa B ligand, which is implicated in inflammation, innate immunity and bone resorption.
Conclusion: We confirm a previously suggested effect of the *TNFRSF11B* locus on T1DM, and identify a novel T1DM association at the *NOTCH2* locus. Although this association, reported previously for T2DM, is stronger for late-onset than early-onset T1DM, it is still detectable in early-onset cases. This adds to the evidence that T1DM and T2DM are part of a continuum, with some genetic features in common.
Supported by: JDRF, Diabetes UK and CSO
Disclosure: **A. Spiliopoulou:** Grants; JDRF, Diabetes UK, CSO.

209

Antibodies to oxidised insulin improve prediction of type 1 diabetes in children with positive standard islet-autoantibodies

R. Strollo[1], C. Vinci[2], N. Napoli[1], E. Fioriti[1], E. Maddaloni[1], L. Åkerman[3], R. Casas[3], P. Pozzilli[1], J. Ludvigsson[3], A. Nissim[2];
[1]Universitá Campus Bio-Medico, Rome, Italy, [2]Queen Mary, University of London, London, UK, [3]Linköping University, Linköping, Sweden.

Background and aims: We have shown that insulin post-translationally modified by reactive oxidants (oxPTM-INS) is a neoantigen in type 1 diabetes (T1D). Most of T1D subjects or prediabetic children have autoantibodies to oxPTM-INS (oxPTM-INS-Ab). However, it is not clear whether oxPTM-INS-Ab can improve early diagnosis and prediction in association with the standard islet-AAB. Here, we evaluated whether oxPTM-INS-Ab can improve T1D prediction in children with positive islet-autoantibodies (AAB).
Materials and methods: We evaluated sensitivity, specificity, accuracy and risk for progression to T1D associated with oxPTM-INS-Ab and the standard islet-AAB that include insulin (IAA), GAD (GADA), and tyrosine-phosphatase 2 (IA-2A) in a cohort of islet-AAB positive (AAB^+) children (median follow-up 8.8 years) from the 'All Babies in Southeast Sweden' (ABIS), a large prospective birth cohort study in the general population. oxPTM-INS-Ab to insulin modified by hydroxyl radical (•OH) were measured by our developed ELISA platform.
Results: oxPTM-INS-Ab was the most sensitive and specific autoantibody-biomarker (74% sensitivity, 91% specificity), followed by IA-2A (71% sensitivity, 91% specificity). GADA and IAA showed lower sensitivity (65%, and 50%, respectively) and specificity (66%, and 68%, respectively). Accuracy (AUC of ROC) of oxPTM-INS-Ab was higher than GADA and IAA ($p = 0.003$ and $p = 0.017$, respectively), and similar to IA-2A ($p = 0.896$). Risk for diabetes was higher ($p = 0.03$) among multiple AAB^+ who were also oxPTM-INS-Ab^+ compared with those who were oxPTM-INS-Ab^-. Importantly, when replacing IAA with oxPTM-INS-Ab diabetes risk increased to 100% in children with oxPTM-INS-Ab^+ in combination with $GADA^+$, and IA-$2A^+$, compared to 84.37% in those with IAA^+, $GADA^+$, and IA-$2A^+$ ($p = 0.04$).
Conclusion: Antibodies to oxidised insulin (oxPTM-INS-Ab), compared to IAA which measure autoantibodies to native insulin, improve T1D risk assessment and prediction accuracy in AAB^+ children.
Supported by: EFSD/JDRF/Lilly; JDRF
Disclosure: **R. Strollo:** None.

210

Mutations in YIPF5 are a novel cause of neonatal diabetes, highlighting the critical role of endoplasmic reticulum-to-Golgi trafficking in human beta cell survival

E. De Franco[1], M. Lytrivi[2], K. Patel[1], M. Igoillo-Esteve[2], M. Wakeling[1], B. Haliloglu[3], E. Unal[4], T. Godbole[5], M. Yildiz[6], S. Ellard[1], M. Cnop[2], A.T. Hattersley[1];
[1]University of Exeter Medical School, Exeter, UK, [2]ULB Center for Diabetes Research, Bruxelles, Belgium, [3]Yeditepe University Hospital, Istanbul, Turkey, [4]Dicle University, Diyarbakır, Turkey, [5]Harmony Health Hub, Nashik, India, [6]Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Turkey.

Background and aims: Neonatal diabetes (NDM) diagnosed before 6 months is caused by mutations that reduce β cell number (reduced formation/increased destruction) or impair β cell function. Understanding the genetic basis of rare NDM subtypes highlights fundamental biological processes in β cells. We investigated the genetic cause of a syndrome characterised by NDM, microcephaly and epilepsy.
Materials and methods: We performed whole genome sequencing for 2 unrelated patients with NDM (diagnosed aged 5 and 9 weeks), epilepsy and microcephaly, born to consanguineous parents. Replication studies were performed in 394 patients with NDM (16 with microcephaly) using a targeted next generation sequencing assay. YIPF5 was silenced in the human β cell line EndoC-βH1 using RNA interference. Cells were exposed to the endoplasmic reticulum (ER) stressors thapsigargin and brefeldin A. Apoptosis was evaluated by staining with DNA-binding dyes or real-time annexin V binding assay. mRNA expression was assessed by qPCR.
Results: The two patients had homozygous likely deleterious variants (missense, p.(Ala181Val) and in-frame deletion p.(Lys106del)) in *YIPF5*. Replication studies identified 2 homozygous *YIPF5* mutations (p.(Trp218Arg) and p.(Ile98Ser)) in 3 patients (2 siblings) with insulin treated diabetes diagnosed before the age of 12 months. All patients had epilepsy and microcephaly. YIPF5 mRNA showed abundant expression in pancreas and islets. As YIPF5 is thought to be key in trafficking between ER and Golgi compartments, we examined the impact of YIPF5 deficiency on β cell survival during ER stress. YIPF5 knockdown did not affect basal β cell survival, but it sensitized β-cells to thapsigargin and brefeldin A (respectively $44 \pm 2\%$ and $29 \pm 5\%$ apoptosis vs $24\text{-}25 \pm 3\%$ in control siRNA-transfected cells, $p < 0.05$, $n = 4$). In time course experiments, ER stressed YIPF5-depleted cells showed increased expression of CHOP, BiP and spliced XBP1 ($p < 0.001$ at 24 h, $n = 5$), indicating activation of the 3 canonical branches of the ER stress response. CHOP silencing protected YIPF5-depleted cells from thapsigargin-induced apoptosis ($p < 0.001$, $n = 3$). Expression of the proapoptotic proteins PUMA and DP5 was enhanced by YIPF5 silencing ($p < 0.001$ at 24 h, $n = 5$). Treatment with forskolin, a potent cAMP inducer, abolished apoptosis induced by thapsigargin in YIPF5-depleted cells ($p < 0.001$, $n = 5$).
Conclusion: Homozygous loss of function mutations in *YIPF5* are a novel cause of a syndrome of microcephaly, epilepsy and NDM, which we suggest is termed MEND syndrome. Functional studies show that YIPF5 deficiency reduces β cell survival by enhancing the ER stress response and sensitizing human β cells to ER stress-induced apoptosis. This is the first report of mutations in a gene affecting ER-to-Golgi trafficking resulting in NDM by increasing β cell ER stress. This study highlights an unexpected critical role of *YIPF5* in the human β cell.
Supported by: Wellcome Trust Senior Investigator Grant to SE and ATH
Disclosure: **E. De Franco:** None.

OP 36 Muscling up on diabetes

211

Functional state of muscle mitochondria reflects exercise-induced changes in insulin sensitivity and cognitive performance in elderly

J. Ukropec[1], M. Nemec[1], D. Maderova[1], V. Tirpakova[2], P. Krumpolec[1], L. Slobodova[1,3], M. Vajda[4], M. Schon[1,3], J. Cvecka[4], Z. Sumbalova[5,6], M. Sedliak[4], B. Ukropcova[1,3];
[1]Institute of Experimental Endocrinology, Biomedical Research Center, Bratislava, Slovakia, [2]Slovak Health University, Bratislava, Slovakia, [3]Institute of Pathological Physiology Faculty of Medicine Comenius University, Bratislava, Slovakia, [4]Faculty of Physical Education and Sport Comenius University, Bratislava, Slovakia, [5]Faculty of Medicine Comenius University, Bratislava, Slovakia, [6]Medical University of Innsbruck, Innsbruck, Austria.

Background and aims: Sedentary ageing accelerates the risk of chronic metabolic and neurodegenerative diseases, while regular exercise could effectively support healthy ageing. Here we report results of the 3-month supervised aerobic/strength training intervention in seniors with/without mild cognitive impairment and prediabetes.
Materials and methods: Fourteen non-obese sedentary seniors with/without mild cognitive impairment and prediabetes were subjected to 3-month training (3x1h/week), consisting of intensive whole-body aerobic (60–70% HRmax) and resistance exercises (60–70% 1RM) of major muscle groups. Whole-body glucose metabolism (oGTT), insulin sensitivity (euglycemic hyperinsulinemic clamp), resting energy expenditure/REE, metabolic substrate preference/RQ and metabolic flexibility/ΔRQ (indirect calorimetry), daily ambulatory activity (accelerometers/Activinsights) and physical fitness/VO_2max (Rockport Test) were determined. Cognitive functions were assessed with a battery of validated cognitive tests (MMSE/ACE-R/CogState/Memtrax). Biopsy of *m. vastus lateralis* was performed in local anesthesia using Bergström needle technique. Functional state of muscle mitochondria was determined by O_2k high-resolution respirometry, applying SUIT protocols RP1&RP2 (Oroboros). Oxygen consumption rate (pmol/s/mg tissue wet weight) was evaluated in saponin-permeabilized muscle fibers.
Results: Exercise intervention increased propensity to voluntary physical activity, insulin sensitivity, metabolic flexibility, mitochondrial ETC complex I activity, as well as memory and executive functions ($p < 0.05$ for all). Muscle mitochondrial oxidative phosphorylation capacity was negatively associated with BMI ($R = -0{,}548$; $p = 0{,}042$) and mitochondrial fatty acid oxidation rate was positively associated with short-term memory (CogState, $R = 0{,}616$; $p = 0.019$). Improvements in physical fitness were associated with cognitive tests' scores (ACE-R; $R = 0.671$, $p = 0.0002$, CogState; $R = 0.561$, $p = 0.003$) and metabolic flexibility (ΔRQ) with the baseline/leak respiration of muscle mitochondria ($R = 0.440$, $p = 0.05$) as well as with the memory and executive functions ($R = 0.721$, $p = 0.001$). Moreover, non-coupled respiration rate of muscle mitochondria correlated positively with learning/working memory (CogState, $R = 0.412$, $p = 0.032$) and with the psychomotor attention score (CogState, $R = 0.471$, $p = 0.011$).
Conclusion: Our preliminary results showed that exercise-induced changes in functional state of muscle mitochondria are tightly linked to BMI, whole-body metabolic flexibility and cognitive performance in seniors with/without mild cognitive impairment and prediabetes. We are extending the population to investigate the exercise-related links between muscle metabolic and functional state, whole-body metabolism and cognition in prediabetic and metabolically healthy seniors.
Clinical Trial Registration Number: NCT03330470
Supported by: VEGA 2/0107/18; APVV-15-0253; SAS-NSC JRC 2013/17
Disclosure: **J. Ukropec:** None.

212

Local-tissue hyperinsulinaemia is a greater risk factor for insulin resistance than hyperglycaemia in type 1 diabetes and MODY2

J.M. Gregory, T.J. Smith, N.N. Abumrad, A.D. Cherrington;
Vanderbilt University, Nashville, USA.

Background and aims: Insulin resistance (IR) is strongly associated with macrovascular disease and occurs consistently in type 1 diabetes (T1DM). Mechanisms underpinning T1DM IR are unclear, however. We hypothesized that hyperinsulinemia resulting from peripheral circulation insulin delivery is a greater contributor to tissue-specific IR than hyperglycemia in T1DM.
Materials and methods: Insulin sensitivity was assessed in 3 cohorts with differing conditions for glycemia and insulinemia: healthy controls (euinsulinemia + euglycemia, $n = 10$), MODY2 (euinsulinemia + hyperglycemia, $n = 9$) and T1DM (hyperinsulemia + hyperglycemia equivalent to MODY2, $n = 9$). A 2-step, hyperinsulinemic, euglycemic, pancreatic clamp and isotopic tracer techniques were used to assess tissue-specific IR. Hyperglycemia's contribution to IR was indicated by differences in insulin sensitivity between control and MODY2. Hyperinsulinemia's effect on IR was shown by differences in insulin sensitivity between MODY2 and T1DM. Insulin sensitivity of the liver and fat were quantified by how much a 12 mU/m^2/min insulin infusion (step 1) suppressed glucose production (ΔR_a) and NEFA levels, respectively, from baseline. Muscle insulin sensitivity was determined by quantifying glucose uptake (R_d) during high insulin infusion (40 mU/m^2/min, step 2). Cohorts were matched for age and BMI. T1DM participants received a variable IV insulin infusion overnight to match glucose with the MODY2 group at the start of the clamp.
Results: Risk factors for IR (BMI, age, etc.) were similar between groups. HbA_{1c} was 4.8 ± 0.1, 6.7 ± 0.2 and 6.1 ± 0.1% for control, T1DM and MODY, respectively. Arterialized plasma insulin concentrations (μU/mL) for control, T1DM and MODY2, respectively, were 8.7 ± 0.5, 20.7 ± 2.1 and 8.7 ± 0.9 at baseline; 21.1 ± 0.8, 28.4 ± 1.5 and 21.0 ± 0.8 during step 1; and 80.5 ± 3.2, 76.6 ± 3.3 and 76.3 ± 2.0 during step 2. Somatostatin infusion suppressed c-peptide to T1DM levels in control and MODY2. Glucagon, epinephrine, norepinephrine and cortisol levels remained basal and equal between cohorts. ΔR_a (mg/kg FFM/min) in step 1 was minimally different between groups (1.7 ± 0.1, 1.8 ± 0.2 and 2.1 ± 0.2 for control, T1DM and MODY2). ΔNEFA (mmol/L) was virtually identical between control and MODY2 (393 ± 52 vs 382 ± 69) but less in T1DM (124 ± 87, $p = 0.02$ vs control, $p = 0.03$ vs MODY2). R_d (mg/kg FFM/min) in step 2 was similar between control and MODY2 (15.4 ± 1.1 vs 13.8 ± 0.8, Δ = 1.6, 95% CI −1.3 to 4.6) but lower for T1DM (11.3 ± 1.3; vs control: $p = 0.03$, Δ = 4.1, 95% CI of Δ 0.6 to 7.7; vs MODY2: $p = 0.1$, Δ = 2.5, 95% CI of Δ −0.6 to 5.7).
Conclusion: Iatrogenic local tissue hyperinsulinemia occurs chronically at muscle and fat in T1DM but not in MODY2 nor control and is associated with tissue-specific IR. Despite having hyperglycemia, when MODY2 is compared with control, insulin sensitivity at fat was nearly identical and insulin sensitivity at muscle was minimally different. The T1DM group had lower insulin sensitivity at fat and muscle than control and MODY2, despite having similar hyperglycemia to MODY2. Based on arterialized insulin concentrations, each group had the same estimated chronic hepatic insulin levels and differences in hepatic insulin sensitivity were indistinguishable. This suggests iatrogenic hyperinsulinemia confers more risk for IR at these tissues than hyperglycemia. Approaches to restore the physiologic insulin distribution between the portal and peripheral circulations should mitigate IR in T1DM.
Clinical Trial Registration Number: NCT02971202
Supported by: NIH K12HD087023
Disclosure: **J.M. Gregory:** None.

213

AAV-mediated FGF21 overexpression in skeletal muscle expands healthspan and counteracts insulin resistance

V. Sacristan Fraile[1], C. Jambrina[2], E. Casana[2], S. Muñoz[2], J. Rodo[1], S. Darriba[1], M. Garcia[2], X. Leon[2], I. Grass[1], V. Jimenez[2], F. Bosch[2];
[1]Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona, Bellaterra, [2]Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona and Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabolicas Asociadas (CIBERDEM), Bellaterra, Spain.

Background and aims: Insulin resistance and weight gain increases with aging, resulting in increased risk of cardiovascular disorders. Fibroblast growth factor 21 (FGF21) has been described as a potential factor that could exert benefitial effects to treat these age-related diseases. The aim of this study was to evaluate the potential of extending healthspan of the long-lasting secretion of FGF21 into the bloodstream following a single administration of adeno-associated viral (AAV) vectors to the skeletal muscle (Skm). Moreover, the anti-obesogenic and anti-diabetic effects of this treatment were also assessed.
Materials and methods: AAV vectors with high tropism for skeletal muscle carrying a murine FGF21 coding sequence (AAV-FGF21) or non-coding AAV-Null vectors were administered into the quadriceps, gastrocnemius, and tibialis cranialis muscles of each hind limb of adult (8- or 19-week-old) or old (1-year-old) C57Bl6 mice. To induce obesity and insulin resistance, 8-week-old C57Bl6 mice were fed a HFD for 12 weeks and afterwards administered intramuscularly with AAV-FGF21 vector. After treatment, mice were maintained on HFD feeding.
Results: Animals treated with FGF21-encoding vectors at 8 weeks of age and fed a chow diet showed a marked increase in circulating FGF21, which was parallel to high levels of expression of vector-derived FGF21 in the 3 injected muscles. At the end of the 10-month follow-up period (12 months of age) mice injected intramuscularly with AAV-FGF21 maintained the body weight they had at the initiation of the study and were slimmer than controls, which steady increased their weight as animals aged. While the weight of the muscles was barely affected by FGF21 gene transfer, the weight of the white and brown depots as well as the liver were considerably reduced. In contrast to null-injected animals, mice treated with AAV-FGF21 showed reduced insulinemia and markedly improved insulin sensitivity. In addition, assessment of improvements in healthspan in a new cohort of mice treated with AAV-FGF21 vectors when aged 1-year is ongoing. Moreover, when Skm was used as source of circulating FGF21 in HFD-fed mice, counteraction of obesity and increased insulin sensitivity were also observed.
Conclusion: Altogether, these results demonstrate that intramuscular administration of AAV vectors that lead to therapeutically-relevant levels of circulating FGF21 is safe in the long-term and highlight the therapeutic potential of this approach to expand healthspan as well as to treat T2D and obesity in the future.
Supported by: EFSD/MSD, SAF2014-54866R, MYOCURE SPH-14-2015-667751,
Disclosure: **V. Sacristan Fraile:** Grants; SAF2014-54866R, 2014-SGR-1669, ICREA Academia Award to F.B, EFSD/MSD, MYOCURE, PHC-14-2015-667751, EFSD/Lilly. Other; Veronica Jimenez, Claudia Jambrina and Fatima Bosch are coinventors on a patent application for the use of AAV vectors for the treatment of metabolic disorders.

214

Insulin and muscle contraction regulate TBC1D1 through phosphorylation and interaction with the cytosolic tail of insulin-regulated aminopeptidase

S. Mafakheri[1,2], R.R. Flörke[1,2], S. Kanngießer[1], S. Hartwig[1,2], T. Schönberger[1], N. Hamker[1], S. Lehr[1,2], A. Chadt[1,2], H. Al-Hasani[1,2];
[1]Institue of Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Düsseldorf, [2]German Center for Diabetes Research (DZD), Düsseldorf, Germany.

Background and aims: In skeletal muscle, the ~1200 aa Rab GTPase-activating (GAP) protein TBC1D1 is phosphorylated by AKT and AMPK in response to insulin and contraction. Mutation of phosphorylation sites for AKT and AMPK impairs GLUT4 translocation from storage vesicles (GSVs) to the plasma membrane. However, the exact phosphorylation pattern and the mechanism how the signal is transmitted to GSVs is unclear.
Materials and methods: We expressed and purified recombinant full-length His6-TBC1D1 in *Sf*9 insect cells via the Baculovirus system. We characterized the enzymatic activity of purified TBC1D1 under various conditions by adding recombinant Rab GTPases and measuring of Gamma-32P-GTP hydrolysis, *in vitro*. Mapping of the phosphorylation sites of TBC1D1 after *in vitro* phosphorylation using purified AKT/AMPK was performed by mass spectrometry and phospho-specific antibodies.
Results: Size-exclusion chromatography of the purified protein reveals a molecular mass of approx. 600 kDa, consistent with formation of TBC1D1 trimers. Similar to the truncated 50 kDa C-terminal GAP domain, full-length TBC1D1 shows RabGAP activity towards GLUT4-associated Rab8a, Rab10 and Rab14 but with a 200-fold increase in velocity compared to the GAP domain expressed in *E. coli*. Full-length TBC1D1 is phosphorylated at Ser^{231} in response to AMPK and at Thr^{590} in response to both AMPK and AKT. While *in vitro* phosphorylation of TBC1D1 by AKT or AMPK increased 14-3-3 binding, it did not alter the RabGAP activity. However, we found that full-length TBC1D1 interacts with the 110 aa cytoplasmic domain of the insulin-regulated aminopeptidase (IRAP), a resident protein in GLUT4 storage vesicles, and this binding is disrupted by phosphorylation of TBC1D1 by AKT or AMPK.
Conclusion: For the first time, we purified and characterized active full-length TBC1D1. Our data indicate that insulin and contraction-mediated activation of AKT/AMPK alters the recruitment of TBC1D1 to GSVs via phosphorylation and interaction with IRAP. In response to insulin/contraction, this makes phosphorylated TBC1D1 unavailable for its Rab substrates, and consequently increases the activation state, i.e., the active GTP-bound form of the Rabs that subsequently triggers GLUT4 translocation from GSVs to the plasma membrane.
Supported by: DFG
Disclosure: **S. Mafakheri:** None.

215

Diabetes modulates microRNAs 29b-3p, 29c-3p, 199a-5p and 532-3p expression in muscle: potential participation in GLUT4 repression

J. Esteves[1], C.Y. Yonamine[1], F. Gerlinger-Romero[1,2], D.C. Pinto-Junior[1], F.J. Enguita[3], U.F. Machado[1];
[1]University of São Paulo, São Paulo, Brazil, [2]Deakin University, Geelong, Australia, [3]Instituto de Medicina Molecular, Universidade de Lisboa, Lisboa, Portugal.

Background and aims: Diabetes is a metabolic disease characterized by hyperglycemia associated with impaired glucose uptake, in which reduced GLUT4 protein expression (encoded by the *SLC2A4* gene) plays an essential role. MicroRNAs (miRNAs), which are small noncoding RNA molecules that regulate gene expression at the posttranscriptional level, have been described as involved in the pathophysiology of diabetes. However, the miRNAs involvement upon skeletal muscle GLUT4 repression in diabetes, consequently impairing the glucose uptake, is unclear. Therefore, the aim of this study was to evaluate the expression of miRNAs potentially involved in the *Slc2a4*/GLUT4 repression in skeletal muscle of diabetic rats.
Materials and methods: Male Wistar rats (70-day old) were rendered diabetic by receiving streptozotocin (50 mg/kg, i.v.). After 13 days, three groups were formed: non-diabetic (ND), and diabetic treated with placebo (D) or with NPH insulin (ID) (6 U/day). Treatments were conducted for 7 days, totalizing 21 days of diabetes. At the end of the experimental period, the animals were anesthetized, blood was collected, and the soleus

skeletal muscles were harvested for evaluation of different mRNAs and miRNAs by RT-qPCR, and proteins by Western blotting. *In silico* analysis was used to select miRNAs predicted as potential regulators of *Slc2a4*/GLUT4 in rat. The comparison among groups was performed by analysis of variance followed by Bonferroni post-test. Correlations analysis were performed by Pearson or Spearman correlation coefficient.

Results: Diabetic rats reduced body and skeletal muscle mass development, and shown hyperglycemia, glycosuria and increased plasma fructosamine; insulin treatment improved these parameters. Diabetes reduced ($P < 0.001$) by ~55% and ~77% the *Slc2a4* mRNA and GLUT4 protein; insulin treatment restored these variables completely. *In silico* analysis revealed that 651 miRNAs were predicted as potential regulators of *Slc2a4*/GLUT4 expression, from these, 16 miRNAs were selected for analysis. Seven miRNAs were modulated by diabetes ($P < 0.05$ to $P < 0.001$), being two upregulated, miR-29b-3p (~118%) and miR-29c-3p (~51%,); and five downregulated (~30%), miR-93-5p, miR-150-5p, miR-199a-5p, miR-345-3p e miR-532-3p. Except for miR-150-5p, insulin treatment reverted these changes. Besides, GLUT4 protein content correlated negatively ($P < 0.05$) with miR-29b-3p and miR-29c-3p, suggesting a causal relationship, and positively with miR-199a-5p and miR-532-3p, suggesting an indirect relationship. Correlations were also detected between these miRNAs and blood glucose, glycosuria and plasma fructosamine, and insulin therapy reversed most of the alterations.

Conclusion: In summary, diabetes leads to upregulation of miR-29b-3p and miR-29c-3p expression and downregulation of miR-199a-5p and miR-532-3p expression in skeletal muscle. These miRNAs are predicted to regulate *Slc2a4*/GLUT4 expression, and their regulations correlated significantly with GLUT4 content variations. The results make these miRNAs as potential targets to control GLUT4 expression in muscle and, consequently, the tissue glucose disposal and blood glucose control in diabetes.

Supported by: FAPESP (#2017/19449-9, #2016/15603-0)

Disclosure: **J. Esteves:** None.

216

Human skeletal muscle mitochondrial dynamics in relation to insulin sensitivity and oxidative capacity

A. Houzelle, J.A. Jörgensen, G. Schaart, S. Daemen, E. Phielix, M.K.C. Hesselink, P. Schrauwen, J. Hoeks;
Nutrition and Movement Sciences, Maastricht University, Maastricht, Netherlands.

Background and aims: Recent insights indicate that mitochondria operate in a highly dynamic network, constantly remodeling itself via fusion and fission mechanisms, termed mitochondrial dynamics, rather than acting as single organelles. Recent studies have started to unravel the link between mitochondrial dynamics regulatory proteins, such as mitofusin-2 (MFN2) and dynamin-related protein 1 (DRP1), mitochondrial function and metabolic diseases. Moreover, manipulation of these proteins via gain or loss-of function studies in animal models resulted in alterations in mitochondrial function. Here, we aim to investigate the relation between mitochondrial dynamics and oxidative capacity and insulin sensitivity in human skeletal muscle over a wide range in insulin sensitivity.

Materials and methods: We collected muscle biopsies from 45 well-phenotyped subjects recruited from 4 metabolically distinct populations (T2DM patients, healthy obese subjects, lean sedentary subjects and endurance-trained athletes) and measured content of proteins involved in regulating mitochondrial dynamics (MFN1/2, OPA1, Fis1 and DRP1) and -quality control (HSP60, PINK1 and LC3) via western blotting. In addition, muscle tissues from a subgroup of athletes and Type 2 diabetes subjects ($n = 3$ in both groups) were used to investigate mitochondrial network morphology and fragmentation using confocal microscopy. Correlation analysis was performed to determine relationship between mitochondrial dynamics and VO_2max or Glucose Infusion Rate (GIR) as assessed by a hyperinsulinemic-euglycemic clamp.

Results: Fis1, involved in mitochondrial fission, and OPA1, promoting fusion of the inner mitochondrial membrane, were 57% and 42% lower in type 2 diabetes when compared to endurance-trained athletes ($p < 0.01$ and $p = 0.01$ respectively). HSP60 protein levels, involved in mitochondrial quality control, were also reduced by 54% in T2DM as compared to athletes ($p < 0.001$). Although it did not reach statistical significance, assessed via Tukey's post-hoc test, obese and lean individuals showed an intermediate phenotype for these proteins. Confocal images showed a higher mitochondrial network fragmentation index in T2DM compared to athletes. This observation was independent of fiber type, as fragmentation was 21% higher in type I fibers and 27% higher in type 2 fibers in T2DM compared to athletes. Finally, in the entire cohort, Fis1 and HSP60 protein levels correlated positively with VO_2max ($n = 45$, $r = 0.57$, $p < 0.001$, and $r = 0.33$, $p = 0.02$). Furthermore, Fis1 protein levels correlated positively with the GIR obtained after the high-insulin phase of a hyperinsulinemic-euglycemic clamp ($n = 45$, $r = 0.45$, $p < 0.01$).

Conclusion: Impaired mitochondrial function has been reported multiple times in type 2 diabetes. Here, we report disturbance in skeletal muscle mitochondrial dynamics. We show, in diabetes patients, that skeletal muscle mitochondrial network and its regulatory proteins point toward a more fragmented mitochondrial network and correlate with oxidative metabolism and insulin sensitivity. Whether this is a mere consequence, or a potential contributing factor, to the development of type 2 diabetes remains to be elucidated.

Supported by: NWO VIDI

Disclosure: **A. Houzelle:** None.

OP 37 Adipose tissue: I have you under my skin

217

Targeting adipose tissue glyoxalase system with GLP-1 to improve capillarisation and insulin sensitivity

P. Matafome[1,2], T. Rodrigues[1], P. Borges[1], C. Carrêlo[3], L. Mar[1], H. Eickhoff[1,4], B. Almeida[5], D. Marques[1], S. Pires[6], M. Abrantes[6], B. Martins[3], C. Uriarte[7], P. Gomes[7], S. Silva[3,8], R. Seiça[1];
[1]Institute of Physiology and Institute of Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra (FMUC), Coimbra, [2]Instituto Politécnico de Coimbra, Coimbra, [3]iCBR, FMUC, Coimbra, [4]Obesity Center, Hospital de Santiago, Setúbal, Portugal, [5]Association for the Protection of Portuguese Diabetic Patients (APDP), Lisbon, [6]Institute of Biophysics and iCBR, Faculty of Medicine, University of Coimbra, Coimbra, [7]Department of Surgery, Universitary Hospital Center of Coimbra, Coimbra, [8]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Background and aims: Adipose tissue capillarization is correlated with insulin sensitivity and metabolic homeostasis. Methylglyoxal was shown to impair adipose tissue capillarization and insulin sensitivity in obese rats leading to the onset of metabolically unhealthy obesity. We hypothesized that decreased glyoxalase 1 (GLO-1) activity in adipose tissue may be correlated with impaired insulin sensitivity in obese patients, thus being a promising therapeutic target in obesity and type 2 diabetes. Given that bariatric surgery was observed to increase adipose tissue angiogenesis and GLP-1 is known to promote angiogenesis, we hypothesized that glyoxalase could be a target of GLP-1 favoring adipose tissue angiogenesis.
Materials and methods: In a cohort of obese patients (diabetic and non-diabetic), we collected samples of visceral adipose tissue, determined insulinemia and calculated insulin resistance indexes. Glyoxalase activity in human visceral adipose tissue was determined using an enzymatic assay. The role of GLP-1 in adipose tissue angiogenesis and glyoxalase modulation was assessed using the adipose tissue angiogenic assay and HUVEC cell line. In order to evaluate the role of GLP-1 *in vivo*, type 2 diabetic GK rats were submitted to sleeve gastrectomy (surgical model) or Liraglutide administration (pharmacological model). The activation of glyoxalase and angiogenic and vasoactive mechanisms were evaluated in the epididymal adipose tissue of GK rats.
Results: Glyoxalase activity in visceral adipose tissue of obese patients was inversely correlated ($n = 82$, Pearson correlation) with plasma insulin levels ($r = -0.27$, $p = 0.013$), HOMA-IR ($r = -0.277$, $p = 0.019$) and HOMA2-IR ($R = -0.276$, $p = 0.012$) indexes, while directly correlated with the insulin sensitivity index QUICKI ($r = 0.248$, $p = 0.024$). GLP-1 increased adipose tissue capillarization in the adipose tissue angiogenic assay in a glyoxalase-dependent manner and increased glyoxalase expression in HUVECs. Moreover, glyoxalase expression in epididymal adipose tissue was increased in both rats submitted to sleeve gastrectomy and treated with Liraglutide, surgical and pharmacological models of increased GLP- 1 levels. Increased glyoxalase activity in Liraglutide-treated rats was associated with increased expression of angiogenic and vasoactive factors, such as VEGF, VEGFR2, FGFR, HIF-2alpha and eNOS, as well as increased insulin receptor phosphorylation (Tyr1163).
Conclusion: Lower adipose tissue glyoxalase is correlated with insulin resistance and may be a target of GLP-1 in order to improve adipose tissue capillarization and insulin sensitivity, which may be a promising therapeutic approach to prevent metabolic dysregulation in obesity and type 2 diabetes.
Supported by: FCT (Pest-C/SAU/UI3282/2011; SFRH/BD/101172/2014; SFRH/BPD/104881/2014)
Disclosure: **P. Matafome:** None.

218

Abdominal subcutaneous adipose tissue gene expression in relation to tissue-specific insulin resistance in human obesity

B.W. van der Kolk[1], M. Kalafati[2,3], M. Adriaens[3], M.M.J. van Greevenbroek[4], W.H.M. Saris[1], C.J.H. van der Kallen[4], C.D.A. Stehouwer[4], G.H. Goossens[1], I.C.W. Arts[3,5], J.W.E. Jocken[1], C.T. Evelo[2,3], E.E. Blaak[1], the CODAM study, the Maastricht Study, the DiOGenes consortium;
[1]Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, [2]Bioinformatics-BiGCaT, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, [3]Maastricht Centre for Systems Biology (MaCSBio), Maastricht University, Maastricht, [4]Department of Internal Medicine, CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, [5]Department of Epidemiology, CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, Netherlands.

Background and aims: Obesity-related insulin resistance (IR) may develop in key metabolic organs, representing different etiologies towards cardiometabolic diseases. This study aimed to identify distinct transcriptome profiles of abdominal subcutaneous adipose tissue (ScAT) in relation to muscle or liver IR.
Materials and methods: Overweight/obese non-diabetic participants of the European DiOGenes project (BMI >27 kg/m^2, $n = 368$) were classified at baseline into 4 groups: i) no-IR ($n = 186$), ii) muscle-IR ($n = 69$), iii) liver-IR ($n = 53$), iv) muscle/liver-IR ($n = 60$). The IR phenotype was based on tertiles of the muscle insulin sensitivity index (MISI) and the hepatic IR index (HIRI), derived from a 5-point OGTT. ScAT RNA sequencing data were compared between groups using DESeq2, adjusted for study center, sex, BMI and waist-to-hip ratio. Based on DiOGenes outcomes, the relationship between systemic low-grade inflammation and IR phenotype was subsequently studied in overweight/obese non-diabetic individuals of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM, BMI >25 kg/m^2, $n = 325$) and Maastricht study (BMI >27 kg/m^2, $n = 792$), using linear regression analyses.
Results: In DiOGenes, ScAT extracellular matrix organization genes (e.g. collagens) were significantly upregulated in the liver-IR *vs* no-IR comparison (Fold change (FC) >1.2, $p < 0.05$). In muscle-IR *vs* no-IR comparison, inflammatory pathways were significantly changed with pronounced upregulation of chemokine and complement genes (FC >1.2, $p < 0.05$). Plasma low-grade inflammation was inversely associated with MISI (CODAM: standardized-ß [95%CI]: −0.108 [−0.205; −0.011] $p = 0.028$; Maastricht Study: −0.131 [−0.193; −0.068] $p < 0.001$), while no association was observed with HIRI (CODAM std-ß [95% CI]: 0.066 [−0.032; 0.165] $p = 0.184$; Maastricht Study: 0.000 [−0.064; 0.064] $p = 0.995$). The association between low-grade inflammation and MISI was adjusted for HIRI, and vice versa.
Conclusion: Muscle and hepatic IR were characterized by distinct abdominal ScAT transcriptome profiles. Extracellular matrix remodeling genes were upregulated in individuals with primarily hepatic IR, whilst inflammatory genes were significantly upregulated in primarily muscle IR individuals. An increased systemic low-grade inflammation profile was specifically related to muscle IR. We propose that increased abdominal ScAT inflammatory gene expression in the muscle IR phenotype may translate into an increased systemic inflammatory profile, putatively linking abdominal ScAT inflammation to muscle IR.
Clinical Trial Registration Number: NCT00390637
Supported by: European Commission (FP6-2005-513946), MUMC+ and Nestle
Disclosure: **B.W. van der Kolk:** Grants; European Commission, Food Quality and Safety Priority of the Sixth Framework Program (FP6-2005-513946). Other; grant from Nestlé Institute of Health Sciences, Lausanne, Switzerland, grant from the Maastricht University Medical Center+.

219

New insights in adipose tissue dysfunctions in diabetic obese patients: a new PPARγ truncated isoform mimicking PPARG dominant negative mutations

M. Aprile[1], S. Cataldi[1], M. Blüher[2], A. Ciccodicola[1], V. Costa[1];
[1]Institute of Genetics and Biophysics "A. Buzzati-Traverso" (IGB-CNR), National Research Council, Naples, Italy, [2]Department of Medicine, University of Leipzig, Leipzig, Germany.

Background and aims: Adipose tissue (AT) dysfunctions are hallmark of insulin resistance and contribute to type 2 diabetes (T2D) onset. PPARγ is a ligand-dependent transcriptional factor essential for differentiation of insulin-sensitive adipocytes and maintenance of whole body insulin sensitivity. *PPARG* loss of function mutations are associated with lipodistrophy, increased BMI, insulin resistance, and dominant negative mutations also block adipocyte differentiation. We identified a new dominant negative PPARγ isoform lacking LBD (PPARγΔLBD) highly expressed in human AT. Its overexpression interferes with the transcription of PPARγ-induced genes involved in lipid, glucose and insulin homeostasis. Our aim is to investigate whether PPARγΔLBD functionally mimics dominant negative mutations and to verify its relevance in the context of human metabolic diseases.
Materials and methods: PPARγΔLBD over-expression (assessed by qPCR, western blot and immunofluorescence) was induced by electroporation in the early stages of adipogenesis of hTERT-immortalized adipose-derived mesenchymal stem cells (AdMSCs), used as *in vitro* model. Terminal differentiation was assessed by Oil Red O staining and expression analysis of specific markers (qPCR). Subcutaneous AT biopsies were obtained from a German cohort of patients ($n = 95$; mean age = 55.5 ± 16.5 y.o.; mean BMI = 35.4 ± 11.8) undergoing bariatric surgery. According to fasting glucose levels and after OGTT, patients were classified as diabetic (T2D, $n = 32$), with impaired glucose tolerance (IGT, $n = 15$) and with normal glucose tolerance (NGT, $n = 47$). Patients were also stratified in lean (BMI <25) and overweight/obese (BMI >25). Gene expression differences were analyzed by Student's t test or Kolmogorv-Smirnov test, and linear models implemented in R language were used for correlation analysis.
Results: PPARγΔLBD over-expression in pre-adipocytes significantly impairs their adipogenic potential, resembling the effects of *PPARG* mutations. PPARγΔLBD has variable expression in human subcutaneous AT. Interestingly, T2D patients display increased PPARγΔLBD/PPARγ ratio ($p < 0.01$) compared to NGT. Additionally, PPARγΔLBD/PPARγ ratio positively correlates with BMI ($n = 95$, $r = 0.37$; $p = 0.0002$), regardless of diabetic state (T2D $r = 0.394$; $p = 0.025$ and NGT $r = 0.33$; $p = 0.02$). Accordingly, significantly higher PPARγΔLBD/PPARγ ratio was measured in the overweight/obese group *vs* lean patients ($p < 0.01$). Indeed, PPARγΔLBD/PPARγ ratio had a positive correlation with BMI only in the overweight/obese group ($r = 0.28$; $p = 0.020$; lean $r = 0.03$; $p = 0.873$). These data reveal for the first time the presence of high levels of a dominant negative isoform of PPARγ, PPARγΔLBD, in AT of diabetic and obese patients, suggesting a role in the impairment of PPARγ that is functionally related to obesity-associated AT dysfunctions.
Conclusion: The new naturally-occurring truncated isoform of PPARγ identified by our group, PPARγΔLBD, is capable to impair the adipogenic potential of precursor cells mimicking the effects of dominant negative mutations in *PPARG* gene. Its increased levels in AT of T2D patients and overweight/obese individuals and its positive correlation with BMI strongly suggest its contribution to the impairment of PPARγ activity in the AT of diabetic obese patients.
Disclosure: **M. Aprile:** None.

220

Elevated plasma proneurotensin levels predict NAFLD and visceral adipose tissue inflammation in adults with and without type 2 diabetes

I. Barchetta[1], F.A. Cimini[1], F. Leonetti[1], D. Capoccia[1], L. Bertoccini[1], V. Ceccarelli[1], G. Silecchia[1], M. Orho-Melander[2], O. Melander[2], M.G. Cavallo[1];
[1]Sapienza University of Rome, Rome, Italy, [2]Lund University, Malmo, Sweden.

Background and aims: Neurotensin (NT) is an intestinal peptide released by fat ingestion and promoting lipids absorption; higher circulating NT levels are associated with the incidence of type 2 diabetes (T2D), cardiovascular disease, breast cancer, and with total and cardiovascular mortality. Reduced intestinal fat absorption, along with protection from obesity and fatty liver, have been shown in NT-deficient mice fed with high-fat diet. NT was also demonstrated to take part to visceral adipose tissue (VAT) inflammation -a leading cause of non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH)- in experimental colitis, by enhancing the preadipocyte-dependent macrophage migration. Whether NT is related to NAFLD and NASH in humans has not been explored. This study aimed at investigating the relationship between plasma proneurotensin1-117 (pro-NT), a stable fragment of the NT precursor hormone, and the presence/severity of NAFLD and NASH and to unravel correlates of increased pro-NT levels.
Materials and methods: For this cross-sectional study, we recruited 320 consecutive individuals (M/F: 158/162; mean ± SD age: 50.7 ± 11.2 years), with and without T2D ($n = 110/210$), referring to the Endocrinology and Diabetes outpatient clinics at Sapienza University of Rome, Italy, for metabolic evaluations. A first cohort (i) of sixty obese patients underwent bariatric surgery for clinical purposes and intraoperative liver biopsies were performed for diagnosing NAFLD/NASH. Moreover, we explored the presence of VAT inflammation in VAT biopsies available for 40 out of these 60 subjects. In the cohort (ii) of individuals not candidate to surgery ($n = 260$), NAFLD was evaluated through liver ultrasonography (US). Circulating pro-NT levels were measured by a chemiluminometric sandwich immunoassay on plasma frozen immediately after separation and stored at −80°C.
Results: Subjects with biopsy-proven NAFLD ($n = 32/60$, 53%) had significantly higher plasma pro-NT than those without NAFLD (183.6 ± 81.4 vs 86.7 ± 56.8 pmol/L, $p < 0.001$). Greater pro-NT correlated with the presence of NASH ($p < 0.001$), higher NAS and SAF score (both $p < 0.001$), age, female gender, T2D and insulin-resistance. Notably, pro-NT positively associated with signatures of VAT inflammation (greater CD68+ macrophage infiltration, reduced microvessel density, higher HIF-1α, WISP-1 and UNC5B expression in VAT; all $p < 0.05$). Higher pro-NT predicted NAFLD with AUROC = 0.836 (C.I.95%:0.73–0.94; $p < 0.001$). At the multivariate logistic regression analysis, pro-NT levels were associated with biopsy-proven NAFLD independently from all the confounders. The positive association between pro-NT and presence of NAFLD was confirmed in the second cohort (US-NAFLD+: $n = 103/260$; 40%), regardless of age and other determinants of greater pro-NT in this population, such as female gender, metabolic syndrome and T2D.
Conclusion: Increased plasma pro-NT levels identify the presence and severity of NAFLD/NASH and are associated with signatures of VAT inflammation; in dysmetabolic individuals, NT may specifically promote hepatic fat accumulation though mechanisms likely related to metabolic impairment and increased insulin-resistance.
Supported by: Sapienza University
Disclosure: **I. Barchetta:** None.

OP 38 Novel actions of metformin and pioglitazone

221

Metformin attenuates the fall in postprandial blood pressure and slows gastric emptying in type 2 diabetes

M.J. Borg, C.K. Rayner, M. Horowitz, K.L. Jones, T. Wu;
Adelaide Medical School, University of Adelaide, Adelaide, Australia.

Background and aims: There is evidence that metformin has cardioprotective benefits in type 2 diabetes (T2DM) independent of improvements in glycaemic control. It is now recognised that exposure of the small intestine to nutrients (determined by the rate of gastric emptying), and the concomitant increase in splanchnic blood flow, may be associated with a reduction in BP and, in some cases, postprandial hypotension. The latter, defined by a fall in systolic BP (SBP) of ≥20 mmHg within 2 hours of a meal, occurs frequently in T2DM, and is associated with syncope, falls, stroke and increased mortality. We recently reported that metformin modulates the cardiovascular response to intraduodenal (ID) glucose in patients with T2DM. We have now evaluated the acute effects of metformin (administered by ID infusion in order to standardise small intestinal exposure) on SBP, diastolic BP (DBP), heart rate (HR) and gastric emptying following oral glucose in T2DM.
Materials and methods: 10 T2DM patients managed by diet alone (5 male and 5 female; 65.6 ± 3.1 years; BMI 30.1 ± 1.7 kg/m^2; HbA1c 6.4 ± 0.2% [45.8 ± 1.6 mmol/mol]; duration of known T2DM 5.5 ± 1.0 years), were studied on two occasions in a double-blind, randomised, crossover design. Participants received either metformin 1 g, or saline control, via an ID catheter ($t = -60$ to -55 min), before ingesting a 50 g glucose drink labelled with ^{13}C-acetate ($t = -2$ to 0 min). SBP, DBP and HR were assessed every 5 min by automatic sphygmomanometer, and breath samples taken at regular intervals to determine the gastric half-emptying time (T50). Data are mean values ± SEM. $P < 0.05$ was considered significant.
Results: The studies were all tolerated without nausea. Basal SBP (control 136 ± 6 mmHg vs. metformin 129 ± 4 mmHg), DBP (control 74 ± 3 mmHg vs. metformin 71 ± 4 mmHg) and HR (control 67 ± 4 beats/min vs. metformin 68 ± 3 beats/min) did not differ between the two days. On both days, SBP and DBP decreased, while HR increased, following oral glucose ($P < 0.01$ for each). The fall in SBP was less after metformin than control (treatment effect: $P = 0.036$, treatment by time interaction: $P < 0.001$; Figure) without any differences in either DBP or HR. Four participants exhibited a sustained fall in SBP ≥20 mmHg with control, and only two with metformin. Metformin also slowed gastric emptying (T50: 183 ± 51.6 vs. 130 ± 21.1 min; $P = 0.008$).
Conclusion: Acute administration of metformin attenuates the hypotensive response to, and slows the gastric emptying of, oral glucose in T2DM. These effects may contribute to both postprandial glucose lowering by metformin, and its favourable cardiovascular profile.

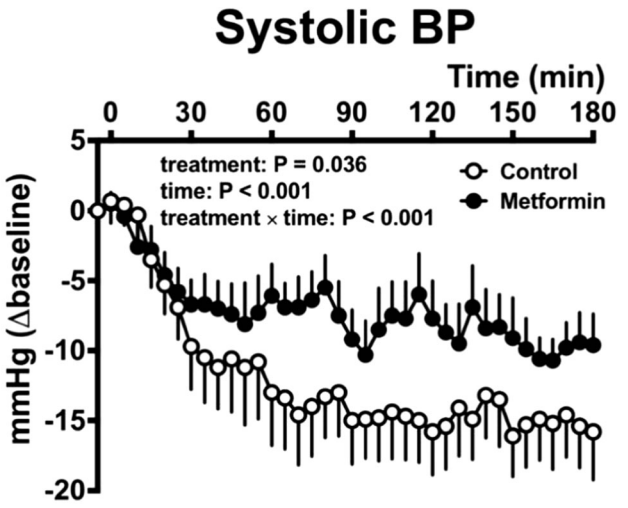


Figure: Effect of metformin 1g, administered acutely by intraduodenal infusion, on the systolic BP response to an oral glucose drink (50g) in patients with type 2 diabetes. Two-factor repeated measures ANOVA, with treatment and time as factors, was used to determine statistical significance. Data are mean values ± SEM.

Clinical Trial Registration Number: ACTRN12617000243314
Supported by: Diabetes Australia (grant number Y17G-WUTO)
Disclosure: **M.J. Borg:** None.

222

Comparative effects of proximal and distal small intestinal administration of metformin on plasma glucose and GLP-1, and gastric emptying after oral glucose in type 2 diabetes

T. Wu, M. Borg, K.L. Jones, M. Horowitz, C.K. Rayner;
Adelaide Medical School, The University of Adelaide, Adelaide, Australia.

Background and aims: The gastrointestinal tract is increasingly recognised as key to the anti-diabetic action of metformin. Emerging evidence indicates a substantial role for glucagon-like peptide-1 (GLP-1), which has pleiotropic glucose-lowering effects, including slowing of gastric emptying (GE). Given that GLP-1 is released predominantly from the ileum and colon, the distal gut may be of greater relevance for the action of metformin, compared with the proximal gut. Accordingly, we evaluated the comparative effects of metformin administered into the proximal and distal small intestine on plasma glucose and GLP-1, and GE, after oral glucose in type 2 diabetes (T2DM).
Materials and methods: 10 diet-managed T2DM patients (5 male; 65.6 ± 3.1 years; BMI 30.1 ± 1.7 kg/m^2; HbA1c 6.4 ± 0.2%; duration of known diabetes 5.5 ± 1.0 years), were studied on three occasions in a double-blind, randomised, crossover design. On each study day, a transnasal multilumen catheter was positioned with proximal and distal infusion ports located at 13 and 190 cm beyond the pylorus, respectively. On separate days, participants received infusions of (i) proximal saline + distal saline (control), (ii) proximal metformin (1000 mg) + distal saline, or (iii) proximal saline + distal metformin (1000 mg), each in a volume of 40 mL over 5 min, followed 60 min later by a 200 mL glucose drink, containing 50 g glucose and 150 mg ^{13}C-acetate. "Arterialised" venous blood was sampled at frequent intervals over 3 hours after the drink for measurements of plasma glucose, GLP-1, insulin and glucagon. Breath samples were collected for the measurement of GE by calculating the half-emptying time (T50).
Results: There were significant treatment effects of metformin on the peak glucose concentrations ($P < 0.001$), the incremental areas under the curves (iAUCs) for plasma glucose ($P < 0.001$) and total GLP-1 ($P = 0.03$), and the T50 ($P = 0.01$), but not on the iAUC for plasma insulin

or glucagon. Compared with control, both proximal and distal metformin reduced the peak and iAUC for plasma glucose ($P < 0.05$ each), with no difference between them. Proximal metformin augmented the iAUC for GLP-1 and slowed GE ($P < 0.05$ each), while the distal metformin was associated with numerically, but not statistically, greater GLP-1 concentrations and longer T50 than control. However, plasma GLP-1 concentrations and the T50 did not differ between proximal and distal metformin.
Conclusion: In diet-controlled T2DM patients, glucose-lowering, resulting from a single dose of metformin administered to the proximal and distal small intestine, was comparable, despite the stimulation of GLP-1 secretion and slowing of GE being modestly greater when metformin was administered to the proximal small intestine. These observations suggest that the site of gastrointestinal administration is not critical to the capacity of metformin to reduce blood glucose.

	Control	Proximal metformin	Distal metformin	P value
Peak glucose (mmol/L)	12.2 ± 0.4	10.8 ± 0.4**	10.9 ± 0.7*	0.007
Glucose iAUC (mmol/L*min)	788.1 ± 48.4	610.6 ± 34.7***	644.1 ± 73.3*	< 0.001
Insulin iAUC (mU/L*min)	4136.3 ± 777.8	3612.6 ± 728.1	3550.5 ± 799.8	0.29
Glucagon iAUC (pg/mL*min)	-2051.3 ± 487.7	-1271.3 ± 445.6	-1590.5 ± 534.7	0.19
Total GLP-1 iAUC (pmol/L*min)	491.2 ± 291.4	1255.9 ± 339.0*	1012.0 ± 368.4	0.03
Gastric emptying T50 (min)	130 ± 21	183 ± 52*	160 ± 47	0.01

One-factor ANOVA, with Bonferroni's correction for post hoc comparisons, was used for statistical analysis. *P < 0.05, **P < 0.01 and *** P< 0.001 for proximal or distal metformin vs. control. Data are means ± SEM.

Clinical Trial Registration Number: ACTRN12617000243314
Supported by: DART
Disclosure: **T. Wu:** None.

223

Pioglitazone treatment reverts diabetes-related abnormalities in mitochondrial proteomic profile of skeletal muscle

T.V. Fiorentino[1], A. Monroy[2], S. Kamath[2], C. Carroll[2], K. Hakala[2], R. Sotero[1], G. Daniele[2], M. Abdul-Ghani[2], M.L. Hribal[1], D. Tripathy[2], G. Sesti[1], R.A. DeFronzo[2], F. Folli[3,2];
[1]University Magna Graecia of Catanzaro, Catanzaro, Italy, [2]University of Texas Health Science Center, San Antonio, USA, [3]University of Milan, Milano, Italy.

Background and aims: Several studies have demonstrated that type 2 diabetes (T2DM) is associated with skeletal muscle (SKLM) mitochondrial dysfunction. Pioglitazone (PIO) is a hypoglycemic agent which improves insulin sensitivity through several and incompletely understood mechanisms, including the promotion of mitochondrial oxidative metabolism. In this study, we aimed to identify the determinants of mitochondrial dysfunction in SKLM of subjects with T2DM and to evaluate whether PIO treatment is able to modulate the SKLM mitochondrial proteomic pattern.
Materials and methods: Two different groups of adults were studied. Group 1 was composed of 16 individuals, 8 with normal glucose tolerance (NGT) and 8 with T2DM, subjected to analysis of SKLM mitochondrial proteome by 2D-gel electrophoresis followed by mass spectrometry-based protein identification. Group 2 included 48 individuals, 24 with NGT and 24 with T2DM. SKLM biopsies of these subjects were lysed and subjected to immunoblot analysis. Of 24 subjects with T2DM, 20 were randomized to receive placebo or PIO (15 mg daily) for 6 months. After 6 months of treatment, SKLM biopsy was repeated.
Results: By performing mitochondrial proteomic analysis on study group 1, we identified ten mitochondrial proteins involved in oxidative metabolism which were differentially expressed between T2DM and NGT groups, with a downregulation of ATP synthase alpha chain (ATPA), electron transfer flavoprotein alpha-subunit (ETFA), cytochrome c oxidase subunit VIb isoform 1 (CX6B1), pyruvate dehydrogenase protein X component (ODPX), dihydrolipoamide dehydrogenase (DLDH), dihydrolipoamide-S-succinyltransferase (DLST), and mitofilin, and an up-regulation of hydroxyacyl-CoA-dehydrogenase (HCDH), 3,2-trans-enoyl-CoA-isomerase (D3D2) and delta3,5-delta2,4-dienoyl-CoA-isomerase (ECH1) in T2DM as compared to NGT subjects. To extend these findings we performed immunoblot analysis on SKLM lysates obtained from a separate, and more numerous, study group (group 2). In comparison to NGT subjects, T2DM exhibited lower SKLM protein levels of ATPA (−30%, $P = 0.006$), ETFA (−50%, $P = 0.02$), CX6B1 (−30%, $P = 0.03$), key factors for mitochondrial ATP biosynthesis, and mitofilin (−30%, $P = 0.01$), a structural protein essential for normal mitochondrial function. Individuals with T2DM displayed reduced expression of the enzymes involved in the Krebs cycle DLST and ODPX (−20%, $P \leq 0.05$) and increased expression of HCDH and ECH1, enzymes implicated in the fatty acid catabolism (+30%, $P \leq 0.05$) in comparison to NGT subjects. Notably, subjects with T2DM treated with PIO for 6 months exhibited increased expression of ATPA (+33%, $P \leq 0.05$), ETFA (+60%, $P \leq 0.05$), CX6B1 (+33%, $P = 0.01$), mitofilin (+20%, $P \leq 0.05$) and DLST (+10%, $P = 0.08$) in comparison to baseline, whereas no change was observed in placebo treated T2DM patients. Furthermore, HCDH and ECH1 protein levels, which were upregulated in SKLM of subjects with T2DM in comparison to NGT individuals, were reduced by −10% and −15% respectively ($P \leq 0.05$ for both) after PIO treatment.
Conclusion: Treatment with PIO exerts positive effects on the abnormal expression of several mitochondrial proteins involved in oxidative metabolism in the SKLM of subjects with T2DM.
Clinical Trial Registration Number: NCT01223196
Supported by: The study was by TAKEDA, and NIH grant DK24092-38
Disclosure: **T.V. Fiorentino:** None.

224

Comparison of ipragliflozin and metformin for bone density and muscle in type 2 diabetes: a prospective, blinded-endpoint, randomised controlled study

K. Ishikawa[1,2], M. Koshizaka[1,2], R. Ishibashi[2], K. Sakamoto[2], A. Kobayashi[1,2], H. Yokoh[1,2], M. Shoji[1,2], S. Ide[1,2], K. Ide[1,2], Y. Baba[1,2], T. Ishikawa[1,2], Y. Maezawa[1,2], T. Horikoshi[3], K. Yokote[1,2];
[1]Division of Metabolism and Endocrinology, Chiba university hospital, CHIBA, [2]Clinical cell biology and medicine, Chiba university graduate school of medicine, Chiba, [3]Diagnostic Radiology and Radiation Oncology, Chiba university graduate school of medicine, CHIBA, Japan.

Background and aims: Sodium glucose transporter 2 (SGLT2) inhibitors are glucose-lowering agents that cause a reduction in body weight and prevent the recurrence of cardiovascular disease. We have reported that ipragliflozin (SGLT2 inhibitor) reduces visceral fat compared with metformin in patients with type 2 diabetes who received dipeptidyl peptidase-4 (DPP-4) inhibitor as a first-line medication for diabetes. The effects of SGLT2 inhibitors on bone and skeletal muscle are not well understood. In this study, we investigated the influence of ipragliflozin on bone mineral content and skeletal muscle in Asian patients with type 2 diabetes that cannot be sufficiently controlled with DPP-4 inhibitors.
Materials and methods: This was a randomized, multicenter, 24-week, open-label, and blinded-endpoint study. Overall, 103 patients with type 2 diabetes treated with sitagliptin 50 mg/day were assigned to receive either ipragliflozin 50 mg/day ($n = 51$) or metformin 1000–1500 mg/day ($n = 52$). The primary outcome was the change in rate of visceral fat area (obtained by computed tomography at the fourth lumbar level and measured by two radiologists blinded to the clinical information) in the two groups at 24 weeks. The secondary outcome was the change in the rate of bone-specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase-5b (TRACP-5b), grip strength, bone mineral density, and muscle measurements in the same slice of the visceral fat image.
Results: The rate of reduction in visceral fat area in the ipragliflozin-treated group was greater than that in the metformin-treated group after 24 weeks (−12.06% vs. −3.65%, group difference [95% CI] −8.40% [−16.4 to −3.38], $P = 0.040$). BAP and TRACP-5b in the ipragliflozin-treated group were significantly higher than those in the metformin-treated group at 24 weeks (BAP; −0.71% vs. −10.4%, group difference [95% CI] 14.76% [6.31 to 23.05], $P = 0.0004$: TRACP-5b; 11.94% vs.

−10.3%, group difference [95% CI] 25.47% [17.46 to 34.19], $P < 0.0001$). There were no differences in bone mineral density, grip strength, and muscle area between ipragliflozin and metformin. Bone fracture was not observed in either group.

Conclusion: The bone mineral density was the same after ipragliflozin and metformin treatment. However, the osteogenic marker BAP and the osteolytic marker TRACP-5b were higher in the ipragliflozin-treated group than in the metformin-treated group; therefore, the bone metabolism in the ipragliflozin-treated group was of the "high turnover" type. No effect of ipragliflozin on skeletal muscle was identified.

Table

		Ipragliflozin		Metformin		Difference between groups		
	Weeks	Rate of change (%)	95% CI	Rate of change (%)	95% CI	Rate of change (%)	95% CI	p value
Visceral fat area	24	-12.06	-20.55, -3.56	-3.65	-11.78, 4.48	-8.4	-16.43, -3.38	0.04
BAP *	12	-4.47	-7.87, 4.88	-10.48	-13.55, -1.5	5.81	-0.42, 13.56	0.064
	24	-0.71	-5.88, 10.24	-10.4	-18.92, -5	14.76	6.31, 23.05	0.0004
TRACP-5b *	12	12.21	1.18, 20.22	-9.3	-15.49, -6.22	21.4	13.5, 29.28	<0.0001
	24	11.94	7.56, 20.08	-10.3	-21.68, -7.17	25.47	17.46, 34.19	<0.0001
Bone density in CT	24	-1.58	-5.38, 2.21	-3.09	-5.54, -0.64	1.51	-2.95, 5.96	0.504
Muscle area in CT	24	-2.59	-3.75, -1.44	-1.71	-3.01, -0.42	-0.88	-2.59, 0.83	0.31
Grip strength	12	1.04	-1.31, 3.39	3.53	-0.51, 7.58	-2.49	-7.1, 2.12	0.285
	24	1.86	-0.35, 4.06	2.81	-1.06, 6.67	-0.95	-5.36, 3.47	0.671

Rate of change is shown as means unless otherwise indicated.
*: The data were not normally distributed and had outliers; nonparametric analysis (Wilcoxon rank sum test and group difference confidence interval by Hodges-Lehmann estimator) was performed. Rate of change are shown as median.
CI, Confidential interval; BAP, bone alkaline phosphatase; CT, computed tomography.

Clinical Trial Registration Number: UMIN 000015170
Supported by: Astellas Pharma Inc.
Disclosure: **K. Ishikawa:** None.

OP 39 Understanding diabetes through registry data

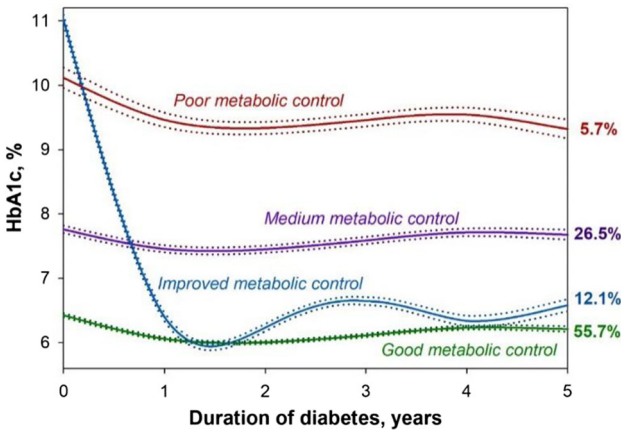
225

Distinct trajectory patterns of HbA_{1c} in adults with type 2 diabetes: a longitudinal group-based modelling approach based on the DPV registry

K. Laubner[1], A. Schwandt[2,3], W. Rathmann[4,3], O. Kuss[4,3], F. Best[5], S. Ebner[6], M. Paulmann[7], R.W. Holl[2,3], DPV initiative and the DZD;
[1]Department of Medicine II, Division of Endocrinology and Diabetology, Freiburg, Germany, [2]Institute of Epidemiology and Medical Biometry (ZIBMT), Ulm, Germany, [3]German Centre for Diabetes Research (DZD), Munich-Neuherberg, Germany, [4]Leibniz Institute for Diabetes Research at Heinrich-Heine-University Düsseldorf, Institute for Biometrics and Epidemiology, Düsseldorf, Germany, [5]Diabetes practice Dr. Best, Essen, Germany, [6]Kepler University Hospital, Medical Campus III, Linz, Austria, [7]Specialist diabetes practice Hannover, Hannover, Germany.

Background and aims: Metabolic control is essential in the management of type 2 diabetes (T2D). This study aimed to identify groups of heterogeneous HbA1c trajectories over time in a large adult T2D cohort.
Materials and methods: A total of 6,470 adults with T2D (≥40 years, 55% males) were selected from the German/Austrian multicenter diabetes prospective registry DPV. Subjects were examined during the first five years after T2D onset (with at least three aggregated HbA1c values per year diabetes duration). Latent class growth modeling as trajectory approach (SAS PROC TRAJ) was applied to determine distinct groups following similar HbA1c patterns over time. The number of groups was determined by BIC and cluster size.
Results: Four longitudinal trajectories of HbA1c were found (Figure). The largest group maintained *good metabolic control* over time, whereas participants within the second group were characterized as *medium metabolic control*. Another group showed initially severe hyperglycemia, but reached good metabolic control after one year diabetes duration (*improving HbA1c*). High HbA1c over time was observed in the fourth group (*poor metabolic control*). Significant differences were observed for age at T2D onset, gender, glucose-lowering therapy, and BMI among all HbA1c groups (all $p < 0.01$). As compared to the *good metabolic control* group, the *improving HbA1c* group comprised more males, younger age at onset, more intensive glucose-lowering treatment and less often nonpharmacological therapy, whereas in the *medium* or *poor metabolic control* group, higher BMI, lower frequency of treatment with glucose-lowering drugs only and higher frequency of nonpharmacological treatment was observed.
Conclusion: Among this large T2D cohort of adults from Germany/Austria, four trajectories with heterogeneous HbA1c patterns over time were found. Glucose-lowering treatment, demographics, and BMI were related to distinct HbA1c trajectories.

Figure Trajectories of HbA1c among adults with T2D.



Supported by: Competence Network for Diabetes Mellitus, EFSD
Disclosure: **K. Laubner:** None.

226

Costs of prevalent and incident cardiovascular disease in patients with type 2 diabetes in Scotland using routinely collected data

P. McMeekin[1], C. Geue[1], O. Wu[1], E. Mocevic[2], C. Stentoft Hoxer[3], A. Ochs[4], S. McGurnaghan[4], H. Colhoun[4], Scottish Diabetes Research Network Epidemiology Subgroup;
[1]HEHTA, University of Glasgow, Glasgow, UK, [2]Novo Nordisk A/S, Søborg, Denmark, [3]Novo Nordisk, West Sussex, UK, [4]MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK.

Background and aims: Patients with type 2 diabetes mellitus (T2DM) are disproportionately affected by cardiovascular disease (CVD). Morbidity associated with CVD places a burden on health services and the wider economy. We estimated annual average cost per patient to the healthcare system and wider economy of treating T2DM patients with established CVD, patients at high risk of CVD, but without established CVD and patients without CVD (Analysis A). We also estimated for patients with established CVD total average costs in the first, second and third year after CVD was diagnosed compared to patients with no CVD (Analysis B).
Materials and methods: Data about all T2DM patients in Scotland were obtained from the Scottish Care Information Diabetes Collaboration (SCI DC) registry: i) for those alive at 1 July 2015 (Analysis A) and ii) those alive between 1 January 2010 and 30 June 2015 (Analysis B). For analysis B, a 10year look-back was used from patient entry to exclude patients with prior CVD. Data linkage was used to retrieve information on secondary care admissions and day cases (Scottish Morbidity Records (SMR01)), prescribed medications (Prescribing Information System) and deaths (National Records for Scotland). Hospital costs were estimated from length of stay using per diem costs by Health Board and Speciality. Prescription costs were estimated using British National Formulary codes. Care home utilisation was determined from SMR01 recorded discharge destination and costs from the Scottish Care Home Census. Indirect costs where estimated for those of non-pensionable age using time in hospital and residential care and average wages. Primary care use and cost were estimated accounting for comorbidities, age and sex.
Results: Analysis A included 73,037 T2DM patients with established CVD, 141,428 at high risk of CVD and 30,287 with no CVD. The annual cost per T2DM patient with established CVD was £6,890 (95% CI; £1,567, £29,705), per T2DM patient with no CVD £2,456 (95% CI; £704, £7,999) and per patient at high risk of CVD £3,346 (95% CI; £1,000, £16,077). Hospital admissions made up the majority of costs in each patient category. Those with established peripheral arterial diseases and hypertensive diseases incurred the largest costs, those with ischaemic heart disease and needing revascularisation the smallest. Costs for each CVD category increased with age. Analysis B included 245,428 T2DM patients, 35,322 of whom had established CVD. By year three the total cost of treating a person with incident CVD was £31,910 (95% CI: £13,449, £76,066) compared to a patient without CVD (£6,863 (95% CI: £2,960, £21,561)). Over two years the cost was £26,024 (95% CI £10,881, £62,983) and £4,874 (£2,130, £15,272) respectively and during the first year £18,927 (95% CI: £7,725, £47,603) and £2,422 (95% CI £1,025, £7,983). Costs increased with age, and cerebrovascular events and hypertensive diseases were associated with the greatest cost.
Conclusion: CVD in T2DM patients places a significant financial burden on health and social care services. The highest costs are incurred during the incident CVD event. Cost differentials between patient groups continue to show beyond the incident event.
Supported by: This study is funded by Novo Nordisk A/S
Disclosure: **P. McMeekin:** Employment/Consultancy; Study is funded by Novo Nordisk A/S.

227

2016/17 national diabetes audit: certain newer to market agents impact positively on glycaemic control at a population level

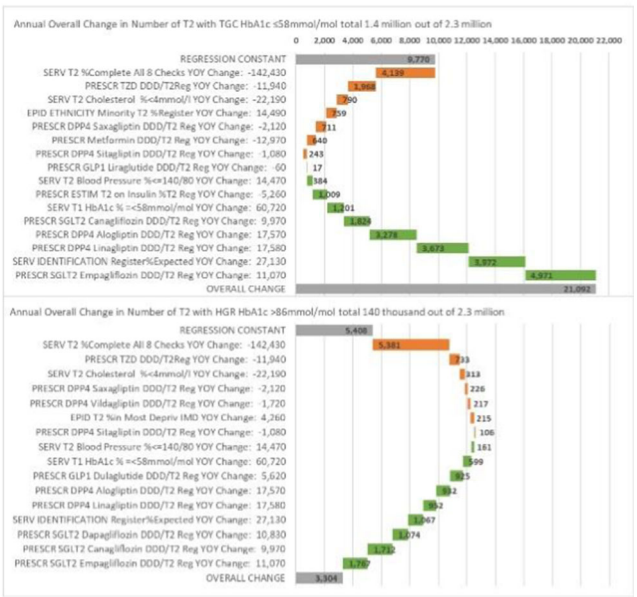
A. Heald[1], A. Fryer[2], S.G. Anderson[1], S. Farman[3], M. Livingston[1], M. Davies[4], G. Moreno[5], R. Gadsby[6], M. Stedman[4];
[1]The School of Medicine and Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK, [2]Institute for Applied Clinical Sciences, Keele University, Keele, Keele, UK, [3]Mersey Deanery Psychiatry Rotation, Liverpool, UK, [4]RES Consortium, Andover, UK, [5]High Specialty Regional Hospital of Ixtapaluca, Mexico City, Mexico, [6]University of Warwick, Warwick, UK.

Background and aims: Spend on Type 2 diabetes mellitus (T2DM) medication is increasing, proportion of people with T2DM achieving target glycaemia outcomes is static or declining. Aim was to determine using public published General Practitioner Practice (GPP) level data, how differences in T2DM prescribing patterns including newer agents relate to achieving glycaemic target levels.
Materials and methods: Multiple linear regression modelling was applied to data from GPP in 2015/16 and latest 2016/17 National Diabetes Audit (NDA) with >100 patients on T2DM register. GPP data included epidemiology, level of diabetes service, and prescribing using defined daily dose (DDD) both by class and BNF chemical including Glucagon-like peptide-1 (GLP1), and Dipeptidyl peptidase-4 inhibitors (DPP4i) and SGLT2i, was linked to proportion achieving target glycaemic control (TGC; HbA1c ≤7.5%, 58 mmol/mol) and at high glycaemic risk (HGR; HbA1c >10.0%, 86 mmol/mol).
Results: T2DM register (5,488 GP practices) grew 6.2% to 2.26 million. Most new patients came in <64 age which grew from 44.4% to 46.7%. %Patients <65 was found to have negative impact on both TGC & HGR. Level of service as % completion of 8 checks fell by 12% from 54.1% to 47.8%. T2DM medication/patient fell by 1.7%, 15% less glitazones (TZD) (0.03DDD/T2 register), 6% less sulphonylurea (SU) (0.61), 2% less insulin and 1% less metformin (0.51). 3% growth in GLP1 (0.03 DDD/T2 register), 12% DPP4i (0.13) and 65% in SGLT2i (0.04). Specifically Dulaglutide grew by 880%, Empagliflozin 440%, Canagliflozin 100%, Alogliptin 175%, and Linagliptin 30%. Vildagliptin declined by 17%, Exenatide 15% and Saxagliptin 10%. Liraglutide and Sitagliptin prescriptions were relatively static. Wide variations were observed between GPP. % TGC increased by 1.4% of total (20,507) to 66.8% and HGR increased by 2.1% of total (3125) to 6.7%. Linking annual change by factor to change in TGC and HGR numbers (Figure) showed a fall in diabetes check completion by 142,430 patients & TZD prescribing by 11,940 patients with associated reduced TGC by 6,107 & increased HGR by 6,114 patients. Provision of various DPP4i

and SGLTi to at least 55,500 patients brought 12,200 into TGC and 6400 out of HGR.

Conclusion: GPP type and service are significant to outcomes. SU use is falling but continues to deliver poor outcomes. Certain newer agents are linked to improved outcome (Figure). The SGLTIs empagliflozin and canagliflozin show benefits in TGC and all SGLTis reduce numbers at HGR. GLP1 seem to have peaked in some practices. There is considerable variation in impact of different DPP4i. TZD still seem to offer some benefits.

Figure 1: Applying Regressions Coefficient from Longitudinal Analysis to the numbers of patients being treated to show the impact of factors with p score <0.01 on overall change in numbers of achieving target Glycaemic control (TGC) or at Higher Glycaemic Risk (HGR)(numbers in bars with orange denoting deterioration and green denoting improvement).



Disclosure: **A. Heald:** None.

228

Are neighbourhood factors associated with diabetes progression in prediabetes patients? Using census data to predict patient-centered health outcomes

J.A. Schmittdiel[1], W. Dyer[1], C. Marshall[1], R. Bivins[2];
[1]Kaiser Permanente Northern. California, Oakland, USA, [2]University of Warwick, Coventry, UK.

Background and aims: Existing prediction models that examine factors associated with developing diabetes are commonly focused on individual level patient factors such as weight, blood sugar levels, and race/ethnicity. Data regarding patient social context at the area level are rarely assessed as predictors in these models, despite the known relationship between a patient's neighborhood environment (*e.g.* the "zip code effect") and a wide range of health outcomes. The purpose of this study is to determine whether United States Census variables on income, education, and receipt of food assistance at the area level are associated with developing diabetes after adjustment for traditional demographic and clinical factors.

Materials and methods: Kaiser Permanente Northern California (KPNC) is an integrated delivery system that provides comprehensive health care to more than 4 million patients in the United States. This retrospective cohort study included adult patients aged ≥18 from KPNC the with laboratory-defined prediabetes (fasting plasma glucose [FPG] 100–125 mg/dL and/or glycated hemoglobin [A1c] 5.7–6.4%) between 1/1/2006 and 12/31/2010. To create an incident prediabetes cohort, we excluded all patients who had tested in this range in the 2 years prior, as well as those with a pre-existing diagnosis of diabetes or prediabetes during this period. The primary outcome was progression to diabetes within 36 months of prediabetes identification. To assess predictors of progression to diabetes, we employed logistic regression using age, gender, race/ethnicity, baseline blood sugar levels, baseline body mass index (BMI), Census block group level education and income, and Census track level percentage of households receiving benefits through the governmental food assistance 'Supplemental Nutrition Assistance Program' (SNAP).

Results: The cohort included 157,752 patients, with a mean age of 57.2 (SD 13.6), 50% female, and 59% non-Hispanic White. In the multivariate regression model greater age, having overweight or obesity, and having a blood sugar value of FPG >110 or A1c >6.0 at baseline were all significantly associated with developing diabetes within 36 months of prediabetes identification. After adjusting for these demographic and clinical variables, patients were also more likely to progress to diabetes if they lived in an area where <50% of the adult population aged ≥25 had obtained a bachelor's degree or higher (OR = 1.2, 95% CI = 1.1, 1.3), or if they lived in an area where SNAP benefits were received by 10% or more of households (OR = 1.2, 95% CI = 1.1, 1.4). Higher median household income at the area level had a protective effect against diabetes progression (OR = 0.98, 95% CI = 0.97, 0.99).

Conclusion: Census information on neighborhood and tract-level education, income, and receipt of food assistance are significant predictors of developing diabetes within a prediabetes population, even after adjusting for traditional individual demographic and clinical factors. Clinical interventions should take these factors into account, and health care systems should consider addressing neighborhood-level resources and social needs as a path to improving community and population-level health outcomes.

Supported by: P30DK092924

Disclosure: **J.A. Schmittdiel:** None.

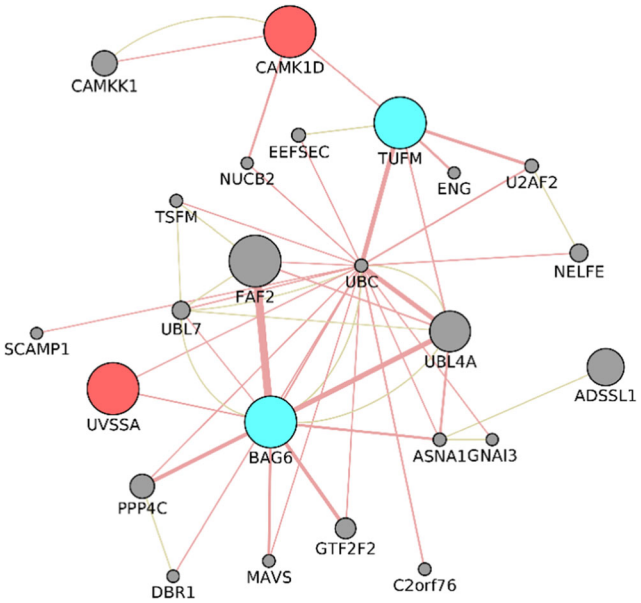
OP 40 Innovation in genetics

229

A common regulatory network for type 1 and type 2 diabetes susceptibility genes in human pancreatic islets

S. Kaur[1], A.H. Mirza[2], F. Pociot[1], J. Størling[1];
[1]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [2]Weill Cornell Medicine, New York, USA.

Background and aims: Genetic risk factors contribute significantly to the etiology of type 1 diabetes (T1D) and type 2 diabetes (T2D) and genome-wide association studies (GWAS) have identified >50 loci for both diseases. Despite the clinical and phenotypical similarities between the two forms of diabetes, there is basically no overlap at the genetic level between the two diseases. Since pancreatic β-cell dysfunction is the principal cause of both forms of diabetes, we hypothesized that a proportion of the risk genes for T1D and T2D interact in common networks and pathways in islets to affect β-cell fragility. In this study, we aimed to identify common functional interaction networks between T1D and T2D risk loci-associated genes by integrating GWAS, human islet gene expression and islet-specific expression quantitative trait locus (eQTL) data.
Materials and methods: The T1D and T2D GWAS data was retrieved from T1Dbase v.4.16 and DIAGRAMv3. All genes located ±100 kb from genome-wide associated significant SNPs were extracted. Publically available human pancreatic islet RNAseq data consisting of 118 islet preparations was used to identify islet expressed genes and islet *cis*-exon eQTLs. ToppGene suite and CytoScape were used to create interaction networks. The interactions between T1D and T2D loci genes were further extended to include neighboring genes (max $n = 20$) based on physical interactions and shared protein domains in GeneMania. Functional annotation based on gene ontology terms and pathways was performed using ClueGO.
Results: In total, 27,772 protein-coding and non-coding transcripts were found expressed in 118 human islet samples, of which 2,339 genes had at least one significant islet exon-eQTL (p value <0.05). Out of the 2,339 islet eQTL genes, 66 and 27 genes were located in T1D and T2D GWAS loci, respectively. These two groups (66 T1D and 27 T2D genes) were further subjected to network analysis. The T1D/T2D islet gene network identified interactions between TUFM ~ CAMK1D and BAG6 ~ UVSSA. The functional annotation analysis of the extended T1D/T2D interaction network identified 3 clusters of significantly-enriched gene ontology terms related to regulation of tumor necrosis factor-mediated signaling pathway, regulation of cell death and cell cycle-related processes.
Conclusion: This study identified a shared islet network consisting of T1D and T2D candidate genes. This network highlighted plausible roles of novel genes associated with cell cycle processes and cell-death regulation that might contribute to pancreatic β-cell impairment in both T1D and T2D.



Disclosure: **S. Kaur:** None.

230

Type 2 diabetes-induced beta cell gene regulatory networks identified using single-cell RNA-sequencing of human islets

N. Wierup[1], J.A. Martínez-López[2], A. Lindqvist[1], R.G. Fred[1], A.B. Muñoz-Manchado[2], S. Chriett[1], L. Shcherbina[1], J. Hjerling-Leffler[2];
[1]Lund University, Malmo, [2]Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.

Background and aims: Deranged islet function is a culprit in type 2 diabetes (T2D). Currently, major focus is on assessing differential expression of genes in T2D vs. nonT2D whole-islet preparations. This strategy is limited by the fact that the islets are composed of at least five different cell types. Therefore, single-cell information on disease vs. control cells is warranted. Single-cell RNA-sequencing has been successfully performed in human islets, but the available data is contradictory, and rely on simple differential expression analysis. We aimed at understanding, and to functionally test, gene regulatory changes in relevant biological processes in beta cells of T2D patients.
Materials and methods: Handpicked islets (100/donor) from cadaver donors were dissociated into single cell suspensions using Accutase. Cells were isolated by unbiased FACS and Smart-seq2 single-cell transcriptomics was used to sequence cells from 6 nonT2D- and 4 T2D donors (obtaining transcriptomes of 3075 cells). Cell identity was determined using the two way unsupervised clustering algorithm BackSPIN and t-SNE technique. We identified affected biological pathways using differential gene correlation network analysis. Insulin secretion and gene expression after siRNA silencing was assessed in INS-1 (832/13) and EndoC-BH1 cells.
Results: BackSPIN identified 12 distinct pancreatic cell populations, including alpha-, beta-, delta-, PP- and ghrelin cells. Clustering was verified manually by comprehensive analysis of differential expression of established markers for each cell type. Numerous genes were differentially regulated in every cell population in T2D donors. To understand the biological processes that fail in T2D beta cells, we assessed networks of genes. Using novel bioinformatic tools we identified 12 gene regulatory networks (GRNs), representing established (e.g. mitochondria function and ER-stress), as well as hitherto unknown biological processes that were affected in T2D beta cells. The GRNs included genes with

established roles in T2D, e.g. NEUROD1, PDX1, NKX6.1, and GLP1R, and our analysis put them in a new disease context. We also identified node genes, i.e. genes potentially regulating many other genes, in the GRNs. Most of the node genes have not been described in beta cell biology and their significance was validated in beta cell lines. 12 out of 14 of the uncovered T2D-genes were found to affect insulin expression and/or glucose- and cAMP-stimulated, but not basal insulin secretion. We also tested selected GRNs functionally by siRNA targeting of node genes, followed by bulk RNAseq of INS-1 (832/13) cells. Immunohistochemistry in pancreatic sections from the same donors confirmed protein expression of the node genes in human beta cells.

Conclusion: We have identified 12 distinct biological processes that are affected in T2D beta cells and identified and functionally tested node genes, many of them with previously unknown function, that are key regulators of each process. This is a major leap forward for the understanding of the deranged characteristics of beta cells in T2D.

Supported by: The Novo Nordisk Foundation, EFSD-AZN. Diabetes Wellness Foundation Sweden,

Disclosure: **N. Wierup:** None.

231

Dietary fat quality and genetic risk of type 2 diabetes

J. Merino[1], M. Guasch-Ferre[2], C. Ellervik[3], H. Dashti[1], C.E. Smith[4], T.O. Kilpeläinen[5], D.I. Chasman[6], J.C. Florez[1];
[1]Center for Genomic Medicine, Massachusetts General Hospital, Boston, USA, [2]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, USA, [3]Department of Laboratory Medicine, Boston Children's Hospital, Boston, USA, [4]Nutrition and Genomics Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, USA, [5]Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, Denmark, [6]Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA.

Background and aims: Type 2 diabetes (T2D) is a complex disease driven by genetic and lifestyle factors. The extent to which T2D genetic burden modifies the association between dietary fat quality and T2D incidence is unknown.

Materials and methods: We used Cox proportional-hazards models to calculate adjusted hazard ratios (HRs) for T2D among 103,206 participants of European descent from 15 prospective cohort studies. T2D genetic risk profile was characterized by a 68-variant genetic risk score (GRS) weighted by published effect sizes. Diet was recorded using validated cohort-specific dietary assessment tools.

Results: During a median follow-up of 12 years, 20,451 participants developed T2D. The relative risk of T2D per increment of 10 risk alleles in the GRS was 1.68 (95% confidence interval [CI] 1.62–1.74). Increasing polyunsaturated or total ω-3 fat intake in place of refined carbohydrates was associated with a lower risk of T2D (HR per 5% of energy 0.92, 95% CI 0.85–1.00; and HR per increment of 1 g/d 0.95, 95% CI, 0.92–0.99, respectively), while increasing monounsaturated fat intake in place of refined carbohydrates was associated with a higher risk of T2D (HR per 5% of energy 1.08, 95% CI 1.02–1.15). We did not observe evidence of significant interactions between dietary fat subtypes and GRS on the risk of T2D.

Conclusion: In the present long-term prospective study including 103,206 participants, our results support that genetic risk profile and monounsaturated fat intake were each associated with a higher risk of T2D, whereas polyunsaturated fat intake was associated with a lower risk of T2D. Findings from this study suggest that dietary fat recommendations do not need to be tailored to individual T2D genetic risk profile for the primary prevention of T2D, and that dietary fat subtypes associate with the risk of T2D across the spectrum of T2D genetic risk.

Supported by: H2020-MSCA-IF- 2015-703787

Disclosure: **J. Merino:** None.

232

Genetic determinants of glucose response patterns during the OGTT: findings from the ADDITION-PRO cohort

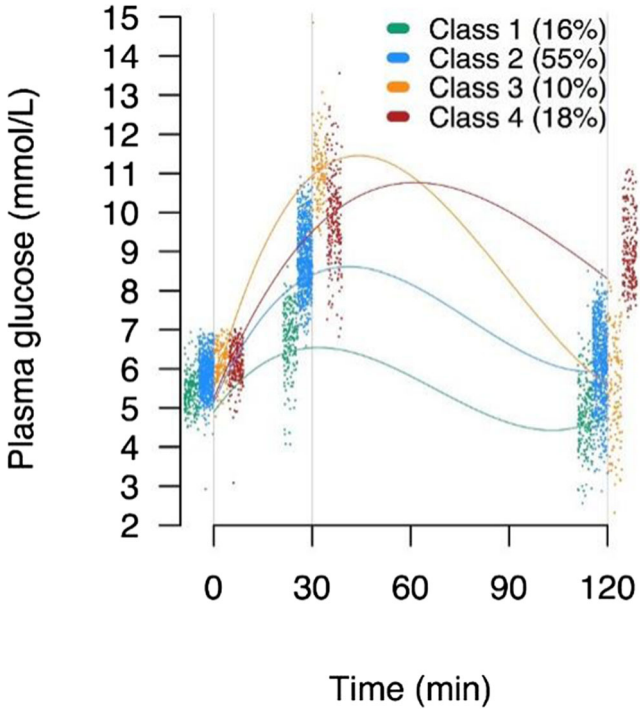
A. Hulman[1,2], A. Jonsson[3], K. Færch[4], D. Vistisen[4], T. Hansen[3], N. Grarup[3], M.E. Jørgensen[4], D.R. Witte[1,2];
[1]Aarhus University, Aarhus, [2]Danish Diabetes Academy, Odense, [3]Novo Nordisk Center for Basic Metabolic Research, Copenhagen, [4]Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: We have previously identified heterogeneous glucose response patterns in the RISC cohort based on glucose measurements at five time points during the oral glucose tolerance test (OGTT) and showed that the four identified groups have different metabolic risk profiles. We aim to understand to which degree group membership is determined by unmodifiable (genetic) factors.

Materials and methods: We analysed data from 1222 participants (628 men and 594 women) without diabetes in the ADDITION-PRO cohort who underwent an OGTT with glucose measurements at three time points (0, 30, 120 mins). Individuals were categorised into four groups using a latent class model developed in the RISC cohort based on their class membership probabilities (Figure: *Glucose response patterns derived in the RISC cohort [lines] and plasma glucose measurements at 0, 30 and 120 minutes in the ADDITION-PRO cohort [scatterplot]*). Genetic risk scores for insulin resistance (GRS-IR) and impaired early insulin secretion (GRS-IEIS) were calculated and compared between the four groups with different glucose response patterns using linear and multinomial regression models with adjustment for age, sex and BMI.

Results: More than half of the cohort belonged to class 2 (55%), while 16%, 10% and 18% were members in classes 1, 3 and 4, respectively. Mean GRS-IR values were similar between classes 1, 2 and 3. Only class 4 showed an indication of GRS-IR (class 4 vs. class 2: +0.26 risk allele, 95% CI: −0.06 to 0.58). Mean GRS-IEIS values were lowest in class 1 (class 1 vs. class 2: −0.66 risk allele, 95% CI: −1.12 to −0.20), highest in class 3 (class 3 vs. class 2: +0.64 risk allele, 95% CI: 0.09 to 1.19) and similar in classes 2 and 4. Individuals with a higher GRS-IEIS were more likely to belong to class 3 (OR: 1.08, 95%CI: 1.01 to 1.16 per risk allele) and less likely to be in class 1 (OR: 0.93, 95%CI: 0.88 to 0.98 per risk allele), than to class 2.

Conclusion: The glucose pattern exhibiting the highest peak followed by a rapid fall in the second hour of the OGTT is determined by impaired genetically determined early insulin secretion rather than insulin resistance. Contrarily, higher glucose concentrations at 2 hours seem to be determined to a higher degree by insulin resistance. Our findings show that the four different classes are partially determined by differences in the balance between insulin resistance and secretion, and that these differences play a role throughout life. The interaction between these genetic determinants and environmental exposures needs to be explored further.



Supported by: Danish Diabetes Academy
Disclosure: **A. Hulman:** None.

OP 41 Novel mechanisms of inflammation in obesity

233

SUCNR1 signalling controls macrophage alternative activation and regulates immune metabolic responses in obesity

S. Fernandez Veledo[1,2], N. Keiran[1,2], E. Calvo[1,2], V. Ceperuelo-Mallafré[1,2], M.I. Hernández-Alvarez[1,2], M. Ejarque[1,2], C. Nuñez-Roa[1,2], D. Horrillo[3], E. Maymó-Masip[1,2], M.M. Rodríquez-Peña[1,2], A. Zorzano[4,2], G. Medina-Gómez[3], C. Serena[1,2], A. Castrillo[5], J. Vendrell[1,2];
[1]Institud d´Investigacio Sanitaria Pere Virgili, Tarragona, [2]CIBERDEM, Madrid, [3]Universidad Rey Juan Carlos, Madrid, [4]IRB Barcelona, Barcelona, [5]IIB Alberto Sols, Madrid, Spain.

Background and aims: Macrophages have a central role in metabolic homeostasis and some metabolites are now known as critical regulators of their functional plasticity. Whereas accumulation of succinate is a hallmark of pro-inflammatory (M1) macrophages, succinate can be released and act through its receptor SUCNR1, which is predominantly expressed in alternative (M2) macrophages. The precise role of this succinate/SUCNR1 axis in macrophages, however, remains unclear.
Materials and methods: Generation and metabolic phenotype of myeloid cell-specific *Sucnr1* knockout mice (LysM-*Sucnr1*$^{-/-}$) fed with a normal chow diet (NCD; 3.1% fat) or challenged to a high fat diet (HFD; 45% fat). Long-term cold exposure and LPS-induced endotoxemia studies. Gene/protein expression and histological analysis. RNAseq and intracellular signalling studies in bone marrow-derived macrophages (BMDMs). Human adipose tissue biopsies obtained from donors undergoing non-acute surgical interventions: lean $n = 15$ (BMI 22.87 ± 1.51); obese $n = 36$ (BMI 31.44 ± 4.55).
Results: Myeloid-specific SUCNR1 deficiency promotes a pro-inflammatory phenotype, disrupting glucose homeostasis in mice under normal chow diet, and exacerbating the metabolic consequences of diet-induced obesity. Intriguingly, succinate signaling via SUCNR1 promotes alternative macrophage phenotype and boosts the IL-4 response through activation of CREB-KLF4 pathway. Accordingly, LysM-*Sucnr1*$^{-/-}$ mice show reduced adipose tissue browning in response to cold-exposure and higher susceptibility to endotoxemia, both M2 immune responses. We also show that succinate/SUCNR1 axis is disturbed in obese humans. SUCNR1 expression in adipose tissue-resident macrophages is significantly lower in obese than in lean individuals, which correlates with a failure of succinate to suppress the inflammatory phenotype of obese adipose tissue.
Conclusion: Our findings establish the succinate/SUCNR1 axis as a novel signal-regulatory mechanism of alternative macrophage activation and assign an unpredicted homeostatic role of succinate in limiting inflammation.
Supported by: SAF2015-65019-R, CPII16/00008, PI14/00228, PI17/01503
Disclosure: **S. Fernandez Veledo:** Grants; Spanish Ministry of Economy and Competitiveness (PI17/01503, SAF2015-65019-R, SAF2014-56819-R, BFU2016-78951-R, PI15/00143, PI15/01562, BFU2015-70454-REDT) co-financed by ERDF.

234

ANKRD26 gene expression depends on the methylation of its promoter and associates with cardio-metabolic risk and altered levels of inflammatory mediators in human obesity

A. Desiderio[1], M. Campitelli[1], G. Cacace[1], A. Leone[1], I. Prevenzano[1], D. Conza[1], F.C. Pignalosa[1], M. Milone[2], C. Miele[1], F. Beguinot[1], G.A. Raciti[1];
[1]URT-GDD, National Council of Research, Department of Translational Medical Sciences, "Federico II" University of Naples, Naples, [2]Department of Public Health, "Federico II" University of Naples, Naples, Italy.

Background and aims: Obesity is now considered to be a major threat to human health and well-being worldwide. This makes the need of achieving a better understanding of its pathogenesis a priority. Furthermore, recent evidence points out that epigenetic modifications have an extraordinary impact on the natural history of this disorder and may explain predisposition for obesity in cases of familial aggregation or as effects of environmental exposure. Thus, understanding how epigenetic changes may contribute to human obesity is of particular importance. We have recognized the *Ankyrin repeat domain 26* (*Ankrd26*) gene as an interesting and proper target. Indeed, we have demonstrated that *Ankrd26* is involved in the development of both obesity and diabetes in mice and is a target sensitive to environment-induced epigenetic modifications in diet-induced obese mice. Here, we aimed at investigating whether impaired *ANKRD26* gene expression and DNA methylation occur in human obesity and may correlate to alteration of metabolic/inflammatory mediators in these subjects.
Materials and methods: Lean ($n = 14$) and obese ($n = 20$) subjects were recruited at the Federico II University of Naples. Gene expression and DNA methylation were evaluated in peripheral blood leucocytes (PBLs) by qRT-PCR and bisulfite sequencing, respectively. Serum mediators were assayed by ELISA. Promoter activity was evaluated by luciferase assay.
Results: *ANKRD26* mRNA levels were reduced by ≈ 25% in PBLs from obese individuals compared with lean subjects ($p < 0.01$). Furthermore, DNA methylation analysis of the *ANKRD26* promoter (−1000/+390 bp from TSS) revealed a 3-fold increased methylation in a restricted region of the *ANKRD26* promoter, containing the CpG dinucleotides, −689 bp, −659 bp and −651 bp, in obese compared with lean individuals ($p < 0.01$). *ANKRD26* gene expression inversely correlates to the percentage of DNA methylation of these 3 CpG sites ($p < 0.05$), as well. Additionally, a luciferase assay performed on the aforementioned region pointed out a cause effect relationship between the DNA methylation of this 3 CpG sites at the *ANKRD26* promoter and its gene expression as shown by a decrease of 30% of the luciferase activity in the methylated compared with the un-methylated *ANKRD26* specific promoter region ($p < 0.01$). Very interestingly, a significant negative correlation was found between *ANKRD26* mRNA levels and both BMI ($p < 0.01$) and TG/HDL-C ratio, a marker of insulin resistance and increased cardio-metabolic risk ($p < 0.001$), as well as with the pro-inflammatory cytokines, IL1β ($p < 0.05$) and TNFα ($p < 0.01$).
Conclusion: The *ANKRD26* gene is sensitive to epigenetic regulation even in humans. Indeed, a site-specific CpG hyper-methylation of its promoter and the down-regulation of its expression represent common abnormalities in obese patients and its gene expression associates to cardio-metabolic risk and altered levels of inflammatory mediators in these subjects.
Supported by: Italian Diabete Ricerca Foundation and Eli Lilly Italy
Disclosure: **A. Desiderio:** None.

235

Loss of fractalkine-CX3CR1 signalling promotes diet-induced adipose tissue inflammation and insulin resistance by controlling the M1/M2 status of macrophage

M. Nagashimada[1], Y. Ni[2], T. Ota[3];
[1]Kanazawa University, Ishikawa, Kanazawa City, Japan, [2]Zhejiang University of Technology, Hangzhou, Zhejiang, China, [3]Asahikawa Medical University, Asahikawa City, Japan.

Background and aims: We previously demonstrated that obesity-induced activates adipose tissue macrophage (ATM) via chemokine of CCR5 system as well as CCR2-MCP-1 system, and is pivotal for the development of insulin resistance. Recently, we found that CX3C chemokine, fractalkine (Cx3cl1) mRNA expression was persistently down-regulated in epididymal white adipose tissue (eWAT) of High fat diet (HFD)-induced obese (DIO) mice, as compared to lean controls. Recent studies reported that fractalkine exerts both negative and positive influences on disease pathogenesis and progression. We observed that *Cx3cr1*$^{-/-}$ (KO) mice exacerbate HFD-induced glucose intolerance, insulin resistance and hepatic steatosis. Here we show that fractalkine-CX3CR1 signaling plays a crucial role in the adipose tissue inflammatory response to HF feeding by regulating M1/M2 status of macrophage. Moreover, we demonstrated that fractalkine attenuated glucose intolerance and insulin signaling in DIO mice.
Materials and methods: The localization of fractalkine and CX3CR1 were examined in the eWAT of DIO mice by immunofluorescence staining. To determine the effect of fractalkine-CX3CR1 signaling on ATM subsets, we performed flow cytometry analysis to quantify M1/M2 ATMs in WT or KO mice fed the NC or HF diet for 16 weeks. In addition, to ascertain whether the therapeutic effect of fractalkine, we performed systemically expression of fractalkine in WT or KO mice fed a HF diet by hydrodynamic tail vein injection and investigated metabolic phenotypes.
Results: Immunofluorescence analysis of eWAT in HF-fed mice revealed that fractalkine and CX3CR1 were F4/80^{+} macrophages, but these are poorly expressed by pelilipin^{+} adipocytes. Interestingly, both CD11c^{+}CD206^{-} (M1-type) and CD11c^{-}CD206^{+} (M2-type) ATM were expressed fractalkine and CX3CR1. To quantify ATM subsets in WT or KO mice on the NC or HF feeding, we performed flow cytometry analysis. KO mice with NC feeding resulted in a 40.3% increase in M1-type macrophages and 17% decrease in M2 type macrophages compared with WT mice. Moreover, KO mice with HF feeding had 39% more M1 type macrophage, 49% fewer M2 type macrophage than WT mice on the same diet, which resulted in predominance of M1 over M2 ATM population. Furthermore, an increased fractalkine concentration in plasma of DIO mice by hydrodynamic tail vein injection, led to attenuate glucose intolerance and enhance insulin sensitivity in both liver and eWAT. While no significant difference was observed in β cell mass between fractalkine administration and control. In addition, an increased fractalkine of KO mice with a HF feeding showed no change in the attenuation of glucose intolerance.
Conclusion: Loss of fractalkine-CX3CR1 signaling exacerbates diet-induced inflammation and insulin resistance by a dynamic M1 shift of ATM. Additionally, a decline in plasma of fractalkine level by obesity is recovery, and, in a consequence, the improvement of insulin resistance. Thus, fractalkine-CX3CR1 signaling plays a critical role in diet-induced insulin resistance by regulating the M1/M2 status of ATM. Fractalkine-CX3CR1 signaling may have a potential clinical utility for type 2 diabetes.
Supported by: Grants-in-Aid for Scientific Research
Disclosure: **M. Nagashimada:** None.

236

Diverse hepatic microbial DNA fingerprints in healthy lean and obese steatotic humans

J.I. Bagger[1], M.P. Suppli[1], T. Nielsen[2], B. Lelouvier[3], A. Broha[3], M. Demant[1], M.J. Kønig[4], C. Strandberg[4], A. Lund[1], T. Vilsbøll[1], F.K. Knop[1,5];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte, Denmark, [2]Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Metabolic Research, Copenhagen, Denmark, [3]Vaiomer, Labege, France, [4]Radiology, Herlev Gentofte Hospital, Gentofte, Denmark, [5]Clinical Medicine, Faculty of Health and Medical Sciences, Copenhagen, Denmark.

Background and aims: Hepatic inflammation associated with non-alcoholic fatty liver disease is generally believed to be a result of steatosis-induced hypoxia, cell death, infiltration of macrophages and expression of chemokines and proinflammatory cytokines. The leaky gut hypothesis links translocating microbial products with hepatic inflammation. We evaluated the 16S rDNA bacterial profiles in liver samples from healthy lean and obese subjects.

Materials and methods: Fifteen lean and 15 obese subjects (body mass index of 18.5–25 and 30–40 kg/m^2, respectively), 25–80 years of age, with normal fasting plasma glucose and HbA1c <40 mmol/mol were recruited for ultrasound-guided transcutaneous liver biopsies. Assessment of steatosis was done by histology. After bacterial DNA extraction from liver biopsies, qPCR targeting the V3-V4 region of the bacterial 16S ribosomal gen was employed to quantify the amount of bacterial DNA. Furthermore, 16SrDNA sequencing of the V3_V4 region was performed using the Illumina Miseq platform. Reads were clustered into operational taxonomic units (OTUs) and taxonomic assignment was performed using the Silva 128 database. Indices of alfa and beta diversity were applied to assess community structure and differentially abundant taxa between the groups were analysed using the LEfSe method.
Results: Histology revealed steatosis in 14 of 15 obese subjects and only 2 of 15 lean subjects ($p > 0.001$). A robust signal from qPCR revealed significantly higher bacterial DNA in liver samples from obese subjects compared to lean (Figure 1). Observed OTUs as well as other indices of alfa diversity were increased in obese subjects compared to lean. No differences between groups were observed in beta diversity. The taxonomic comparison between groups showed increased abundance of *Proteobacteria* in the group of obese subjects.
Conclusion: We provide evidence for the presence of bacterial DNA in the human liver and show significantly higher amounts of bacterial 16S rDNA copies in liver samples from obese compared to samples from lean subjects. Furthermore, we provide a unique window to the hepatic relative proportions of taxa in the two groups. Further studies investigating the clinical implications of our findings are warranted.

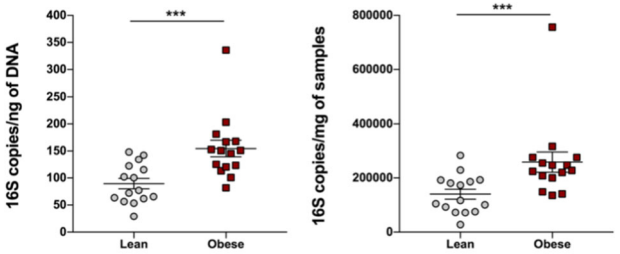


Figure 1. Quantification of 16S copies per ng DNA (left) and 16S copies per mg liver tissue (right) in lean (open symbols) and obese (filled symbols). Asterisks indicate significant difference (Man Whitney test P<0.001)

Clinical Trial Registration Number: NCT02337660
Disclosure: **J.I. Bagger:** None.

OP 42 Intercellular interactions and islet function

237

Bottom-up islet engineering

Q.P. Peterson[1,2], A. Veres[2], D.M. Gonzalez[2], J. Ryu-Kenty[2], B. Tyrberg[3], M. Sörhede-Winzell[3], D.A. Melton[2];
[1]Physiology and Biomedical Engineering, Mayo Clinic, Rochester, USA, [2]Stem Cell and Regenerative Biology, Harvard University, Cambridge, USA, [3]Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca Gothenburg, Mölndal, Sweden.

Background and aims: Diabetes Mellitus results from dysfunction of pancreatic islets leading to elevation of blood glucose levels and an increase in morbidity and mortality. In type 1 diabetics, the precipitating event is the loss of insulin producing pancreatic β cells through autoimmune attack. As such, the *in vitro* production of β cells for use as a cell transplantation therapy has been a major focus of type 1 diabetes research. However, it is unlikely that β cells by themselves will recapitulate the complex biology involved in islet function. Indeed, the three major approaches proposed by the field to regain glycemic control in diabetic patients (bionic pancreas, transplantation of *in vitro* derived β cells, and production of β cells *in vivo* through replication or reprogramming) fail to fully account for the complexity of islet endocrine function and focus almost exclusively on the function of the β cell.
Materials and methods: To this end, we seek to generate human islet organoids from component parts using a bottom-up tissue engineering approach. Here we report the development of separate protocols for generating stem cell-derived α, β and δ cells and subsequently combine these cell types to create islet organoids of defined composition by dispersing and reaggregating in various proportions (β only, 4:1 β to α, 1:1 β to α, and α only). Organoids were cultured for 4 days before assessing glucose stimulated insulin secretion. Organoids were transplanted under the kidney capsule in mice and assessed for survival and human insulin secretion *in vivo*.
Results: Stem cell-derived α, β and δ cells exhibit many of the characteristics of their *bona fide* counterparts including gene expression, hormone secretion, ultrastructure and *in vivo* function. We also find that combination of stem cell-derived α and β cells in islet organoids exhibit improved function *in vitro* and *in vivo* as compared to β cells alone. *In vitro* β cell insulin secretion in response to glucose was increased by 92% in the presence of α cells compared to controls ($p < 0.05$). Upon transplantation, combined α and β cell grafts resulted in a reduced fasting blood glucose (125 ± 7 mg/dl vs. 152 ± 9 mg/dl, $p < 0.05$), increased human insulin secretion after glucose challenge and an improved glucose tolerance (264 ± 23 mg/dl/h AUC vs. 341 ± 32 mg/dl/h AUC, $p < 0.05$) compared to transplantation of β cells alone. Combination of β cells with δ cells resulted in an overall decrease in insulin secretion and a decreased stimulation index *in vitro* (0.8 ± 0.2 for β and δ cells vs. 1.4 ± 0.2 for β cells only, $p < 0.05$).
Conclusion: These studies suggest that stem cell-based products more closely resembling the endogenous architecture and composition of the human islet may be better suited for cell replacement therapy, disease modeling and drug screening efforts.
Supported by: NIDDK and AZ
Disclosure: **Q.P. Peterson:** None.

238

The DPP4 inhibitor sitagliptin increases intra-islet active GLP-1 levels in human islets and may confer additional protection from cell death

S.A. Campbell, M. Hubert, N. Salamon, K. Ondrusova, A. Barr, W. Long, M. Fatehi, K. Philippaert, P. Light;
Pharmacology, University of Alberta, Edmonton, Canada.

Background and aims: Recent studies in genetic mouse models suggest that intra-islet GLP-1 is required for proper glucose homeostasis,

highlighting the importance of paracrine GLP-1 signalling within the islet. However, information on intra-islet GLP-1 secretion and action within human islets is lacking, despite the fact that 30–40% of islet cells are alpha cells and are capable of secreting GLP-1. Moreover, as DPP4 is found in human islets, treatment with the DPP4 inhibitor sitagliptin should increase levels of active GLP-1 within the islet and have consequences for insulin secretion and cell survival. Therefore, we confirmed that DPP4 is expressed within human islets and then studied the effects of sitagliptin on 1) active and total GLP-1 levels 2) cell survival and 3) glucose-stimulated insulin secretion.

Materials and methods: DPP4 protein was analyzed using Western blot. GLP-1 positive cells were identified with immunofluorescence microscopy on paraffin embedded human islet sections. Human islets were cultured with sitagliptin 200 nmol/L or vehicle control for the GLP-1 secretion assays, dead cell assay, and islet perifusions. Glucose concentrations for the experiments were: dead cell assay (6.1 mmol/L), glucose-stimulated insulin secretion (2.8, 28 mmol/L), glucose-stimulated GLP-1 secretion (2.8, 11 mmol/L), and islet perifusions (2.5, 11 mmol/L). Islet cell death was induced by culture time (48 hour) and detected with Sytox Green. Active and total GLP-1 levels were measured with an immunoassay (Meso Scale Discovery), while insulin levels were measured with an ELISA (Alpco).

Results: Western blot analysis demonstrated that human islets express significant levels of DPP4. In addition, using immunofluorescence microscopy of human islet sections, we identified a sub-population alpha cells that contained cleaved GLP-1. When normalized to islet size, our human islet cultures secrete 50X more active GLP-1 than mouse islets. Furthermore, active GLP-1 levels were significantly increased by ~7-fold ($N = 3$, $P < 0.05$) when islets were cultured with sitagliptin and this negatively correlated with increased cell death ($r = -0.983$, $P < 0.05$). Active GLP-1 levels negatively correlated with the stimulation index for insulin secretion ($r = -0.969$, $P < 0.05$). However, insulin secretion from perifused non-diabetic islets treated with sitagliptin did not differ from controls ($N = 6$). Preliminary results from glucose-stimulated GLP-1 secretion experiments show that active GLP-1 secretion decreases at high glucose with non-diabetic islets, but not with type 2 diabetic islets.

Conclusion: Our results support the concept that intra-islet GLP-1 may play an important physiological role in human islets as alpha cells are more numerous in human islets than mouse islets and also contain a sub-population of GLP-1 positive alpha cells. Moreover, we have determined that human islets secrete much larger amounts of active GLP-1 compared to mouse islets. Our novel results with sitagliptin suggest that DPP4 inhibitors may exert some of their glucose-lowering therapeutic effects via upregulation of intra-islet active GLP-1 levels. Although, the sitagliptin-mediated increase in active GLP-1 is correlated with enhanced islet cell survival, it did not increase insulin secretion in non-diabetic islets. Therefore, experiments are underway in islets from donors with type 2 diabetes.

Supported by: CIHR, ADI

Disclosure: **S.A. Campbell:** None.

239

Acetate stimulates insulin secretion of human pancreatic microislets

E. Lorza Gil[1,2], F. Gerst[1,2], U. Deschl[3], H.-U. Häring[1,2], S. Ullrich[1,2], M. Beilmann[3];
[1]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Zentrum München at the University of Tübingen (IDM), Tübingen, [2]German Center for Diabetes Research, Tübingen, [3]Boehringer Ingelheim Pharma GmbH & Co. KG, Nonclinical Drug Safety, Biberach, Germany.

Background and aims: The effect of short chain fatty acids (SCFA), i.e. acetate, butyrate and propionate on glucose-induced insulin secretion (GIIS) depends on FFAR2 and FFAR3 expression. In isolated rodent islets conflicting results have been published, including stimulatory, inhibitory or no effects of SCFA on GIIS. Due to species-related differences in islet architecture and function, a reliable and reproducible testing in human islet cell preparations is indispensable for the translation to the human situation. However, isolated human islets are poorly glucose-responsive and vary largely in basal secretion, size and architecture. This study aims to establish and validate a standardized in vitro test with islet cells from human donors. To this end microislets were generated by dissociation of isolated human islets into single cell suspension with subsequent reaggregation of defined cell numbers and equal composition of endocrine and non-endocrine cells. The microislets were cultured up to two weeks and functionally characterized.

Materials and methods: Human pancreatic islets were received from ECIT Centers and microislets purschased from InSphero. Islets were cultured overnight, digested with trypsin and reaggregated into microislets. Reaggregation of islet cells was performed using the hanging-drop method. Isolated islets and microislets were tested in parallel for insulin secretion using static incubation and RIA or ELISA for insulin and glucagon measurement.

Results: Basal insulin secretion at 2.8 mM glucose of isolated human islets ($n = 4$) was $3.2 \pm 0.6\%$ of content. At 12 mM glucose the secretion increased to $4.6 \pm 0.8\%$ of content. Forskolin (5 μM) further augmented GIIS to $6.6 \pm 1.2\%$ and palmitate (0.6 mM) to $6.1 \pm 0.8\%$. Basal secretion of microislets was significantly lower (1–2% of content) than of islets, while GIIS was largely improved (13-, 10- and 9-fold in microislets consisting of 1000, 2000 and 4000 cells, respectively). Acetate significantly augmented GIIS. The adrenoceptor agonist adrenaline inhibited GIIS indicating that receptor function is at least partially maintained in the microislets. The secretory capacity did not correlate to insulin content since islets contained significantly more insulin (23.0 ± 1.2 ng/islet) than microislets. The cell aggregates of 1000, 2000 and 4000 cells contained 1.6 ± 0.2, 2.7 ± 0.3, 10.1 ± 1.4 ng insulin/microislet, respectively. Insulin content of microislets and GIIS was maintained after an additional 2 week culture period. Glucagon secretion was not significantly different between 2.8 and 12 mM glucose and was less controllable than insulin secretion. However, glucagon release was lowest at 12 mM glucose and 2-fold higher upon addition of 5 mM arginine to 2.8 mM glucose.

Conclusion: The dissociation and reaggregation of human islet cells into microislets reduced basal insulin secretion and largely improved GIIS. The glucose responsiveness inversely correlated to the microislet size and was maintained over 14 days. FFAR2 and FFAR3 agonists significantly augmented GIIS. Thus, dissociation and reaggregation of human islet cells improves the functional integrity of beta-cells and it can be used as a tool for long term functional test and for standardization of islet cell transplantation.

Supported by: DZD Grant.

Disclosure: **E. Lorza Gil:** None.

240

Using a novel perifusion platform to investigate real-time crosstalk effects of contracting skeletal muscle on pancreatic beta cell function in vitro

J. Barlow, T. Solomon;
University of Birmingham, Birmingham, UK.

Background and aims: It is suggested that mediators liberated by contracting skeletal muscle play a role in mediating the beneficial effects of exercise on β-cell function in patients with type 2 diabetes (T2D). Examining this inter-organ crosstalk in vitro is limited to static co-culture incubation methods which are cumbersome and time limiting. Therefore we have developed a novel in vitro perifusion system to investigate skeletal muscle to β-cell crosstalk in real-time. Using this system, we aimed to examine real-time effects of mediators released by contracting skeletal muscle on insulin release from INS-1E pseudoislets and cells under healthy and T2D-like conditions.

Materials and methods: Krebs Ringer HEPES buffer (pH 7.4) containing 5 mM glucose was perifused from C2C12 myotubes to INS-1E pseudoislets for 270 minutes at a flow rate of 0.5 mL/min. After an initial 30 minute equilibration, C2C12 myotubes were contracted for 60 minutes by electrical pulse stimulation (EPS - 40V, 1.0 Hz, 2 ms) - control myotubes were left unstimulated. To assess for post contraction effects, after EPS, perifusion was maintained for a further 120 minutes, at which stage the glucose concentration of the perifusion buffer was raised to 20 mM for the final 80 minutes of the perifusion. Perifusate was collected every 4 minutes and assayed for insulin by homogenous time-resolved fluorescence. Differences in insulin secretion from INS-1E pseudoislets perifused with perifusate from C2C12 myotubes ± EPS averaged over each 30 minute period were examined by two-way ANOVA. To examine effects of contracted skeletal muscle on β-cell function under T2D-like conditions, perifusate was collected for 2-hours post ± EPS from C2C12 myotubes at 5 or 20 mM glucose and used to assess acute insulin release from INS-1E cell monolayers pre-exposed to 5 or 20 mM glucose ± palmitate (20 nM).
Results: Insulin release from pseudoislets remained similar over each 30 minute period upto 120 minutes of perifusion irrespective of whether pseudoislets were perifused with perifusate from contracting myotubes. Within the 2-hour period post contraction, insulin secretion increased significantly from 0.94 (±0.27) pg/ngDNA to 3.4 (±0.87) pg/ngDNA ($P < 0.0001$) by pseudoislets perifused with EPS-treated myotube perifusate - insulin release from perifused pseudoislets with non-treated myotube perifusate was not significantly different ($P = 0.93$). Specifically, the average amount of insulin secreted between 150–180 minutes ($P < 0.05$) and 180–210 minutes ($P < 0.01$) was significantly more from pseudoislets perifused with myotube perifusate after EPS. Increasing the glucose concentration of the perifusate to 20 mM stimulated insulin secretion further irrespective of EPS. Perifusate from EPS-treated myotubes also potentiated insulin secretion from INS-1E cell monolayers in the presence and absence of palmitate ($P < 0.01$). Pre-treatment of cells to 24-h high glucose ± palmitate dampened this potentiating effect. Moreover, 24-h palmitate plus high glucose lowered acute glucose-stimulated insulin release to a similar extent when induced by perifusate collected from EPS-treated or non-treated myotubes.
Conclusion: Mediators released by contracting C2C12 myotubes potentiate acute insulin release from normal and palmitate treated INS-1E pseudoislets and/or cells at 5 mM but not 20 mM glucose and fail to improve insulin release by INS-1E cells exposed to T2D-like conditions.
Supported by: Diabetes UK
Disclosure: **J. Barlow:** None.

OP 43 Do not let bugs pass by you

241

Low-calorie sweeteners disrupt the gut microbiome in healthy subjects in association with impaired glycaemic control

R.L. Young[1,2], D. Kreuch[1,2], F.M. Mobegi[3], L. Leong[3], G. Schober[1,2], N.J. Isaacs[1,2], T. Wu[1,4], N. Cvijanovic[1,2], N. Pezos[1,2], M.M. Bound[1,4], M. Horowitz[1,4], D.J. Keating[5,2], C.K. Rayner[1,4], G.B. Rogers[3];
[1]Adelaide Medical School, The University of Adelaide, Adelaide, [2]Nutrition & Metabolism, South Australian Health & Medical Research Institute, Adelaide, [3]Infection & Immunity, South Australian Health & Medical Research Institute, Adelaide, [4]Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide, Adelaide, [5]Medicine & Public Health, Flinders University of South Australia, Bedford Park, Australia.

Background and aims: Epidemiological studies indicate that regular high intake of beverages sweetened with low-calorie sweeteners (LCS) increase the risk of developing type 2 diabetes mellitus (T2DM), but the underlying mechanisms are unknown. We recently showed that 2 week dietary supplementation with LCS in healthy non-diabetic subjects led to clinically relevant increases in glycaemic responses to enteral glucose. Augmented glucose absorption (serum 3-O-methyl glucose, 3-OMG) and attenuated release of glucagon-like peptide-1 (GLP-1) contribute to this dysglycaemia, however it is unclear whether gut dysbiosis due to LCS also contributes to dysglycaemia, as occurs in rodents.
Materials and methods: 29 non-diabetic subjects (age 30 ± 2 years, body mass index 24 ± 3 kg/m^2, HbA1c 32 ± 1 mmol/mol (5.2%), 16 male) were randomised, in double-blind fashion, to diet supplementation with a LCS combination (92 mg sucralose +52 mg acesulfame-K, equivalent to ~1.5 L of diet beverage consumption/day, $N = 14$) or placebo ($N = 15$); these were taken in capsules three times daily over 2 weeks. The gut microbiome was assessed by shotgun metagenomic sequencing in stool collected before and after treatment. Differences in taxonomic and functional microbiome characteristics were determined using MetaPhlAn2 and HUMAnN2 abundance, respectively.
Results: LCS-treated subjects exhibited a greater variation in faecal microbiota composition, along with a significant reduction in the health-associated bacterium *Eubacterium cylindroides* (−11 $\log_2$ fold change, FC) and an increased abundance of 11 opportunistic gut pathogens, including *Klebsiella* (17 FC), *Porphyromonas* (15 FC) and *Finegoldia* (12 FC; all $P \leq 0.001$). A decrease in beneficial and fermentative *Bifidobacterium*, *Lactobacillus* and *Bacteroides* populations correlated with augmented glucose absorption (3-OMG), while a decrease in *Butyrivibrio* populations correlated with attenuated GLP-1 release (Spearman correlation: $\rho \geq \pm 0.37$; $P \leq 0.05$). Finally, shifts in the abundance of microbial genes involved in sucrose degradation and pyruvate metabolism correlated with a deterioration in glucose regulation in LCS-treated subjects.
Conclusion: In healthy non-diabetic subjects 2 weeks of LCS supplementation (i) causes gut dysbiosis and (ii) increases the abundance of gut pathogens normally absent in health. Moreover, a decrease in fermentative microbial populations and shifts in bacterial energy harvesting pathways due to LCS predict a deterioration in glucose regulation. Our findings support the concept that LCS disrupt glycaemic responses in healthy humans via dysregulation of glucose uptake and disposal, and secondary to dysbiosis of gut commensal bacteria. This highlights the clinical relevance of dietary LCS patterns to overall glycaemic control.
Clinical Trial Registration Number: ACTRN12615000866505
Supported by: NHMRC
Disclosure: **R.L. Young:** None.

242

Gut microbiome induced by intra-intestinal carbohydrates suppresses glucose-dependent insulinotropic polypeptide secretion

E. Lee[1], J. Miyamoto[2], I. Kimura[2], T. Miki[1];
[1]Chiba University, Chiba, [2]Tokyo University of Agriculture and Technology, Fuchu, Japan.

Background and aims: Dietary carbohydrate triggers secretion of the two major incretins: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 and GIP are secreted from enteroendocrine L-cells and K-cells in response to carbohydrate ingestion. We previously reported that co-administration of maltose plus the α-glucosidase inhibitor miglitol (maltose/miglitol) potentiates GLP-1 secretion through the activation of SGLT3 expressed in serotonin-secreting enterochromaffin cells. Although the mechanism of carbohydrate sensing and incretin secretion has been considered to be mostly similar between L-cells and K-cells, we found that maltose/miglitol administration evokes the opposite response; stimulatory for GLP-1 but inhibitory for GIP. In the present study, we examined the regulatory mechanism of carbohydrate-induced GIP secretion.
Materials and methods: Maltose (2 g/kg) and miglitol (10 mg/kg) were administered orally to mice. At 30 minutes after oral ingestion, the mice were anesthetized and the blood sample was drawn from the portal vein and was subjected to the measurement of plasma GIP and short chain fatty acid (SCFA) concentrations. Involvement of the gut microbiota and its product SCFAs in GIP secretion was evaluated by oral antibiotics (Abs)-treatment and by using mice lacking the SCFA receptor FFAR2 (also known as GPR43) (*Ffar2*$^{-/-}$) or FFAR3 (also known as GPR41) (*Ffar3*$^{-/-}$). Plasma SCFA levels were determined by gas chromatography-mass spectrometry (GC-MS).
Results: GIP secretion in wild-type mice was significantly decreased by maltose/miglitol administration (GIP concentrations in portal vein; 73.2 ± 4.5 pmol/l in vehicle administration and 45.5 ± 3.0 pmol/l in maltose/miglitol administration, $p < 0.001$). We found that plasma SCFAs in the portal vein was acutely increased after a single administration of maltose/miglitol administration. In addition, oral Abs-treatment for 4 weeks significantly attenuated the maltose/miglitol-induced suppression of GIP secretion, suggesting that the gut microbiome is involved in the GIP suppression by maltose/miglitol. We next examined the suppressive effect of maltose/miglitol administration on GIP secretion in *Ffar2*$^{-/-}$ and *Ffar3*$^{-/-}$ mice. Although GIP secretion was suppressed normally by maltose/miglitol administration in *Ffar2*$^{-/-}$ (GIP concentrations in portal vein; 78.8 ± 6.3 pmol/l in vehicle administration and 57.3 ± 5.2 pmol/l in maltose/miglitol administration, $p < 0.05$), but not in *Ffar3*$^{-/-}$ (GIP concentrations in portal vein; 87.7 ± 6.6 pmol/l in vehicle administration and 76.9 ± 5.9 pmol/l in maltose/miglitol *Ffar3*$^{-/-}$, not significant). Similarly, co-administration of glucose plus the sodium glucose transporter inhibitor phloridzin (glucose/phloridzin) decreased GIP secretion in a microbiome-dependent manner.
Conclusion: Intra-intestinal retention of carbohydrate facilitates its usage by gut flora and release of SCFAs, which inhibits GIP secretion through their binding to FFAR3.
Supported by: The Ministry of Education, Culture, Sports, Science and Technology, Japan
Disclosure: **E. Lee:** None.

243

GLP-1 secreting enteroendocrine cells co-release ATP as a fast acting transmitter to modulate vagal afferent neuronal activity

F. Reimann, V.B. Lu, J. Rievaj, C.A. Smith, R. Pais, G. Tolhurst, F.M. Gribble;
University of Cambridge, Cambridge, UK.

Background and aims: Glucagon-like peptide-1 (GLP-1), now widely used in the treatment of diabetes and obesity, is secreted from enteroendocrine L-cells found scattered in the intestinal epithelium. Whilst many of the benefits of GLP-1 rely on its incretin activity on pancreatic beta cells there is also considerable evidence that L-cells directly activate the afferent vagus nerve, thereby triggering signals from the gut to the brainstem. We found previously that GLP-1 itself was a poor stimulus of vagal neurons from the nodose ganglion but strongly sensitised them to other stimuli such as ATP. The aim of this study was to investigate if L-cells co-secrete ATP as a fast transmitter capable of directly activating vagal nerve endings.
Materials and methods: ATP-containing vesicles were visualised by quinacrine (5 μM). ATP concentrations in supernatants of GLUTag-cells, an L-cell model line, were measured by a bioluminescence-assay and ATP release from GLUTag and primary L-cells was also demonstrated by "sniffer patches", detecting ATP through activation of heterologously expressed $P2X_2$ receptors. Quinacrine release was monitored by total internal reflection fluorescence (TIRF) microscopy as a proxy of ATP-containing vesicle fusion. Changes in cytosolic Ca^{2+}-concentration in co-cultured GLUTag cells and nodose ganglion-derived (ND) neurons were monitored after Fura-2 loading.
Results: Quinacrine staining was detected in vesicular structures in GLUTag and primary cultured L-cells. By TIRF microscopy, these vesicles showed transient increases and subsequent dissipation of fluorescence intensity and the frequency of such events approximately doubled in response to a known L-cell stimulus angiotensin-II (1 μM), consistent with enhanced vesicular ATP secretion. This was also demonstrated by "sniffer patch"-currents, which increased in response to AngII, when brought into close proximity of GLUTag or primary L-cells, or by measurement of ATP in cell supernatants. To test for ATP-dependent communication between L-cells and vagal neurons, we co-cultured GLUTag-cells expressing clozapine-N-oxide (CNO)-sensitive designer receptors exclusively activated by designer drugs (Gq-DREADD) and mCherry together with ND neurons labelled with EYFP. Upon stimulation of GLUTag cells with CNO, ~30% of ND neurons showed a Ca^{2+}-elevation that was not seen with CNO in the absence of co-cultured GLUTag cells. This response was sensitive to the broad-spectrum P2Y/P2X inhibitor PPADS (100 μM).
Conclusion: ATP is co-secreted with GLP-1 from intestinal L-cells and can act as a fast local neurotransmitter, triggering activation of vagal neurons and potentially synergising with locally elevated peptide hormones. Co-secretion of small molecular "neurotransmitters" from enteroendocrine cells should be considered more widely in the gut-brain axis.
Supported by: Wellcome (106262/Z/14/Z and 106263/Z/14/Z) & MRC (MRC_MC_UU_12012/3)
Disclosure: **F. Reimann:** Grants; Wellcome joint investigator award to FR/FMG (106262/Z/14/Z and 106263/Z/14/Z), FR/FMG joint MRC programme within Metabolic Diseases Unit (MRC_MC_UU_12012/3).

244

L cell secretory responses after gastric bypass surgery can be imitated in un-operated individuals by modulating carbohydrate digestion

C. Martinussen[1,2], K.N. Bojsen-Møller[1,2], C. Dirksen[1,2], M.S. Svane[1,2], J.J. Holst[2], S. Madsbad[1,2];
[1]Dept. of Endocrinology, Hvidovre Hospital, [2]NNF center for Basic Metabolic Research and Dept. of Biomedical Sciences, University of Copenhagen, Denmark.

Background and aims: Exaggerated postprandial secretion of the L-cell hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) seems important for the metabolic benefits of gastric bypass (GB) surgery and may be explained by the accelerated arrival of carbohydrates to the distal small intestine where L-cell density is high. Therefore, individuals with intact gastrointestinal anatomy could potentially achieve similar enhancements of L-cell secretion if carbohydrates could be delivered to the distal gut. The disaccharides sucrose and isomaltulose are hydrolyzed by alpha glucosidase enzymes to the absorbable monosaccharides glucose (glu) and fructose (fru), but the rate of hydrolysis is much slower for isomaltulose than for sucrose, resulting in prolonged and distal

absorption. Distal absorption may also be obtained for sucrose by inhibiting the action of alpha glucosidases with acarbose. We measured secretion of GLP-1 and PYY, the latter of which is primarily secreted from the distal L-cells, in response to simple saccharides differing in digestibility after GB and in un-operated matched controls (CON).
Materials and methods: 10 GB patients and 10 CON matched on BMI, age and sex. On 4 separate days, participants ingested isomolar oral loads of glu and fru, either given as separate monosaccharides (25 g glu + 25 g fru) or as disaccharides in the form of 47.5 g isomaltulose or 47.5 g sucrose ± acarbose. Blood was sampled frequently for 4 hours and plasma was analyzed for GLP-1, PYY and glucose.
Results: Digestibility of the carbohydrate loads was reflected in the glycemic profiles with glu+fru and sucrose inducing the highest peaks and largest excursions, while more moderate responses were seen for isomaltulose and sucrose + acarbose in both groups. GLP-1 responses (positive iAUC) to rapidly absorbed glu+fru and sucrose were similar within groups, but 3-fold higher in GB than in CON ($p < 0.01$). Intake of the slowly digested isomaltulose led to prolonged and increased GLP-1 secretion especially in CON (+50% in GB, $p = 0.11$, and +160% in CON, $p = 0.01$, vs. sucrose) so that the response to isomaltulose in CON was similar to the glu+fru ($p = 0.91$) and sucrose ($p = 0.79$) induced responses in GB. Acarbose reduced GLP-1 by 50% in GB ($p < 0.01$ vs. sucrose alone), but had no effect on GLP-1 responses in CON. Secretion of PYY after glu+fru and sucrose was 2–4 fold higher in GB than in CON ($p = 0.03$, and $p = 0.06$, respectively), while the response to isomaltulose were similar to glu+fru in GB and comparable between GB and CON. Acarbose increased PYY nearly 3-fold in both GB ($p = 0.08$) and CON ($p = 0.23$), but the response was still lower in CON than in GB.
Conclusion: The exaggerated GLP-1 and PYY secretion after GB in response to rapidly absorbed carbohydrates was mimicked in un-operated matched subjects by ingestion of the slowly digested and distally absorbed disaccharide isomaltulose. Acarbose was effective in increasing the secretion of PYY in both GB and CON but inhibited GLP-1 responses in GB, perhaps due to extensive blockage of sucrose hydrolysis. The results support that the exaggerated L-cell secretion after gastric bypass is due to rapid delivery of nutrients to the distal gut and suggest that modified carbohydrates with slower digestion and distal absorption may be used to produce similar responses in un-operated individuals.
Clinical Trial Registration Number: NCT02879955
Supported by: European Research Council (grant No 695069) and Danish Diabetes Academy
Disclosure: **C. Martinussen:** Grants; Danish Diabetes Academy, Novo Nordisk Foundation, European Research Council, Hvidovre Hospital Research Foundation.

OP 44 Clinical use of insulin: What works and what doesn't

245

Quantitative ultrasound characteristics of insulin-induced lipohypertrophy in subjects with diabetes

M.M. Lazarev, V.V. Klimontov, A.A. Makhotin, D.M. Bulumbaeva, E.A. Koroleva, A.Y. Letyagin;
Research Institute of Clinical and Experimental Lymphology – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russian Federation.

Background and aims: Lipohypertrophy at injection sites is one of the most frequent complications of insulin therapy. Recent studies demonstrated the applicability of ultrasound scan for diagnostics of insulin-induced lipohypertrophy in diabetic subjects. Sonoelastography and 3D power Doppler ultrasound could provide new opportunities for quantitative characterization of lipohypertrophy. The aim of our study was to assess the relationships between quantitative ultrasound parameters of lipohypertrophy areas, characteristics of insulin therapy and metrics of glycemic control in insulin-treated diabetic subjects.
Materials and methods: Eighty two adult subjects, 27M/55F, with duration of insulin therapy for more than 3 months, were consecutively recruited. Among them, 26 ones had type 1 diabetes and 56 individuals had type 2 diabetes. The sites of insulin injections were inspected by palpation and ultrasound. The visualization protocol included gray-scale densitometry with Mean Gray Value (MGV) index estimation, strain elastography imaging with Strain Ratio (StR) calculation, and 3D power Doppler ultrasound with Vascularization Index (VI), Flow Index (FI), and Vascularization Flow Index (VFI) assessment. Glucose variability (GV) indices: Standard Deviation, Mean Amplitude of Glucose Excursions, Low Blood Glucose Index, and High Blood Glucose Index were calculated from 6-point glucose profiles on three consequent days. Serum levels of insulin antibodies were determined by ELISA.
Results: Lipohypertrophy was revealed by palpation and ultrasound in 57 and 80 patients (70% and 98%) respectively. The aggregated ultrasound-verified lipohypertrophy square (LS) varied from 50 to 1847 mm^2 (median 370 mm^2). Most of the lipohypertrophy sites demonstrated hyperechogenicity and increased stiffness when compared to surrounding subcutaneous fat (MGV and StR indices: $p < 0.001$). The reduced vascularity in lipohypertrophy areas were confirmed by 3D power Doppler ultrasound vascular indices (all $p < 0.05$). Total LS and MGV showed weak positive correlations with daily insulin dose (both $r = 0.3$, $p = 0.006$), however in patients with type 1 diabetes LS correlated with insulin dose more closely ($r = 0.47$, $p = 0.02$). Patients receiving insulin analogues had smaller aggregated LS than those on human insulin ($p = 0.03$). The LS demonstrated positive correlations with mean postprandial glucose ($r = 0.35$, $p = 0.001$) and triglycerides levels ($r = 0.4$, $p = 0.0002$). The FI index, but not VI and VFI, correlated negatively with mean postprandial glucose ($r = -0.29$, $p = 0.01$). The GV indices, levels of HbA1c and insulin antibodies showed no association with ultrasound parameters.
Conclusion: The gray-scale densitometry, strain elastography and 3D power Doppler ultrasound provide comprehensive quantitative characteristic of the areas of lipohypertrophy in insulin-treated diabetic subjects. Ultrasound indices of insulin-induced lipohypertrophy demonstrate some associations with daily insulin dose and metabolic parameters in these subjects.
Disclosure: **M.M. Lazarev:** None.

246

Favourable effects of insulin treatment for latent autoimmune diabetes in adults do not outweigh autoimmunity-induced decline in insulin release during 21 months of intervention

I. Hals[1,2], V. Grill[2], H. Fiskvik Fleiner[3], N. Reimers[4], M. Astor[5], Z. Ma[6], K. Filipsson[7], A. Björklund[6];

[1]Dep. of Endocrinology, St Olavs University Hospital, Trondheim, Norway, [2]Dep. of Clinical and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway, [3]Dep. of Clinical Pharmacology, St Olavs University Hospital, Trondheim, Norway, [4]Sjøsiden Legesenter, Trondheim, Norway, [5]Dep. of Medicine, Haukeland University Hospital, Bergen, Norway, [6]Dep. of Molecular Medicine and Surgery, Endocrine and Diabetes Unit, Karolinska University Hospital Solna, Karolinska Institutet, Stockholm, Sweden, [7]Dep. of Clinical Sciences, Unit of Molecular Metabolism, Lund University Diabetes Centre, Malmö, Sweden.

Background and aims: The optimal beta cell preserving treatment of LADA patients is unclear - should one treat with insulin from diagnosis as for type 1 diabetes or by peroral drugs, including insulin enhancing ones, as for type 2 diabetes? This uncertainty is due to a lack of randomized clinical trials in LADA. We compared beta cell function during treatment with either insulin or sitagliptin, a DDP4-inhibitor, taking into account a variable degree of autoimmunity in LADA.
Materials and methods: Inclusion criteria included GADA positivity, age 30–75 years, no clinical need for insulin treatment and diabetes diagnosed <3 years before inclusion. Participants were stratified by age, BMI and degree of GADA positivity. Beta cell function was evaluated by C-peptide, insulin and proinsulin recorded during C-peptide glucagon tests performed after 3, 9 and 21 mo. following randomization, always after a 48 h temporary withdrawal of study medication.
Results: In the 61 participants the median age at randomization was 53 years and BMI 27 kg/m^2. These and other parameters (male/female, HbA1c, fasting C-peptide) were similar between the study arms. All participants were treated with metformin before and during the intervention. HbA1c was similar at baseline and after 21 mo. of intervention (in the insulin arm median 6.8% at baseline and 6.6% at 21 mo., in the sitagliptin arm 6.5% and 6.6%). Stimulated C-peptide after 21 mo. of intervention decreased similarly in both arms (median −0.09 nmol/L in the insulin, −0.08 nmol/L in the sitagliptin arm). Stimulated insulin was unaltered at 21 mo. of insulin treatment (median 24.5 μU/ml at randomization, 24.1 μU/ml at 21 mo.), whereas levels decreased following treatment with sitagliptin (from 22.4 μU/ml to 15.2 μU/ml, $p < 0.03$ vs. insulin). The ratio proinsulin/insulin (a marker of beta cell stress) did not change following insulin treatment (median 0.13 at randomization, 0.11 at 21 mo.) but increased following sitagliptin (0.13 at randomization, 0.21 at 21 mo, $p < 0.02$ vs. insulin). We assessed effects of apparent autoimmunity in the whole study population after dichotomizing titers (low/high) of GADA. Stimulated C-peptide did not change in low GADA (median 0.77 at randomization, 0.78 at 21 mo.) but decreased in high GADA participants by 27% (from 0.87 to 0.60 nmol/l, $p < 0.05$ vs. low GADA). Reciprocally the proinsulin/insulin ratio increased from 0.13 to 0.21 in high GADA but was unaffected with low GADA participants (from 0.14 to 0.15, $p < 0.04$ for difference high vs. low GADA). Further analysis did not detect a modulating effect by insulin treatment on these parameters.
Conclusion: Early insulin treatment may be advantageous in LADA but does not protect against an autoimmune assault on beta cells.
Clinical Trial Registration Number: NCT01140438
Supported by: NRC, SRC, NTNU, St Olavs Hospital, The Norwegian Diabetes Association
Disclosure: **I. Hals:** None.

247

Comparison of canagliflozin and liraglutide as a replacement for bolus insulin in type 2 diabetes patients well-controlled by basal-bolus insulin

N. Kumashiro, Y. Ando, F. Shigiyama, H. Igarashi, F. Yoshikawa, H. Uchino, H. Yoshino, M. Miyagi, K. Ikehara, T. Hirose;
Toho University Graduate School of Medicine, Tokyo, Japan.

Background and aims: When we select anti-diabetic drugs, prevention of hypoglycemia, weight gain, and cardiovascular events has been taken into consideration based on the recent results of large clinical trials such as ACCORD, LEADER, and CANVAS. Basal-bolus insulin therapy is available in various situation, but it has risks of hypoglycemia and weight gain that may lead to cardiovascular events. In contrast, canagliflozin, a sodium glucose cotransporter 2 inhibitor (SGLT2i), and liraglutide, a glucagon-like peptide-1 receptor agonist (GLP1RA), are reported to reduce body weight and cardiovascular events. Combination therapy of basal insulin plus GLP1RA is well-known for its effectiveness, but that of basal insulin plus SGLT2i remains to be tested. The aim of this study was to address the efficacy of canagliflozin as a replacement for bolus insulin compared to that of liraglutide in type 2 diabetes patients who were well-controlled by basal-bolus insulin therapy.
Materials and methods: This study was a prospective, randomized, open-label, parallel-group, comparative study. A total of 40 type 2 diabetes patients treated by basal-bolus insulin over 1 year (HbA1c <7.5%) were randomized to either canagliflozin group (Group Cana) or liraglutide group (Group Lira). After consent, bolus insulin was totally switched to canagliflozin or liraglutide. Dose of canagliflozin was fixed at 100 mg/day, but liraglutide was increased from 0.3 to 0.9 mg/day. Basal insulin was continued and its dose was adjusted using algorism. At 24 weeks, changes from baseline in HbA1c, hypoglycemia, glucose fluctuation assessed by continuous glucose monitoring system, body weight, BMI, insulin dose, and diabetes-treatment-related quality of life (DTR-QOL) were compared between the groups. Adverse events were also monitored for 24 weeks.
Results: Subjects were aged 58.1 ± 11.9 years, male/female; 32/8, known duration of diabetes; 9.5 ± 6.9 years, body weight; 74.8 ± 9.9 kg, BMI; 26.6 ± 3.3 kg/m^2, HbA1c; 6.7 ± 0.6%, daily basal insulin dose; 14.4 ± 8.5 U, and daily bolus insulin dose; 18.2 ± 7.1 U at baseline. Finally, 17 patients per each group were analyzed. As results, HbA1c maintained good levels in both groups (from 6.8 ± 0.7 to 6.7 ± 0.7% *vs.* from 6.4 ± 0.6 to 6.2 ± 0.8%, Group Cana *vs.* Lira). No sever hypoglycemia was observed in all patients nor there was no difference in hypoglycemia between the groups, and nocturnal hypoglycemia tended to decrease in both groups. Glucose fluctuation did not change from baseline in both groups. Body weight and BMI tended to decrease in both groups. Basal insulin dose increased significantly in both groups (+44.5% *vs.* +39.2% from baseline in Group Cana *vs.* Lira), but total daily insulin dose decreased significantly in both groups (−34.5% *vs.* −47.6% from baseline in Group Cana *vs.* Lira). Regarding DTR-QOL, total score improved significantly in both groups, but while all domain scores improved in Group Cana, no improvement was observed in domains of anxiety and dissatisfaction with treatment and hypoglycemia in Group Lira.
Conclusion: Replacement from bolus insulin to either canagliflozin or liraglutide was safe and effective on glycemic control, hypoglycemia, body weight, and QOL in type 2 diabetes patients well-controlled by basal-bolus insulin therapy. Supported by basal insulin, canagliflozin as well as liraglutide is a good therapeutic option as a replacement for bolus insulin.
Clinical Trial Registration Number: UMIN000019382
Disclosure: **N. Kumashiro:** Grants; AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Inc., Ono Pharmaceutical Co., Ltd., Novo Nordisk Inc, Sanofi-Aventis Deutschland GmbH, Daiichi-Sankyo Co., Ltd., Eli Lilly Japan K.K., Takeda Pharmac. Lecture/other fees; Novo Nordisk Inc, Takeda Pharmaceutical Company Limited, and Sanofi-Aventis Deutschland GmbH.

248

Reasons for discontinuation of insulin therapy: results from the International Diabetes Management Practices Study (IDMPS)

J.-M. Chantelot[1], P. Aschner[2], J.J. Gagliardino[3], H. Ilkova[4], F. Lavalle[5], A. Ramachandran[6], G. Kaddaha[7], J.C. Mbanya[8], M. Shestakova[9], J.C.N. Chan[10];

[1]Sanofi, Paris, France, [2]Javeriana University School of Medicine and San Ignacio University Hospital, Bogotá, Colombia, [3]CENEXA, Center of Experimental and Applied Endocrinology (La Plata National University – La Plata National Scientific and Technical Research Council), La Plata, Argentina, [4]Istanbul University, Cerrahpasa Medical Faculty, Department of Internal Medicine, Division of Endocrinology, Metabolism and Diabetes, Istanbul, Turkey, [5]Facultad de Medicina de la Universidad Autónoma de Nuevo León, Monterrey, Mexico, [6]India Diabetes Research Foundation, Dr. A. Ramachandran's Diabetes Hospitals, Chennai, India, [7]Government of Dubai, Dubai Health Authority, Dubai, United Arab Emirates, [8]Biotechnology Center, Doctoral School of Life Sciences, Health and Environment, and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, [9]Endocrinology Research Center, Moscow, Russian Federation, [10]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, Hong Kong SAR, China.

Background and aims: Adherence to insulin therapy is often suboptimal, and understanding patients' perspectives on drug adherence is essential for identification of barriers to therapy. We studied the reasons given for discontinuation of insulin therapy by people with diabetes in the developing world.

Materials and methods: The IDMPS is a global observational survey on the management of people with type 1 (T1D) and type 2 (T2D) diabetes. In 2016–17, participants were enrolled from 24 countries in the Middle East, South Asia, Eurasia and Africa.

Results: In people with T1D ($N = 2000$; mean (SD) age 34.0 (12.3) years; 48.8% male; time since diagnosis 13.1 (9.9) years), 14% (273/1955) discontinued insulin for 1 month (median), without physician indication. The nature of discontinuation, e.g. missed injections either of 1 prandial or 1 basal insulin [basal-bolus regimen] or 1 premixed insulin, is unknown. The main reasons given were impact on social life, cost, fear of hypoglycaemia, and lack of support (**Table**). In people with T2D ($N = 2596$; age 57.2 (11.1) years; 47.8% male; time since diagnosis 13.5 (8.8) years for insulin alone, 12.8 (7.5) years for insulin + oral glucose-lowering drugs [OGLD]), insulin discontinuation for ≥2 months (median), without physician indication, was reported by 13.4% of people treated with insulin alone (86/642) and 13.8% of people who received insulin + OGLD (261/1936). Reasons for discontinuation included impact on social life, fear of hypoglycaemia, lack of support, and cost. The pattern of insulin (e.g., a definitive or temporary stop, or missed injection[s]) was not documented.

Conclusion: These observations are cause for concern, in particular the proportion of people with T1D who discontinue, and indicate the need for a multi-pronged strategy including patient education and access to therapy to improve treatment adherence and optimise outcomes.

Table 1. Reasons for insulin discontinuation

Reasons for discontinuation, %	T1D n=273	T2D	
		Insulin alone n=86	Insulin + OGLD n=261
Lack of efficacy	3.3	5.8	9.2
Fear of hypoglycaemia	26.7	24.4	29.1
Occurrence of side effects	8.4	12.8	10.3
Impact on social life	41.0	29.1	31.0
Lack of experience in insulin dosing	20.9	30.2	24.1
Lack of experience in insulin administration	9.5	16.3	14.2
Cost of medicine and strips	34.4	22.1	25.3
Weight gain	8.8	4.7	10.3
Lack of support	26.4	29.1	24.9
Occurrence of hypoglycaemia	14.7	15.1	11.9

OGLD, oral glucose-lowering drug

Supported by: Sanofi

Disclosure: **J. Chantelot:** Employment/Consultancy; Sanofi. Stock/Shareholding; Sanofi.

OP 45 Memory and mood

249

Biliverdin reductase-A mediates the beneficial effects of intranasal insulin administration in Alzheimer disease: a novel molecular mechanism

E. Barone[1], F. Triani[1], A. Tramutola[1], T. Cassano[2], M. Perluigi[1]; [1]Department of Biochemical Sciences, Sapienza University of Rome, Rome, [2]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy.

Background and aims: Biliverdin reductase-A (BVR-A) - known for its role in the degradation of heme - is also a Ser/Thr/Tyr kinase regulating insulin signalling. BVR-A controls IRS1 activation and favours the activation of the protein kinase B (PKB/Akt). Previous studies from our group reported about reduced BVR-A activation in Alzheimer disease (AD) human brain. Furthermore, in a longitudinal study we showed that reduced BVR-A activation precedes the inhibition of IRS1 and the onset of brain insulin resistance in the brain of 3xTg-AD mice, independently from amyloid beta (Aβ) accumulation and TNF- α elevation. Intranasal insulin administration (INI) is under evaluation as therapeutic strategy to mitigate brain insulin resistance in AD, however the molecular mechanisms underlying INI effects are still unknown. Our goal was to clarify whether INI delays the onset of brain insulin resistance by both preventing and rescuing BVR-A impairment in the brain of adult and aged 3xTg-AD mice.

Materials and methods: We evaluated changes of (1) cognitive functions; (2) insulin signalling activation and (3) AD neuropathology in the hippocampus of 3xTg-AD and WT mice that received insulin (2U, 3 times/week) or vehicle for 2 months through the intranasal route. The role of BVR-A was strengthened by evaluating age-associated changes in BVR-A knock-out (KO) mice. Furthermore, cell-based experiments to support *in vivo* data were performed in SH-SY5Y neuronal cells.

Results: INI significantly improved learning and memory functions evaluated through the Morris water maze and the novel object recognition tasks in 3xTg-AD mice both at 6 and 12 months. At 6 months, insulin prevented the reduction of BVR-A activation ($p < 0.01$). Furthermore, INI fostered the activation of the insulin receptor (IR, $p < 0.05$), prevented the IRS1 hyper-activation ($p < 0.01$), improved the activation of Akt ($p < 0.001$) and prevented the downregulation of mTOR ($p < 0.05$). At 12 months, INI recovered BVR-A activation ($p < 0.01$) along with an improvement of the activation state of IR ($p < 0.01$), IRS1 ($p < 0.05$), ERK1/2 ($p < 0.01$) and Akt ($p < 0.05$). The amelioration of the insulin signalling was also associated with reduced Aβ levels and Tau phosphorylation (pTau) both at 6 (Aβ $p < 0.05$; pTau $p < 0.05$) and 12 months (Aβ $p < 0.05$; pTau $p < 0.05$) of age in insulin-treated 3xTg-AD with respect to vehicle-treated mice. BVR-A KO mice showed similar alterations observed in 3xTg-AD mice with age. Indeed, we found an early significant hyper-activation of the IR/IRS1/Akt axis at 2 months, followed by a consistent inhibition of the axis at 6 months, which persists until 12 months. Furthermore, brain insulin resistance was associated with an increased Aβ production in BVR-A KO mice with respect to WT. Finally, we showed that neuronal cells lacking BVR-A (i) rapidly develop insulin resistance [increased IRS1 inhibition ($p < 0.05$), reduced Akt activation ($p < 0.01$)] if treated with insulin (100 nM, 2 h); and (ii) can be rescued from insulin resistance only if insulin is co-administered with the BVR-A mimetic peptide, thus supporting findings about the effects of INI treatment in 3xTg-AD mice.

Conclusion: These findings shed light on the molecular mechanisms underlying the development of brain insulin resistance in AD and suggest BVR-A as potential therapeutic target to develop future treatments.

Supported by: FP7/2007-2013; Banca d'Italia n° 1286; SIR program n° RBSI144MT 8/17;

Disclosure: **E. Barone:** None.

250

Activation of LXR-β improves cognitive function of diabetic mice through ABCA1

R. Cai[1], S. Wang[2];
[1]Southeast University Medical School, Nanjing, [2]The Affiliated ZhongDa Hospital of Southeast University, Nanjing, China.

Background and aims: The disorder of cholesterol metabolism is the common pathological basis of type 2 diabetes and alzheimer's disease, and may be associated with the occurrence of cognitive dysfunction in diabetes. Liver X receptor (LXR) is an oxidized steroid-activated nuclear receptor that regulates intracellular cholesterol levels by regulating genes involved in cholesterol transport, such as ATP-binding cassette transporter A1 (ABCA1). LXR agonists can activate LXR-β, regulate cholesterol efflux and decrease β-amyloid protein (Aβ) levels through upregulate ABCA1 gene expression. ABCA1 levels in diabetic patients decrease significantly, looking for effective methods to up-regulate the expression of ABCA1 may be a new target for the prevention and treatment of diabetic cognitive dysfunction. This study aims to observe whether LXR-β agonist improves cognitive function of KKA^y mice by regulating ABCA1 protein and its effect on Aβ metabolism.
Materials and methods: This study used KKA^y mice as the animal model of cognitive dysfunction of T2DM. Different doses of T0901317 were applied to KKA^y mice as prevention or treatment method. The prevention group were given T0901317 at the age of 9 week; the treatment group received daily administration of T0901317 at the age of 15 week. The experiments of dark-avoidance test and the Morris water maze test were used to detect the behavioral changes of mice. The morphological changes in brain tissue of mice were detected by HE and Nissl staining. Immunohistochemistry was used to detect the expression of LXR-β, ABCA1 and $Aβ_{42}$ in hippocampus. RT-PCR and Western Blot were used to determine the mRNA and protein expression levels of LXR-β and ABCA1 in hippocampus of KKA^y mice.
Results: 1. In the dark-avoidance experiment, compared to the control group, mice in the model group showed shorter dark-avoidance latency and increased number of errors than the control group. In the water maze test, the latency period of the model group was prolonged; while the number of crossing the platform and the target quadrant residence time were all reduced. After prevention or treatment of T0901317, the learning and memory functions were significantly improved in KKA^y mice. 2.Compared to the control group, the structure of neurons in hippocampus of T0901317 intervention groups were improved to some extent. Neuron morphology damage in the intervention groups were reduced, and the Nissl bodies were increased. 3. Immunohistochemical staining results showed that the IOD values of LXR-β and ABCAl in model group were significantly decreased compared with those in control group, and the $Aβ_{42}$ staining was statistical deeper. After T0901317 prevention or treatment, the expression of LXR-β and ABCA1 increased, and the amount of $Aβ_{42}$ was significantly reduced. 4. The expression of LXR-β and ABCA1 at mRNA and protein levels were significantly decreased in diabetic model group than those in control group. The prevention and treatment groups all showed higher mRNA and protein levels of LXR-β and ABCA1 than the model group.
Conclusion: ABCA1 is involved in the pathogenesis of cognitive impairment in diabetes, which may have a protective effect on cognitive function. LXR-β agonist may improve diabetes associated cognitive dysfunction by increasing ABCA1 levels and decreasing Aβ deposition.
Supported by: National Natural Science Foundation of China (No.81570732)
Disclosure: **R. Cai:** None.

251

Dissecting shared pathophysiology of type 2 diabetes and depressive symptoms using multi-phenotype genome-wide association study

Z. Balkhiyarova[1], M.A. Kaakinen[1], H.H.M. Draisma[1], M. Timonen[2], J. Veijola[2], M.-R. Jarvelin[1], A. Nouwen[3], I. Prokopenko[1];
[1]Imperial College, London, UK, [2]University of Oulu, Oulu, Finland, [3]Middlesex University, London, UK.

Background and aims: Type 2 diabetes and depression are among the most prevalent non-communicable diseases, affecting quality of life and well-being. Epidemiological studies suggest shared aetiology between these conditions. Genetic variants, reportedly associated with these conditions, belong to pathways involved in lipid metabolism, cell proliferation, immune and inflammatory response, and oxidative stress, supporting the shared pathophysiology between them. However, the phenotypic variance responsible for type 2 diabetes and depression captured by genome-wide association studies (GWAS) explains only ~5% of susceptibility to these conditions. Our aims were: (1) to elucidate shared pathophysiological mechanisms between depression and type 2 diabetes; and (2) to identify genetic factors contributing to their co-morbidity using multi-variable analytical framework.
Materials and methods: We performed a multi-phenotype GWAS (MP-GWAS) for a combination of two diseases/disease symptoms evaluated at age 46 years: diagnosis of type 2 diabetes and a sum score of depressive symptoms (the Beck Depression Inventory) using GWAS data from the Northern Finland Birth cohort 1966 (NFBC1966, $N = 3597$). Participants were genotyped using the Illumina HumanCNV370DUO platform and imputed to the 1000G reference panel. After quality control of genetic data, >10 M autosomal single nucleotide polymorphisms (SNPs) were available for analysis. We performed the MP-GWAS by fitting a "reverse regression" model between each SNP and the linear combination of residuals for type 2 diabetes and depressive symptoms score, obtained after adjusting for sex and three principal components to control for population structure. We used the SCOPA software for the analysis.
Results: The single locus at *GPR12* (rs9507787) reached genome-wide significance ($P = 3.98 \times 10^{-8}$). It has been suggestively ($P < 10^{-7}$) associated with antipsychotic drug-induced QTc interval change in schizophrenia (PMID:29064910). Three loci at *MICAL2* (rs10765927), *INPP5K* (rs145536147), and near *ATXN8OS-KLHL1* (rs9599553) genes demonstrated suggestive effects and were in regions with previously associated with neuroticism, mean platelet volume, and schizophrenia/major depressive disorder. Replication of these findings in additional datasets is ongoing.
Conclusion: The results of this MP-GWAS provide first evidence for a possible shared aetiology between type 2 diabetes and depressive symptoms.
Disclosure: **Z. Balkhiyarova:** None.

252

Pragmatic lifestyle modification programme reduces depression and perceived stress in south Asian adults with pre-diabetes: a randomised controlled trial

S. Drignath[1], K. Winkley[2], M. Wijesuriya[3], L. Vasantharaja[3], D. Thamlini[3], J. Karalliedde[4];
[1]TU Dresden, Dresden, Germany, [2]Florence Nightingale School of Nursing & Midwifery, King's College London, London, UK, [3]Diabetes Association of Sri Lanka, Colombo, Sri Lanka, [4]Faculty of Life Sciences and Medicine, King's College London, London, UK.

Background and aims: Depression and high perceived stress are associated with increased risk for incident type 2 diabetes mellitus diabetes (T2DM). Previous research suggests that pragmatic lifestyle modification programme (trimonthly/P-LSM) versus less-intensive 12-monthly control LSM (C-LSM) is effective in improving cardio-metabolic outcome in a South-Asian prediabetic adult population. This was a secondary analysis of trial data to determine the effectiveness of LSM on depression and stress at 3 years follow-up.
Materials and methods: This was a randomised controlled trial conducted at the National Diabetes Centre, Sri-Lanka, 4672 participants at risk of

T2DM were randomised, (2596 were adults mean age 30.55, range 18–46 years, 52.2% males) to receive trimonthly (P-LSM $n = 1287$) or 12-monthly (C-LSM $n = 1309$) peer educator advice aimed at reducing weight, improving diet, psychological stress and increasing physical activity. Patient Health Questionnaire (PHQ9) was used to determine depressive symptoms and perceived stress scale (PSS) to determine psychological stress.

Results: Regression analysis was conducted with 3 year PHQ9 and PSS scores as the dependent variable. PHQ9 analysis was adjusted for baseline PHQ9 and PSS score, plus age, gender, marital status and BMI. PSS analysis was adjusted for baseline PSS and PHQ9 score, plus age, gender, marital status and BMI. There was significant improvement in depressive symptoms at 3 years ($p < 0.001$) and in perceived stress ($p < 0.001$) in the P-LSM versus the C-LSM group.

Conclusion: In a at-risk South-Asian adult population, a pragmatic LSM programme significantly reduced stress and depressive symptoms. The results presented here are important for the development of future LSM interventions to improve well-being and reduce risk of T2DM for adults with pre-diabetes.

Clinical Trial Registration Number: SLCTR/2008/003

Disclosure: **S. Drignath:** None.

OP 46 Neuropathy: nervy eyes

253

Corneal confocal microscopy detects greater reduction of small fibers in patients with painful neuropathy

A. Kalteniece[1], M. Ferdousi[1], S. Azmi[1], S. Adam[1], A. Marshall[1], A.J.M. Boulton[1], H. Soran[1], R.A. Malik[2];
[1]Institute of Cardiovascular Sciences, University of Manchester, Manchester, UK, [2]Weill Cornell Medicine-Qatar, Research Division, Qatar Foundation, Education City, Doha, Qatar.

Background and aims: About 50% of patients are affected by diabetic peripheral neuropathy (DPN), which is associated with high morbidity, foot ulceration and mortality. Corneal confocal microscopy (CCM) is a rapid non-invasive ophthalmic technique, which has been increasingly utilized to quantify corneal nerve morphology and established as surrogate end point for the assessment of diabetic neuropathy (DN). We aimed to assess corneal nerve morphology, keratocyte and Langerhans cells (LC's) density in patients with and without painful DN.

Materials and methods: A total of 87 patients with DN underwent detailed assessment of neuropathy disability score, quantitative sensory testing (including vibration perception threshold (VPT)), electrophysiology (peroneal motor nerve amplitude (PMNA) and velocity (PMNCV)), quality of life (QoL) using SF-36 questionnaire. Based on visual analogue score (VAS), patients were divided into those with painless (VAS ≤4) ($n = 35$) and painful (VAS >4) ($n = 52$) neuropathy age-matched to each other. CCM was performed in all participants. Corneal nerve fiber density (CNFD), branch density (CNBD), length (CNFL), corneal inferior whorl length (IW), LC's density and keratocyte density were quantified.

Results: CNFD ($p = 0.01$) and IW ($P = 0.01$) were significantly reduced in patients with painful compared to painless neuropathy. However, no significant difference was found for CNBD, keratocytes and LC's density, and nerve conduction studies comparing painful and painless neuropathy. QoL ($P < 0.0001$) was significantly reduced in patients with painful compared to painless neuropathy. There was a significant association between QoL and IW ($r = 0.721$, $P < 0.0001$), CNFD ($r = 0.246$, $P = 0.03$), CNBD($r = 0.312$, $P = 0.007$), CNFL ($r = 0.28$, $P = 0.01$).

Conclusion: This study shows significant reduction of small fibers and quality of life in patients with painful compared to painless DN.

	Painless (n=35)	Painful (n=52)	P value
Duration of diabetes	28.03 ±3.91	19.7 ±1.97	0.06
IFCC (mmol/mol)	58.12 ± 3.55	56.87 ± 1.85	0.7
CNFD, (no./mm^2)	23.28 ± 1.07	19.61 ± 1.02	0.01*
CNBD (no./mm^2)	52.84 ± 4.8	43.35 ± 3.92	0.1
CNFL, (mm/mm^2)	21.82 ± 1.07	19.43 ± 1.32	0.1
IW, (mm/mm^2)	25.08 ± 2.23	17.66 ± 1.74	0.01*
LC's density (no./mm^2)	50.52 ± 15.96	53.88 ± 17.21	0.8
PMNCV (m/s)	40.01 ± 0.8	40.67 ± 0.81	0.5
PMNA (m/V)	2.86 ± 0.35	2.97 ± 0.19	0.7
VPT (V)	25.67 ± 2.2	22.92 ± 1.6	0.3
SF-36 (0-100)	73.23 ± 2.44	51.9 ± 2.7	<0.0001*

Supported by: FP7

Disclosure: **A. Kalteniece:** None.

254

High RAGE expression might be responsible for early corneal nerve fibre damage in diabetic individuals

J. Leckelt[1], F. Thomas[1], A. Kott[1], R. Waterstradt[1], O. Stachs[2], A. Jünemann[2], M. Tiedge[1], S. Baltrusch[1];
[1]Institute of Medical Biochemistry and Molecular Biology, Rostock, Germany, [2]Department of Ophthalmology, Rostock, Germany.

Background and aims: In the assessment of diabetic neuropathy, in vivo corneal confocal microscopy (IVCCM) is currently a matter of

investigation to be established as a non-invasive diagnostic tool. Clinical and experimental animal studies demonstrated a loss of corneal nerve fibres already at the onset of diabetes mellitus. The underlying mechanisms are still unknown. However, neuronal damage could be mediated by increased binding of advanced glycation end products (AGEs) to the AGE receptor (RAGE). In various mouse strains we found that the RAGE expression in the cornea is significantly higher than in other tissues. The aim of this study was to investigate the sub-epithelial corneal nerve plexus in B6J-RAGE knockout mice during diabetes manifestation.

Materials and methods: Diabetes mellitus was induced in 8–10 weeks old male homozygote B6J-RAGE knockout mice by multiple low-dose-injections of streptozotocin (STZ) ($n = 6$). Sodium citrate treated B6J-RAGE knockout mice ($n = 6$) served as controls. Using IVCCM corneal nerve fibre length (CNFL) of the sub-epithelial nerve plexus was determined after 10 and 20 days. The region of the inferior whorl was analysed ex vivo at the endpoint by PGP.9.5 immunostaining and fluorescence microscopy. Expression of the extracellular and membrane RAGE domain was investigated in cornea and liver with quantitative RT-PCR.

Results: Gene expression of the membrane RAGE domain was 30-fold higher in cornea than in liver. Expression of the extracellular RAGE domain was not detectable confirming the knockout. At the endpoint STZ treated B6J-RAGE knockout mice developed significant signs of diabetes compared to controls (blood glucose concentration in mmol/l 6.9 ± 0.3 vs. 21 ± 1.0, $p < 0.0001$; body weight in g 26 ± 0.8 vs. 23 ± 0.6, $p < 0.02$; HbA1c in mmol/mol 21.0 ± 0.7 vs. 48.8 ± 1.7, $p < 0.0001$) and baseline. B6J-RAGE knockout mice showed only a minor reduction in CNFL after diabetes manifestation, which was comparable to the age-dependent nerve fibre loss in controls. The texture of the inferior whorl of the corneal sub-epithelial nerve plexus appeared identical in diabetic and control B6J-RAGE knockout mice.

Conclusion: After diabetes manifestation neither the CNFL nor the appearance of the inferior whorl of the corneal sub-epithelial nerve plexus changed in B6J-RAGE knockout mice. In contrast, in STZ diabetic B6J-thy1 YFP mice we could show a significant loss of corneal nerve fibres. Thus, significant RAGE expression in the cornea could explain the high susceptibility of corneal nerves to the increase in AGEs in diabetes mellitus.

Disclosure: **J. Leckelt:** None.

255

Corneal confocal microscopy shows nerve regeneration after treatment with exenatide/pioglitazone or basal/bolus insulin in patients with poorly controlled type 2 diabetes

R. Malik[1], G. Ponirakis[1], A. Khan[1], H. Al-muhannadi[1], I. Petropoulos[1], O. Migahid[2], A. Jayyousi[2], M. Abdul-Ghani[2];
[1]Weill Cornell Medicine, Doha, [2]Academic Health System, Hamad Medical Corporation, Doha, Qatar.

Background and aims: There are no approved therapies for diabetic peripheral neuropathy (DPN), a major risk factor for diabetic foot ulceration and amputation. The LEADER trial has recently shown a reduction in the incidence of foot ulceration and amputation in T2DM patients treated with Liraglutide. The aim of the study is to compare the effects of combination therapy with Exenatide once weekly/Pioglitazone vs basal plus prandial insulin on structural and functional measures of DPN in patients with poorly controlled T2DM.

Materials and methods: In a sub-group of the Qatar Study ($n = 46$; age = 50 ± 1, female = 47%, BMI = 30.9 ± 1.1, diabetes duration = 11.1 ± 1.0 years), patients with T2DM and poor glycemic control (HbA1c = 10.5 ± 0.3) despite treatment with maximal dose of sulfonylurea and metformin were randomly assigned to receive pioglitazone (30 mg) plus weekly Exenatide (2 mg) (combination) or basal plus prandial insulin (insulin), and underwent corneal confocal microscopy, assessment of painful neuropathy using DN4, vibration perception threshold using a Neurothesiometer and sudomotor function using Sudoscan.

Results: Participants were examined at baseline and at 12 months follow up. HbA1c was markedly reduced in the combination ($10.6 \pm 1.8\%$ to $7.1 \pm 1.4\%$, $P < 0.0001$) and insulin ($10.4 \pm 1.3\%$ to $7.2 \pm 0.9\%$, $P < 0.0001$) therapy groups and combination therapy also reduced triglycerides ($P = 0.01$). Subjects on insulin showed a significant increase in corneal nerve branch density (CNBD) ($P < 0.001$) and fibre length (CNFL) ($P < 0.001$) but not fibre density (CNFD), whereas subjects on combination therapy showed an increase in CNBD ($P = 0.04$) and DN4 ($P = 0.06$). Both therapies showed no improvement in vibration perception ($P = 0.5$–0.7) or sudomotor function ($P = 0.2$–0.8). There was no relationship between the degree of HbA1c reduction and improvement in corneal nerve parameters.

Conclusion: Exenatide/pioglitazone combination therapy and insulin therapy equally effectively reduce HbA1c in poorly controlled T2DM patients and induce corneal nerve fibre regeneration and a reduction in painful neuropathic symptoms, without an improvement in vibration perception or sudomotor function.

Disclosure: **R. Malik:** None.

256

Corneal confocal microscopy in screen-detected type 2 diabetes: ADDITION-Denmark

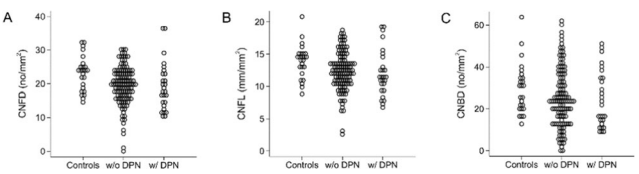
S. Toft Andersen[1], K. Grosen[2], H. Tankisi[3], M. Charles[1], N.T. Andersen[1], H. Andersen[4], I.N. Petropoulos[5], R.A. Malik[5], T.S. Jensen[2], P. Karlsson[2];
[1]Department of Public Health, Aarhus University, Aarhus, Denmark, [2]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark, [3]Aarhus University Hospital, Aarhus, Denmark, [4]Department of Neurology, Aarhus University Hospital, Aarhus, Denmark, [5]Central Manchester University Hospitals Foundation Trust, Manchester, UK.

Background and aims: We aimed to compare corneal confocal microscopy (CCM) measures between participants with type 2 diabetes with and without confirmed diabetic polyneuropathy (DPN) and controls without diabetes.

Materials and methods: CCM, nerve conduction studies, and assessment of symptoms and deficits of DPN were undertaken in 144 participants with type 2 diabetes and 25 controls without diabetes. DPN was defined according to the Toronto criteria for confirmed DPN. We used ANOVAs to compare CCM measures between groups and regression analyses to determine clinical variables associated with CCM measures.

Results: Corneal nerve fiber density (CNFD) was lower in participants with confirmed DPN ($n = 27$) ($P = 0.04$) and without confirmed DPN ($n = 117$) ($P = 0.01$) compared with controls. No difference between participants with and without confirmed DPN was observed ($P = 0.98$). There were no differences in corneal nerve fiber length and corneal nerve branch density between participants with and without confirmed DPN ($P = 0.06$ and $P = 0.29$, respectively). CNFD was associated with age (b −0.150 [95% CI 0.297; 0.003]), height (b 0.154 [95% CI 0.049; 0.260]), total cholesterol (b1.090 [95% CI 0.067; 2.112]), and LDL cholesterol (b 1.358 [95% CI 0.022; 2.694]). The effect of age on CNFD remained stable in multiple linear regressions including DPN status, sex, and diabetes duration.

Conclusion: This study supports CNFD being lower in participants with type 2 diabetes compared with controls. CCM measures were unable to differentiate between participants with type 2 diabetes with and without confirmed DPN.



Supported by: Novo Nordisk Foundation Challenge Programme grant
Disclosure: **S. Toft Andersen:** None.

OP 47 New insights in diabetes from mouse studies

257

Determining the contribution of the G319S variant of HNF1a to beta cell dysfunction and type 2 diabetes development

C.A. Doucette[1,2], T. Morriseau[1,2], P. Agarwal[3], M. Fonseca[3,2], C. Nian[4,5], B.A. Wicklow[6,2], E. Sellers[6,2], V.W. Dolinsky[3,2], F.C. Lynn[4,5]; [1]Physiology and Pathophysiology, University of Manitoba, Winnipeg, [2]Children's Hospital Research Institute of Manitoba, Winnipeg, [3]Pharmacology and Therapeutics, University of Manitoba, Winnipeg, [4]Surgery, University of British Columbia, Vancouver, [5]British Columbia Children's Hospital Research Institute, Vancouver, [6]Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada.

Background and aims: In Canada, Manitoba has the highest rate of youth-onset type 2 diabetes (T2D), which is amongst the highest in the world. Disproportionately affected are Indigenous youth with Oji-Cree heritage. Nearly 40% of Oji-Cree youth with T2D in Manitoba carry a single nucleotide polymorphism in the hepatocyte nuclear factor 1α (*HNF1α*) gene, causing replacement of a highly conserved glycine with a serine at codon 319 ("G319S"). Youth with T2D who harbour the G319S variant have higher $HbA1_c$, lower fasting insulin and less insulin resistance at diagnosis compared to youth with T2D who carry the wild type (WT) allele. These observations suggest that T2D in G319S carriers is primarily driven by a β cell defect; however, the mechanistic impact of the G319S variant on the β cell has not yet been explored. Here, we aimed to 1) develop novel animal and cell models to assess the functional impact of the G319S variant on insulin secretion; 2) determine if the G319S variant alone causes diabetes in a rodent model; and 3) elucidate the mechanism(s) whereby the G319S variant triggers β cell dysfunction and diabetes development.
Materials and methods: Using CRISPR-Cas9 to knock-in the g>a.955 mutation into MIN6 clonal β cells and C57BL6 mice, we have generated novel *in vitro* and *in vivo* model systems of the G319S variant. We assessed insulin secretion capacities in response to low and high glucose stimulation and examined how the G319S variant influenced glucose tolerance and metabolic health in mice. Finally, we performed targeted quantitative PCR (qPCR) to elucidate the mechanism(s) by which the G319S variant influences β cell function.
Results: Upon exposure to 16.7mM glucose, WT- and G319S-MIN6 secreted similar amounts of insulin; however, at low glucose, basal insulin secretion was reduced 3.2-fold in G319S-MIN6 compared to WT-MIN6 cells (0.8748 vs 2.764 ng insulin/μgDNA/hr; $p < 0.0001$). A similar phenotype of reduced basal insulin secretion was observed in islets isolated from heterozygous (G/S) male mice compared to G/G mice (0.4794 vs 1.850 ng insulin/μgDNA/hr; $p = 0.0032$). *In vivo* glucose tolerance tests revealed that G/S male mice developed glucose intolerance and fasting hyperglycemia by 3 months old, indicative of diabetes and consistent with reduced/insufficient basal insulin. Targeted qPCR was performed on of a number of genes with key roles in the β cell and found a 2-fold ($p = 0.0012$) upregulation of *Cpt-1a*, which has been shown to play a role in regulating basal insulin secretion capacity.
Conclusion: Our studies suggest that the G319S variant does not impair GSIS, which is typically observed in T2D; but rather, the G319S variant drives T2D via impaired basal insulin secretion, which may involve altered fatty acid metabolism. Future studies will examine if restoration of basal insulin secretion can prevent the diabetes phenotype in S allele-expressing mice as well as to determine how modern dietary influences interact with the G319S variant to influence T2D susceptibility.
Supported by: Research Mantioba; CHRIM
Disclosure: **C.A. Doucette:** None.

258

Restoration of insulin secretion after bariatric surgery in leptin deficient mice independently of weight loss

C. Amouyal[1], J. Castel[2], C. Guay[3], A. Lacombe[4], J. Denom[2], S. Migrenne[2], J. Sobel[3], C. Rouault[1], F. Marquet[1], S. Luquet[2], R. Scharfmann[5], K. Clement[1], R. Regazzi[3], C. Magnan[2], F. Andreelli[1,6]; [1]Sorbonne Université, INSERM, UMRS1166, NutriOmicsTeam, PARIS, France, [2]Unit of Functional and Adaptive Biology, Paris Diderot University-Paris 7, PARIS, France, [3]Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland, [4]PreclinICAN, Institute of Cardiometabolism and Nutrition (IHU-ICAN), PARIS, France, [5]INSERM, URMS1016, Institut Cochin, Paris Descartes Université, PARIS, France, [6]APHP, Pitié Salpétrière Hospital, Sorbonne université, ICAN, diabetology departement, Paris, France.

Background and aims: Bariatric surgery has demonstrated metabolic improvement especially in type 2 diabetes. We have previously published in high fat diet mice a bariatric procedure (entero-gastro-anastomosis with pyloric ligature, EGA) that recapitulates the beneficial effects on glucose homeostasis of the Roux-en-Y gastric bypass procedure in humans. Here, we studied the effects of EGA procedure on beta cell functionality in leptin deficient ob/ob mice.
Materials and methods: We performed EGA on ob/ob mice (Ob-EGA) and compared them with ob/ob mice before surgery (Ob-Ob), sham operated ob/ob mice (Ob-sham) and Ob-EGA group intraperitoneally infused by exendin [9-39] amide (Ex9) or saline. Metabolic exploration was performed before and 30 days post surgeries. Immunohistological staining of pancreas, pancreatic insulin content, expression of microRNAs (microarrays profiling and qPCR) and pancreatic islet cell composition by flow cytometry were performed. We used ANOVA or Mann-Whitney tests to compare data (mean ± SD) from different groups.
Results: OGTT dramatically improved after EGA despite persistent obesity and hyperphagia. Indeed, EGA procedure did not induce changes in food intake, body weight, body composition and energy expenditure in these mice. Furthermore, insulin and C peptide secretions were significantly increased after EGA (insulinemia, time 15 min (T15) of OGTT: Ob-Sham: 7.29 ± 3.7 ng/ml Ob-EGA: 28,8 ± 21 ng/ml, $p = 0.03$) whereas whole-body insulin sensibility (insulin tolerance test 0.75 and 2 UI/kg) and systemic inflammation (Il6, TNFa, MCP1, resistine) were unchanged. Infusion of Ex9 during 30 days after EGA resulted in lowering insulin secretion at T15 of OGTT without significant changes in glucose excursion. After EGA, pancreas immunohistology revealed a significantly higher level of insulin positive cell area (Ob-ob: 1,03 ± 0,5%; Ob-Sham: 1,42 ± 1%; Ob-EGA: 1,92 ± 1,16%, $p < 0,001$). and pancreatic insulin content increased. In contrast, ratio alpha/beta cells, alpha and beta cell proliferation rate (Ki67 index) and immune cell infiltration of pancreatic islets were unchanged by EGA. In comparison to Ob-sham mice, EGA procedure modified in pancreatic islets the expression of 5 microRNAs with known functions on apoptosis and/or glucose stimulated insulin secretion (up regulation of miR-184, $p = 0.0269$; down regulation of miR-106b, $p < 0.001$; miR-199a-3p, $p = 0.014$; miR-708, $p < 0.001$; and miR-34a, $p < 0.001$).
Conclusion: Resolution of diabetes after EGA was primarily explained by improvement of beta cell functionality in ob/ob mice and not exclusively by an incretin effect. This was associated with correction of defects in microRNA expression profiles observed in pancreatic islets of Ob-sham diabetic mice. Importantly, our model demonstrated that resolution of diabetes after bariatric surgery can be independent to body weight loss.
Supported by: SFD, INSERM
Disclosure: **C. Amouyal:** None.

259

A mutation in the NADH-dehydrogenase subunit 2 in the mitochondrial genome protects against diet-induced hepatosteatosis

M. Wietzke, M. Fischer, S. Baltrusch, M. Tiedge;
University of Rostock, Rostock, Germany.

Background and aims: The mitochondrial genome of the diabetes-resistant B6-mtALR (ALR) mouse strain is characterized by a mtDNA encoded ND2 mutation in complex I of the respiratory chain. ALR mice were less prone to liver steatosis despite higher mitochondrial ROS production and showed an effective hepatocellular energy metabolism. In this study, we focused on the susceptibility to hepatosteatosis after feeding a high fat diet. Therefore, we investigated metabolic parameters in serum and tissue, the NAFLD activity score in conplastic B6-mtALR mice compared to B6-mtAKR control strain. Furthermore we analysed expression of metabolic and inflammatory genes in liver.
Materials and methods: Conplastic B6-mt^{AKR} (AKR, control) and B6-mt^{ALR} (ALR, mutant) mice were fed with a high fat diet (HFD, western style diet) or control diet for 3 months. Liver tissue was collected and gene expression was quantified by real-time RT-PCR analysis. Liver histology was assessed using hematoxylin and eosin and Goldner`s trichrome stains. A blinded assessment of NASH was made according to the established NAFLD activity score. Lipid contents of the liver and collagen amounts reflected by hydroxyproline concentrations were quantified by commercial assays.
Results: Fasting blood glucose, serum triglyceride and serum cholesterin levels showed no differences in both strains before and after feeding a HFD for 3 months. However, hepatic triglyceride contents were lower in the ALR mouse strain. In line with these data histological analysis revealed lower hepatic lipid deposition (steatosis score) and ballooned hepatocytes in ALR mice after HFD. Hepatic collagen content was also lower in AKR mice indicating a milder grade of fibrosis. After 3 months of HFD feeding gene expression of glucokinase, Ppara and AcadI were significantly higher in ALR mice. Expression of Atp8, mtND2 and Ndufb4 as key genes for mitochondrial energy metabolism were also significantly increased in ALR mice after HFD. Interestingly, expression of the proinflammatory indicators TNFα and IL1b was increased in the liver from ALR mice. Histological analysis of liver tissue showed no signs of inflammation irrespective of mouse strain and diet.
Conclusion: The mtND2 mutation in complex I of the respiratory chain correlates with a lower risk to develop a hepatosteatosis after feeding a high fat diet. Despite high production of ROS, mitochondrial polymorphism ensuring adequate ATP production to metabolic demands confers protection against NAFLD. Our data suggest that analysis of mitochondrial polymorphisms in combination with functional analysis of energy metabolism in liver will help to stratify the risk for NAFLD in diabetic patients.
Disclosure: **M. Wietzke:** None.

260

The effect of kidney lipotoxicity on renal dysfunction in a model of metabolic syndrome

H. Malinska[1], M. Huttl[1], I. Markova[1], O. Oliyarnyk[1], J. Trnovska[1], P. Kačer[2], L. Kazdova[1];
[1]Institute for Clinical and Experimental Medicine, Prague, [2]Biocev, First faculty of medicine, Charles University, Prague, Czech Republic.

Background and aims: The development of metabolic syndrome-associated renal dysfunction can be exacerbated by dyslipidaemia, ectopic deposition of lipids and their toxic metabolites, impairment of lipid metabolism as well as insulin resistance. Renal dysfunction can also be affected by the production of pro-inflammatory and pro-fibrotic factors secreted from adipose tissue, which can directly impair kidney cells and potentiate insulin resistance. However, the aggravation mechanism of renal function is not fully understood. In our study, we investigated the manifestation of kidney lipotoxicity and its effect on renal dysfunction in a model of metabolic syndrome - hereditary hypertriglyceridaemic rats (HHTg) - by assessing microalbuminuria and target urine proteomics.
Materials and methods: Wistar control rats and a strain of HHTg rats, which served as a model of insulin resistance and metabolic syndrome, were fed a standard diet and observed over the course of ageing at 3, 12 and 20 months of age. Microalbuminuria was determined using the HPLC-method, while assessment of urine proteomics was performed using the mass spectrometry method. Gene expression was measured by quantitative RT-PCR.
Results: Chronically elevated levels of triglycerides in HHTg rats were associated with increased levels of FFA during OGTT as well as over a period of 24 hours (+80%, $p < 0.01$). In the case of the FFA lipid class, animals exhibited qualitative changes in FFA fatty acid composition, which was represented by an increased profile of saturated fatty acid ($p < 0.05$) and a decreased profile of n3-PUFA ($p < 0.01$). Ectopic lipid deposition in the kidneys of HHTg rats - triglycerides (+30%) and cholesterol (+10%) - was associated with markedly elevated microalbuminuria as ageing increased, despite the absence of microalbuminuria at the young age of 3 months in these animals. The presence of neutral lipids in the renal tubule and glomerulus was verified based on histological observation of HHTg rats. According to targeted proteomic analysis, 3-month-old HHTg rats (in comparison to age-matched controls) exhibited increased urinary secretion of pro-inflammatory parameters (MCP-1, IL-6, IL-8, $p < 0.01$) together with decreased urinary secretion of epidermal growth factor (EGF, $p < 0.01$) before manifestation of microalbuminuria. Elevation in the urinary secretion of inflammatory cytokines can be affected by increased weight in perirenal adipose tissue ($p < 0.01$) and increased relative expression of MCP-1 in perirenal adipose tissue ($p < 0.05$) as well as in kidney cortex ($p < 0.05$).
Conclusion: Our results confirm that dyslipidaemia and ectopic lipid accumulation play key roles in the development of metabolic syndrome-associated renal dysfunction. Assessing urinary secretion of pro-inflammatory cytokines and epidermal growth factor can help detect the early development of metabolic syndrome-associated renal dysfunction.
Supported by: the MH CZ – DRO grant (IKEM, IN 00023001)
Disclosure: **H. Malinska:** None.

OP 48 Functional imaging of insulin secretion

261

Glucose-induced changes of granules and actin in beta cells as visualised by TIRF-microscopy

D. Bruening, K. Reckers, I. Rustenbeck;
TU Braunschweig, Braunschweig, Germany.

Background and aims: Recently we have noticed that in contrast to potassium depolarization glucose diminishes the granule turnover in the submembrane space. Here, we verified this observation by gradually increasing the glucose concentration. In this context, the role of the actin cytoskeleton for the granule mobility was explored.
Materials and methods: All parameters were measured in perifused single beta-cells from NMRI mice. $[Ca^{2+}]_i$ was measured by the Fura technique, the mobility of granules in the submembrane space and the structure of the actin cytoskeleton were imaged by TIRF microscopy. Actin was visualized by tagRFP-Lifeact and the insulin granules were visualized by the cargo-directed label, Insulin-EGFP. Adenoviral transduction was used for labelling.
Results: Single pancreatic beta cells were continuously perifused during TIRF microscopy. After a 45 min adaption period the glucose concentration was raised stepwise from 5 to 15 mM and then to 30 mM, each step lasted for 10 min. For control purpose measurements of $[Ca^{2+}]_i$ were performed which showed an increase by 15 mM glucose starting after 2 min and reaching 115 nM at 5 min, followed by a slow decrease until 30 mM glucose raised it to about 140 nM at which it remained stable. At 2 and 7 min in the course of each step sequences of 200 TIRF images were acquired at 8 Hz. The number of granules in the first image of each sequence decreased throughout the perifusion as did the total number of granules identified per sequence. The number of granules which arrived at the plasma membrane and the number of granules which stayed for a short time only (≤1 s) increased slightly. However when net values were calculated by subtracting the values obtained during control perifusions (5 mM glucose throughout) from those of the test perifusions, a different picture emerged. The granule number in the first image and the total number was increased at 15 mM glucose and remained at the same level at 30 mM glucose. The net number of arriving granules and short-term granules, in contrast, showed a decrease with high glucose. At basal glucose the actin cytoskeleton in beta cells double transfected with tagRFP-LifeAct and hIns-EGFP showed a heterogeneous pattern. In one third of the cells stress fibers were prominent, in about half of the cells filopodia were visible. Areas with stress fibers were nearly devoid of granules. In cells with a predominant cortical actin web the granules were concentrated in the void spaces between the actin fibers. In the course of glucose stimulation the stress fibers remained virtually unchanged whereas the fiber structure of the cortical actin became blurred and dot-like shapes appeared.
Conclusion: Stimulatory glucose diminished the turnover of granules in the submembrane space. The distribution of the granules in this space appeared to be determined by the cortical actin network, which became loosened during exposure to high glucose. The occurrence of stress fibers and filopodia may be a reaction to the unphysiological condition of single cell existence.
Supported by: DDG, DFG
Disclosure: **D. Bruening:** None.

262

Probing the dynamic fingerprint of insulin secretory granules in living beta cells by spatiotemporal fluctuation spectroscopy

G. Ferri[1,2], M. Bugliani[3], L. Digiacomo[4], Z. Lavagnino[5], M. Occhipinti[3], P. Marchetti[3], G. Caracciolo[4], D.W. Piston[5], F. Cardarelli[1];
[1]NEST - Scuola Normale Superiore, Istituto Nanoscienze - CNR (CNR-NANO), Pisa, Italy, [2]Nanoscopy, Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy, [3]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [4]Department of Molecular Medicine, University of Rome "La Sapienza", Rome, Italy, [5]Department of Cell Biology and Physiology, Washington University Scholl of Medicine, St Louis, USA.

Background and aims: Insulin secretory granules (ISGs) exert their complex tasks through tight regulation of key structural and functional properties, such as size, diffusivity, mode of motion, etc. Quantitative description of these parameters in living β-cells is crucial for our understanding of insulin granule function in physiology and pathology. Despite the efforts, however, this goal remains a challenge in the field. We addressed this issue by a fluorescence-based spatiotemporal fluctuation spectroscopy of fluorescently-labelled granules in living cells. The method extracts quantitative information directly from imaging, in the form of a mean square displacement (MSD) versus time-delay plot (named iMSD), with no need for a-priori knowledge on the system, no need for complex labelling, no need to calculate trajectories. Relevant parameters, such as granule average diffusivity, anomalous coefficient, and size are readily accessible by this approach. Their clustering in a multidimensional parametric space defines the "fingerprint" of ISGs at the cell-population level.
Materials and methods: INS-1 E cell culturing. Plasmid transient transfection. Advanced imaging techniques (e.g. time-lapse confocal microscopy, iMSD analysis). Human pancreatic islets; human β-cells.
Results: To start, a reference granule structural and dynamic fingerprint is built by using INS-1 E cells expressing Proinsulin fused to a fluorescent protein (FP) under standard cell-culture conditions and validated under well-known stimuli, such as cholesterol overload, cytoskeleton disruption, glucose stimulation,. Then, the effect of different fluorescent labelling strategies is assessed, by using alternative granule-specific proteins such as Phogrin-FP, Syncollin-FP or IAPP-FP. While the latter two yield a ISG fingerprint very similar to that of the Proinsulin-FP reference, an hitherto-neglected neat alteration of both the structural and dynamic properties of the granule are observed upon the expression of Phogrin-FP, a widely used transmembrane ISG marker. To provide a better characterization of ISGs fingerprint in physiological conditions, we also dissociated human pancreatic islets and transfected the obtained isolated cells with Syncollin-FP. The iMSD-based comparison of INS-1 E and Human β-cells expressing Syncollin-FP highlights differences both in ISGs average size and diffusivity (i.e. ISG in human cells are smaller and slower).
Conclusion: Spatiotemporal fluctuation spectroscopy emerges as a powerful new platform for cell-based rapid and robust screening of the average properties of ISGs, allowing to easily highlight any alteration affecting their structural and/or dynamic characteristics. Reported results suggest caution in the definition of an experimental standard for the study of granule properties, both in terms of labelling strategies and of the cellular model selected.
Supported by: DRINN Project
Disclosure: **G. Ferri:** None.

263

Control of insulin secretion by basement membrane proteins

P. Thorn, O.H. Do, L. Cottle, E. Kosobrodova, M. Bilek, W.J. Gan;
University of Sydney, Sydney, Australia.

Background and aims: Loss of insulin secretion is a recognised characteristic of diabetes. It is therefore an important goal to determine how insulin secretion is controlled and what goes wrong in disease. Our recent work, in intact islets, shows that beta cells are structurally polarised and that insulin secretion is selectively targeted towards the vasculature. One of the factors that might regulate this targeting of secretion is the extracellular matrix. Here we test the hypothesis that extracellular matrix proteins, secreted by endothelial cells of the vasculature, induce the formation of focal adhesions which provide an important cue for beta cell orientation.

Materials and methods: A PDMS stamp was fabricated and used as a template to stamp basement membrane protein patterns onto coverslips. Isolated mouse pancreatic beta cells were cultured onto the coverslips and the cells imaged using a custom-built 2 photon microscope employed with a 60x objective. SRB was used as an extracellular dye excited at 940 nm with emission light collected >550 nm. A piezo objective stage (PI) rapidly moved the objective in z while collecting images at 3 frames/s.Cells were stimulated with 15 mM glucose and insulin granule exocytosis was detected as the sudden entry of the extracellular SRB into each fusing granule. Image analysis was performed with ImageJ.
Results: Using 3D live-cell two-photon microscopy, we showed that the spatial distribution of glucose-induced fusion of insulin granules was enriched towards the culture coverslips coated with matrix substrates (laminin 511, fibronectin or collagen IV). In contrast, granule fusion occurred all over the cell surface on poly-l-lysine coated control coverslips. Using microcontact printing to further pattern basement membrane proteins on the coverslips surface, we manipulated the area where the granules fused on the coverslips surface. To identify the mechanism of beta cell interaction with the substrate, we bathed the cells in drugs that affect the formation of focal adhesion such as the FAK inhibitor and beta-1 integrin blocking antibody. We showed that the targeting of secretory response is focal adhesion dependent. As the ongoing work, we are investigating the distribution of insulin granule fusion in beta cells following diabetic-induced modification of basement membrane proteins
Conclusion: In summary, our results provide evidence that basement membrane proteins are functionally important for targeting of insulin secretion and the process is focal adhesions dependent.
Supported by: NHMRC, SREI Sydney University, Diabetes Australia, JDRF Australia
Disclosure: **P. Thorn:** None.

264

The positive effect of apolipoprotein A-I on insulin secretion involves priming of insulin granules

O. Nilsson, R. Del Giudice, J.O. Lagerstedt;
Experimental Medical Science, Lund University, Lund, Sweden.

Background and aims: Increasing plasma levels of high density lipoproteins (HDL) and Apolipoprotein A-I (ApoA-I), its main protein component, have been shown to have an anti-atherogenic effect as well as positive action on glucose disposal in type 2 diabetic patients. ApoA-I directly increases insulin synthesis and secretion in insulin resistant mouse model, isolated pancreatic islets and clonal β-cells. The current study investigates ApoA-I's unexplored function to prime β-cells to increase insulin secretion and dissects mechanism behind this phenomenon.
Materials and methods: Rat insulinoma β-cell line INS1-E or isolated murine islets were used as experimental models for insulin secretion. Briefly, INS1-E or islets were pre-incubated for 2 h with low glucose in the presence or absence of ApoA-I, followed by 1 h challenge with glucose or other secretagogues, in the absence of the protein. Insulin was measured using ELISA (Mercodia) and normalized towards total protein content (BCA assay). Confocal and electron microscopy were applied to visualize the distribution of insulin granules in β-cells.
Results: Pre-incubation of β-cells and isolated murine islets with ApoA-I resulted in a significantly higher insulin secretion in response to high glucose (50% $n = 6$ ****$p < 0.0001$ and 30% $n = 12$ *$p < 0.05$ increase in insulin release for INS1-E and islets, respectively, as compared to high glucose control). ApoA-I's priming effect was even more pronounced when cells were challenged with secretagogues that lead to direct cell membrane depolarisation (four-fold higher insulin release in the presence of either Tolbutamide or KCl, as compared to cells incubated with ApoA-I in low glucose $n = 6$, ***$p < 0.001$ ****$p < 0.0001$, respectively). Increased reservoir of insulin granules at the cell membrane, resulting from ApoA-I's action, was confirmed by confocal and negative stain electron microscopy. Electron microscopy allowed for estimation of distances of the granules from the cell membrane, revealing that the incubation with ApoA-I resulted in a higher number of granules in the immediate proximity of the membrane (0–0.4 μm). Proinsulin levels, determined by Western blot, after stimulation with ApoA-I were found not to be changed with respect to controls. At the same time, insulin secretion as well as total insulin levels were significantly higher suggesting a regulatory function of ApoA-I on proinsulin processing as well as on insulin synthesis. Immunoblotting was applied to measure the expression of key regulators of proinsulin processing, protein convertase 1 (PC1/3) and carboxypeptidase E (CPE), showing significantly higher levels of PC1/3 as well as of CPE when ApoA-I was added ($n = 3$ *$p < 0.05$). Finally, inhibitors of endocytosis were used to block cellular uptake of ApoA-I. Incubation with inhibitors partially blocked ApoA-I endocytosis in INS1-E cells, resulting in increased accumulation of the protein at the cell membrane. Preliminary data on functional significance of the partially blocked uptake show an increase in ApoA-I's positive priming effect.
Conclusion: In the presented study, a novel function for ApoA-I has been identified, *i.e.*, its ability to prime β-cells to increase glucose-stimulated insulin secretion. The proposed mechanisms include increased number of insulin granules close to the cell membrane, increased processing of proinsulin and enhanced insulin synthesis. These findings are of interest for future anti-diabetic treatments with added anti-CVD effect.
Disclosure: **O. Nilsson:** None.

PS 001 Diabetes health burden

265

Health burden in type 2 diabetes and prediabetes: the Maastricht Study

M. Veugen, V. Onete, A. Koster, P. Dagnelie, N. Schaper, C. van der Kallen, M. Schram, R. Henry, C. Stehouwer;
On behalf of The Maastricht Study group; Cardiovascular Research Institute Maastricht (CaRIM); MUMC+, Maastricht, Netherlands.

Background and aims: Premature mortality in type 2 diabetes (T2D) is determined not only by classical complications (e.g., cardiovascular disease), but also by comorbidities (e.g., depression). A similar but less strong pattern is seen in prediabetes. However, prevalent comorbidities in T2D and prediabetes have not been comprehensively and quantitatively examined and compared to normal glucose metabolism (NGM) in a population-based study. Therefore, we investigated health burden in type 2 diabetes and prediabetes as compared to NGM, as defined by the presence of a diverse set of non-classical comorbidities in addition to the presence of classical complications and cardiometabolic risk factors.
Materials and methods: 3,410 participants (mean age: 59.8 ± 8.3 years; 52% men; 975 T2D, 511 prediabetes, and 1,924 NGM) of the population-based Maastricht Study underwent extensive phenotyping to determine presence of 15 comorbidities, 6 classical complications, and 10 cardiometabolic risk factors. These were added up into individual sum scores (for each category) and a combined sum score (with the 80th percentile in NGM as the highest sum score category cut-off). We used (multinomial) regression analyses adjusted for age and sex to study group differences.
Results: Individuals with T2D and prediabetes, as compared to NGM, more often had a comorbidities sum score of ≥3 (frequencies (95% CI): 49.4% (45.3; 53.4) and 28.9% (24.7; 33.2) vs 19.5% (17.8; 21.3), *p*-trend <0.001); a classical complication sum score of ≥2 (26.6% (23.1; 30.1; $p < 0.001$ vs NGM) and 10.1% (7.8; 12.7; $p = 0.065$ vs NGM) vs 8.0% (6.9; 9.3)); a cardiometabolic risk factors sum score ≥6 (39.7% (35.9; 43.4) and 28.5% (24.5; 32.6) vs 14.0% (12.5; 15.6); *p*-trend <0.001); and a combined sum score of ≥8 (60.7% (56.7; 56.7; 64.7) and 38.2% (33.8; 42.7) vs 21.0% (19.2; 22.9), *p*-trend <0.001).
Conclusion: Our results show, independently of age and sex, and in a population-based setting, a considerably greater health burden in both T2D and prediabetes, which to an important extent is related to non-classical co-morbidities. These results emphasize the need for awareness of comorbidities in (pre)diabetes and for further investigation of the potential aetiological role of so-called mild hyperglycaemia.
Supported by: EFRO, MUMC+
Disclosure: **M. Veugen:** None.

266

Circulating cardiac stress, vascular dysfunction and inflammatory biomarkers predict acute kidney injury in French type 2 diabetes patients: the SURDIAGENE cohort

P. Saulnier[1], B. Sautenet[2], E. Gand[1], M. Fraty[1], J. Halimi[2], E. Thorin[3], O. Meilhac[4], E. Burillo[4], G. Velho[5], R. Roussel[5], S. Ragot[1], S. Hadjadj[1], for the SURDIAGENE study group;
[1]INSERM CHU Poitiers, Poitiers, France, [2]CHU Tours, Tours, France, [3]Montreal Heart Institute, Montreal, Canada, [4]INSERM, Saint Denis de la Réunion, France, [5]INSERM, Paris, France.

Background and aims: Acute kidney injury (AKI) is a related to chronic kidney disease and death in patients from the general population, with or without type 2 diabetes. Nevertheless AKI biomarkers are rarely validated in diabetes population. We aimed to explore the individual and combined prognostic value of 8 circulating candidate markers for AKI. This include markers of cardiac and endothelial dysfunction (mid-regional-pro-adrenomedullin [MRproADM], angiopoietinlike-2 [ANGPTL2], N-terminal prohormone brain natriuretic peptide [NTproBNP]) oxidative stress (fluorescent advanced glycation endproducts [AGEs], carbonyls), cardio-renal pathways (copeptin [CTproAVP]), and inflammation (soluble TNF receptor 1 [TNFR1]).
Materials and methods: We prospectively followed-up 1345 (565 women/780 men) type 2 diabetes participants of a French single-centre hospital-based cohort (SURDIAGENE) with baseline GFR ≥30 ml/min/1.73 m^2 and no renal replacement to onset of AKI, death, or December 31, 2015, whichever came first. Intrahospital AKI was diagnosed and staged using the KDIGO criteria (increase in serum creatinine concentration by 0.3 mg/dL or increase in serum creatinine to ≥1.5 times baseline). Cox models were used to estimate the association between time to AKI and baseline value of each biomarker after adjustement for usual risk factors: sex, diabetes duration, HbA1c, systolic blood pressure, GFR, ACR, use of antihypertensive, and history of cardiovascular disease. Hazard ratios were reported per 1 SD increment of the logarithm of the biomarker concentration.
Results: At baseline, mean ± SD age was 64 ± 11 years, diabetes duration 14 ± 10 years, HbA1c 7.8 ± 1.6%, and eGFR 77 ± 21 ml/min/1.73 m^2, and median (IQR) ACR 3 (1–10) mg/mmol. During a median follow-up of 4.7 years, 449 (33%) patients developed an AKI. In univariate analysis, each biomarker was significantly associated with AKI, and 6 remained associated after multivariable adjustment (Table). The addition of a multimarker score summing standardized and weighted values of these 6 markers to the model including usual risk factors significantly improved C-statistics (0.724 to 0.759, $P < 0.0001$), and 5-year risk-predictive performance (relative integrated discrimination improvement index = 0.435, $P < 0.0001$).
Conclusion: A panel of 6 biomarkers representing cardiac, vascular and inflammatory pathways improved the prediction of AKI over usual risk factors in patients with type 2 diabetes.

	Univariate		Multivariate	
Biomarkers	Hazard Ratio (95% CI)	*P*-value	Hazard Ratio (95% CI)	*P*-value
AGEs	**1.30 (1.18-1.43)**	**<0.0001**	**1.12 (1.02-1.24)**	**0.020**
Carbonyls	**1.09 (1.00-1.20)**	**0.055**	1.03 (0.95-1.12)	0.519
ANGPTL2	**1.60 (1.47-1.75)**	**<0.0001**	**1.29 (1.16-1.43)**	**<0.0001**
NTproBNP	**1.85 (1.69-2.03)**	**<0.0001**	**1.51 (1.35-1.68)**	**<0.0001**
CTproAVP	**1.63 (1.49-1.78)**	**<0.0001**	**1.33 (1.20-1.48)**	**<0.0001**
MRproADM	**2.13 (1.95-2.33)**	**<0.0001**	**1.93 (1.70-2.18)**	**<0.0001**
TNFR1	**2.12 (1.93-2.33)**	**<0.0001**	**1.79 (1.57-2.03)**	**<0.0001**
Biomarker risk score	**2.38 (2.17-2.61)**	**<0.0001**	**2.35 (2.05-2.70)**	**<0.0001**

Risk of acute kidney injury according to biomarkers in patients of the SURDIAGENE
HRs per 1 SD increase

Supported by: PHRC
Disclosure: **P. Saulnier:** None.

267

Glycaemic and cardiovascular risk factor burden post therapy intensification in patients with type 2 diabetes in the USA

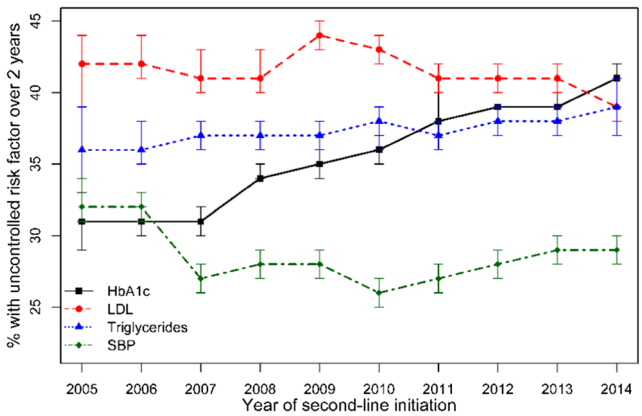
S. Paul, O. Montvida;
Melbourne EpiCentre, University of Melbourne, Melbourne, Australia.

Background and aims: The recent drive for holistic management of cardiovascular (CV) and glycaemic risk factors for individualized treatment of patients with type 2 diabetes (T2D) requires a detailed evaluation of population-level risk factor dynamics. Aim is to evaluate the persistent glycaemic and CV risk factor burden over 2 years post therapy intensification (TI).
Materials and methods: From US Centricity Electronic Medical Records, 276,884 patients with T2D aged 18–80 years, who intensified metformin with a second-line anti-diabetic drug (ADD), had longitudinal prescription information for lipid-modifying and blood pressure (BP) control therapies, were selected. Second-line ADDs were sulfonylurea

(SU), DPP-4 inhibitor (DPP-4i), GLP-1 receptor agonist (GLP-1RA), thiazolidinedione (TZD) and insulin. For those with/without history of CV disease (CVD) at TI, systolic BP (SBP) ≥130/140 mmHg and LDL ≥70/100 mg/dL were defined as uncontrolled. Triglycerides (Trig) ≥150 mg/dL and HbA1c ≥7.5% were defined as uncontrolled. Based on 6-monthly longitudinal measures over 24 months post TI, the burden was defined as continuously uncontrolled levels of risk factors.

Results: With 3.7 years mean follow-up, patients were 59 years old, 70% obese, 22% had history of CVD, 60/30/50/48% had uncontrolled HbA1c/SBP/LDL/Trig at TI. 191,883 (69% of cohort) had follow-up ≥2 years. Among patients without a history of CVD, 77% and 63% were receiving therapies for BP and lipid control respectively. Among those with a history of CVD, these proportions were 94% and 84% respectively. The proportion of patients with HbA1c ≥7.5% consistently over 2 years ranged between 31–41% when evaluated by year of TI, with increasing pattern from 2005 to 2014 (Figure). Among those on lipid-modifying drugs, 40–42% continued to have high LDL and around 37% consistently had high Trig over time. Being on BP control therapies, 27–32% continued to have SBP burden over 2 years. Among patients receiving lipid or BP control therapies, 62% failed to achieve control in HbA1c + LDL, 62% failed to achieve control in HbA1c + Trig, and 55% failed to achieve control in HbA1c + SBP over 1 year post TI. These proportions were similar at 2 year follow-up. Compared to patients intensified with SU, those intensified with DPP-4i, GLP-1RA and TZD were 22%, 33% and 35% significantly less likely to fail in HbA1c + LDL control over 2 years respectively (all $p < 0.05$). Patients treated with DPP-4i and GLP-1RA were 32% and 45% less likely to fail in HbA1c + SBP control over 2 years respectively, compared to those with SU (all $p < 0.05$).

Conclusion: More than a third of patients with T2D continued to have clinically unacceptable HbA1c and lipid levels, and about 28% failed to control blood pressure over 2 years post therapy intensification for the multiple risk factor control. The glycaemic risk burden has been increasing over last decade. Treatment with incretins and TZD appears to have residual beneficial association with simultaneous glycaemic and CV risk factor control, compared to SU.



Disclosure: **S. Paul:** None.

268

Prevalence of cardiovascular disease and treatment pattern in patients with type 2 diabetes in a real world setting

W. Weng[1], Y. Tian[1], S.X. Kong[2], R. Ganguly[1], M. Hersloev[2], J. Brett[3], T. Hobbs[4];
[1]Health Economics & Outcomes Research, Novo Nordisk, Inc, Plainsboro, [2]Clinical Development & Outcomes Research, Novo Nordisk, Inc, Plainsboro, [3]Medical Affairs, Novo Nordisk, Inc, Plainsboro, [4]CMR, Novo Nordisk, Inc, Plainsboro, USA.

Background and aims: Primarily based on the results of two large cardiovascular outcomes trials, the American Diabetes Association (ADA) issued its 2017 Standards of Medical Care in Diabetes (SOC) which recommends, "for patients with long-standing sub-optimally controlled Type 2 Diabetes (T2D) and established atherosclerotic cardiovascular disease (ASCVD) - empagliflozin or liraglutide should be considered as they have been shown to reduce cardiovascular and all-cause mortality when added to standard care." The current study assessed the prevalence of ASCVD associated with patient characteristics, medication usage, and healthcare utilisation patterns in a real-world T2D population prior to the 2017 ADA standard care guidelines.

Materials and methods: This was a retrospective, cross-sectional analysis of a large US administrative claims database in 2015. Inclusion criteria were: ≥2 diagnoses for T2D or ≥1 T2D diagnosis + ≥1 oral antidiabetic drug (OAD) claim, and no more than 1 T1D diagnosis according to ICD-9/-10 codes, ≥18 years of age, and continuous health plan enrolment in 2014 & 2015. Eligible patients were divided into two groups: T2D patients with ASCVD (with-ASCVD) and those without ASCVD (without-ASCVD). ASCVD was defined based on the ICD-9/-10 codes corresponding to ADA 2017 SOC of atherosclerotic CVD: Acute Coronary Syndrome, Myocardial Infarction, Peripheral Arterial Disease, Stroke, Transient Ischaemic Attack, and Coronary or other arterial particularisation. Sub-group analyses were conducted for 3 age groups (18–44, 45–64, and 65+ years).

Results: A total of 1,202,596 T2D patients were identified and 45.2% had established ASCVD. The with-ASCVD group was older than the without-ASCVD group (mean age 67 vs. 56 years) and had a slightly higher percentage of males (52.9% vs. 49.2%). Less than 10% of all T2D patients had visited an endocrinologist. About 40% of T2D patients with-ASCVD had visited a cardiologist, compared to 11% in the without-ASCVD group. Generally, the use of glucagon-like peptide-1 receptor agonists (GLP-1RA) and sodium-glucose co-transporter 2 inhibitors (SGLT-2i) was very low overall (<11%); and even lower in the with-ASCVD group (<9%). The prevalence rate of ASCVD among 3 age groups (18–44, 45–64, and 65+ years) were 15%, 36%, and 71%, respectively, and use of GLP-1RA and SGLT-2i was 5% or lower among the 65+ subgroup, regardless of ASCVD status.

Conclusion: This analysis of a large, real world claims database showed a high prevalence of ASCVD among T2D patients. It also confirmed as a baseline assessment, the low use of GLP-1RA and SGLT-2i in these at-risk patients prior to the ADA recommendations. It would be of interest to assess changes in the use of GLP-1RA and SGLT-2i among T2D patients with ASCVD based on these new recommendations in the coming years.

Supported by: Novo Nordisk, Inc

Disclosure: **W. Weng:** None.

269

High LDL cholesterol levels and risk of peripheral vascular diseases: a Mendelian randomisation study including 116,419 individuals from the general population

F. Emanuelsson[1], B. Nordestgaard[2], A. Tybjaerg-Hansen[1], M. Benn[1];
[1]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, [2]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark.

Background and aims: High LDL cholesterol levels are causally involved in the pathogenesis of atherosclerosis and are causally related to an increased risk of cardiovascular disease. It is unknown whether high LDL cholesterol levels are causally related to an increased risk of microvascular diseases, such as retinopathy and neuropathy, and peripheral vascular diseases also involving larger arteries, such as chronic kidney disease (CKD), and peripheral arterial disease (PAD). We hypothesized that high LDL cholesterol levels are causally related to the risk of retinopathy, neuropathy, CKD and PAD in the general population.

Materials and methods: We included 116,419 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study and used Mendelian randomization to examine causality between high LDL cholesterol levels and peripheral vascular endpoints. As genetic instrument we selected and genotyped eleven variants in the *LDLR*, *APOB*, *HMGCR*, *NPC1L1* and *PCSK9* genes. To test whether we could replicate the findings in another general population cohort, we performed a 2-sample Mendelian randomization analysis using genetic variants associated with high LDL cholesterol levels in the Global Lipid Genetic Consortium, and peripheral vascular endpoints from the UK Biobank.

Results: Observationally we found no association between high LDL cholesterol levels and risk of retinopathy (P trend = 0.12) or neuropathy (P trend = 0.005). We found a stepwise increase in the hazard rate of CKD and PAD with higher LDL cholesterol levels, with a hazard ratio (HR; 95% confidence interval) of 1.06(0.99–1.14) for CKD and 1.37(1.20–1.57) for PAD in individuals with LDL cholesterol levels above the 95th percentile *versus* below the 50th percentile. In the genetic, causal analyses the risk ratio (95% confidence interval) of disease for a 1 mmol/L higher LDL cholesterol level was 1.06 (0.24–4.58) for retinopathy, 1.05 (0.25–1.72) for neuropathy, 3.10 (1.79–5.39) for CKD and 1.96 (1.26–3.06) for PAD. Summary level data from the UK Biobank using the weighted median of instrumental variable estimates Mendelian randomization gave a risk ratio of 0.65 (0.25–1.70) for retinopathy, 0.86 (0.51–1.46) for neuropathy, 0.88 (0.66–1.18) for CKD and 1.57 (0.91–2.71) for PAD.

Conclusion: Our study suggests that LDL cholesterol has no causal effect on peripheral microvascular diseases such as retinopathy and neuropathy; but may have a causal effect on peripheral arterial diseases involving larger arteries such as PAD and CKD. The findings were replicated in the UK Biobank cohort with similar results for retinopathy, neuropathy and PAD, but with conflicting results for CKD.

Supported by: Danish Council of Independent Research

Disclosure: **F. Emanuelsson:** None.

270

More adverse differences in cardiometabolic risk factor levels in women with prediabetes and diabetes compared with men: the Maastricht study

R. de Ritter[1,2], S.J.S. Sep[1,2], M.T. Schram[1,2], E. Derksen[1], C.J.H. van der Kallen[1,2], A. Koster[3,4], N. Schaper[2,4], A.A. Kroon[1,2], R.M.A. Henry[1,2], M.M.J. van Greevenbroek[1,2], S.A.E. Peters[5], P.C. Dagnelie[2,4], C.D.A. Stehouwer[1,2];
[1]Dept. of Internal Medicine, Maastricht University Medical Centre +, Maastricht, Netherlands, [2]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, Netherlands, [3]Dept. of Social Medicine, Maastricht University, Maastricht, Netherlands, [4]School for Public Health and Primary Care (CAPHRI), Maastricht University, Maastricht, Netherlands, [5]The George Institute for Global Health, University of Oxford, Oxford, UK.

Background and aims: Type 2 diabetes (T2D) attenuates, or may even reverse, the protective effect of female sex on the risk of cardiovascular disease. This may in part be explained by a relatively more adverse cardiometabolic profile in women with versus without T2D compared with men with versus without T2D. However, the mechanisms responsible for these sex differences remain to be elucidated. The metabolic sequences that eventually lead to T2D precede the development of hyperglycemia by years or even decades. Whether women before the onset of T2D already suffer from a relatively more adverse cardiovascular risk profile has only scarcely been investigated. We aimed to evaluate sex differences in metabolic syndrome-related markers of cardiovascular risk both before and after the onset of T2D .

Materials and methods: We analyzed cross-sectionally in a T2D-enriched population-based cohort (age 40–75 y), by means of linear regression analyses adjusting for age, whether the association of prediabetes and T2D with metabolic syndrome-related markers of cardiovascular risk differed between women (n = 1536) and men (1666). Differences in associations between men and women were tested by incorporating interaction terms of glucose metabolism status with sex into the regression models. Sex-stratified analyses were performed with normal glucose metabolism as reference category.

Results: Compared to normal glucose metabolism, women with prediabetes had more adverse age-adjusted mean differences in systolic BP (3.52 (95%-CI 0.73; 6.31) mmHg larger, i.e. mean difference in women exceeds that in men by 3.52), HDL cholesterol (0.10 (0.01; 0.18) mmol/L smaller) and triglycerides (0.14 (0.05; 0.24) mmol/L larger) than men with prediabetes. Mean differences for prediabetes in women equaled those for T2D in men. Compared to normal glucose metabolism, the more adverse mean differences in body mass index and waist circumference as observed for T2D in women versus men (2.27 (1.66; 2.89) kg/m^2, and 5.41 (3.74; 7.08) cm higher, respectively), were less evident for prediabetes (0.53 (−0.16; 1.22) kg/m^2, and 0.99 (−0.89; 2.87) cm, respectively).

Conclusion: The novel finding of this study is that there are already sex differences in cardiometabolic risk factors before the onset of T2D, to women's disadvantage. Besides the relatively more adverse cardiometabolic profile in women with versus without T2D as compared to their male counterparts, sex differences in cardiometabolic risk factors, which were less favourable for the female sex, were also observed in their association with prediabetes. This suggests that the cardiometabolic risk profile of women has deteriorated to a larger extent even before the onset of T2D.

Supported by: ERDF, PL, SDW, PSID, CVC, CARIM, CAPHRI, NUTRIM, SA, HFL, JC, NNF, SAN, ZMW

Disclosure: **R. de Ritter:** None.

271

Effect of number of achieved targets for risk factors on coronary artery disease (CAD) in those with and without diabetes

K. Fujihara, M. Harada, M. Yamamoto, Y. Mathubayashi, M. Kaneko, T. Osawa, T. Yamada, K. Kato, S. Kodama, H. Sone;
Niigata University, Niigata, Japan.

Background and aims: Although control of multiple risk factors is essential to prevent CAD in persons with and without DM, longitudinal studies are scarce that directly and quantitatively compared effects of such control in DM and non-DM.

Materials and methods: We investigated effects on subsequent CAD of the number of controlled risk factors among blood pressure, LDL cholesterol and HbA1c using a nationwide claim-based database (median follow-up 4.8 y) in Japan. Targets were based on ADA and Japanese guidelines.

Results: Of 207,029 non-DM persons, 42.8% and 37.9% were at target for 1 and 2 factors, respectively. Of 13,471 persons with DM, 39.5%, 32.1%, and 10.0% were at target for 1, 2, and 3 factors, respectively. Multivariate Cox analysis showed reduced CAD risk with increased numbers of risk factor targets reached in DM compared to non-DM (Table, upper). However, in DM, although fulfillment of the target for only 1 risk factor significantly elevated the hazard ratio (HR) (i.e., 1.98 (1.28–3.07)) to non-significance (i.e., 1.03 (0.68–1.66)), no further significant HR reduction below reference (i.e., non-DM with no target achieved) was found (Table, lower).

Conclusion: These findings show that composite control of modifiable risk factors has a larger effect in DM compared to non-DM, but the effect was not sufficient to bring CAD risk in DM below that for non-DM persons with none of 3 risk factors being at target based on current target levels.

Adjusted HRs (95% CI) for CAD events among persons with and without DM according to individual and composite risk factor targets achieved

events/n	Non-DM (200/207,029)	DM (91/13,471)
	Adjusted HR (95% CI)	Adjusted HR (95% CI)
No. risk factors with target achieved		
None	Ref	Ref
Any one target achieved vs. none	0.79 (0.58-1.07)	0.61 (0.37-0.996)
Any two targets achieved vs. none	0.63 (0.41-0.96)	0.51 (0.30-0.89)
All three targets achieved vs. none	NA	0.28 (0.10-0.80)
None	Ref	1.98 (1.28-3.07)
Any one target achieved	0.77 (0.57-1.05)	1.03 (0.68-1.66)
Any two targets achieved	0.60 (0.39-0.91)	0.81 (0.51-1.30)
All three targets achieved	NA	0.49 (0.18-1.45)

Adjusted for age, sex, BMI, HDLC, current smoking, antihyperglycemic drug therapy, antihypertensive drug therapy and antihyperlipidemic drug therapy.

Target levels were blood pressure <130/80 mmHg, LDL-Cholesterol <3.1 mmol/L, and HbA1c l <7%.

Disclosure: **K. Fujihara:** None.

272

The use of computer simulation modelling to estimate complications in patients with type 2 diabetes: validation of the cornerstone diabetes simulation model

Z.T. Su[1], L. Sauriol[2], J. Bartelt-Hofer[3], S. Brown[1], E. Lew[3], L. Annemans[4], D.T. Grima[1];
[1]Cornerstone Research Group, Burlington, Canada, [2]Sanofi, Laval, Canada, [3]Sanofi, Chilly-Mazarin, France, [4]Ghent University, Ghent, Belgium.

Background and aims: Decision analytic models aim to extend clinical trial (CT) findings to predict long-term outcomes, compare alternative therapies, and test exploratory scenarios. This requires credible models, as such the ability of models to predict long-term complications and mortality should be validated as suggested by the American Diabetes Association (ADA) guidelines for diabetes computer modelling. This study aimed to validate the Cornerstone Diabetes Simulation Model (CDSM), a Microsoft Excel-based, non-product specific, patient level simulation for type 2 diabetes mellitus (T2DM) based on the revised United Kingdom Prospective Diabetes Study Outcomes Model (UKPDS-OM2, aka UKPDS82) risk equations, following the ADA guidelines for diabetes computer modelling.
Materials and methods: Three levels of validation were conducted. Internal validation was assessed through independent review and model stress-testing. External validation was addressed by populating the CDSM with baseline characteristics and treatment effects from 4 major diabetes CTs that were used in the 5th Mount Hood Diabetes Challenge (MH5) for computer simulation models; simulated vs. observed clinical outcomes were compared via coefficient of determination (R^2). Cross-validation of predicted outcomes was tested vs. 8 diabetes models that participated in the MH5. Results are presented for the absolute risk of each clinical outcome and the difference in absolute risk between control and intervention arm in each CT.
Results: There were 45 single and composite endpoints across the 4 CTs. The CDSM could predict 18 of these endpoints, including 15 for macrovascular complications (myocardial infarction, stroke, congestive heart failure, ischemic heart disease, and composite endpoints based on these events) and 3 for all-cause mortality. While the CDSM could predict renal failure, blindness, amputation, and ulcer, it could not predict any CT-specific microvascular complications due to differences in event definitions. Compared to other diabetes models, the CDSM achieved a high level of validity in predicting all endpoints (R^2 0.637), macrovascular complications (R^2 0.822), and all-cause mortality (R^2 0.975). The CDSM ranked in the top 4 of all models in predicting absolute risks for all endpoints, macrovascular complications, and all-cause mortality. The CDSM performed especially well in predicting risk differences for all endpoints and macrovascular complications (Table).
Conclusion: The CDSM for T2DM provides good prediction of diabetes-related complications when compared to actual CT outcomes and alternative models. Future research to assess predictions of costs and cost-effectiveness would complement this validation and provide useful information for decision-makers to optimize health resource allocation.

Table. Summary of external validation and cross-validation results for diabetes clinical trials and simulation models reported in the Fifth Mount Hood Diabetes Challenge.

	All endpoints		Macrovascular complication endpoints		All-cause mortality endpoints		Other endpoints	
Diabetes model	R^2 for absolute risks (n)	R^2 for absolute risk differences (n)	R^2 for absolute risks (n)	R^2 for absolute risk differences (n)	R^2 for absolute risks (n)	R^2 for absolute risk differences (n)	R^2 for absolute risks (n)	R^2 for absolute risk differences (n)
CDSM	0.637 (18)	0.442 (18)	0.822 (15)	0.767 (15)	0.975 (3)	0.586 (3)	N/A (0)	N/A (0)
IMS CORE	0.496 (34)	0.113 (34)	0.789 (24)	0.304 (24)	0.877 (3)	0.677 (3)	0.480 (7)	0.025 (7)
MICHIGAN	0.212 (23)	0.041 (23)	0.260 (13)	0.288 (13)	0.995 (2)	1.000 (2)	0.014 (8)	0.029 (8)
ECHO-T2DM	0.519 (33)	0.325 (33)	0.805 (23)	0.338 (23)	0.968 (3)	0.103 (3)	0.247 (7)	0.096 (7)
UKPDS-OM1	0.616 (21)	0.290 (21)	0.843 (17)	0.470 (17)	0.959 (3)	0.007 (3)	1.000 (1)	N/A (1)
UKPDS RE	0.733 (4)	0.665 (4)	0.941 (3)	0.991 (3)	N/A (0)	N/A (0)	1.000 (1)	N/A (1)
CDC-RTI	0.724 (20)	0.212 (20)	0.741 (10)	0.602 (10)	0.935 (3)	0.349 (3)	0.743 (7)	0.008 (7)
CARDIFF	0.505 (21)	0.028 (21)	0.819 (17)	0.076 (17)	0.951 (2)	1.000 (2)	0.835 (2)	N/A (2)
EBMI	0.134 (17)	0.418 (17)	0.896 (11)	0.562 (11)	0.982 (3)	0.900 (3)	0.734 (3)	0.614 (3)

Abbreviations: CARDIFF, Cardiff Research Consortium Model; CDC-RTI, the Centers for Disease Control and Prevention-RTI Diabetes Cost-effectiveness Model; CDSM, Cornerstone Diabetes Simulation Model; EBMI, the Evidence-Based Medicine Integrator Simulator; ECHO-T2DM, The Swedish Institute of Health Economics model titled Economics and Health Outcomes in Type 2 Diabetes Mellitus Model; IMS CORE, IMS CORE Diabetes Model; MICHIGAN, the Michigan Model for Diabetes; n, number of endpoints predicted; R^2, coefficient of determination; risk Δs, risk differences; UKPDS-OM1, the United Kingdom Prospective Diabetes Study Outcomes Model; UKPDS RE, the United Kingdom Prospective Diabetes Study Risk Engine.

Disclosure: **Z.T. Su:** Employment/Consultancy; employee of Cornerstone, which received consulting fees from Sanofi.

PS 002 Diabetes prevalence

273

Prevalence of undiagnosed diabetes and hypertension in high-risk adults across Europe: Feel4Diabetes study

G.E. Dafoulas[1], O. Androutsos[2], C. Mavrogianni[2], C.-P. Lambrinou[2], S. Liatis[1], G. Cardon[3], J. Lindstrom[4], V. Iotova[5], T. Tankova[5], R. Mateo-Gallego[6], L.A. Moreno[7], C. Semanova[8], M. de Sabata[9], K. Makrilakis[1], Y. Manios[2];
[1]National and Kapodistrian University of Athens, Athens, Greece, [2]Department of Nutrition and Dietetics, Harokopio University, Athens, Greece, [3]Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium, [4]National Institute for Health and Welfare, Helsinki, Finland, [5]Department of Pediatrics, Medical University Varna, Varna, Bulgaria, [6]Instituto Investigacion Sanitaria Aragon (IISA), CIBERCV, Zaragoza, Spain, [7]Universidad De Zaragoza, Zaragoza, Spain, [8]University of Debrecen, Department of Family and Occupational Medicine, Debrecen, Hungary, [9]International Diabetes Federation European Region, Brussels, Belgium.

Background and aims: The prevalence of type 2 diabetes (T2D) and hypertension has increased rapidly in recent decades and this trend will continue as the global population ages. Since these chronic diseases have an asymptomatic preclinical phase, the prevalence of undiagnosed cases is also of clinical and public health concern. The present study presents the prevalence of undiagnosed diabetes and hypertension using the baseline data obtained from the Feel4Diabetes study, which is an ongoing school- and community-based intervention aiming to tackle obesity and obesity-related metabolic risk factors among families from vulnerable groups in six European countries (i.e. Belgium, Bulgaria, Finland, Greece, Hungary, Spain).
Materials and methods: In total 24,306 adults (parents of primary school children in selected municipalities) were screened for their diabetes risk using the FINDRISC questionnaire. The adults with increased risk were invited and about 3,000 participated in a medical check-up, including the assessment of fasting blood glucose levels and blood pressure.
Results: The population of the current work was 2,443 adults (mean age 41.2 ± 5.5 years, 64.7% females) with available data for all the variables used in the current analysis. The distribution of subjects in the following FINDRISC score categories <10, 10–14 and ≥15, was 38%, 48% and 14% respectively. The prevalence of self-reported diabetes diagnosis was 3.3%, while among participants without diagnosed diabetes the prevalence of undiagnosed diabetes based on fasting glucose levels ≥126 mg/dl was 1.7%, ranging from 0.8% and 1.4% to 5.5% for participants with FINDRISC score <10, 10–14 and ≥15, respectively. A large percentage (36.4%) of adults with diabetes was undiagnosed. Regarding blood pressure (BP), medication-treated hypertension was reported by 8.2% of adults while among the remaining participants the prevalence of systolic BP ≥140 mmHg and/ or diastolic BP ≥90 mmHg was 15.6%, ranging from 12.1% and 18.2% to 18.6% for participants with FINDRISC score <10, 10–14 and ≥15, respectively. A large percentage (63.7%) of adults with hypertension was undiagnosed.
Conclusion: Considering the young age of this population, the current study revealed a relatively high prevalence of undiagnosed diabetes and hypertension in low-middle income countries and in low socioeconomic status municipalities in high income countries.
Clinical Trial Registration Number: NCT02393872
Supported by: EU's Horizon 2020 research and innovation programme
Disclosure: **G.E. Dafoulas:** None.

274

A high glucose visit-to-visit variability is associated with a poor metabolic profile in individuals in the Hoorn Diabetes Care System cohort

R.C. Slieker[1,2], A.W.A. van der Heijden[3], P.J.M. Elders[3], G. Nijpels[4], L.M. 't Hart[1,5], J.W.J. Beulens[6,7];
[1]Cell and Chemical Biology, Leiden University Medical Center, Leiden, [2]Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, [3]Department of General Practice and Elderly Care Medicine, VU University Medical Center, Leiden, [4]Department of General Practice and Elderly Care Medicine, VU University Medical Center, Amsterdam, [5]Molecular Epidemiology, Leiden University Medical Center, Leiden, [6]VU University Medical Center, Amsterdam, [7]Julius Center for Health Sciences and Primary Care, Utrecht, Netherlands.

Background and aims: The disease trajectory of individuals with type 2 diabetes is characterized by dynamic glycaemia. Previous studies have identified glycaemic variation as a risk factor for diabetes-related complications. Here we aimed to identify determinants of glycemic visit-to-visit variability within individuals with type 2 diabetes.
Materials and methods: Individuals with type 2 diabetes ($n = 6770$) from the Hoorn Diabetes Care System cohort were included in this study when they had at least five years follow-up data and an age of onset over 35 years. Measurements within the first six months after diagnosis were excluded to reduce the first treatment effect. The coefficient of variation (CV, σ/μ) was used for the visit-to-visit variability of fasting glucose (FG). CVs were calculated over 5-year sliding interval (I), that is over 1–5 years, 2–6, $n–n + 5$ years until the end of the follow-up. Intervals were aligned based on the diabetes duration and within the interval divided in glucose-CV quintiles (Q). The 5-year average triglycerides, HDL, BMI and the age of disease onset and any insulin use were tested against the quintiles using an ANOVA with adjustment for (the interval average) of glucose, sex, smoking, systolic blood pressure, BMI and use of antihypertensive agents.
Results: Individuals with low or high FG-CV largely remained in the same quintile across their follow-up, i.e. 74.4% had at least 50% of their intervals (I) in one quintile and 72.0% had at least 75% of their intervals in two adjacent quintiles. No difference was observed between sexes, in FG-CV at any time point ($P_{bonf} > 0.05$). Individuals with a high FG-CV were those with an early age of diabetes onset ($I_{1\text{-}12,14}$, average difference between Q5 and Q1 ($D_{Q5\text{-}Q1}$) -3.9 years, $P_{bonf} \leq 0.01$). A high FG-CV was associated with a higher BMI ($I_{1\text{-}11,15\text{-}20}$, $D_{Q5\text{-}Q1} = +2.7$ kg/m^2, $P_{bonf} \leq 0.05$). Higher FG-CV was associated with an unfavourable lipid profile, i.e. lower levels of HDL in individuals with high FG-CV ($I_{1\text{-}20}$, −0.2 mmol/L, $P_{bonf} \leq 0.05$) and higher triglycerides levels ($I_{1\text{-}14,16,17,19,20}$, $D_{Q5\text{-}Q1} = +0.3$ mmol/L, $P_{bonf} \leq 0.05$). For total cholesterol, blood pressure and eGFR, no consistent associations were observed across quintiles. Also, individuals in Q5 versus Q1 showed a higher risk of insulin initiation (HR = 4.07, CI: 3.07–5.40, $P < 0.0001$). In line with that, individuals with high variation were often on insulin with ORs ranging from 19.4 (95%CI = 12.9–30.6, $P = 2.1 \cdot 10^{-39}$) in the first interval to 17.5 (95%CI = 6.0–58.4, $P = 5.9 \cdot 10^{-05}$) in the 20th interval.
Conclusion: Individuals with higher a glucose visit-to-visit variability tend to have a less favorable metabolic profile and are at increased risk of insulin initiation. Whether glucose visit-to-visit variability adds to prediction of vascular complications needs to be further investigated.
Disclosure: **R.C. Slieker:** None.

275

Diabetes prevalence, mortality and healthcare expenditure in 2017 and 2045 in Europe: data from the IDF Diabetes Atlas

S. Karuranga[1], Y. Huang[1], A. F Moura[1], W. Rathmann[2], B. Malanda[1];
[1]International Diabetes Federation, Brussels, Belgium, [2]German Diabetes Center, Düsseldorf, Germany.

Background and aims: Diabetes is among the leading causes of death in the IDF Europe Region (EUR), imposing high human, social and economic costs. Therefore, robust estimates of its prevalence are required for effective allocation of resources. The International Diabetes Federation

(IDF) Diabetes Atlas illustrates the continuing burden of diabetes estimates and projections in this region. Aim: To estimate diabetes prevalence, mortality and health care expenditure in the IDF Europe Region (EUR) for the years 2017 and 2045.

Materials and methods: A total of 63 population-based, high-quality data sources from 35 countries were used to generate age- and sex-specific adult diabetes prevalence for the 57 countries in the IDF Europe Region (EUR). Countries without good quality data sources were extrapolated based on data from countries with similar geography, economics and ethnicity. The UN population projections in 2045 were used to project the diabetes prevalence in 2045 for each country.

Results: Approximately 66 million people, 9% of the population aged 18–99, were living with diabetes in the IDF Europe region in 2017. This represents 1 in 11 adults living with diabetes. The age-adjusted prevalence was 6.7% (5.4–9.7%). About 38% of those people living with diabetes were estimated to be undiagnosed. If the trend continues, the number of people with diabetes is projected to reach 81 million in 2045 in the IDF Europe region. The 5 countries with the highest diabetes prevalence were Russian Federation, Germany, Turkey, Spain and Italy. In addition, a further 41 million people, 5.6% of the adult population, were estimated to be living with impaired glucose tolerance (IGT). The number of deaths attributed to diabetes from age 18 to 99 years was about 693,000 in 2017. The total healthcare expenditure related to diabetes was USD 208 billion, representing 25% of the expenditure worldwide.

Conclusion: Our findings demonstrate that diabetes exerts a heavy burden in European Region. Effective management programs should focus on diabetes prevention, in order to avoid harmful and costly consequences in the coming decades.

Supported by: Lily Diabetes; MSD; novo nordisk

Disclosure: **S. Karuranga:** None.

276

BMI z-score trajectories in youth with type 1 diabetes: an international analysis from Australia, Germany/Austria and USA

A. Schwandt[1,2], N. Foster[3], H. Phelan[4], C. Steigleder-Schweiger[5], M. Wu[3], J.J. Couper[6], P. Kroschwald[7], S.M. Willi[8], T.W. Jones[9], N. Prinz[1,2], D. Maahs[10], M.E. Craig[11];

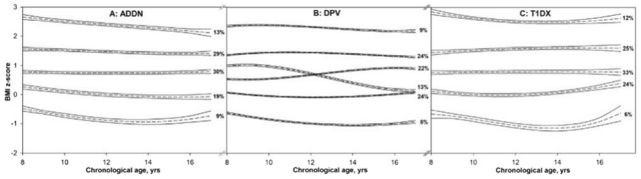
[1]Institute of Epidemiology and Medical Biometry, ZIBMT, University of Ulm, Ulm, Germany, [2]German Center for Diabetes Research (DZD), Munich-Neuherberg, Germany, [3]Jaeb Center for Health Research, Tampa, USA, [4]John Hunter Children's Hospital, Newcastle, Australia, [5]Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria, [6]Department of Diabetes and Endocrinology, Women's and Children's Hospital, and University of Adelaide, Adelaide, Australia, [7]Ruppiner Clinics, Department for Pediatrics and Adolescent Medicine, Neuruppin, Germany, [8]Children's Hospital of Philadelphia and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, USA, [9]Department of Diabetes and Endocrinology, Princess Margaret Hospital, and Telethon Kids Institute, Western Australia, Australia, [10]Stanford University, Stanford, USA, [11]Institute of Endocrinology, Childrens Hospital Westmead, and University of Sydney, Sydney, Australia.

Background and aims: Data on individual change in BMI during puberty are limited in T1D cohorts. We aimed to analyze international differences in individual BMI z-score (BMIz,WHO) patterns over time among youth with T1D.

Materials and methods: Longitudinal data from 11,513 youth from the Australasian Diabetes Data Network (N_{ADDN} = 1073, female: 46%), German/Austrian Diabetes Prospective Follow-up (N_{DPV} = 8722, 46%), and US T1D Exchange registry (N_{T1DX} = 1718, 45%) were examined. Subjects with follow-up from age 8–17 years, T1D duration >1 year and >5 aggregated BMI values were included. Children with celiac or thyroid disease were excluded. Latent class growth modeling (PROC TRAJ) was applied to identify distinct BMIz trajectories.

Results: International BMIz trajectories are presented in the figure. The most dramatic BMIz change during puberty were observed in DPV. Obese subjects (BMIz ≥2) from ADDN had a BMI decrease over time, while obese individuals in T1DX exhibited decreasing BMIz from age 8 to 12 years, followed by increasing BMIz during puberty. Comparing the reference group (BMIz ~0) with the other groups, higher BMIz was associated with older age at T1D onset, racial/ethnic minority, elevated HbA1c and lower insulin pump use ($p < 0.05$). In DPV boys were more likely to follow a low BMIz or had a decreasing BMIz from childhood to young adulthood, whereas girls more often experienced pubertal BMIz increase. In ADDN, a preponderance of boys was found in lower and near-normal patterns, while elevated BMI trajectories were more likely in girls. However, in the T1DX data sex ratio did not differ.

Conclusion: In three registries, the majority of T1D youth have a BMI pattern above the age- and gender-specific norms. The large international and individual differences in BMIz trajectories among youth with T1D likely result from diverse genetic and therapeutic factors, and suggest the need for personalized treatment regimens that consider healthy weight in addition to glucose control.



Disclosure: **A. Schwandt:** None.

277

Comparison of the incidence of diabetes in U.S. and Indian youth: an international harmonisation of youth diabetes registries

E.T. Jensen[1], D. Dabelea[2], P.A. Praveen[3], A. Anandakumar[4], C.W. Hockett[2], S. Isom[5], T.C. Ong[6], V. Mohan[4], R. D'Agostino, Jr.[5], M.G. Kahn[6], R.F. Hamman[2], E. Mayer-Davis[7], N. Tandon[3];

[1]Epidemiology and Prevention, Wake Forest School of Medicine, Winston-Salem, USA, [2]Epidemiology, University of Colorado, Denver, USA, [3]All India Institute of Medical Sciences, New Delhi, India, [4]Madras Diabetes Research Foundation, Chennai, India, [5]Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, USA, [6]Pediatrics, University of Colorado, Denver, CO, USA, [7]Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Background and aims: Diabetes in youth has increased in both India and the United States (U.S.). To date, few data exist for comparing population-based estimates of the burden of diabetes in contemporary youth cohorts in the U.S. and India. Such comparisons not only offer context for understanding the relative burden of disease, but may also provide insight into the pathogenesis of diabetes.

Materials and methods: We harmonized data elements from the SEARCH for Diabetes in Youth registry (SEARCH) from five sites in the U.S. and the Registry of People with Diabetes with Youth Age at Onset (YDR - Chennai and New Delhi regions) in India to the structure and terminology in the Observational Medical Outcomes Partnership (OMOP) Common Data Model (v5). Data analyzed were from youth with incident type 1 (T1D) and type 2 (T2D) diabetes cases age <20 diagnosed between 2006 and 2012. Denominators were obtained from Census data for the demographic regions corresponding to the registry case ascertainment areas. We compared diabetes incidence across registries by type and within age and sex categories using a 2-sided, skew-corrected inverted score test.

Results: Overall, the incidence of both T1D and T2D was significantly higher in SEARCH as compared to YDR (Table). Age at peak incidence of T1D was similar across registries, whereas T2D incidence was higher at an earlier age in SEARCH. Comparing the incidence of T1D in Asian

and Pacific Islanders (API) in SEARCH to the overall incidence of T1D in YDR, the difference in rates was reduced (7.8 [95% CI: 5.2, 11.8] in SEARCH vs 4.0 [95% CI: 3.6, 4.5] in YDR). Sex and age differences existed, with a higher rate of T2D among females in SEARCH (7.5/100,000, females vs 4.4/100,000. males) as compared to YDR, where the distribution of T2D by sex was similar in YDR (0.5/100,000, females vs 0.4/100,000, males) (Table).

Conclusion: Comparison of India-based and U.S.-based youth-onset diabetes registries indicated that the incidence of T1D and T2D in youth was significantly different. The proportion of females with T2D was higher in SEARCH and the age distribution at diagnosis for T2D was older in YDR. Examination of the distribution of risk factors for T2D is needed to elucidate whether the differences observed represent a diagnostic delay or differences in distribution of risk factors.

Table. Average annual incidence rates (per 100,000 per year) by type and, within type, by sex, age at diagnosis

	SEARCH			YDR – New Delhi and Chennai			
Type 1	Average Annual Cases	Denominator (Millions)	Rate/100,000 (95% CI)	Average Annual Cases	Denominator (Millions)	Rate/100,000 (95% CI)	p
Sex							
Female	498	2.459	20.2 (18.5,22.1)	141	3.475	4.1 (3.4, 4.8)	<0.0001
Male	566	2.566	22.0 (20.3,23.9)	158	3.970	4.0 (3.4, 4.6)	<0.0001
Age at diagnosis							
0-4	172	1.222	14.1 (12.1,16.3)	50	1.676	3.0 (2.3, 3.9)	<0.0001
5-9	345	1.228	28.1 (25.3,31.2)	92	1.821	5.0 (4.1, 6.2)	<0.0001
10-14	383	1.250	30.7 (27.8,33.9)	97	1.956	4.9 (4.1, 6.0)	<0.0001
15-19	162	1.325	12.3 (10.5,14.3)	60	1.992	3.0 (2.4, 3.9)	<0.0001
Crude Incidence	1,063	5.025	21.2 (19.9,22.5)	299	7.445	4.0 (3.6, 4.5)	<0.0001
Type 2	Cases	Denominator (Millions)	Rate/100,000 (95% CI)	Cases	Denominator (Millions)	Rate/100,000 (95% CI)	p
Sex							
Female	185	2.459	7.5 (6.5,8.7)	16	3.475	0.5 (0.3, 0.7)	<0.0001
Male	112	2.566	4.4 (3.6,5.2)	17	3.970	0.4 (0.3, 0.7)	<0.0001
Age at diagnosis							
0-4	1	1.222	0.0 (0.0,0.4)	0	1.676	0.0 (0.0, 0.3)	0.47
5-9	14	1.228	1.1 (0.7,1.9)	2	1.821	0.1 (0.0, 0.4)	<0.0001
10-14	134	1.250	10.7 (9.1,12.7)	9	1.956	0.5 (0.2, 0.9)	<0.0001
15-19	148	1.325	11.2 (9.5,13.2)	21	1.992	1.1 (0.7, 1.6)	<0.0001
Crude Incidence	297	5.025	5.9 (5.3,6.6)	32	7.445	0.4 (0.3, 0.6)	<0.0001

Supported by: NIH/NIDDK R21DK105869-02

Disclosure: **E.T. Jensen:** Grants; Study supported by NIH/NIDDK R21DK105869-02.

278

Prevalence of pre-diabetes, undiagnosed and diagnosed diabetes among adults aged 18 to 70 years in France: the CONSTANCES cohort

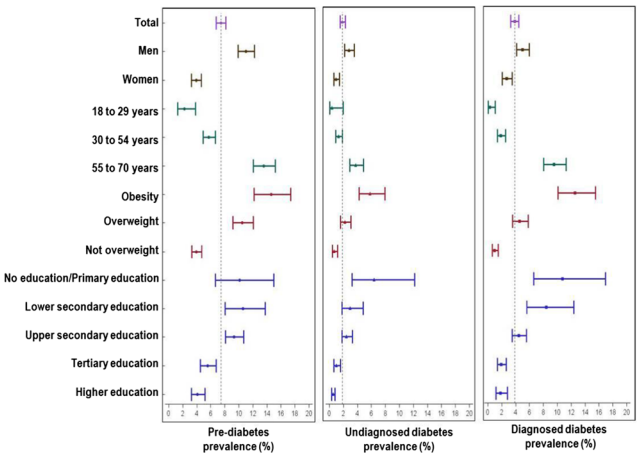
S. Fuentes[1], S. Fosse-Edorh[1], N. Regnault[1], M. Goldberg[2,3], E. Cosson[4,5];
[1]Santé Publique France, Paris, France, [2]INSERM UMS 11, Population-based Epidemiological Cohorts Unit, Villejuif, France, [3]Paris Descartes University, Paris, France, [4]Direction de maladies non transmisibles, Department of Endocrinology-Diabetology-Nutrition, AP-HP, Jean Verdier Hospital, Paris 13 University, Sorbonne Paris Cité, CRNH-IdF, CINFO, Bondy, France, [5]Sorbonne Paris Cité, UMR U1153 Inserm/U1125 Inra/Cnam/Université Paris 13, Bobigny, France.

Background and aims: There is a lack of knowledge on rates of pre-diabetes and undiagnosed diabetes. This study aimed to update the prevalence of different dysglycemic states in France among adults aged 18 to 70 years according to gender, age, body mass index (BMI - kg/m^2-) categories and socioeconomic status.

Materials and methods: The CONSTANCES cohort is a randomly selected representative sample of French adults. In 2013, 16,340 participants were recruited. Based on data from self-administered questionnaires , medical examination (including fasting plasma glucose (FPG) measurement) and data from the French National Health Insurance Information System(antidiabetic drugs consumption and hospitalization), three states were defined: pre-diabetes (WHO definition), diagnosed and undiagnosed (FPG ≥126 mg/dL) diabetes. Weighted prevalence of each dysglycemic state was estimated for all population and by gender, age, BMI categories and educational level. Confidence intervals were calculated using logit transformation.

Results: In 2013, the prevalence of pre-diabetes, undiagnosed diabetes and diagnosed diabetes was 7.4%, 1.9% and 3.9%, respectively. All these rates were higher in males, increased steadily with age and BMI category, while decreasing with education (Figure).

Conclusion: Our results show that the prevalence of prediabetes and unknown diabetes are still very high in developed countries, like France. They highlight the need to increase primary prevention, and to reinforce secondary prevention of diabetes, especially through promotion of screening in population at risk.



Disclosure: **S. Fuentes:** None.

279

DIAGESTCAT. Trends in prevalence of diabetes in pregnancy and perinatal outcomes: a large, population-based study in Catalonia, Spain, 2006–2015

L. Gortazar[1], A. Goday[1,2], D. Benaiges[1,2], E. Sarsanedas[1], L. Mañé[1], G. Llauradó[1,2], J.J. Chillarón[1,2], M. Prados[1], A. Paya[3], J.A. Flores[1,2];
[1]Department of Endocrinology and Nutrition, Hospital del Mar, Barcelona, Spain, [2]Department of Medicine, Universitat Autònoma de Barcelona, Campus del Mar, Barcelona, Spain, [3]Department of Gynecology and Obstetrics, Hospital del Mar, Barcelona, Spain.

Background and aims: There are no recent epidemiological studies of Diabetes Mellitus (DM) in Pregnancy in Catalonia. Our aims were to explore trends in the prevalence of diabetes in pregnancy and examine whether the risk of adverse perinatal outcomes has changed during the period of 2006–2015 in Catalonia.

Materials and methods: Retrospective epidemiological study about prevalence of DM and pregnancy in Catalonia. All hospital admissions for singleton births during the study period were collected from The Minimum Basic Data Set for Hospital Discharge. Cases of Gestational Diabetes (GDM), type 1 DM (T1DM) and type 2 DM (T2DM) were identified in every hospital delivery discharge report using ICD-9-MC codes. Data regarding maternal characteristics and obstetric complications (pre-eclampsia, prematurity, macrosomia, Large for gestational age (LGA), small for gestational age (SGA) and caesarean deliveries) were analysed. Crude and age-adjusted annual prevalences were calculated for every DM type. Poisson regression model was used to assess trends in prevalence and in obstetric outcomes during the study period, adjusting for age.

Results: Data from 743,762 hospital deliveries were collected. From 2006 to 2015, there was an increase in crude and age-adjusted prevalence of GDM, T2DM and T1DM. A rising in crude and age-adjusted rates of pre-eclampsia was observed in non diabetic, T2DM and T1DM during the study period. Prematurity only showed and increasing rate in women

without DM. An increment in cesarean deliveries was detected in non-diabetic and GDM but none of them was statistically significant when adjusted for age. We observed a decrease in crude and age-adjusted rates of macrosomia during the study period in non-diabetic and in all diabetes categories but a reduction of LGA was only observed in non-diabetic women and T2DM. The rates of SGA decreased in non-diabetic women, no significant changes were detected for any type of DM.

Conclusion: Prevalences of T1DM, T2DM and GDM have progressively increased during the study period in Catalonia. There has been a decrease in some of the adverse maternal-fetal outcomes.

	Women without DM n=704,148		GDM n=35,729		T2DM n=2,586		T1DM n=1,299	
	2006	2015	2006	2015	2006	2015	2006	2015
Prevalence % (CI)			3.99 (3.85-4.14)	6.22 $^{\alpha}$ Ω (6.03-6.40)	0.31 (0.27-0.35)	0.35 $^{\alpha}$ Ω (0.30-0.39)	0.14 (0.11-0.17)	0.20 $^{\alpha}$ Ω (0.16-0.24)
Pre-eclampsia % (CI)	1.28 (1.20 - 1.37)	1.76 α Ω (1.65 - 1.86)	2.25 (1.76 - 2.86)	2.52 (2.09 - 3.03)	4.63 (2.53 - 8.31)	6.78 β π (4.22 - 10.73)	3.65 (1.51 - 9.47)	12.88 β π (8.20 - 19.66)
Prematurity % (CI)	12.39 (12.14 - 2.64)	13.22 β Ω (12.95 - 3.49)	14.98 (13.68 - 6.39)	15.76 (14.70 - 6.88)	32.00 (25.93 - 38.75)	28.09 (22.73 - 34.15)	44.57 (34.83 -54.74)	35.61 (27.95 - 44.08)
Cesarean d. % (CI)	24.57 (24.25 - 4.89)	26.01 Ω (25.66 - 6.36)	28.01 (26.38 - 29.70)	31.02 π (29.65 - 2.42)	43.52 (37.08 - 50.19)	40.66 (34.61 - 47.05)	57.69 (48.09 - 66.75)	51.52 (43.07 - 59.88)
Macrosomia % (CI)	5.94 (5.77 - 6.13)	5.86 β π (5.67- 6.05)	9.15 (8.12 - 10.32)	7.84 16 β π (7.07 - 8.68)	14.71 (10.50 -20.22)	11.06 β π (7.66 - 15.72)	25.81 (18.00 - 35.53)	13.64 π (8.80 - 20.53)
β π LGA % (CI)	12.51 (12.26 -12.75)	13.58 α Ω (13.31-13.85)	18.10 (16.72 - 19.57)	20.01 (18.84 - 21.23)	32.87 (26.96 - 39.39)	27.54 β π (22.24 - 33.57)	44.23 (35.06 - 53.81)	43.94 (35.76 - 52.46)
SGA % (CI)	1.78 (1.68 - 1.88)	1.41 α Ω (1.32 - 1.51)	1.53 (1.14 - 2.06)	1.14 (0.87 - 1.51)	3.24 (1.58 - 6.54)	0.00 (0.00 - 1.60)	0.00 (0.00 - 3.56)	0.76 (0.13 - 4.17)

β Significant association (p <0.05) for time trend during the study period, adjusting for age categories

α Significant association (p <0.001) for time trend during the study period, adjusting for age categories

π Significant association (p < 0.05) for time trend during the study period

Ω Significant association (p < 0.001) for time trend during the study period

Disclosure: **L. Gortazar:** None.

280

Regional burden of obesity and diabetes in adults: projections from 2017–2045

S. Nielsen[1], D. Napier[2], B.B. Jensen[3], A. Moses[4], N. Lund[1];
[1]Health Advocacy, Novo Nordisk A/S, Bagsværd, Denmark, [2]University College London, London, UK, [3]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [4]Novo Nordisk Inc, Plainsboro, NJ, USA.

Background and aims: Obesity and type 2 diabetes (T2D) are rising at alarming rates. T2D is a complex disease influenced by multiple diverse factors and long delays between causes and effects. The most significant modifiable driver of T2D is excess bodyweight. Currently, around 650 million people worldwide have obesity and more than 400 million have diabetes. To support the WHO target 7 "Halt the rise in diabetes and obesity" we have previously demonstrated that targeting a global diabetes prevalence stabilised at 10% by 2045 requires 25% reduction of obesity prevalence. The study is part of Cities Changing Diabetes established to improve the understanding of diabetes in urban settings. It is a partnership between Novo Nordisk, Steno Diabetes Center Copenhagen, University College London and local partners. The study shows how regional prevalence of T2D is affected from 2017 to 2045 in a *past trend* scenario assuming that future increase in obesity prevalence is extrapolated linearly and in a *target* scenario assuming that obesity prevalence is reduced by 25% in 2045.

Materials and methods: BMI data for all countries in the world 2000–2014 were obtained from the Non-communicable Disease Risk Factor Collaboration. For each country the adult population was divided into age and BMI groups and share of people in each BMI class was projected depending on scenario. Risks of T2D for age and BMI obtained from the literature were applied allowing estimates of prevalence of T2D for each country each year.

Results: North America and Caribbean (NAC) shows the highest current obesity and T2D prevalence (35.7% and 13.2%) and continues to do so in 2045 in both *past trend* (51.5% and 16.8%) and *target* scenario (26.8% and 13.1%). The *target* scenario results in 15.3 million fewer people with T2D in 2045 compared to *past trend* scenario. Despite moderate current obesity prevalence (9.0%) Africa (AFR) shows the lowest current T2D prevalence (3.3%) and this pattern persists in 2045 for *past trend* (16.4% and 4.2%) and *target* scenario (6.4% and 3.5%). In this region, 7.2 million fewer people have T2D in 2045 if the current obesity prevalence is reduced by 25%.In contrast, the lowest current obesity prevalence is found in South-East Asia (SEA) (4.0%) despite relatively high current T2D prevalence (8.7%). This tendency proceeds in 2045 for *past trend* (7.9% and 12.0%) and *target* scenario (3.1% and 10.7%) resulting in 17.1 million fewer people with T2D in 2045 in *target* compared to *past trend* scenario. AFR is the most dynamic with growth of people with T2D in 2045 between 150 and 180% while Europe (EUR) which has a high prevalence of obesity and T2D (26.3 and 9.2%) is the most stable with growth between 23 and 3% depending on scenario. Share of people with T2D also suffering from obesity differs substantially among regions. In NAC 60% of people with T2D also have obesity while it is only 10% in SEA.

Conclusion: NAC and EUR where obesity has been on the rise for decades have the highest T2D prevalence but also the slowest future increases. In regions with lower T2D prevalence like AFR, the number of people with T2D will increase up to three fold in the coming three decades unless obesity prevalence is reduced. To realise the *target* scenario, health should be integrated into all policies in order to contribute to reduce the obesity and T2D burden. Not doing so represents a lost opportunity to improve peoples' health, well-being and economic productivity.

Supported by: Novo Nordisk A/S

Disclosure: **S. Nielsen:** Employment/Consultancy; Employer of Novo Nordisk. Grants; A grant from Novo Nordisk was given to the research. Stock/Shareholding; Shareholder of Novo Nordisk.

PS 003 Type 2 diabetes prediction

281

Are the normal glucose tolerance individuals totally outside of the diabetes spectrum?

A.F. Pina[1,2], R.S. Patarrão[1,3], R.T. Ribeiro[4,5], C. Penha-Gonçalves[3], J.F. Raposo[1,5], R.M. de Oliveira[1], L. Gardete-Correia[5], R. Duarte[5,6], J.M. Boavida[5], R. Andrade[5], I. Correia[5,6], J.L. Medina[6], R. Henriques[7], M. Macedo[1,5];
[1]CEDOC-NOVA Medical School, Lisbon, Portugal, [2]Departamento de Ciências Médicas, Universidade de Aveiro, Aveiro, Portugal, [3]IGC - Instituto Gulbenkian e Ciência, Lisbon, Portugal, [4]Departamento de Ciências Médicas, Universidade de Aveiro, Lisbon, Portugal, [5]APDP - Associação Protectora dos Diabéticos de Portugal, Lisbon, Portugal, [6]SPD - Sociedade Portuguesa de Diabetologia, Lisbon, Portugal, [7]NovaIMS - Nova Information Managment School, Lisbon, Portugal.

Background and aims: Type 2 diabetes (T2D) and prediabetes (PD) glycemia cut-off values are established by convention, and for the latter there is no agreement about fasting glycemia cut-off values. Some normal glucose tolerant individuals (NGT's) presents a higher risk of developing diabetes and its micro and macrovascular complications. In the PD group there are cases that don't progress to T2D. Glycemia, *per si*, is insufficient to an accurate diabetes risk assessment and diagnosis. Also, association of multiple factors, involved in T2D pathophysiology, can lead to diverse phenotypes. Cluster analysis, as Self Organizing Maps (SOM), is used to identify populations patterns. Analyzing complex data, identifies clusters, highlighting relevant data structures to the understanding of the aggregation. We hypothesize that by analyzing glycemia levels, not considering the predefined cut-offs, together with parameters classically associated with T2D, will reveal novel clusters, reflective of different pathological phenotypes.
Materials and methods: We applied cluster analysis to 1010 individuals, from PREVADIAB2 cohort (Portuguese Diabetes Prevalence Study: 73% NGT, 22% PD and 5% diabetic). We first reduced data to 27 units, applying superSOM algorithm, using variables distributed over 8 grids (g1 - Glycemia OGTT profile, g2 - Insulin OGTT profile, g3 - C-peptide OGTT profile, g4 - Insulin clearance OGTT profile, g5- free fatty acids OGTT profile, g6- Fasting cholesterol LDL, HDL, triglycerides, g7-HOMA IR, HOMA B, g8 - BMI, waist circumference). The units were analysed and clustered (hierarchical clustering algorithm, in R).
Results: We found 10 clusters with different C peptide, insulin levels and insulin *clearance* along the OGTT (0′, 30′ and 120′). Most of the clusters group together NGT, PD and diabetic individuals. However, there are clusters where the majority are NGT, while others group a greater proportion of hyperglycemic people. The proportions differ with the profile patterns. We found diabetic patients in 5 clusters, which differ in parameters classically associated with T2D.
Conclusion: We found cluster with different insulin, C-peptide and insulin clearance profiles, not based solely on glycemia. Surprisingly, most clusters group together NGT, PD and diabetic individuals. We know that there are individuals with prediabetes that already show T2D-assocaited microvascular complications, while others never progress in the disease spectrum. In the same line of thought, and in view of our results, are the NGT, who we found to group together with a greater proportion of hyperglycemic individuals, totally outside of the diabetes spectrum? Cluster analysis reveals new risk factors for the NGT individuals. These clusters can represent different phenotypes and contribute to clarify the pathophysiological mechanisms responsible for glycemia changes that will imprint a precision medicine approach.
Supported by: FCT-PD/BD/136887/2018
Disclosure: **A.F. Pina:** Grants; FCT-PD/BD/136887/2018, PTDC/BIM/MET/2115/2014, Sociedade Portuguesa de Diabetologia PREVADIAB Grant support.

282

A simple clinical risk score in detecting diabetes in the Chinese populations: insights from two population-based Chinese cohorts

Y.C. Woo[1], B. Gao[2], C.H. Lee[1], C.H.Y. Fong[1], J. Ming[2], S. Lin[1], T.H. Lam[3], E. Janus[4], Q. Ji[2], K.S.L. Lam[1];
[1]Department of Medicine, The University of Hong Kong, Hong Kong, Hong Kong, [2]Department of Endocrinology, Fourth Military Medical University, Xi'an, China, [3]Department of Public Health, The University of Hong Kong, Hong Kong, Hong Kong, [4]Department of General Medicine, University of Melbourne, Melbourne, Australia.

Background and aims: Diabetes is a major health problem in China. Early diagnosis and intervention should be beneficial for prevention of diabetes-related complications. We aim to develop and validate a simple clinical parameter-based diabetes risk score by two independent Chinese cohorts.
Materials and methods: 3132 subjects (age 18–84) without known diabetes who had oral glucose tolerance tests (OGTT) performed during the China National Diabetes and Metabolic Disorders Study in Shaanxi Province (Shaanxi cohort) were investigated for risk factors independently associated with diabetes by multiple logistic regression analysis. The risk factors identified were used to develop a categorisation point scoring system, the Chinese Diabetes Score (CDS). The application of CDS for diabetes screening was validated in 2694 subjects without known diabetes (age: 25–74) assessed at the baseline visit of the Hong Kong Cardiovascular Risk Factors Prevalence Study (Hong Kong cohort). The area under the receiver operating characteristic curve (AUROC) of CDS was calculated to assess the accuracy of the model. The optimal cut-off of CDS was determined by Youden's index. Diabetes was defined as fasting glucose ≥7 mmol/L or 2 hours post OGTT glucose ≥11.1 mmol/L.
Results: Among the Shaanxi cohort, 229 (7.31%) subjects were screened to have diabetes. Age (O.R. 1.05, $P < 0.05$), family history of diabetes (O.R. 1.62, $P < 0.05$), hypertension (O.R. 1.42, $P < 0.05$) and body mass index (O.R. 1.14, $P < 0.05$) were independently associated with diabetes and were included in the CDS. The CDS showed good accuracy in detecting diabetes with an AUROC at 0.752. Subjects with ≥17 points out of 47 were considered at risk of having diabetes. Validation of CDS in the Hong Kong cohort also showed good accuracy (AUROC 0.804). The CDS showed good negative predictive value when applied to both cohorts (Shaanxi 97.5%, Hong Kong 98.1%). The sensitivity (82.1 vs. 86.5) and specificity (55.5 vs. 54.5) of the model was comparable when used in Shaanxi and Hong Kong. Applying the CDS, 8 subjects were needed to screen to detect one case of diabetes in both regions.
Conclusion: The CDS is an effective screening tool for Chinese residing in northwest (Shaanxi) and southern (Hong Kong) China. The difference in geographic location and composition of rural and urban subjects appear to have minimal impact on the performance of the CDS. It involves only four clinical parameters and can be adopted as a public health strategy for identifying Chinese individuals with undiagnosed diabetes for early intervention.
Disclosure: **Y.C. Woo:** None.

283

The predictive role of endothelial progenitor cells and asymmetric dimethylarginine in the onset of type 2 diabetes: a 10-year prospective study

A. Angelidi[1], A. Papazafiropoulou[1], A. Gritzapis[2], T. Sergentanis[3], E.-M. Tzouganatou[1], S. Papantoniou[1], E. Efstratiadi[1], S. Matsagos[4], P. Spyropoulou[4], J. Protopsaltis[1], E. Boutati[5], G. Dimitriadis[5], A. Melidonis[1];
[1]First Department of Internal Medicine and Diabetes Center, Tzanio General Hospital, Piraeus, Greece, [2]Department of Pathology, Locus-Medicus SA, Athens, Greece, [3]Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National University of Athens, Athens, Greece, [4]Blood Bank Service, & Molecular Biology

Department, Tzanio General Hospital, Piraeus, Greece, [5]Second Department of Internal Medicine & Research Institute and Diabetes Center, "Attikon" University Hospital, Athens University Medical School, Athens, Greece.

Background and aims: Endothelial Progenitor Cells (EPCs) take part in postnatal neovascularization and promote vascular homeostasis. Asymmetric dimethylarginine (ADMA) is a major endogenous inhibitor of nitric oxide synthase. EPCs and ADMA have been associated with endothelial dysfunction, cardiovascular disease (CVD) and diabetic vascular complications. According to a hypothetical scenario, EPC alterations may precede and determine the development of both CVD and type 2 diabetes (T2DM). The aim of the present study is to investigate possible associations of EPCs, ADMA and other cardiometabolic risk factors with the development of T2DM.
Materials and methods: A total of 57 volunteers, without a previous history of a cardiovascular, renal or metabolic disease, undergone a 75 gr OGTT (42 subjects with prediabetes and 15 controls) and they were followed up for a 10-year period. All participants were retested after performing a new 75 gr OGTT. Several anthropometric and cardiometabolic risk factors (including HOMA-IR insensitivity index, high sensitivity-CRP) and the initial levels of EPCs and ADMA were determined in each participant both at baseline and at 10-year follow up of the study. Flow cytometry identified and quantified the EPCs (CD34+ CD133+ KDR+ cells), while ADMA levels were determined by immunoenzymatic method (ELISA). Statistical analysis was performed using SPSS 20.0 software.
Results: T2DM was developed in 30 participants from the prediabetes group and two subjects from the control group. The median age of the participants was 58.47 ± 11.28 years. Univariate analyses performed separately for all estimated parameters examining their association with the onset of T2DM. Parameters statistically associated with T2DM onset were included in the stepwise multivariate analysis. From the multivariate analysis statistically significant association was observed between the development of T2DM and age (OR: 1.052, 95% CI: 1.002–1.1104, $p < 0.001$), Body Mass Index (OR: 1.186, 95% CI: 1.065–1.322, $p = 0.003$), Impaired Glucose Tolerance (OR: 12.00, 95% CI: 2.944–48.907, $p = 0.001$), HbA1c (OR: 3.213, 95% CI: 1.575–6.555, $p = 0.006$), hypertension (OR: 10.267, 95% CI: 2.953–35.693, $p < 0.001$), ADMA (OR: 6.616, 95% CI: 2.676–16.356, $p < 0.001$) and EPCs (OR: 0.990, 95% CI: 0.984–0.995, $p = 0.001$).
Conclusion: According to the results of this 10-year prospective study, it appeared that in addition to the presence of some traditional risk factors, the incidence of T2DM was independently associated with ADMA and low EPC levels. These two parameters may reflect a disrupted pathophysiological microenvironment, which may precede and potentially associated with the more unfavorable cardiovascular profile of these individuals and the development of T2DM.
Disclosure: **A. Angelidi:** None.

284

Mathematical modelling of glucose tolerance tests describing glucose, insulin and C-peptide levels in different cohorts: an IMI DIRECT study

C. Dings[1], N. Scherer[1], V. Nock[2], A.M. Hennige[2], E.R. Pearson[3], P.W. Franks[4], T. Lehr[1], for the IMI DIRECT consortium;
[1]Clinical Pharmacy, Saarland University, Saarbrücken, Germany, [2]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany, [3]Medical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, UK, [4]Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Sweden.

Background and aims: The oral glucose tolerance test (OGTT) and mixed meal tolerance test (MMTT) are well-established tests for the diagnosis and study of diabetes. However, comparing the resulting data, such as post challenge levels and AUCs of glucose and insulin, is impossible, as the amount of glucose intake differs between the two types of tests. To address this problem, we aimed to develop a mathematical model that simultaneously describes glucose, insulin and c-peptide levels during OGTT and MMTT and to estimate individual model parameters which allow comparison independently of the type of the tolerance test.
Materials and methods: The model was developed on data from the Diabetes Research on Patient Stratification (DIRECT) study using nonlinear mixed effects methods implemented in the software NONMEM (version 7.3.0). 2247 pre-diabetic as well as 821 recently diagnosed type 2 diabetic participants with frequently sampled OGTT and MMTT measurements, respectively, were included.
Results: The developed model simultaneously describes glucose, insulin and c-peptide using a one compartment turn-over model for each entity. The data was best described when oral glucose uptake was implemented as a transit model with a first-order absorption rate constant and one and two transit compartments for the OGTT and MMTT, respectively. Glucose utilization followed a second-order process. Endogenous glucose release was decreased exponentially by change in insulin levels. A hill function described the release of c-peptide and was multiplied by a bioavailability factor to describe the release of insulin, accounting for its pre-systemic hepatic clearance. The effect of incretin hormones influenced the hill function. C-peptide elimination followed a first-order, and insulin degradation a saturable process. The precision of all parameter estimates was excellent (relative standard error <17%). The population estimate for fasting glucose (FG) was 5.61 mmol/l and 7.07 mmol/l for the pre-diabetic and the diabetic participants, respectively. Fasting c-peptide was lower in the pre-diabetic population (808 vs 1010 pmol/l in diabetics). However, their maximum c-peptide release rate was higher (5630 vs 4110 pmol/l/h in diabetics). The insulin sensitivity was higher in the pre-diabetic population (54.2 vs 30.4 nmol/h in diabetics). FG measurements were used as a surrogate parameter for disease status. Especially the model parameters for insulin sensitivity and maximum c-peptide release depict the same trend when correlated against FG ($p < 0.0001$), indicating that they are meaningful and comparable descriptors of disease status.
Conclusion: A mathematical model describing OGTT and MMTT in prediabetic and diabetic participants was developed successfully. The derived model parameters of both populations show physiologically plausible differences, while their correlation to FG is similar. We will further test the parameters for their comparability, hopefully providing a powerful tool to identify disease status independently of the used tolerance test.
Supported by: IMI DIRECT
Disclosure: **C. Dings:** None.

285

Performance of existing risk assessment models for prevalent or undiagnosed type 2 diabetes in a multi-ethnic population

M. Obura[1], I.G.M. van Valkengoed[2], L. 't Hart[1,3], S.P. Rauh[1], R.J. Peters[4], F. Rutters[1], M.B. Snijder[2], J.W.J. Beulens[1];
[1]Epidemiology and Biostatistics, VU Medical Center, Amsterdam, Netherlands, [2]Department of Public Health, Academic Medical Center, Amsterdam, Netherlands, [3]Molecular Cell Biology and Dept. of Molecular Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [4]Department of Cardiology, Academic Medical Center, Amsterdam, Netherlands.

Background and aims: Different ethnicities have varying risks for type II diabetes mellitus (T2DM). Little is known about the performance of risk assessment models for prevalent (undiagnosed) T2DM in ethnicities other than the development population. We therefore aimed to identify existing models for the risk of prevalent or undiagnosed T2DM and externally validate them in a large multiethnic population.
Materials and methods: A systematic literature search in PubMed was performed until December 2017 to identify risk assessment models for

prevalent or undiagnosed T2DM. We cross-sectionally validated these scores in 4,547 Dutch, 3,035 South-Asian Surinamese, 4,119 African Surinamese, 2,326 Ghanaians, 3,598 Turkish, and 3,894 Moroccans from the HELIUS (Healthy LIfe in an Urban Setting) study carried out in Amsterdam. T2DM was defined as having fasting glucose level ≥7.0 mmol/l, and/or using glucose-lowering medication, and/or if the participant self-reported to have been diagnosed with diabetes by a health care professional. Model performance was assessed in terms of discrimination (C-statistic) and calibration (Hosmer-Lemeshow test).

Results: We identified 27 studies containing 30 risk assessment models for prevalent or undiagnosed T2DM. The prevalence of T2DM among the participants was 3.9% ($n = 179$), 22.2% ($n = 675$), 14.4% ($n = 593$), 14.4% ($n = 334$), 11.4% ($n = 410$) and 12.4% ($n = 482$) for Dutch, South-Asian Surinamese, African Surinamese, Ghanaians, Turkish, and Moroccans, respectively. The C-statistic varied between 0.77–0.92 among the Dutch, 0.66–0.83 in the South-Asian Surinamese, 0.70–0.82 in African Surinamese, 0.61–0.81 in Ghanaians, 0.69–0.86 in the Turkish and 0.69–0.87 in the Moroccan populations. The C-statistics were consistently lower among the Ghanaians, compared to other ethnicities. One model with Hba1c as a predictor had C-statistics varying between 0.92–0.98 across ethnicities. Calibration was poor (Hosmer-Lemeshow $p < 0.05$) for all models except one.

Conclusion: In general, existing risk assessment models show moderate to good discriminatory ability in different ethnic populations, but poor calibration. Furthermore, these models show heterogeneous discrimination per ethnicity.

Supported by: Dutch Heart Foundation, ZonMw, European Union (FP-7)

Disclosure: **M. Obura:** Grants; Dutch Heart Foundation (grant number 2010T084), Netherlands Organization for Health Research and Development (ZonMw) (grant number 200500003), European Union (FP-7) (grant number 278901). Non-financial support; Academic Medical Center, Amsterdam and the Public Health Service of Amsterdam.

286

Triglycerides, triglyceride-rich lipoprotein subfractions and genetic predisposition for type 2 diabetes in the Women Genome's Health Study (WGHS)

S. Ahmad, S. Mora, P.M. Ridker, F.B. Hu, D.I. Chasman;
Harvard University Boston, USA, Boston, MA, USA.

Background and aims: Higher triglyceride (TG) is an independent risk factor for the development of type 2 diabetes mellitus (T2DM). Previously genetic-susceptibility for TG has shown to be paradoxically protective for the development of T2DM and further a genetic risk score has shown interaction with baseline TG for incident T2DM. Whether such an interaction is present in our prospectively ascertained sample and the interaction pattern is selective with respect to TG-rich lipoprotein particle (TRLP) subfractions remains to be explored.

Materials and methods: The prospective sample of WGHS cohort consists of 15,813 participants with fasting status including 1453 incident T2DM cases. A weighted genetic risk score (TG-wGRS) was calculated based upon the 40-TG associated published genetic variants.

Results: The TG-wGRS was inversely associated with incident T2DM (HR 0.66, 95%CI (0.58, 0.75), P value ≤ 0.0001 per 10-TG associated risk alleles) when the Cox model was adjusted for baseline BMI, HDL-C and TG. TG was associated with higher risk of incident-T2DM in individuals within the low TG-wGRS tertile (HR [95%CI] = 1.98 [1.83, 2.14]) per mmol/L compared to the high TG-wGRS tertile (HR [95%CI] = 1.68 [1.58, 1.80] per mmol/L, $P_{interaction} = 0.0007$). Similarly, in TG-adjusted analysis, large and medium but not the small-TRLPs associated with higher T2DM-incidence in the low TG-wGRS tertile compared to the middle and higher TG-wGRS tertiles, $P_{interaction}$ = 0.014, 0.012 and 0.6203, respectively.

Conclusion: Our results confirm the original findings and further suggest that increased TG-risk for the incident-T2DM among low genetic risk individuals compared to higher TG-associated genetic risk individuals, and these interaction effects are mostly observed through larger TRLP subfractions.

Supported by: NCI (CA047988, UM1CA182913); NHLBI (HL043851, HL080467); AHA (0670007)

Disclosure: **S. Ahmad:** None.

287

A prospective study on fasting glucagon prior to OGTT and mixed meal and 7-year change of fasting glucose: the Hoorn Meal Study

A.D.M. Koopman[1], F. Rutters[1], M. Alssema[1,2], A. van der Heijden[3], P. Elders[3], J. Beulens[1,4], J. Dekker[1];
[1]Department of Epidemiology and Biostatistics, Amsterdam Public Health Institute, Amsterdam, Netherlands, [2]Unilever Research and Development, Vlaardingen, Netherlands, [3]Department of General Practice and Elderly Care, Amsterdam Public Health Institute, Amsterdam, Netherlands, [4]Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, Netherlands.

Background and aims: Several cross-sectional studies observed that abnormal glucagon responses already exist in individuals with impaired glucose tolerance. However, prospective data on elevated fasting glucagon levels possibly preceding glucose deterioration is limited.Therefore, the aim of this study was to examine the association of fasting glucagon preceding an oral glucose tolerance test (OGTT) and a mixed meal test (MTT) at baseline with changes in fasting glucose levels 7 years later, in individuals who were non-diabetic at baseline.

Materials and methods: We used data from the Hoorn Meal Study; a cohort study among 121 persons without diabetes (age 61.0 ± 6.7, 50% men), who were subjected to a 5-point 75 g-OGTT and 7-point MMT, in random order, at baseline. After 7 years, fasting glucose levels were determined. The association of fasting glucagon at baseline with fasting glucose levels after 7y, corrected for baseline glucose levels, age, sex, follow-up duration, BMI and fasting incretin levels, was determined using linear regression analysis.

Results: Median (IQR) fasting glucagon level prior to OGTT was 9.55 (3.4) pmol/l and prior to MTT, 8.81 (3.4) pmol/l. As this was systematically different, fasting glucagon was examined as the average of OGTT and MMT and separately for OGTT and MMT. Whether this is a chance finding or reflects anticipatory mechanisms needs to be evaluated. For OGTT, compared to the lowest tertile of fasting glucagon levels, those in the middle and highest tertiles had a higher change in fasting glucose levels at follow-up, respectively 0.17 (95%CI, −0.1; 0.4) and 0.14 (95%CI, −0.1; 0.4) mmol/l. For MMT, compared to the lowest tertile, those in the middle and highest tertiles had a change in fasting glucose levels of −0.11 (95%CI, −0.4; 0.1) and 0.06 (95%CI, −0.2; 0.3) mmol/l at follow-up. For fasting glucagon averaged for OGTT and MMT, for those in the middle and highest tertiles, compared to the lowest tertile, the change in fasting glucose levels at follow-up was 0.001 (95%CI, −0.2; 0.3) and 0.02 (95%CI, −0.3; 0.3) mmol/l.

Conclusion: Within our non-diabetic cohort, fasting glucagon levels at baseline were not associated with changes in fasting glucose levels after 7-year follow-up, suggesting that high fasting glucagon levels are not associated with glucose deterioration over time.

Disclosure: **A.D.M. Koopman:** None.

288

Plasma acylcarnitines and risk of type 2 diabetes in a Mediterranean population at high cardiovascular risk

M. Guasch-Ferré[1], M. Ruiz-Canela[2], J. Li[3], M. Bullo[1], D. Wang[3], E. Toledo[2], C. Clish[4], D. Corella[5], R. Estruch[6], E. Ros[6], M. Fitó[7], L. Liang[3], M.A. Martinez-Gonzalez[2], F.B. Hu[3], J. Salas-Salvado[1];

[1]Rovira i Virgili University, Reus, Spain, [2]Department of Preventive Medicine and Public Health, Pamplona, Spain, [3]Harvard TH Chan School of Public Health, Boston, MA, USA, [4]The Broad Institute, Cambridge, MA, USA, [5]University of Valencia, Valencia, Spain, [6]Hospital Clinic, Barcelona, Spain, [7]Hospital del Mar Research Institute (IMIM), Barcelona, Spain.

Background and aims: Elevated concentrations of acylcarnitine metabolites may be indicative of impaired β-oxidation and mitochondrial dysfunction and have been associated with insulin resistance and type 2 diabetes (T2D). We aimed to evaluate the associations between baseline and 1-year changes in acylcarnitines and their diabetes predictive ability beyond traditional risk factors in individuals at high cardiovascular risk.
Materials and methods: We designed a case-cohort study within the PREDIMED Study including all incident cases of T2D ($n = 251$) after a median of 3.8-years of follow-up, and a random subsample of participants free of diabetes at baseline with available metabolomics data (641 non-cases). Plasma acylcarnitines were measured using a targeted approach by LC-MS/MS. We tested the associations between baseline and 1-year changes in individual acylcarnitines and diabetes risk using weighted proportional hazards Cox regression models. We used elastic net regressions to select acylcarnitines for T2D prediction and compute a weighted score using a cross-validation approach. We evaluated the prediction capability of acylcarnitines beyond conventional risk factors.
Results: An acylcarnitine profile, especially including short-chain and long-chain, was significantly associated with a higher risk of T2D independent of traditional risk factors. The relative risk of T2D per SD increase of the predictive model score were 4.03 (95%CI, 3.36–4.83; $P < 0.001$) for the conventional model, and 4.85 (95%, 3.97–5.94; $P < 0.001$) for the model including acylcarnitines, with a HR of 1.33 (95%CI, 1.13–1.56; $P < 0.001$) attributed to the acylcarnitines. Although the area under the receiver operator characteristic curve improved only slightly after including acylcarnitines (0.86 to 0.88, $P = 0.53$), the net reclassification index (0.19 [95% CI, 0.03–0.34; $P = 0.02$]) and integrated discriminatory improvement (0.04 [95%CI 0.02–0.05]) were significantly improved. One-year increase in C4OH-carnitine was associated with higher risk of T2D after adjustment for potential confounders [per SD increase: 1.44 (1.03–2.01)].
Conclusion: An acylcarnitines profile, mainly including short- and long-chain acylcarnitines, was significantly associated with higher T2D risk in a Mediterranean population at high cardiovascular risk.
Clinical Trial Registration Number: ISRCTN 35739639
Supported by: Lilly Foundation European Association of Diabetes (EASD) through the IISPV
Disclosure: **M. Guasch-Ferré:** None.

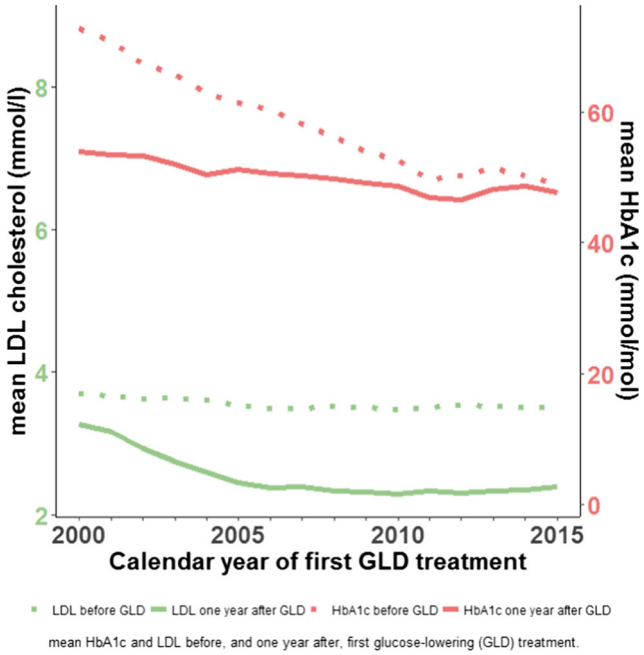
PS 004 Diabetes: therapeutic approaches

289

Improvements in HbA_{1c} and LDL-cholesterol in type 2 diabetes in Denmark, 2000–2015: a population based study

J.S. Knudsen[1], A. Hulman[2,3], D.R. Witte[2,3], R.W. Thomsen[1];
[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus University Hospital, Aarhus, Denmark, [2]Department of Public Health, Aarhus University, Aarhus, Denmark, [3]Danish Diabetes Academy, Odense, Denmark.

Background and aims: Guidelines for monitoring and treating LDL cholesterol and HbA_{1c} in adults with type 2 diabetes have changed in recent decades. The implementation of these guidelines in a clinical setting has not been properly documented. We examined 16-year time trends of blood lipid and HbA_{1c} testing, test results, and the use of lipid lowering drugs in people with type 2 diabetes in Denmark.
Materials and methods: Sequential population-based cross-sectional analysis. We used routine clinical care databases to identify all people living in Northern Denmark from 2000–2015 who initiated first (ever) glucose-lowering treatment (GLD) for type 2 diabetes ($n = 94{,}162$). Within one year following GLD start we assessed whether each patient: (1) had one or more HbA_{1c} tests; (2) had one or more blood lipid tests; (3) received any lipid lowering drug. For each calendar year 2000–2015, we examined pre-treatment HbA_{1c} and LDL cholesterol values, and post-treatment values achieved at 12 months after GLD start. We assessed the proportion of patients achieving post-treatment HbA_{1c} targets below 6.5% and 7%, and LDL values below 1.8 mmol/l and 2.0 mmol/l.
Results: The proportion of patients with at least one HbA_{1c} test within 12 months after GLD start increased from 53% in 2000 to 92% in 2015. The chance of having at least one blood lipid test increased from 82% to 98% and lipid lowering drug therapy within 12 months increased from 12% to 60%. The mean pre-treatment HbA_{1c} declined two percentage points (95% CI: −2.2; −2.0) from 8.8% to 6.6% between 2000 and 2015 (Figure 1). For the mean post-treatment HbA_{1c}, a much smaller decline was seen (−0.6 percentage point (95% CI: −0.7; −0.6), from 7.1% to 6.5%). Contrastingly, pre-treatment LDL cholesterol remained stable, but the mean post-treatment value declined by 1.0 mmol/l (95% CI: −1.0; −0.9) from 3.3 mmol/l to 2.4 mmol/l (Figure 1). From 2000 to 2015, the proportion of patients who achieved a post-treatment HbA_{1c} target <6.5% increased from 37% to 59% and for <7% increased from 54% to 83%. The proportion of patients achieving LDL cholesterol <1.8 mmol/L increased from 5% to 26% and for <2.0 mmol/l increased from 7% to 35%.
Conclusion: Monitoring and treatment of HbA_{1c} and LDL cholesterol in type 2 diabetes have improved substantially in the past 15 years. HbA_{1c} levels before first GLD therapy have decreased substantially and likely drive improvements in meeting HbA1c targets. In contrast, decreasing lipid levels over time are likely related to more intensive lipid lowering therapy.



Disclosure: **J.S. Knudsen:** None.

290

Socioeconomic factors, gender and adherence to lipid-lowering therapy in type 1 diabetes

C. Hero[1], S. Axia Karlsson[1], S. Franzén[2], A.-M. Svensson[2], M. Miftaraj[2], K. Andersson Sundell[1,3], S. Gudbjörnsdottir[1], B. Eliasson[1], K. Eeg-Olofsson[1];
[1]University of Gothenburg, Gothenborg, Sweden, [2]National Diabetes Register, Centre of Registers Västra Götaland, Gothenborg, Sweden, [3]AstraZeneca AB, Medical Evidence and Observational Research, Gothenburg, Sweden.

Background and aims: High adherence and persistence to lipid-lowering therapy (LLT) are important to reduce risk of cardiovascular disease (CVD) in patients with and without diabetes. The aim of this study was to assess the impact of socioeconomic factors and gender on the level of refill adherence and persistence to LLT in persons with type 1 diabetes (T1D).
Materials and methods: We included 6192 T1D persons, 18 years or older, registered in the Swedish National Diabetes Register who initiated novel use of LLT between 1 July 2006 and 31 December 2010. Information on socioeconomic factors were collected from Statistics Sweden and comorbidity from the National patient register. Age and income were divided into quartiles. Marital status was defined as married, single, divorced or widowed and education into compulsory school or lower, upper secondary and post-secondary school. Country of origin was born in Sweden or not. We followed the patients for 36 months estimating adherence to LLT after 18 months and 36 months, by calculating the medication possession ratio (MPR), i e. the proportion of days with medicines on hand, divided into two categories, MPR above 80% and MPR below or equal to 80%. Non-persistence, referred to as discontinuation, was defined as being without medication on hand for more than 180 days. A logistic regression was performed, the models were adjusted for gender, age, socioeconomic factors and previous CVD.
Results: Mean age was 45 ± 12 years and diabetes duration 30 ± 13.5 years. 57% were male. 9% had previous CVD. 93% were born in Sweden. 45% were married. 83% had an education above elementary school. After 18 and 36 months the mean MPR was 72.5 ± 28% and 69.3 ± 28% respectively. 52% had an MPR above 80% at 18 months and 48% after 36 months. 27% discontinued their LLT within 18 months and 42% within 36 months. After 18 months women were more likely to be adherent (MPR above 80%) then men (odds ratio (OR) = 1.18, $p = 0.002$) and less prone to discontinue LLT (OR = 0.81, $p = 0.0006$). At 36 months there was no difference between genders. Adherence increased by age OR = 2.62, $p < 0.0001$ at 18 months, among persons above 53 years compared to those under 36 years, slightly attenuated at 36 months (OR = 3.00, $p < 0.0001$). Discontinuation of therapy decreased by age with OR = 0.43, $p < 0.0001$ at 18 months. Divorced persons were less adherent compared to married with OR = 0.73, $p = 0.001$ at 18 months and OR = 0.71, $p < 0.0001$ at 36 months and discontinued their medication more often OR = 1.39, $p = 0.0002$ after 18 months and OR = 1.52, $p < 0.0001$ after 36 months. Persons who were not born in Sweden discontinued their medication more often (OR = 1.36, $p = 0.0042$) at 18 months. Neither educational level nor income was associated with adherence at 18 or 36 months but persons with high income were less prone to discontinuation.
Conclusion: This nationwide register-based cohort study with data from routine care showed that refill adherence to novel use of LLT in T1D was associated with gender, age, marital status and whether born in Sweden or not. Lower adherence was associated with male gender, younger age and if not born in Sweden. Level of Income and education did not affect adherence. These factors should be taken into consideration when evaluating adherence to medication in clinical practice.
Disclosure: **C. Hero:** None.

291

Investigation of an association of the anti-inflammatory drug sulfasalazine on HbA_{1c} in a large cohort of individuals with type 2 diabetes

S.M.S. N'Dow, L.A. Donnelly, E.R. Pearson, G. Rena;
University of Dundee, Dundee, UK.

Background and aims: Earlier studies including a recent case series have suggested that the anti-inflammatory drug sulfasalazine falsely lowers HbA_{1c}, attributed to hemolytic effects of the drug. In the current study we have investigated possible mechanisms of this effect by comparing sulfasalazine with other structurally related aminosalicylate (5-ASA) drugs (mesalazine, olsalazine and balsalazide) for associations with changes in HbA_{1c} and other hematological parameters.
Materials and methods: An observational cohort study was performed using comprehensive electronic medical records from individuals in the Scottish Care Information Diabetes Collaboration (SCI-Diabetes) in Tayside and Fife, Scotland. Individuals with type 2 diabetes and an incident prescription for a 5-ASA drug between 1st January 2006 and 30th April 2017 were eligible for the study. To allow assessment of HbA1c change, individuals were required to have a baseline HbA_{1c} (defined as closest measure between 6 months prior and 7 days after drug start date) and a treatment HbA_{1c} (defined as the measure closest to 6 months after drug start but within a 3 to 9 months window). To investigate the hemolytic association, change in the constituents of the full blood count were also assessed between baseline and 6 months, where measures were available. As sulfasalazine is used to treat rheumatoid arthritis and mesalazine, olsalazine and balsalazide are used to treat inflammatory bowel disease, we compared patient characteristics at baseline between the drug groups. Variables of interest included gender, age, duration of diabetes, HbA_{1c}, diabetes therapy and constituents of the full blood count. Comparison of baseline characteristics by drug group was by t-test for continuous variables and Chi-square test for categorical variables. Paired t-tests were used to compare the difference in baseline and treatment measures.
Results: A total of 216 individuals were eligible for the study. This was split by 113 individuals on sulfasalazine, 103 on mesalazine with no eligible individuals on olsalazine or balsalazide. There were no significant differences in patient characteristics between the sulfasalazine and mesalazine groups at baseline. We observed a mean(SD) HbA_{1c} reduction of −0.85(1.4)% ($p < 0.0001$) in the sulfasalazine group. There was no significant association in the mesalazine group (0.17(1.4)% ($p = 0.23$)). Sulfasalazine was associated with a mean(SD) increase in mean cell corpuscular volume of 3.7(5.9)fL ($p < 0.0001$) and a decrease in red blood cells of $-0.23(0.4) \times 10^{-12}$/L ($p < 0.0001$).
Conclusion: In this large, observational, population-based study we show that sulfasalazine was associated with a significant decrease in HbA_{1c} of 0.85%

within a 6 month period. In contrast, mesalazine use was associated with a non-significant increase in HbA_{1c}. Our results are consistent with hemolytic effects of sulfasalazine contributing to HbA_{1c}-lowering. These may be contributed by the sulfapyridine pharmacophore in sulfasalazine, which is absent in mesalazine. We do not exclude, owing to the observational nature of our study, that the difference in effect of these two drugs on HbA_{1c} may also be related to differences in absorption or prescribing patterns. This study provides numerous future research opportunities that could guide future clinical practice in diabetes.
Disclosure: **S.M.S. N'Dow:** None.

292

Long-term relapse of type 2 diabetes after bariatric surgery: prediction and clinical relevance

J. Debédat[1], N. Sokolovska[1,2], M. Coupaye[3], L. Genser[4], G. de Turenne[1], J.-L. Bouillot[5], C. Poitou[1,6], J.-M. Oppert[6], S. Ledoux[3], J.-D. Zucker[1,2], K. Clément[1,6], J. Aron-Wisnewsky[1,6];
[1]NutriOmics team, Sorbonne Universités, INSERM, Paris, France, [2]Integromics team, Institute of Cardiometabolism and Nutrition, ICAN, Paris, France, [3]Explorations Fonctionnelles Department, Assistance Publique Hôpitaux de Paris, Louis Mourrier Hospital, Colombes, France, [4]Visceral Surgery Department, Assistance Publique Hôpitaux de Paris, Pitié-Salpêtrière Hospital, Paris, France, [5]Visceral Surgery Department, Assistance Publique Hôpitaux de Paris, Ambroise Paré Hospital, Paris, France, [6]Nutrition Departement, Assistance Publique Hôpitaux de Paris, Pitié-Salpêtrière Hospital, Paris, France.

Background and aims: Roux-en-Y gastric bypass (RYGB) induces 1-year type 2 diabetes remission (DR) in 60–80% of patients, yet relapse occurs in roughly half of them on the longer-term. One-year DR-predictive scores such as the DiaRem or the Ad-DiaRem, including solely baseline parameters, lack to accurately predict 5-years DR (5y-DR). We aimed to develop a new score better at predicting 5y-DR.
Materials and methods: Among our bariatric surgery cohort, we retrospectively included all patients with type-2 diabetes (T2D) who underwent RYGB before May 2013 ($n = 175$; 48 ± 10 years, 29% male, BMI 47 ± 7 kg/m², follow-up 5 ± 0.7 years). An extensive bioclinical phenotyping was performed (i.e. medical history and comorbidities, body composition, bioclinical data, treatments) before RYGB and 3, 6, 12 and 60 months after. We used the 2009 ADA's definition of DR. Using machine-learning algorithms, we developed the "5y-Ad-DiaRem" by integrating variables strongly associated 5-year diabetic outcomes (remission, relapse or non-remission). The variable selection was based on odd-ratios values and statistical significance between our three groups (i.e. 5y-DR, 5-years non-remission (5y-NDR) or 5-years relapse (5y-Relapse)). We examined this score in an independent French RYGB cohort ($n = 54$) in a confirmation purpose.
Results: 61% ($n = 106$) of our patients were in DR 1-year post-RYGB (concordant with the literature), and 25% ($n = 27$) relapsed between the 1st and 5th year. Compared to 5y-DR patients, 5y-Relapse patients exhibited a more severe T2D condition at baseline, as seen with higher HbA1C values and increased anti-diabetic treatments and insulin usage. Besides, they lost significantly less weight during the 1st year post-RYGB (−22% vs −30%, $p < 0.0001$) and regained more afterwards (+8% vs +2.5%, $p < 0.01$). In the 5y-Ad-DiaRem, we included baseline (T2D duration, number of anti-diabetic treatments and HbA1C) and 1-year parameters (fasting glycaemia, number of anti-diabetic treatments, 1-year remission status and weight lost during the first-year). The 5y-Ad-DiaRem was more accurate (AUROC 0.90, accuracy 85%) at predicting long-term T2D outcomes (5y-DR vs 5y-NDR) than both the DiaRem and Ad-DiaRem (AUROC 0.81 and 0.84, accuracy 79% and 78%, respectively). Below a score of 12, patients were predicted to enter in 5y-DR at 5-year (accuracy >0.90). Overall, this improved predictive power translated in the correction of one third (13/39) of the misclassifications of the DiaRem.
Conclusion: The 5y-Ad-DiaRem performed significantly better at predicting 5-year DR status than the published scores and appears relevant to identify patients at risk of relapse (score >11). Using this score could help intensify patient care following the first-year post-surgery, to maximize weight-loss or limit weight regain to prevent DR relapse.

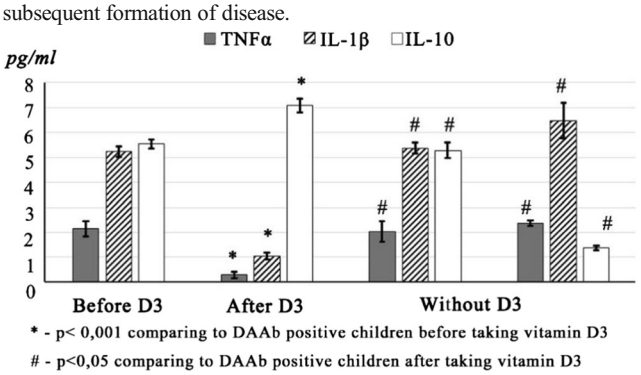
Supported by: IBD, ANR, EU-Metacardis, ICAN
Disclosure: **J. Debédat:** None.

293

Effect of vitamin D_3 therapy on immunological parameters at stages of development of type 1 diabetes in children and adolescents

N.M. Muz, V.V. Popova, V.L. Orlenko, K.Y. Ivaskiva, Y.A. Sayenko, K.M. Tron'ko, O.V. Furmanova, O.V. Bolshova, K.P. Zak, M.D. Tron'ko; State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of Natl. Acad. Med. Sci. of Ukraine», Kyiv, Ukraine.

Background and aims: Dysregulation of the immune processes is the basis of autoimmune development of type 1 diabetes (T1D). Active participation of vitamin D3 in modulating the functions of the immune system in T1D development was established by numerous clinical studies to date. However, the mechanism of this effect is not fully understood. Therefore, the aim of this study was to analyze the prospective immunological data in children positive for the presence of diabetes-associated autoantibodies (DAAb) at the preclinical stage and debut of disease with oral administration of vitamin D3 in comparison with similar children without taking vitamin D3.
Materials and methods: We prospectively studied 37 children positive for DAAb aged 13.43 ± 2.16 years at the preclinical stage with prognostic duration of the preclinical stage of T1D development for 3 years, established on the basis of determining DAAb titers - decarboxylase glutamic acid (GADA) and autoantibodies against the protein tyrosine phosphatase (IA-2A) and 21 children positive for DAAb with predicted debut of T1D. The control group consisted of 17 healthy children of the same age. Oral administration of vitamin D3 at a daily dose of 2000 IU/day for 6 months in children at the preclinical stage and in T1D debut with detected vitamin D deficiency. The titre of DAAb - IA-2A and GADA - was determined by the radioimmunoassay method, the immunophenotype of lymphocytes - by FACS analysis, and the level of cytokines (IL-1β, TNFa, IL-10) - by ELISA.
Results: The maximum significant decrease in elevated titers of DAAb in 6 months after oral administration of vitamin D3 was determined in DAAb positive children at the preclinical stage of the disease development after taking vitamin D3: IA-2A (21.76 ± 3.36 vs 4.03 ± 1.03 U/ml, $p < 0.001$) and GADA (18.31 ± 2.43 vs 5.98 ± 1.57 U/ml, $p < 0.05$) regarding DAAb-positive children at latent stage of T1D formation without taking vitamin D3 and similar DAAb-positive children with T1D debut. At the same time, a predominant decrease in the levels of proinflammatory cytokines IL-1β (5.22 ± 0.21 vs 1.04 ± 0.13 pg/ml, $p < 0.001$), TNFa (2.14 ± 0.32 vs 0.30 ± 0.12 pg/ml, $p < 0.001$), and an increase in the level of the protective cytokine IL-10 (5.53 ± 0.19 vs 7.07 ± 0.27 pg/ml, $p < 0.001$), as well as an increase in the absolute number of CD3 + (0.79 ± 0.05 vs 1.27 ± 0.06 10^9/l, $p < 0.001$), CD4 + (0.52 ± 0.03 vs 0.84 ± 0.04 10^9/l, $p < 0.05$), CD56 + (0.18 ± 0.02 vs 0.84 ± 0.04 10^9/l, $p < 0.05$) were observed in DAAb-positive children at the preclinical stage of T1D after 6-months of cholecalciferol therapy comparing to DAAb-positive children without cholecalciferol use, and similar groups of DAAb-positive children with T1D debut and healthy children.
Conclusion: The use of vitamin D3 at the stages of T1D developmen can serve as pathogenetic-protective therapeutic factor with respect to the subsequent formation of disease.



Disclosure: **N.M. Muz:** None.

294

Change over 12 months in HbA_{1c}, fasting plasma glucose and weight among patients with type 2 diabetes in 37 countries: DISCOVER

P. Fenici[1], F. Bonnet[2], H. Chen[3], J. Cid-Ruzafa[4], M.B. Gomes[5], N. Hammar[6], L. Ji[7], K.F. Kennedy[8], K. Khunti[9], M. Kosiborod[8], S. Pocock[10], W. Rathmann[11], M.V. Shestakova[12], I. Shimomura[13], B. Charbonnel[14];
[1]AstraZeneca, Cambridge, UK, [2]University of Rennes, Rennes, France, [3]AstraZeneca, Gaithersburg, MD, USA, [4]Evidera, Barcelona, Spain, [5]Rio de Janeiro State University, Rio de Janeiro, Brazil, [6]AstraZeneca Gothenburg, Mölndal, Sweden, [7]Peking University People's Hospital, Beijing, China, [8]Saint Luke's Mid America Heart Institute, Kansas City, MO, USA, [9]University of Leicester, Leicester, UK, [10]London School of Hygiene and Tropical Medicine, London, UK, [11]German Diabetes Center, Duesseldorf, Germany, [12]Endocrinology Research Center, Diabetes Institute, Moscow, Russian Federation, [13]Osaka University, Osaka, Japan, [14]University of Nantes, Nantes, France.

Background and aims: DISCOVER is a 3-year, observational study of patients with type 2 diabetes initiating second-line glucose-lowering therapy in 37 countries. We report change from baseline in HbA_{1c}, fasting plasma glucose (FPG) and weight over 12 months.
Materials and methods: HbA_{1c} and weight were assessed in patients who had values recorded for these variables at baseline and 12 months (HbA_{1c}, $N = 7225$; weight, $N = 10\ 332$). FPG was assessed in patients who had FPG values at baseline and 12 months, but who had HbA_{1c} unreported at either time point ($N = 1788$). Patients were categorized by second-line therapy, and changes over 12 months were adjusted for baseline values using least-squares means.
Results: Overall mean (SD) changes over 12 months were: HbA_{1c}, −1.1% (1.6%); FPG, −34.2 mg/dL (59.4 mg/dL); and weight, −0.5 kg (5.5 kg). At 12 months, the overall mean (SD) HbA_{1c} value was 7.3% (1.2%), ranging from 7.0–7.3% in all treatment categories except insulin (8.0%; Table). After initial baseline adjustment, changes in HbA_{1c} were comparable across treatment categories. Weight increased in patients receiving a sulphonylurea or insulin, and decreased in patients receiving a dipeptidyl peptidase 4 inhibitor.
Conclusion: HbA_{1c} and FPG were reduced substantially after 12 months in all treatment categories, with overall mean values only slightly above guideline-recommended targets. Mean weight increases in patients receiving insulin or a sulphonylurea were moderate.

Table. HbA_{1c}, FPG and weight at baseline and 12 months, and baseline-adjusted change over 12 months, according to second-line therapy

	Second-line therapy					
	SU[a]	DPP4i[a]	MET + SU[f]	MET + DPP4i[f]	Insulin[a]	All other[h]
HbA_{1c}, %						
No. of patients[a]	226	299	1461	1960	526	2753
Baseline, mean (SD)	8.0 (1.9)	7.5 (1.2)	8.4 (1.6)	8.0 (1.3)	9.9 (2.0)	8.4 (1.6)
12 months, mean (SD)	7.1 (1.4)	7.0 (0.9)	7.3 (1.2)	7.1 (1.0)	8.0 (1.5)	7.3 (1.3)
Adj. Δ 12 months, lsmean (SE)[b]	−1.06 (0.07)	−1.08 (0.06)	−1.05 (0.03)	−1.20 (0.03)	−0.84 (0.05)	−1.06 (0.02)
FPG, mg/dL						
No. of patients[c]	40	30	480	349	153	736
Baseline, mean (SD)	162.3 (57.5)	141.0 (47.9)	165.5 (50.9)	153.0 (45.2)	198.5 (59.6)	166.7 (57.0)
12 months, mean (SD)	123.5 (40.9)	133.5 (38.0)	134.5 (38.6)	129.0 (37.4)	136.9 (42.9)	130.4 (34.7)
Adj. Δ 12 months, lsmean (SE)[b]	−41.9 (5.8)	−28.9 (6.7)	−31.3 (1.7)	−35.1 (1.7)	−33.6 (3.0)	−35.6 (1.4)
Weight, kg						
No. of patients[d]	324	395	2648	2381	784	3800
Baseline, mean (SD)	80.1 (18.3)	79.9 (17.2)	78.2 (17.0)	83.3 (17.9)	78.5 (18.3)	81.6 (19.4)
12 months, mean (SD)	80.3 (18.1)	79.6 (17.1)	78.3 (17.0)	82.4 (17.4)	78.9 (17.6)	80.7 (18.5)
Adj. Δ 12 months, lsmean (SE)[b]	0.15 (0.30)	−0.33 (0.27)	−0.09 (0.10)	−0.66 (0.11)	0.22 (0.19)	−0.87 (0.09)

[a]Patients with data on HbA_{1c} at both baseline and 12 months; [b]Adjusted for baseline values; [c]Patients with data on FPG at both baseline and 12 months, but unreported data on HbA_{1c} for either baseline or 12 months; [d]Patients with data on weight at both baseline and 12 months; [e]Monotherapy; [f]Dual therapy; [g]May include other therapies in addition to insulin; [h]Monotherapy or combination therapy
Adj., adjusted; DPP4i, dipeptidyl peptidase 4 inhibitor; FPG, fasting plasma glucose; lsmean, least-squares mean; MET, metformin; SD, standard deviation; SE, standard error; SU, sulphonylurea

Clinical Trial Registration Number: NCT02322762
Supported by: AstraZeneca
Disclosure: **P. Fenici:** Employment/Consultancy; AstraZeneca.

295

Can a visit at the dentist's help prevent type 2 diabetes?

L. Czupryniak[1], M. Czerniuk[1], E. Szymańska-Garbacz[2], P. Bijoś[3];
[1]Medical University of Warsaw, Warsaw, Poland, [2]Military Medical Academy Memorial Teaching Hospital - Central Veterans' Hospital, Lodz, Poland, [3]Teva Pharmaceuticals Polska Sp. z o.o., Warsaw, Poland.

Background and aims: Type 2 diabetes should be diagnosed and optimally treated as early as possible, because it is the best evidence-based way to prevent long term vascular complications. Despite universally increasing knowledge of risk factors for diabetes development as well as better understanding of its pathogenesis, with growing public awareness of diabetes and its deadly consequences, still at least a third of cases remain undiagnosed globally. Importantly, the rate of diagnosed individuals with prediabetes is even lower, which decreases global chances for diabetes prevention. Therefore, identifying any means which would facilitate early diagnosis of glucose intolerance conditions is of utmost importance for current public health strategies worldwide. As diabetes is a well known risk factor for periodontal and dental disease, we assumed that a visit at the dentist's might be a valuable opportunity to assess one's glucose tolerance. The aim of the study was to evaluate whether visiting a dentist by a person at risk of developing diabetes may help diagnose glucose metabolism disturbances.
Materials and methods: Between March and June 2017 we conducted a nationwide cross-sectional study with thirty individual dental surgeries involved. Each dentist, having received basic training in diabetes pathophysiology and diagnosis delivered by the authors of the study, was given 20 laboratory referral notes for free fasting plasma glucose (FPG) measurement to be handed over to 20 consecutive eligible individuals who gave their consent to take part in the study. The referred patients were asked to perform FPG test during the following 4 weeks. The study inclusion criteria were: age ≥ 18 yrs, any degree of any dental or periodontal disease, presence of at least one diabetes risk factor (i.e. BMI ≥25 kg/m^2, family history of diabetes, sedentary lifestyle, past gestational diabetes, hypertension, dyslipidemia, cardiovascular disease, policystic ovary syndrome) and negative history of any persistent glucose metabolism disturbances.
Results: Out of 600 referred patients, 469 (78.2%, 330 [70.4%] women, mean [±SD] age 53.7 ± 15.4 yrs) had FPG assessed. Mean FPG in all subjects was 5.4 ± 1.16 mmol/l [97.2 ± 20.9 mg/dl] (range 3.5–20.9 mmol/l [63–377 mg/dl]). In 140 subjects (29.9% of those tested and 23.3% of those referred) impaired fasting glucose (IFG; i.e. FPG ≥5.55 mmol/l [100 mg/dl] and <7.0 mmol/l [126 mg/dl]) was diagnosed. 19 subjects had FPG ≥7.0 mmol [126 mg/dl]. IFG subjects were significantly older than those with normal FPG: 60.1 ± 12.6 vs 50.9 ± 15.7 yrs ($p < 0.001$), and were more often men (40% vs 27%; $p < 0.01$). Odds ratio for IFG in subjects aged ≥60 vs those <60 yrs was 3.413 (95% CI 2.215–5.264). All individuals with any degree of glucose intolerance were referred to diabetes care outpatient centres for further assessment and treatment.
Conclusion: Approximately every fourth patient at the dentist's with at least one diabetes risk factor presents with IFG. Dentists should be particularly aware of prediabetes risk in their male patients aged ≥60 yrs. This population may consitute an important group to whom diabetes prevention programmes should be addressed. In general, increasing awareness of glucose metabolism disturbances among the dentists may help diagnose prediabetes and thus lead to more effective diabetes prevention.
Supported by: Teva Pharmaceuticals Polska Sp. z o.o.
Disclosure: **L. Czupryniak:** None.

296

Burden of illness associated with generalised lipodystrophy (GL) in leptin replacement therapy-naive patients: a longitudinal medical chart review study

B. Akinci[1], E. Oral[2], A. Neidert[3], D. Rus[3], W. Cheng[4], P. Thompson-Leduc[5], T. Salinardi[6], E. Cochran[7], R. Brown[7];
[1]Department of Endocrinology, Dokuz Eylul University Hospital, Izmir, Turkey, [2]Department of Internal Medicine, University of Michigan

Medical School and Health Systems, Ann Arbor, MI, USA, [3]University of Michigan, Ann Arbor, MI, USA, [4]Analysis Group, Boston, MA, USA, [5]Analysis Group, Montreal, QC, Canada, [6]Employee at the time of study, Aegerion Pharmaceuticals, A Novelion Therapeutics Company, Cambridge, MA, USA, [7]National Institutes of Health, Bethesda, MD, USA.

Background and aims: GL is an ultra-rare disorder characterized by lack of adipose tissue, hyperphagia, altered physical appearance, and is associated with increased risk of organ abnormalities and potentially accelerated death. While severe GL patients may receive leptin therapy in research studies, little is known about GL patients that do not receive this treatment. This study assessed the burden of illness associated with GL among leptin-naïve patients using longitudinal, multi-center medical chart review data.

Materials and methods: Medical records of patients (pts) with confirmed non-HIV-related GL, never treated with leptin replacement therapy, from the National Institutes of Health, the University of Michigan, and Dokuz Eylul University, Turkey, were reviewed. Pts were observed from birth to loss to follow-up, death, or end of chart abstraction. Date of first symptoms was defined as the onset of GL-related evidence (1st of symptoms/diagnosis). Physical characteristics were assessed at last visit. Lifetime prevalence of organ abnormalities of the liver, pancreas (including diabetes), kidney, and heart was determined. Kaplan-Meier curves were used to describe 1) time to first organ abnormality from the date of first symptoms, 2) time to progression: from first to second organ abnormality and 3) time to death from birth. A time-varying Cox model was used to describe the association between number of organs with abnormalities and death. HR and 95% CI are reported.

Results: Among 56 pts included in the study 41.1% were male, which is a larger proportion than that found among treated patients. Pts experienced first symptoms at mean age 11.5 y; diagnosis of GL occurred 3.9 years later, at age 15.4 y (SD = 14.4). Most pts (87.5%) had congenital GL, 8.9% had acquired GL and 3.6% had generalized progeroid lipodystrophy. The five most common physical characteristics were muscular appearance, hepatomegaly, lack of facial fat, prominent veins and acanthosis nigricans. Lifetime prevalence of organ abnormalities was 89.3% for liver, 67.9% for pancreas (53.6% diabetes, 12.5% pancreatitis), 50.0% for kidney, and 39.3% for heart. 92.9% of pts had ≥1 organ with abnormalities after appearance of first symptoms, with a median (IQR) time to first organ abnormality of 5.0 (0.8–12.7) y. 53.8% had a second organ abnormality a median (IQR) of 5.7 (2.0–10.4) y later. Among the 14.3% of pts who died, median (IQR) age at death was 31.7 (28.2–52.4) y. A positive association existed between number of organs with abnormalities and death (HR = 3.8, 95%CI = 2.2–6.6, $p < 0.0001$).

Conclusion: This study documents the high burden of GL among leptin-naïve patients, and is the first to quantify the high risk of organ abnormalities, survival/mortality patterns, and the association between organ abnormalities and death. Since more severe patients may have sought leptin therapy under ongoing research studies, evaluation of leptin-naïve patients may underestimate the impact of GL on all patients..

Supported by: Aegerion Pharmaceuticals, A Noevlion Therapeutics Company

Disclosure: **B. Akinci:** Honorarium; Aegerion Pharmaceuticals. Lecture/other fees; AstraZeneca, Eli Lilly, Novartis, Novo Nordisk, Boehringer Ingelheim, Servier, Sanofi-Aventis.

PS 005 Prediction of type 1 diabetes

297

CVB5 proteases 2A reduces insulin granule maturation only indirect

K.-P. Knoch[1], A. Petzold[1], C. Wegbrod[1], A. Sönmez[1], C. Münster[1], A. Friedrich[1], Z. Zuzana Marinicova[1], J.-P. Merl-Pham[2], S. Hauck[2], M. Solimena[1];
[1]Molecular Diabetology, Paul Langerhans Institute Dresden of the Helmholtz Center Munich at Univ. Hospital and Faculty of Medicine, TUD, Dresden, Germany, [2]Molecular Diabetology, Research Unit Protein Science Helmholtz Center Munich, München, Germany.

Background and aims: Regulation of insulin translation is a key process in pancreatic islet beta cells. We previously showed that Coxsackieviruses B (CVBs), which are among the potential environmental factors for triggering/accelerating the autoimmune destruction of beta cells in type 1 diabetes (T1D), exploit for translation of their genome the same cap-independent translation machinery used by beta cells for expression of secretory granule (SG) cargoes, including insulin. CVB Infection on MIN6 cells, however, strongly reduces the stores and release of insulin SGs. Therefore, in these studies we aimed at gaining insight into how CVBs affect the turnover of insulin SGs.

Materials and methods: MIN6 cells were transfected with either CVB5 protease 2A or 3C or infected with CVB5. Levels of insulin and other granule cargoes in control and CVB expressing cells were assessed by western blotting, ELISA, immunostaining and quantitative proteomics.

Results: The levels of insulin and other SG markers were unaffected in MIN6 cells expressing CVB5 3C, while they were reduced in cells infected with CVB5 or expressing CVB5 2A. SG cargo depletion in CVB5 and CVB 2A-expressing cells correlated neither with increased ER stress nor apoptosis. Proteomic analysis of CVB 2A-expressing MIN6 cells revealed the depletion of several factors involved in post-Golgi vesicular transport, including Arf1, GGA2, Rab3b and Sytl4. Knockdown of Rab3b and GGA2, but not of Arf1 or Sytl4, reduced the levels of SG cargoes similarly to the expression of CVB5 2A. No cleavage products attributable to GGA2 or Rab3b were detected in CVB5 2A-expressing cells. On the other hand, time-course studies in MIN6 cells treated with the translation inhibitor cyclohexamide indicated that the half-life of GGA2 and Rab3b is approximately only 12-hours. Hence, reduced cap-dependent translation of GGA2 and Rab3b rather than cleavage could account for their depletion upon CVB 2A expression.

Conclusion: We propose that CVB 2A protease leads to depletion of SG stores by altering their biogenesis/traffic from the Golgi complex, possibly by inducing their premature targeting to lysosomes, hence reducing insulin secretion. Massive intracellular degradation of SG cargoes may in turn affect their antigen presentation.

Supported by: INNODIA

Disclosure: **K. Knoch:** None.

298

Relationship of C-peptide persistence and HbA_{1c} in type 1 diabetes

M. Colombo[1], T.J. McDonald[2,3], P.M. McKeigue[1], H.M. Colhoun[4,5], on behalf of the SDRNT1BIO Investigators;
[1]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, UK, [2]University of Exeter Medical School, Exeter, UK, [3]Blood Sciences, Royal Devon and Exeter Hospital, Exeter, UK, [4]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK, [5]Department of Public Health, NHS Fife, Kirkcaldy, UK.

Background and aims: To show if C-peptide persistence is associated with average longitudinal glycated haemoglobin A_{1c} (HbA_{1c}) in type 1 diabetes mellitus (T1DM) patients.

Materials and methods: The study comprised 5608 adult patients with T1DM recruited from diabetes clinics and primary care into the Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO). Retrospective and prospective clinical record measures of risk factors, eGFR and $HbA_{1c}$and direct measures of eGFR, C-peptide and glucose on day of recruitment were available. We investigated associations of random serum C-peptide with recruitment day HbA_{1c} and average of all retrospective and prospective HbA_{1c} readings through linear regression in models incrementally adjusted for age, sex, diabetes duration and in-sample glucose. Models were also stratified for diabetes duration.
Results: At recruitment median (interquartile range) age was 44.1 (32.6, 53.9) years, duration 21 (11.6, 31.6) years, HbA_{1c} 69 (61, 80) mmol/mol. C-peptide was below 5 pmol/l in 3459 patients (61.6%), while the rest had a median of 91 (21, 321) pmol/l. C-peptide was inversely associated with both recruitment day HbA_{1c} and average longitudinal Hba_{1c}: having a C-peptide of at least 5 pmol/l was associated with an average longitudinal HbA_{1c} that was 2.0 mmol/mol lower (95% CI: −2.8, −1.2; $p = 3.8 \times 10^{-7}$) in models adjusted for age, sex and duration. There was evidence of a linear effect ($p = 4.9 \times 10^{-6}$), with those having C-peptide above 100 pmol/l having an average longitudinal Hba_{1c} that was 3.6 mmol/mol lower (95% CI: −4.5, −2.6; $p = 4.7 \times 10^{-13}$). Models stratified by diabetes duration revealed strongly significant effects in the group of 1901 patients with fewer than 15 years of diabetes, in which having a C-peptide of at least 5 pmol/l was associated with an average longitudinal HbA_{1c} that was 4.2 mmol/mol lower (95% CI: −5.7, −2.8; $p = 2.1 \times 10^{-8}$), while the association was weaker for those with longer duration (−1.1 mmol/mol, 95% CI: −2.0, −0.2; $p = 1.6 \times 10^{-2}$), in models adjusted for age, sex, duration and in-sample glucose.
Conclusion: The effect of persistent C-peptide secretion on average glycaemia is small but independent of confounders and easily detected in this large sample. Possible clinical impact of C-peptide persistence on risk of severe hypoglycaemia is a subject for future study.
Supported by: Diabetes UK and CSO
Disclosure: **M. Colombo:** Grants; Diabetes UK, CSO.

299

Population screening of type 1 diabetes and coeliac disease: Autoimmunity Screening for Kids (ASK)

M. Rewers, C. Geno-Rasmussen, J. Baxter, K. Waugh, L. Yu, B. Frohnert, A.K. Steck, E. Liu;
Univ of Colorado School of Medicine, Aurora, CO, CO, USA.

Background and aims: Early detection can prevent morbidity associated with type 1 diabetes (T1D) and celiac disease (CD). ASK is a 4-year program designed to screen 50 000 children for islet autoantibodies (IA) and transglutaminase autoantibodies (TGA), increase public awareness of T1D and CD, and provide evidence for universal screening. We are reporting first-ever prevalence data for IA and TGA in the U.S. general population children aged 2–17 y.
Materials and methods: ASK has approached ~24,000 children for consent, screened 7295, with results available for 7021. Study participants' age, sex, and race/ethnicity closely reflected Denver's general population; 4% had a first-degree relative (FDR) with T1D. Standard radiobinding assays (RBA) and more specific electrochemiluminescence (ECL) assays for autoantibodies to insulin, GAD, IA-2, ZnT8 and TGA were used for screening and confirmation 2–6 weeks later. Children with confirmed persistent IA received follow-up with education to prevent DKA, metabolic monitoring, psychological support, and referrals to prevention trials or clinical services.
Results: Multiple IA, predicting a 44% 5-y risk of T1D, were found in 37 (0.5%; 95% CI 0.4–0.7%) children. A single positive IA, confirmed as high-affinity by ECL and predicting a 29% 5-y risk of T1D, was found in an additional 36 (0.5%; 0.4–0.7%) children. Nearly 90% (64/73) of the screening-detected high-risk children did not have an FDR with T1D. Of the 73 high-risk children, all remained persistently IA positive, 12 have developed dysglycemia and four have already progressed to clinical T1D. The prevalence of TGA varied from 2.7% (2.4–3.1%) using the most sensitive ECL assay (detects IgA, IgG and IgM antibodies) to 2.1% (1.8–2.5%) using standard RBA assay and 0.6% (0.5–0.8%) using a cutoff 10 times the upper limit of normal range. Only 30% of TGA-positive children reported symptoms usually associated with CD.
Conclusion: This novel population-based screening program for the two most common autoimmune diseases of childhood reports high prevalence of pre-symptomatic T1D and CD in Denver children. Prospective follow-up of screening-detected cases for clinical outcomes and cost-effectiveness analysis will inform potential future universal screening.

Prevalence (%) and the binominal 95% confidence interval of islet or transglutaminase autoantibodies by family history of T1D or CD.

First degree relative		Islet autoantibodies (IA)		Transglutaminase autoantibodies (TGA)		
		Multiple *5-y T1D risk 44%*	Single high-affinity# *5-y T1D risk 29%*	IgA, IgG, IgM# by ECL	IgA by RBA&	IgA by RBA > x10 nl
with T1D	Yes, n=368	1.4% (0.4-3.1%)	1.1% (0.3-2.8%)	5.7% (3.6-8.6%)	3.8% (2.1-6.3%)	0.8% (0.2-2.4%)
	No, n=6653	0.5% (0.3-0.7%)	0.5% (0.3-0.7%)	2.6% (2.2-3.0%)	2.0% (1.7-2.4%)	0.6% (0.4-0.8%)
with CD	Yes, n=298	0.3% (0.0-1.9%)	0% (0.0-1.2%)	7.7% (5.0-11.4%)	4.7% (2.6-7.8%)	1.3% (0.4-3.4%)
	No, n=6723	N=0.5% (0.4-0.7%)	0.6% (0.4-0.8%)	2.5% (2.2-2.9%)	2.0% (1.7-2.4%)	0.6% (0.4-0.8%)

detected by both electrochemiluminescence (ECL) assay and radiobinding assay (RBA)
& normal limit = the 100th percentile of TGA distribution in a healthy population

Supported by: JDRF HCT 3-SRA-2018-564-M-N
Disclosure: **M. Rewers:** None.

300

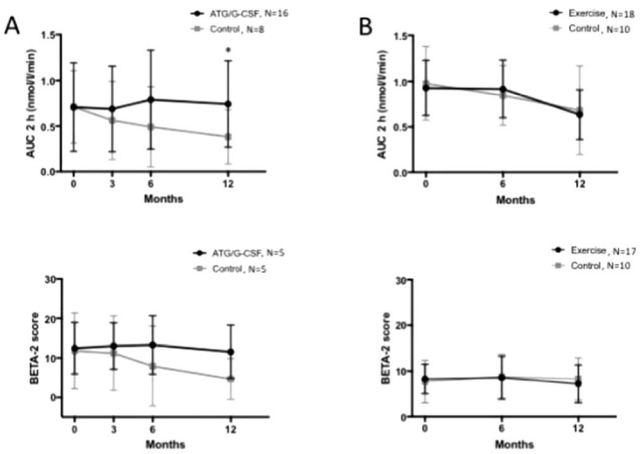
The BETA-2 score: a novel measure of beta cell function in type 1 diabetes intervention trials

A. Lam[1], R. Oram[2], R. Andrews[2], P. Narendran[3], M. Haller[4], P. Senior[1]; [1]University of Alberta, Edmonton, AB, Canada, [2]University of Exeter, Exeter, UK, [3]University of Birmingham, Birmingham, UK, [4]University of Florida, Gainesville, FL, USA.

Background and aims: Stimulated C-peptide following mixed meal tolerance test (MMTT) is the gold standard endpoint in type 1 diabetes (T1D) intervention trials. Unfortunately, MMTTs are costly, time consuming and fail to capture the clinical benefit expected with beta cell preservation. The BETA-2 score is a validated index of beta cell function in clinical islet transplantation. The score is conveniently calculated from a single fasting blood sample based on C-peptide, HbA_{1c}, blood glucose and insulin dose. It may therefore be useful as a practical and clinically relevant endpoint in T1D trials. This was a proof of concept study comparing BETA-2 score with AUC C-peptide as measures of beta cell function in T1D trials.
Materials and methods: Data from 2 RCTs were analyzed separately post-hoc. The primary or secondary endpoint of each trial was change in AUC C-peptide following 2 h MMTT at 12 months. The anti-thymocyte globulin (ATG)/pegylated granulocyte CSF (GCSF) trial included T1D subjects (age 12–45 years, duration 4–24 months) randomized to ATG/GCSF ($n = 17$) or placebo ($n = 8$). The EXTOD trial included T1D subjects (age 16–60 years, duration <12 weeks) randomized to exercise ($n = 27$) or usual care ($n = 17$). The BETA-2 score were calculated before, during and end of study where data were available. Data are expressed as mean ± SD (control vs. treated).
Results: In both trials, the BETA-2 score significantly correlated with AUC C-peptide at 0, 6 and 12 months (ATG/G-CSF trial $r = 0.685$–0.853, $P < 0.01$; EXTOD trial $r = 0.391$–0.657, $P = 0.00$–0.01).

Intriguingly, BETA-2 measured at both 3 and 6 months correlated significantly with AUC C-peptide at 12 months in the ATG/G-CSF trial (3 months: $r = 0.60$, $P = 0.02$; 6 months: $r = 0.80$, $P = 0.00$). Compared with control, ATG/GCSF treatment was associated with superior beta cell function at 12 months measured by AUC C-peptide (0.43 ± 0.32 vs. 0.74 ± 0.47 nmol/l/min, $P = 0.05$) or BETA-2 although this difference was not statistically significant (5.2 ± 5.4 vs. 9.7 ± 5.4, $P = 0.11$). In the EXTOD trial, there were no differences between control or intervention using either AUC C-peptide (0.69 ± 0.44 vs. 0.61 ± 0.30 nmol/l/min, $P = 0.51$) or BETA-2 score (7.8 ± 4.8 vs. 7.1 ± 4.1, $P = 0.69$) at 12 months.

Conclusion: The BETA-2 score allows for more convenient and frequent assessment of both beta cell function and metabolic outcome. It may therefore be useful as a surrogate endpoint in T1D intervention trials allowing for smaller and shorter duration trials. We show here that the BETA-2 score correlates well with AUC C-peptide and that early BETA-2 score appears to be predictive of AUC C-peptide at 1 year. Furthermore, we found that the BETA-2 score showed a trend in response to treatment that was similar to AUC C-peptide. Our study was limited in terms of missing data limiting statistical power and thus, further prospective studies evaluating the reliability and responsiveness of the BETA-2 score in T1D trials are warranted.



AUC C-peptide and BETA-2 score in subjects from the ATG/G-CSF trial (A) and the EXTOD trial (B). AUC C-peptide are shown as total AUC divided by 120 minutes. *P=0.05.

Disclosure: **A. Lam:** None.

301

Type 1 diabetes leading to severe insulin deficiency occurs after 30 years of age and is commonly treated as type 2 diabetes in clinical practice

N. Thomas, A. Grubb, T. McDonald, A. Hill, M. Weedon, R. Oram, A. Hattersley, A. Jones;
Exeter University, Exeter, UK.

Background and aims: Severe loss of endogenous insulin secretion defines type 1 diabetes treatment requirements. We aimed to determine the prevalence and characteristics of type 1 diabetes leading to severe endogenous insulin deficiency after age 30 in patients with insulin treated diabetes.

Materials and methods: We assessed the characteristics of type 1 diabetes defined by rapid insulin requirement (within 3 years) and severe endogenous insulin deficiency (non-fasting C-peptide <200 pmol/l) in a population cohort of 583 participants with insulin treated diabetes diagnosed after age 30. We compared characteristics with participants with retained endogenous insulin secretion (>600 pmol/L) and 220 participants with severe insulin deficiency diagnosed under age 30.

Results: 21% (18–25) of insulin treated diabetes diagnosed after 30 had severe insulin deficiency. 39% of these participants did not receive insulin at diagnosis, of whom 46% self-reported type 2 diabetes. Rapid insulin requirement was highly predictive of late onset type 1 diabetes, with 84% requiring insulin within 1 year. 44% of participants progressing to insulin within 3 years develop severe endogenous insulin deficiency. Clinical, biochemical and genetic characteristics were comparable to participants diagnosed before age 30. In contrast patients with retained endogenous insulin secretion had substantially lower type 1 diabetes genetic risk scores (0.268 vs 0.229, $p < 0.001$), antibody positivity (82% vs 6%, $p < 0.001$) and higher BMI (26.0 vs 31.6, $p < 0.001$). 39% of participants with late onset type 1 diabetes and severe insulin deficiency did not receive insulin at diagnosis as shown in table 1, these participants were a median of 12 months from diagnosis before insulin treatment was started. Those where insulin commencement was delayed (compared to immediate insulin) were older at diagnosis, 48 vs 41 years $p < 0.049$ but had similar BMI (25.6 vs 26.9) $p = 0.69$, T1DGRS (0.267 vs 0.268) $p = 0.88$ and islet autoantibodies positivity (78% vs 85%, positive for any antibody) $p = 0.37$. Of those with delayed commencement of insulin only 50% self-reported as having type 1 diabetes vs 96% in those immediately starting insulin ($p < 0.001$), these participants were also more likely to receive oral hypoglycaemic agents: 29% vs 7% $p = 0.001$.

Conclusion: Type 1 diabetes leading to severe insulin deficiency has similar clinical and biological characteristics to that occurring at younger ages, but is frequently not identified. Clinicians should be alert to the possibility of type 1 diabetes in patients requiring insulin within three years of diagnosis.

Table 1

Variable % (95% CI), or median (interquartile range)	Insulin at diagnosis n= 76	Delayed insulin treatment n=48	p value
Current age (years)	60 (49-64)	64 (56-73)	p=0.002
Age diagnosed (years)	41 (36 -51)	48 (39-56)	p=0.049
Duration of diabetes at recruitment (years)	11 (5-22)	17 (8-23)	p=0.11
BMI (kg/m^2)	26.9 (23.1 – 29.5)	25.6 (23.0-28.9)	p=0.69
Gender (% male)	59 (47-70)	50 (35, 65)	p=0.32
T1DGRS	0.268 (0.241-0.285)	0.267 (0.243-0.282)	p=0.88
C-peptide (pmol/l)	7 (3-49)	10 (3-139)	p=0.43
Islet autoantibody positive (%)	85 (72-94)	78 (61-90)	p=0.37
Treated with concurrent Oral hypoglycaemic agent (%)	7 (2-15)	29 (17-44)	p=0.001
Insulin regimen basal bolus or pump (%)	88 (77-94)	82 (66-92)	p=0.41
Insulin dose (units/kg)	0.61 (0.44-0.78)	0.66 (0.49-0.97)	p=0.30
HbA1c (mmol/mol), (%)	69 (57-83), 8.5 (7.4-9.7)	67 (63-80), 8.3 (7.9-9.5)	p=0.98
Self-reported type 1 diabetes (%)	96 (89-99)	50 (35-65)	p<0.001
Self-reported type 2 diabetes (%)	3 (0-9)	46 (31-61)	p<0.001

Disclosure: **N. Thomas:** None.

302

Impact of routine C-peptide screening in individuals with a clinician diagnosis of type 1 diabetes

E. Foteinopoulou, C. Clarke, R. Pattenden, J. McKnight, M.W.J. Strachan;
Western General Hospital, Edinburgh, UK.

Background and aims: Type 1 diabetes (T1D) is typically diagnosed on clinical grounds using criteria such as younger age at presentation, low body mass index, rapid onset of symptoms and the presence of ketosis. It is well recognised that these criteria are not perfect and that some

individuals with other causes of diabetes, such as Type 2 diabetes (T2D) and monogenic diabetes, may be incorrectly diagnosed as having T1D. Such individuals may be commenced unnecessarily on insulin. C-peptide provides a measure of endogenous insulin secretion and can be used to distinguish individuals with severe insulin deficiency (T1D) from those with substantial insulin secretion (T2D and monogenic diabetes).

Materials and methods: In July 2017, C-peptide screening was introduced in our centre as part of routine clinical care for patients with a clinician diagnosis of T1D (1,205 registered patients). Patients with a duration of T1D of ≥3 years were considered for C-peptide screening. Plasma C-peptide was measured on a random sample using the ARCHITECT immunoassay (Abbott). Blood glucose was measured contemporaneously. Severe insulin deficiency was defined as C-peptide <200 pmol/L, when blood glucose was >4 mmol/L. C-peptide ≥200 pmol/L prompted evaluation for other aetiologies of diabetes (measurement of anti-GAD and IA-2 autoantibodies and where appropriate monogenic diabetes screening).

Results: Data for the first 390 patients screened are reported. 335 (85.9%) patients had C-peptide <200 pmol/L, and so were considered to be appropriately managed with insulin, with a probable diagnosis of T1D. 55 patients (14.1%) had C-peptide ≥200 pmol/L and their initial diagnosis of T1D was re-evaluated. 8 of these patients (14.5%) had C-peptide >900 pmol/L, consistent with significant insulin resistance. All had negative autoantibodies and their diagnosis was revised to T2D. Two of these patients were Caucasian and had presented with ketoacidosis at diagnosis. So far 4 patients have switched from insulin to alternative anti-diabetic therapy (median [range] duration of insulin therapy was 10 [4–28] years) and 3 other patients are in the process of insulin withdrawal. 11 patients (20.0%) had C-peptide ranging from 600–900 pmol/L. Of these, 3 patients had at least one autoantibody positive in high titre. 1 patient had an isolated anti-GAD antibody titre of 12.5 U/ml and a high T1D genetic risk score (93rd centile); this patient has successfully stopped insulin after 6 years. These 4 patients were all considered to have T1D; their median (range) duration of diabetes was 6 (6–11) years. 7 had negative autoantibodies, of whom 3 were considered to have T2D. 3 patients are undergoing further investigation; 1 patient has confirmed HNF1α monogenic diabetes and has now discontinued insulin after 10 years. 36 patients (65.5%) had C-peptide 200–600 pmol/L. 11 of these patients had positive autoantibodies, 10 had negative autoantibodies (and are undergoing further investigation) and 15 are awaiting confirmation of antibody status.

Conclusion: The introduction of C-peptide testing has permitted cessation of long duration insulin therapy in some patients. We have identified that ketoacidosis can occur in Caucasians with T2D, while substantial endogenous insulin secretion can persist for many years in some patients with T1D. The current clinical criteria used to diagnose T1DM are insufficient. C-peptide testing should be performed in all individuals with a diagnosis of T1D of at least 3 years duration from secondary care service.

Disclosure: **E. Foteinopoulou:** None.

303

Serum 25-hydroxyvitamin D concentration at birth in children screened for HLA-DQB1 conferred genetic risk for type 1 diabetes

M. Mäkinen[1], E. Löyttyniemi[2], M. Koskinen[1], M. Vähä-Mäkilä[1], H. Siljander[3], M. Nurmio[1], J. Mykkänen[2], S.M. Virtanen[4,5], O. Simell[2], H. Hyöty[5,6], J. Ilonen[1], M. Knip[3,5], R. Veijola[7], J. Toppari[1];
[1]University of Turku and Turku University Hospital, Turku, Finland, [2]University of Turku, Turku, Finland, [3]University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [4]National Institute of Health and Welfare, Helsinki, Finland, [5]Tampere University and University Hospital and Science Centre, Tampere, Finland, [6]Pirkanmaa Hospital District, Tampere, Finland, [7]University of Oulu and Oulu University Hospital, Oulu, Finland.

Background and aims: Vitamin D has several effects on the immune system that might be of relevance for the pathogenesis of type 1 diabetes. The aim of this study was to evaluate whether cord serum concentration of 25-hydroxy-vitamin D (25[OH]D) differ in children developing either islet autoimmunity or type 1 diabetes during childhood and adolescence.

Materials and methods: Umbilical cord serum samples from 764 children born 1994–2004 with HLA-DQB1 conferred risk for type 1 diabetes participating in the Type 1 Diabetes Prediction and Prevention (DIPP) study were analyzed for 25(OH)D using enzyme immunoassay. The participants comprised 250 case children who developed type 1 diabetes at a median age of 6.7 years (Interquartile range [IQR] 4.0–10.1 years) and 132 additional case children who developed islet autoimmunity, i.e. turned positive for multiple islet autoantibodies. Cases were matched for date of birth, gender and area of birth with 382 control children, who remained autoantibody negative. The median duration of follow-up was 9.8 years (IQR 5.7–13.1 years)

Results: The median 25(OH)D concentration in cord serum were low (31.1 nmol/L [IQR 24.0–41.8]; 88% <50 nmol/L), and not statistically different between children who developed type 1 diabetes or islet autoimmunity and their control groups ($P = 0.70$). The levels were associated mainly with geographical location, year and month of birth, age of the mother and maternal intake of vitamin D during pregnancy.

Conclusion: The 25(OH)D concentration at birth is not associated with the development of type 1 diabetes during childhood.

Supported by: JDRF, AF, FUHF, FDRF

Disclosure: **M. Mäkinen:** Grants; Juvenile Diabetes Research Foundation, Academy of Finland, Funds for University Hospitals in Finland, the Finnish Diabetes Research Foundation.

304

Gene expression profile of peripheral blood mononuclear cells of recent-onset type 1 diabetes

A.S. Santos[1], C. Chevillard[2], N.V. Gonfinetti[3], J. Kalil[4], E. Cunha-Neto[4], M.R. Silva[1];
[1]Endocrinologia, Laboratório de Carboidratos e Radioimunoensios- LIM 18 -Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR, Sao Paulo, Brazil, [2]Aix-Marseille Université/ INSERM/AMU UMR_1090 – France, Marselha, France, [3]Endocrinologia, Instituto Castro de Medicina, Sao Paulo, Brazil, [4]Imunologia, Disciplina de Imunologia e Alergia- LIM 60 e Laboratório de Imunologia - Instituto do Coração- FMUSP-Universidade de São Paulo, Sao Paulo, Brazil.

Background and aims: Type 1 diabetes mellitus (T1D) is characterized by the autoimmune destruction of insulin-secreting β-cells, mediated by T auto reactive infiltrating cells, inflammatory cytokines and immunological mechanisms. However, little is known about the expression of genes and pathways dysregulated in peripheral blood mononuclear cells (PBMC) of patients with T1D. Aims: to investigate the gene expression profiles of circulating PBMC in recent-onset T1D patients (up to 6 months of diagnosis) in comparison with controls in the context of putative disease-related pathobiological processes and pathways

Materials and methods: expression of mRNA from PBMC of 12 T1D patients, 16.4 ± 8.9 years and age-matched 12 healthy controls, 15.0 ± 8.1 years, ($p > 0.05$) was evaluated with the Whole Human Genome Microarray Kit Agilent (58341 probes) and analyzed by GeneSpring software with a fold change cutoff of 1.5 and adjusted P value <0.05; pathways analysis was performed with the software Ingenuity Pathway Analysis (IPA).

Results: 223 genes (259 probes) were differentially expressed between T1D patients and controls, of which 129 (58%) were upregulated, while 94 (42%) genes were downregulated in T1D. The interactions between the differentially expressed genes evidenced expression patterns of 30 networks. The 10 most significantly enriched (most discriminating) canonical pathways between groups were those related to tumor necrosis factor (TNF) pathway and cell cycle regulation (cellular growth, mitosis, survival, apoptosis, DNA repair and genomic stability). Pathways

analysis indicated there was a trend for activation of inflammatory pathways like TNF receptors (TNFR1 and TNFR2) associated with increased expression of genes TNFAIP3 (TNP alpha induced protein 3), TNFRSF12A (OX40 receptor,TNF receptor superfamily member 12A), CDC42 (cell division cycle 42) and reduction of IKBKB (inhibitor of nuclear factor kappa B kinase subunit beta) , favoring also NFkB signaling.

Conclusion: our data suggest a proinflammatory activation profile dependent on TNF receptor pathway and inflammatory NFkB signaling in peripheral blood mononuclear cells, which could play a role in T1D pathogenesis.

Supported by: EFSD

Disclosure: **A.S. Santos:** None.

PS 006 Diabetes progression

305

Impact of visit-to-visit fasting plasma glucose variability on the development of type 2 diabetes: a nationwide population based cohort study

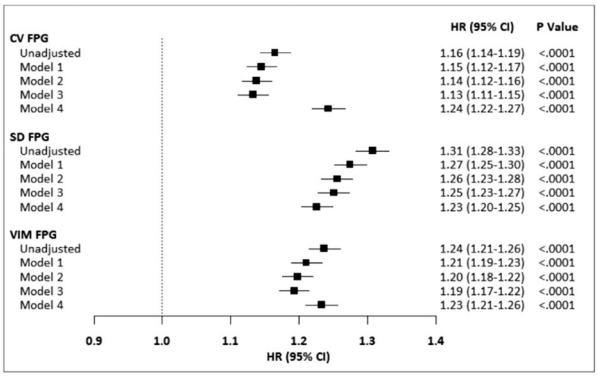
J. Kim, Y. Lee, E. Roh, S. Hong, N. Kim, H. Yoo, J. Seo, S. Kim, N. Kim, S. Baik, K. Choi;
College of Medicine, Korea University, Seoul, Republic of Korea.

Background and aims: Glucose variability is the deviation from steady state of glucose concentration. Glycemic variability have recently draw attention as an another aspect of glycemic control and may contribute to additional risk for diabetic complications independent of HbA1c. Previous studies demonstrated that fluctuations in blood glucose levels had a greater impact on oxidative stress, inflammatory cytokines, and endothelial function than sustained hyperglycemia. On the other hand, oxidative stress-activated signaling pathway leads to both insulin resistance and impaired insulin secretion, which are main pathogenic mechanism of diabetes. Although these evidences suggest the relationship between long-term glycemic variability and development of type 2 diabetes, there has been no previous study. Therefore, we examined the impact of visit-to-visit FPG variability on the development of type 2 diabetes using a large population-based cohort data from the National Health Insurance Service-National Health Screening Cohort.

Materials and methods: The current study analyzed 131,744 Korean men and women without diabetes using the Korean National Health Insurance System cohort with periodic health examination program. FPG variability was calculated using the coefficient of variance (FPG-CV), standard deviation (FPG-SD), and variability independent of the mean (FPG-VIM). The number of FPG measurements per subject ranged from 3 to 6: 3 measurements (n = 68,027, 52%), 4 measurements (n = 14,078, 11%), 5 measurements (n = 18,663, 14%), and 6 measurements (n = 30,976, 24%). Kaplan-Meier and Cox proportional hazard analyses were used to evaluate the association between FPG variability and type 2 diabetes.

Results: During the median follow-up of 8.3 years, Kaplan-Meier curves demonstrated lower disease-free probability in higher FPG variability group compared to lower FPG variability group. Multivariable Cox proportional hazard analysis exhibited that the hazard ratio (HR) for incident T2DM was 1.67 (95% confidence interval [CI] = 1.58–1.77; P < 0.001) in the highest quartile of FPG-CV compared to the lowest quartile of FPG-CV after adjusting for confounding variables. The association between FPG variability and the risk of T2DM was consistent when modelling using FPG-SD and FPG-VIM in both normal and impaired fasting glucose (IFG) group. An increase of 1 SD in the FGP-CV was associated with a 24% increased risk of T2DM in the fully adjusted model.

Conclusion: Increased variability of FPG is associated with the development of type 2 diabetes independent of diverse risk factors. These results suggest that long-term FPG variability may be useful for risk stratification of type 2 diabetes in the general population.



Hazard ratios and 95% confidence intervals for every one SD increase of fasting plasma glucose variability
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, income, family history of diabetes, hypertension, dyslipidemia, and body mass index (BMI)
Model 3: adjusted for age, sex, income, family history of diabetes, hypertension, dyslipidemia, BMI, smoking, alcohol intake, and exercise
Model 4: adjusted for age, sex, income, family history of diabetes, hypertension, dyslipidemia, BMI, smoking, alcohol intake, exercise, and mean FPG

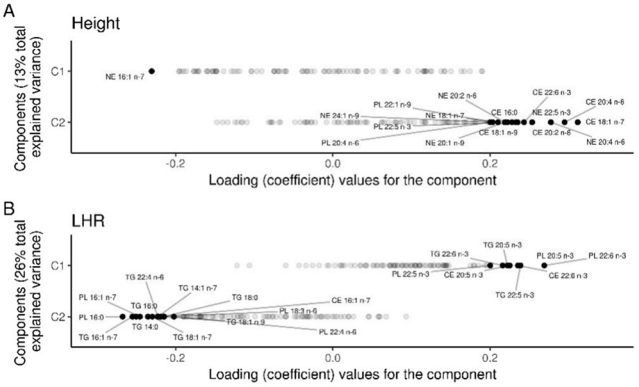
Disclosure: **J. Kim:** None.

306

Leg length, a marker of early childhood conditions, associates with specific clusters of serum fatty acids

L.W. Johnston[1], Z. Liu[2], R. Retnakaran[2], S.B. Harris[2], R.P. Bazinet[2], A.J. Hanley[2];
[1]Aarhus University, Aarhus, Denmark, [2]University of Toronto, Toronto, ON, Canada.

Background and aims: Adverse early childhood conditions have been associated with greater risk for adult chronic diseases such as type 2 diabetes (T2DM) and cardiovascular disease. However, the specific mechanism of action is not well elucidated. Adult leg length is an established biomarker of early childhood conditions. We aimed to explore distinct clusters of a broad spectrum of serum fatty acids (FA) by height and leg length.
Materials and methods: Canadian adults ($n = 453$) at risk for T2DM from the Prospective Metabolism and Islet Cell Evaluation (PROMISE) cohort had detailed personal and metabolic data collected, including the measurement of FA and stature. The concentrations of 22 FA in the cholesteryl ester (CE), phospholipid (PL), triacylglycerol (TG), and non-esterified (NE) fractions were quantified. Height and sitting height were measured, which were used to compute leg to height ratio (LHR). To identify clusters in the FA profile, we used the supervised dimensionality reduction method partial least squares (PLS) with the stature components as the constraining variables and the FA as the predictor variables. Separate models were analyzed for height and LHR.
Results: The participants were mostly female (72%) and of European-ancestry (71.6%). Mean (SD) age was 50.4 (10.0) years, height was 166.1 (9.1) cm, and LHR was 0.47 (0.02). The four FA with the largest proportion in serum were CE 18:2 n-6 (20.6%), PL 16:0 (11.5%), PL 18:2 n-6 (8.2%), TG 18:1 n-9 (7.8%). For each PLS model, we extracted the first two components (C1 and C2; Figure). Higher LHR tended to load with a higher C1 cluster (i.e. more 20:5n-3 and 22:6n-3 in multiple lipid fractions) and loaded with a lower C2 cluster (i.e. less 14:1n-7, 14:0, 16:0, 16:1n-7, 18:0 in primarily the TG and PL fractions). There were no well defined specific cluster of FA in C1 for height, which may reflect that higher height correlates positively with total FA concentration. Height tended to load with a higher C2 cluster (e.g. 20 or more carbon long FA in multiple fractions).
Conclusion: We found that shorter adult leg length had a distinct lipid profile compared to shorter height, reflecting more omega-3 long chain FA and less of the 14 and 16 chain FA. Previous research has shown that these 14–16 chain FA associate with greater de novo lipogenesis and exert lipotoxic effects. Our results suggest that early childhood conditions, as reflected in adult leg length, may lead to changes in lipid production and usage.



PLS loadings of C1 and C2 by height (A) and LHR (B). Higher loading values indicate a stronger contribution of the FA to the component. Interpretation is that a higher loading score correlates with a higher height or LHR, and that higher FA corresponds to its loading value. For example, in Figure B, one unit increase in PL 22:6 n-3 contributes to the C1 score by 0.269.

Supported by: CDA, CIHR, BBDC
Disclosure: **L.W. Johnston:** None.

307

Individuals fulfilling criteria for type 2 diabetes display transient evidence of autoimmunity preceding diagnosis with possible clinical implication: the HUNT study

E.P. Sørgjerd[1], B.O. Åsvold[2,1], P.M. Thorsby[3], V. Grill[4];
[1]Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology, Levanger, Norway, [2]Department of Endocrinology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway, [3]Hormone Laboratory, Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway, [4]Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology, Trondheim, Norway.

Background and aims: Type 2 diabetes is heterogeneous, and phenotype, prognosis and efficacy of treatment can vary greatly between individuals. In this context, we examined if some individuals may have transient evidence of autoimmunity preceding the diagnosis of type 2 diabetes, and if so, if clinical characteristics differed from those of other individuals with type 2 diabetes.
Materials and methods: We used data and serum samples from the second (HUNT2, 1995–1997) and third (HUNT3, 2006–2008) surveys of the Nord-Trøndelag Health Study (HUNT). We included 794 individuals diagnosed with type 2 diabetes between HUNT2 and HUNT3. Classification criteria of type 2 diabetes were autoantibody-negativity (GADA and IA-2A) and no insulin treatment within one year after diagnosis. From these individuals, serum samples from HUNT2 (prior to diabetes diagnosis) were assayed for GADA, IA-2A, ZnT8A and IAA.
Results: Among 794 individuals, 26 (3.3%) were positive for at least one autoantibody before type 2 diabetes diagnosis. Autoantibody-positive individuals were younger at the time of diagnosis (53 vs. 61 years of age, $p < 0.001$) than autoantibody-negative individuals. There was a tendency of more symptomatic onset of diabetes and also lower insulin production (fasting C-peptide 861 vs. 1006 pmol/L), lower beta-cell function (HOMA2-%B 60.6 vs 78.1) and higher HbA1c (7.1 vs 6.7%) after diagnosis (HUNT3) in previously autoantibody positive participants.
Conclusion: To our knowledge, we are the first to demonstrate transient evidence of autoimmune activity prior to diagnosis in a subgroup of type

2 diabetes. These individuals were diagnosed at a younger age and might have reduced beta-cell function and poorer glycemic control than other diagnosed with type 2 diabetes.

Supported by: Liaison Committee for education, research and innovation in Central Norway

Disclosure: **E.P. Sørgjerd:** None.

308

Longitudinal analyses of serum metabolite phenotypes in the EarlyBird cohort identify metabolic readouts associated with childhood insulin resistance

J. Hosking[1], M. Lauria[2], O. Cominetti[3], A. Jeffery[1], J. Hager[3], J. Pinkney[1], F.-P. Martin[3];
[1]University of Plymouth, Plymouth, UK, [2]University of Trento, Trento, Italy, [3]Nestle Institute of Health Sciences, Lausanne, Switzerland.

Background and aims: Metabolite signatures have emerged as biomarkers associated with insulin resistance (IR) and type 2 diabetes (T2D). These biomarkers have potential to elucidate the mechanisms linking obesity to IR, and identify individuals at risk of T2D, but there are few longitudinal data in children through adolescence, when IR undergoes profound changes. Longitudinal studies in healthy children are essential to resolve whether altered metabolite signatures precede the development of IR. We conducted longitudinal modelling of metabonomic and clinical data from the Earlybird cohort to determine whether metabolite signatures are associated with IR.

Materials and methods: EarlyBird is a non-intervention prospective cohort study (347 healthy UK children followed throughout childhood and puberty). Annual fasting serum samples from a sub-group of 150 children underwent metabonomic profiling by proton nuclear magnetic resonance spectroscopy. Subjects were chosen to represent the range of blood glucose observed in the cohort from 5y to 16y. We applied a combination of methods: consensus based clustering (CClust), and mixed effects modelling (MEM). CClust was used to group children according to their temporal profile of IR (HOMA-IR). Non-parametric testing was performed at each time point, and results aggregated according to the Fisher method to identify influential variables. MEM was used to assess the association between HOMA-IR and individual metabolites, taking into account age, BMI, physical activity and pubertal timing.

Results: MEM identified several metabolites that were significantly and inversely associated ($p < 0.05$) with IR across childhood independently of BMI sds, physical activity and age at peak height velocity. Amongst the most influential metabolites were ketone bodies, branched amino acids, histidine, glutamine, lysine, and creatine. CClust analyses of HOMA-IR trajectories were performed at pre-puberty and adolescence, for both genders (f, females, m, males). This enabled the identification of metabolic patterns of high IR status at specific biological ages. At puberty IR was associated with reduced concentrations of amino acids histidine (f adj. $p = 0.09$, m adj. $p = 0.02$), glutamine (f adj. $p = 0.09$, m adj. $p = 0.03$), lysine (f adj. $p = 0.09$, m adj. $p = 0.007$) and valine (f adj. $p = 0.09$, m adj. $p = 0.02$). Additional patterns in central energy metabolism at puberty were increased 3D-hydroxybutyrate (f adj. $p = 0.09$, m adj. $p = 0.003$) and reduced creatine (f adj. $p = 0.08$, m adj. $p = 0.007$).

Conclusion: The integrative approach developed here enabled us to explore the interactions between metabolite signatures and IR over time, during pubertal development. Several metabolites were associated with IR in children independently of pubertal development, adiposity and physical activity. This longitudinal analysis in healthy children confirms that IR is associated with complex but changing metabolite signatures. Amino acid signatures observed in more obese populations may be consequences rather than antecedents of IR. Ketogenesis was inversely associated with IR throughout childhood, but became positively associated with IR in puberty. Longer term observational studies may show whether these metabolite signatures predict adult health risks such as T2D, or could be used as a basis for intervention.

Supported by: NIHS

Disclosure: **J. Hosking:** Grants; Nestle Institute of Health Sciences.

309

Quantification of individual disease progression in type 2 diabetes patients using a semi-mechanistic model: an IMI DIRECT study

N. Scherer[1], C. Dings[1], V. Nock[2], A.M. Hennige[2], E.R. Pearson[3], P.W. Franks[4], T. Lehr[1], for the IMI DIRECT consortium;
[1]Clinical Pharmacy, Saarland University, Saarbruecken, Germany, [2]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany, [3]Medical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, UK, [4]Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Sweden.

Background and aims: Fasting plasma glucose (FPG) and HbA1c are biomarkers to diagnose type 2 diabetes mellitus (T2DM) and to observe disease progression. Intensive glycemic management by body weight (BW) loss or medication intake results on average in decreased HbA1c levels leading to reduced morbidity and mortality. Likewise, the underlying mechanisms of T2DM progression are still not completely understood, and predictive biomarkers associated with fast and slow rates of glycemic deterioration are currently not available.

Here, we firstly aim to develop a mathematical model to describe the interaction between FPG and HbA1c over time, quantifying the effects of BW changes and pharmacological intervention. Secondly, the model is used to identify the intrinsic disease progression independent of medication intake or BW changes.

Materials and methods: Data from 795 recently diagnosed T2DM patients fulfilling the inclusion criteria of the Diabetes Research on Patient Stratification (DIRECT) study were used for the model development. FPG and HbA1c concentrations and information on BW and pharmacological interventions were reported every nine months during an observation period of 36 months. For model development, nonlinear mixed-effects methods implemented in the software NONMEM (version 7.3.0) were used.

Results: The dataset includes 2745 FPG, 2767 HbA1c concentrations and 2760 BW measurements from 795 patients. FPG is described by a turn-over model with zero-order production and first-order elimination. The formation of HbA1c from hemoglobin (Hb0) is described by a second-order process dependent on FPG and Hb0. The degradation of Hb0 and HbA1c is described by a first-order process assuming an erythrocyte half-life of 120 days. Pharmacotherapy and changes in BW significantly influence the production rate of FPG ($p < 0.001$) and lead to a decreased production rate under medication intake or BW loss. Despite therapeutic intervention, T2DM is known to progress over time at different rates across individuals. Therefore, we estimate an individual progression factor that is multiplied by the production rate of FPG to evaluate the progression of the disease. We observed that during the observation period, 45% of our participants show an increase of their HbA1c levels despite therapy. Assuming no medication and BW changes, our model predicts that 60% are participants progressing in their disease status. The mean observed difference in HbA1c was 1.46 mmol/l (range from −26.0 to 52.0), while the expected difference without any intervention was 1.73 mmol/l (range from −9.54 to 28.2).

Conclusion: We developed a model that successfully describes progression of T2DM considering two key clinical biomarkers (FPG and HbA1c) simultaneously. In addition, it allows to determine intrinsic disease progression not affected by the influence of body weight and other clinical interventions. Our approach presents a new strategy to determine new parameters that can be used in predicting disease progression.

Supported by: IMI DIRECT

Disclosure: **N. Scherer:** None.

310

Breastfeeding effect on insulin and adipokines secretion in women during postpartum period

L. Tchapmi Wandji, S. Leumi, M. Guewo-Fokeng, E. Djahmeni, M. Dehayem, M. Etoa, J. Mbanya, E. Sobngwi;
Molecular medicine and metabolism, University of Yaounde 1, Yaoundé, Cameroon.

Background and aims: Health benefits of breastfeeding have so far focused mainly on infants. However, lactating women could be concerned including weight loss and improved lipid and glycemic metabolism. Because of the energy expenditure associated with breastfeeding and the possible stimulation of insulin secretion by prolactin, we aim to determine the effects of breastfeeding on insulin and adipokines secretion in lactating women.
Materials and methods: We conducted a prospective observational study in a cohort of 38 women over the first six weeks postpartum. They were classified into two groups including exclusive breastfeeding, and mixed or bottle feeding, according to the self-reported predominant infant feeding practice over the observation period. In all participants, we calculated the BMI, measured serum lipids, and performed a 75-g oral glucose tolerance test (OGTT) at two days postpartum, and at the six weeks postpartum vaccination visit. At the second visit, serum levels of C-peptide, adiponectin and TNF-alpha (tumor necrosis factor alpha) were measured. Eating habits and physical activity were recorded using an interviewer-administered standardized questionnaire.
Results: The median age of the participants was 27 [22–29] years old. No participants had a glycemic abnormality at both visits, however, the "exclusive breastfeeding" group had a lower fasting blood glucose at the second visit (4.31 ± 0.55 mmol/L vs. 4.59 ± 0 25 mmol/L, $p = 0.03$). There was no significant difference in the anthropometric and lipid profile between the two groups at the first visit and after six weeks of observation. At the second visit, compared to the mixed or artificial breastfeeding group, the exclusive breastfeeding group had lower levels of C-Peptide (14.50 ± 1.04 pmol/L vs. 14.56 ± 0.73 pmol/L) a higher HOMA-B (54.28 [52.93; 56.86] vs 53.44 [52.96; 54.19]) and a lower HOMA-IR (1.522 ± 0.003 vs. 1.524 ± 0.002). In addition, this group had lower TNF-alpha levels (5.53 ± 3.82 pg/mL vs. 7.48 ± 7.28 pg/mL) and higher adiponectin levels (66.99 ± 4.45 ng/mL vs 65.28 ± 6.13 ng/mL) compared with mixed and artificial breastfeeding group. These associations were significant after adjustment for age, diet and physical activity.
Conclusion: Exclusive breastfeeding is associated with better insulin sensitivity, better insulin secretion, higher levels of adiponectin and lower levels of TNF-alpha compared to mixed or artificial breastfeeding during the first six weeks postpartum. Thus, exclusive breastfeeding has metabolic benefits in postpartum women and may be useful in the prevention of diabetes and related diseases.
Clinical Trial Registration Number: 270/CRERSHC/2016
Disclosure: **L. Tchapmi Wandji:** None.

311

Effects of preoperative hepatitis B virus infection, hepatitis C virus infection, and co-infection on the development of new-onset diabetes after kidney transplantation

M. Yu, J. Liang, C. Lv, M. Xu, J. Gao, R. Rong, T. Zhu;
Zhongshan hospital, Fudan university, Shanghai, China.

Background and aims: The effects of preoperative hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, and co-infection with both viruses on the development of new-onset diabetes after transplantation (NODAT) remains unexplored and controversial in kidney transplant recipients (KTRs). We aimed to examine the associations between different preoperative viral statuses, including HBV infection, HCV infection, as well as co-infection and incident NODAT in a large population of Chinese KTRs. Important confounders were adjusted.
Materials and methods: We conducted a retrospective cohort study of 915 KTRs from Zhongshan Hospital (1993–2014). Exclusion criteria included pre-transplant diabetes, incomplete information, graft loss or death within the first post-transplant year, multiple organ transplantation and renal retransplantation, and the remaining 557 KTRs were enrolled in present study. Pre- and postoperative data were extracted and analyzed. NODAT was diagnosed following the American Diabetes Association guidelines (2014). Viral status was defined by the serological results for hepatitis B surface antigen and anti-HCV antibody. The cumulative incidence of NODAT was compared across 4 groups of KTRs with different viral statuses. Multivariate COX regression models were used to estimate the effects of HBV infection, HCV infection, and co-infection on incident NODAT; potential confounders including sex, age, family history of diabetes, body mass index, donor type, type of dialysis, primary polycystic kidney disease, preoperative levels of biochemical indicators, preoperative viral status, occurrence of acute rejection, medication for immunity induction and immunosuppression were adjusted.
Results: HCV seropositive KTRs, including those with HCV monoinfection and co-infection, presented with a significantly higher cumulative incidence of NODAT than the uninfected KTRs (both $P < 0.05$). Multivariate regression analysis showed that only HCV infection was a notable risk factor for NODAT, increasing the NODAT risk by 3.03 times (95% CI 1.77–5.18, $P < 0.001$). HBV infection and co-infection were not independently correlated with incident NODAT in KTRs.
Conclusion: Preoperative HCV infection remarkably increased the risk of NODAT in Chinese KTRs, while HBV infection and co-infection were not correlated with NODAT development.
Disclosure: **M. Yu:** None.

312

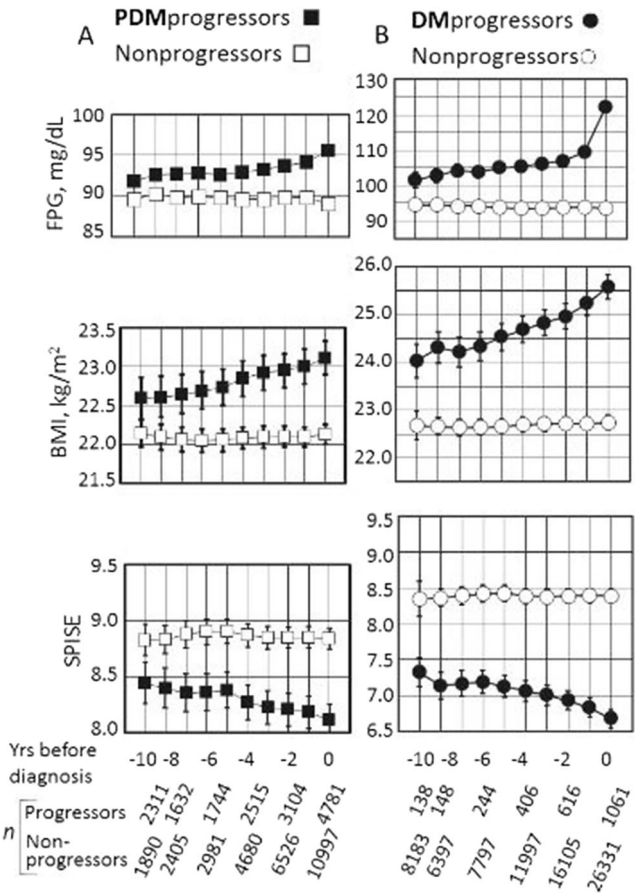
Type 2 diabetes: When does it start?

H. Sagesaka[1], Y. Sato[1], K. Yamashita[1], K. Hirabayashi[1], H. Koike[1], Y. Someya[2], Y. Tamura[2], H. Watada[2], M. Komatsu[3], T. Aizawa[1];
[1]Aizawa Hospital, Matsumoto, Japan, [2]Juntendo University, Tokyo, Japan, [3]Shinshu University, Matsumoto, Japan.

Background and aims: Timeline of type 2 diabetes (DM) has not fully been understood. Above all, it remains unclear when it begins. To disclose the onset of dysglycaemia leading to DM, we assessed trajectories of fasting plasma glucose (FPG) in individuals developed DM, and those developed prediabetes (PDM), separately.
Materials and methods: Data from 27,392 non-diabetic health examinees (male/female ratio, 15,897/11,495; mean age 49 years and body mass index (BMI) 22.6 kg/m^2) containing 15,778 with normal glucose regulation (NGR) and 11,614 with PDM, was retrospectively analyzed with a 5.3 year (mean)-observation. DM was diagnosed with fasting plasma glucose (FPG) ≥126 mg/dL and/or HbA1c ≥6.5%, PDM with FPG 110–125 mg/dL and/or HbA1c 5.7–6.4%, and NGR with FPG <110 mg/dL and HbA1c <5.7%. Trajectories of FPG, BMI, the single point insulin sensitivity estimator (SPISE) and HbA1c, prior to diagnosis of PDM or DM were assessed separately by mixed effects model. SPISE, $[(600 \times \text{HDL-c}^{0.185})/(\text{TG}^{0.2} \times \text{BMI}^{1.338})]$, is an index of insulin sensitivity (Si), where HDL-c is high-density lipoprotein cholesterol and TG triglycerides, both in mg/dL. Validity of SPISE in the Japanese subjects was confirmed in an independent non-diabetic cohort at Juntendo University: correlation between the rate of disappearance of glucose upon hyperinsulinaemic, euglycaemic clamp and SPISE was robust (Spearman rho 0.688, $P < 0.01$, $n = 111$).
Results: Out of 27,392 non-diabetic individuals, 1,061 developed diabetes in whom FPG and BMI were higher and SPISE lower than in those who did not, already at −10 years (all $P < 0.01$): the differences were progressively greater to year 0 (the time of DM diagnosis) (Figure, the right panels). In those developed diabetes, 986 were with PDM and 75 with NGR at baseline, and FPG at −10 years were significantly higher than in newly diagnosed subjects with PDM. Out of 15,778 with NGR,

4781 developed prediabetes, in whom FPG, BMI and SPISE were slightly but significantly different from those who did not at −10 years (all $P < 0.01$). Again, the differences were progressively greater to year 0 (the time of PDM diagnosis).

Conclusion: Glucose dysregulation, increased BMI and lowered Si were detectable at −10 years of diagnosis of DM, and the same abnormalities, albeit mild in degree, existed at least 10 years before diagnosis of PDM. Vast majority of patients with DM gone through the stage of PDM, so that diabetes may have commenced >20 years before its diagnosis. *Figure Legend.* Trajectories of FPG, BMI and SPISE. The right panels, subjects developed and not developed diabetes; the left panels, those developed and not developed prediabetes. Differences between the two groups at each year were all statistically significant ($P < 0.01$). Data are estimated marginal means and 95%CI obtained by mixed effects model. Variation in the graphs for FPG was so small that it is not visible. Years and the number of subjects (every two years for the sake of visibility) shown at the bottom.



Disclosure: **H. Sagesaka:** None.

PS 007 Diet and lifestyle influences

313

Vitamin D, prediabetes and diabetes: bi-directional Mendelian randomisation analysis

N. Wang, L. Zhao, F. Xia, Y. Chen, C. Chen, B. Han, Q. Li, B. Hu, Y. Lu; Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, China.

Background and aims: Vitamin D deficiency is associated with prediabetes and diabetes in many observational studies. However, the causality between them has not been well established. We used bi-directional mendelian randomization (MR) analysis to explore the causal relationship between 25-hydroxyvitamin D [25(OH)D] and glycemic status and indices.

Materials and methods: Participants were included from a survey in East China from 2014–2016 (10,338 and 10,655 participants having diabetes and vitamin D related genotyping information). We calculated weighted genetic risk scores (VD_GRS and DM_GRS) as the instrumental variables for 25(OH)D concentration and diabetes based on related single nucleotide polymorphisms (four SNPs for 25(OH)D and eighteen for diabetes and prediabetes). Fasting plasma glucose and HbA1c were measured. Diagnosis of diabetes and prediabetes was based on American Diabetes Association criteria.

Results: Mendelian randomization analysis showed no significant associations of 25(OH)D with FPG, HbA_{1c} and risk of type 2 diabetes and prediabetes. The causal OR of genetically determined 25(OH)D for risk of diabetes and prediabetes was 0.985 (95%CI 0.940, 1.032) and 0.982 (95%CI 0.948, 1.016) respectively. Using $VD\_GRS_{metabolism}$ and $VD\_GRS_{synthesis}$, the results were not significantly changed. Moreover, the causal regression coefficient of genetically determined diabetes and prediabetes for 25(OH)D was 0.448 (–0.395, 1.291) and 1.303 (–1.210, 3.816). Thus, both directions showed no significant association.

Conclusion: Our results support the conclusion that there is no causal association between vitamin D and diabetes and prediabetes using a bi-directional MR approach.

	Effect size (95% CI)
VD_GRS	
Diabetes	0.985 (0.940, 1.032)
Prediabetes	0.982 (0.948, 1.016)
FPG, mmol/L	-0.015 (-0.035, 0.006)
HbA_{1c}, %	-0.003 (-0.017, 0.011)
$VD\_GRS_{metabolism}$	
Diabetes	0.994 (0.944, 1.047)
Prediabetes	1.002 (0.966, 1.040)
FPG, mmol/L	-0.005 (-0.027, 0.018)
HbA_{1c}, %	-0.002 (-0.017, 0.013)
$VD\_GRS_{synthesis}$	
Diabetes	0.976 (0.901, 1.056)
Prediabetes	0.962 (0.907, 1.020)
Fasting glucose, mmol/L	-0.024 (-0.059, 0.010)
HbA_{1c}, %	-0.005 (-0.027, 0.017)
DM_GRS	
25(OH)D (from diabetes)	0.448(-0.395, 1.291)
25(OH)D (from prediabetes)	1.303(-1.210, 3.816)

Bidirectional mendelian randomization estimates of the association between 25(OH)D concentrations and glycemic status Data are presented as odds ratio (diabetes and prediabetes) or regression coefficient (fasting glucose, HbA_{1c}, 25(OH)D) (95% confidence interval).

Clinical Trial Registration Number: ChiCTR-ECS-14005052
Supported by: NSFC, SJTU, STCSM
Disclosure: **N. Wang:** None.

314

FGF21 regulates insulin sensitivity following long-term chronic stress

M. Dille[1,2], T. Jelenik[1,2], S. Müller-Lühlhoff[1,2], D.G. Kabra[1,2], Z. Zhou[1,2], C. Binsch[1,2], A. Chadt[1,2], M. Roden[1,2], T.R. Castañeda[1,2], H. Al-Hasani[1,2];
[1]German Diabetes-Center, Düsseldorf, Germany, [2]German Center for Diabetes Research (DZD), München, Germany.

Background and aims: Chronic variable stress (CVS) may cause post-traumatic stress disorder (PTSD) and has been linked to increased risk for type 2 diabetes. However, the impact of stress on tissue-specific insulin sensitivity and metabolic long-term adaptations to stress are unknown. We investigated the influence of early life exposure to CVS on long-term insulin sensitivity and glucose metabolism.
Materials and methods: Twelve weeks old male, body weight matched C57BL/6J mice on standard chow were exposed to a 15 day intervention of CVS (swimming, cold exposure, shaking, restraint and isolation). The unstressed control (Ctrl) mice were housed separately. One group of Ctrl and CVS mice were analyzed directly after the CVS intervention. Another group of mice was kept three months without any further stress application (Ctrl3m, CVS3m). Whole-body insulin sensitivity was determined by hyperinsulinemic-euglycemic clamps. Additionally, insulin-stimulated glucose uptake and fatty acid oxidation was measured in intact isolated skeletal muscle. Insulin-stimulated glucose uptake was also determined in isolated primary white adipose cells. Tissues and plasma were collected for molecular and biochemical analyses. Organ-specific insulin signaling was analyzed following i.p. injection of insulin (1 unit/ kg body weight) or saline as a control.
Results: Acutely after stress intervention CVS mice showed markedly increased plasma corticosterone levels (Ctrl: 36.57 ± 8.27 ng/ml, CVS: 186.19 ± 47.49 ng/ml, mean ± SEM, One-Way ANOVA with Bonferroni post-test) and hepatic insulin resistance compared to the Ctrl (Ctrl: 117.1 ± 6.7% of basal, CVS: 91.7 ± 6.5% of basal, mean ± SEM, unpaired student's T-test). However, three months after the stress intervention (CVS3m) mice exhibited improved whole-body insulin sensitivity (Ctrl3m: 275.8 ± 16.7% of basal, CVS3m: 363.3 ± 28.7% of basal, mean ± SEM, unpaired student's T-test), increased insulin stimulated glucose uptake in adipose cells (Ctrl3m: 268.6 ± 35.16 CPM/mg lipid, CVS3m: 432.2 ± 63.1 CPM/mg lipid, mean ± SEM, One-Way ANOVA with Bonferroni post-test) and enhanced mitochondrial function. Plasma levels of fibroblast growths factor 21 (FGF21) were substantially elevated (Ctrl3m: 119.9 ± 26.26 pg/ml, CVS3m: 283 ± 73.55 pg/ml, mean ± SEM, One-Way ANOVA with Bonferroni post-test). Moreover, adipose tissue from CVS3m mice showed increased expression of genes involved in fatty acid oxidation (*Pgc1a*, *Cpt1a*) and formation of brown-like adipocytes (*Ucp1*, *Prdm16*, *Bmp7*).
Conclusion: Early life exposure to CVS leads to long-term improvements in insulin sensitivity, oxidative metabolism and adipose tissue remodeling. These improvements are related to the increase in FGF21 plasma levels and linked to a physiological memory mechanism for maintenance of metabolic homeostasis after early life CVS intervention.
Disclosure: **M. Dille:** None.

315

Did decreasing prevalence of obesity and intensifying regular exercise reduce the number of people at high risk of developing diabetes? Results from nationwide survey

S. Katoh[1], H. Toshima[1,2], Y. Sakamoto[1], K. Utsunomiya[1];
[1]Jikei University, Tokyo, Japan, [2]Chiba Prefectural University of Health Sciences, Chiba, Japan.

Background and aims: The annual National Health and Nutrition Survey (NHNS) is a nationwide population-based survey composed of a questionnaire, physical examination and blood test, conducted by the Japanese Ministry of Health, Labour and Welfare (MHLW). Geographic areas covered by the survey are randomly selected every year. According to the NHNS, the number of people treated with antidiabetic medication or with glycated hemoglobin (HbA1c) ≥6.5% has increased since 1997, while the number of people with 6.5 > HbA1c ≥ 6.0%, who are at high risk of developing diabetes, has decreased since 2007. The MHLW in 2008 began an annual specific medical examination (SME) program for all Japanese workers, followed by lifestyle interventions among those who are at high risk of developing obesity and diabetes. We examined the effectiveness of the SME program by using NHNS data to investigate relationships among trends in the prevalence of obesity, regular exercise, and the number of people who are at risk of developing diabetes.
Materials and methods: As individual data are unavailable, we conducted retrospective analyses using publically available NHNS age group data from 2016 (n = 26,354) and 2015 (n = 6,655) to investigate the longitudinal trend in the prevalence of obesity (body mass index ≥25.0) in Japan. Participants were divided into groups according to sex and age group (20–29, 30–39, 40–49, 50–59, and 60–69 years; and 70 years or older). For example, the population aged 50–59 years in the 2016 survey was aged 40–49 years in 2006 and aged 30–39 years in 1996. In this way, we extrapolated population data and compared 10-year age groups by 10-year survey intervals as if they were longitudinal quasi-cohorts (i.e., we compared NHNS group data from 2016 with data from 2006 and 1996, as well as NHNS group data from 2015 with that from 2005 and 1995; in total, 12 male and 12 female groups). Ratio of persons engaged in regular exercise (RPERE) was calculated, with regular exercise defined as ≥30-min sessions at least 2 times a week, continued for 1 year or more. The same method was applied to the Korean National Health and Nutrition Examination Survey (KNHANES) and the US National Health and Nutrition Examination Survey (NHANES) for reference.
Results: Prevalence of obesity significantly increased from NHNS 1996/1997 to 2006/2007 (25.6 ± 3.2 to 29.3 ± 3.2, $p < 0.0001$) but significantly decreased from 2006/2007 to 2016/2017 (29.3 ± 3.2 to 26.0 ± 3.7, $p = 0.0041$). The delta of prevalence of obesity from NHNS 1996/1997 to 2006/2007 was +6.49 ± 4.6, and that from 2006/2007 to 2016/2017 was +0.13 ± 4.5. These delta values were significantly different ($p = 0.0001$) and were significantly correlated with the corresponding delta of RPERE ($r = 0.447$, $p = 0.0097$ for a total of 32 group data sets). A similar decreasing trend in the prevalence of obesity was observed in KNHANES among only Korean men aged 40–59 years from 1998 to 2013/2014, while an increasing trend was observed among Korean women in KNHANES and among American men and women in NHAMES from the 1960s to 1999/2000.
Conclusion: Prevalence of obesity in the Japanese population decreased (or at least, leveled off), and this was associated with intensified regular exercise. A nationwide lifestyle intervention program may successfully contribute to this change, followed by a decrease in the number of people at high risk of developing diabetes (6.5 > HbA1c ≥ 6.0%).
Disclosure: **S. Katoh:** None.

316

Insomnia and incident risk of diabetes related complications in Hong Kong Chinese patients with type 2 diabetes

C. Ding[1], J. Zhang[2], E. Lau[1], A. Luk[1,3], E. Chow[1], R. Ma[1,3], J. Chan[1,3], Y. Wing[2], A. Kong[1,3];
[1]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong, [2]Department of Psychiatry, The Chinese University of Hong Kong, Shatin, Hong Kong, [3]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong.

Background and aims: Insomnia is associated with worse glycaemic control in patients with type 2 diabetes (T2D). However, whether insomnia has impact on the development of diabetes related complications is under-explored. We hypothesized that insomnia might be associated with increased risk of incident clinical endpoints. Here, we tested the

hypothesis by examining the associations between insomnia and incident clinical endpoints in patients with T2D.

Materials and methods: Participants of Hong Kong Diabetes Register were assessed for sleep habits and insomnia by questionnaires between July 2010 and June 2015 and were prospectively followed up for outcomes. Insomnia was defined as the Insomnia Severity Index score >14. Clinical outcomes including incident cardiovascular disease (CVD), chronic kidney disease (CKD), end-stage renal disease (ESRD), cancer and all-cause death were censored on 30th June 2017.

Results: Among 3407 patients with T2D [mean (standard deviation) age was 54.4 (8.5) years, 57.5% were men], 9.5% had insomnia. Compared to T2D without insomnia, T2D with insomnia had more women (52.2 vs 41.5%), anxiety/depressive symptoms (51.6 vs 15.4%), habitual snoring (44.5 vs 34.9%), worse glycaemic control [glycated haemoglobin, HbA_{1c} 7.72 (1.74) vs 7.50 (1.46) %; fasting plasma glucose 7.91 (3.21) vs 7.54 (2.37) mmol/l] despite the fact that there were higher percentage of T2D with insomnia were put on insulin treatment (31.1 vs 24.6%) than those without insomnia (all $p < 0.05$). At baseline, T2D with insomnia had a higher percentage of sensory neuropathy (7.5 vs 3.2%, $p < 0.001$) and macroalbuminuria (13.1 vs 9.2%, $p = 0.024$) We excluded snorers ($n = 1360$) who might have obstructive sleep apnoea. Among 2047 non-snorers, after a mean follow-up of 4.74 (1.22) years, T2D with insomnia had a higher incidence of ESRD (1.51% versus 0.50%, $p = 0.001$) than T2D without insomnia. After adjusted for potential confounders including age, sex, disease duration of diabetes, HbA_{1c}, low density lipoprotein cholesterol, triglyceride, body mass index, blood pressure, mean sleep duration, anxiety and depression, smoker and drinking habits and medications (anti-diabetic drugs, insulin, anti-hypertensives and lipid-lowering agents), insomnia remained to be significantly associated with increased risk of incident ESRD in patients with T2D (Hazard ratio, HR 2.36, 95% confidence interval, CI 1.02–5.45, $p = 0.044$). However, the effect became attenuated after further adjustment for macroalbuminuria and sensory neuropathy (HR 1.99, 95% CI 0.83–4.75, $p = 0.121$).

Conclusion: T2D patients with co-morbid insomnia may have increased risk of ESRD when compared to their counterparts without insomnia. Our findings suggest a possible link between mental and physical health and call for a holistic approach to improving diabetes care. Long-term prospective studies including T2D with insomnia but without complications at baseline would be required to examine the impact of insomnia on the development of diabetic complications.

Disclosure: **C. Ding:** None.

317

Effects of dietary and physical activity interventions on type 2 diabetes risk in South Asians: individual participant data meta-analysis of randomised controlled trials

A.K. Jenum[1], I. Brekke[2], I. Mdala[1], M. Muilwijk[3], A. Ramachandran[4], M. Kjøllesdal[5], K.R. Richardsen[2], G. Cezard[6], A. Sheikh[6], C.A. Celis-Morales[7], J.M.R. Gill[7], N. Sattar[7], K. Stronks[3], E.J.A. Beune[3], I.G.M. van Valkengoed[3];

[1]1. General Practice Research Unit, Dept. of General Practice, University of Oslo, Oslo, Norway, [2]Oslo Metropolitan University, Oslo, Norway, [3] Dept. of Public Health, Academic Medical Center, Amsterdam, Netherlands, [4]Dr.A.Ramachandran's Diabetes Hospital, Chennai, India, [5]Dept. of Community Medicine and Global Health, University of Oslo, Oslo, Norway, [6]University of Edinburgh, Edinburgh, UK, [7]University of Glasgow, Glasgow, UK.

Background and aims: People of South Asian origin are at higher risk of type 2 diabetes. Effectiveness of lifestyle modification (LSM) interventions incorporating diet and/or physical activity to prevent diabetes in South Asians is unclear, given the small number of studies, the relatively low numbers of cases, and variation in outcome reporting across studies. Therefore, we performed an individual participant data (IPD) meta-analysis of randomised controlled trials (RCTs) in these high-risk populations.

Materials and methods: We searched PUBMED, Embase, Cochrane Library and Web of Science (to September 30th 2017), and obtained IPD on 1816 participants from all six eligible RCTs on LSM in high-risk South Asian adults, both from Europe and India. The quality according to the Quality Assessment Tool for Quantitative Studies was strong for five studies, and moderate for one. Applying a 2-step approach, we generated hazard ratio (HR) estimates for incident diabetes and mean differences for fasting glucose, 2-hour glucose, weight and waist circumference, using fixed-effects meta-analysis overall, and by pre-specified subgroups. We applied the GRADE system to rate the quality of evidence. (PROSPERO registration CRD4217078003).

Results: Incident diabetes was observed in 118 of 932 (12.6%) participants in the intervention group and in 176 of 876 (20.0%) participants in the control group. The adjusted HR was 0.65 (95% CI 0.51 to 0.82; $I^2 = 0\%$) in the intervention compared with control groups; the absolute difference was 7.4% (95% CI 1 to 16), with no subgroup differences for sex, age, BMI, study duration or region. The GRADE quality of evidence was rated as moderate. Mean difference for intervention versus control groups for 2-hour glucose was −0.35 mmol/l (95% CI −0.63 to −0.06; $I^2 = 51\%$); for weight −0.76 kg (95% CI −1.36 to −0.15; $I^2 = 72\%$) and for waist −1.16 cm (95% CI −2.15 to −0.17; $I^2 = 74\%$). Findings were also similar across subgroups for these measures, except for weight by region. No effect was found for fasting glucose.

Conclusion: In high-risk South Asian populations, LSM interventions resulted in a 35% relative reduction in diabetes incidence, which was consistently present across pre-specified subgroups. This suggests that LSM interventions should be more widely used in these populations across different contexts.

Disclosure: **A.K. Jenum:** None.

318

Investigation of vitamin D metabolites, vitamin D genes, and risk of type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY)

J.M. Norris[1], N.L. Zwick[1], B.C. DeFelice[2], R.K. Johnson[1], F. Dong[3], L. Vanderlinden[1], P. Carry[1], J. Seifert[1], B.I. Frohnert[3], K. Kechris[1], T. Fingerlin[4], O. Fiehn[2], M. Rewers[3];

[1]Colorado School of Public Health, Aurora, CO, USA, [2]University of California Davis, Davis, CA, USA, [3]The Barbara Davis Center, Aurora, CO, USA, [4]National Jewish Health, Denver, CO, USA.

Background and aims: Vitamin D has been inconsistently identified as a protective factor for type 1 diabetes (T1D). Lack of evidence regarding the timing of the effect of vitamin D, i.e., before or after islet autoantibody seroconversion, and the influence of genetic variation on the effect of vitamin D may explain these inconsistencies. This study investigated the associations between several metabolites in the vitamin D pathway and T1D, and examined whether the effect of these metabolites was modified by alleles in vitamin D pathway genes.

Materials and methods: Children at increased genetic risk of T1D have been followed from birth by the Diabetes Autoimmunity Study in the Young (DAISY). We conducted a nested case-control study in 74 T1D cases and 72 controls, frequency matched on age at autoantibody seroconversion (in the case) and ethnicity. Plasma vitamin D metabolites (vitamin D_3, $25(OH)D_3$, $25(OH)D_2$, 3-epi $25(OH)D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$) were quantified by LC-MS/MS analysis in samples collected prior to the development of T1D at time points both before and after autoantibody seroconversion for the T1D case and age-similar time points for the control. Variants in vitamin D pathway genes (*GC*, *CYP27B1*, *CYP24A1* and *VDR*) were genotyped using the Taqman OpenArray platform. Multivariable logistic regression analyses were conducted examining vitamin D metabolite levels from pre- and post-

autoantibody seroconversion time points on the odds of T1D, adjusting for HLA-DR3/4. Gene variant x metabolite interaction terms were used to test for effect modification.

Results: Significant interactions between the rs4588 variant in *GC* and post-seroconversion levels of vitamin D_3 ($p = 0.016$), $25(OH)D_3$ ($p = 0.017$) and $24,25(OH)_2D_3$ ($p = 0.033$) on the odds of T1D were detected. Higher vitamin D_3 (OR: 0.80; CI: 0.66–0.96), $25(OH)D_3$ (OR: 0.84; 95%CI: 0.73–0.97) and $24,25(OH)_2D_3$ (OR: 0.87; CI: 0.79–0.97) levels were inversely associated with T1D in children carrying at least one minor allele at rs4588 but not in children with no minor alleles. No consistent associations or interactions were observed for levels of vitamin D metabolites measured prior to autoantibody seroconversion.

Conclusion: The *GC* gene encodes the vitamin D binding protein, which is responsible for transporting all vitamin D pathway metabolites and therefore plays an important role in the action of vitamin D. Moreover, the rs4588 variant of *GC* has been associated with differences in protein glycosylation and levels of binding protein. These results suggest that *GC* may modify the effect of multiple vitamin D pathway metabolites on T1D development in children at increased genetic risk of the disease, particularly later in the disease process.

Supported by: NIH DK32493 and DK104351

Disclosure: **J.M. Norris:** None.

319

Adherence to the Dutch dietary guidelines 2015 and the risk of prediabetes and type 2 diabetes in Dutch adults: the New Hoorn study

N.R. den Braver[1], A.L.J. van der Spek[1], M. Looman[2], M.M.E. Geelen[2], J. Lakerveld[1], F. Rutters[1], J. Brug[1,3], S.S. Soedama-Muthu[2,4], J.W.J. Beulens[1,5];

[1]Epidemiology & Biostatistics, VU Medical Center, Amsterdam, Netherlands, [2]Division of Human Nutrition, Wageningen University and Research, Wageningen, Netherlands, [3]Faculty of Social and Behavioural Science, University of Amsterdam, Amsterdam, Netherlands, [4]Department of Medical and Clinical Psychology, Tilburg University, Tilburg, Netherlands, [5]Julius Center for Health Sciences and Primary Care, Univesity Medical Center Utrecht, Utrecht, Netherlands.

Background and aims: In order to prevent ten major chronic diseases including type 2 diabetes (T2D), new Dutch dietary guidelines were developed with a focus on food groups rather than nutrients. In this study, we investigated whether adherence to these Dutch dietary guidelines was associated with prediabetes and T2D.

Materials and methods: In this prospective cohort 1624 participants, with an average age of 53.5 ± 6.5 years, 46.7% male and without T2D at baseline, were included in the analyses. Information on baseline dietary intake was assessed using a validated 104-item Food Frequency Questionnaire and classified in tertiles of adherence to the Dutch Healthy Diet 2015 (DHD15) index (range: 0–140). Prediabetes and T2D were classified according to the WHO criteria 2011. Multivariable multinomial regression analyses were performed to estimate odds ratios for prediabetes and T2D, adjusted for follow-up duration, energy intake, baseline prediabetes, sociodemographic- and lifestyle factors. In addition, fasting plasma glucose (FPG) levels (mmol/L) were analysed using linear regression analyses, additionally adjusted for baseline value and medication use.

Results: During a mean follow-up of 7.2 ± 0.7 years, 419 participants developed prediabetes and 143 participants developed T2D. Highest adherence to the DHD15 was associated with prediabetes, compared to lowest adherence ($OR_{T3\text{-}T1} = 0.72$ (0.53; 0.99), $p_{trend} = 0.05$), while there was no association with T2D incidence ($OR_{T3\text{-}T1} = 1.01$ (0.60; 1.72), $p_{trend} = 0.98$). Each 15 point increase in DHD index was not associated FPG levels (0.014 mmol/L (−0.035; 0.007)).

Conclusion: In this Dutch population-based study, adhering to the Dutch dietary guidelines 2015 was associated with lower prediabetes, but not association with T2D, during seven years of follow-up. These results indicate that prevention strategies should focus on early stage disease development.

Disclosure: **N.R. den Braver:** None.

320

Plasma ceramides and dairy consumption in the D.E.S.I.R. study

F. Fumeron[1,2], A. Nicolas[1,3], L. Wigger[4,5], M. Ibberson[4], C. Cruciani-Guglielmacci[2,6], H. Le Stunff[6], G. Velho[1], C. Magnan[2,6], M. Marre[2,7], B. Balkau[8,9], R. Roussel[2,7];

[1]INSERM UMRS 1138, Paris, France, [2]Université Paris Diderot, Sorbonne Paris Cité, Paris, France, [3]UPMC Univ Paris 6, Sorbonne Universités, Paris, France, [4]Vital-IT Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland, [5]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland, [6]Unité de Biologie Fonctionnelle et Adaptative (BFA), CNRS UMR 8251, Paris, France, [7]Diabetology, Endocrinology, Nutrition, APHP - Bichat Hospital, Paris, France, [8]Centre for research in Epidemiology and Population Health (CESP), INSERM, U1018, Villejuif, France, [9]Umr-s 1018, University Paris-Sud, University Versailles Saint-Quentin, Villejuif, France.

Background and aims: In the D.E.S.I.R. cohort, a higher consumption of dairy products is associated with lower 9-year incidence of the metabolic syndrome and of impaired fasting glycemia/type 2 diabetes. In the same population, plasma dihydroceramide concentrations are higher in people who progress to diabetes, up to 9 years before disease onset. Our aim is to study relationships between dairy consumption and concentrations of dihydroceramides and ceramides.

Materials and methods: In total, 5212 volunteers from western-central France were included in the D.E.S.I.R. cohort. At baseline they reported the frequency and level of consumption of different foods; two items concerned dairy products (cheese, other dairy products). For these two items, we divided participants into two groups (high, low) according to the consumptions. At baseline, then at years 3, 6 and 9, dihydroceramides and ceramides were determined by mass spectrometry in a subset of the cohort ($n = 295$); we analyzed the 105 people who did not progress to diabetes, because disease *per se* might be a confounding factor. The associations between plasma lipids (log values) over follow-up with baseline dairy intake, sex and their interaction, were tested by analysis of covariance (ANCOVA) for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, plasma cholesterol, triglycerides and fasting plasma glucose.

Results: A higher consumption of dairy products (other than cheese) was associated with total plasma dihydroceramides during follow-up, in interaction with sex ($P = 0.01$): dihydroceramide levels were lower in women with high consumption as compared to the low consumers ($P = 0.03$), with a significant increase in dihydroceramides during the follow-up ($P = 0.01$) in low consumers only. In men, no significant association was found. There was also a trend for lower plasma ceramides in women with a high intake of dairy (other than cheese) ($P = 0.08$). Cheese intake was associated with plasma dihydroceramides and ceramides during follow-up ($P = 0.04$ for both), with trends for lower levels in high consumers, and for higher levels in low consumers (non-significant).

Conclusion: These results show, in women, an inverse association between fresh dairy product consumption and plasma predictive markers of type 2, dihydroceramides.

Plasma total plasma dihydroceramide concentration (µmol/l, median [25-75%]) in 51 women without diabetes at follow-up, according to intake of dairy (except cheese)

	Baseline	3 years	6 years	9 years
Low intake	0.135 [0.114-0.158]	0.137 [0.118-0.167]	0.155 [0.124-0.218]	0.164 [0.131-0.236]
High intake	0.125 [0.101-0.175]	0.122 [0.090-0.167]	0.125 [0.103-0.189]	0.135 [0.088-0.170]

P (intake)=0.03; in low intake group, P (time)=0.01; in high intake group, P (time)=0.95 by ANCOVA for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, plasma cholesterol, triglycerides, fasting plasma glucose

Supported by: CNIEL
Disclosure: **F. Fumeron:** Grants; from CNIEL.

PS 008 Diabetes complications

321

Factors predicting participation in the Diabetes Prevention Program (DPP) among people with prediabetes

W.H. Herman, K.L. Joiner, M.B. Stites, L.N. McEwen;
University of Michigan, Ann Arbor, MI, USA.

Background and aims: Since September 2015, the University of Michigan has paid for interventions for diabetes prevention for its employees, dependents, and retirees with prediabetes. We studied individuals with prediabetes who chose to participate or not participate in the DPP to identify strategies to better target interventions to improve uptake.
Materials and methods: As a first implementation strategy, nondiabetic individuals ≥18 years of age with BMI ≥25 kg/m² (23 if Asian) and a claims diagnosis of prediabetes or an HbA1c 5.7–6.4% (39–46 mmol/mol) were identified using insurance claims data. They were then mailed one letter of invitation and one reminder letter. After human subjects approval, surveys were mailed to individuals who enrolled in the DPP and a random sample of those who did not. T-tests and chi-square tests were used to compare the demographic characteristics and domains of the Health Belief Model between DPP enrollees and non-enrollees and a multivariable model was subsequently constructed.
Results: By January 2018, 7,389 individuals with prediabetes were invited to participate and 565 (8%) enrolled. 356 of 510 DPP enrollees and 472 of a random sample of 1,200 non-enrollees completed surveys (response rates 74% and 39% respectively). Women (73% vs. 61%) and individuals with higher education (68% vs. 59% ≥ college) were significantly more likely to enroll. Enrollment did not differ by age (55 ± 11 yrs) or race (79% white). Enrollees reported higher perceived susceptibility based on family history of diabetes (60% vs. 50%), greater knowledge of diabetes risk factors, higher body mass index (32 vs. 30 kg/m²), higher perceived risk of developing diabetes, more worry about diabetes, and less optimistic bias. Both enrollees and non-enrollees perceived diabetes to be a serious disease. Enrollees and non-enrollees did not differ with respect to perceived benefit of engaging in lifestyle interventions or perceived barriers to action. Self-efficacy as assessed by sense of personal control was greater in enrollees but confidence in ability to eat a healthy diet and engage in regular physical activity did not differ between groups. Cues to action and social support were the major factors distinguishing enrollees and non-enrollees. 74% of enrollees but only 24% of non-enrollees were aware that they had prediabetes. 62% of enrollees vs. 15% of non-enrollees recalled receiving a letter encouraging them to enroll. 30% of enrollees indicated that their doctor and 17% indicated that a family member, friend, or coworker had encouraged them to enroll. Among non-enrollees, these rates were only 10% and 4%. In multivariate analysis, enrollees were significantly more likely to be women, have more knowledge of diabetes risk factors, have higher BMI, report more personal control over their health, be aware of their prediabetes status, recall receiving a letter encouraging them to enroll, and receiving support from a physician, family member, friend, or coworker to enroll in the DPP.
Conclusion: Making the DPP available at no cost to people with prediabetes has been effective in increasing enrollment above previously reported rates. Efforts to increase enrollment should target men and focus on increasing individual awareness of prediabetes and support for participation from physicians, family, friends, and coworkers.
Supported by: NIH/NIDDK 1 R01 DK109995
Disclosure: **W.H. Herman:** Grants; NIH/NIDDK 1R01DK109995.

322

Phthalates exposure as determinant of albuminuria in type 2 diabetes subjects: a cross-sectional study

A. Mengozzi[1], F. Carli[2], V. Della Latta[2], E. Buzzigoli[2], M. Seghieri[1], E. Biancalana[1], A. Solini[1], A. Gastaldelli[2];
[1]University of Pisa, Pisa, Italy, [2]National Research Council, Pisa, Italy.

Background and aims: Albuminuria is considered an independent risk factor for cardiovascular (CV) disease. Recent epidemiological studies have reported an association between exposure to phthalates, a group of environmental and dietary contaminants, and states of higher CV risk like insulin resistance, obesity and type 2 diabetes (T2D). No studies have so far addressed the presence of these compounds in urines of T2D individuals with different degrees of renal function.

Materials and methods: We enrolled 209 T2D patients (75.5% normoalbuminuric, 19.7% microalbuminuric, 4.8% macroalbuminuric) consecutively referring to Pisa hospital outpatient diabetes clinic. Routine blood laboratory tests were centrally performed. Total concentrations of three different phthalate urinary metabolites of di-2-ethylhexylphthalate (DEHP), *i.e.* mono 2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono 2-ethyl-5-hydroxyhexyl phthalate (MEHHP), were quantified in a spot morning urine sample by ultra-HPLC coupled with electrospray ionization/quadrupole time-of-flight MS. Data were normalized for creatinine urinary excretion and related to clinical and biochemical parameters, adjusting for potential confounders by univariable and multivariable regression models.

Results: Mean age of the participants was 67.8 ± 12.4 years; 59% males. Median T2D duration was 10.5 ± 9.7 years, HbA_1c was 7.18 ± 1.31%; 29% of them were insulin-treated. The three metabolites were detected in 92% (MEHP), 96% (MEOHP), 94% (MEHHP) of the subjects. Creatinine-adjusted urinary concentrations medians of MEHP, MEOHP, MEHHP were 7.53 [4.84–12.60] μg/g, 3.04 [1.03–5.14] μg/g and 10.70 [7.02–17.40] μg/g. Data from DEMOCOPHES, a recent European human biomonitoring study, showed similar ranges of exposure. Using the median values as cut-off, no difference emerged according to age, sex, BMI, T2D duration, smoking, BP, HBA_1c; GFR (estimated with CDK-EPI equation) did not influence urinary levels of these metabolites, even in subjects below 45 ml/min/1.73 m^2 (n = 22). This was also true after adjusting for confounders. Exposure to MEHP and MEOHP was significantly higher in micro/macroalbuminuric than in normoalbuminuric individuals ($p < 0.02$ for MEHP; $p < 0.04$ for MEOHP). When stratified for quartiles of each urinary metabolite, after adjustment for clinical variables (age, sex, BMI, T2D duration, smoking, BP, HBA_1c and GFR), 4th vs 1st quartile of MEHP and MEOHP showed a significant higher risk of albuminuria (OR 4.83 [CI 1.45–16.06], $p < 0.03$ for trend for MEHP; OR 3.29 [CI 1.08–10.04], $p < 0.04$ for trend for MEOHP). Comparing their levels in subjects with and without previous CV events, MEOHP was significantly higher in the former ($p = 0.034$), with a positive trend ($p = 0.061$) for MEHP too.

Conclusion: These findings point out for the first time an association between exposure to specific metabolites of DEHP and degree of AER in T2D subjects; the lack of relation with GFR suggests their urinary levels to be independent of renal function, rather representing a potential early hallmark of widespread vascular damage. Though with the limitations of a cross-sectional study, a long-term exposure to these contaminants might mark a higher CV risk, thus implying the need for prospective studies addressing the pathophysiologic mechanisms underlying such association.

Disclosure: **A. Mengozzi:** None.

323

Selected osteoprotegerin gene variants as diabetic foot risk factors

P. Nehring[1], B. Mrozikiewicz-Rakowska[2], K. Szymański[3], A. Sobczyk-Kopcioł[4], N.A. Acharya[5], R. Płoski[3], W. Drygas[6], J. Krzymień[2], L. Czupryniak[2];

[1]Department of Gastroenterology and Internal Medicine, Medical University of Warsaw, Warszawa, Poland, [2]Department of Diabetology and Internal Medicine, Medical University of Warsaw, Warszawa, Poland, [3]Department of Medical Genetics, Medical University of Warsaw, Warszawa, Poland, [4]Department of General Biology and Parasitology, Medical University of Warsaw, Warszawa, Poland, [5]Medical University of Warsaw, Warszawa, Poland, [6]Department of Epidemiology, Cardiovascular Disease Prevention and Health Promotion, Institute of Cardiology, Warszawa, Poland.

Background and aims: Diabetic Foot (DF) ulceration and Charcot Neuroarthropathy (CN) are end point complications in type 1 and type 2 diabetes mellitus affecting up to 6% of population. Osteoprotegerin (OPG) is a key protein in bone metabolism. An emerging contribution of signaling pathway RANKL/RANK/OPG in the pathogenesis of DF and CN has been proposed. Here, we aimed to elucidate the role of selected OPG gene variants and their role in the risk of DF occurrence in diabetic patients.

Materials and methods: We enrolled 1268 individuals; 300 cases with diabetes mellitus and DF and 968 healthy controls. Of these, there were 252 subjects with type 2 diabetes, 43 with type 1 diabetes, 166 with DF of neuropathic origin, 102 with DF of neuroischemic origin and 77 with CN. Anthropometric analyses were obtained on all subjects and segregated for vascular and neuropathic complications due to diabetes. Genotyping for 15 OPG variants was performed using real time polymerase chain reaction.

Results: Compared to controls, rs1872426 and rs1485286 showed correlation with DF in diabetic subjects (OR = 1.4; $P = 0.05$ and OR = 0.46; $P = 0.003$, respectively). Significant association between rs2073618, rs1872426, rs7464496 and rs1485286 in men were reported (OR = 1.57; $P = 0.02$, OR = 1.92; $P = 0.005$, OR = 1.46; $P = 0.05$ and OR = 0.40; $P = 0.008$; respectively). The aforementioned correlations were also present in type 2 diabetes patients' subgroup. Between type 2 and type 1 diabetes patients, there was a difference in distribution of rs7464496 and rs2073618 alleles. Variant rs1485286 was associated to DF of neuropathic origin (OR = 0.36; $P = 0.007$). Sex-specificity for females was present for rs6993813 in patients with DF of neuropathic origin and type 1 diabetes (OR = 2.89; $P = 0.05$). In males with DF of neuropathic origin and type 2 diabetes were correlated with rs2073617 (OR = 1.76; $P = 0.04$), and males with DF of neuroischemic origin and type 2 diabetes with rs1872426 (OR = 3.05; $P = 0.02$). Variants rs1872426, rs2073617and rs1485286 were correlated with CN. There was a sex-specificity for woman of rs6993813 in patients with CN and type 1 diabetes. Anthropometric analyses proved that except height, the patients' age, body weight, body mass index, waist circumference, hip circumference and waist-hip ratio were among the basic risk factors of DF, except for diabetes itself.

Conclusion: Our findings suggest that the variants *TNFRSF11B* (rs2073618, rs2073617, rs1872426, rs1032128, rs7464496 and rs1485286), *COLEC10* (rs6993813) and *TNFSF11* (rs9533156) influence diabetic foot occurrence. This correlation is specific for sex, diabetes type and DF etiology. Altogether, it contributes towards the use of these OPG variants in genetic panels assessing in assessing risk of DF in the diabetic population alongside adequate correlation with clinical data, such as diabetes type, gender, age and other features.

Supported by: NCN grant 2013/11/N/NZ5/00208

Disclosure: **P. Nehring:** None.

324

Changes in glycaemic control and rates of diabetes-related complications in type 2 diabetes: 10 years of the International Diabetes Management Practices Study (IDMPS)

P. Aschner[1], J.J. Gagliardino[2], H. Ilkova[3], F. Lavalle[4], A. Ramachandran[5], G. Kaddaha[6], J.C. Mbanya[7], M. Shestakova[8], J.-M. Chantelot[9], J.C.N. Chan[10];

[1]Javeriana University School of Medicine and San Ignacio University Hospital, Bogotá, Colombia, [2]CENEXA, Center of Experimental and Applied Endocrinology (La Plata National University – La Plata National Scientific and Technical Research Council), La Plata, Argentina, [3]Istanbul University, Cerrahpasa Medical Faculty, Department of Internal Medicine, Division of Endocrinology, Metabolism and Diabetes, Istanbul, Turkey, [4]Facultad de Medicina de la Universidad Autónoma de Nuevo León, Monterrey, Mexico, [5]India Diabetes Research Foundation, Dr. A. Ramachandran's Diabetes Hospitals, Chennai, India, [6]Government of Dubai, Dubai Health Authority, Dubai, United Arab Emirates, [7]Biotechnology Center, Doctoral School of Life Sciences, Health and Environment, and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde,

Cameroon, [8]Endocrinology Research Center, Moscow, Russian Federation, [9]Sanofi, Paris, France, [10]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, Hong Kong SAR, China.

Background and aims: New therapies and technologies mean that diabetes treatment has evolved over the last decade. We investigated whether this evolution has had any impact on the degree of glycaemic control and the frequency of diabetes-related complications in people with T2D in the developing world over this period.

Materials and methods: From 2005 to 2017, the IDMPS, a global observational survey on the management and patterns of care of people with type 1 (T1D) and T2D diabetes in the developing world, has collected data in 7 individual waves. Each wave enrolled different participants who were recruited from 48 countries across Africa, the Middle East, South Asia, Latin America, Asia and Eurasia.

Results: Mean disease duration ranged from 6.8 to 7.5 years across waves. From wave 1 to wave 7, no improvement in the proportion of people achieving HbA_{1c} <7% was seen. This was the case for all groups, i.e. those receiving oral glucose-lowering drugs (OGLDs) plus injectable treatments, and those receiving insulin (**Table**). The proportions of participants with micro- and macrovascular complications remained relatively unchanged. Throughout all waves, the proportion of participants receiving insulin remained constant (≈20%). For those treated with OGLDs plus basal insulin, the median daily dose of insulin was 0.21 U/kg in 2005 and 0.32 U/kg in 2017; over this period, the median BMI at diagnosis increased from 27 kg/m^2 to 29 kg/m^2.

Conclusion: Despite the introduction of new drugs/technologies, there has only been a small improvement in degree of metabolic control or experience of chronic complications for people with T2D in the developing world. For optimum outcomes, provision of new therapeutic tools should be accompanied by education of people with diabetes and their medical teams on proper use.

Table. Achievement of HbA_{1c} <7 % and incidence of micro- and macrovascular complications in people with T2D enrolled in the IDMPS

T2D OGLDs	Wave 1 2005 N=3836	Wave 2 2006 N=8135	Wave 3 2008 N=6003	Wave 4 2010 N=2933	Wave 5 2011–12 N=4486	Wave 6 2013–14 N=3067	Wave 7 2015–16 N=3330
Age (mean), years	59.0	58.3	58.1	58.1	57.6	57.1	56.9
Disease duration (mean), years	7.1	6.9	7.1	6.8	6.7	7.2	7.5
HbA_{1c}							
Mean, %	7.5	7.6	7.5	7.5	7.6	7.7	7.6
<7 %, %	48.0	46.7	49.6	48.5	45.1	41.0	44.7
Blood pressure, mean							
Systolic, mmHg	132.6	132.2	129.2	128.6	131.6	132.5	132.9
Diastolic, mmHg	79.6	79.2	78.3	78.4	79.7	80.2	80.7
Total serum cholesterol (mean), mmol/l (mg/dl)	4.32 (167.0)	4.95 (191.3)	4.86 (188.0)	4.92 (190.3)	4.91 (189.9)	4.92 (190.2)	4.76 (183.9)
Complications, %							
≥1 microvascular	40.6	33.7	24.6	27.1	30.3	36.4	32.3
≥1 macrovascular	20.7	18.9	14.7	13.8	13.3	13.3	8.6
T2D insulin + OGLDs	**Wave 1 2005 N=2028**	**Wave 2 2006 N=3693**	**Wave 3 2008 N=3036**	**Wave 4 2010 N=1449**	**Wave 5 2011–12 N=2737**	**Wave 6 2013–14 N=1870**	**Wave 7 2015–16 N=2365**
Age (mean), years	59.6	59.7	59.4	59.6	59.7	59.8	58.9
Disease duration (mean), years	12.6	13.1	13.0	13.2	12.6	13.1	13.1
Duration of insulin use, years	3.5	3.6	3.5	2.0	3.8	4.5	4.7
HbA_{1c}							
Mean, %	8.3	8.6	8.6	8.6	8.6	8.6	8.7
<7 %, %	28	23	26	23	21	15	16
Blood pressure, mean							
Systolic, mmHg	134.3	133.9	130.8	130.3	134.8	135.3	135.2
Diastolic, mmHg	79.8	79.3	77.8	78.1	80.3	80.8	80.5
Total serum cholesterol (mean), mmol/l (mg/dl)	3.85 (149.0)	5.00 (193.4)	4.88 (188.8)	4.94 (191.1)	5.02 (194.2)	4.98 (192.4)	4.81 (186.0)
Complications, %							
≥1 microvascular	68	65	57	57	66	68	64
≥1 macrovascular	36	31	29	25	33	33	24

OGLD, oral glucose-lowering drug

Supported by: Sanofi

Disclosure: **P. Aschner:** Honorarium; Sanofi. Other; Sanofi (travel sponsorship).

325

The impact of blood pressure on retinal vascular traits in patients with type 2 diabetes: a Mendelian Randomisation study

Y. Huang[1], T. Li[1], S. Hogg[2], G. McKay[3], E. Trucco[2], C. Palmer[1], A. Doney[1];

[1]Ninewells Hospital and Medical School, School of Medicine, University of Dundee, Dundee, UK, [2]Computing, School of Science and Engineering, University of Dundee, Dundee, Dundee, UK, [3]Centre for Public Health, Queen's University Belfast, Belfast, UK.

Background and aims: In patients with Type 2 Diabetes (T2D), Retinal Vascular Traits (RVTs) have potential for use as biomarkers for T2D complications. However, their clinical use in this context depends on their relationship to modifyable risk factors. In previous observational studies, BP has been associated with several RVTs. However, in patients with T2D this association may be confounded by other clinical factors such as dyslipidaemia or dysglycaemia etc. Therefore we used a Mendelian Randomization (MR) approach employing a genomic Instrumental Variable (IV) comprising a weighted Genetic Risk Score (wGRS) for systolic BP (SBP) to determine whether there is a true association between SBP and RVTs in patients with T2D.

Materials and methods: 3950 patients with T2D from Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) were selected for this study. RVTs were measured by VAMPIRE software from standard Diabetes Retinal Screening photographs. We considered central retinal artery/vein equivalent (CRAE, CRVE), their ratio (AVR), arterial/ venular fractal dimensions (d0a, d0v), tortuosity of the largest arterioles/venules (TORTA, TORTV). For each individual, the median SBP of all available measures before retinal image date was determined as the overall estimation of SBP exposure. A 262 SNP wGRS for SBP was constructed based on previous GWAS for BP by summing the number of risk alleles multiplied by their corresponding effect size. Linear regression was used to model the association of SBP with each RVT adjusting for sex and age (Observational Study). Two-stage least square regression was used for the MR study - wGRS for SBP as IV, adjusted for sex and age. The Wu-Hausman statistic was used to illustrate the difference between the observational and MR models. The 'ivreg' package in R was used for MR study.

Results: The median age at date of image was 70.9 years, 45.8% were female and median SBP was 140 mmHg. Overall, the wGRS could explain 1.36% of the variance of SBP after adjusting for sex and age. In the observational analysis SBP was found to be associated with CRAE, AVR, d0v and VTORT. However, in the MR model, SBP was only found to be associated with AVR and ATORT. Comparing observational model with the MR model, the Wu-Hausman statistic was significant when using AVR and ATORT as outcome variable. Meanwhile, in observational study, the effect size of SBP for AVR and ATOR are smaller than MR studies, which suggest the existence of confounding factors in observational study (Table 1).

Conclusion: According to this MR study, SBP is causally linked with ATORT and AVR. Thus these RVTs may be of particular relevance in therapeutic stratification of T2D risk of complications with respect to SBP management.

Table1. Results from observational study model and MR model

	Observational Study		MR Model		
	β - SBP	p	β - SBP	p	p (Wu-Hausman)
CRAE	**-0.02**	**<0.001**	-0.05	0.17	0.39
CRVE	-0.008	0.09	0.04	0.33	0.24
AVR	**$-3.08e^{-04}$**	**<0.01**	**-0.002**	**0.01**	**0.02**
d0a	$-8.152e^{-05}$	0.47	-0.001	0.21	0.23
d0v	**$2.32e^{-04}$**	**0.03**	$-5.41e^{-05}$	0.95	0.76
ATORT	0.003	0.08	**0.043**	**<0.001**	**<0.01**
VTORT	**0.005**	**<0.001**	0.006	0.51	0.88

*Adjusted for sex and age in both observational and MR model

Disclosure: **Y. Huang:** None.

326

Diabetic kidney disease occurrence in a large cohort of patients with incident diabetes in the UK

D. Vizcaya[1], A. Gonzalez-Perez[2], M.E. Saez[2], L.A. Garcia Rodriguez[2]; [1]Epidemiology, Bayer Hispania SL, Barcelona, Spain, [2]Spanish Centre for Pharmacoepidemiologic Research (CEIFE), Madrid, Spain.

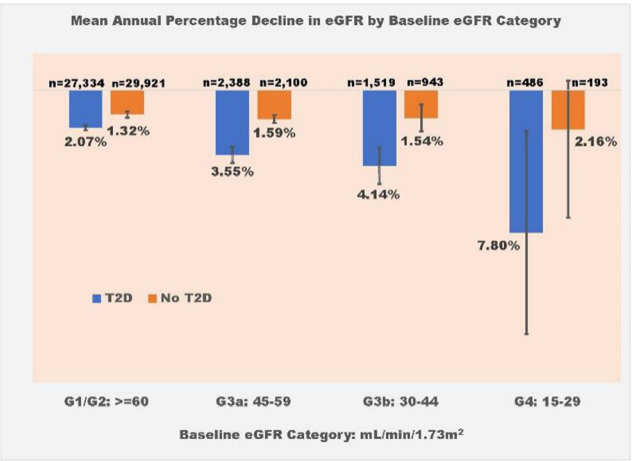
Background and aims: Chronic kidney disease (CKD) is a common and life-threatening complication of diabetes. We aimed to evaluate the incidence and time to CKD onset by stage in a large cohort of newly diagnosed type 1 and type 2 diabetes mellitus (T1D and T2D, respectively) patients.
Materials and methods: We identified a cohort of incident T1D and T2D patients aged 2 to 90 years old between 2002 and 2014 registered in the Health Improvement Network (THIN) database (UK). We followed this cohort from first diabetes mellitus (DM) diagnosis until the first of the following endpoints: occurrence of CKD, last data collection, 31 December 2015, or death. We defined CKD based on Read codes, eGFR and albuminuria values, using a modified version of a previously published algorithm. We assessed CKD stage based on eGFR values. We used two alternative definitions of CKD: CKD stages 3–5, and CKD/proteinuria (that includes CKD stages 3–5 and/or presence of proteinuria). Proteinuria was ascertained using Read codes, UACR >30 mg/g, and/or Albumin in urine >20 mg/L. We calculated age- and sex-adjusted incidence rates of CKD by using direct standardization.
Results: We identified 5,116 T1D and 161,852 T2D patients. As expected, the latter were noticeably older at time of first diagnosis, and consequently had greater number of comorbidities. Among the subcohort of DM patients free of CKD/proteinuria at baseline (n = 115,437), a total of 29,833 (25.8%) developed overall CKD/proteinuria during follow-up. Of these, 468 (1.57%) reached stage 5 and 71 (0.24%) reached end-stage renal disease (ESRD). Crude incidence rates of CKD/proteinuria per 100 person-years were 1.6 in T1D and 5.7 in T2D, with a mean follow-up of 5.6 (interquartile range: 2.6–8.1) and 4.7 (interquartile range: 2.0–6.8) years, respectively. The corresponding age- and sex-adjusted incidence rates were 6.1 and 5.5. In this cohort, average time from DM onset to CKD/proteinuria was 4.3 years among T1D and 3.6 years among T2D. On the other hand, crude incidence rates of CKD stages 3–5 were 0.4 and 2.8 per 100 person-years respectively in T1D and T2D. The corresponding age- and sex-adjusted incidence rates were 3.3 and 2.6 per 100 person-years.
Conclusion: According to our results, crude incidence rates of CKD are markedly higher among T2D patients as compared to T1D. In contrast after adjustment, in particular for age, results showed similar estimates between the two types of DM. In any case, caution is warranted when directly comparing DM types given inherent differences in disease etiology and patients' characteristics.
Disclosure: **D. Vizcaya:** Employment/Consultancy; Full-time employee at Bayer.

327

Larger and faster decline in eGFR among patients with vs without type 2 diabetes

G.A. Nichols[1], A. Deruaz-Luyet[2], S.J. Hauske[2], K.G. Brodovicz[3]; [1]Kaiser Permanente, Portland, OR, OR, USA, [2]Boehringer Ingelheim GmbH, Ingelheim am Rhein, Germany, [3]Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA.

Background and aims: Type 2 diabetes (T2D) is a well-known risk factor for chronic kidney disease (CKD) and for progression to end-stage renal disease (ESRD). Baseline kidney function is associated with rate of decline, which in turn increases ESRD risk. To our knowledge, no large study has evaluated baseline kidney function and rate of eGFR decline by T2D status. We aimed to compare the annual rate of eGFR decline for up to 11 years among patients with and without T2D.
Materials and methods: We used the electronic medical records of an integrated delivery system in the USA to identify 31,727 patients with and 33,157 patients without diagnosed T2D for whom we could calculate eGFR (CKD-EPI formula) from the first available serum creatinine value from 2006–2012 (baseline), confirmed by a second eGFR at least three months later if the first was <60 mL/min/1.73 m^2. We defined kidney function by KDIGO stages of eGFR (G1: ≥90 mL/min/m^2; G2: 60–89; G3a: 45–59; G3b: 30–44; G4: 15–29) and followed patients until their last available eGFR through December 2016. We calculated the annual decline in eGFR by subtracting the last eGFR from the baseline value, dividing by months between the two measures and multiplying by 12. Percentage change was calculated by dividing the change in value by the baseline value. We compared the mean percentage changes by baseline eGFR category between patients with and without T2D in total, by age strata (<65/≥65 years), and by sex.
Results: Mean percentage decline in eGFR (±SE) was greater among patients with T2D across all eGFR categories (Figure). Among those with T2D, the percentage change was substantially greater as baseline kidney function declined but was relatively constant across eGFR categories among those without T2D. As a result, the relative difference in eGFR decline between patients with vs. without T2D increased by category. For the category G1/G2, T2D was associated with a 1.56 times greater percent decline in eGFR (2.07% vs. 1.32%, $p < 0.001$). For G3a, G3b, and G4, the differences were 2.23 (3.55% vs. 1.59%, $p < 0.001$), 2.69 (4.14% vs. 1.54%, $p < 0.001$), and 3.61 (7.80% vs. 2.16%, $p = 0.217$) times greater, respectively. Mean percentage changes were somewhat greater among patients who were <65 or male with but not without T2D, resulting in larger relative differences in these strata (not shown).
Conclusion: Poorer baseline kidney function is associated with largerdeclines in eGFR among patients with T2D while declines by kidney function were much more modest among those without T2D. Thus, T2D is a major risk factor for eGFR decline and the rate of decline accelerates as kidney function worsens, especially among younger and male patients. Analyses did not account for proteinuria/albuminuria, which may play an important role in eGFR decline. This warrants further study.



Disclosure: **G.A. Nichols:** Grants; Boehringer Ingelheim, Amarin Pharma, Sanofi, Janssen Pharmaceuticals.

PS 009 Epigenetics and gene regulation

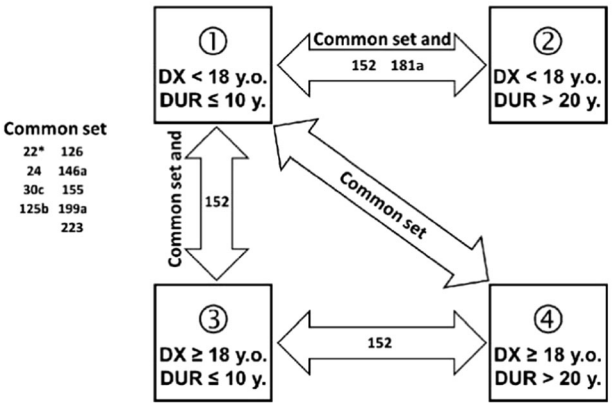
328

MicroRNAs associated with insulin secretion in type 1 diabetes

A.S. Januszewski[1,2], M. Joglekar[1], Y. Cho[3], E. Scott[1], R. Farr[1], Y. Loh[1], D.N. O'Neal[2], P. Benitez-Aguirre[3], M. Craig[3], K. Donaghue[3], A. Hardikar[1], A.J. Jenkins[1,2];
[1]NHMRC Clinical Trials Centre, University of Sydney, Camperdown, NSW, Australia, [2]Department of Medicine, University of Melbourne, Fitzroy VIC, Australia, [3]Children's Hospital at Westmead, University of Sydney, Westmead NSW, Australia.

Background and aims: Low level insulin secretion can persist even in long-term Type 1 diabetes (T1D) and may reduce risks of hypoglycaemia and chronic complications.
Materials and methods: Plasma C-peptide and microRNAs were quantified in a cross-sectional study of 211 healthy controls (CON, F/M 113/98) age (mean ± SD) 35 ± 16 yo and 304 T1D patients (F/M 170/134), age 30 ± 16 yo; T1D duration 15 ± 12 y; 99 with vascular complications (CX+). HbA1c 8.4 ± 1.6% and 5.1 ± 0.4% in T1D and CON respectively ($p = 0.00001$). C-peptide was by ultra-sensitive ELISA (Mercodia, Sweden). Cycle threshold (CT) values of 50 miRs were used. T1D subjects were divided by age of diagnosis (DX, <18 and ≥18 yo) and duration (DUR: ≤10, 10–≤20 and >20 y).
Results: C-peptide was detectable in all CON and in 55% T1D. Median (LQ, UQ) C-peptide levels were 535 (401, 762) and 5.0 (2.6, 31.2) pmol/L in T1D with detectable levels respectively. Young age of T1D DX (<18 yo) and short T1D DUR (≤10 y) vs adult DX and short DUR was associated with lower rates of detectable C-peptide (39% vs 88%; $p = 0.008$). There was a positive relationship in young DX for percentage with detectable C-peptide by T1D duration (Cochrane-Armitage $p = 0.009$). miRs: Upregulated (lower CT values) in T1D included (Mann-Whitney p value): miR-24 ($p < 0.0001$), miR-146A ($p < 0.0001$), miR-155 ($p = 0.007$), miR-199a-3p, miR-30c, miR-223 ($p < 0.0001$), miR-22*, miR-125a-5p, miR-125b ($p < 0.0001$) and miR-126 ($p < 0.0001$) (all $p < 0.05$ unless otherwise stated). In T1D with undetectable (vs. detectable) C-peptide miRs with lower CT values (Mann-Whitney p value) included: *miR-24* (<0.0001), *miR-146a* (0.003), *miR-155* (<0.0001), *miR-199a* (0.03), *miR-223* (0.0002), *miR-126* (0.0006), *miR-22* (<0.001), *miR-125b* (0.02), *miR-152* (0.006), miR-181a (0.01). Higher CT value: miR-326 (0.03). *C-peptide and miR value correlations included*: Positive: (Spearman (R; p -value)) *miR-24* (0.25; <0.0001), *miR-155* (0.28; <0.0001) and *miR-223* (0.21; 0.0003). Penalized logistic regression: CT values positively associated with detectable C-peptide included: *miR-24, miR-155, miR-199a, miR-223*; Negatively associated: *miR-22*, miR-152, miR-181a*. Exhaustive search: miRs associated with detectable C-peptide level values included: *miR-30c and miR-125a-5p*. Adjusted R of best model 0.37.
Conclusion: Residual C-peptide is more common in LONGER T1D diagnosed in youth vs. later, even if of similar T1D DUR. Data support more beta cell loss in young onset T1D, and potential beta cell regeneration. Many MiR differences exist by C-peptide status and T1D DX age and DUR. Most differences are between T1D DX young and of short DUR vs. those DX as adults, irrespective of T1D DUR. There were no miR difference between T1D DX young and long DUR vs. those DX late and of short DUR. Longitudinal studies are merited.

Figure: miRs significantly different (Mann-Whitney) between T1D subgroups. For clarity only 4-groups shown: ① - DX <18 yo and DUR ≤10 y, ② - DX ≥18 yo and DUR >20 y, ③ - DX ≥18 yo and DUR ≤10 y. and ④ - DX ≥18 yo and DUR >20 y.



Supported by: DART General Grant
Disclosure: **A.S. Januszewski:** None.

329

Impact of family history on the phenotype and genotype of type 1 diabetes at diagnosis

M. Turtinen[1], T. Härkönen[2], A. Parkkola[1], J. Ilonen[3], M. Knip[1];
[1]Children's hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [2]Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland, [3]Immunogenetics laboratory, Institute of Biomedicine, University of Turku, Turku, Finland.

Background and aims: In previous studies, the risk of developing familial type 1 diabetes (T1D) has been about two times higher when the father is affected by T1D compared to the affected mother. We tested the hypothesis of more severe clinical features and a more aggressive humoral autoantibody profile at diagnosis in index cases with an affected father compared to the other familial subgroups.
Materials and methods: A cross-sectional, observational study was performed based on the Finnish Pediatric Diabetes Register. Clinical and metabolic characteristics, β-cell autoantibodies and HLA class II genetics were analyzed from the index cases diagnosed under the age of 15 years, between January 2003 and December 2016 in Finland. The information on the presence of T1D in first-degree relatives (FDR) was collected at diagnosis by a structured questionnaire.
Results: Out of 4,993 newly diagnosed index cases, 523 (10.5%) had familial T1D. More than 5% ($n = 254$, 5.1%) had an affected father, 2.9% ($n = 143$) an affected mother, 1.9% ($n = 95$) an affected sibling and 0.6% ($n = 31$) two or more affected family members. All the clinical and metabolic parameters were markedly poorer in the sporadic cases compared to the familial cases. The index cases with an affected father or mother were younger than those with an affected sibling ($p < 0.001$). After age- and sex-adjusted analyses, index cases with an affected father presented more often with ketoacidosis ($p = 0.03$) and had greater weight loss before diagnosis ($p = 0.001$) than those with an affected mother. There was a trend, that those with more than one affected family members had a lower number of positive autoantibodies at diagnosis, and this group tended to test negative for all autoantibodies more frequently than the sporadic cases. The absence of both major HLA risk haplotypes (DR3-DQ2 and

DR4-DQ8) was more common in the group of sporadic cases than in the familial group ($p = 0.015$). In contrast, the DR4-DQ8 haplotype was more frequent in the familial vs. the sporadic group ($p = 0.002$) and especially among those with an affected father when compared to sporadic cases ($p = 0.029$).

Conclusion: The more severe metabolic derangement at diagnosis in sporadic cases compared to those with familial T1D was confirmed. The higher frequency of diabetic ketoacidosis and increased weight loss at diagnosis in index cases with an affected father support the hypothesis that paternal T1D is associated with a more severe disease in the offspring than maternal diabetes.

Supported by: Academy of Finland, HUH Research Funds, Sigrid Juselius Foundation

Disclosure: **M. Turtinen:** None.

330

Circulating miRNAs as predictive biomarkers for type 2 diabetes development in individuals at risk: outcomes of a 5-year prospective observational cohort study

M. Niemira[1], K. Maliszewska[2], M. Paczkowska-Abdulsalam[1], A. Bielska[1], A. Szalkowska[1], E. Adamska[1], A. Citko[1], L. Szczerbinski[2], E. Siewiec[1], M. Gorska[2], M. Ciborowski[1], A. Kretowski[2];
[1]Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland, [2]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Background and aims: Due to a global increase in prevalence to type 2 diabetes (T2DM), there is an urgent need to take preventive actions among populations with risk factors like prediabetic state, obesity, family history of diabetes or significant lack of physical activity. Identification of early biomarkers, which would help to predict an increased risk of progression to T2DM a few years before disease fully develops, can improve the chance for effective prevention. Epigenetic modifications, along with genetic predisposition, play significant role in T2DM development. Here, we postulate that circulating serum-derived microRNAs may serve as biomarkers for early T2DM diagnosis and help to identify individuals with predisposition to develop diabetes.

Materials and methods: For the present case-control study 33 subjects were selected from the large 1000PLUS cohort study (Bialystok, Poland), including 18 patients (mean age: 48.7 ± 8.2 yrs, mean BMI: 33.6 ± 6.7 kg/m²) who developed type 2 diabetes during the 5-year prospective observation (the study group) and 15 healthy individuals with matching age, sex and BMI for control group, without carbohydrate tolerance disturbances detected during the follow up period. The presence of diabetes based on WHO criteria was excluded at the beginning of study (baseline) and was examined after 60 months or during the follow if clinical symptoms were observed. The microRNA profiling was performed using Nanostring nCounter Technology in serum collected at baseline from both T2DM patients and healthy controls.

Results: A total of 21 miRNAs were differentially expressed between the healthy and diabetic individuals (fold change >1.5 or <−1.5; $P < 0.05$). The serum expression levels of miR-1307, miR-1287, miR-548n, miR-548z, miR-548ah, miR-3144, miR-221, miR-509, let-7b, let-7g, miR-1269a, miR-1245b, miR-125a, miR-4707, miR-519e were higher in patients with T2DM, compared with healthy subjects, however, the levels of miR-1200, miR-216b, miR-508, miR-3614, miR-615, miR-372 were lower. The predicted target genes were identified using Ingenuity Pathway Analysis (Qiagen). The results indicated 482 putative target genes for the upregulated and downregulated miRNAs. Based on the above we constructed four interactive signalling networks. Top functions of target genes were related to muscle atrophy and insulin resistance (CASP3, PTGR2, SMARCD3), insulin signalling and translocation of GLUT4 (TBCC, PRKCZ, CD42EP3), beta-mass cell (CCND1), insulin resistance (PPARG, FOXO1) and several cancer-associated pathways.

Conclusion: The results of the present study indicate that circulating miRNAs may be used as potential biomarkers for early diagnosis of T2DM.

Disclosure: **M. Niemira:** None.

331

Epigenetic regulation of Slc2a4 gene in skeletal muscle of type 2 diabetic mice: participation of post-translational modifications of histone H3

C.Y. Yonamine[1], A.B. Alves-Wagner[1], J.V.D. Esteves[1], M.M. Okamoto[1], M.L. Corrêa-Giannella[2], D. Giannella-Neto[3], U.F. Machado[1];
[1]Physiology and Biophysics, University of Sao Paulo, Sao Paulo, Brazil, [2]University of Sao Paulo, Sao Paulo, Brazil, [3]University Nove de Julho, Sao Paulo, Brazil.

Background and aims: The main characteristic of diabetes mellitus is the loss of glycemic homeostasis. In this process, skeletal muscle plays a key role and maintenance of the GLUT4 glucose transporter (encoded by the *Slc2a4* gene) expression is fundamental. Epigenetic regulations of *Slc2a4*have never been investigated in diabetes; and resveratrol, suggested as an insulin sensitizer, might be a modulator of these regulations, as it is an activator of the deacetylase sirtuin 1 (SIRT1). The present study aimed to evaluate in type 2 diabetic mice (T2D) the effect of resveratrol treatment on glycemic homeostasis, *Slc2a4*/GLUT4 expression in skeletal muscle, epigenetic regulations of *Slc2a4* such as lysine acetylation (ac) or tri-methylation (me3) of histone 3 (H3Kac and H3K9me3), and the possible participation of SIRT1.

Materials and methods: T2D was induced by neonatal subcutaneous injection of monosodium glutamate (MSG) from day 1 to day 5 (2 mg/kg body weight) and day 7 (4 mg/kg body weight). Control mice received only the vehicle (0.9% NaCl). At the age of 19 weeks, T2D mice were treated or not with resveratrol (30 mg/kg body weight) for 60 days. Resveratrol was offered in the drinking water. On the 53rd day of treatment, insulin tolerance test (ITT) was performed. On the 60th day of treatment the animals were anesthetized with sodium thiopental (7 mg/kg body weight), blood was collected from the left ventricle and the gastrocnemius muscle was removed for analysis of: *Slc2a4* mRNA (RT-qPCR), total GLUT4 and nuclear SIRT1 (Western blotting), acetylation of H3K9,14,18,23 and 27 and tri-methylation of H3K9 (ChIP assay).

Results: T2D mice developed obesity, increased concentrations of plasma glucose (by 1.8 folds, $P < 0.001$), fructosamine (by 1.5 folds, $P < 0.05$) and insulin (by 6.8 folds, $P < 0.001$), and decreased rate of glucose decay in the ITT; resveratrol treatment reversed all these alterations, except the obesity. The *Slc2a4*/GLUT4 expression was reduced in muscle of T2D (by 30%, $P < 0.01$ and 50%, $P < 0.05$, respectively), and that was partially reversed by resveratrol. ChIP assay evinced that diabetes increased the content of both H3Kac (1.5 folds, $P < 0.01$) and H3K9me3 in the *Slc2a4* promoter (by 1.4 folds, $P < 0.001$); and the later was reversed by resveratrol. Additionally, although the nuclear SIRT1 content did not alter in T2D, the resveratrol treatment increased it (by 1.7 folds, $P < 0.05$).

Conclusion: T2D induced epigenetic regulations in skeletal muscle *Slc2a4* gene, such as increased H3Kac and H3K9me3, the later potentially participating on the *Slc2a4* gene repression. Resveratrol treatment improved the metabolic control of T2D mice and partially reversed the *Slc2a4*/GLUT4 expression. These regulations could not be explained by deacetylase activity of SIRT1 upon *Slc2a4* gene, but may be related to modulations of H3K9me3. These results point out the histone H3 post-translational modifications as potential targets to control GLUT4 expression and, consequently, to improve glycemic homeostasis in T2D.

Supported by: CNPq (142187/2013) and FAPESP (2012/04831)

Disclosure: **C.Y. Yonamine:** None.

332

Epigenetic response to bariatric surgery in human skeletal muscle

M. Ouni[1,2], S. Gancheva[3,4], C. Koliaki[4,5], J. Szendroedi[4,3], T. Jelenik[4,2], D. Markgraf[2,4], M. Jähnert[1,2], M. Schlensak[6,2], A. Schürmann[1,2], M. Roden[4,2];
[1]Department of Experimental Diabetology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany, [2]German Center for Diabetes Research (DZD), München-Neuherberg, Germany, [3]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany, [4]Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, Germany, [5]Laikon University Hospital, Athens, Greece, [6]General Surgery, General Surery, Schön Clinic, Düsseldorf, Germany.

Background and aims: Epigenetic states are highly metastable and reversible in response to different environmental signals such as drugs, aging and diet. Rapid weight loss after bariatric surgery can be considered as an environmental stimulus and provides an interesting system to investigate DNA methylation flexibility. Although, skeletal muscle insulin sensitivity (IS) is known to increase after bariatric surgery, the underlying molecular mechanisms remain unclear. We hypothesized that DNA methylation contributes to improvement of insulin sensitivity and therefore tested whether epigenetic changes participate in the modulation of skeletal muscle metabolism in response to bariatric surgery.
Materials and methods: Genome-wide gene expression and DNA methylation were analyzed in skeletal muscle biopsies of 16 obese humans OBE (OBE; 38 ± 10 yrs, BMI 44 ± 10 kg/m^2), who also underwent detailed metabolic phenotyping, at baseline, 2 and 52 weeks after bariatric surgery.
Results: At 2 w, excessive adipose tissue lipolysis with subsequent accumulation of specific muscle diacylglycerols prevented from rapid improvement of IS despite slightly increased muscle oxidative capacity. At the same time, alteration of 1,287 genes of the skeletal muscle was found when compared to baseline levels. Gene ontology analysis showed a significant enrichment in cAMP biosynthesis and lipid metabolism. None of differentially expressed genes exhibited differences in DNA methylation. After 52 w, IS had markedly improved along with normalization of myocellular mitochondrial function and lipid species levels in OBE when compared to lean humans. The improvement of the muscle metabolism was associated with DNA methylation changes in 1,467 CpGs of 430 differentially expressed genes. These genes were related to metabolic processes such as cAMP biosynthesis and oxidative stress. Interestingly, among those genes with the highest changes in DNA methylation, only a few were described previously to be epigenetically regulated in obese subjects such as *KCNQ1* (potassium voltage-gated channel subfamily KQT member 1), *ATP10A* (ATPase phospholipid transporting 10A) and *MYO7A* (myosin VIIA).
Conclusion: While initial metabolic alterations after bariatric surgery do not associate with epigenetic changes, the long-term beneficial changes in muscle insulin sensitivity are related to distinct changes in muscle DNA methylation.
Supported by: DZD
Disclosure: **M. Ouni:** None.

333

Increased serum expression of miR-518d-3p and miR-618 in individuals with type 1 diabetes with microvascular chronic complications

D.P. Santos-Bezerra[1], A.S. Santos[1], G.C. Guimarães[1], S.N. Admoni[1], R.V. Perez[1], T.S. Pelaes[1], C.G. Machado[2], M. Passarelli[3], U.F. Machado[4], M.S. Queiroz[5], M.E.R. Silva[1], M.L.C. Corrêa-Giannella[1,6];
[1]Laboratório de Carboidrato e Radioimunoensaio, LIM-18, Hospital das Clinicas HCFMUSP, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [2]Divisão de Oftalmologia do Hospital das Clinicas HCFMUSP, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [3]Laboratório de Lípides, LIM-10, Hospital das Clinicas HCFMUSP, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [4]Departamento de Fisiologia e Biofisica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil, [5]Divisão de Endocrinologia do Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, SAO PAULO, Brazil, [6]Programa de Pós-Graduação em Medicina, Universidade Nove de Julho (UNINOVE), Sao Paulo, Brazil, São Paulo, Brazil.

Background and aims: Epigenetic changes have been recognized in the pathogenesis of chronic diabetic complications; several studies have shown that periods of hyperglycemia result in permanent abnormalities in the target tissues of complications, a phenomenon known as "metabolic memory". One of the epigenetic mechanisms is the control of gene expression by microRNAs (miRNAs), which are small non-coding RNAs which repress the translation of messenger RNAs (mRNAs). The hypothesis of the present study is that the serum profile of miRNAs is different between individuals with type 1 diabetes (T1D) with and without chronic complications.
Materials and methods: In order to characterize and compare serum miRNAs profile, blood from 10 pre-selected patients from the Diabetes Outpatient Clinic was collected; Group 1 ($n = 5$) patients without microvascular complications (without diabetic kidney disease [DKD], without distal sensory-motor polyneuropathy [PN], without cardiovascular autonomic neuropathy [CAN] and without diabetic retinopathy [DR]) and Group 2 ($n = 5$) patients with microvascular complications (DKD, PN, CAN and severe DR), matched for sex, diabetes duration and degree of glycemic control. The profile of miRNAs was characterized with the use of the commercial kit *Taqman® Human MicroRNA Array A* that use hydrolysis probes for the analysis of 381 miRNA expression. Five out of 25 miRNAs differentially expressed between the two groups (the ones with the highest statistical difference: 518-3p, 34a-5p, 126-5p, 425-5p and 618) were validated in the serum of 47 individuals with T1D, 20 without microvascular complications and 27 with all microvascular complications.
Results: A total of 193 out of 381 evaluated miRNAs was expressed in the serum of patients with T1D; 21 miRNAs were found overexpressed in the group with microvascular complications (ANOVA test with a fold change >1.50 and $P < 0.05$ considered as significant). Of the 5 validated miRNAs, two were confirmed as differentially expressed between the two groups of patients studied, 518d-3p and 618 ($P = 0.02$ for both).
Conclusion: miR-518d-3p targets the peroxisome alpha proliferator (*PPARA*) mRNA, which plays a critical role in lipid homeostasis and inflammation, and whose low expression has been demonstrated in the retina of animals with diabetes. One of the targets mRNA of miR-618 is *TXNIP*, which interferes with the antioxidant activity of thioredoxin and whose low expression has already been observed in peripheral blood mononuclear cells from individuals with T1D and chronic complications.
Supported by: FAPESP #2012/04831-1 and #16/15603-0, CNPq - #162789/2015-7
Disclosure: **D.P. Santos-Bezerra:** None.

334

Hyperglycaemia changes the miRNA expression pattern during differentiation and maturation of human visceral adipocytes

J. Strycharz, E. Swiderska, A. Wroblewski, M. Podolska, J. Drzewoski, J. Szemraj, A. Sliwinska;
Medical University of Lodz, Lodz, Poland.

Background and aims: MiRNAs are short endogenous non-coding RNAs and negative epigenetic regulators. Adipogenesis is a process of differentiation of mesenchymal stem cells to adipocytes. Obesity, especially visceral one, increases the risk of developing type 2 diabetes. The latter is preceded by hyperglycemia, which exerts deleterious effects on various tissues, including visceral adipose tissue. Thus, the aim of our study was to examine the effect of exposure to high glucose during

differentiation and maturation of human visceral adipocytes on the expression profile of selected miRNAs.
Materials and methods: miRNA expression pattern was determined during adipogenesis of HPA-v cells performed under normoglycemia and hyperglycemia. The differentiation and maturation of adipocytes involved three culture stages: (1st) preadipocytes stage, (2nd) differentiated and (3rd) mature adipocytes stages. To obtain hyperglycemia, D-(+) glucose was added to medium to provide 30 mM concentration. Expression of 78 miRNAs was determined by Real-time PCR after completion of each culture stage using ΔCt method. Statistical significance was evaluated using ANOVA with Tukey post hoc test with $p \leq 0.05$ considered as significant. We performed hierarchical clustering/Pearson's correlation for studied comparisons of variants (Gitools2.3.1) and pathway enrichment analysis for significant miRNAs (DIANA mirpath v3).
Results: miRNAs expression changes detected during adipogenesis are presented in Table 1. The completion of 2nd stage in normoglycemia (NN vs N) revealed the majority of miRNAs to be downregulated and the reverse was observed for the transition from 2nd to 3rd stage (NNN vs NN). There was no strongly dominant direction of miRNAs expression changes for NNN vs N, yet the expression level of above one third of studied miRNAs was reduced. In hyperglycemia, 2nd stage (HH vs H) completion evoked the increase of a small majority of miRNAs, while a similar number of miRNAs was decreased in HHH to H. A large majority of miRNAs was declined in HHH in relation to HH. Several comparisons of variants were strongly and positively correlated ($r = 0.8$), being confirmed by hierarchical clustering. We revealed 22 miRNAs differentially expressed during adipogenesis in both conditions, which were enriched in pathways connected with fatty acids, ECM, cell cycle and Hippo, p53, PI3K/AKT and TGF-β signaling. miR-140-5p, miR-31-3p, miR-376c-3p were changed during normo- and hyperglycemic adipogenesis.
Conclusion: The numbers of miRNAs being upregulated, downregulated and unchanged in adipogenesis revealed that the miRNAs expression pattern is pronouncedly disparate in hyperglycemia comparing to normoglycemia. Differentially expressed miRNAs may be important for visceral adipogenesis independently of glycemia, possibly via regulating genes indispensable for adipocytes functionality.

Table 1. The miRNA expression pattern reported during adipogenesis of HPA-v carried out in normoglycemia and hyperglycemia

Culture variant	upregulation	downregulation	No changes
NN vs N	14	50	14
NNN vs N	23	32	23
NNN vs NN	47	2	29
HH vs H	36	22	20
HHH vs H	17	39	22
HHH vs HH	15	50	13

Supported by: no. 2015/17/B/NZ7/03019 National Science Centre
Disclosure: **J. Strycharz:** None.

335

Familial diabetes status and the risk of incident type 2 diabetes in Denmark

O. Silverman-Retana[1,2], A. Hulman[1,2], J. Nielsen[3,4], C.T. Ekstrøm[5], B. Carstensen[6], R.K. Simmons[7], D.R. Witte[1,2];
[1]Department of Public Health, Aarhus University, Aarhus, Denmark, [2]Danish Diabetes Academy, Odense, Denmark, [3]Global Health Section, Department of Public Health, University of Copenhagen, Copenhagen, Denmark, [4]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA, [5]Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark, [6]Clinical Epidemiology Department, Steno Diabetes Center Copenhagen, Gentofte, Denmark, [7]The Healthcare Improvement Studies Institute, University of Cambridge School of Clinical Medicine, Cambridge, UK.

Background and aims: Individuals with a family history of diabetes have an increased risk of developing the same disease. Despite known effects of the shared environment and genetic determinants, familial aggregation of diabetes at the population level is not fully understood. This study aims to quantify the association between parental and sibling diabetes status and individual's risk of developing the disease at the level of the entire Danish population.
Materials and methods: We performed a register-based analysis of all individuals in Denmark who were 30 years or older and did not have diabetes on January 1st 1995, following them until the end of 2012. We restricted the analysis to those individuals who had available information on their parents. Parental and sibling diabetes status (exposure) and date of diabetes onset in the index individual (outcome) were defined from the Danish National Diabetes Register. We used Poisson models to calculate the age and sex-adjusted incidence rate of diabetes among individuals whose parents and siblings did not have diabetes (reference) and incidence rate ratios (IRR) for those with maternal, paternal diabetes and/or siblings with diabetes. We tested interaction terms to determine whether the risk for those with diabetes in both parents and siblings exceeded the contribution from each family member.
Results: We included 877,807 individuals (53.7% male, median age at baseline: 36 years [IQR: 33–40]) who contributed 14,226,923 person-years of follow up (median duration: 17 years). Paternal diabetes was present in 132,468 (15.0%) individuals; maternal diabetes was found in 142,414 (16.2%) and 35,004 (3.9%) individuals had one or more siblings with diabetes. In 26,746 (3.0%) individuals, both parents had diabetes. The number of incident diabetes cases was 60,256; the age and sex-adjusted (for a 50-year-old male) incidence rate of diabetes for those without familial diabetes was 4.1/1000 person-years (95%CI: 4.0; 4.2). The IRR for individuals with maternal diabetes was 1.89 (95%CI: 1.83; 1.96), for individuals with paternal diabetes was 1.79 (95%CI: 1.73; 1.87) and for those individuals with a sibling with diabetes was 2.02 (95% CI: 1.93; 2.11) compared to individuals without familial diabetes. Among individuals whose both parents had diabetes, the IRR was 2.95 (95% CI: 2.79; 3.13). We found no indication of a deviation from the multiplicative effect when both parents and siblings had diabetes.
Conclusion: Family history of diabetes confers a marked risk elevation at the population level. The ability to extract valid familial diabetes data from population-wide registers offers the opportunity to hone and improve population-based strategies for prevention and early detection of diabetes.
Supported by: Danish Diabetes Academy
Disclosure: **O. Silverman-Retana:** None.

PS 010 Monogenic diabetes

336

Patients with Down syndrome who present with neonatal diabetes are unlikely to have a monogenic aetiology

M.B. Johnson, K.A. Patel, E. De Franco, S. Ellard, S.E. Flanagan, A.T. Hattersley;
Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK.

Background and aims: Autoimmune diabetes is 4–6 times more prevalent in individuals with Down syndrome compared to the general population. Diabetes typically presents early in patients with Down syndrome with 1 in 5 presenting before the age of 2 years. Neonatal diabetes, diagnosed in the first 6 months of life, is usually monogenic with >82% of patients having a mutation in a known gene. We aimed to discover the aetiology of diabetes in patients with Down syndrome and diabetes diagnosed in the first 6 months of life.
Materials and methods: We studied 12 individuals with Down syndrome (reported by the referring clinician) diagnosed with diabetes in the first 6 months of life (median age of diagnosis 2 weeks (range 1 day–26 weeks). All patients required insulin therapy from diagnosis. We screened all known monogenic diabetes genes by targeted next generation sequencing. We genotyped all patients for the top 9 risk alleles for type 1 diabetes to generate a type 1 diabetes genetic risk score. This was compared to gold-standard type 1 diabetes ($n = 1963$) and non-diabetic ($n = 2938$) controls from the Wellcome Trust Case Control Consortium. Islet autoantibody testing (GAD, IA2 and ZnT8) was performed by commercially available ELISA assays where serum was available ($n = 8$).
Results: We did not identify a pathogenic mutation in the known monogenic diabetes genes in any of the 12 individuals. This finding is unlikely to have occurred by chance ($p < 0.0005$) considering the prior probability of identifying a monogenic aetiology in neonatal diabetes is >82%. This suggests that the diabetes in these individuals is unlikely to be monogenic. 5/8 (63%) patients were positive for 1 anti-islet antibody (4 anti-GAD and 1 anti-IA2) supporting an autoimmune aetiology in these individuals. We found evidence that the aetiology of the diabetes in individuals with Down syndrome is distinct to that of type 1 diabetes. The type 1 diabetes genetic risk score was lower in the diabetic patients with Down syndrome than in type 1 diabetes controls (median 0.53 v 0. 0.67, $p = 0.002$) and similar to population controls without diabetes (median 0.53 v 0.50, $p = 0.32$).
Conclusion: Individuals with Down syndrome and neonatal diabetes do not have a mutation in a known monogenic diabetes gene. Our data suggest that the aetiology of the diabetes in these patients is autoimmune but is not due to polygenic susceptibility for type 1 diabetes. These individuals are likely to represent the extreme phenotype of autoimmunity in Down syndrome. Further studies to determine the underlying aetiology of diabetes in these individuals is warranted.
Supported by: Wellcome Trust
Disclosure: **M.B. Johnson:** None.

337

Excess insulin secretion with a high protein meal in sulphonylurea treated KCNJ11 neonatal diabetes patients shows the limitations of amplifying insulin secretion pathways

P. Bowman[1,2], M.H. Shepherd[1,2], T.J. McDonald[1,2], R.C. Andrews[1,2], S.R. Spaull[1,2], S. Statton[1,2], S. Hammersley[1,2], M. Leveridge[3], B.M. Shields[1,2], S.E. Flanagan[1], B.A. Knight[1,2], K.A. Patel[1,2], A.T. Hattersley[1,2];
[1]University of Exeter Medical School, Exeter, UK, [2]Exeter NIHR Clinical Research Facility, Exeter, UK, [3]Royal Devon and Exeter NHS Foundation Trust, Exeter, UK.

Background and aims: *KCNJ11* mutations cause permanent neonatal diabetes (PNDM) by preventing ATP binding and closing the pancreatic ATP-dependent potassium (K_{ATP}) channel. This hyperpolarises the beta-cell and stops all insulin secretion. High dose oral sulphonylureas (SUs) repolarise the beta-cell allowing insulin secretion in response to the amplifying pathways including incretins, but the classical pathway of insulin secretion where glucose acts through ATP plays a minimal role. Despite this patients with SU-treated *KCNJ11* PNDM have excellent glycaemic control with no severe hypoglycaemia. These patients represent a unique opportunity to assess the physiological response to different food types mediated by the amplifying pathways of insulin secretion with minimal response to glucose via the classical ATP pathway. We aimed to assess for the first time the insulin, glucose and glucagon response to both a protein and a carbohydrate-rich meal in patients with SU-treated *KCNJ11* PNDM.
Materials and methods: 6 adults with SU-treated *KCNJ11* PNDM and 6 non-diabetic controls matched for age, gender and BMI, were given a high protein breakfast and an isocaloric high carbohydrate breakfast on 2 separate days. Individuals with *KCNJ11* PNDM took SU as normal with each meal. Blood insulin, glucagon, and glucose were measured 12 times in 4 hours and compared between the different meals in each group and between cases and controls. Non-parametric statistical methods were used.
Results: *KCNJ11* patients had similar insulin secretion with protein vs. carbohydrate (median 0–4 hour insulin incremental area under the curve ($iAUC_{0\text{-}4h}$) 230 vs 228 pmol/L, $p = 0.75$). Controls without diabetes had lower insulin secretion with protein vs. carbohydrate (median insulin $iAUC_{0\text{-}4h}$ 121 vs 577 pmol/L, $p = 0.03$). These different patterns of insulin secretion were associated with different glycaemic responses: *KCNJ11* patients had much lower glucose values after a protein rich meal compared to controls (glucose $iAUC_{0\text{-}4h}$ −9.3 vs −1.1 mmol/L, $p = 0.004$) and much higher glucose values after a carbohydrate meal (glucose $iAUC_{0\text{-}4h}$ 16.2 vs. 1.4 mmol/L, $p = 0.02$). Counter-regulatory glucagon secretion was higher after protein than carbohydrate in both *KCNJ11* patients and controls (median glucagon $iAUC_{0\text{-}4h}$ with protein 7.7 vs 13.3 pmol/L, $p = 0.63$ and with carbohydrate 0.5 vs 0.2 pmol/L, $p = 0.42$).
Conclusion: In patients with SU-treated *KCNJ11* PNDM insulin secretion is stimulated to a similar extent by a protein rich meal and carbohydrate rich meal leading to relatively lower blood glucose after a protein rich meal and a potential risk of moderate postprandial hypoglycaemia. The high protein meal is likely to result in excessive postprandial insulin secretion as a result of the marked stimulation of insulin secretion by amplifying pathways such as incretins which, unlike normal subjects, is not moderated by the glucose responsive classical ATP pathway. This has implications for the dietary advice offered to patients with *KCNJ11* PNDM and also shows that nutrient detection by the amplifying pathways of insulin secretion is imperfect in the absence of glucose regulation by the classical pathway.
Clinical Trial Registration Number: NCT02921906
Supported by: Diabetes UK, Wellcome Trust
Disclosure: **P. Bowman:** None.

338

The diagnostic utility of urinary C-peptide/creatinine ratio (UCPCR): insights from a review of the local use of UCPCR in the diabetes clinic

A. Poddar[1], J.S. Clark[1], V. Nayyar[1], S. Zachariah[1], N. Maghsoodi[1], B.C.T. Field[1,2];
[1]Diabetes and Endocrine, East Surrey Hospital, Redhill, UK, [2]Department of Clinical and Experimental Medicine, School of Health and Medical Sciences, University of Surrey, Guildford, UK.

Background and aims: UCPCR is a less burdensome measure of endogenous insulin secretion compared to traditional serum C-peptide, and 24-hour urinary C-peptide. It can be used to diagnose maturity onset

diabetes of the young (MODY), and helps identify absolute insulin deficiency.

Materials and methods: All UCPCR results at our NHS Trust from September 2015 to September 2017 were identified. Electronic notes were reviewed to collect the following information: clinical justification, age, time to initiating insulin, antibody serology, family history and HbA_{1c}. Changes in diagnosis and management following UCPCR quantification were recorded.

Results: Eighty UCPCR requests were identified. The diagnosis for 40 patients was changed after consideration of UCPCR and other clinical data. Ten people with a clinical diagnosis of type 1 diabetes were reclassified as type 2, and one as HNF1A MODY. Eight people with apparent type 2 diabetes were reclassified as type 1. There was a change in management in 32 cases. Ten individuals have restarted oral medication of which five patients are off insulin and five now on basal only; four such patients had been on insulin for over 15 years, of whom two were on insulin pump therapy. Three patients with LADA are under surveillance using serial UCPCR as well as HbA_{1c} and glucose measurements to inform decision-making on the need for exogenous insulin therapy.

Conclusion: UCPCR is a convenient tool for classifying diabetes and guiding management in the absence of serum or 24-hour urinary C-peptide.

Disclosure: **A. Poddar:** None.

339

Functional characterisation of HNF1A variants identified in Norwegian diabetes registries can be important for precision medicine in diabetes clinics

I. Aukrust[1,2], A. Kaci[1,2], J. Molnes[1,2], H.U. Irgens[1,3], B.B. Johansson[1], L. Bjørkhaug[4], P.R. Njølstad[1,3];
[1]KG Jebsen Center for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, Norway, [2]Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway, [3]Department of Pediatrics and Adolescents, Haukeland University Hospital, Bergen, Norway, [4]Department of Biomedical Laboratory Sciences and Chemical Engineering, Western Norway University of Applied Sciences, Bergen, Norway.

Background and aims: Genetic variants in *HNF1A* encoding the transcription factor hepatocyte nuclear factor-1 alpha (HNF-1A) can cause Maturity-Onset Diabetes of the Young type 3 (MODY3; HNF1A-MODY). The aim of this study was to investigate possible pathogenic effects of 16 *HNF1A*variants of unknown clinical significance identified in the Norwegian MODY Registry and the Norwegian Childhood Diabetes Registry, using functional protein analyses, and to correlate findings with family history and clinical characteristics of *HNF1A* variant carriers.

Materials and methods: All *HNF1A* variants were classified using a five-tier score system commonly used in clinical diagnostic laboratories. To investigate the effect of *HNF1A* variants on normal HNF-1A transcriptional activity, we used a Dual-Luciferase assay system in transfected HeLa cells.

Results: 15 of the *HNF1A* variants investigated were classified as variants of unknown clinical significance, while one variant was classified as likely pathogenic, i.e. *HNF1A* (NM_000545.5) c.1640_1641del p.(T547Rfs*5). This variant was found in a patient diagnosed with diabetes from 11 years of age, initially thought to have type 1 diabetes. He was later established to be GAD- and IA-2 autoantibody negative. Interestingly, his non-diabetic mother (age 38) also carries the variant. The transcriptional activity of this variant was significantly reduced to ~37% ± 6.3 ($p < 0.0001$) compared to wild-type (WT) HNF-1A (100%), but not as severely as for classical MODY3 variants (20–25%). Another variant, c.346G>A p.(A116T), also demonstrated significantly reduced transcriptional activity (~36% ± 4.9 ($p < 0.0001$)). This variant was found in two sisters diagnosed with diabetes at 16 and 20 years of age. One of the sisters had a slightly elevated BMI, and their father (not genetically tested), of normal weight, was diagnosed with type 2 diabetes at age 30–35. Further, three variants caused severe reduction in transcriptional activity (<25%), thus functionally resembling a MODY3 variant. These were c.797A>G p.(N266S), c.666_668del p.(K222del) and c.428A>C p.(H143P). Interestingly, p.(K222del) was found in a family including four affected family members in two generations (mother and three children), all with diabetes onset from 11–25 years of age, supporting this variant to be causative for MODY3. The two other variants, p.(N266S) and p.(H143P), were both found in patients with early onset diabetes (13 and 15 years, respectively) and who are currently treated with insulin. The patient carrying p.(N266S) has normal BMI, is negative for GAD autoantibody, and has no known family relatives with diabetes. The patient carrying p.(H143P) is negative for both autoantibodies GAD and IA-2.

Conclusion: Functional investigation of *HNF1A* variant effects should support precision medicine in diabetes clinics and could help to distinguish neutral variants from variants causing type 2 or MODY3 diabetes, in combination with family history and clinical characteristics.

Clinical Trial Registration Number: REK Vest (2009/2080)
Supported by: Helse Vest, KG Jebsen Foundation, Norwegian Diabetes Association, ERC, UoB

Disclosure: **I. Aukrust:** None.

340

Double monogenic diabetes of the young: implications for treatment in pregnancy

H. Khan[1], A.T. Hattersley[2], D.L. Browne[1];
[1]Endocrine and Diabetes, Royal Cornwall Hospitals NHS Trust, Truro, Cornwall, UK, [2]Molecular Medicine, University of Exeter Medical School, Exeter, UK.

Background and aims: Co -inheritance of HNF 1alpha and Glucokinase (GCK) -maturity-onset diabetes of the young (MODY) mutation is extremely rare. The effects of two simultaneous heterozygous pathogenic mutations on pregnancy and optimal management strategy are not known.

Materials and methods: A 29 year old female in her first pregnancy presented with persistent glycosuria. She had a strong family history of diabetes in her paternal grandfather, father and two siblings. One of her brothers and his son had been diagnosed with HNF1A recently. Her pre-pregnancy body mass index was 22.3.Her oral glucose tolerance test (OGTT) at 8 weeks of gestation demonstrated fasting blood glucose (FBG) of 8.8mmol/L and two hour glucose of 16.5mmol/L. HbA1c at that time was 58mmol/mol. Gene Sequencing for MODY confirmed two heterozygous disease causing mutations; GCK missense variant, c.676G>A, p.Val226Met and HNF1A splicing variant, c.526+1G>A. She was initiated on basal bolus insulin regimen (Isophane and Insulin Aspart). To date, 29 weeks in pregnancy she is requiring 17–20 units total daily Insulin with mean FBG/pre-prandial and 1 hour post prandial capillary blood glucose (CBG) of 4.89 ± 0.7 mmol/L and 6.5 ± 0.98 mmol/L respectively. (Target range: FBG 4.0–5.3 mmol/L, 1 hour Post prandial CBG 4.0–7.8 mmol/L). The foetal growth is normal.

Results: The effects of MODY on pregnancy and foetus largely depend on maternal glycaemic control and whether or not the foetus has inherited the mutation. Treatment during pregnancy usually depends on the type of mutation. In isolated HNF1A MODY outside pregnancy, sulphonylureas are effective but currently transfer to insulin is recommended in pregnancy. In isolated GCK mutation treatment is not required unless there is evidence of foetal macrosomia in the third trimester where insulin is indicated but has little impact on glycaemia. Co-existence of GCK mutation with HNF1A could make it difficult to control FBG due to higher set point of glucose homeostasis and increased gluconeogenesis.

Conclusion: It is the first patient in 2,531 probands with MODY mutations who is known to have a definite pathogenic mutation in both HNFA1 and GCK genes. This case suggests that insulin may be required earlier in pregnancy. Whilst conventional experience shows GCK mutation would make it difficult to achieve optimal control, our patient has progressed well in pregnancy with excellent control on a modest dose of insulin.

Disclosure: **H. Khan:** None.

341

"De Novo" Maternally Inherited Diabetes and Deafness (MIDD)? Variable genetic transmission in MIDD

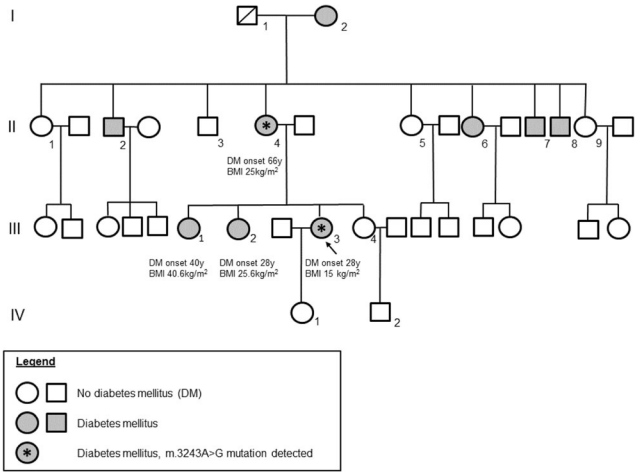
A. Lam[1], Y. Zhao[2], L. Ong[2], W. Sim[2], L. Zhu[1], S. Rama Chandran[1], D.-L. Gardner[1];
[1]Department of Endocrinology, Singapore General Hospital, Singapore, Singapore, [2]Department of Clinical Translational Research, Singapore General Hospital, Singapore, Singapore.

Background and aims: The m.3243A>G mutation is the most prevalent pathogenic mitochondrial (mt) mutation resulting in Maternally Inherited Diabetes and Deafness (MIDD). Maternal inheritance of diabetes (DM) should trigger consideration of the presence of mtDNA mutations, but its absence should not negate it. We aimed to describe the variable transmission of MIDD in a proband and four immediate relatives.

Materials and methods: We describe a 40 year old Chinese woman (III-3) with MIDD and mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) from m.3243A>G mutation. The mother-child and inter-sibling genotype-phenotype relationship is examined, using blood leucocyte and urine epithelial cell (UEC) DNA. Real-time PCR (Taqman assay) was used to quantify heteroplasmy levels, through absolute and relative quantification, and digital PCR.

Results: III-3 had gestational DM during her pregnancy (28y). At 33y, she was diagnosed with DM despite a lean BMI of 15 kg/m^2 and absent features of insulin resistance. Beta cell autoantibodies were negative. Bilateral sensorineural hearing loss was present since childhood. On metformin alone, she maintained HbA1c levels 6.2–7.7% (44–61 mmol/mol) until age 40y when she developed seizures and high lactate levels. C-peptide was 2.62 μg/L (plasma glucose 11.2 mmol/L), suggesting adequate beta cell function. MRI brain showed inflammation in multiple cerebral territories. She had no cardiac, renal or ophthalmologic manifestations. III-3's maternal grandmother (I-2), now 96y, had late onset DM and bilateral hearing loss in her 50s. III-3's mother (II-4) had late onset DM (66y), was overweight (BMI 25 kg/m^2) and was diet-controlled. 4 of 8 maternal siblings have DM (II-2, II-6, II-7, II-8). III-3's eldest sister (III-1) was obese (BMI 40.6kg/m^2) at DM diagnosis (40y) and is on oral glucose lowering agents (OGLA). III-2 had DM at age 28y (BMI 25.6 kg/m^2), and is on OGLA. The youngest sister (III-4, 37y, BMI 23.2 kg/m^2) does not have DM. III-3's daughter (now 12y) has no manifestations. Only I-2 and the proband (III-3) have hearing loss. Genotyping of blood leucocytes from III-3, her mother and 3 sisters revealed the presence of the m.3243A>G mutation in III-3 (19% heteroplasmy), but not in her mother or sisters. UEC from all 5 were analyzed using real-time and digital PCR. The m.3243A>G mutation was detected at lower heteroplasmy levels in the mother (II-4) (3.9%) but remained negative in the 3 sisters, indicating that III-1 and III-2 likely have type 2 DM.

Conclusion: The transmission of m.3243A>G mutation from mother to child is variable, and female carriers do not necessarily transmit the mutation to all offspring. This highlights the challenges of genetic counselling in MIDD. Genotyping of UEC DNA should be performed in those who are negative for mutations in blood leucocytes before dismissing the diagnosis of MIDD.



Disclosure: **A. Lam:** None.

342

The utility of MODY probability calculator among HNF1A- and GCK-MODY Polish patients: a retrospective analysis

M. Szopa, D. Ucieklak, S. Mrozinska, I. Solecka, B. Zapala, J. Hohendorff, B. Matejko, M. Sani, M.T. Malecki;
JUMC, Krakow, Poland.

Background and aims: The most common form of monogenic diabetes is MODY (Maturity Onset Diabetes of the Young). The optimal approach to identify MODY families is still a challenge as there is no ideal non genetic biomarker. An easy-to-use MODY prediction model for identifying genetic-test indicated patient cases was developed in 2012 by the Hattersley's group from Exeter, UK (www.diabetesgenes.org/content/mody-probability-calculator). The aim of this study was to validate the utility of this tool with MODY patients in the Polish population.

Materials and methods: Our MODY patient database was established 18 years ago at the Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland. Patient selection into the database has been based on the following criteria: a) autosomal dominant inheritance pattern of diabetes mellitus; b) presence of the disease in at least three subsequent generations; c) at least two diabetic family members diagnosed at or before the age of 30 who have received at least two years of treatment with diet, oral drugs, or insulin in a dose lower than 0.5 U/kg. Patients in the database who provided all answers to the MODY probability calculator questionnaire were included in the current study. This patient sub-population currently includes 85 GCK-MODY and 74 HNF1A-MODY patients. The control group was established with 100 randomly selected T1DM (Type 1 Diabetes Mellitus) individuals from our outpatient clinic. The control group does not include any T2DM patients due to insufficient number of T2DM cases diagnosed at or before the age of 35 from our outpatient clinic - as required in the calculator model.

Results: The mean predictive value using the calculator was 61.42% for GCK-MODY and 40.38% for HNF1A-MODY patients. This is in contrast with the mean calculator predictive value of 4.48% for T1DM patients. Only one T1DM patient out of 100 obtained a calculator predictive value higher than 20% – the minimum suggested criteria for genetic testing referral. Only 8 GCK-MODY patients and 23 HNF1A-MODY patients received a sub-20% score. More than 60% of GCK-MODY and almost 30% HNF1A-MODY patients obtained a positive predictive value greater than 75.5%.

Conclusion: The model based on the Hattersley's group calculator reliably indicated genetic testing for GCK-MODY patients among our sub-population of Polish patients. The obtained results for HNF1A-MODY patients were also satisfactory. The results are surprising as the probability calculator was initially focused on transcription-factor mutation MODY

rather than GCK ones. The model also efficiently helped to rule out unnecessary genetic testing for patients with T1DM. The usage of this tool will be expanded among our database MODY patients in the near future, at least partly to assess for potential fine-tuning of its predictive power among Polish MODY patients.

Supported by: Polish Diabetes Association Grant 2016

Disclosure: **M. Szopa:** None.

343

Paradoxical worsening of glucose tolerance after metformin in a patient with insulin resistance due to the SHORT syndrome

K.C. Lewandowski[1,2], K. Dabrowska[2], M. Brzozowska[1], J. Kawalec[2], A. Lewinski[1,2];
[1]Department of Endocrinology & Metabolic Diseases, The Medical University of Lodz, Lodz, Poland, [2]Department of Endocrinology & Metabolic Diseases, "Polish Mother's" Memorial Hospital Research Institute, Lodz, Poland.

Background and aims: Metformin is regarded as the first line treatment for type 2 diabetes and insulin resistant states. Hereby, we present a case of an unexpected response to metformin in a 21 year patient (BMI 17.5 kg/m²) with a history of SHORT syndrome *(**S**hort stature, **H**yperextensibility, **O**cular depression, **R**ieger anomaly, **T**eething delay),* an autosomal dominant disorder, that was also diagnosed in her father and younger brother. SHORT syndrome is also characterised by partial lipoatrophy and severe insulin resistance (IR) due to post-receptor defect in insulin signaling (Phosphoinositide-3-Kinase Regulatory Subunit 1 - PIK3R1).

Materials and methods: She had normal thyroid function, prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and dehydroepiandrosterone sulfate (DHEAS), Free Androgen Index 4.52 ($N < 8$), while pelvic ultrasound showed polycystic ovaries. Vitamin D concentration was low (11.0 ng/ml, frank deficiency<20 ng/ml). Lipid concentrations were normal (total cholesterol 156 mg/dl, triglycerides 99 mg/dl). Extended OGTT was performed and showed severe IR. She was then started on Metformin 850 mg bd, and had repeated OGTT (Table 1).

Results: Glucose and insulin concentrations during an extended 75 gram OGTT, before and on Metformin treatment, are presented in Table 1. After three days of Metformin treatment there was a dramatic worsening of glucose tolerance, with insulin concentrations above the upper assay detection limit, at 120, 150 and 180 minutes post glucose load. Metformin was discontinued and she was discharged home on Dydrogesterone and vitamin D supplementation.

Conclusion: The precise cause of such profound and paradoxical worsening of glucose tolerance post metformin remains unknown. As metformin is, however, known to partially inhibit PIK3, then we speculate that further inhibition of this already mutated enzyme might have prevented any beneficial effects of metformin, as these generally affect further steps of insulin signaling. In contrast, further inhibition of PIK3R1 could have worsened insulin resistance.

Table 1: 75 gram OGTT before and after metformin treatment

		0 min.	60 min.	120 min.	150 min.	180 min.	240 min
Initial OGTT	Glucose [mg/dl]	82	146	96	68	57	64
	Insulin [µIU/ml]	37.74	688.8	488.6	246.8	100.6	30.1
OGTT post Metformin 850 mg.	Glucose [mg/dl]	72	169	187	204	176	42
	Insulin [µIU/ml]	29.27	902.7	>1000	>1000	>1000	341.8

Disclosure: **K.C. Lewandowski:** None.

PS 011 Type 2 diabetes therapy intensification

344

Quality of life in patients with type 2 diabetes initiating a second-line glucose-lowering therapy: the global DISCOVER study

A. Nicolucci[1], M.B. Gomes[2], K. Khunti[3], M. Kosiborod[4], S. Pocock[5], W. Rathmann[6], M.V. Shestakova[7], I. Shimomura[8], H. Watada[9], H. Chen[10], J. Cid-Ruzafa[11], P. Fenici[12], N. Hammar[13], F. Tang[4], L. Ji[14];
[1]Center for Outcomes Research and Clinical Epidemiology, Pescara, Italy, [2]Rio de Janeiro State University, Rio de Janeiro, Brazil, [3]University of Leicester, Leicester, UK, [4]Saint Luke's Mid America Heart Institute, Kansas City, MO, USA, [5]London School of Hygiene and Tropical Medicine, London, UK, [6]German Diabetes Center, Duesseldorf, Germany, [7]Endocrinology Research Center, Diabetes Institute, Moscow, Russian Federation, [8]Osaka University, Oaska, Japan, [9]Juntendo University, Tokyo, Japan, [10]AstraZeneca, Gaithersburg, MD, USA, [11]Evidera, Barcelona, Spain, [12]AstraZeneca, Cambridge, UK, [13]AstraZeneca Gothenburg, Mölndal, Sweden, [14]Peking University People's Hospital, Beijing, China.

Background and aims: DISCOVER is a 3-year, global, observational study of patients with type 2 diabetes (T2D) initiating a second-line glucose-lowering therapy in 37 countries. Here, we report health-related quality of life (HRQoL) at baseline.

Materials and methods: HRQoL was assessed using the 36-item Short-Form Health Survey version 2 (SF-36v2) and the Hypoglycaemia Fear Survey II (HFS-II) in 32 countries ($N = 13\,320$) and 24 countries ($N = 10\,264$), respectively. The SF-36v2 is divided into Physical Component Summary (PCS) and Mental Component Summary (MCS) scores, which were normalized to 50 (SD: 10) using the 2009 US population (lower scores indicate decreased HRQoL). The HFS-II is divided into two subscales (higher scores indicate increased fear of hypoglycaemia): behaviour (HFS-B; maximum score 60) and worry (HFS-W; maximum score 72). Factors associated with decreased SF-36v2 scores and increased HFS-II scores were assessed using multivariable regression models.

Results: For the 9449 patients (70.9%) with SF-36v2 data, mean scores for PCS and MCS were 48.3 (SD: 7.8; across-country range [ACR]: 43.6–53.0) and 46.0 (SD: 10.4; ACR: 41.1–53.5), respectively. Lower scores for both PCS and MCS were associated with being female, having a history of macrovascular disease, abstaining from alcohol, using a sulphonylurea (vs metformin) as first-line therapy, and higher HFS-B and HFS-W scores. Lower PCS (but not MCS) scores were significantly associated with older age, lower education level, higher BMI and a history of chronic kidney disease (Table). For the 7090 patients (69.1%) with HFS-II data, mean HFS-W and HFS-B scores were 6.9 (SD: 11.4; ACR: 2.2–26.1) and 7.8 (SD: 9.7; ACR: 5.8–55.9), respectively. Higher scores for both HFS-B and HFS-W were significantly associated with being female, receiving diabetes education and a history of vascular complications. Higher HFS-B (but not HFS-W) scores were significantly associated with older age and higher BMI.

Conclusion: Factors associated with lower HRQoL in patients with T2D are related to both disease (e.g. history of complications) and treatment (e.g. use of sulphonylureas). Fear of hypoglycaemia varied greatly across countries and was associated with lower HRQoL.

	SF-36v2 PCS (difference in score [95% CI])	SF-36v2 MCS (difference in score [95% CI])
Male (vs female)	1.84 (1.52 to 2.16)	2.09 (1.64 to 2.54)
Age (per 10-year increment)	−0.80 (−0.95 to −0.66)	0.29 (0.09 to 0.49)
Time since T2D diagnosis (per 1-year increment)	0.00 (−0.03 to 0.03)	0.02 (−0.02 to 0.06)
HbA_{1c} at baseline (per 1% increment)	−0.01 (−0.10 to 0.09)	−0.20 (−0.32 to −0.07)
BMI (per 5-kg/m² increment)	−0.84 (−0.99 to −0.69)	−0.08 (−0.28 to 0.12)
Education level (vs 0–6 years of education)		
Secondary education (7–13 years)	0.51 (0.09 to 0.94)	0.56 (−0.01 to 1.13)
Higher education (> 13 years)	1.52 (1.05 to 1.98)	0.59 (−0.05 to 1.24)
T2D education in the past year (yes vs no)	1.38 (1.01 to 1.75)	0.86 (0.35 to 1.37)
Health insurance (yes vs no)	−0.35 (−0.80 to 0.09)	0.89 (0.29 to 1.49)
Current smoker (yes vs no)	−0.38 (−0.86 to 0.11)	−0.61 (−1.28 to 0.06)
Alcohol drinking (vs lifetime abstainer)		
Former drinker	0.03 (−0.54 to 0.61)	−0.15 (−0.92 to 0.61)
Current drinker	0.76 (0.31 to 1.22)	1.32 (0.69 to 1.95)
Medical history		
Macrovascular complications	−1.88 (−2.34 to −1.42)	−0.93 (−1.55 to −0.30)
Microvascular complications	−0.02 (−0.36 to 0.31)	1.02 (0.57 to 1.48)
Chronic kidney disease	−1.46 (−2.30 to −0.63)	1.30 (0.17 to 2.44)
First-line treatment (vs metformin)		
Sulphonylurea	−0.42 (−0.78 to −0.06)	−0.71 (−1.19 to −0.22)
DPP-4 inhibitor	0.03 (−0.65 to 0.72)	−0.32 (−1.25 to 0.62)
Sulphonylurea + DPP-4 inhibitor	−0.51 (−1.56 to 0.54)	−3.59 (−5.01 to −2.17)
Other	−0.52 (−1.24 to 0.20)	−1.75 (−2.73 to −0.77)
HFS-II subscale scores (per 5-unit increment)		
Behaviour	−0.91 (−1.01 to −0.80)	−0.93 (−1.09 to −0.77)
Worry	−0.23 (−0.33 to −0.14)	−0.66 (−0.78 to −0.53)

Table. Factors associated with changes in quality of life in patients initiating a second-line glucose-lowering therapy, as measured by the SF-36v2 PCS and MCS scores adjusted for regions according to the World Health Organization classification (data not shown; no significant associations) and all variables in the table, using multivariable regression models. BMI, body mass index; CI; confidence interval; DPP-4, dipeptidyl peptidase-4; HbA1c, glycated haemoglobin; HFS-II, Hypoglycaemia Fear Survey II; PCS, Physical Component Summary; MCS, Mental Component Summary; SF-36v2, 36-item Short-Form Health Survey version 2; T2D, type 2 diabetes.

Clinical Trial Registration Number: NCT02322762
Supported by: AstraZeneca
Disclosure: **A. Nicolucci:** Grants; Novo Nordisk, Sanofi-Aventis, Artsana, Dexcom. Honorarium; Novo Nordisk, Medtronic, AstraZeneca, Eli Lilly.

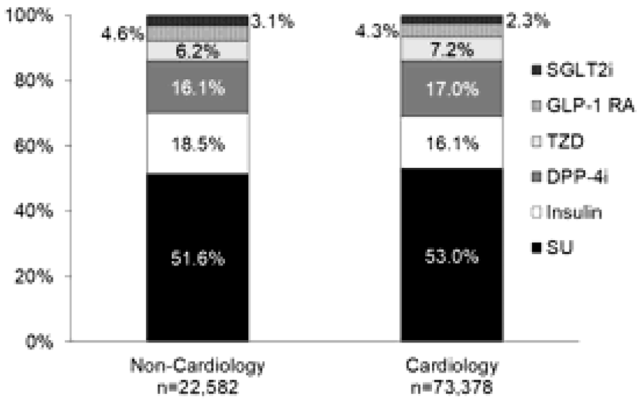
345

Second line glucose lowering treatment therapies as chosen by cardiologists vs non-cardiologists: an analysis of the Diabetes Collaborative Registry (DCR)

D. Koehn[1], E. Repetto[2], I. Brookes-Smith[2], J. Eudicone[2], K. Olsson[2], T. Schutt[3], S. Arnold[4];
[1]Internal Medicine, Virginia Commonwealth University Health System, Richmond, VA, USA, [2]AstraZeneca, Gaithersburg, MD, USA, [3]American College of Cardiology, Washington, DC, USA, [4]Cardiology, University of Missouri-Kansas City School of Medicine, Kansas City, MO, USA.

Background and aims: While metformin is the recommended first agent for diabetes, guidelines suggest a patient-centered approach to choosing second line agents according to side effect profile, weight, comorbidities, and patient preference. Given the results of recent cardiovascular outcomes trials, we sought to better understand the real-world prescribing patterns for second line therapy in type 2 diabetes (T2D) and how this may differ between cardiologists and non-cardiologists.
Materials and methods: We used data from the Diabetes Collaborative Registry (DCR), a US-based outpatient registry of patients across the spectrum of diabetes care and currently includes 11,847 providers across 47 states. Our analytic cohort included adults with T2D on metformin and 1 other glucose-lowering medication. We compared the demographics, comorbidities, and prescribed medications between patients seen by cardiology vs. non-cardiology providers.
Results: Among 95,960 adults with T2D already on metformin in DCR (76% cardiology patients), we found a predominance of sulfonylureas used as 2nd line therapies (Figure). Glucose-lowering medications with CV positive outcomes (SGLT-2i or GLP-1RA) were used less often in patients with established CVD (MI/Stroke/HF) vs in patients without CVD (5.7% vs 7.6%; $p < 0.001$). SGLT-2i were used more commonly in patients with better renal function compared to other second line combinations (GFR in SGLT2i vs other: 86.4 vs 71.8 mL/min/1.73 m^2; $p < 0.001$). GLP-1RA were used more frequently in obese patients (GLP-1RA vs other: 78% vs. 60%; $p < 0.001$). Insulin was used more often in patients with worse glycemic control (A1c in insulin vs other: 8.4 vs 7.5%; $p < 0.001$). There were no clinically relevant differences in practice patterns between cardiologists and non-cardiologists.
Conclusion: Despite greater number of patients with DM and CVD being seen by cardiologists, practice patterns for second-line therapies for patients with T2D appear to be driven more by renal function, obesity, and glycemic control as opposed to CV risk. As these patients may benefit from more targeted treatment for CV risk reduction, greater dissemination of guideline statements and educational efforts to cardiologists and non-cardiologists might optimize these decisions.

Figure. Use of Different 2nd Line Glucose-Lowering Medications by Specialty



Disclosure: **D. Koehn:** Non-financial support; AstraZeneca. Stock/Shareholding; Merck Stock, Roche Stock.

346

The characterisation of people with type 2 diabetes and polypharmacy in the Netherlands: the Diabetes Pearl cohort

F. Rutters[1], S. van Oort[1], P. Elders[1], K. Kramers[2], on behalf of the Diabetes Pearl from the Parelsnoer Initiative;
[1]VU University Medical Center, Amsterdam, Netherlands, [2]Radboud University Medical Center, Nijmegen, Netherlands.

Background and aims: Polypharmacy in people with type 2 diabetes mellitus (T2DM) is highly prevalent and a risk factor for suboptimal glycemic control. The aim of this study was to describe the prevalence of polypharmacy, as well as the subject characteristics and drug types associated with polypharmacy in the general Dutch T2DM population.
Materials and methods: The study population consisted of people with T2DM, treated in different geographical areas and all types of care, from

the Dutch Diabetes Pearl cohort. Data on drug use, as well as sociodemographic, metabolic and complication characteristics were gathered. Logistic regression analyses were performed, stratified by mild polypharmacy (5–9 drug types) and hyperpolypharmacy (≥10 drug types).

Results: We included 6447 participants (60% men, aged 62 ± 10 years). The prevalence of mild polypharmacy and hyperpolypharmacy was 48% and 19%, respectively. Compared to those with mild polypharmacy or no polypharmacy, people with hyperpolypharmacy were characterized by a higher age, female sex, lower educational level, longer diabetes duration, treatment in tertiary care, obesity, suboptimal glycemic control (HbA1c >53 mmol/mol) and more diabetes complications. The use of cardiovascular and diabetes drugs was similar in the three groups, while people with hyperpolypharmacy more often used other drugs than those with mild or no polypharmacy.

Conclusion: Hyperpolypharmacy and mild polypharmacy were highly prevalent in the general Dutch T2DM population and were associated with poorer metabolic control. As the other drugs rather than cardiovascular or diabetes drugs were causing hyperpolypharmacy, this could provide focus for development of future deprescribing guidelines in the T2DM population

Disclosure: **F. Rutters:** None.

347

Individualised HbA_{1c} targets in people with type 2 diabetes initiating second-line therapy: the global DISCOVER study

K. Khunti[1], H. Chen[2], J. Cid-Ruzafa[3], P. Fenici[4], M.B. Gomes[5], N. Hammar[6], K.F. Kennedy[7], S. Pocock[8], M.V. Shestakova[9], I. Shimomura[10], F. Surmont[11], L. Ji[12];
[1]University of Leicester, Leicester, UK, [2]AstraZeneca, Gaithersburg, MD, USA, [3]Evidera, Barcelona, Spain, [4]AstraZeneca, Cambridge, UK, [5]Rio de Janeiro State University, Rio de Janeiro, Brazil, [6]AstraZeneca Gothenburg, Mölndal, Sweden, [7]Saint Luke's Mid America Heart Institute, Kansas City, MO, USA, [8]London School of Hygiene and Tropical Medicine, London, UK, [9]Endocrinology Research Center, Diabetes Institute, Moscow, Russian Federation, [10]Osaka University, Osaka, Japan, [11]AstraZeneca, Luton, UK, [12]Peking University People's Hospital, Beijing, China.

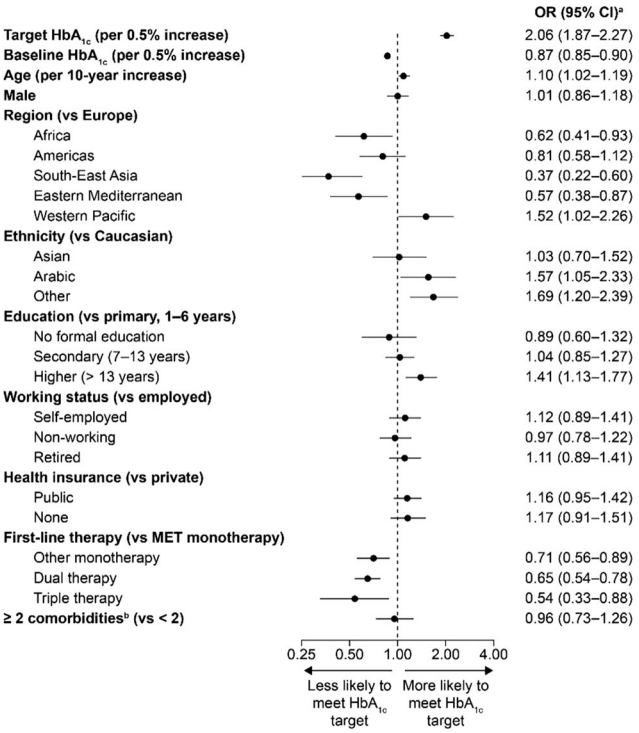
Background and aims: International guidelines recommend setting individualized HbA_{1c} targets for people with type 2 diabetes (T2D). DISCOVER is an observational study of patients with T2D initiating second-line glucose-lowering therapy in 37 countries. We report the proportion of patients set individualized targets, the proportion meeting these targets after 1 year, and factors associated with meeting these targets.

Materials and methods: Patients included had HbA_{1c} data at baseline and at 1 year. Factors associated with meeting targets were assessed using multivariable logistic regression.

Results: Of the 7225 patients with complete HbA_{1c} data who remained in the study after 1 year, 5070 (70.2%) had been set an individualized glycaemic control target. Targets were 7.0% for 2513 patients (49.6%), <7.0% for 2073 patients, (40.9%) and >7.0% for 484 patients (9.6%). Overall, 1744 of these patients (34.4%) met their target after 12 months of follow-up (range across regions: 21.4–42.7%). Factors associated with meeting HbA_{1c} targets are shown in the Figure.

Conclusion: Twelve months after initiating second-line therapy, only one third of patients with set individualized HbA_{1c} targets had met their targets, with considerable variation between regions. Older age, higher education level, lower baseline HbA_{1c} level and higher target were factors associated with meeting targets.

Figure. Factors associated with meeting individualized HbA_{1c} targets.



CI, confidence interval; MET, metformin; OR, odds ratio.
[a]OR adjusted for all variables shown in the figure.
[b]Cormorbidities: heart failure, angina, myocardial infarction, stroke, transient ischemic attack, peripheral artery disease, atrial fibrillation, ventricular arrhythmia, severe valve disease, respiratory disease, depression, dementia, sleep apnea, thyroid disease.

Clinical Trial Registration Number: NCT02322762
Supported by: AstraZeneca

Disclosure: **K. Khunti:** Grants; AstraZeneca, Boehringer Ingelheim, Lilly, Merck Sharpe & Dohme, Novartis, Novo Nordisk, Roche, Sanofi, National Institute for Health Research Collaboration for Leadership in Applied Health Research and Care – East Midlands (NIHR CLAHRC – EM). Honorarium; AstraZeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck Sharpe & Dohme, Novartis, Novo Nordisk, Roche, Sanofi.

348

Treatment thresholds for patients with newly diagnosed diabetes: an application of dynamic marginal structural models in the Clinical Practice Research Datalink

R.E. Farmer[1], D. Ford[2], L. Smeeth[1], N. Chaturvedi[3], K. Bhaskaran[1];
[1]Non Communicable Diseases Epidemiology, London School of Hygiene and Tropical Medicine, London, UK, [2]Medical Research Council Clinical Trials Unit at University College London, London, UK, [3]Institute of Cardiovascular Science, University College London, London, UK.

Background and aims: Current guidelines for newly diagnosed type 2 diabetes (T2DM) advocate treatment initation once HbA_{1c} exceeds 6.5%. However, whether this threshold is optimal for minimising adverse clinical endpoints is unclear. There is a lack of trial data examining differing treatment thresholds, but using large-scale routinely collected observational data sources, people initiating at different thresholds can be compared. A naïve comparison would likely be confounded by time-varying factors that cannot be handled by standard statistical models, but dynamic marginal structural models (dMSMs) use weighting to appropriately

remove such confounding. We used this methodology to compare the effects of different HbA_{1c} thresholds for treatment initiation, on time to subsequent target HbA_{1c}, myocardial infarction (MI), stroke and all-cause mortality.

Materials and methods: A cohort of adults with newly diagnosed T2DM were identified from the Clinical Practice Research Datalink. dMSMs were fitted to compare strategies of initiating treatment at HbA_{1c} thresholds between 6.5% and 10%: each patient's follow up was duplicated for each of the strategies of interest, censoring once the patient became non-compliant with the given strategy. Specifically, follow-up was censored if they initiated treatment below the given threshold, or if they did not initiate within one month of exceeding the given threshold. Patients were considered at risk until the earliest of 10 years; the event of interest; death; administrative censoring; or initiation of any other therapy than metformin or sulfonylureas. Inverse probability of censoring weights were then used to adjust for informative censoring. The association between treatment strategy and outcome was evaluated within the weighted population using pooled logistic regression to approximate a Cox model.

Results: 47,950 patients with incident T2DM were included (median follow up 4.7 years). Initiation at higher thresholds of HbA_{1c} was associated with lower rates of reaching target HbA_{1c} of 6.5%. The estimated proportion of patients reaching target by 1 year were 0.36 (0.35, 0.38), 0.32 (0.31–0.32), 0.30 (0.30–0.31) and 0.30 (0.30–0.31) for thresholds of 7, 8, 9 and 10% respectively. Higher thresholds of initiation showed a trend for increased risk of MI, though observed incidence of MI was low and CI's overlapped (4 year cumulative incidence (%) of 1.13 (0.85–1.44), 1.16 (0.96–1.41), 1.41 (1.17–1.68), 1.52 (1.25–1.79) and 1.53 (1.31–1.85) for thresholds of 6.5, 7 , 8 , 9 and 10% respectively). Longer term differences in MI incidence between strategies had the same pattern but lacked precision. There was no evidence of differences in cumulative incidence of stroke or all-cause mortality at 4 years. Cumulative incidence curves to 10 years also showed no long term differences between strategies.

Conclusion: This analysis suggests a benefit of early intervention in terms of subsequent glucose control and risk of MI. A limitation of the study was limited follow up and low overall observed cardiovascular events. dMSMs are a useful tool for examining clinically important treatment strategy questions using routinely collected medical records.

Supported by: Funded by the MRC London Hub for clinical trials methodology research

Disclosure: **R.E. Farmer:** None.

349

Impact of treatment intensification in patients with type 2 diabetes suboptimally controlled on basal insulin in The Health Improvement Network UK primary care database

E. Lew[1], M. Myland[2], M. Nixon[2], N. Gooch[3], C. O'Leary[2], A. Shaunik[4], E.B. Jude[5];
[1]Sanofi, Chilly-Mazarin Cedex, France, [2]IQVIA, London, UK, [3]Sanofi, Guildford, UK, [4]Sanofi, Bridgewater, NJ, USA, [5]Diabetes Center, Tameside General Hospital, Ashton-under-Lyne, UK.

Background and aims: Despite an abundance of antidiabetes therapies and guidelines, treatment intensification is often delayed in patients with suboptimal glycaemic control. The aim of this study was to assess the effect of treatment intensification on change from baseline HbA_{1c} in patients with type 2 diabetes (T2D) suboptimally controlled on basal insulin (BI).

Materials and methods: Patients with T2D and uncontrolled HbA_{1c} (>8.0% high-risk, >7.5% otherwise) prescribed BI between 1 January 2005 and 16 May 2017 were identified in The Health Improvement Network (THIN) database. Treatment intensification (Intensifiers) was defined as the addition of a glucagon-like peptide-1 receptor agonist (GLP-1 RA) or rapid-acting insulin (RAI) within 12 months of uncontrolled HbA_{1c} (Index). Intensifiers were matched 1:1 on key demographics to patients who did not intensify treatment within 12 months of Index (Non-intensifiers). The difference between baseline and earliest recorded HbA_{1c}, greatest change, and proportion of patients achieving target HbA_{1c} in the 12 months post intensification were calculated. Descriptive statistics and mixed models repeated measures were used to compare HbA_{1c} changes between Intensifiers and Non-intensifiers. Adjusted logistic regression was used to compare proportions of patients achieving target HbA_{1c}.

Results: The study included 1,342 BI users (646 Intensifiers; 696 Non-intensifiers). In the Intensifier group, 8.5% added a GLP-1 RA and 91.5% a RAI to BI therapy. Baseline characteristics and outcomes in the 12 months post intensification are shown in **Table 1**. In the 12 months post intensification, the magnitude of reduction for the earliest change in HbA_{1c} for Intensifiers was twice that for Non-intensifiers; this difference was statistically significant (mean −0.54% versus −0.26%, respectively; adjusted $P = 0.0004$). Greatest HbA_{1c} change in the 12 months post intensification was also more substantial for Intensifiers compared with Non-intensifiers (mean −0.81% versus −0.49%, respectively; adjusted $P = 0.0077$). In addition, a greater (and statistically significantly) proportion of Intensifiers achieved 12-month target HbA_{1c} compared with Non-intensifiers (33% Intensifiers vs. 28% Non-intensifiers; $P < 0.0001$).

Conclusion: Our study shows that timely intensification of BI therapy in patients with T2D and uncontrolled HbA_{1c} provided clinically meaningful glycaemic reductions within 12 months post intensification. In addition, more patients achieved target HbA_{1c} after intensifying antidiabetes therapy; however, the proportions were small, which may be a reflection of high HbA_{1c}. Our results emphasise the need for improved therapeutic options to more effectively manage glycaemic control.

Patient characteristics	Intensifiers (n = 646)	Non-intensifiers (n = 696)
Age at baseline, years, mean (SD)	58.3 (12.9)	59.0 (13.3)
Male, n (%)	343 (53.1)	375 (53.9)
BMI, kg/m², mean (SD) [n]	31.8 (7.3) [436]	32.1 (7.4) [463]
Diabetes diagnosis duration, n (%)		
< 5 years	51 (7.9)	56 (8.0)
5-9 years	200 (31.0)	233 (33.5)
≥ 10 years	373 (57.7)	407 (58.5)
Comorbidities, n (%)		
Hypertension	63 (9.8)	74 (10.6)
Dyslipidaemia	8 (1.2)	15 (2.2)
Obesity[a]	238 (36.8)	262 (37.6)
Baseline HbA_{1c} , %, mean (SD) [n]	9.38 (1.45)	9.40 (1.57)
Earliest change in HbA_{1c}, 12 months post intensification		
Mean (SD)	−0.54 (1.47)	−0.26 (1.55)
Estimated treatment difference (95% CI)[b]	−0.38 (−0.59, −0.17)*	
Greatest change in HbA_{1c}, 12 months post intensification		
Mean (SD)	−0.81 (1.99)	−0.49 (2.13)
Estimated treatment difference (95% CI)[b]	−0.32 (−0.55, −0.08)	

* $P = 0.0004$. [a] Including baseline BMI > 30 kg/m². [b] Adjusted treatment difference in HbA_{1c} change using mixed models repeated measures. Bonferroni adjustments were made ensuring $P < 0.001$ indicates a statistically significant finding.

Supported by: This study was sponsored by Sanofi.

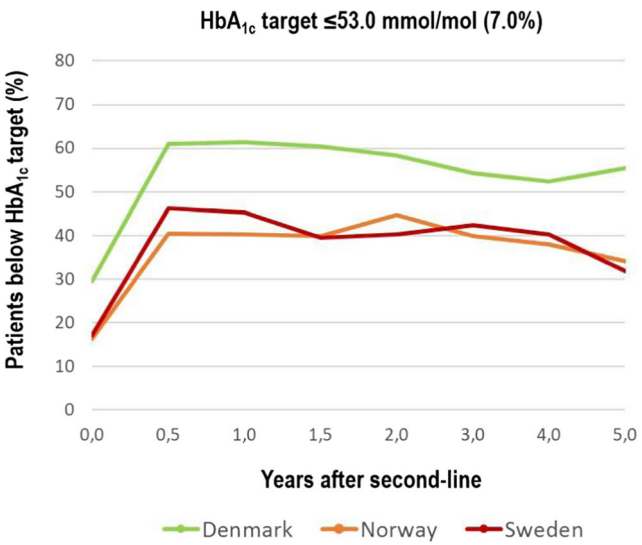
Disclosure: **E. Lew:** Employment/Consultancy; Sanofi.

350

Proportion of patients reaching HbA_{1c} targets related to second-line treatment initiation: a Nordic observational study comparing type 2 diabetes management in primary care

F. Persson[1], J. Bodegard[2], S.T. Knudsen[3], H.L. Gulseth[4], K. Furuseth[5], M. Thuresson[6], A. Lindh[7], M. Alvarsson[8], M.E. Jørgensen[1], J. Søndergaard[9], K.I. Birkeland[4], P.M. Nilsson[10];
[1]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [2]AstraZeneca Nordic-Baltic, Oslo, Norway, [3]Aarhus University, Aarhus, Denmark, [4]Oslo University Hospital, Oslo, Norway, [5]Solli Klinikk, Jessheim, Norway, [6]Statisticon AB, Uppsala, Sweden, [7]., Åkersberga, Sweden, [8]Karolinska University Hospital, Stockholm, Sweden, [9]University of Southern Denmark, Odense, Denmark, [10]Lunds University, Lund, Sweden.

Background and aims: Second line treatment with glucose lowering drugs (GLD) is an important part of type 2 diabetes (T2D) management. Previous research has shown that the Nordic countries differ with respect to proactivity when initiating second-line treatment, despite that guidelines argue for early intervention of uncontrolled HbA_{1c}. The aim of this study was to describe proportion of patients successfully below HbA_{1c} target levels below 47.5 mmol/mol (DCCT 6.5%), 53 (7.0) and 58.5 (7.5) at initiation of second-line and up to 5 years after using data from Denmark (DK), Norway (NO) and Sweden (SE).
Materials and methods: Electronic medical records (EMR) data on T2D patients was extracted from 60 primary care clinics in DK, NO and SE, and linked with national Prescribed Drug-, National Patient- and Cause of Death Registry data in respective country. Second line treatment (index date) was defined as dispense of new GLD class after ≥6 months metformin monotherapy.
Results: Between 2010–2015, 2861 patients were identified in DK, NO and SE; 646, 635 and 1580 patients, respectively. Mean age 60, 62 and 64 years; females 42, 42 and 39%; established CVD 19, 21 and 26%; and chronic kidney disease 1, 4 and 3%, respectively. Use of sulphonylurea and insulin as second line treatment was 2-fold greater in SE compared with NO and DK. In 2015, the greatest initiation of either DPP-4i, SGLT-2i or GLP-1a was observed in DK (70%) and NO (75%) compared to SE (48%). At index date, DK had the lowest HbA_{1c} (61.7 mmol/mol, 95%CI [59.6–62.8]) compared to NO (67.2 [65.0–68.3]) and SE (66.1 [63.9–67.2]). In DK, initiation of second-line treatment showed the greatest proportion with HbA_{1c} below all targets compared to NO and SE (Figure). During follow-up, the proportion of patients below targets was also greatest in DK compared to the other countries. Norway and Sweden demonstrated similar target patterns.
Conclusion: Despite similar demographics and health care systems in three Nordic countries, we have shown marked differences in drug treatment patterns and HbA1c target strategies related to second-line treatment. In Denmark, second-line treatment was initiated earlier, i.e. in patients with lower mean HbA_{1c}, which also resulted in an observed better glycaemic control over the next two years compared to Norway. These observations may indicate a more proactive disease management in the included general practices in Denmark in a primary care setting compared to the other countries.



Supported by: AstraZeneca
Disclosure: **F. Persson:** Honorarium; AstraZeneca. Lecture/other fees; AstraZeneca.

351

Initiation of combination therapy in type 2 diabetes patients with high HbA_{1c} at diagnosis

R.M. Klok[1], A. Kumar[2], S. Rajpathak[1], A.Z. Fu[2,3], K. Khunti[4];
[1]Merck & Co., Inc., Kenilworth, NJ, USA, [2]Complete HEOR Solutions (CHEORS), North Wales, PA, USA, [3]Georgetown University Medical Center, Washington, DC, USA, [4]Diabetes Research Centre, University of Leicester, Leicester, UK.

Background and aims: Clinical guidelines for type 2 Diabetes (T2DM) recommend that patients with high HbA_{1c} (≥HbA_{1c} of 9.0%) at the time of diagnosis, should be initiated with a combination therapy. In this study we evaluated the proportion of patients that start with a combination therapy in this patient population.
Materials and methods: A retrospective study of adult T2DM patients using the UK Clinical Practice Research Datalink (CPRD). The study population was T2DM patients aged 18 years and older, with a first prescription of an antidiabetic agent (index date) from January 2010-December 2015. Patients with continuous enrolment in the database one year prior to the index date, having an HbA_{1c} lab value between 3 months before to 2 weeks after index date were included. Patients were excluded if they had type 1 diabetes mellitus, other forms of secondary diabetes, gestational diabetes mellitus, or had taken any of the antidiabetic drugs in the baseline period. Patients were classified as initiated on combination therapy if they received a prescription for 2 or more different antidiabetic agents in the first month post index date, and both drug classes had at least 1 follow-up prescription after the initial prescription.
Results: There were totally 19,409 eligible patients initiated on monotherapy or combination therapy, among which 42.5% were female. Their median age was 61 years and a median baseline HbA_{1c} 8.2%. Of these new users, 38.7% had an initial HbA_{1c} ≥9.0%, of whom 14.5% were initiated on a combination therapy, while 85.5% were initiated on mono-therapy.
Conclusion: Despite clinical guidelines only 14.5% of newly diagnosed patients with T2DM and an HbA_{1c} of ≥9.0% are initiated on a combination therapy.
Disclosure: **R.M. Klok:** Employment/Consultancy; Merck & Co., Inc., Kenilworth, NJ, USA. Stock/Shareholding; Merck & Co., Inc., Kenilworth, NJ, USA.

PS 012 Diabetes mortality

352

Microvascular disease and all-cause mortality in a single-center cohort of individuals with type 1 diabetes: a 10-year follow-up study

M. Garofolo, R. Giannarelli, M. Aragona, D. Lucchesi, L. Giusti, V. Sancho Bornez, G. Daniele, R. Miccoli, G. Penno, S. Del Prato;
Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

Background and aims: The incidence rate of type 1 diabetes (T1DM) is increasing worldwide. Despite improvements, T1DM continues to be associated with a sustantially increased risk of cardiovascular (CV) events and premature death. The effect of microvascular disease (MD) burden on all-cause mortality in T1DM is still poorly explored.
Materials and methods: The relationship between the cumulative burden of retinopathy, nephropathy and peripheral neuropathy (diabetic microvascular triopathy) and all-cause mortality was evaluated in 774 T1DM recruited in an observational, single-center study (age 40.2 ± 11.7 years; BMI 24.8 ± 3.6 kg/m^2; diabetes duration (DD) 19.4 ± 12.2 years; HbA1c 7.8 ± 1.2%) over a mean follow-up of 10.7 ± 2.5 years. Hazard ratios for the risk of all-cause mortality associated with MD was calculated by unadjusted and adjusted Cox regression. EURODIAB PCS risk score was calculated for all patients.
Results: Out of 774 T1DM 425 (54.9%) had no MD, 250 had 1 MD (32.3%); 75 had 2 MD (9.7%) and: 24 had 3 MD (3.1%). Distribution was unchanged after esclusion of 41 T1DM (5.3%) with prior CV events (57.0%, 32.2%, 8.5% and 2.3%, respectively). Compared to no-MD, 1–3 MD had an adverse CV risk profile with steeply increase in age, DD, BMI, WHR, sBP, dBP, HbA1c, uric acid, and EURODIAB PCS risk score for major vascular outcomes ($p < 0.0001$); differences were observed for total- and LDL-cholesterol, and triglycerides as well ($p < 0.05$). Genders and smoking habits (in particular current smokers) were equally distributed between groups. As expected, eGFR (CKD-EPI) decreased and albuminuria progressively increased ($p < 0.0001$). Rates of CV events and EURODIAB PCS risk score ≥20 increased with MD: 1.6%, 5.6%, 17.3%, and 29.2%; 4.0%, 14.4%, 41.3% and 79.2%, respectively ($p < 0.0001$). As expected, number of subjects on BP-lowering agents, RAS-blockers, statins and anti-platelet drugs increased wirh MD number ($p < 0.0001$). A total of 52 deaths occurred over a 8,184 person-years follow-up (6.7%; 6.36 ×1000 person-years). Death rate increased with number of MD: no-MD 1.9%; 1 MD, 6.8% (HR: 3.75, 95%CI 1.62–8.69); 2 MD, 14.7% (HR: 7.10, 2.85–17.67); 3 MD 66.7% (HR: 45.64, 19.50–106.79; $p < 0.0001$). Death rate did not change after exclusion of the 41 subjects with prior CV events (1.9%, 6.4%, 12.9%, and 64.7%; $p < 0.0001$). After adjustment for age and sex, HRs were: 1 MD: 2.61, 1.11–6.14; 2 MD: 3.42, 1.29–9.06; 3 MD: 16.21, 6.20–42.35 ($p < 0.0001$). In fully adjusted model, HRs were: 1 MD: 2.51, 95%CI 1.01–6.23; 2 MD: 2.97, 1.07–8.25; 3 MD 9.68, 3.19–29.36; ($p = 0.001$), with independent effects for age (HR 1.06; 95%CI 1.04–1.09), uric acid (1.37; 1.15–1.64), and smoking (2.45; 1.28–4.70). In a different fully adjusted model including the EURODIAB risk score, the MD burden as well the EURODIAB score were strong and independent predictors of all-cause mortality.
Conclusion: The development of microvascular disease increases in a dose-dependent manner the risk of all-cause mortality in type 1 diabetes individuals. This effect is independent of conventional cardiovascular risk factors and risk score for major vascular outcomes. In conclusion, presence and number of microvascular complications should be considered in stratifying cardiovascular risk in type 1 diabetes.
Disclosure: **M. Garofolo:** None.

353

Diabetes increases the mortality in myocardial infarction, heart failure and stroke: results from a longitudinal study over 40 years

E. Lampa[1], J. Ärnlöv[2], J. Sundström[3], U. Risérus[4], L. Lind[3];
[1]Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden, [2]Dept of Neurobiology, Care Sciences and Society, Karolinska Institutet, Huddinge, Sweden, [3]Department of Medical Sciences, Uppsala University, Uppsala, Sweden, [4]Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden.

Background and aims: It is well known that diabetes increase the risk of the main cardiovascular diseases, myocardial infarction (MI), ischemic stroke or congestive heart failure (HF), as well as mortality. Since it is less known how diabetes influences mortality risk once a diabetic individual has obtained a main cardiovascular diagnosis, we investigated this using data from a 40-year longitudinal study.
Materials and methods: In the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort, participants were physically examined at 50 ($n = 2322$), 60 ($n = 1860$), 70 ($n = 1221$), 77 ($n = 839$) and 82 ($n = 530$) years of age. Hospital diagnoses on diabetes, acute myocardial infarction (MI), ischemic stroke or congestive heart failure (HF) and death were obtained during 40 years of follow-up. A multi-state model was used to model transition rates between the different cardiovascular states and mortality as a function of the covariates. The model was adjusted for blood pressure, LDL and HDL cholesterol, triglycerides, smoking, diabetes duration and use of antihypertensive and lipid lowering medication.
Results: During a median of 31 years follow-up (interquartile range 22–38 years, max 43 years) 458 MI events, 331 stroke events and 177 HF events were observed as the first event. 1916 deaths occurred of which 1109 occurred without prior CV event. Diabetes ($n = 360$) was associated with an increased risk for a first CV event at 60 years of age (Rate Ratio (RR) = 1.76 (95% CI: 1.05–2.94) for MI, 1.61 (0.74–3.51) for stroke and 2.94 (1.10–7.83) for HF). The association with HF was attenuated, but still relevant, as age increased (RR = 1.95 (1.25–3.06) at age 82. The associations for MI and stroke were almost unchanged at age 82; RR = 1.93 (1.20–3.10) for MI and 1.86 (1.21–2.87) for stroke. Diabetes was also associated with death at higher age; RR = 1.87 (1.51–2.32) at age 82. In individuals experiencing a CV event, diabetes was associated with increased mortality; RR = 4.48 (2.17–9.24) at age 60 to 1.30 (0.87–1.94) at age 82 following an MI and RR = 2.81 (1.11–7.14) at age 60 to 1.60 (1.13–2.26) following a HF. The association between diabetes and death following a stroke was less conclusive; RR = 1.58 (0.62–4.05) at age 60 to RR = 1.79 (1.20–2.69) at age 82.
Conclusion: Diabetes was associated with a first main cardiovascular event and mortality both in middle-age and in the elderly. Diabetes was also associated with an increased mortality risk following a cardiovascular event, especially in heart failure, middle-aged individuals with a myocardial infarction and elderly with stroke.
Disclosure: **E. Lampa:** None.

354

Decreasing trend in years of life lost due to diabetes: a nationwide cohort study

L. Niskanen[1], T. Partonen[2], A. Auvinen[3], J. Haukka[4], CARING Project;
[1]Endocrinology and Diabetology, Helsinki University Hospital, Helsinki, Finland, [2]Public Health Solutions, National Institute for Health and Welfare, Helsinki, Finland, [3]Epidemiology and Health Sciences, University of Tampere, Tampere, Finland, [4]Public Health, University of Helsinki, Helsinki, Finland.

Background and aims: We analyzed all-cause mortality and mortality from main causes of death in a large national cohort.
Materials and methods: Nationwide cohort of patients with diabetes (DM): individuals who received reimbursement for ≥1 prescription between 1.1.1997–31.12.2010 and sex, age and area-matched reference (Ref) group. All the insulin users were included and 50% random sample of the OAD (oral antidiabetic drug) users. DM-groups were divided as OAD only, OAD + insulin or insulin only. The prescription, cancer and mortality data was obtained by register linkages from national registries.

Follow-up started on the date of the first purchase of diabetes medication prescription, till 31.12.2012 or the date of death. End-points were death from any cause, CHD, cerebrovascular diseases (CVD), and cancer. The cohort data were analyzed using Poisson regression models separately for each end-point and reported as mortality rate ratios (MRR). Competing risks of four causes of death were analyzed by competing risks regression. Years of life lost (YLL) were calculated as the difference of the areas under the survival curves.

Results: The study population consisted of 434,629 individuals (226,372 men). The mean follow-up time was 6.9 years. We observed total 85,180 deaths (23,236 from IHD, 7671 from CVD, and 19,765 from cancer). The mortality rates were elevated among the patients with diabetes. Mortality was highest on those with OAD+insulin, followed by insulin only, and lowest in subjects with OAD only. CVD mortality was significantly higher in women than in men. Unadjusted survival was clearly lower in all diabetes groups compared to the reference population. In the OAD group, survival was substantially higher than in the OAD+insulin and to the only-insulin groups. When comparing the OAD+insulin and the insulin only groups, survival was higher in the former for about five years after start of the follow-up, but reversed after that. Proportions of different causes of death remained similar. We detected a significant interaction between start of the follow-up year and diabetes group in Poisson regression models for all-cause mortality, as well as cause-specific mortalities. All-cause mortality in the OAD group approached that of the reference population by 2009. A similar trend was observed for CHD mortality, but not for cancer mortality or CVD mortality. Estimated YLL diminished considerably in the OAD group, with smaller decreases in the other medication groups.

Conclusion: Difference in mortality between the OAD group and the reference population in Finland has decreased in the 14-year period from 1997 to 2010.

Supported by: EU Grant 282526

Disclosure: **L. Niskanen:** None.

355

Association between postprandial hyperglycaemia at clinic visits and cancer mortality in patents with type 2 diabetes

T. Takao[1], K. Takahashi[1], K. Kimura[1], M. Suka[2], H. Yanagisawa[2], Y. Iwamoto[1];
[1]The Institute for Adult Diseases Asahi Life Foundation, Tokyo, Japan, [2]The Jikei University School of Medicine, Tokyo, Japan.

Background and aims: There is evidence that patients with diabetes have an increased risk of several site-specific cancers and a high rate of cancer mortality. Acute glucose excursions rather than sustained hyperglycemia trigger oxidative stress. Oxidative stress generates DNA damage, increased inflammation, and vascular endothelial damage, which may contribute to cancer progression and atherosclerosis. However, few studies have examined the relationship between postprandial hyperglycemia and cancer mortality in patients with type 2 diabetes. Thus, we aimed to evaluate the impact of postprandial hyperglycemia at clinic visits on all-cause and cancer mortality in patients with type 2 diabetes independent of the HbA_{1c} level in a real-world setting.

Materials and methods: Blood glucose (BG) and HbA_{1c} levels were measured at each clinic visit. Postprandial time intervals were calculated and classified, according to 15-min units, by a medical technologist. BG levels at 2 h ± 30 min after breakfast were defined as 2-h post-breakfast BG (2h-PBBG). The intrapersonal mean values of 2h-PBBG, HbA_{1c}, and other clinical data during the 3 years after the first visit were used as baseline data. This retrospective observational cohort study included 1089 patients with type 2 diabetes who first visited our clinic between 1995 and 1998, had been followed up for at least 3 years, and had undergone 2h-PBBG measurements during the initial 3 years. They were followed up through 2017, and then questionnaires were mailed. The association between the intrapersonal mean 2h-PBBG during the initial 3 years and death from all causes and cancer were determined by multivariate Cox regression analysis. SAS software (version 9.4) was used.

Results: During follow-up, 167 patients died. The cause was cancer in 57 and cardiovascular disease in 47 patients. The overall follow-up rate was 73.3% (798/1089). During the 3 years in which baseline values were established, the median (interquartile range) number of 2h-PBBG measurements was 3 (2–7) and the median follow-up period was 16.7 (10.8–18.2) years. At baseline, the mean patient age was 58.4 years; 81% were male, and 42% were smokers. Cox regression analysis showed the mean 2h-PBBG significantly predicted all-cause and cancer mortality, after adjusting for mean HbA_{1c}, number of 2h-PBBG measurements (ln-transformed), age, sex, and smoking status. In the model in which the duration of diabetes, mean BMI, mean systolic BP, and mean total cholesterol/HDL cholesterol were added, similar results were obtained (Table). Using a stepwise method adjusted for mean HbA_{1c} and the number of 2h-PBBG measurements, the results were the same.

Conclusion: Postprandial hyperglycemia at clinic visits is associated with all-cause and cancer mortality in type 2 diabetes patients, independent of the HbA_{1c} level. Further studies are warranted to confirm our findings.

Table Multivariate Cox proportional hazard models for mortality from all-causes and cancer.

	All-cause mortality (Event/Patients 167/1089)				Cancer mortality (Event/Patients 57/1089)			
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Mean 2h-PBBG (1 mmol/l)	1.07 (1.02–1.13)	0.007	1.07 (1.01–1.12)	0.016	1.09 (1.01–1.18)	0.024	1.09 (1.01–1.18)	0.032
Mean HbA1c (%)	1.29 (1.03–1.61)	0.025	1.21 (0.95–1.53)	0.12	1.18 (0.81–1.73)	0.39	1.17 (0.79–1.74)	0.44
Number of 2h-PBBG measurements (ln-transformed)	1.11 (0.95–1.30)	0.19	1.11 (0.95–1.29)	0.21	1.02 (0.78–1.34)	0.87	1.02 (0.78–1.34)	0.87
Age (10 years)	3.74 (3.07–4.56)	<0.0001	3.55 (2.89–4.37)	<0.0001	2.93 (2.09–4.11)	<0.0001	2.73 (1.91–3.90)	<0.0001
Women/Men	0.81 (0.53–1.24)	0.33	0.80 (0.52–1.24)	0.32	1.17 (0.51–2.66)	0.71	1.16 (0.50–2.70)	0.72
Current smoker	1.56 (1.10–2.20)	0.013	1.57 (1.10–2.24)	0.012	3.62 (1.92–6.84)	<0.0001	3.75 (1.96–7.17)	<0.0001
Diabetes duration (5 years)	—	—	1.08 (0.98–1.19)	0.11	—	—	1.09 (0.91–1.30)	0.36
Mean BMI (kg/m^2)	—	—	0.99 (0.93–1.05)	0.70	—	—	1.02 (0.92–1.13)	0.73
Mean SBP (10 mmHg)	—	—	1.10 (0.99–1.23)	0.08	—	—	1.10 (0.91–1.32)	0.34
Mean TC/HDL-C	—	—	1.06 (0.91–1.24)	0.47	—	—	0.79 (0.60–1.04)	0.10

Disclosure: **T. Takao:** None.

356

Socioeconomic status and mortality risk among patients with hypertension and diabetes: a cohort study from the Swedish Primary Care Cardiovascular Database (SPCCD)

T. Andersson[1,2], M. Pikkemaat[3], L. Schiöler[2], P. Hjerpe[4], A.C. Carlsson[5], P. Wändell[5], K. Manhem[6], T. Kahan[7], J. Hasselström[5], K. Bengtsson Boström[4];
[1]Närhälsan Norrmalm Health Centre, Skövde, Sweden, [2]Department of Public Health and Community Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [3]Department of Clinical Sciences, Lund University, Malmö, Sweden, [4]Närhälsan R&D Centre Skaraborg Primary Care, Skövde, Sweden, [5]Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Huddinge, Sweden, [6]Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [7]Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden.

Background and aims: Hypertension and diabetes are important risk factors for cardiovascular complications and premature death, often coexist, and are mostly managed in primary care. No studies with a primary care setting seem to have estimated the mortality risk among hypertensive patients who develop diabetes, and how this risk is associated with socioeconomic status. Thus, the aim of the study was to estimate mortality risk among hypertensive patients with diabetes (DM+) versus without diabetes (DM-), and how the risk associated with level of income, education, and country of birth.

Materials and methods: The SPCCD is an observational database of patients diagnosed with hypertension in primary care 2001–2008. The

medical records have been linked with five national population-based registers. The current study cohort consisted of 66 659 patients, of whom 10 577 were subsequently diagnosed with predominantly type 2 diabetes. Patients were included at the date of the first registration of hypertension, and were followed until end of study (31 December, 2012) or date of death, retrieved from the Cause of Death Register. Mortality hazard ratios (HR) were calculated by Cox regression models with diabetes as a time-updated variable, and were adjusted for quintiles of income, level of education, country of birth, sex, attained age, cardiovascular comorbidity, cancer, smoking, BMI, BP, lipids, and creatinine. Missing data was handled by multiple imputation.

Results: During 455 443 person-years 12 014 deaths (26.4/1000 person-years, 95% CI 25.9–26.9) occurred among DM- and 2 927 deaths occurred during 68 675 person-years (42.6/1000 person-years, 41.1–44.2) among DM+. The overall adjusted HR for mortality was 1.56, 1.49–1.63, for DM+, as compared to DM-. Low versus high income was associated with increased adjusted mortality risk (reference DM- highest income quintile: HR 1.00; DM+ highest income quintile: HR 1.96, 1.64–2.34; DM- lowest income quintile: HR 2.59, 2.33–2.87; DM+ lowest income quintile: HR 3.84, 3.41–4.32). Level of education was not associated with adjusted mortality risk among either DM+ or DM-. Compared to Sweden as country of birth, the adjusted mortality risk was increased for Finland among DM- (HR 1.25, 1.14–1.36) but not among DM+ (HR 1.09, 0.92–1.29) and decreased for country of birth outside Europe (DM-: HR 0.66, 0.57–0.77; DM+: HR 0.60, 0.48–0.75).

Conclusion: Adding diabetes to hypertension was associated with 56% excess mortality risk in primary care patients. The mortality risk was 3.8 and 2.6-fold increased among hypertensive patients in the lowest income quintile with and without diabetes, as compared to patients with hypertension in the highest quintile. Risk of mortality varied with country of birth.

Supported by: The Skaraborg Institute R&D, Närhälsan R&D Skaraborg

Disclosure: **T. Andersson:** None.

357

Thirty-day mortality following admission with lactic acidosis in diabetes patients using metformin: a Danish nationwide study

A. Aharaz[1], R.W. Thomsen[2], K. Berencsi[2], T. Frøslev[2], H. Beck-Nielsen[1,3];
[1]Dpt. of Endocrinology, Odense University Hospital, Odense, Denmark, [2]Dpt. of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark, [3]The Danish Centre for Strategic Research in Type 2 Diabetes (DD2), Department of Endocrinology,, Odense University Hospital, Odense, Denmark.

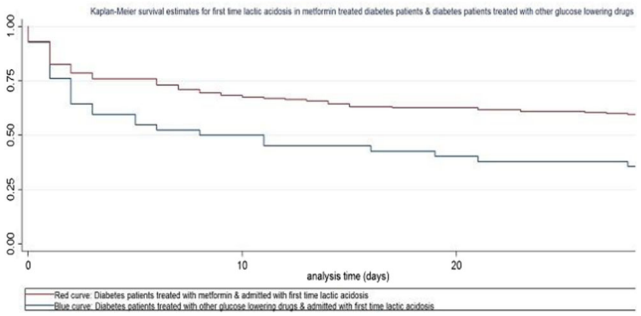
Background and aims: Few population-based data are available on the prognosis of lactic acidosis associated with diabetes treatment. We examined seven-day and 30-day mortality following hospital admission with lactic acidosis associated with metformin and other glucose-lowering drug (GLD) use in 2004–2012.

Materials and methods: We linked three different Danish population-based registers to obtain data for this study: The Civil Registration System, The National Patient Register and The National Prescription Register. We identified 233 lactic acidosis admissions in GLD users, of which 191 events were in metformin-treated individuals and 42 in users of other GLDs. We compared mortality following lactic acidosis in metformin and other GLD users using Kaplan-Meier survival estimates, and using Cox regression analysis to adjust for differences in age, sex, renal disease and other comorbidity.

Results: We identified 233 lactic acidosis admissions in GLD users, of which 191 events were in metformin-treated individuals and 42 in users of other GLDs.Among metformin-treated patients with lactic acidosis, 77 of 191 (40.3%) died within the first 30 days of admission, whereas in patients using other GLDs admitted with lactic acidosis, 27 out of 42 (64.3%) died. After controlling for potential confounders, seven-day and 30-day mortality for metformin-associated lactic acidosis was lower than for lactic acidosis associated with other GLDs: adjusted seven-day hazard ratio (HR) = 0.50 (95% confidence interval (CI) 0.28–0.87) and adjusted 30-day HR = 0.51 (95% CI 0.31–0.81). A lower lactic acidosis mortality associated with metformin than other GLD use was consistently found in all subgroups regardless of age, sex, presence or absence of comorbidity or renal disease.

Conclusion: Mortality 30 days after admission with lactic acidosis is high among metformin users in a population-based setting, yet prognosis is even worse in users of other GLDs.

Figure 1: Kaplan-Meier estimates for survival in diabetes patients admitted with their first time lactic acidosis



Clinical Trial Registration Number: jr-nr: 2011-41-5755
Supported by: phd stpendiate for Abdellatif Aharaz by The University of Southern Denmark

Disclosure: **A. Aharaz:** None.

358

Haemoglobin glycation index and all-cause mortality in individuals with type 2 diabetes: the Pisa Mortality Study

G. Penno[1], M. Garofolo[1], D. Lucchesi[1], L. Giusti[1], V. Sancho Bornez[1], R. Miccoli[1], C. Bianchi[1], G. Sesti[2], S. Del Prato[1];
[1]Department of Clinical and Experimental Medicine, University of Pisa, PISA, Italy, [2]Department of Medical and Surgical Sciences, "Magna Graecia" University of Catanzaro, Catanzaro, Italy.

Background and aims: Data from RCTs have proposed hemoglobin glycation index (HGI), the difference between measured HbA1c and HbA1c predicted from fasting plasma glucose (FPG), as an independent marker of higher risk for diabetic micro- and macrovascular complications. HGI accounts for interindividual variation in the association between HbA1c and plasma glucose concentrations. We have tested the association between HGI (as a continuous or a categorized variable) and all-cause mortality in a large group of outpatient with type 2 diabetes mellitus (T2DM).

Materials and methods: The linear relationship between FPG and HbA1c [predicted HbA1c = 0.015*FPG (mg/dl) + 5.169; $r = 0.547$, $r^2 = 0.299$] was determined in a large sample of 9,696 consecutively recruited T2DM subjects after exclusion of 335 "outliers" (FPG and/or HbA1c values below 1st or above 99th percentile). HbA1c was measured with HPLC using a National Glycohemoglobin Standardization Program (NGSP) and FPG determined by enzymatic method. We have then identified 1019 T2DM individuals recruited in an observational, cross-sectional, single-center study with baseline cardio-metabolic risk stratification and prospective assessment of all-cause death.

Results: HGI (0.036 ± 1.011; mean ± SD) was normally distributed, ranging from −2.66 to 3.82. Study population was stratified as HGI tertiles (low ≤−0.474, intermediate −0.475 to 0.331, high ≥0.332). At baseline, intermediate and high HGI individuals had similar age, BMI, FPG, blood pressure, LDL-cholesterol, HDL-cholesterol, eGFR (CKD-

EPI), albumin-to-creatinine ratio, gender, and active smoking, but longer diabetes duration (DD), higher HbA1c and triglycerides than low HGI. Rates of retinopathy (23.0%, 26.1%, 38.3%; $p < 0.0001$), peripheral neuropathy (19.5%, 21.8%, 27.1%, $p = 0.017$) and peripheral artery disease (3.5%, 6.5%, 8.3%; $p = 0.035$) were the highest in high HGI. Finally, in high HGI more subjects were on glucose-lowering agents including insulin. A total of 234 deaths occurred over 13,131 person-years and 12.9 ± 2.7 year mean follow-up (23.0%; 17.8 ×1000 person-years) with no difference in mortality rate among HGI tertiles: 24.2%, 21.7%, and 23.1% (K-M; Log Rank, $p = 0.777$). In Cox regression model gender (HR 1.53, 95%CI 1.16–2.01; $p = 0.003$), age (1.08, 95%CI 1.06–1.11; $p < 0.0001$), DD (1.02, 95%CI 1.00–1.03; $p = 0.014$) and HbA1c (1.28; 95%CI 1.06–1.56, $p = 0.011$) were associated with increased risk of all-cause mortality while this risk decreased per unit HGI increase (HR 0.76; 95%CI 0.60–0.95; $p = 0.017$). The opposite effects of HbA1c and HGI were lost after adjustment for confounders. In a second Cox model including both HbA1c and HGI tertiles the opposite effects of HbA1c and HGI on all-cause death were confirmed but, once again, the associations were lost after adjustment for confounders.

Conclusion: In conclusion, in a large population of individuals with type 2 diabetes the predictive effect on all-cause mortality of both HbA1c and HGI is less powerful than gender, age, and duration of diabetes. The present findings do not support the hypothesis that HGI may identify a subset of individuals with type 2 diabetes at high risk for long-term all-cause mortality.

Disclosure: **G. Penno:** None.

PS 013 Diabetes across ethnicities

359

Aryl-hydrocarbon receptor binding and the incidence of type 2 diabetes: the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

B.B. Duncan[1], C.D. Castilhos[1], P.A. Bracco[1], M.I. Schmidt[1], H.K. Lee[2], A. Vigo[1], Y.K. Pak[3];
[1]UFRGS, Porto Alegre, Brazil, [2]Eulji University, Seoul, Republic of Korea, [3]Kyung Hee University, Seoul, Republic of Korea.

Background and aims: Persistent organic pollutants (POPs) are a suspected cause of diabetes, but investigation of their risk has been hampered by the large number of different molecules, and the difficulty and cost of analyses. Much of their toxicity may result from their binding to the aryl hydrocarbon receptor (AhR), a transcriptional enhancer which affects various regulatory proteins. Ensuing mitochondrial dysfunction is postulated as a mediator of their action. Our aim is to investigate the association of POPs exposure with incident and prevalent diabetes indirectly by bioassaying the degree of AhR binding and mitochondrial inhibiting potential by *in vitro* incubation of cellular bioassays with plasma samples.

Materials and methods: We conducted incident and prevalent case-cohort analyses of participants from the Rio Grande do Sul center of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), an ongoing cohort study. Of 2061 subjects enrolled between 2008 and 2010, 1598 were free of diabetes at baseline, returned for a follow-up visit 4 years later, and had complete information to classify diabetes. AhR binding was determined by a cell-based AhR-dependent luciferase activity bioassay and mitochondrial function with a serum mitochondria inhibiting activity bioassay. Diabetes was ascertained by self-report, medication use, OGTT or HBA_{1c}.

Results: 366 (17.8%) participants had diabetes at baseline and 106 (6.6%) developed diabetes during follow-up. In logistic regression analyses adjusting for sex, age, ethnicity, educational attainment, parental history of diabetes, hypertension, BMI, and waist-hip ratio, those with above-median values for AhR binding and for mitochondrial inhibiting potential had 72% and 187% greater odds developing diabetes (OR = 1.72; 95%CI 1.04–2.86 and 2.87; 1.66–4.96), respectively. Restricted spline analyses, when similarly adjusted, demonstrated tapering of associations at highest levels of AhR binding. Associations with prevalent diabetes, however, were less consistent. Although statistically significant cross-sectional associations for AhR binding were seen in restricted spline analyses (e.g. OR = 1.53; 1.12–2.08 for an AhR binding level of 3.0 vs. a reference level of 1.5), no association was found for mitochondrial inhibiting potential.

Conclusion: The increased incidence of diabetes associated with higher AhR binding and with greater mitochondrial inhibiting potential in this cohort suggest that ambient exposure to POPs could be an important contributing cause to the current diabetes epidemic.

Clinical Trial Registration Number: NCT02320461

Supported by: Ministries of Health, and Science and Technology, Brazil

Disclosure: **B.B. Duncan:** None.

360

Local traditions and conventions impact vulnerability to type 2 diabetes

A.-M. Volkmann[1], L. Hesseldal[2], M. Bagger[2], D. Napier[1];
[1]University College London, London, UK, [2]Health Advocacy, Novo Nordisk A/S, Bagsværd, Denmark.

Background and aims: More than half of the world's population live in cities, and two thirds of the more than 400 million people with diabetes live in urban areas. That makes cities an important focal point for

researching diabetes and for improving its prevention, care and management. To reduce the global diabetes burden, a variety of interventions have been implemented all over the world. However, the success of any intervention is significantly influenced by inter-related geographical, economic, environmental, social, and cultural factors, which up until now have been under-explored. Cultural factors, i.e. shared traditions and conventions, influence, among others, health beliefs and food practices, gender attitudes, and local practices to care seeking and health management, and therefore impact vulnerability to type 2 diabetes. For example, where foods and beverages that are high in calories and low in nutrition play an important role in local traditions sustaining social bonds, vulnerability may emerge as preventive lifestyle change is harder to implement and management more difficult to sustain.

Materials and methods: In order to obtain data about relevant environmental, social and cultural factors that make certain people vulnerable to type 2 diabetes, Vulnerability Assessments were carried out in five highly diverse cities around the world, namely Copenhagen, Houston, Mexico City, Shanghai, and Tianjin. In total, 740 individual semi-structured interviews were conducted across the five cities as part of the assessments, which also included demographic and ethnographic data collection. The research was part of the Cities Changing Diabetes programme established by Novo Nordisk together with Steno Diabetes Center Copenhagen, University College London and local partners. The aim of the programme is to understand the complexity of urban diabetes and to provide concrete guidance for policy change, urban planning, and public health interventions through collaborative research.

Results: The research identified a set of social factors and cultural determinants relevant to type 2 diabetes vulnerability that were evident across all cities with considerable local variations. 'Traditions and Conventions' was one such factor and was exemplified as follows: Copenhagen: standard medical referral practices acted as barriers to preventive care and services. Houston: food traditions were interwoven with heritage and culture, and therefore carried meaning beyond nutrition and diet. Food rituals were often perceived as providing 'comfort' central to a common culture and heritage. Mexico City: traditional gender roles limited effective self-care in men when there was no female household member present, and some men were unable or unwilling to provide diabetes support to others. Shanghai: denying hardship was culturally valorised. This attitude prevented people from seeking help from family, friends and healthcare professionals. Tianjin: belief in 'miracle cures' among some in the population created biological risks and psychological ill health.

Conclusion: 'Traditions and Conventions' impact vulnerability to type 2 diabetes. Where local traditions and conventions encourage specific individual behaviours, community-based interventions may prove ineffective, even if there are good local care providers present. Taking this cultural determinant into consideration and identifying any local variants may be a prerequisite for successful interventions.

Supported by: Novo Nordisk A/S

Disclosure: **A. Volkmann:** Employment/Consultancy; Consultant in Cities Changing Diabetes. Grants; A grant from Novo Nordisk was given to the research.

361

Adherence to diabetes care process indicators in migrants as compared to non-migrants with diabetes: a population study

G. Seghieri[1], L. Policardo[1], P. Francesconi[1], C. Seghieri[2];
[1]ARS Tuscany, Pistoia, Italy, [2]Laboratorio Management e Sanità , Istituto di Management, Scuola Superiore Sant'Anna, Pisa, Italy, Pistoia, Italy.

Background and aims: According to previous studies the prevalence rate of diabetes is higher among migrants who have moreover been found to face a worse quality of care as compared with non_migrants. Whether migrants are correctly inserted into a standard quality of process care for diabetes by the regional health system and whether processes of care are adequately accomplished at follow up after being taken in charge, are the questions addressed by the present study which compares migrants with non-migrants (Italian residents) followed up since January 1st 2011 to December 31st2015 in Tuscany, a region of central Italy

Materials and methods: Diabetic patients living in Tuscany in the period January 1th 2011-31st December 2015 have been recruited according to a validated algorithm by administrative databases. Migrants were compared with Italian residents for the probability of fulfilling along this period at least one GCI (Guideline Composite Indicator, a process indicator including one annual assessment of HbA1c and at least two among eye examination, serum lipids measurement and microalbuminuria), through a logistic regression analysis and, for those with at least one GCI, the probability of having further GCIs through a truncated Poisson regression analysis. The analysis was finally completed by comparing two cohorts of migrants and Italian residents with diabetes, fully matched for main confounders by means of a *propensity score* ($N = 3{,}766$ for both).

Results: On January 1st 2011 a cohort of 130,648 diabetic patients has been identified of whom 3,766 composed by migrants. Migrants were on average younger (age: 51 ± 12 yr vs. 67 ± 13 yr; $p < 0.0001$), had a lower number of males (47% vs. 50%), a lower amount of previous comorbidities as testified by a higher Charlson Index (>1 in 9% vs. 26%) and finally a lower compliance to GCI significantly lower than among Italian residents (43% vs. 50%; OR = 0.776; 95%CI: 0.724–0.832); $p < 0.0001$ for all comparisons. After matching the two cohorts by propensity score, however, the probability of complying with at least one GCI was similar in migrants compared to Italian residents: OR:0.966 (95%IC:0.900–1.035; p = NS). On the contrary, even after matching cohorts by the propensity score the expected number of GCIs in those with at least one GCI was significantly reduced in migrants than in Italian residents with an Incidence Rate Ratio (IRR):0.86 (0.83–0.89); $p < 0.0001$.

Conclusion: These findings suggest that in Tuscany the regional health system can similarly intercept migrants and Italian residents with diabetes with regard to the expected adherence to guidelines. However, the quality of care seems to be impaired among migrant patients with diabetes, since they have a lower probability (by around 15%) of continuing to adhere to guidelines as evidenced by compliance to GCI.

Disclosure: **G. Seghieri:** None.

362

Area level deprivation and quality of care in type 2 diabetes: results from a disease management programme in North Rhine-Westphalia, Germany

B. Hagen, S. Groos, J. Kretschmann, C. Macare, A. Weber;
DMP-Projektbuero, Zentralinstitut fuer die kassenaerztliche Versorgung in Deutschland, Cologne, Germany.

Background and aims: Area level deprivation or socioeconomic status and its association with quality of care in type 2 diabetes (T2D) were addressed in a number of recent studies. Correlations were confirmed between low income and diabetes prevalence and diabetes-related mortality on the one hand, but higher referral rates on the other. In the context of a disease management programme (DMP) for T2D former analyses of the association between markers of socioeconomic status on a regional level and DMP-related indicators of quality of care showed ambiguous results. Therefore in a series of new analyses more detailed regional information was made accessible.

Materials and methods: For the 14 cities forming the metropolitan region of the central Ruhr area (approx. 3.36 millions of inhabitants, average population density 2.049 inhabitants/square kilometre) information on area level deprivation (income, migration status, educational level) was available on the level of small area city quarters. From the DMP documentation location of the practices as well as the results in the indicators of quality of care were known. An index of area level deprivation (1 = lowest grade of deprivation, 7 = highest) was used as a predictor of

specific indicators of quality of care in independent multivariate logistic regression models, adjusted for age, sex, comorbidities, duration of participation in DMP, and type of specialised care. All patient records of the years 2015 and 2016 from the region were analysed (*n* of patients = 206.903, mean age 68.1 + −12.6 yrs., female 50%).

Results: 4.0% of patients were assigned to the lowest level of area deprivation (1), 4.2% to the highest (7). 62.7% of patients were living in quarters characterised by medium level area deprivation (3–5). Comparing patients in index areas 1–5 with those assigned to 6 and 7 only marginal differences were seen with regard to HbA1c (<8,5%: 90.7% vs. 89.0%), blood pressure (<140/90 mmHg: 59.7% vs. 60.8%), assessment of renal function (92.4% vs. 91.6%) and prescription of metformin (87.8% vs. 87.7%). By contrast higher rates in patients from areas of lower deprivation were observed with regard to the achievement of the individual HbA1c aim (64.5% vs. 60.3%), regular eye examination (67.8% vs. 63.9%), referral to a specialist practice in case of severe feet lesions (50.6% vs. 44.3%; index areas 1–5 vs. 6–7, respectively), taking part in a recommended patient education (57.8% vs. 48.7%), and continuity of DMP participation (≥70% of all visits expected: 90.7% vs. 82.5%; index areas 1 vs. 7, respectively). Controlling for covariates patients in deprived areas had a significant lower chance to achieve their HbA1c aim (OR 0.90, 95%CI 0.88–0.92), to get an eye examination (0.84; 0.82–0.86), to take part in a patient education (0.67; 0.61–0.73) or to take part continuously (0.54; 0.48–0.61), and by trend to be referred to a specialist practice in case of feet lesions (OR 0.73; 0.50–1.05).

Conclusion: Data from a DMP for patients with T2D confirmed a strong influence of area level deprivation on specific indicators of quality of care, especially with regard to patient education and continuity of participation. As a consequence an intensification of supporting DMP patients in areas with high deprivation is required and intensified, cultural fair, and multilingual patient education programs must be implemented in those areas.

Disclosure: **B. Hagen:** None.

363

Diabetes knowledge, self-care behaviours, and metabolic control in Arabic-speaking adults with type 2 diabetes in Edmonton, Canada

R.O. Yeung[1], A. Belag[1], M. Alfaituri[2], F. Al-Sayah[3];
[1]Division of Endocrinology and Metabolism, University of Alberta, Edmonton, AB, Canada, [2]University of Alberta, Edmonton, AB, Canada, [3]School of Public Health, University of Alberta, Edmonton, AB, Canada.

Background and aims: The number of Arabic-speaking immigrants in Canada is growing, as is the prevalence of T2D (T2D) in this population. Understanding of T2D knowledge and self-care management in the Arabic-speaking population in Canada is lacking. The objective of this study was to examine diabetes knowledge, self-care behaviors, and health outcomes in Arabic-speaking adults with T2D in Canada.

Materials and methods: We conducted a cross-sectional study in Edmonton, AB between July 2017 and January 2018. Eligible adult Arabic-speaking participants with T2D were recruited from primary care settings and community centers in Edmonton. Data collection involved face-to-face interviews to complete an online survey via Research Electronic Data Capture (REDCap). The interviews were conducted in Arabic. Survey measures included diabetes knowledge (Michigan Diabetes Knowledge Test), self-care behaviours (Summary of Diabetes Self-Care Activates, SDSCA), medication adherence (Morisky medication adherence scale, MMAS8), and depressive symptoms (Patient Health Questionnaire 2, PHQ-2).

Results: A total of 114 individuals participated in this study. The mean age(±SD) of participants was 56 ± 16 years, and the majority were male (61.0%). The largest subset (37.7%) were of Lebanese origin. The mean diabetes duration was 11.8 ± 8.6 years. The majority (60.9%) had a BMI ≥25 kg/m^2 and 71.9% had a family history of diabetes. More than half of respondents reported having hypertension (58.8%) and dyslipidemia (54.4%). Less than the half (47.3%) had glycated hemoglobin less than the target range of 7%. Half (53.6%) had an average score (7 to 11 points) on their diabetes knowledge, and 25.4% scored poorly (<7 points). The poorest domain of self-care behaviours was exercise (mean 2.1 days/week). For those taking medication, 42.7% had moderate medication adherence (6–7), and 26.2% had poor medication adherence (score <6). One in five participants (19.3%) screened positive for depressive symptoms.

Conclusion: To the best of our knowledge, this is the first study describing diabetes-related knowledge, self-care management, depressive symptoms, medication adherence, and health outcomes in the growing Arabic-speaking population with T2D in Canada. We found significant gaps in knowledge and self-care behaviours. These findings have implications for the creation and implementation of culturally-tailored interventions to enhance diabetes knowledge, self-care behaviors, and clinical outcomes in the Arabic-speaking diaspora in Canada.

Disclosure: **R.O. Yeung:** None.

364

The impact of black ethnicity on performance of HbA_{1c} and 1 hour plasma glucose as screening modalities for impaired glucose tolerance

A. Ghafar, O. Bello, M. Ladwa, G. Alberti, S.A. Amiel, L. Goff;
Diabetes Research Group, School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK.

Background and aims: With a two-fold higher prevalence of type 2 diabetes (T2D) in people of Black compared with white European ethnicity, effective screening in this high-risk community is a public health priority. In the UK, HbA1c is used to detect risk of progression to T2D, with 42–47 mmol/mol as a criterion for entry into the new National Diabetes Prevention Programme. Ethnic-specific variations in HbA1c *vs* plasma glucose make these cut-offs of HbA1c for identification of pre-diabetes in people of Black ethnicity controversial. Furthermore, recent evidence favouring the 1 hour rather than the conventional 2 hour oral glucose tolerance test (OGTT) has not considered the possibility of ethnicity-related disparities in 1 hour plasma glucose concentrations (1hr PG). We aimed to compare the sensitivity and specificity of HbA1c and 1hr PG for the detection of impaired glucose tolerance (IGT) in men of Black West African (BWA) and White European (WE) ethnicity.

Materials and methods: Data were collected from participants undertaking a 75 g 2 hour OGTT during screening for entry into the South London Diabetes and Ethnicity Phenotyping Study between October 2015 and February 2018. Participants were male, aged 18–65 years and of BWA or WE ethnicity. Those with overt T2D or isolated impaired fasting glucose were excluded from analysis. IGT was diagnosed using WHO criteria (2 hour PG ≥7.8 mmol/L and <11.1 mmol/L). Sensitivity and specificity of HbA1c (42–47 mmol/mol) and 1hr PG (≥8.6 mmol/l) for detecting IGT in each ethnic group was tested using 2 hr PG values from the OGTT as the reference standard.

Results: 39 BWA and 56 WE men were recruited; IGT was found in 7.7% and 17.9% respectively. In the IGT group, HbA1c was significantly higher in BWA compared to WE men (45.0 ± 1.77 mmol/mol *vs* 38.0 ± 1.67 mmol/mol, $p = 0.014$), despite similar baseline characteristics (age 43.00 ± 3.61 years *vs* 52.50 ± 12.27 years, $p = 0.224$; BMI 33.35 ± 6.19 *vs* 29.93 ± 3.27, $p = 0.217$). In BWA men, sensitivity of HbA1c for detecting IGT was not significantly different in comparison to WE men (66.7%; 95% CIs [12.9, 98.2] *vs* 20.0%; 95% CIs [3.7, 40.9] $p = 0.125$) but specificity was lower (77.8%; 95% CIs [73.8, 80.4] *vs* 93.5%; 95% CIs [89.9, 98.0], $p = 0.038$). In BWA men, sensitivity of 1 hr PG for detecting IGT was not significantly different in comparison to WE men (66.7%; 95% CIs [13.0, 98.2] vs 88.9%; 95% CIs [53.5, 99.4] $p = 0.054$, but specificity was higher (81.8%; 95% CIs [76.9, 84.7] *vs* 60.9%; 95% CIs [54.0, 62.9] $p = 0.046$).

Conclusion: HbA1c levels of 42–47 mmol/mol have similar sensitivity but lower specificity in detecting IGT in BWA compared to WE men, whereas 1hr PG is more specific in detecting IGT in BWA compared to WE men and may offer a useful screening modality in people of Black ethnicity. Further work is needed to determine whether ethnic-specific HbA1c cut-offs are indicated.

Clinical Trial Registration Number: IRAS 178092
Supported by: Diabetes UK
Disclosure: **A. Ghafar:** Grants; Diabetes UK.

365

Integration increases the risk of diabetes and obesity in the Filipino population resident in Rome

S. Pieralice[1], R. Del Toro[1], G. Pesce[1], K. Jansen[2], M. Khazrai[1], A. Maurizi[1], S. Manfrini[1], P. Pozzilli[1];
[1]Unit of Endocrinology and Diabetes, Department of Medicine, Campus Bio-Medico University, Rome, Italy, [2]The Hague University of Applied Sciences, Alphen aan den Rijn, Netherlands.

Background and aims: Italy is home to over 166,000 Filipino immigrants. However, dietary habits and predictors of risk of diabetes and metabolic disease within this immigrant group are not well investigated. Aims of the present study were to assess the dietary intakes and to evaluate the anthropometrical and metabolic characteristics of the Filipino population migrant to the Southern European city of Rome, Italy. Moreover, changes in metabolic characteristics between first-generation immigrants and their descendants were examined.
Materials and methods: A cross-sectional study was carried out in the city of Rome. A total of 132 first-generation immigrants (42 M/90 F, mean age: 49.3 ± 11.2 years, mean residence in Italy: 15.6 ± 10.6 years) and 27 descendants (12 M/15 F, mean age: 15.7 ± 3.2 years) were studied. Data were collected by standardized questionnaires; anthropometrical parameters and fasting capillary blood glucose (FCG) were measured. Paired t test (two tailed) and analysis of variance were used to evaluate differences in metabolic characteristics between the two groups. A Pearson's correlation analysis was performed to identify any significant correlation between BMI and life-style/eating habits.
Results: Impaired fasting glucose (FCG ≥110 mg/dl) was observed in 22.6% of first-generation immigrants and in 8% of descendants' group. Limited to the first-generation group, Toumilehto questionnaire showed a 10-year risk of developing diabetes >50% in 20% of subjects. BMI ≥25 kg/m^{2}was found in 41.7% of first-generation immigrants, while children's BMI-percentile-for-age >85% was recorded in 48.1% of second-generation group, with a significant different between the two populations ($p < 0.05$). Evaluation of dietary intake pattern showed that first-generation immigrants consumed traditional food more days x week compared to their descendants, who preferred western food, with a large consumption of eggs (>4 times/week: 96%), processed meat (>4 times/week: 74%) and sweet foods or beverages (>4 times/week: 96%). Body Mass Index (BMI) showed a positive correlation with the years spent in Italy ($R = 0.19$, $p = 0.03$) and with educational level ($R = 0.19$, $p = 0.04$). Limited to the first-generation women, the size of household was directly related to weight gain ($R = 0.40$, $p < 0.001$).
Conclusion: In the present study, substantial risk of developing diabetes within Filipino community was found. Moreover, the prevalence of obesity is higher in the second-generation, probably due to westernization in eating habits linked to an unfavourable socio-economic context.
Disclosure: **S. Pieralice:** None.

PS 014 Genetics of diabetes

366

WITHDRAWN

367

Identification of novel loci associated with lipid levels in recent-onset type 1 diabetes

A.J. Overgaard[1], S. Kaur[1], A. Ali[1], J.M. Weir[2], K. Jayawardana[2], H.B. Mortensen[3], P.J. Meikle[2], F. Pociot[1];
[1]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [2]Baker IDI Heart and Diabetes Research Institute, Melbourne, Australia, [3]Herlev Gentofte Hospital, Herlev, Denmark.

Background and aims: Multiple studies have identified panels of lipidomic biomarkers for increased cardiovascular risk and declining beta cell function in type 1 diabetes (T1D) patients. So far, single nucleotide polymorphisms (SNPs) at 157 loci have been robustly associated with blood lipids and several of these lipid loci are associated with cardiovascular and metabolic traits. However, shared genetic components between dyslipidemia and T1D are largely unknown. Here we aim to identify lipid-associated SNPs in children with recent onset T1D and examined their effects on glucose-related traits using global Lipidomics profiling.
Materials and methods: The plasma lipidome (352 lipids) in a cohort of children diagnosed with T1D from the Danish Remission Phase Study ($n = 106$) was profiled at 4 different time-points (1, 3, 6 and 12 months) using Agilent 1290 liquid chromatography system coupled to an Agilent 6490 triple quadrupole mass spectrometer. The cases were genotyped using ImmunoChip, a custom-made Illumina Infinium array, described previously. The lipid levels were quantile normalized and adjusted for covariates. The association with individual SNPs with lipids was tested using linear mixed models (FastLmm). Linkage disequilibrium (LD) analysis was performed to identify lead SNPs for each lipid-SNP association. Associations between lipid-associated SNPs and HbA1c were computed. Known lipid-loci were retrieved from Global Lipid Genetics Consortium (GLGC) cohort.
Results: We found 7 loci significantly associated with specific lipids at minimum 2 time-points (Table1). Among these, EVI5 locus has been previously shown to be associated with total cholesterol (TC) levels in GLGC cohort. Specific lipids that associated with EVI5 lead variants rs2065916 (OR = 1.31, p value = 2.9e-07), rs11164778 (OR = 1.29, p value = 7.0e-07) and rs6658232 (OR = 1.14, p value = 3.2e-06) in our analysis included CE:18:2 and COH at 6 months; DG(36:2), TG(50:2), TG(51:0), TG(54:1) and CE:22:0 at 12 months from lipid classes cholesteryl esters, free cholesterol, diacylglycerols, and triacylglycerols respectively. Interestingly, each copy of the risk alleles for EVI5 variant rs2065916 corresponded to up to 30% increase in the associated lipid levels. Furthermore, lead variants at 3 loci (EVI5, MAP3K8 and USP34) were associated with increased HbA1c at different time-points. Overlap with known lipid-loci identified FADS1/FADS2 lead variant rs174537 which has been previously shown to be associated with triglycerides, total cholesterol, LDL and HDL. In our analysis, rs174537 significantly associated with PC:36:4b at 6 months.
Conclusion: This study identified 7 loci that associate with lipid levels at multiple time-points in T1D patients. Three loci showed pleiotropic associations with lipids and HbA1c suggesting a complex genetic regulation and metabolic interplay.

Table: Lipid-SNP associations significant in at least 2 time-points

Lead SNP	MAF	Locus	Associated lipids, timepoints (months)	P-value	Odds Ratio	HbA1c ~ SNP
rs2811796	T:0.05	ADAMTSL1	CE:18:2, COH (3, 6)	3.5e-07	1.17	
rs34266322	G:0.07	MTPAP, MAP3K8	LPC:24:0 (3); LPC:24:0, LPC:22:0, SM:38:1 (6)	5.5e-09	1.02	0.03, 0.009 (3 and 12 month)
rs4959079	A:0.05	PPIAP9, MICB	CE:24:4 (6); CE:22:0, PE:40:4, PC(O-36:0) (12)	2.2e-07	1.04	
rs9267487	G:0.05	DDX39B	CE:24:4 (6); CE:22:0 (12)	2.2e-07	1.04	
rs1019113	A:0.12	CCL2	LPC(O-22:1) (1, 12)	1.8e-06	1.01	
rs9906713	A:0.12			1.8e-06	1.01	
rs72815516	C:0.08	USP34	CE:18:2,COH (3, 6)	5.0e-07	1.15	0.04 (6 month)
rs2065916	A:0.08	EVI5	CE:18:2, COH (6); DG(36:2), TG(50:2), TG(51:0), TG(54:1), CE:24:0 (12)	2.9e-07	1.31	1.6e-08 (12 month)
rs11164778	A:0.08			7.0e-07	1.29	2.3e-08 (12 month)
rs6658232	A:0.08			3.2e-06	1.14	2.1e-08 (12 month)

Supported by: JDRF: PNF and SRA grants
Disclosure: **A.J. Overgaard:** None.

368

Genetic determinants of glycated haemoglobin in type 1 diabetes

A. Syreeni[1,2], N.K.A. Sandholm[1,2], J. Cao[3], I. Toppila[1,2], C. Forsblom[1,2], V. Harjutsalo[1,4], M. Parkkonen[1,2], E. Valo[1,2], A.D. Paterson[3], P.-H. Groop[1,2], the FinnDiane Study Group;
[1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, Finland, [2]Abdominal Center, Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [3]Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Canada, [4]The Chronic Disease Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland.

Background and aims: HbA_{1c} is an important measure of glycemia in diabetes. Notably, HbA_{1c} is influenced not only by environmental but also by genetic factors, both in people with and without diabetes. For example, single nucleotide polymorphisms (SNPs) that affect erythrocyte turn-over or that modify glucose homeostasis may influence HbA_{1c}. Genetic variants for HbA_{1c} have mainly been studied in non-diabetic individuals, and only one genome-wide association study (GWAS) has previously been conducted in individuals with type 1 diabetes in the DCCT/EDIC cohort.
Materials and methods: Here we performed a GWAS for HbA_{1c} in The Finnish Diabetic Nephropathy Study (FinnDiane). A total of 4,622 individuals with type 1 diabetes were included. Human Core Exome Bead Chips were used for genotyping and imputation was performed. SNPs were analyzed with linear regression with covariates age, duration, sex, number of HbA_{1c} measurements, genotyping batch and principal components 1–10. Replication was performed in DCCT/EDIC divided into individuals with conventional (CON, $n = 667$) or intensive (INT, $n = 637$) treatment of diabetes during DCCT. The association between top SNPs and HbA_{1c} was further studied in non-diabetic cohorts from European, East Asian and South Asian origin with the data from a large meta-GWAS for HbA_{1c}.
Results: In the FinnDiane population, three tightly correlated SNPs on chromosome 13 near relaxin/insulin like family peptide receptor 2 (*RXFP2*) with minor allele frequencies (MAFs) 3.0–4.1% were associated with HbA_{1c} (Beta = 0.42 [95% CI 0.27, 0.56], $p = 1.5 \times 10^{-8}$ for the lead SNP rs2085277). The chromosome 13 top SNPs were not significantly associated with HbA_{1c} in the DCCT/EDIC and the MAFs were low. For example, MAF for rs2085277 was 0.2% and 0.5% in the DCCT/EDIC CON and INT cohorts, respectively. Interestingly, rs2085277 had a minor T-allele frequency of 38.5% in the 1000 Genomes East Asian population and this SNPs was associated with HbA_{1c} in non-diabetic subjects from East Asia (Beta = 0.02 [0.006, 0.03], $p = 0.005$). Further, rs1360072 on the chromosome 13 locus had MAF = 34.6% in East Asian and MAF = 9.0% in South Asian individuals and it significantly associated with HbA_{1c} in both Asian populations (Beta = 0.02 [0.003, 0.03], $p = 0.013$) with the consistent direction of the effect.
Conclusion: We identified variants near *RXFP2* that were associated with HbA_{1c} in Finnish individuals with type 1 diabetes as well as in individuals without diabetes. Detailed analysis of the involved genes and pathways may help understanding how this locus affects HbA_{1c} values and glycemia in diabetes.
Supported by: Folkhälsan Research Foundation, W&E Stockmann, JDRF, Academy of Finland
Disclosure: **A. Syreeni:** None.

369

Type 1 diabetes genetic risk score discriminates between monogenic and type 1 diabetes in patients with diabetes presented below five years of age in Iranian population

H. Yaghootkar[1], K. Patel[1], K. Colclough[1], A. Moleirinho[1], N. Ghaemi[2], S. Heidari[3], S. Vakili[2], S. Enayati[3], A. Rabbani[3], F. Abbasi[3], T. McDonald[1], S. Ellard[1], M.M. Amoli[3], R. Vakili[2], A.T. Hattersley[1];
[1]University of Exeter, Exeter, UK, [2]Mashhad University of Medical Science, Mashhad, Islamic Republic of Iran, [3]Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

Background and aims: Previous studies of non-consanguineous white Europeans have indicated a type 1 diabetes genetic risk score (T1D-GRS) as a tool to discriminate between T1D and other non-autoimmune causes of diabetes. We aimed to assess the utility of T1D-GRS to distinguish monogenic from type 1 diabetes in predominantly consanguineous population.
Materials and methods: We collected 91 patients with diabetes diagnosed between 9 months and 5 years old from two centres in Iran. We analysed clinical features at referral, measured three islet autoantibodies (IA2, GAD and ZnT8) and generated T1D-GRS using 30 common genetic variants associated with T1D. All patients underwent a targeted next-generation sequencing of all known monogenic diabetes genes (35 genes).
Results: Our cohort included 44 females and 47 males, from 32 consanguineous families (37.7%). We identified monogenic diabetes in 6 patients (7%). Of these, 5 had homozygous mutations (4 in *WFS1* and 1 in *SLC19A2*) and 1 had heterozygous mutation in *GCK*. The frequency of mutations was different from monogenic diabetes in European population where mutations in *GCK* are the most common cause especially in children under 5 years old. T1D-GRS of the monogenic patients was lower (mean GRS 8.2, 95% CI [7–9.1]) than the rest of the patients (10.4 [7.4–12.9], $p_{difference} = 0.006$). Patients with monogenic cause did not have any antibodies but 80% of non-monogenic patients were positive for at least one of the antibodies. Age of diagnosis, birth weight, syndromic features, consanguinity and gender were similar in patients with monogenic cause and rest of the cohort (all $p > 0.05$). The T1D-GRS was highly discriminatory between monogenic and non-monogenic group (ROC area under the curve (AUC) 0.90 [0.82–0.98]). This is additive to the discriminatory power of islet autoantibodies (RUC AUC autoantibodies alone 0.81 [0.65–0.98] vs ROC AUC T1D-GRS and autoantibodies 0.94 [0.86–1.00], $p_{difference} = 0.018$).
Conclusion: Our study provides the first evidence that T1D-GRS can be used in the predominantly consanguineous Iranian population to distinguish monogenic from type 1 diabetes. Importantly, T1D-GRS was independent and additive to the widely available islet autoantibodies for identifying monogenic diabetes. This highlights the clinical utility of T1D-GRS in routine clinical practice.
Supported by: Wellcome Trust Seed Award, Diabetes UK RD Lawrence fellowship
Disclosure: **H. Yaghootkar:** None.

370

IRS1genetic variants associated with glucose control and insulin resistance in type 2 diabetic patients from Bosnia and Herzegovina

L. Mahmutovic[1], T. Bego[2], M. Sterner[3], G. Gremsperger[3], E. Ahlqvist[3], Z. Velija Asimi[2], B. Prnjavorac[4], N. Hamad[1], A. Causevuc[2], L. Groop[5], S. Semiz[1];

[1]International University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [2]University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [3]Lund University Diabetes Centre, Malmoe, Sweden, [4]General hospital Tesanj, Tesanj, Bosnia and Herzegovina, [5]Lund University Diabeter Centre, Malmoe, Sweden.

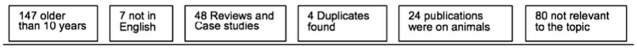
Background and aims: Previous studies reported conflicting results regarding association of variation insulin receptor substrate 1 (*IRS1*) gene variation with Type 2 diabetes (T2D) and markers of insulin resistance (IR) in different ethnic groups. Here we examined the association of rs7578326 (G>A), rs2943641 (T>C), and rs4675095 (A>T) with T2D risk and its related traits in a population from Bosnia and Herzegovina (BH), which is among European countries with the highest T2D prevalence of 12.3%.
Materials and methods: Our study included 390 T2D patients and 252 unrelated nondiabetic control subjects. Biochemical parameters, including fasting glucose (FG), fasting insulin (FI), HOMA-IR, and HbA1c levels, were measured in all participants. We performed sensitivity analysis in a subgroup of 96 T2D patients not treated with any therapy (NT-T2D) to dissect the potential drug effects on phenotypic measures. Genotyping analysis was performed by Mass Array Sequenom iPlex platform in cooperation with Lund University Diabetes Centre, Malmo, Sweden.
Results: Our results demonstrated that upon adjustment for BMI, age, and gender, rs7578326 and rs4675095 variants were positively associated with FG ($B = 0.05295$% CI 0.004; 0.099, $p_{dom} = 0.034$; $B = 0.029$ 95% CI 0.002; 0.054, $p_{add} = 0.037$, respectively), thus, in opposite direction as compared to rs2943641 ($B = -0.17$ 95% CI −0.033; −0.002, $p_{add} = 0.030$). Strikingly, the risk allele of both, rs7578326 and rs2943641, were also associated with higher HbA1c ($B = 0.034$ 95% CI 0.003;0.065, $p_{dom} = 0.035$; and $B = 0.032$ 95% CI 0.002; 0.065, $p_{dom} = 0.040$, respectively). Furthermore, the risk A and C allele of these two polymorphisms were associated with HOMA-IR ($B = 0.316$, 95% CI 0.026; 0.607, $p_{rec} = 0.033$) and higher levels of FI ($B = 0.350$ 95% CI 0.022; 0.487, $p_{rec} = 0.033$) in NT-T2D patients. Interestingly, the rs7578326/rs2943641 haplotype was associated with FG ($p = 0.024$) and waist circumference ($p = 0.029$) in control subjects, while in NT-T2D patients it was associated with HOMA-IR ($p = 0.024$), thus confirming the observed individual effects of the risk alleles on FG and IR. Logistic regression analysis demonstrated that the odds of developing T2D in this sample of BH population were not associated with the presence of *IRS1* rs7578326, rs2943641, and rs4675095 variants after adjustment for gender and age.
Conclusion: Our results showed the association of the common *IRS1* genetic variants with insulin resistance markers and fasting glucose levels in the population of Bosnia and Herzegovina, indicating that *IRS1* is the common locus for insulin resistance across different populations. Importantly, here we report first here the association of *IRS1* variation with HbA1c levels, further strengthening its role in blood glucose control.
Supported by: Council of Ministers BH/MCA BH and FMES awarded to S.S.
Disclosure: **L. Mahmutovic:** None.

371

Does low birth weight and type 2 diabetes share a common genetic background?

O. Al-Allaf;
Exeter Medical School, Exeter, UK.

Background and aims: It has been shown, in observational epidemiological studies, that a low birth weight (BW) increases the susceptibility of type 2 diabetes (T2D) later in life. The Foetal Insulin Hypothesis proposes that these two phenotypes are of one genotype. We reviewed the literature providing evidence for the genetic factors linking low BW to T2D. Aim: To improve our understanding on the relationship of low BW and T2D at the genome level.
Materials and methods: We conducted a literature search on PubMed, Google Scholar and Springer-Link, using the search terms; 'Genetics', 'Birth Weight' and 'Type 2 Diabetes'. We found 314 publications and after applying our exclusion criteria we left with 4 papers to review (Figure 1).
Results: The publications we looked at had tested whether common genetic variants that predispose to T2D are also linked to low BW. Over the 4 papers (including 63,332 candidates in total) we looked at, we found a total of 6 Single Nucleotide Polymorphisms (SNPs) on 4 different loci that increase susceptibility to T2D and are associated with low BW. The CDKAL1-rs10946398 variant was associated with a reduction in BW by 41 g and 21 g in 2 different papers with *P* values of 0.034 and 2×10^{-5} respectively. The CDKAL1-rs7756992 variant showed a reduction in BW by 36 g and 22 g with *P* values of 0.048 and 0.04 respectively. The rs900400 SNP near CCNL1 reduced BW by 40 g ($P = 2 \times 10^{-35}$). The rs9883204 SNP on the *ADCY5 loci showed a 30 g reduction in BW* ($P = 7 \times 10^{-15}$) and the rs11708067 variant on the same loci showed a 33 g reduction with $P = 0.004$. Lastly, the HHEX-IDE-rs1111875 reduced BW by 14g ($P = 0.004$). (All figures for BW reduction stated are 'per risk allele'). Discussion: Insulin is vital for foetal growth and metabolism through life. SNPs in CDKAL1, HHEX-IDE, ADCY5 and near CCNL1 can affect the pancreatic beta cell function, resulting in low insulin mediated foetal growth and the increased risk of T2D in later life, which is strong evidence for the foetal insulin hypothesis. Clearly, the above-mentioned gene variants are associated with low BW, which was statistically significant ($P < 0.05$ in all of the above studies). On the other hand, these SNP's were found to increase susceptibility to T2D according to other studies previously done. This demonstrates that there is a clear overlap between the genetics of T2D and foetal growth which suggests that lower BW and T2D may be two phenotypes of one genotype. Also, low birth weight might be a predictor for future T2D.
Conclusion: Current studies showed clearly that low birth weight and future susceptibility to T2D share a common genetic background. This supports the Foetal Insulin Hypothesis.



Disclosure: **O. Al-Allaf:** None.

372

Variants in genes regulating vitamin D metabolism (DHCR7, CYP2R1 and GC) determine low vitamin D levels in type 2 diabetic patients

L. Bertoccini[1], D. Bailetti[1], I. Barchetta[1], C. Pibiri[1], F. Bagella[1], T. Filardi[1], E. Alessi[1], M. Incani[2], S. Carletti[3], S. Pezzilli[1], P. Piscitelli[4], G. Leanza[5], U. Di Folco[6], SUMMER Study Group, M.G. Baroni[1];
[1]Sapienza University of Rome, Rome, [2]University of Cagliari, Cagliari, [3]Pertini Hospital, Rome, [4]IRCCS CSS, S. Giovanni Rotondo, [5]Campus Biomedico, Rome, [6]San Camillo Forlanini Hospital, Rome, Italy.

Background and aims: Hypovitaminosis D is associated with an increased prevalence and incidence of metabolic syndrome and type 2 diabetes (T2D). Low vitamin D levels are constantly present in T2D patients. A large meta-analysis of GWAS of serum 25-hydroxyvitamin D identified variants in 3 genes involved in vitamin D metabolism (DHCR7, CYP2R1 and GC). So far, the association between these variants and vitamin D levels has been studied only in the general population, while no robust data are available in the context of T2D. Our aim was to investigate the role of variants in DHCR7, CYP2R1 and GC genes, considered either individually or in combination, on serum vitamin D concentrations in a large and very homogeneous cohort of Italian patients with T2D.
Materials and methods: The first 2165 consecutive study subjects of the "Sapienza University Mortality and Morbidity Event Rate (SUMMER)

study in diabetes" cohort were studied. Clinical data of all participants were collected. Centralised measurements of serum vitamin D levels were carried out in all patients. The following SNPs were studied: DHCR7 rs12785878 T>G, CYP2R1 rs10741657 G>A, GC rs4588 G>T.

Results: The rs12785878 SNP of DHCR7 gene was significantly associated with vitamin D levels (23.8 ± 10.2, 22.6 ± 10.1 and 21 ± 9.5 ng/ml in TT, TG and GG individuals, respectively, $p = 1.7 \times 10^{-4}$). The allelic OR for vitamin D insufficiency (<30 ng/ml) was 1.28 [CI = 1.09–1.51], $p = 3 \times 10^{-3}$. A similar tendency toward association was observed between the CYP2R1 rs10741657 SNP and vitamin D levels (24.7 ± 12.2, 23.3 ± 10.8 and 22.6 ± 9.4 ng/ml in AA, AG and GG individuals, respectively, $p = 0.20$). The allelic OR for vitamin D insufficiency was 1.18 [CI = 1–1.38], $p = 0.042$. The GC rs4588 SNP was significantly associated with vitamin D levels (24.1 ± 10.7, 22.3 ± 9.5 and 20.8 ± 8.7 ng/ml in GG, GT and TT individuals, respectively, $p = 3.5 \times 10^{-5}$). The allelic OR for vitamin D insufficiency was 1.36 CI = [1.14–1.61], $p = 4.7 \times 10^{-4}$. A weighted genotype risk score (w-GRS) was then calculated by summing the risk alleles of the 3 SNPs in each individual, weighting each risk allele with the effect size for risk of hypovitaminosis D (<30 ng/ml). We observed a strong association with vitamin D levels, decreasing significantly from the first to the last w-GRS category (24.3 ± 11, 24.8 ± 11.5, 22 ± 8.9, 21 ± 9 ng/ml respectively, $p = 1.0 \times 10^{-7}$), with an OR for the subgroup with 3+ risk alleles (vs. 0 alleles carriers) of 1.24 CI = [1.13–1.37], $p = 1.1 \times 10^{-5}$.

Conclusion: In this study, we observed a significant association between DHCR7 rs12785878 and GC rs4588 variants with lower vitamin D levels in T2D patients. When the 3 variants were considered together as GRS, a highly significant association was observed both with vitamin D levels and with the risk of hypovitaminosis D. These results provide strong evidence of the effects of variability in genes involved in vitamin D metabolism on vitamin D levels in patients with T2D.

Clinical Trial Registration Number: NCT02311244
Supported by: SID 2016; Sapienza 2015
Disclosure: **L. Bertoccini:** None.

373

Association of CUBN gene variants with type 2 diabetes and vitamin D levels in an elderly Greek population

X. Tsekmekidou[1], F. Tsetsos[2], S. Karras[1], M. Georgitsi[3,2], A. Roumeliotis[4], S. Panagoutsos[4], E. Thodis[4], M. Theodoridis[4], N. Papanas[5], D. Papazoglou[5], P. Pasadakis[4], E. Maltezos[5], P. Paschou[2], K. Kotsa[1];
[1]Division of Endocrinology and Metabolism, 1st Dept of Internal Medicine, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, [2]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, [3]Laboratory of General Biology-Genetics, Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki, [4]Department of Nephrology, Democritus University of Thrace, Alexandroupolis, [5]Diabetes Centre, Second Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece.

Background and aims: Vitamin D has been shown to influence both insulin secretion and sensitivity. Genetic variations of *VDR* have been implicated in diabetes mellitus (DM) pathogenesis. Cubilin, encoded by the *CUBN* gene in humans, is involved in the conversion of 25-hydroxyvitamin D [25(OH)D] to biologically active 1α,25-dihydroxyvitamin D in the kidney. Thus, in this study, we aim to explore potential differences of *CUBN* variants in an elderly Type 2 DM (T2DM) population compared to non-diabetic subjects and assess potential effect of *CUBN* variants on 25(OH)D levels.

Materials and methods: In this case-control study, 1258 participants were categorized as T2DM patients ($n = 716$) by diabetes history, HbA1c and fasting plasma glucose levels and non-DM controls ($n = 542$) by absence of history of T2DM, HbA1c <6.5%, fasting glucose <126 mg/dl and age >65 years. Subjects on vitamin D supplementation were excluded. Informed consent was obtained and whole blood was collected for DNA extraction. Samples were analysed on Illumina Infinium PsychArray. After individual and SNP quality control, polymorphisms of *CUBN* were selected. For compound analysis, the PLINK software suite (v1.9) was used. Permutation test analysis was implemented to determine statistical significance ($p < 0.05$). Vitamin D levels [25(OH)D] were measured in a sub-group ($n = 276$) and *CUBN* variants were further analyzed. We also employed a correction method based on effective SNPs instead of Bonferroni, resulting to a $p = 0.05/115 = 4.434e^{-4}$ as significance cut-off.

Results: Female subjects predominated in study population (55.5%). Our results indicated a potential association of *CUBN* variants with T2DM. Permutation analysis associated rs11254375 ($p = 0.00049$, OR = 1.482), rs6602175 ($p = 0.016$, OR = 0.822), rs1801224 ($p = 0.025$, OR = 0.830), rs4366393 ($p = 0.028$, OR = 0.829) and rs7071576 ($p = 0.04$, OR = 1.219) with disease. Mean 25(OH)D levels were significantly lower in patients with T2DM than in the control group (16.705 ± 6.69 ng/ml vs 18.51 ± 6.71 ng/ml, $p < 0.05$), although both groups were vitamin D deficient. In a further quantitative analysis, rs41301097 was strongly associated with higher 25(OH)D levels ($p = 5.233e^{-6}$, beta = 15.95).

Conclusion: Our results indicate a potential role of *CUBN* in T2DM pathogenesis. Rs11254375 and rs7071576 exhibited a significant association with disease, whereas other variants show a potential protective role. These results may suggest an indirect effect of vitamin D metabolism in the pathogenesis of T2DM. Interestingly, in our quantitative analysis rs41301097 was associated with higher vitamin D levels, exhibiting a protective role against vitamin D deficiency. Further studies are required to replicate our findings and to further explore the role of cubilin in T2DM pathogenesis.

Supported by: THALES. ESF (MIS 380273)
Disclosure: **X. Tsekmekidou:** None.

PS 015 Environment and beta cell damage

374

Novel roles of alpha-4, a non-canonical scaffolding subunit of protein phosphatase 2A, in the onset of beta cell dysfunction under glucotoxic conditions

A. Kowluru;
Wayne State University, Detroit, USA.

Background and aims: Despite a growing body of evidence suggesting key roles for protein kinases in islet beta-cell function, protein phosphatases remain an under studied class of signaling proteins in islet biology. The protein phosphatase 2A (PP2A), which accounts for 80% of total serine/threonine phosphatases, has been implicated in the regulation of cell proliferation, survival and apoptosis. The PP2A is a hetrotrimeric holoenzyme consisting of the structural (A), regulatory (B) and catalytic (C) subunits. Post-translational methylation (at Leu-309) and phosphorylation (at Tyr-307) of PP2Ac have been shown to increase and decrease the catalytic function of PP2A, respectively. We recently demonstrated sustained PP2A in beta-cell models of glucotoxicity, and proposed that hyperactivation of PP2A could lead to dephosphorylation of key proteins requisite for physiological insulin secretion and beta cell proliferation. Herein, we determined putative regulatory roles for alpha-4, a non-canonical adaptor regulatory subunit of PP2A, in the sustained activation of PP2A under glucotoxic conditions.
Materials and methods: Islets were isolated from Sprague-Dawley, Zucker lean control, and Zucker Diabetic rats by the collagenase digestion method. Human islets were from Prodo Labs (Aliso Viejo, CA). INS-1 cells, rat and human islets were cultured under basal (2.5 mM glucose) or glucotoxic (20–30 mM glucose; 24–48 hrs.) conditions. PP2A activity assay kit was from Millipore. Degree of cell death was detected by Annexin V/Propidium fluorescence staining.
Results: Glucotoxic conditions significantly promoted PP2A activity in INS-1 832/13 cells (~3 fold), rodent islets (~1.8 fold) and human islets (~2.2 fold). Sustained activation of PP2A was also seen islets derived from the pre-diabetic (7 weeks; ~1.4 fold) and diabetic (13 weeks; ~2 fold) Zucker diabetic rats. Western blot analysis indicated that alpha-4 is expressed in INS-1 832/13 cells, rat islets and human islets. Furthermore, glucotoxic conditions increased the expression of alpha-4 in INS-1 cells and human islets. siRNA-mediated knockdown of endogenous expression of alpha-4 resulted in significant inhibition (−60%) of high glucose-induced PP2A activity in INS-1 832/13 cells suggesting key regulatory roles for this scaffolding protein in the sustained activation of PP2A under glucotoxic conditions. Lastly, silencing of endogenous alpha-4 expression markedly attenuated high glucose-induced cell death in INS-1 832/13 cells.
Conclusion: Glucotoxic conditions promote activation of PP2A in *in vitro* and *in vivo* models of glucotoxicity and impaired insulin secretion. Alpha-4 is expressed in a variety of insulin-secreting cells including human islets and rat islets. Alpha-4 plays novel regulatory roles in promoting sustained activation of PP2A and associated dysfunction of pancreatic beta-cells. Studies are underway to further define putative signaling mechanisms underlying alpha-4 mediated regulation of PP2A activation including its role in facilitating post-translational modifications of PP2Ac and the holoenzyme assembly of PP2A.
Supported by: Department of VA
Disclosure: **A. Kowluru:** None.

375

Glutamine deprivation induces metabolic adaptations associated with beta cell dysfunction and exacerbate lipotoxicity

R. Carlessi, J. Rowlands, G. Ellison, H.H. de Oliveira Alves, P. Newsholme, C. Mamotte;
School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Australia.

Background and aims: Circulating levels of the amino acid L-glutamine have been reported to be significantly reduced in type 2 diabetes (T2D) patients. Recent studies have demonstrated that supplementation with L-glutamine or the dipeptide L-alanyl-L-glutamine (Ala-Gln) has the potential to improve glycaemic control, although the mechanisms are still largely unknown. We hypothesized that L-glutamine is essential for a healthy beta cell bioenergetics, and deprivation of this amino acid could induce cell metabolic alterations capable of promoting insulin secretory dysfunction. Thus, we evaluated the bioenergetic and insulin secretory responses of beta cells to chronic L-glutamine deprivation in vitro. In addition, we also endeavoured to determine the impact of L-glutamine deprivation to the pathological mechanisms induced by lipotoxicity.
Materials and methods: BRIN-BD11 rat insulin secreting cells were submitted to 24h of either L-glutamine deprivation or treatment in the presence of the non-hydrolysable L-glutamine analogue 6-Diazo-5-oxo-L-norleucine (DON), a potent inhibitor of intracellular L-glutamine metabolism due to irreversible binding to glutaminases. Cellular bioenergetics was monitored by extracellular flux analysis using the XFe96 seahorse analyser. Several other cell metabolic outcomes, including glucose uptake and consumption, gene and protein expression of glycolytic enzymes, as well as determination of insulin production and secretion were evaluated. In addition, the responses of rat islets and BRIN-BD11 cells to palmitate treatment in combination with different levels of L-glutamine deprivation were assessed in terms cell viability, insulin secretion and activation of the apoptotic and endoplasmic reticulum (ER) stress response pathways.
Results: L-glutamine deprivation or inhibition of its intracellular metabolism for 24h induced striking metabolic adaptations. These included a potent reduction in mitochondrial oxidative phosphorylation, evident by marked decrease in oxygen consumption rates (OCR). Measurements of glucose consumption and uptake, however, indicated an increase in glucose utilization. This observation was supported by gene expression analysis, which evidenced a significant increase in several glycolytic enzymes, indicative of a compensatory metabolic adaptation to absence of L-glutamine as a fuel source. Such adaptations were paralleled by activation of the PERK/eIF2α/ATF4 pathway, responsible to drive global inhibition of protein synthesis during nutrient restriction and ER stress. Accordingly, this phenotype was associated with a striking impairment in β-cell function, as demonstrated by a markedly decreased insulin production and secretion. Treatment with palmitate, as expected, led to insulin secretory dysfunction, induction of ER stress, loss of viability and apoptosis in our experimental system. L-glutamine deprivation significantly exacerbated these phenotypes, which could be rescued by supplementation with Ala-Gln in a concentration dependent manner.
Conclusion: Altogether our data suggest that excessively low L-glutamine levels could participate in the process of beta cell dysfunction in T2D, warranting future nutritional strategies in order to re-establish normal levels of this amino acid as a therapeutic approach in T2D.
Supported by: Study supported by funds from the DART and Heart Foundation of Australia
Disclosure: **R. Carlessi:** None.

376

Proteasomal degradation of the histone acetyl transferase p300 contributes to beta cell injury in a diabetes environment

S. Costes[1], L. Ruiz[1], T. Gurlo[2], M.A. Ravier[1], A. Wojtusciszyn[3], J. Mathieu[1], M.R. Brown[4], C. Broca[3], G. Bertrand[1], P.C. Butler[2], A.V. Matveyenko[4], S. Dalle[1];
[1]Institute for Functional Genomics, Montpellier, France, [2]Larry L. Hillblom Islet Research Center, Los Angeles, USA, [3]Laboratory of Cell Therapy for Diabetes, Montpellier, France, [4]Mayo Clinic School of Medicine, Rochester, USA.

Background and aims: In type 2 diabetes (T2D), amyloid oligomers, chronic hyperglycemia, lipotoxicity and pro-inflammatory cytokines are detrimental to beta-cells, causing apoptosis and impaired insulin

secretion. The histone acetyl transferase p300, involved in remodeling of chromatin structure by epigenetic mechanisms, is a key activator of the transcriptional machinery. Whereas p300 appears as a central integrator of various signaling pathways, the regulation and biological actions of p300 in pancreatic beta-cells remain elusive. Here, we aimed to study the potential role of p300 in beta-cell survival and to investigate its mechanism of regulation in beta-cells exposed to stress situations known to be associated with T2D.

Materials and methods: Experiments were performed with the pancreatic beta-cell line (INS-1E), isolated mouse pancreatic islets and human pancreatic islets. p300, Pdx-1 and Nkx6.1 levels were evaluated by western blot. p300 mRNA levels were analyzed by RT-PCR. p300 protein levels were evaluated by immunofluorescence in pancreatic tissue obtained from human subjects with T2D versus BMI-matched control subjects. Apoptosis was evidenced by cleaved caspase-3 emergence and TUNEL staining in isolated mouse and human islets.

Results: Inhibition of p300 acetyl transferase activity by the inhibitor C646 in INS-1E cells and mouse islets led to an increase in caspase-3 cleavage ($p < 0.05$ and $p < 0.01$, respectively). The frequency of TUNEL staining in mouse beta-cells and human beta-cells was increased by 2.7 fold and 1.7 fold, respectively, in islets treated with C646 ($p < 0.05$). Inhibition of p300 also led to an alteration of beta-cell function as shown by the decreased insulin stimulation index and the diminished levels of the transcription factors Pdx-1 and Nkx6.1 ($p < 0.05$). Knock-down of p300 by siRNA further confirmed an altered beta-cell function and survival. Diabetes-related conditions (amyloid, glucolipotoxicity, pro-inflammatory cytokines) as well as T2D itself induced a loss of p300 protein levels in mouse and human beta-cells, whereas p300 mRNA levels remained unchanged under these conditions. Treatment of INS-1E cells with the proteasome inhibitor MG-132 prevented the decrease in p300 content induced by high glucose or pro-inflammatory cytokines exposure. Altogether these data point to a proteasomal degradation involved in p300 loss in pathological beta-cells. Finally, we found that activation of melatonin signaling restored p300 levels in beta-cells exposed to diabetic situations.

Conclusion: Our study demonstrates for the first time a key role of p300 in beta-cell survival and function and its alteration under pathological conditions in T2D. We further show that p300 proteasomal degradation plays a role in the pathophysiology of diabetes and constitutes a potential site for therapeutic intervention. Finally, melatonin signaling may represent a strategy for the maintenance of p300 integrity in order to preserve a functional beta-cell mass in T2D.

Supported by: SFD, Paris, France

Disclosure: **S. Costes:** None.

377

Omentin-1, a new adipokine influencing islet cells survival and function

S. Rutti[1], P. Heckman[1], W. Bietiger[1], C. Widmann[2], M. Pinget[1], K. Bouzakri[1];
[1]Centre Européen d'Etude du Diabète, Strasbourg, France, [2]Physiology Departement, University Lausanne, Lausanne, Switzerland.

Background and aims: Adipose tissue secretes a variety of bioactive molecules called adipokines that are now recognized as part of an "adipo-insular axis" whose dysregulation may contribute to beta-cell failure and hence to the development of type 2 diabetes. Omentin-1 is an adipokine that is predominantly expressed in visceral fat where it stimulates glucose uptake in response to insulin. Omentin-1 has also been shown to exert beneficial actions on endothelial cell function and survival and on vascular smooth muscle cells. In a recent study, we have shown that omentin expression in islet cells is positively regulated by IL-13, an interleukin protecting beta-cells from IL-1β induced apoptosis. However, the impact and mode of action of omentin-1 on primary human or murine beta-cell function and survival remain to be explored.

Materials and methods: Human and mouse dispersed islet cells were maintained in culture for 48 h in the presence of omentin-1 alone or in combination with a cytokine cocktail (IL-1β, IFNγ, and TNFα) known to be deleterious to islet cells. Insulin and glucagon secretion in response to glucose were measured by ELISA, proliferation by incorporation of BrdU over 48 h, cell death by TUNEL assay.

Results: Omentin-1 (100 ng/ml) decreased basal cell death in human (normalized to control: 0.62 ± 0.27) and mouse beta-cells (normalized to control: 0.56 ± 0.07) and protected them against cytokine induced cell death (normalized to control: cytokines: 2.24 ± 0.5; cytokines + omentin-1: 1.35 ± 0.4), without affecting beta-cell proliferation. Omentin-1 decreased glucose stimulated insulin secretion of mouse beta-cells (decreased to $1.6 \pm 0.3\%$ of total insulin content/h at 16.7mM from $3.4 \pm 0.7\%$) but did not affect human insulin secretion. Interestingly, omentin-1 increased glucagon secretion of human islets.

Conclusion: We show for the first time that omentin-1 improves primary beta-cell survival and protects them against cytokine induced beta-cell death without affecting proliferation. Omentin-1 also modulates insulin and glucagon secretion suggesting that this adipokine might influence beta and alpha cells secretory and survival capacities.

Disclosure: **S. Rutti:** None.

378

Activation of Ang II type 2 receptor (AT2R) protects pancreatic islets function via regulation of apoptosis and autophagy in obese rats

M. Liu[1], X. Li[2], A. Wang[2], S. Yin[1], Y. Mu[2];
[1]The First Affiliated Hospital of Chinese PLA General Hospital, Beijing, [2]Chinese PLA General Hospital, Beijing, China.

Background and aims: Activation of AT2R has been examined as a potential therapeutic strategy in cardiovascular and central nervous systems. However, there is few findings regarding the role of activation of AT2R in islets. In the current study, we evaluated the effects of Compound 21 (C21), a nonpeptide AT2R agonist, on the islets in obese rats induced by high-fat diet (HFD) to investigate the role of activation of AT2R in pancreatic islet.

Materials and methods: Adult male Sprague-Dawley (SD) rats were randomly assigned into four groups: normal (fed with normal diet), HFD and HFD respectively plus C21 (1 mg/kg/d) and telmisartan (Tel, AT1R antagonist, 1 mg/kg/d). Tel and C21 were continually given by oral administration for four weeks. After treatment, the rats received an intraperitoneal glucose tolerance test (IPGTT), and the pancreases were harvested to examine islet morphology and biochemical parameters of insulin secretion, apoptosis and autophagy by immunohistochemical, immunofluorescence and Western Blotting. Mitochondria and autophagy were observed by Electron microscope technique.

Results: We found that, compared with control HFD rats and HFD rats treated with Tel, those HFD rats treated with C21 displayed lower blood glucose lever, higher serum insulin concentration and improved glucose tolerance. These rats had more integrated islets, larger positive insulin-staining islet mass ratio and higher PDX-1, GLUT2 and GCK protein expressions. Western Blotting showed that anti-apoptosis factor Bcl-2 and p-Akt expression is up-regulated accompanied with down-regulated cleaved caspase-3 expression in the pancreas of those HFD rats treated with C21. Autophagy markers including LC-3B and Beclin-1 expression are also increased in those rats. Electron microscope showed that C21 treatment increased the autophagy and ameliorated the mitochondrial vacuolation against lipotoxicity in obese rats induced by HFD.

Conclusion: These data suggest that C21 protects pancreatic islets function against lipotoxicity via regulation of apoptosis and autophagy.

Supported by: 2016 Beijing Nova Program

Disclosure: **M. Liu:** None.

379

Overexpression of sphingosine-1 phosphate lyase sensitises insulin-secreting INS1E cells to lipotoxicity

E. Gurgul Convey, D. Kindler, H. Möller-Gnangra;
Institute of Clinical Biochemistry, Hannover School of Medicine, Hannover, Germany.

Background and aims: Lipotoxicity plays an important role in pancreatic beta cell dysfunction and failure in type 2 diabetes. Lipid oversupply has been shown to dysregulate formation of bioactive lipids and to induce oxidative stress in many cell types. An imbalance in the sphingolipid metabolism is a common feature during the development of many inflammatory diseases. The sphingolipid pathway is tightly regulated by a network of enzymes controlling generation and metabolism of various sphingolipids. The final step in this complex pathway is catalyzed by the enzyme sphingosine-1 phosphate lyase (SPL). The aim of this study was to analyze the role of SPL in insulin-secreting INS1E cells exposed to lipotoxic conditions.
Materials and methods: Insulin-secreting INS1E cells were stably transfected either with an empty pcDNA3.1 vector (INS1E-control) or with the pcDNA3.1-SPL vector (INS1E-SPL). Cells were treated with 250 μM palmitate (PA), 250 μM oleate (OA) or a combination of both fatty acids (FFA) for 24 h. Thereafter cell viability was estimated by a MTT assay, cell proliferation by BrdU ELISA, oxidative stress by DCFDA oxidation and gene expression analyses were performed by qRT-PCR.
Results: The expression of SPL was strongly increased by PA in INS1E cells. Exposure of INS1E-control cells to PA resulted in ~40% decrease of cell viability, an effect that was strongly potentiated by SPL overexpression (~70% cell viability loss, $p < 0.01$). OA was not toxic to INS1E-control cells and protected against PA-mediated cell viability loss. Interestingly, exposure of INS1E-SPL cells to OA led to a significant ~45% decrease of cell viability. Furthermore, in INS1E-SPL cells PA inhibited cell proliferation significantly more strongly than in INS1E-control cells. PA-exposed INS1E-SPL cells were also characterized by a higher level of oxidative stress as compared to INS1E-control cells. The toxicity of OA in INS1E-SPL cells was linked with reduced cell proliferation and increased oxidative stress. The expression of sphingolipid-4-delta-desaturase (SPL4dDes), an enzyme catalyzing the formation of ceramides, was enhanced by PA and OA in INS1E-SPL cells.
Conclusion: Our results showed that SPL overexpression sensitized insulin-secreting INS1E cells to PA toxicity and, interestingly, also induced OA toxicity. The mechanism was dependent on the reduction of cell viability and proliferation. These effects strongly correlated with the induction of oxidative stress and SPL4dDes expression, suggesting the involvement of SPL in the FFA-mediated ceramide generation. Thus, an imbalance in the sphingolipid metabolism may be an important mechanism of lipotoxicity in insulin-secreting cells.
Disclosure: **E. Gurgul Convey:** None.

380

High-fat diet accelerates pancreatic stellate cell activation and initiates islet fibrosis during pancreatogenic diabetes

X. Zhu[1], J. Sun[2], L. Li[1];
[1]Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, [2]Shaoxing University Medical School, Shaoxing, China.

Background and aims: Epidemiological studies support strong links between obesity, diabetes, and chronic pancreatitis (CP). Risk factors for type 2 diabetes include overweight and obesity, which may accelerate the presentation of diabetes in the context of pancreatic disease. However, no direct evidence has supported the causal association between high-fat diet (HFD) and the risk of pancreatogenic diabetes. Here, we investigated the effects of HFD on the activation of pancreatic stellate cells (PSCs), development of pancreatic fibrosis, and destruction of pancreatic islets by using a CP mice model.
Materials and methods: A total of 40 male C57BL/6 mice were randomly selected and were divided into four groups, namely, Alc-Cer plus HFD, Alc-Cer plus normal chow diet (ND), saline plus HFD, and control (saline plus ND). CP was induced by intraperitoneally injecting mice with ethanol (3.2 g/kg, 30% v/v) and cerulein (50 μg/kg) for 6 weeks. The serum amylase activity was assayed after the last injection. HFD was given starting on the 56th day up to the fifth month in two cohorts (HFD and Alc-Cer plus HFD). The body weights and blood glucose levels were monitored every two weeks. At the end of the experiment, glucose tolerance and insulin-resistance tests were performed. Blood samples were collected to measure the lipid metabolism, insulin, and transforming growth factor β (TGF-β). The pancreas was collected to observe the pancreatic morphology by HE staining and to characterize the pancreatic fibrosis by Sirius red staining. Tissues were analyzed using immunofluorescence for insulin and α-smooth muscle actin (α-SMA) to visualize β cells and activate PSCs, respectively. The expression levels of collagen type I (Col-I), fibronectin (FN), and TGF-β were assayed using immunohistochemistry. The expression levels of Col-I, FN, TGF-β and α-SMA in the pancreas were assessed using real-time PCR or Western blot.
Results: Significant difference was observed in the amylase levels of the CP and control groups ($P < 0.05$). After 19 weeks, all mice fed with HFD gained significant increase in weight compared with those mice fed with ND ($P < 0.05$). Only a significant increase in total cholesterol concentrations in the plasma was observed in all HFD groups ($P < 0.05$). The mice in the Alc-Cer plus HFD cohorts developed hyperglycemia and hypoinsulinemia and exhibited elevated levels of TGF-β ($P < 0.05$). The mice with CP fed with HFD remained glucose intolerant and insulin resistant compared with the control mice. All mice that received Alc-Cer injections exhibited histopathological signs of CP with significant pancreatic atrophy, severe acinar architectural damage, and edema, especially the Alc-Cer plus HFD group. In comparison with the control group, the mice with CP fed with HFD for 3 months displayed extensive extracellular matrix deposition and activated PSCs in the islet and peri-islet exocrine pancreas. A decrease in the pancreatic islet numbers and size was exhibited in the pancreatic tissues of the Alc-Cer plus HFD mice. Western blot and RT-qPCR confirmed the enhanced expression of Col-I, FN, α-SMA, and TGF-β in the Alc-Cer plus HFD mice compared with those in the other three groups ($P < 0.05$).
Conclusion: HFD exerts a detrimental influence on the progression of pancreatogenic diabetes by inducing PSCs activation, pancreatic β cell dysfunction, and islet fibrosis.
Disclosure: **X. Zhu:** None.

381

MSCs improved insulin resistance and beta cell function in type 2 diabetes through modulation of macrophage polarisation and restoration of autophagy in insulin-targeted organs and islets

Y. Mu;
Department of Endocrinology, Chinese PLA General Hospital, Beijing, China.

Background and aims: Type 2 diabetes(T2D) has become one of the leading health problems in the world. T2D often is controlled by oral or injected therapeutics. However, till now, a considerable amount of diabetic patients didn't have adequate glycemic control. Thus alternative therapeutic approaches are urgent to be investigated. Insulin resistance and progressive β-cell dysfunction are recognized as fundamental pathologies of T2D. Mesenchymal stem cells (MSCs) are multipotent, and can be easily isolated from several human tissues such as bone marrow, adipose tissue, and umbilical cord. Small sample clinical trials showed that MSC infusion significantly decreased the levels of blood glucose and HbA1c and reduced daily insulin doses. Inspiringly, animal experiments

showed that MSCs infusion improved insulin resistance and β-cell function in T2D animal models. All this data makes MSCs a promising tool for the treatment of T2D. So our team was devoted to reveal the underlying mechanisms for MSCs in improving insulin resistance and β-cell function, and to develop new strategies to assist MSCs and enhance their efficiency in improving metabolic control in subjects with T2D.

Materials and methods: We induced a T2D model using a combination of a high-fat diet (HFD) with low-dose streptozotocin (STZ). Intravenously infused human umbilical cord-derived MSCs (UC-MSCs) promoted insulin sensitivity and islet function.

Results: On one hand, M1 macrophages in both adipose tissue and islets were directed towards an anti-inflammatory M2-like state after UC-MSC infusion. In vitro study also proved that UC-MSCs inhibited the activation of the M1 phenotype and induced the generation of the M2 phenotype via secretion of interleukin (IL)-6, blocking which by small interfering RNA (siRNA) largely abrogated the UC-MSCs effects on macrophages both in vitro and in vivo and resulted in dampened insulin sensitivity and β-cell function in T2D mice. On the other hand, MSC infusion upregulated LAMP2 expression and enhanced formation of autophagosomes and autolysosomes in hepatic cells and β-cells, which probably also contributed in the promotion of insulin sensitivity and β-cell function. In addition, combination of low-dose decitabine, an FDA-approved DNA methylation inhibitor, or increasing IL-6 secretion by preconditioning MSCs with high glucose and LPS, further promoted M2 macrophage polarization in adipose tissue and islets, enhancing the effects of MSCs on insulin sensitivity and β-cell function in T2D mice. Last but not the least, we first use low-dose STZ combined with long-term HFD to establish a late-stage T2D complication rat model, and multiple infusions of MSCs promoted the recovery of various targeted organs such as kidney, heart, lens, lung and liver in morphology and function.

Conclusion: In conclusion, MSCs improved insulin resistance and β-cell function in T2D through modulation of macrophage polarization and restoration of autophagy in insulin-targeted organs and islets. Combining MSCs with low-dose decitabine and pre-conditioning of MSCs prove to be effective strategies to improve metabolic control by promoting M2 macrophage polarization. Multiple infusions of MSCs also showed stunning therapeutic effects in late-stage T2D complications.

Supported by: 2013AA020105 and 2012AA020502

Disclosure: **Y. Mu:** None.

PS 016 Regulation of functional beta cell mass

382

Characterisation of enteroendocrine transcription factors during development in the human duodenum and pancreas

J. Li, R. Wang;
Physiology & Pharmacology, The University of Western Ontario, London, Canada.

Background and aims: Pancreatic bud formation arises from the duodenal endoderm. Both the duodenum and pancreas share similar transcription factors (TFs) and hormone-secreting endocrine cells, and their major development stages occur from 8 to 21 weeks of fetal age. Growth factors produced by the enteroendocrine cells (EECs) of the duodenum and pancreas play a critical role in regulating islet cell proliferation and glucose metabolism. However, the temporal expression of shared EECs and TFs during human duodenal and pancreatic development are not fully documented. This study aimed to characterize common TFs and EECs present during development in the human fetal duodenum and pancreas

Materials and methods: Human fetal duodenum and pancreas samples were collected during the 1st (8–10 weeks of fetal age) and 2nd (19–21 weeks of fetal age) trimester of pregnancy. Microarray was performed with Affymetrix Gene Chip Human Genome U133 Plus 2.0 Array chips, and data for EECs and TFs were verified with qRT-PCR. The co-localization of TFs in EECs and epithelial cells were examined and quantified.

Results: 11 common TFs were expressed in both organs, with 7 TFs (ie. *PDX1, NGN3, PAX4*) expressed at a consistent level during development and only *NEUROD1* increased in both organs from the 1st to 2nd trimester. *PAX6*, *ISL1*, and *NKX2-2* increased significantly in the pancreas during the 2nd trimester, but reduced *ISL1* and *NKX2-2* expression was observed in the duodenum as development progressed. *NKX2-3*, required for duodenal development, was barely detected in the pancreas, while *ARX* expression increased 5-fold in the pancreas at the 2nd trimester. Chromogranin-A^{+} (EEC populations) staining showed EECs present in small villi and underdeveloped crypts of the duodenum and pancreatic duct-buds during the 1st trimester. The population of EECs increased in the crypt-villus structure and adult-like islet clusters at the 2nd trimester of development. 10 subtypes of EECs were found in both the duodenum and pancreas, while gastrin and CCK expression were exclusive to the developing duodenum. Co-localization of TFs with EECs was identified in both organs throughout the 1st and 2nd trimester of development and included GIP and GLP1 with PAX6, PC1/3 and PC2 with NKX2-2, and SST and PYY with ISL1. The percent of NKX2-2 in EEC populations was significantly reduced in the duodenum but increased in the pancreas by the 2nd trimester. The percent of PDX1^{+}/CK19^{+} cells was increased in the duodenum and reduced in the pancreas from the 1st to 2nd trimester of the development. A high number of SST^{+} and PC1/3^{+} cells was present in both organs, and clusters composed of SST or PC1/3 EECs were found in the submucosa of the developing duodenum.

Conclusion: This study has generated an extensive EEC gene expression profile of the human fetal duodenum and pancreas, confirming that common TFs are necessary for EEC development in both organs. The results from this study represent a small step towards the full understanding of all factors required for the regulation of duodenal and pancreatic endocrine development. Since duodenal and pancreatic hormone regulation are important for maintaining glucose homeostasis in postnatal life, understanding the shared transcription factors that regulate duodenal and pancreatic EEC development may benefit anti-diabetic and stem cell therapeutic targets.

Supported by: Canadian Diabetes Association

Disclosure: **J. Li:** None.

383

A pancreas-specific ECM scaffold - human iPS cell culture and pancreatic differentiation goes 3D

C. Berger[1], Y. Bjørlykke[2], M. Mühlemann[1], H. Ræder[2,3], H. Walles[4,1], M. Metzger[4], D. Zdzieblo[1];
[1]Chair Tissue Engineering & Regenerative Medicine (TERM), University Hospital Wuerzburg, Wuerzburg, Germany, [2]Department of Pediatrics, Haukeland University Hospital Bergen, Bergen, Norway, [3]KG Jebsen Center for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, Norway, [4]Translational Center Regenerative Therapies (TLC-RT), Fraunhofer Institute for Silicate Research ISC, Wuerzburg, Germany.

Background and aims: Human induced pluripotent stem cell- (hiPSC) derived ß-cells display a great hope to treat diabetes mellitus. Current *in vitro* differentiation protocols enable the generation of immature ß-cells, which require additional *in vivo* maturation to achieve full functionality. Structural 3-dimensional (3D) characteristics, biophysical cues and biological components of the organ-specific extracellular matrix (ECM) are known to play important roles during organogenesis *in vivo* supporting cell survival, differentiation and function. Therefore, we hypothesize that β-cell differentiation of iPSCs could be improved using ECM-based cell culture systems mimicking the *in vivo* microenvironment. In this study, we report about a pancreas-specific ECM (PanMa) and its use as biological scaffold in 3D static bioreactor cultures for hiPSC maintenance culture and β-cell differentiation.
Materials and methods: The PanMa was generated by perfusion-decellularization of porcine pancreata with a chemical detergent. PanMa characterization was performed qualitatively by (immuno-)histochemical analyses and quantitatively by proteomics. Scanning electron microscopy (SEM) was performed to analyze PanMa ultrastructure. The generated matrix was used as liquid supplement in monolayer cultures (2D) or as biological scaffold in 3D bioreactor cultures. PanMa influence on hiPSC pluripotency and β-cell differentiation was assessed by analyzing cell type-specific gene and protein expression profiles under PanMa-based conditions.
Results: Matrix characterization studies demonstrate that the decellularized PanMa scaffold maintains key ECM components found in native tissue, such as Elastin, Laminin, Collagen I and IV. Furthermore, SEM analysis revealed preservation of ultrastructural features such as vascular structures. Human iPSCs cultured on PanMa scaffolds were viable for at least 12 days retaining cellular characteristics such as colony formation and proliferation. However, PanMa-based maintenance culture of iPSCs resulted in loss of pluripotency characterized by a diminished NANOG protein expression. Notably, iPSCs cultured on PanMa scaffolds under conditions allowing spontaneous differentiation revealed an altered gene expression pattern in comparison to typical embryoid body differentiation. Furthermore, β-cell differentiation under PanMa conditions resulted in the formation of insulin^{+}cell clusters and altered gene or protein expression for characteristic β-cell markers with a tendency for improved expression under ECM conditions.
Conclusion: In summary, we were able to establish a biological ECM from porcine pancreata termed PanMa that retains important ECM characteristics. Further, it is suitable as biological scaffold for hiPSC culture and differentiation in 3D. First *in vitro* experiments suggest that the organ-specific PanMa affects pluripotency and promotes differentiation towards the pancreatic lineage. Together, we hypothesize positive impacts of the organ-specific ECM on quality and function of iPSC-derived β-cells.
Supported by: Elitenetzwerk Bayern, BayFS
Disclosure: **C. Berger:** None.

384

The epigenetic characteristics of liver to pancreas transdifferentiation

S. Ferber[1,2], H. Cohen[1], A. Har-Zahav[1,2], M. Szyf[3], D. Cheishvili[3], I. Meivar-Levy[1];
[1]The Sheba Regenerative Medicine, Stem Cell and Tissue Engineering Center, Sheba Medical Center, Tel-Hashomer, Israel, [2]Dept. of Human Molecular Genetics and Biochemistry, Tel-Aviv University, Tel-Aviv, Israel, [3]McGill University, Montreal, Canada.

Background and aims: Transdifferentiation is the direct reprogramming of adult cells into alternate cell types with different function. The efficiency of the process between most tissues analyzed is generally low. Liver to pancreas transdifferentiation (TD) induced by ectopic expression of pancreatic transcription factors (pTFs) is limited to up to 15% of the pTFs treated human liver cells in culture. Our previous data indicated that; The process is restricted to a sub-population of human liver cells that are persistently predisposed to undergo reprogramming while >85% of the cells resist TD. TD-resistant can be converted into TD-prone cells by overcoming the identified epigenetic barriers. Currently, we are analyzing the epigenetic characterization of the TD prone cells and of the TD process.
Materials and methods: A method for preselecting TD-predisposed liver cells by a lineage tracing approach based on the activation of a WNT response element, is suggested.
Results: The TD-propensity of the liver cells is stable and inherited to daughter cells upon proliferation. The separated WNT-responsive cells undergo efficient transdifferentiation and up to 70% of the pTFs treated cells produce and secrete insulin in a glucose-regulated manner. Continuously active WNT signaling is obligatory but insufficient for TD propensity to pancreas, since reconstruction of active WNT signaling does not allow the activation of the pancreatic lineage in TD-resistant liver cells. Our data suggest that pancreatic genes' chromatin is more transcrpition-permissive in transdifferentiation-predisposed than in recalcitrant liver cells. TD-predisposed liver cells display a reduced level of DNA methylation which further decreases upon the induction of reprogramming. Our data suggest a crucial role for epigenetic alterations induction in extending the transdifferentiation capacity to originaly transdifferentiation resistant cells.
Conclusion: Our results suggest that transdifferentiation is restricted to a specific population within the tissue, which harbors obligatory signaling patterns and specifically permissive epigenome. The efficient generation of insulin producing cells by adult cells reprogramming relies on the epigenetic landscape modulation. The extension of transdifferentiation capacity to most of the cells in culture, dramatically increase in transdifferentiation efficiency allowing the diabetic patient to serve also as the donor of his own therapeutic tissue
Supported by: ISF-JDRF programme, ISF, EFSD, Dia-Cure P37, Orgenesis LTD
Disclosure: **S. Ferber:** Employment/Consultancy; Orgenesis. Stock/Shareholding; Orgenesis.

385

The global identification of glucose-regulated RNA binding proteins (RBPs) in pancreatic beta cells and their role in beta cell function and diabetes

T.P. Herbert, R. Wardman, J. Whitehead;
University of Lincoln, Lincoln, UK.

Background and aims: RNA-binding proteins (RBPs) play an essential role in regulating insulin gene expression, and coordinating complex biological and metabolic processes including cell replication, differentiation, and division. The aim of this study was to identify all RBPs within pancreatic β-cells and to quantify changes in their binding to RNA in response to glucose. We hypothesise that this will reveal unique insights into the role of RBPs in β-cell physiology and uncover hitherto unknown connections between RBPs and diabetes.
Materials and methods: Using 'interactome capture' RBPs (from 3 independent experiments) were isolated and purified from mouse clonal pancreatic β-cells (MIN6). The RBPs were identified and quantified

using Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH-MS) and the data verified by Western blot analysis in both MIN6-cells and primary rodent islets. Ontological analysis and interrogation of published data sets were used to identify the RBPs potential roles in controlling metabolic pathways and molecular processes, and their putative importance in the development of β-cell dysfunction in diabetes.

Results: Using interactome capture we have determined system-wide changes in RBP interactions with RNA in response to glucose in β-cells. This revealed 398 RBPs of which the RNA binding activity of 55 was signficantly increased by glucose (>1.5 fold, $p < 0.05$). 122 RBPs were identified as having known function in ribosome biogenesis, RNA processing, export, or translation. Interestingly, 29 RBPs are enzymes involved in intermediatary metabolism, such as hexokinase, alpha enolase, and LDH, and over 22 RBPs were identified as being involved in secretion, such as RAB10 and SEC22. There is a precedent for enzymes 'moonlighting' as RBPs. For example, aconitase is an iron regulatory protein that has been shown to bind to the mRNA of genes involved in iron metabolism, including ferritin, to modulate their expression. Intriguingly, the expression of some of the RBPs identified, such as DDX5 an RNA helicase, LDH, and RAB10, are dysregulated in human β-cells isolated from subjects with type 2 diabetes.

Conclusion: As each RBP can potentially regulate the expression of a subset of mRNAs to coordinate a biological/metabolic process or pathway, our data has revealed new regulatory links between gene expression, nutrient metabolism and insulin secretion. We are now in the process of verifying changes in RBP binding in human EndoC-βH1 cells and identifying the RNAs that bind to specific RBPs using Photoactivatable Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) in concert with RNA sequencing.

Disclosure: **T.P. Herbert:** None.

386

The E3 SUMO ligase PIASy is a novel interaction partner regulating the activity of diabetes associated hepatocyte nuclear factor-1 alpha

A. Kaci[1,2], M. Keindl[1], P. Njølstad[1,3], L. Bjørkhaug[4], I. Aukrust[1,2];
[1]KG Jebsen Center for Diabetes Research, University of Bergen, Bergen, [2]Department of Medical Genetics, Haukeland University Hospital, Bergen, [3]Department of Pediatrics and Adolescents, Haukeland University Hospital, Bergen, [4]Department of Biomedical Laboratory Sciences and Chemical Engineering, Western Norway University of Applied Sciences, Bergen, Norway.

Background and aims: The transcription factor hepatocyte nuclear factor-1 alpha (HNF-1A) is involved in normal pancreas development and function. Rare variants in the *HNF1A* gene can cause monogenic diabetes, while common variants confer type 2 diabetes risk. The precise mechanisms for regulation of HNF-1A, including the role and function of post-translational modifications, are still largely unknown. Since SUMOylation is a post-translational regulatory mechanism previously shown to regulate key proteins in glucose homeostasis, the aim of this study was to investigate the functional relevance of HNF-1A SUMOylation in various cell models.

Materials and methods: HNF-1A SUMOylation was assessed in HEK293 cells by immunoprecipitation analysis. A rat albumin promoter-linked luciferase reporter assay in MIN6 β-cells measured the effect of SUMOylation on HNF-1A transcriptional activity. The interaction between protein inhibitor of activated STAT (PIASγ) and HNF-1A, and its subsequent effect on nuclear distribution was investigated in HEK293 cells by co-immunoprecipitation and immunofluorescence experiments, respectively.

Results: Here, we present the first evidence for HNF-1A being a substrate for SUMOylation *in cellulo* identifying three lysine (K) residues (K205, K273 and K506) as SUMOylation sites. Overexpression of the E3 SUMO ligase, PIASγ, repressed the transcriptional activity of HNF-1A and was independent of HNF-1A SUMOylation. Furthermore, PIASγ was demonstrated to interact with HNF-1A, and sequestrate HNF-1A in the nuclear periphery.

Conclusion: SUMOylation of HNF-1A represents a novel post translational regulatory mechanism affecting HNF-1A function. Further, we have identified PIASγ is a new HNF-1A interaction partner that enhances HNF-1A SUMOylation and leads to re-localization of HNF-1A to the nuclear periphery, presumably restricting access to its target genes and thereby reducing the overall HNF-1A transcriptional activity. Our findings reveal potential new targets for drug development and precision medicine in diabetes.

Supported by: Helse Vest, KG Jebsen Foundation, Norwegian Diabetes Foundation, UiB, ERC

Disclosure: **A. Kaci:** None.

387

The absence of melatonin during pregnancy impairs energy metabolism and maternal pancreatic remodelling: Is it the onset of type 2 diabetes in the mother?

P.R.L. Gomes[1], E.A. Leite[1], C.F. Lucena[1], A.C.M. Bonassa[1], E.A. Vilas-Boas[1], F.G. Amaral[2], A.R. Carpinelli[1], J. Cipolla-Neto[1];
[1]Physiology and Biophysics, University of Sao Paulo, Sao Paulo, [2]Physiology, Federal University of Sao Paulo, Sao Paulo, Brazil.

Background and aims: Circulating pineal melatonin, which is elevated at night, acts to synchronize physiological functions, including energy metabolism. Among its actions, this hormone promotes the timing of central and peripheral metabolic functions, playing an important role in the action of insulin and its secretion, as well as local action on trophism and endocrine pancreatic survival. Considering the maternal organism, studies indicate increasing serum concentrations of maternal melatonin during the gestational period in both humans and rodents, due to stimuli of placental hormones. In addition, there is a fine regulation of maternal-fetal energy metabolism, with the main consequence being the expansion of the maternal pancreatic islets during pregnancy, a result of the increase of the beta cell proliferation, returning to pre-gestational conditions around childbirth time. Thus, this study aims to evaluate the role of melatonin on energy metabolism and the remodeling of maternal pancreatic islets during pregnancy.

Materials and methods: Pregnant Wistar rats were divided into: P - pregnant rats, Pinx - pinealectomized pregnant rats, Mel.Fixed - pinealectomized pregnant rats with melatonin replacement at fixed concentrations (0.1 mg/kg for 21 days) and Mel.Dinamic - Pinealectomized pregnant rats with melatonin replacement at variable concentrations (0.1 mg/kg, 1–7 day of pregnancy, 0.2 mg/kg, 8–14 day of pregnancy and 0.5 mg/kg, 15–21 day of pregnancy). The animals were submitted to GTT, ITT on the 7th and 14th days. At the 21th day all groups were euthanized at ZT14 and pancreatic islets were isolated for GSIS, cell viability, apoptosis and cell death and production of ROS. The Ethics Committee for Animal Use approved the study, and complies with the Brazilian Society of Laboratory Animal Science.

Results: Improvement in glucose tolerance was observed in Pinx and Mel.Fixed in G7 ($P = 16718 \pm 1257$; Pinx = 13964 ± 502.4; Mel-Fixed = 13537 ± 1213 and Mel.Dinamic = 15392 ± 1530, $p < 0.05$) and in G14 ($P = 1013 \pm 72.4$; Pinx = 735 ± 85.4; Mel.Fixed = 779.2 ± 64.1 and Mel.Dinamic = 1043 ± 353.4, $p < 0.05$), as well as improvement in insulin sensitivity in G14 ($P = 1.9 \pm 0.8$; Pinx = 2.4 ± 0.3; Mel.Fixed = 3.2 ± 0.3 and Mel.Dinamic = 2.0 ± 0.6, $p < 0.05$). There was no difference in cell viability ($P = 73.9 \pm 7.8$; Pinx = 74.5 ± 8.4; Pinx.Fixed = 71.1 ± 8.2 and Pinx.Dinamic = 71.3 ± 3.9) and cell death ($P = 19 \pm 6$; Pinx = 19.1 ± 6.1; Pinx.Fixed = 21.7 ± 5.6 and Pinx.Dinamic = 18.9 ± 5.6) of the islets among the groups. However cellular apoptosis in the Mel.Dinamic presented apparent increase in relation to the others groups ($P = 7 \pm 3.5$; Pinx5 $\pm$ 1.1=; Pinx.Fixed = 6.3 ± 2.1 and Pinx.Dinamic = 9.7 ± 3.2). Lastly, maximum insulin response to glucose was higher in P rats

compared to the other groups, independent of replacement melatonin or not ($P = 0.2 \pm 0.1$; Pinx = 0.02 ± 0.00; Pinx.Fixed = 0.01 ± 0.00 and Pinx.Dinamic = 0.02 ± 0.00, $p < 0.05$).
Conclusion: Melatonin during pregnancy seems to be responsible for the fine regulation of energy metabolism to preserve adequate substrate for both mother and conception, characterizing it as a delicately architected period. In addition, the absence of melatonin impairs pancreatic remodeling at the end of pregnancy to the return of the tissue to non-gravid conditions, outlining a future setting for the onset of DM2 in the mother.
Supported by: FAPESP, CAPES
Disclosure: **P.R.L. Gomes:** None.

388

Identification of pancreatic elastase inhibitors with a potential to stimulate beta cell proliferation

G. Basile[1], A. Vetere[2], J. Hu[1], K.-C. Liu[3], Y. Zhang[1], O. Andersson[3], B.K. Wagner[2], R.N. Kulkarni[1];
[1]Islet Cell and Regenerative Biology, Joslin Diabetes Center, Boston, USA, [2]Chemical Biology and Therapeutics Science Program, Broad Institute of Harvard and MIT, Cambridge, USA, [3]Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden.

Background and aims: The identification of novel small molecules that can target endogenous factors to regulate β-cell proliferation is a desirable goal to counter Type 1 or Type 2 Diabetes. Recently, we have reported that serpinB1 (SB1), an endogenous protease inhibitor, can promote human β-cell proliferation by inhibiting elastase activity, and demonstrated that the elastase inhibitor, sivelestat, also induced β-cell proliferation. Hypothesizing the importance of blocking elastase activity within islet cells in order to selectively activate the proliferative pathways in β-cells, we employed high-throughput screening(HTS) assays to identify additional novel elastase inhibitors to evaluate their potential for inducing β-cell proliferation.
Materials and methods: We screened 16,320 compounds using a fluorescence-based porcine pancreatic elastase (pPE) assay, and confirmed inhibition of human neutrophil (hNE) and pancreatic (hPE) elastases using an absorbance-based assay. Mouse islets were isolated from ~10 week old C57BL6 mice and human islets were obtained from three healthy control donors from the IIDP. After treatment with increasing doses of the elastase inhibitors, β-cell proliferation was assessed using immunofluorescence techniques.
Results: We identified several compounds among which two emerged as powerful inhibitors of pancreatic elastase (PE): 1) an antiviral drug belonging to the protease inhibitor family, and 2) an antibiotic from the group of β-lactam antibiotics, which had not been previously associated with elastase inhibition and/or β-cell proliferation. Both compounds inhibited porcine PE with greater potency than sivelestat, a known elastase inhibitor, used as a positive control (IC_{50}: antiviral 33.9 nM, antibiotic 1.1 μM, sivelestat 2.4 μM; $n = 3$). The results were confirmed on hPE (IC_{50}: antiviral 15.7 nM, antibiotic 3.6 μM, sivelestat 2.3 μM; $n = 3$), while neither the antiviral nor the antibiotic blocked hNE activity. Evaluation of the mitogenic properties of the compounds in *in vitro* cultures of islets revealed that the antiviral and the antibiotic drugs each promoted proliferation of β-cells in rodent (~3-fold and ~1-fold increase, respectively; $n = 3$) and human islets (~5-fold and ~2.5-fold increase, respectively; $n = 3$) at 100 μg/ml concentration, compared to vehicle-treated islets. Furthermore, in an independent study, both compounds (at 10 μM), stimulated β-cell regeneration in a zebrafish model resulting in ~1.5-fold increase in insulin-positive cells in treated versus non-treated groups.
Conclusion: Taken together, these data, from independent studies, confirm the identification of novel compounds that can enhance mammalian β-cell proliferation, and highlight the therapeutic potential of pancreatic elastase inhibitors to stimulate human β-cell proliferation to treat all forms of diabetes.
Disclosure: **G. Basile:** None.

389

The impact of cilia-genes on pancreatic beta cell replication and the risk of type 2 diabetes

M. Stadion[1,2], O. Kluth[1,2], P. Gottmann[1,2], H. Aga[1,2], U. Krus[3], J. Gerdes[4,2], C. Ling[3], A. Schuermann[1,2];
[1]Experimental Diabetology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Potsdam, Germany, [2]German Center for Diabetes Research (DZD), Neuherberg, Germany, [3]Department of Clinical Sciences, Lund University, Malmö, Sweden, [4]Institute for Diabetes and Regeneration Research, Helmholtz Center Munich, Garching, Germany.

Background and aims: Type 2 diabetes (T2D) results from a complex interplay between environmental stimuli and predisposing genes and is characterized by an insufficient adaptive beta-cell proliferation. Recent data suggest that primary cilia are implicated in beta-cell fuction and the susceptibiltly to T2D. Cilia are dynamic mikrotubule-based hair-like organelles located on almost all polarised mammalian cell types. They function as versatile sensory antennae regulating numerous cellular processes, but their role on beta-cell replication has not been investigated. This study aimed to examine islet cell ciliation and cilia-gene expression in two obese mouse models that differ in their susceptibility to T2D as well as in human pancreatic islets of non-diabetic and diabetic donors.
Materials and methods: Diabetes-resistant B6-ob/ob and diabetes-prone New Zealand Obese mice were fed a carbohydrate-free diet for 15 weeks followed by a diabetogenic carbohydrate-containing diet for two days. Islet ciliation and cilia-gene expression were analysed by immunohistochemistry and transcriptomics. Human pancreatic islets of 124 non-diabetic donors and 78 donors diagnosed with T2D were provided by the Nordic Network for Islet Transplantation and gene expression profiles were analysed by RNA sequencing. To directly investigate the impact of cilia on beta-cell replication, we repressed KIF3A, a subunit of the kinesin-II motor protein, via an adenovirus expressing *Kif3a*-specific shRNA in MIN6 beta-cells.
Results: Upon the carbohydrate challenge, exclusively B6-ob/ob mice exibited a massive induction of beta-cell division accompanied with cilia disassembly. Comparative islet transcriptomics identified a significant enrichment of 327 differentially expressed cilia-annotaded genes of which 81 human orthologues were also affected in islets of diabetic donors. Interestingly, in islets of non-diabetic mice and humans, we found a huge overlap of upregulated cilia-genes involved in cell-cycle progression. The shRNA-mediated supression of the cilia-gene KIF3A in MIN6 cells resulted in a decreased proliferation capacity in comparison with scrambled-shRNA transfected control cells as detected by a reduced incorporation of BrdU.
Conclusion: These findings provide direct functional evidence for the substantial role of cilia on compensatory beta-cell proliferation. We postulate that an impaired regulation of cilia-genes and a restricted capacity of cilia disassembly participate in the development of T2D in mice and men.
Supported by: 82DZD00302; B31 5631/2006; DDG 2017/02/21
Disclosure: **M. Stadion:** None.

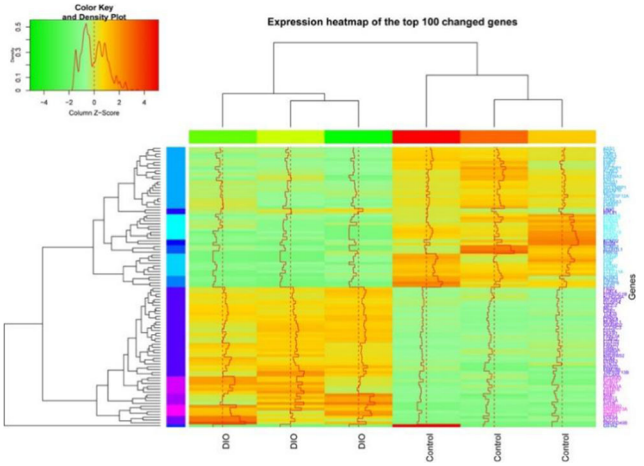
390

Protective effects of Clec11a on lipotoxicity induced islets injury via modulation of proliferation and secretion in mice

R. Shi[1], S. Zhao[2], Z. Sun[1];
[1]Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, Medical School, Southeast University, Nanjing, [2]Department of Biochemistry and molecular biology, Medical School, Southeast University, Nanjing, China.

Background and aims: Glycemic dysregulation and insulin resistance are common threads in the progression from obesity to diabetes. The growth of beta cells and insulin secretion are essential to maintain blood

glucose homeostasis. Here we attempted to explore a new molecular mechanism mediated by Clec11a (C-type lectin domain family 11, member A) in prevention of islets disfunction during obesity.
Materials and methods: C57BL/6 mice aged 5 weeks fed a high-fad diet or standard diet for 14 weeks. Transcriptomic sequencing was performed with the islets of diet-induced obesity (DIO) C57BL/6 mice and normal food fed control mice. Differentiatial gene transcription was confirmed by real-time PCR, and protein translation was verified by western blot and immunofluorescence. The proliferation of MIN6 cells was measured by CCK8. The insulin secretion was measured by ELISA.
Results: The body weight (49.29 ± 0.79 g vs. 29.30 ± 0.54 g, $P < 0.01$, Fig 1.A), fasting blood glucose (7.58 ± 0.58 mmol/L vs. 3.90 ± 0.38 mmol/L, $P < 0.01$, Fig 1.B), and insulin level in plasma (1240.95 ± 92.30 pg/mL vs. 723.56 ± 74.33 pg/mL, $P < 0.01$, Fig 1.C) were higher in DIO mice than those of control mice. Moreover, the results of oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) exhibited the impaired glucose tolerance and insulin resistance in DIO mice ($P < 0.05$, Fig. 1D,E). The mRNA of Clec11a significantly decreased in islets of DIO mice in RNA-Seq experiment by 5 folds (Fig. 1F) and confirmed by real-time PCR (Fig. 1G). The expression heatmap of the top 100 changed genes is shown in Fig. 1H. As shown in Fig. 2, Clec11a staining is localized in islets. Palmic acid (PA) treated cultured isolated islets expressed less Clec11a in a dose dependent manner (Fig. 3A,B). We then chose 0.5mM PA as the concentration in time course experiment. The protein expression of Clec11a demonstrated an waved trend with upregulated peak at 12 hours and then declined until a reversed level comparing to free fat acid (FFA-BSA) at 48h (Fig. 3C,D). The expression of Clec11a in MIN6 is also downregulated by PA in a dose dependent manner measured by real-time PCR (Fig. 4A) and western blot (Fig. 4B,C). Additionally, PA treatment inhibited the proliferation (Fig. 5A) and the secretion of insulin (Fig. 5B) in MIN6. The treatment of Clec11a protein containing medium rescued the proliferation (0.66 ± 0.05 vs. 0.42 ± 0.04, $P < 0.01$, Fig. 5C), the secretion of insulin in high glucose (22708.16 ± 2275.08 pg/mg/h vs. 11911.69 ± 1421.1 pg/mg/h, $P < 0.01$, Fig. 5D). Moreover, the phosphorylation level of Akt was elevated by Clec11a protein containing medium in PA treated MIN6 cells by 2 folds ($P < 0.05$, Fig. 5E).
Conclusion: Clec11a might protects the islets function injured by lipotoxicity via a mechanism of regulating pancreatic beta cell proliferation and secretion.



Disclosure: **R. Shi:** None.

PS 017 Insulin secretion

391

Evidence for differential role of beta-arrestin2 in GLP-1 and GIP signalling in mouse pancreatic beta cells

M.A. Ravier[1], J. Obeid[1], M. Leduc[1], S. Costes[1], P. Gilon[2], S. Dalle[1], G. Bertrand[1];
[1]Institut de Genomique Fonctionnelle, Montpellier, France, [2]Université Catholique de Louvain, Brussels, Belgium.

Background and aims: The scaffold protein beta-arrestin2 (ARRB2) is known to uncouple G protein coupled receptors (GPCR) from G protein and to recruit new signaling pathways (such as ERK1/2) to the activated GPCR. Several groups have reported a direct interaction of the GLP-1 receptor (GLP-1R) but not of the GIP receptor (GIPR) with ARRB2 in non beta cells. Nevertheless, we and others failed to detect any differences for insulin secretion in response to 20–100 nM GLP-1 from Arrb2$^{-/-}$ mouse pancreatic islets. Our aim was to determine if ARRB2 could be involved in GLP-1R and GIPR signaling in mouse beta cells.
Materials and methods: The experiments were carried out in beta cells from four-month-old Arrb2$^{+/+}$ and Arrb2$^{-/-}$ male mice. cAMP production (CAMPS-epac), endogeneous PKA (AKAR3) and ERK1/2 (EKAR) activations were measured after adenoviral infection of cells with FRET-based sensors of interest by live microscopy. ERK1/2 phosphorylation was also determined by immunofluorescence.
Results: Compared to Arrb2$^{+/+}$ mice, Arrb2$^{-/-}$ mice displayed a better oral glucose tolerance despite an impaired i.p. glucose tolerance and a decrease in beta cell mass. This was associated with a significant increase in plasma insulin concentration after the oral glucose administration ($p < 0.05$), suggesting a greater incretin effect in Arrb2$^{-/-}$ mice. Insulin secretion was then measured from isolated islets in response to both incretin hormones: GIP and GLP-1. Unexpectedly, whereas insulin secretion from Arrb2$^{-/-}$ isolated islets was significantly reduced in response to GIP (100 pM, $p < 0.05$), insulin secretion was larger with physiological concentrations of GLP-1 (1 and 10 pM GLP-1; $p < 0.05$). By contrast, supra-physiological concentrations of GLP-1 (1 nM–10 nM) induced similar insulin secretion in Arrb2$^{+/+}$ and Arrb2$^{-/-}$ islets. The larger insulin release with physiological concentrations of GLP-1 was associated with an increased cAMP production and PKA activation in Arrb2$^{-/-}$ beta cells, while cytosolic [Ca2+] increases and emptying of the endoplasmic reticulum were both similarly affected in Arrb2$^{+/+}$ and Arrb2$^{-/-}$ beta cells. Conversely, GLP-1-induced activation of ERK1/2 was strongly decreased (~50%, $p < 0.05$) in Arrb2-/- beta cells, but the re-expression of ARRB2 in Arrb2$^{-/-}$ beta cells, using an adenovirus encoding ARRB2-GFP, completely restored ERK1/2 phophorylation induced by GLP-1 ($p < 0.01$).
Conclusion: Our study revealed in living mouse beta cells, a differential role of ARRB2 in GLP-1R and GIPR signalling. For GLP-1R, ARRB2 contributes to a partial uncoupling for the production of cAMP, the activation of PKA and consequently insulin secretion in the pM range of GLP-1, which is the physiological circulating concentration of the incretin. On the other hand, ARRB2 is required for GLP-1-induced full activation of ERK1/2. For GIPR, ARRB2 is required for GIP-induced amplification of insulin secretion. Therefore, any variation in the expression of ARRB2, as observed in diabetic states, should affect differentially the signaling of GLP-1R and GIPR.
Disclosure: **M.A. Ravier:** None.

392

CB1 and CB2 antagonists stimulate insulin secretion and regulate human and mouse islet viability

I. Ruz-Maldonado[1], B. Liu[1], P. Atanes[1], A. Pingitore[1], G.C. Huang[1], D. Baker[2], S.J. Persaud[1];

[1]Department of Diabetes, King's College London, London, [2]Blizard Institute, Barts and The London School of Medicine and Dentistry, London, UK.

Background and aims: CB1, CB2 and GPR55 cannabinoid receptors are expressed by islets where they regulate insulin secretion. Some CB1 and CB2 antagonists are reported to also act as GPR55 agonists. In this study we evaluated the functional effects of the CB1 antagonists/GPR55 agonists SR141716A and AM251 and the CB2 antagonist JTE 907 in human and mouse islets.
Materials and methods: Islets from WT and GPR55KO mice and human donors were perifused in the absence and presence of 10 μM SR141716A, AM251 or JTE 907 alone, or SR141716A or AM251 in combination with JTE 907 and insulin secretion was determined by RIA. $[Ca^{2+}]_i$, cAMP and apoptosis were also quantified in islets by standard techniques.
Results: SR141716A potentiated insulin secretion from both WT and GPR55KO mouse islets (WT: 2.7 ± 0.3 pg/islet/min maximum increase (MXI) above 20mM glucose response (20GR); KO: 6.4 ± 0.3, $n = 20$). Similar effects were obtained with AM251 (WT: 4.4 ± 0.6 MXI above 20GR; KO: 2.3 ± 0.8, $n = 20$), JTE 907 (WT: 1.5 ± 0.8 MXI above 20GR; KO: 1.4 ± 0.3, $n = 4$) and SR141716A or AM251 in combination with JTE 907 (WT: 2.0 ± 0.1 MXI above 20GR; KO: 1.9 ± 0.7 and WT: 2.1 ± 0.9 MXI above 20GR; KO: 1.7 ± 0.7 respectively, $n = 4$). Stimulation of glucose-induced insulin secretion from human islets was also observed in response to SR141716A (control AUC: 671 pg insulin/20 min; +SR141716A: 1391, $p < 0.001$, $n = 4$); AM251 (control AUC: 524; +AM251: 1124, $p < 0.01$, $n = 4$); JTE 907 (control AUC: 16; +JTE 907: 29, $p < 0.05$, $n = 4$); SR141716A + JTE 907 (control AUC: 16; +SR141716A + JTE 907: 34, $p < 0.01$, $n = 4$) and AM251 + JTE 907 (control AUC: 16; +AM251 + JTE 907: 31, $p < 0.05$, $n = 4$). SR141716A and AM251 also elevated $[Ca^{2+}]_i$ in mouse islets (WT, SR141716A: 0.02 ± 0.01 fluorescence 340/380, MXI above 20GR, AM251: 0.04 ± 0.01; KO, SR141716A: 0.01 ± 0.01, AM251 0.05 ± 0.01, $n = 25$). However, SR141716A and AM251 did not promote cAMP generation (basal: 5 ± 0.8 nM cAMP; +SR141716A: 2 ± 0.7; +AM251: 6 ± 0.9, $p > 0.2$, $n = 6$) nor did they reduce elevated cAMP (1 μM forskolin: 90 ± 16.8 nM cAMP; +SR141716A: 122 ± 12.9; +AM251: 115 ± 11.1, $p > 0.2$, $n = 6$), indicating that they do not signal through G_s or G_i. The lack of effect of SR141716A and AM251 on cAMP was also observed in human islets, whereas 20 nM exendin-4 and 1 μM clonidine elevated ($p < 0.001$) and decreased ($p < 0.05$) cAMP respectively, $n = 6$. SR141716A and AM251 reduced cytokines-induced apoptosis in WT and GPR55KO islets (control WT: 8.9×10^4 LU/islet; +SR141716A: 4.6×10^4; +AM251: 5.8×10^4, $p < 0.001$; control KO: 6.2×10^4; +SR141716A: 3.9×10^4, $p < 0.001$; +AM251: 4.4×10^4, $p < 0.05$, $n = 8$). Conversely, in human islets, both antagonists induced apoptosis (control: 5.2×10^5 LU/islet; +SR141716A: 1.2×10^6, $p < 0.0001$; +AM251: 9.7×10^5, $p < 0.05$, $n = 8$). JTE 907 had no effect on apoptosis in human islets but it did induce apoptosis in mouse islets in a GPR55-dependent manner (WT: 13.2×10^4 LU/islet, $p < 0.001$, KO: 5.2×10^4, $p > 0.2$; $n = 8$).
Conclusion: Our data demonstrate that the CB1 and CB2 antagonism enhances glucose-induced insulin secretion from mouse and human islets in a GPR55-independent manner. This could be useful in the development of novel therapeutics that have dual actions to peripherally antagonise the obesogenic effects of cannabinoids while directly stimulating insulin secretion. However, the CB1 antagonists under investigation induced apoptosis in human islets, indicating that such compounds are not appropriate for therapeutic development.
Supported by: Diabetes UK
Disclosure: **I. Ruz-Maldonado:** None.

393

The role of ER Ca^{2+} sensor, stromal interaction molecule 1 (STIM1) in GPR40-mediated potentiation of glucose-induced insulin secretion

R. Usui, D. Yabe, H. Goto, M. Fauzi, S. Tokumoto, H. Tatsuoka, Y. Tahara, M. Ogura, K. Nagashima, N. Inagaki;
Department of Diabetes, Endocrinology and Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Background and aims: Long-chain fatty acid receptor GPR40 plays an important role in potentiation of glucose-induced insulin secretion (GIIS) from pancreatic β-cells. It was previously shown that GPR40 agonist fasiglifam (FAS) enhances Ca^{2+}-release from the endoplasmic reticulum (ER) by activating inositol 1,4,5-triphosphate (IP3) receptor 1 and enhances GIIS. However, it remains unknown how the enhanced Ca^{2+} release from the ER is linked to GIIS potentiation. We thus hypothesized that ER Ca^{2+}-sensor STIM1 senses decline of Ca^{2+} levels in the ER, translocate to the plasma membrane and triggers extracellular Ca^{2+} influx through Ca^{2+}-channel Orai1 to potentiate GIIS. To test the hypothesis, we examined the role of STIM1 in insulin-secreting MIN6 cells as well as isolated islets derived from β-cell specific STIM1-deficient mice.
Materials and methods: MIN6 cells were transfected with STIM1, IP3 receptor 1 (IP3R1) or scrambled siRNA; and analyzed for insulin secretion or intracellular Ca^{2+}-dynamics. MIN6 cells were also transfected with pEX-SP-YFP-STIM1(23-685) to monitor intracellular trafficking of STIM1 in response to glucose and FAS. Islets were also isolated from the β-cell specific STIM1-deficient (βSTIM1 cKO) or C57BL6/J mice; and analyzed for insulin secretion. In addition to FAS, Xestospongin C and GSK7975A were used as inhibitors for IP3 receptor and Orai1, respectively.
Results: STIM1 or IP3R1 knockdown in MIN6 cells similarly abolished FAS-mediated potentiation of GIIS, while they had little effects on GIIS itself. STIM1 knockdown in MIN6 cells also attenuated FAS-induced elevation of intracellular Ca^{2+} levels. STIM1-YFP was rapidly translocated from the ER to the plasma membrane in response to FAS. FAS-mediated potentiation of GIIS was also severely impaired in isolated islets from βSTIM1 cKO mice. In addition, FAS-mediated potentiation of GIIS was also severely impaired by Xestospongin C and GSK7975A.
Conclusion: STIM1 is essential for FAS-mediated potentiation of GIIS, possibly sensing decline of Ca^{2+} levels in the ER, translocate to the plasma membrane and triggers extracellular Ca^{2+} influx through Ca^{2+}-channel Orai1.
Disclosure: **R. Usui:** None.

394

A genetically encoded low-affinity Ca^{2+}sensor unmasks autocrine purinergic signalling in beta cells

M. Yang, O. Idevall-Hagren, E. Gylfe, A. Tengholm;
Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden.

Background and aims: Changes of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) play a critical role in the regulation of various cell functions including insulin secretion from β-cells. Ca^{2+} signalling has been studied for many years using low molecular-weight, organic fluorescent dyes. More recently, genetically encoded, fluorescent-protein-based Ca^{2+} sensors have been developed. Whereas the latter sensors have potential advantages, experience from their use in islet cells is limited. The aim of the present study was to compare the response of the red fluorescent protein-based sensor R-GECO with that of an organic dye with low affinity (fluo-5F; K_d 2.3 μM) to clarify if there are differences in the way they report temporal dynamics of $[Ca^{2+}]_i$ in β-cells.
Materials and methods: MIN6 β-cells, mouse islets and human islets were transfected with the red fluorescent protein-based sensor R-GECO and/or loaded with the organic fluorescent dye fluo-5F by 30 min incubation with 2 μM of its acetoxymethyl ester. Changes of $[Ca^{2+}]_i$ in response to glucose or K^+ depolarization were recorded using total internal reflection fluorescence microscopy. The Ca^{2+} dependence of the fluorescent sensor responses was determined in cells permeabilized with α-toxin.
Results: In all cell preparations, R-GECO and fluo-5F showed a rapid increase of fluorescence when voltage-gated Ca^{2+} influx was triggered by

depolarization of the β-cell membrane with 30 mM K^+ in the presence of 3 mM glucose. However, while fluo-5F most often reported stable elevations of $[Ca^{2+}]_i$, R-GECO usually showed repeated, transient, high-amplitude increases (spikes) from a sustained elevated level. Accordingly, protein sensor and dye remaining in the cytoplasm of MIN6-cells after plasma membrane permeabilization responded to elevations of the medium Ca^{2+}concentration with half-maximal increases of fluorescence at 3.0 ± 0.3 μM for R-GECO and 1.7 ± 0.1 μM for fluo-5F. This *in situ* value for R-GECO is around 6-fold higher than expected from previous studies of purified protein *in vitro*, but it seems unlikely that the distinct $[Ca^{2+}]_i$ signalling patterns reflect the relatively small differences in recorded Ca^{2+} affinities. The depolarization-triggered $[Ca^{2+}]_i$ spikes depended on release of the ion from intracellular stores and was consequently inhibited by depletion of endoplasmic reticulum Ca^{2+} with the SERCA pump inhibitor cyclopiazonic acid. The spiking was also suppressed by the purinergic $P2Y_1$-receptor antagonist MRS2179, indicating involvement of IP_3-gated Ca^{2+} mobilization in response to extracellular adenine nucleotides. Similar, MRS2179-sensitive $[Ca^{2+}]_i$ spikes were observed in R-GECO-expressing β-cells in mouse and human islets stimulated by a rise of the glucose concentration from 3 to 20 mM.

Conclusion: Voltage-gated Ca^{2+} influx in β-cells is tightly coupled to Ca^{2+} release from the endoplasmic reticulum triggered by autocrine feedback from adenine nucleotides co-released with insulin. The resulting $[Ca^{2+}]_i$ spiking with apparent significance for insulin secretion often escapes detection by traditional organic Ca^{2+} dyes but is readily revealed by R-GECO with apparent low affinity for Ca^{2+} in the cytoplasm.

Disclosure: **M. Yang:** None.

395

The lipid phosphatase INPP5F regulates insulin secretion

P.M. Nguyen, N. Gandasi, O. Idevall-Hagren;
Uppsala University, Uppsala, Sweden.

Background and aims: NPP5F (Sac2) is a phosphatase that localizes to endocytic membranes where it dephosphorylates the lipid phosphatidylinositol 4-phosphate (PI[4]P), a key step in receptor recycling. PI(4)P levels are high in the Golgi membrane from which insulin granules are formed, and recent studies show changes in the phospholipid composition of insulin granules in response to glucose stimulation. It is not known if these changes are important for insulin granule maturation and release. The aim of this study was to investigate the potential involvement of INPP5F in these processes.

Materials and methods: A mouse β-cell line (MIN6) and live cell fluorescence microscopy was used to investigate the dynamic distribution of fluorescently tagged INPP5F and its colocalization with various markers of the secretory pathway. To assess the role of INPP5F in the regulation of insulin secretion, overexpression or siRNA/shRNA-mediated knockdown of INPP5F was combined with single cell TIRF microscopy imaging of insulin granule exocytosis and biochemical detection of insulin release using the AlphaLISA technique.

Results: Confocal microscopy imaging showed that GFP-tagged wild type INPP5F localized to small mobile structures. This localization became more pronounced when the enzyme was catalytically inactivated. Colocalization analysis revealed that INPP5F, in addition to localizing to early endosomes (60 ± 14% enrichment compared to random, $n = 96$, $P < 0.001$), was present on a subset of insulin granules positive for Rab3 (53 ± 9% enrichment, $n = 212$, $P < 0.001$) and Rab27 (46 ± 8% enrichment, $n = 220$, $P < 0.001$). siRNA-mediated knockdown of INPP5F resulted in 45 ± 9% ($n = 4$, $P < 0.01$) inhibition of glucose-stimulate insulin secretion, but was without effect on basal secretion or insulin content. Visualization of insulin granules with NPY-mCherry revealed 51 ± 3% ($n = 25$, $P < 0.001$) reduction in granule density at the plasma membrane in INPP5F knockdown cells. These cells also showed 70 ± 8% ($n = 87$, $P < 0.01$) reduction in K^+-stimulated exocytosis. This suppression was also observed when exocytosis was visualized by the plasma membrane insertion of the pH-sensitive, granule-localized protein VAMP2-pHluorin (34 ± 7% reduction, $n = 100$, $P < 0.01$).

Conclusion: INPP5F localizes to insulin granules and loss of this enzyme leads to reduced insulin granule density at the plasma membrane and to impaired insulin secretion. The exact mechanism of action remains to be determined, but the observation are consistent with INPP5F-mediated dephosphorylation of PI(4)P on the insulin granule membrane being a key step in granule maturation.

Supported by: VR

Disclosure: **P.M. Nguyen:** None.

396

The inhibiton of protein biosynthesis diminishes insulin secretion in freshly isolated islets but not in cultured islets

T. Schulze, M. Morsi, I. Rustenbeck;
TU Braunschweig, Braunschweig, Germany.

Background and aims: In contrast to purely depolarizing insulin secretagogues nutrient secretagogues like glucose stimulate protein biosynthesis concomitantly with the stimulation of insulin release. The question is whether this dual role of nutrient secretagogues affects the kinetics of insulin secretion.

Materials and methods: NMRI mouse islets were used either freshly isolated or after overnight culture in RPMI 1640 with 10% FCS and 5 mM glucose. The kinetics of insulin secretion and the oxygen consumption rate (OCR) were measured in the same batch of perifused islets. Additionally, the kinetics of NAD(P)H- and FAD-autofluorescence and of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) were measured. The protein biosynthesis was inhibited by 10 μM cycloheximide (CHX), which was present during the entire perifusion, starting 60 min prior to the glucose stimulation.

Results: Raising the glucose concentration from 5 to 25 mM in the perifusion medium of freshly isolated islets led to a nearly continuous increase of the secretion rate (70 $pg \times min^{-1} \times islet^{-1}$ after 60 min). In the presence of CHX the secretion rates upon stimulation were smaller from the beginning on and reached only 57% of the control value. With cultured islets no such difference was noted. The diminishing effect of CHX on freshly isolated islets was accompanied by a retarded and less steep increase of the OCR during the first 15 min. Thereafter, the OCR of the control islets showed a continuous increase, whereas that of the CHX-exposed islets remained constant. After culture control islets and CHX-exposed islets showed a virtually identical pattern: the OCR increase was particularly steep during the first 10 min, remained constant thereafter and decreased only slowly. In freshly isolated islets the presence of CHX affected the mitochondrial energetics in response to high glucose as was visible by a continued increase of the NAD(P)H/FAD ratio. This was predominantly caused by a more marked decrease of the FAD-fluorescence. In cultured islets, in contrast, CHX had no appreciable effect on the NAD(P)H/FAD ratio. Finally, it was tested whether the effect of CHX on insulin secretion involved changes of $[Ca^{2+}]_i$. Both in freshly isolated and cultured islets CHX slightly diminished the steady state of elevated $[Ca^{2+}]_i$ by 25 mM glucose, whereas the resting $[Ca^{2+}]_i$ and the velocity of $[Ca^{2+}]_i$ changes was unaffected.

Conclusion: Glucose-induced insulin secretion is markedly diminished by the inhibition of protein biosynthesis in freshly isolated, but not cultured islets. This effect involves changes in mitochondrial energetics, but is not directly related to $[Ca^{2+}]_i$. Apparently, the relation between mitochondrial metabolism and insulin secretion (amplifying pathway) is changed by culturing isolated islets, in that a more stable supply of signalling compounds and metabolites is generated. It is unclear whether this reflects the in vivo working condition of the islets.

Supported by: DDG

Disclosure: **T. Schulze:** None.

397

Selective disruption of the very long chain fatty acid elongase 2 (ELOVL2) in the pancreatic beta cell impairs insulin release during obesity

K. Meneyrol[1], L. Bellini[1], E. Georgiadou[2], B. Blondeau[3], N. Kassis[1], B. Thorens[4], G.A. Rutter[2], C. Cruciani-Guglielmacci[1], C. Magnan[1], H. Le Stunff[1];
[1]Université Paris Diderot, Sorbonne Paris Cité, Unité Biologie Fonctionnelle et Adaptative - CNRS UMR 8251, Paris, France, [2]Section of Cell Biology and Functional Genomics, Division of Diabetes, Endocrinology and Metabolism, Imperial College London, London, UK, [3]Inserm, Saint-Antoine Research Centre, Saint-Antoine Hospital, Sorbonne University, Pierre-and-Marie-Curie University Paris 06, Paris, France, [4]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.

Background and aims: Dietary ω3-polyunsaturated fatty acids, especially docosahexaenoic acid (DHA), are known to influence glucose homeostasis by modulating peripheral insulin sensitivity. We recently showed that expression of the very long chain fatty acid elongase 2 (*Elovl2*) gene in pancreatic beta cells, whose gene product ELOVL2 regulates the synthesis of endogenous DHA, is associated with glucose tolerance in mice. Moreover, we found that down-regulation of *Elovl2* in various beta cell lines is associated with a defect in glucose-induced insulin secretion. However the *in vivo* role of *Elovl2* in beta cells in the regulation of glucose homeostasis is not known. In this study, we developed mice inactivated selectively in the beta cell for *Elovl2* to determine its role in the regulation of insulin secretion and glucose homeostasis during the development of obesity.
Materials and methods: Homologous recombination of floxed *Elovl2* alleles was achieved by breeding carrier mice to animals bearing a beta cell-selective Cre recombinase (Ins1-Cre mice). Control and *Elovl2*-BKO mice were fed either a regular chow or a high fat diet (HFD) for 3 months. Body weight gain, glucose homeostasis (oral glucose tolerance test and insulin tolerance test) and insulin secretion in vivo and in vitro, gene expression and islet cell mass were measured using standard techniques.
Results: Ins1-Cre-based recombination led to efficient beta cell-targeted deletion of Elovl2 (87.5% of reduction $p < 0.01$) without affecting expression of the other elongases. Elovl2-BKO mice displayed normal body weight, fasting glycaemia and islet cell mass after maintenance on a HFD. Similar insulin resistance developed in control and Elov2-BKO mice under HFD. In contrast, after 3 months of HFD, Elovl2-BKO mice displayed a slightly impaired glucose tolerance compared to control mice. Control mice under HFD displayed increased basal and glucose-stimulated insulin secretion *in vivo*. In contrast, both basal and glucose-stimulated insulin secretion were significantly decreased by 48.7% and 30,9% ($p < 0.02$), respectively in Elovl2-BKO mice under HFD compared to control mice. Finally, glucose-stimulated insulin secretion *in vitro* was also significantly decreased (46.2% $p < 0.01$) in isolated islets from Elovl2-BKO mice maintained on HFD versus WT controls.
Conclusion: Taken together, these results demonstrate a cell-autonomous role for *Elovl2* in the control of pancreatic beta cell insulin secretion. We are presently exploring the potential mechanisms involved by assessing Ca^{2+} dynamics and beta cell connectivity.
Supported by: IMI2 Rhapsody
Disclosure: **K. Meneyrol:** None.

398

Interactions between statins and the farnesoid-X-receptor inhibit positive effects of chenodeoxycholic acid on insulin secretion

T. Hoffmeister[1], J. Kaiser[2], G. Drews[2], M. Düfer[1];
[1]Institute of Pharmaceutical and Medicinal Chemistry, Department of Pharmacology, Münster, [2]Institute of Pharmacy, Department of Pharmacology and Clinical Pharmacy, Tübingen, Germany.

Background and aims: It is reported that statins affect expression and signaling of the farnesoid-X-receptor (FXR) in the liver. This study focuses on the interaction between statins and FXR in pancreatic islets. Bile acids are endogenous agonists of FXR and increase glucose-stimulated insulin secretion (GSIS). For this acute effect the cytosolic localization of FXR is required. Since statins increase the risk of developing type 2 diabetes mellitus, this study aims to elucidate whether they interact with FXR in pancreatic β-cells and thereby influence the insulinotropic effect of chenodeoxycholic acid (CDC).
Materials and methods: Islets or β-cells were isolated from wildtype and FXR-deficient mice of a C57BL/6N background. For culture (24 h), standard conditions (10 mM glucose) were used in the presence or absence of atorvastatin (1.5 and 15 μM) or pravastatin (50 and 200 μM). Insulin secretion was measured by radioimmunoassay and $[Ca^{2+}]_c$ was determined by fluorescence microscopy. Statistical significance was assessed by Student's t test or ANOVA followed by Student-Newman-Keuls test.
Results: Treatment of islets with atorvastatin for 24 h decreased insulin secretion stimulated by 15 mM glucose in a concentration-dependent manner (control: 4.9 ± 0.6 ng insulin/islet*h vs. 15 μM atorvastatin: 3.3 ± 0.3 ng insulin/islet*h, $n = 10$, $p \leq 0.05$). Atorvastatin abolished the stimulatory effect of CDC (500 nM) on GSIS (15 μM atorvastatin: 3.7 ± 0.3 ng insulin/islet*h vs. 15 μM atorvastatin + CDC: 3.1 ± 0.4 ng insulin/islet*h, $n = 6$, n.s.) and diminished the rise in $[Ca^{2+}]_c$ induced by acute application of CDC (CDC-mediated increase in $[Ca^{2+}]_c$, control: 36 ± 6%, $n = 55$ vs. 15 μM atorvastatin: 17 ± 5%, $n = 37$, $p \leq 0.05$). In addition, the bile acid receptor seems to be involved in negative effects of atorvastatin on GSIS as well: WT islets showing no elevation of insulin release in response to CDC ($n = 4$), pointing to translocation of FXR from the cytosol to the nucleus, were more sensitive to the inhibitory effect of atorvastatin (15 μM) compared to CDC-responsive preparations (statin-induced reduction of GSIS: 49 ± 8%, $n = 4$ vs. 10 ± 10%, $n = 6$, $p \leq 0.05$). Since the inhibitory effect of atorvastatin on GSIS persisted in islets of FXR-knockout mice (control: 5.2 ± 0.6 ng insulin/islet*h vs. 15 μM atorvastatin: 3.3 ± 0.5 ng insulin/islet*h, $n = 8$, $p \leq 0.05$), FXR is not required for the detrimental effect of atorvastatin but seems to influence it. The more hydrophilic pravastatin also decreased GSIS (control: 5.2 ± 0.6 ng insulin/islet*h vs. 200 μM pravastatin: 3.4 ± 0.4 ng insulin/islet*h, $n = 10$, $p \leq 0.05$) and abolished the stimulatory effect of CDC on GSIS (200 μM pravastatin: 3.6 ± 0.7 ng insulin/islet*h vs. 200 μM pravastatin + CDC: 3.1 ± 0.5 ng insulin/islet*h, $n = 5$, n.s.) or $[Ca^{2+}]_c$ (CDC-mediated increase in $[Ca^{2+}]_c$, control: 27 ± 7%, $n = 27$ vs. 200 μM pravastatin: 11 ± 4%, $n = 33$, $p \leq 0.05$).
Conclusion: Lipophilic and hydrophilic statins both reduce insulin release. This impairing effect does not require FXR but seems to be modulated by the nuclear receptor. In addition to their inhibitory effect on islet function, statins also prevent the positive effects of bile acids on $[Ca^{2+}]_c$ and insulin secretion indicating an interaction between statins and FXR. In patients treated with statins these two mechanisms would act in concert and progressively impair glycemic control.
Supported by: DDG
Disclosure: **T. Hoffmeister:** Grants; DDG.

399

Roles for the type 2 diabetes-associated genes C2CD4A and C2CD4B in the control of insulin secretion

N. Mousavy Gharavy, A. Martinez-Sanchez, G. Rutter;
Medicine, Imperial College London, London, UK.

Background and aims: Single nucleotide polymorphisms near the human *C2CD4A* and *C2CD4B* genes on chromosome 15q are associated with altered pro-insulin levels and Type 2 diabetes risk at genome-wide significance. Altered expression of both *C2CD4A* and *C2CD4B* has been reported in human islets in association with risk variants, particularly in female subjects. Both genes encode putative Ca^{2+} and phospholipid-

binding proteins thought to be localised to the nucleus. Here, we address their roles in the regulation of insulin secretion *in vitro* and of glucose homeostasis *in vivo.*

Materials and methods: *C2cd4b* null mice were generated by the International Mouse Phenotyping Consortium using CRISPR/Cas9-mediated genome editing. Intraperitoneal glucose tolerance (IPGTT) and glucose-stimulated insulin secretion (GSIS) were examined using standard protocols. Subcellular analysis of Flag-tagged constructs was performed by immunocytochemistry and confocal fluorescence microscopy using an inverted optics spinning disk microscope (Nikon ECLIPSE Ti).

Results: Animals deleted globally for *C2cd4b* (KO) showed mild dysglycaemia, and this effect was clearest in females (at 12 weeks: 15 min. IPGTT: WT, 11.5 ± 0.8 mmol/L; KO, 14.78 ± 1.05 mmol/L, $n = 7–11$, $p = 0.0129$, two-way ANOVA test). Whilst differences were still observed in female KO mice *versus* WT animals at 20 or 22 weeks of age, none were observed in males of the same age. Correspondingly, a tendency towards lower glucose-stimulated insulin secretion from isolated islets was observed in female, but not male, null mice compared to controls ($n = 6–9$ animals aged 24 weeks per genotype, $P = 0.177$, Student's t-test). Examined in human foetal pancreas-derived EndoC BH1 β-cells, C2CD4A (92.3% ± 3.3) and C2CD4B (79.22% ± 10.1) were located in both the cytoplasm and nucleus ($n = 100$ and 80 cells, respectively). Additional localisation to the plasma membrane was observed for C2CD4A in 3.6% ± 3.6, and for C2CD4B in 6.1% ± 3.1, of cells. Exclusive localisation to the nucleus was observed in only 0.37% ± 0.4 (C2CD4A) and 5.7% ± 2.1 (C2CD4B) of cells.

Conclusion: Our data suggest that altered *C2CD4B* expression may contribute to exaggerated disease risk conferred by variants at this locus by altering glucose homeostasis. This action may, at least in part, be due to altered glucose-regulated insulin secretion, particularly in females. Analysis of the sub-cellular localisation of C2CD4B suggests that this protein may play a role in extra-nuclear events in beta cells, conceivably including Ca^{2+} signalling, in contrast to earlier findings in other cell types.

Supported by: Diabetes UK

Disclosure: **N. Mousavy Gharavy:** None.

PS 018 Pathogenesis and treatment of type 1 diabetes

400

Shotgun proteomic analysis and protein lysine-acetylation in cytokine exposed human pancreatic islets

F. Ciregia[1], F. Grano[1], M. Mazzoni[1], S. Mossuto[1], A. Lucacchini[1], M. Suleiman[1], L. Giusti[1], C. De Luca[1], S. Lacerenza[1], L. Marselli[1], M. Ronci[2], A. Urbani[3], P. Marchetti[1], M. Bugliani[1];
[1]University of Pisa, Pisa, [2]University G. d'Annunzio of Chieti-Pescara, Chieti, [3]Università Cattolica, Rome, Italy.

Background and aims: Type 1 diabetes is characterized by beta cell destruction also due to the action of proinflammatory cytokines. To shed further light on how cytokine-exposure affects the proteome of human islets and post-translational protein modifications, we performed a study using label free shotgun proteomics and 2D electrophoresis (2DE), and also evaluated the presence of protein lysine-acetylation dysregulation.

Materials and methods: Human islets were prepared by collagenase digestion and gradient centrifugation from the pancreas of five non-diabetic multiorgan donors and then cultured for 48h with or without 50 U/ml IL-1beta + 1,000 U/ml IFN-gamma. We used 250 μg of islet protein extracts for 2DE (n: 5) and 40 μg of extracts for shotgun (n: 3) analysis. Samples were loaded onto 12% acrylamide resolving gel. After separation, gel pieces (13 for lane) were excised and the proteins were identified by shotgun methodology after in-gel trypsin digestion and mass spectrometry analysis. To determine protein lysine-acetylation, 2DE of human islet protein samples coupled with Western blot was performed using specific anti-acetylated lysine antibody.

Results: Around 3,000 proteins were identified by the shotgun analysis, of which 307 resulted differentially expressed after cytokine exposure [184 upregulated (including chemokines, oxidative stress-related proteins and immunoproteasome proteins) and 123 downregulated (including cathepsins, antioxidant proteins, Krebs cycle enzymes). Ingenuity pathways analysis showed that some upstream regulators such as STAT1 and 2, NF-kB, JAK1 were activated, and others, such as MAPK1, atypical chemokine receptor 2, transcription intermediary factor 1-alpha and small ubiquitin-related modifier 3 were inhibited. The search for lysine-acetylated proteins revealed 151 spots; 6 of them showed statistically significant changes after cytokine culture (2 upregulated and 4 downregulated). Mass spectrometry identified peroxiredoxin 3 and superoxide dismutase 1 as the upregulated acetylated proteins and glutamate dehydrogenase, cathepsin D, acyl-CoA dehydrogenase family member 9 and EIF4A1 as the downregulated ones.

Conclusion: Novel human islet proteins regulated by cytokine exposure were found in the present study and the role of cytokines was shown in affecting post-translational lysine acetylation of proteins involved in cell function and survival.

Supported by: EU-IMI2 INNODIA project

Disclosure: **F. Ciregia:** None.

401

An integrated multi-omics approach identifies the type I interferon-induced signature of human beta cells

M.L. Colli[1], E.S. Nakayasu[2], M. Ramos-Rodriguez[3], J.-V. Turatsinze[1], A. Coomans de Brachène[1], M. Lopes[1], R.S. dos Santos[1], J. Juan-Mateu[1], H. Raurell-Vila[3], R. Scharfmann[4], P. Marchetti[5], L. Pasquali[3], T.O. Metz[2], D.L. Eizirik[1];
[1]ULB Center for Diabetes Research, Universite Libre de Bruxelles (ULB), Brussels, Belgium, [2]Pacific Northwest National Laboratory, Richland, USA, [3]IGTP Health Sciences Research Institute of the Germans Trias i Pujol Foundation, Barcelona, Spain, [4]INSERM U1016, University Paris-Descartes, Cochin Institute, Paris, France, [5]Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy.

Background and aims: Overexpression of HLA class I, presence of markers of endoplasmic reticulum (ER) stress and beta cell apoptosis are hallmarks of the pancreatic islets in early type 1 diabetes (T1D). Interferon-α (IFNα) is expressed in islets from T1D patients, and we have recently shown that human beta cells exposed to it recapitulate all the hallmarks observed during T1D development (PMIDs 28062922, 29305625). We presently used an integrated multi-omics approach to systematically characterize the IFNα responses induced in pancreatic human beta cells.
Materials and methods: Human EndoC-βH1 insulin-producing cells were exposed or not to IFNα (2000 U/ml) for 2h, 8h and 24h ($n = 5$). These cells were evaluated in parallel by: a. ATAC-seq to determine the chromatin accessibility and regulatory regions; b. RNA-seq with a high coverage (>220 millions reads) to determine gene expression, including spliced variants; c. Proteomics using liquid chromatography-mass spectrometry (LC-MS). Key findings were validated in EndoC-βH1 cells and primary human islet cells by real-time RT-PCR, Western Blot, and additional chromatin studies. Baricitinib (0.1–4 μM) was added to the cells 1h before IFNα exposure.
Results: IFNα-treated EndoC-βH1 cells showed >4400 gained open chromatin regions at 2h (False Discovery Rate (FDR) <0.05, fold change (FC) >2) but only 1000 at 24h. IFNα changed the mRNA expression (Up/Down) of 489 (411/78), 1659 (1173/486) and 1416 (872/544) genes at 2h, 8h and 24h, respectively, (FDR <0.05, FC >1.5). There were around 20.000 alternative splice events modified by IFNα (FDR <0.05, Percentage Splicing Index (PSI) >|5%|, minimal exon counts: 5). Analysis of the differentially abundant proteins by protein-protein interaction (PPI) tools identified ER protein processing, antigen presentation, type I interferon signaling, apoptosis and MDA5/RIG-I activation. Integration of the PPI networks with the DrugBank database identified the kinase JAK1 as both a hub for IFNα signaling and a target for the JAK inhibitor baricitinib, in test for rheumatoid arthritis treatment. Pretreatment of EndoC-βH1 or human islet cells with Baricitinib decreased IFNα-induced expression of HLA class I, the chemokine CXCL10 and the ER stress marker CHOP ($P < 0.01$).
Conclusion: Exposure of human beta cells to IFNα induces early modifications in chromatin structure and consequent modifications in the expression of thousands of mRNAs and proteins. IFNα activates signaling pathways promoting beta cell autoantigen(s) presentation and potentially neoantigen generation by parallel activation of the alternative splicing machinery and ER stress. The use of an integrated omics approach, coupled to novel bioinformatics tools, allowed us to identify Baricitinib as a potential new drug for beta cell protection in T1D.
Supported by: NIH-NIDDK-HIRN Consortium 1UC4DK104166-0; INNODIA grant agreement 115797
Disclosure: **M.L. Colli:** None.

402

The type 1 diabetes candidate gene CTSH protects against cytokine-induced beta cell apoptosis via Rac2 GTPase

T. Fløyel[1], A.H. Mirza[2], S. Kaur[1], J. Størling[1], F. Pociot[1];
[1]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [2]Department of Pharmacology, Weill Cornell Medicine, New York, USA.

Background and aims: Genome-wide association studies have identified more than 50 risk loci for type 1 diabetes (T1D), including chr15q25.1 with the candidate gene *CTSH* (lysosomal protease cathepsin H). We previously showed that T1D-associated risk variants in *CTSH* affect the expression of CTSH, and that CTSH regulates β-cell function and disease progression in children with newly-diagnosed T1D. Overexpression of *CTSH* protected INS-1 cells against cytokine-induced apoptosis. By global gene expression analysis, the aim of this study was to identify the genes and mechanisms through which CTSH mediates its protective effect.
Materials and methods: INS-1 cells ($n = 3$) stably transfected with a plasmid encoding CTSH (pCTSH) or an empty vector (pcDNA) were stimulated with IL-1β and IFNγ for 0, 6 and 16 hours. Microarray analysis was performed on the extracted RNA using Affymetrix Rat Genome 230 array. Data were analyzed using the Bioconductor affy package and limma in R. Pathway analysis was performed using ROntoTools in R which takes into account the log fold changes and p values for the differentially expressed genes as well as pathways topology in order to assess the abnormal perturbation of the pathways in the condition under study. Two pathways were validated functionally by siRNA-mediated knockdown of *Gna15*, *Cav1* and *Rac2* in the pcDNA and pCTSH cells ($n = 5$) to examine the effect on CTSH-mediated protection against apoptosis using the Caspase-Glo 3/7, the CytoTox-Fluor, and the Cell Death Detection ELISA assays. Knockdown was verified by real-time qPCR.
Results: A total of 56 annotated genes were differentially expressed between the pCTSH and pcDNA cells (abs fold change >1.2 and FDR-adjusted $p < 0.05$). Six pathways were significantly perturbed by *CTSH* overexpression ($p < 0.05$): "Serotonergic synapse" with the differentially expressed gene *Ptgs1*, "Calcium signaling" with *Gna15*, "Amoebiasis" with *C9* and *Gna15*, "Chagas disease" with *Ccl5* and *Gna15*, "Focal adhesion" with *Cav1* and *Rac2*, and "Insulin resistance" with *Ppargc1a*, *Ppp1r3c* and *Trib3*. The three genes from the "Calcium signaling" and "Focal adhesion" pathways (*Gna15*, *Cav1* and *Rac2*) were all upregulated in response to *CTSH* overexpression ($p < 0.05$) and therefore knockdown experiments were done individually to evaluate their potential as mediators of the protective effect of CTSH against apoptosis. Knockdown of *Rac2* reversed the protective effects of *CTSH* overexpression on cytokine-induced apoptosis ($p < 0.05$), whereas knockdown of *Gna15* and *Cav1* had no effect.
Conclusion: The current data suggest that CTSH may mediate its protective effect on cytokine-induced apoptosis through the Rho family small GTPase Rac2, which itself is a T1D candidate gene located at the chr22q12.3 risk locus. Interestingly, Rac1, which has 92% sequence homology with Rac2, is also expressed in insulin-secreting cells, where it has been implicated in both glucose-stimulated insulin secretion and apoptosis. Further experiments are needed to evaluate if higher CTSH and Rac2 levels in β-cells protect against immune-mediated damage and preserve β-cell function, thereby representing possible targets for β-cell therapy in T1D.
Supported by: EFSD, DFF-4183-00031
Disclosure: **T. Fløyel:** None.

403

The type 1 diabetes candidate gene Src kinase associated phosphoprotein 2 (SKAP2) controls beta cell sensitivity to pro-inflammatory cytokines

J. Størling[1], K. Meyerovich[2], T. Fløyel[1], H.B. Mortensen[3], L.B. Nielsen[3], D.L. Eizirik[2], A.K. Cardozo[2], F. Pociot[1];
[1]T1D Biology, Steno Diabetes Center Copenhagen, Gentofte, [2]ULB Center for Diabetes Research, Université Libre de Bruxelles, Bruxelles, [3]Department of Pediatrics E, University Hospital Herlev, Herlev, Denmark.

Background and aims: The gene encoding *Src kinase associated phosphoprotein 2 (SKAP2)* is a type 1 diabetes (T1D) candidate gene, but the causal mechanisms are unknown. We previously showed that pro-inflammatory cytokines believed to cause β cell destruction in T1D modulate the expression of *SKAP2* in human pancreatic islets. Here, we aimed to establish the functional role of SKAP2 in pancreatic β cells with focus on its potential regulatory effect on cytokine-mediated β cell apoptosis.
Materials and methods: Rat INS-1E cells, purified primary rat β cells, and the newly established insulin-secreting human cell line 1.1B4 were used to study the function of SKAP2. Cells were transfected with small interfering RNAs (siRNAs) to knockdown the expression of SKAP2. Overexpression experiments were performed on either stably-

transfected clones of INS-1E cells or in transiently-transfected cell pools. Gene expression and protein analyses were examined by real time PCR and immunoblotting, respectively. Apoptosis in response to cytokines (IL-1β + IFNγ ± TNFα) was determined by caspase 3/7 activation, nucleosome detection or Hoechst/propidium iodide staining. Nitric oxide (NO) production was determined by Griess assay. The clinical relevance of SKAP2 genotypes was evaluated in a cohort of newly-diagnosed children with T1D by HbA1c and insulin dose-adjusted HbA1c (an estimate of residual β cell function) analyses.

Results: Knockdown of SKAP2 aggravated cytokine-induced apoptosis in both INS-1E cells, primary rat β cells and 1.1B4 cells. On the contrary, SKAP2 overexpression in INS-1E cells afforded protection against cytokine-induced apoptosis. The protective effect of SKAP2 overexpression correlated with reduced iNOS expression and NO production and decreased the expression of the endoplasmic reticulum (ER) stress marker CHOP. In a cohort of children with newly diagnosed T1D, we found that the single nucleotide polymorphism (SNP) *rs7804356* in *SKAP2* could predict residual β cell function at 12 months after diagnosis.

Conclusion: The present findings reveal that the T1D candidate gene *SKAP2* plays a critical role in the β cells by controlling their vulnerability to cytokine-mediated apoptosis possibly by regulating ER stress.

Supported by: DFF-1333-00163A, DFF-7016-00282

Disclosure: **J. Størling:** None.

404

Characterisation of CXCL10 expression pattern in pancreatic islets of NOD mice and type 1 diabetic patients: a new role for alpha cells in T-lymphocytes recruitment

L. Nigi[1], F. Mancarella[1], L. Krogvold[2], L. Marselli[3], G. Sebastiani[1], G. Ventriglia[1], G.E. Grieco[1], N. Brusco[1], C. Gysemans[4], P. Marchetti[3], C. Mathieu[4], K. Dahl-Jørgensen[2], F. Dotta[1];
[1]Diabetes Unit, Dept. of Medicine, Surgery and Neuroscience, University of Siena; Umberto Di Mario Foundation ONLUS, Toscana Life Sciences, Siena, Italy, [2]Division of Pediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway, [3]Dept. of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [4]Laboratory of Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium.

Background and aims: in type 1 diabetes (T1D) the pro-inflammatory chemokine CXCL10 is involved in recruitment of autoreactive T-lymphocytes in pancreatic islets, contributing to beta(β)-cells destruction. CXCL10 has been described to be expressed by murine and human pancreatic islets in autoimmune diabetes. However, the specific expression pattern distribution among pancreatic endocrine cell subtypes has not been clarified yet. Therefore, the purpose of our study was to shed light on the pancreatic islet expression pattern of CXCL10 both in NOD mice and in T1D patients.

Materials and methods: we analyzed formalin-fixed paraffin embedded pancreatic sections obtained from C57Bl/6J 8 weeks ($n = 4$), C57Bl/6J 15–20 weeks ($n = 3$), NOD-SCID 2–3 weeks ($n = 4$), NOD normoglycemic (NG) 22 weeks ($n = 4$), NOD recent diabetic (RD) 12–21 weeks old ($n = 4$) mice and from new-onset T1D patients ($n = 6$) participating in Diabetes Virus Detection (DiViD) study, compared to non-diabetic organ donors ($n = 3$) from European Network for Pancreatic Organ Donors with Diabetes (EUnPOD) cohort. Immunofluorescence and confocal microscopy analysis were performed to study the expression of CXCL10, insulin and glucagon. Colocalization analysis between insulin and CXCL10 (INS-CXCL10) and between glucagon and CXCL10 (GCG-CXCL10) was performed by using LAS AF software. In mice, total CXCL10 positive volume was analyzed and normalized per islet volume; moreover, extent of islet inflammation (measured by lymphocytic infiltrates area) was correlated to CXCL10 expression.

Results: CXCL10 was not expressed in pancreatic islets of C57Bl/6J and NOD-SCID mice indicating that CXCL10 expression occurs together with inflammation. Total CXCL10 positive volume was increased in pancreatic islets of RD compared to NG NOD mice ($p < 0.01$) and was positively correlated to extent of islet inflammation ($r = 0.44$, $p = 0.02$). Both in NG and RD NOD mice CXCL10 was expressed in pancreatic islets and in RD NOD mice GCG-CXCL10 colocalization rate was increased compared to INS-CXCL10 ($p < 0.01$). Surprisingly, INS-CXCL10 colocalization rate was similar between NG and RD NOD mice, while GCG-CXCL10 was increased in RD compared to NG NOD mice ($p < 0.001$). The analysis of CXCL10 expression pattern performed on human sections revealed that this chemokine was expressed in pancreatic islets of T1D patients but not in non-diabetic donors. GCG-CXCL10 colocalization rate was increased compared to INS-CXCL10 ($p = 0.003$) and, interestingly, was similar between insulin-containing and insulin-deficient islets ($p = 0.6$); it suggests that CXCL10 expression in alpha(α)-cells is not driven by residual β-cells and may represent an independent effect.

Conclusion: we showed that CXCL10 is preferentially expressed by α-cells both in NOD mice and in T1D patients, thus opening to the possibility of a new role for α-cells in the recruitment of autoreactive T-lymphocytes in pancreatic islets and a primary function in β-cell damage.

Disclosure: **L. Nigi:** None.

405

Studies of insulin-related peptides in pancreas and plasma support the existence of two distinct aetiological subtypes of type 1 diabetes associated with age at diagnosis

P. Leete, R. Oram, T.J. McDonald, C. Ziller, B. Shields, T. Tigi Study Team, A.T. Hattersley, S.J. Richardson, N.G. Morgan;
University of Exeter, Exeter, UK.

Background and aims: Immunopathological analysis of insulitis in pancreas tissue studied soon after the onset of type 1 diabetes (T1D) in patients diagnosed <7 y or ≥13 y has suggested two different aetiological subtypes. We sought to assess whether the distribution of proinsulin and mature insulin in the insulin-containing islets (ICI) remaining at disease onset in these two groups, as well as the secretion of insulin-related peptides in people with longstanding T1D, further support the existence of 2 discrete subtypes.

Materials and methods: 4 μm sections of pancreas tissue from 21 patients studied within 1y of T1D onset (4 < 7 y, 11 7–12 y; 6 ≥ 13 y) and 8 controls, were stained with antisera directed specifically against proinsulin or insulin and visualised via confocal microscopy. Images were analysed in a blinded manner to assess the extent of antigen co-localisation. In parallel, 90 minute plasma C-peptide and proinsulin levels were measured after a mixed meal tolerance test in 191 T1D patients (87 diagnosed <7 y and 84 ≥ 13 y) studied >5 y (median 13.3 y) post diagnosis.

Results: In common with earlier work revealing that two immune cell phenotypes can be distinguished at diagnosis of T1D, two distinct patterns of proinsulin/insulin localisation were also seen, which differed markedly between children diagnosed <7 y and those ≥13 y. Prohormone processing was aberrant in the younger group, as evidenced by abnormally high co-localisation of proinsulin and insulin (72 ± 4% vs 28 ± 1% in controls; $p < 0.0001$) in almost all islets. This phenomenon was much less evident in those ≥13 y where, in 78% of islets, proinsulin did not co-localise with mature insulin and was restricted solely to a perinuclear (Golgi) compartment within beta cells ($p < 0.0001$ vs <7 y). Importantly when the islets of children who had been diagnosed in the middle age range (between 7–12 y) were studied in a blinded manner, islets with aberrant proinsulin processing were found, but their proportion correlated precisely with the assigned immune cell profile of each individual patient; as expected if two distinct disease processes are operative. In patients with longstanding T1D and diagnosed <7 y, C-peptide levels were much lower than in those diagnosed ≥13 y (median (IQR) <3 (<3–<3) v 34.5 (<3, 151)pmol/l; $p < 0.0001$) despite a similar duration (12.4 v

15.8 y $p = 0.07$). In contrast, the proinsulin/C-peptide ratio (PI/CP) was increased in those with onset <7 y compared to people diagnosed ≥13 y (0.18 (0.10, 0.31) v 0.01 (0.009, 0.10) nmol/mmol; $p < 0.0001$). No differences in C-peptide or PI/CP ratio were found in those diagnosed 13–18 y or >19–30 y (all $p > 0.5$), arguing against a continuous effect of age at diagnosis.

Conclusion: These findings strongly support the proposition that two distinct aetiological subtypes of Type 1 diabetes exist, which can be identified clinically by age at diagnosis. These subtypes differ according to their insulitic profiles, numbers of residual insulin-containing islets and the proportion of islets with aberrant proinsulin processing, at diagnosis. These differences are reflected over the longer term in the circulating C-peptide and proinsulin concentrations. Understanding the link between the differences in immune cell infiltration and proinsulin processing may provide clues as to the distinct disease processes in the 2 subtypes.

Supported by: Diabetes UK; JDRF

Disclosure: **P. Leete:** None.

406

Prevascularisation of pseudo-islets: a new strategy to improve the engraftment of transplanted islets

L. Nalbach[1], E. Ampofo[1], V. Becker[1], W. Metzger[2], N. Wilhelm[3], M.D. Menger[1], M.W. Laschke[1];
[1]Institute for Clinical and Experimental Surgery, Saarland University, Homburg/Saar, [2]Department of Trauma, Hand and Reconstructive Surgery, Saarland University, Homburg/Saar, [3]Biomedical Engineering, Frauenhofer Institute, St. Ingbert, Germany.

Background and aims: Pancreatic islet transplantation is a clinically applied strategy to restore physiological blood glucose levels in diabetic patients. However, successful islet engraftment still represents the main critical issue for this surgical approach. To overcome this, we incorporate adipose tissue-derived microvascular fragments (adMVF) into pseudo-islets (PI) to ameliorate the revascularization process of transplanted islets

Materials and methods: Prevascularized PI were generated by liquid overlay cultivation of pancreatic islet cells and adMVF isolated from C57BL/6 donor mice. Native islets and non-prevascularized PI served as controls. Scanning electron microscopy was performed to analyze the morphology of the grafts. The cellular composition was determined by immunhistochemical stainings of insulin, glucagon and CD31. Neutral red/trypane blue stainings, flow cytometric analyses and immunhistochemical stainings of KI67- and caspase3-positive cells were used to investigate the cellular viability. The angiogenic activity of the grafts was evaluated by sprouting assays. In vivo, prevascularized PI, non-prevascularized PI and native islets were transplanted into dorsal skinfold chambers of C57BL/6 mice to investigate their engraftment and vascularization. All values are presented as mean ± SD.

Results: The liquid overlay technique allowed the generation of stable prevascularized and non-prevascularized PI within 5 days. Scanning electron microscopy revealed a more heterogeneous surface pattern of prevascularized PI when compared to non-prevascularized PI and native islets. The analysis of cellular composition indicated that the fraction of CD31-positive endothelial cells was significantly higher in prevascularized PI (23 ± 6%) when compared to native islets (10 ± 2%) and non-prevascularized PI (0 ± 0%). The number of insulin-, glucagon- and somatostatin-positive cells was not affected. Neutral red/trypane blue stainings demonstrated that the incorporation of adMVF did not reduce the grafts viability, which was confirmed by additional flow cytometric and immunhistochemical analyses. In addition, 23 ± 6% of the endothelial cells were positive for KI67 indicating a high proliferative potential. In vitro, prevascularized PI exhibited a high number of sprouts in a length of 120 μm, whereas no sprouts were detected in the control groups after 24 hours. Importantly, the incorporation of adMVF in PI markedly accelerated the process of vascularization and the onset of microvascular perfusion when compared to non-prevascularized PI and native islets in vivo.

Conclusion: The present study demonstrates that the incorporation of adMVF in PI markedly enhances the angiogenic activity of the grafts in vitro and in vivo. Accordingly, this prevascularization approach may represent a promising future strategy to increase the success rate of clinical islet transplantation.

Disclosure: **L. Nalbach:** None.

407

Donor body mass index does not affect short term clinical islet transplant outcomes

K.J. Potter[1], A. Lam[1], D. O'Gorman[2], T. Kin[2], S. Forbes[3], A. Malcolm[1], A. Haqq[1], A. Shapiro[1], P. Senior[1];
[1]University of Alberta, Edmonton, Canada, [2]Alberta Health Services, Edmonton, Canada, [3]University of Edinburgh, Edinburgh, UK.

Background and aims: Clinical islet transplantation is limited by availability and quality of donor organs. Obese donors are generally avoided in whole pancreas transplants. They have, however, been suggested as ideal for islet transplantation based on isolation outcome studies demonstrating improved islet yields. There remains a concern that islets isolated from obese donors have suboptimal function. Further, the effect of donor obesity on clinical outcomes post islet transplantation remains unknown. The aim of this study was to compare short term clinical outcomes in islet transplant recipients who received islets from obese (BMI >30 kg/m^2) compared to non-obese (BMI 18.5–30 kg/m^2) donors.

Materials and methods: Subjects receiving their first single-donor islet infusion at a single center, between 1999 and 2017, were included. We excluded recipients of islets from a donor with BMI <18.5 kg/m^2 or a second islet transplant after less than a month. There were 233 donor-islet pairs. 128 subjects received islets from non-obese BMI donors and 90 from obese donors. Clinical outcomes at one month post-transplant included insulin independence, insulin dose reduction, fasting C-peptide, blood glucose and BETA-2 score. Time to second transplant was compared using Kaplan-Meier analysis.

Results: There was no difference between obese and non-obese donors in terms of either donor age (49.7 ± 12.4 v 47.3 ± 12.8, $p = 0.174$) or donor sex (41.4% v 37.8% female, $p = 0.590$). The average BMI for non-obese donors and for obese donors were 25.6 ± 2.6 kg/m^2 and 35.1 ± 5.2 kg/m^2, respectively. The number of islet equivalents (IEQ) isolated from obese donors was significantly greater (437,588 ± 12,004 v 496,939 ± 19,701 IEQ, $p = 0.007$) but there was no difference in islet particle number (IPN) (412,193 ± 12,966 v 429,723 ± 18,777 particles, $p = 0.428$). The correlation between donor BMI and islet yield (IEQ) was moderate ($r = 0.30$, $p < 0.0001$) and weak for IPN ($r = 0.15$, $p = 0.02$). The number of islets transplanted per kilogram of recipient body weight was similar in non-obese and obese donors (6185 ± 142 vs 6662 ± 264 IEQ, $p = 0.08$). The proportion of islet recipients who achieved single-donor insulin-independence at one month was similar between recipients of non-obese and obese donor islets (13.3% vs 20.2%, $p = 0.1934$). Similarly, reduction in insulin dose was not associated with donor BMI ($r = 0.12$, $p = 0.095$). At one month following transplantation, there was no relationship between donor BMI and fasting plasma glucose ($r = 0.094$, $p = 0.1515$), fasting C-peptide ($r = 0.111$, $p = 0.1027$), or HbA1c ($r = 0.118$, $p = 0.0827$). In recipients of obese donor islets there was a relationship between BMI and BETA2 score ($r = 0.2$, $p < 0.0001$) but not after controlling for number of islets transplanted per kg recipient body weight. Kaplan-Meier analysis of time to second transplant was similar between recipients of islets from non-obese and obese donors ($p = 0.8991$).

Conclusion: This large dataset provides robust confirmation that pancreata from obese donors yield greater islet mass (IEQ) but similar numbers of islet particles. Reassuringly, short-term clinical outcomes were similar between obese donors and non-obese donors and suggest that an elevated BMI in carefully selected donors does not adversely affect islet graft function. The relative importance of islet mass or particle number should be examined in more detail.

Disclosure: **K.J. Potter:** None.

408

DPP6 as a new biomarker suitable for human islet in vivo imaging

R.S.G. Ribeiro[1], S. Demine[2], J. Kerr-Conte[3], F. Pattou[3], A. Balhuizen[2], J. Thevenet[3], D. Pacitte[2], R. Scharfmann[4], P. Marchetti[5], S. Goldman[6], T. Lahoutte[1], L. Bouwens[7], D. Eizirik[2], N. Devoogdt[1];
[1]Faculty of Medicine and Pharmacy, In vivo Cellular and Molecular Imaging Lab, Brussels, Belgium, [2]ULB-Center for Diabetes Research, Brussels, Belgium, [3]CHRU, UMR1190, Lille, France, [4]Institut Cochin, INSERM U1016, Paris, France, [5]Dept of Clinical and Experimental Medicine, Pisa, Italy, [6]Center for Microscopy and Molecular Imaging, Gosselies, Belgium, [7]Faculty of Medicine and Pharmacy, Cell Diferentiation Laboratory, Brussels, Belgium.

Background and aims: Type 1 diabetes is an autoimmune disease characterized by progressive decline in pancreatic beta cell mass (BCM) that culminates in beta cell destruction and insulin deficiency. The extent and evolution of BCM is difficult to quantify due to lack of accurate techniques for *in vivo* determination of pancreatic islet loss. We have recently identified, by a combined RNA sequencing and systems biology approach, Dipeptidyl Peptidase 6 (DPP6) as a new biomarker suitable for *in vivo* imaging of human pancreatic cells. We presently validated its use for the imaging of different amounts of human beta cells implanted in immunodeficient mice.
Materials and methods: The identification of new biomarkers was based on RNA-sequenced human pancreatic islets, treated and untreated with IL-1β and IFN-γ, and on 16 healthy human tissues (Illumina Body Map 2.0:GSE30611). The specificity of the expression of the target was validated at the protein level by immunohistochemistry on human tissue arrays. A ^{99m}Tc- camelid single-domain antibody (nanobody) targeting this protein was developed and used to quantify different kinds of intramuscular or subcutaneous grafts in NOD-SCID mice by SPECT-CT, including insulin-producing human EndoC-βH1 cells and primary human islets. The linearity of SPECT signal was evaluated by performing imaging on mice transplanted with different EndoC-βH1 amounts (2.5, 5 and 10 million cells). The effect of the nanobody on cell viability and basal insulin secretion was assessed in EndoC-βH1 cells by using nuclear dyes (Hoechst/Propidium Iodide) and anti-insulin ELISA, respectively.
Results: DPP6 is a human-specific biomarker present only in beta and alpha cells. At the protein level, DPP6 was detected in alpha and beta cells, as well as in some brain regions, but not in other tissues. By using a ^{99m}Tc-nanobody for SPECT-CT imaging we successfully detected grafts composed of EndoC-βH1 cells, or primary human islets. Graft radioactivity levels were specific, with tumour-to-blood and tumour-to-muscle ratios of 2.5 ± 0.4 and 9.9 ± 2.2 respectively, in EndoC-βH1-bearing mice ($n = 5$, $P \leq 0.05$). Similar results were obtained for primary human islets, while exocrine tissue provided limited or no signal. The SPECT-CT signal seemed to be linearly correlated to the number of cells transplanted ($n = 5$, $R^2 = 0.95$). The probe did not induce beta cell death or alter basal insulin secretion of EndoC-βH1 cells ($n = 4$–5, $P > 0.05$).
Conclusion: We have identified and validated a novel beta and alpha cell biomarker and developed a tracer for its use in *in vivo* islet imaging. This represents a useful tool to non-invasively follow up islet grafts and, pending future development, islet mass in diabetic patients.
Supported by: JDRF Strategic Research Agreement
Disclosure: **R.S.G. Ribeiro:** None.

PS 019 Beta cell signal transduction

409

Orphan G-protein coupled receptors (GPC) expression profiling in human islets revealed novel genes for type 2 diabetes

J. Taneera[1], A. Mohammed[1], N. Sulaiman[1], M. Hamad[1], A.S. Salehi[2];
[1]University of Sharjah, Sharjah, United Arab Emirates, [2]Lund University Diabetes Center, Malmoe, Sweden.

Background and aims: G-protein coupled receptors (GPCRs) regulate pancreatic insulin secretion through various signaling pathways. To date there are several hundred GPCRs have been identified, of them 100 are known to lack a defined ligand and are named as orphan receptors. Little is known about the roles of these receptors, expression or implication in insulin secretion in human pancreatic. As almost 50% of the current pharmaceutical agent are based on GPCRs, therefore, studies on uncharacterized orphan receptors are of great importance to expand the therapeutic targets for diabetes. In here, we used microarray gene expression data to compare the expression level of orphan GPCRs in isolated human pancreatic islets from non-diabeitc (ND) and type 2 diabetic (T2D) organ donors. The expression magnitude of orphan GPCRs in ND islets was also compared to frequently used clonal rat INS-1 (832/13) cells.
Materials and methods: Human Islets from cadaver donors were provided by the Nordic Islet Transplantation Program, Uppsala University. The microarrays (GeneChip® Human Gene 1.0 ST and Rat Gene 2.0 ST) were performed using the Affymetrix standard protocol. The array data were summarized and normalized with Robust Multi-array Analysis method. Gene silencing was done using siRNA and transfection efficiency was assessed by qRT-PCR. Secreted insulin was measured in the incubation medium (1 h) with an ELISA assay. Insulin gene expression was analyzed by qRT-PCR and cAMP by ELISA assay.
Results: Of the 90 analyzed orphan GPCR genes, 50 were in the boundary of detection in human islets, 36 had moderate expression level and 4 (GPR125, GPR56, GPRC5B, and LGR4) displayed a high expression. In INS-1 cells, GPR158 was the highest expressed orphan GPCR transcript. (for more details see Figure 1). Differential expression analysis in diabetic vs. non-diabetic islets revealed 14 differently expressed GPCR transcripts, while 4 GPCRs showed a differential expression pattern in hyperglycemic vs. norm-glycemic donors. GPR75 and GPR183 were found to overlap in both comparisons. GPR75 expression was correlated significantly with insulin secretion and with HbA1c, while GPR183 expression was correlated only with HbA1c. siRNA knockdown of GPR75 and GPR183 in INS-1 cells was associated with a significantly decreased in glucose-stimulated insulin secretion, insulin mRNA expression and cAMP content.
Conclusion: Our study provides a comprehensive expression pattern for orphan GPCRs transcripts in human pancreatic islets and INS-1 cells. The modulatory impact of GFP75 and GPR183 activation on pancreatic B-cell function by specific agonists is of utmost interest for the future treatment of T2D although more functional studies are required.

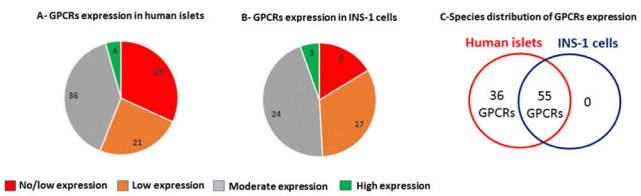


Figure 1: Summary of orphan GPCRs mRNA expression in human pancreatic islets (A) and rat INS-1 (832/13) (B). The color coding represents the expression abundance of GPCRs for each species. (C) Distribution of GPCRs expression in human islets and INS-1 cells. 55 GPCRs were overlapped between the two species, while 35 GPCRs were restricted only to human islets.

Supported by: Dr. Taneera is supported from Aljalila foundation and University of Sharjah
Disclosure: **J. Taneera:** None.

410

Proteomic analysis on human islets shows-up new markers of cellular and metabolic dysfunction

C.M.A. Cefalo[1], T. Mezza[1], S. Alfieri[2], W.-J. Qian[3], R.N. Kulkarni[4], A. Giaccari[1];
[1]Policlinico A. Gemelli, Rome, Italy, [2]Department of digestive an Hepatobiliary surgery, Policlinico A. Gemelli, Rome, Italy, [3]Pacific Northwest National Laboratory, Richland, USA, [4]Joslin Diabetes Center, Boston, USA.

Background and aims: The pathogenesis of type 2 diabetes is characterized by a progressive beta cell dysfunction resulting in both quantitative and qualitative loss of insulin secretion. However, the molecular mechanisms underlying this progressive functional loss are still unknown. The aim of the study is to highlight changes in the proteome of pancreatic islets that can anticipate and eventually predict the onset of diabetes.
Materials and methods: High performance liquid chromatography-mass spectrometry (HPLC-MS) analysis was applied to islets isolated by laser capture microdissection (LCM) from human samples of both diabetic and no diabetic subjects, underwent to duodeno-cefalopancreatectomy for extra-pancreatic and low grading tumors. The subjects were classified on the basis of glucose tolerance assessed by a oral glucose tolerance test before surgery in normal tolerant (NGT, n:7), glucose intolerant (IGT, n:5) and diabetic subjects (T2DM, n:2). Qualitative and quantitative analysis were performed to detect differential protein expression among the three study's groups.
Results: Sixty-seven proteins were found to be significantly different regulated in diabetic subjects compare to NGT, with 29 upregulated and 38 downregulated proteins, while ninety-five proteins were differentially expressed in IGT compare to NGT with 49 upregulated and 46 downregulated. These proteins are mainly involved in cellular and metabolic processes. In particular IGT and DM, compared to NGT, showed a lower expression of proteins involved in cell proliferation such as PURA and NAP1L1, as well as proteins involved in the insulin cleavage process, like ERO1B or in gluconeogenesis process like PG3 and PGK1. While other proteins involved in endoplasmic reticulum stress, CASP14, ERP27 and PDIA3, resulted upregulated in both IGT and DM compare to NGT. A protein, SE1L1, already shown to be potentially involved in the differentiation of pancreatic epithelium resulted upregulated in IGT and downregulated in the DM2 subjects.
Conclusion: Our data suggest that metabolic and cellular processes of islet cells are already dysregulated in patients with impaired glucose tolerance, highlighting some proteins as potential early markers of beta cell dysfunction and novel therapeutic targets to slow the progression to type 2 diabetes.
Supported by: SID-diabete ricerca fellowship
Disclosure: **C.M.A. Cefalo:** None.

411

The glutamate receptor GLUK2 plays a role in glucose homeostasis

M. Abarkan[1], F. Lebreton[1,2], R. Perrier[1], M. Jaffredo[1], J. Gaitan[1], C. Magnan[3], M. Raoux[1], J. Lang[1];
[1]Cell Biology and Biosensors, Univ. Bordeaux, Pessac, France, [2]Université de Genève, Genève, Switzerland, [3]Unité de Biologie Fonctionelle et Adaptative (BFA), Univ. Bordeaux, Paris, France.

Background and aims: Islet hormone secretion is fine-tuned by neurotransmitters and A- as well as B-cells express the ionotropic glutamate receptor Gluk2 according to immunocytochemistry, qPCR and RNAseq. To gain insight into the potential role of Gluk2 in glucose homeostasis we have investigated corresponding knockout mice. These mice have been previously characterized only at the level of the central nervous system and without regard to glucose homeostasis.
Materials and methods: Wild-type C57BL/6 GLUK2$^{+/+}$ mice born on similar dates as C57BL/6 GLUK2$^{-/-}$ mutant litter mice were analyzed (both genotyped by PCR). Adult (week 14–20) and old (week 40–52) mice were investigated. Glucose, pyruvate or insulin tolerance tests were performed according to standard procedures, hormones determined by ELISA. For electrophysiological analysis, islets were isolated and seeded on multielectrode arrays (MEA, 60 electrodes; MCS, Tuebingen). 4 days later signals were recorded (10 kHz) and analyzed offline in terms of slow potential frequency with a commercial software (MC_Rack, MCS).
Results: No statistical differences were apparent in islet mass, beta- or alpha-cell volume or liver glycogen stores between the WT and KO mice. The subsequent functional experiments suggested in essence that old WT mice handled glucose less well than adult (either WT or KO) mice, whereas old KO mice ressembled more to adult mice (WT or KO). Indeed, ip glucose tolerance tests (GTTs) yielded similar values in adult WT or KO mice, whereas old WT mice showed diminished glucose tolerance (1.5 increase in AUC, $P < 0.001$, $n = 7–10$) from 30 min onwards. In contrast, old KO mice behaved as adult WT or KO mice. Insulin levels in-vivo were similar in old mice, except for lower basal values in KO animals (1.18 vs. 0.75 pmol/l; $2p < 0.02$, $n = 7$). Interestingly, fasting glucagonemia was reduced by 39% in old KO mice (compared to old WT). In OGTTs, old KO mice exhibited a far better glucose tolerance than old WT mice from 30 min onwards with a 50% decrease in glycemia (AUC, $n = 14–16$, $2p < 0.05$). Similar results were obtained in pyruvate tolerance test (which mainly reflects neoglucogenesis) where again old KO behaved as adult WT or KO mice, in contrast to augmented glycemia in old WT mice (old WT vs KO, $P < 0.05$ or smaller, $n = 7–9$). Insulin tolerance tests (ip) showed no differences among adult mice (KO or WT) but a marked loss in sensitivity in old WT mice ($P < 0.02$ vs young mice, WT or KO), whereas old KO mice remained as sensitive as the adult ones ($P < 0.001$, all $n = 6$ as compared to old WT). Islet activity of old mice was directly tested in-vitro using MEAs. Stimulation with glucose (8.2 mM, 50 min) resulted in a biphasic pattern of electrical activity in terms of slow potential (SP) frequencies. SPs mainly translate activity of coupled intra-islet beta-cell units. In the second phase, KO mice islets exhibited a 40% higher frequency. Islet insulin and glucagon secretion assays are underway.
Conclusion: Old GLUK2$^{-/-}$ mice exhibited an improved glucose tolerance and insulin sensitivity as compared to WT mice. This is accompanied by a slightly higher glucose-induced electrical islet activity in-vitro mainly during the second phase. These observations suggest a negative effect of Gluk2 on glucose homeostasis especially during aging and which may be due to effects on islets although extra-islet actions are not excluded.
Supported by: MENRT PhD Support
Disclosure: **M. Abarkan:** None.

412

Inhibition of insulin secretion by the chemokine Cxcl14 via a cAMP-independent pathway

P. Atanes, R.G. Hawkes, I. Ruz-Maldonado, O. Olaniru, B. Liu, S. Amisten, S.J. Persaud;
Diabetes Research Group, Department of Diabetes, King's College London, London, UK.

Background and aims: Cxcl14 is a secreted peptide that belongs to the CXC-class chemokine family, acting as a ligand for an unknown receptor. Previous studies have shown that Cxcl14 levels in serum and white adipose tissue are increased in obese mice, whilst Cxcl14 knockout mice are protected from obesity-induced hyperglycaemia and show improved insulin responsiveness. We have identified CXCL14 mRNA expression by islets, but its function in islets has not been elucidated yet. We here explore the role of Cxcl14 on islet and β-cell function and the underlying mechanisms mediating these effects, and attempt to identify the GPCR through which it acts.

Materials and methods: Cxcl14 mRNA expression and the mRNA expression profiles of all CXC-receptors were quantified by qPCR in MIN6 β-cells and mouse islets. Distribution of Cxcl14 in mouse islets was determined by immunohistochemistry (IHC), with insulin, glucagon and somatostatin co-staining. The effects of exogenous Cxcl14 on glucose-stimulated insulin secretion (GSIS) were quantified by radioimmunoassay. Cxcl14 effects on cAMP accumulation, glucose uptake, ATP generation and intracellular calcium levels were assessed by standard techniques using mouse islets and/or MIN6 cells. Activation of Cxcr4 and Cxcr7 receptors was assessed using β-arrestin assays.

Results: Cxcl14 mRNA was not detected in MIN6 β-cells but it was present in mouse islets, and IHC indicated that it was mainly localised to δ-cells. Cxcl14 induced a concentration-dependent inhibition of GSIS from mouse islets (20 mM glucose: 1.93 ± 0.4 ng/islet/hr; +Cxcl14: 1, 2, 10, 20 and 40 ng/mL: 1.59 ± 0.5, 1.31 ± 0.2, 1.08 ± 0.2, 1.00 ± 0.2 and 0.43 ± 0.1 ng/islet/hr, $n = 8$, $p < 0.05$), and a similar trend was observed in MIN6 cells. Cxcl14 (0.01–512 ng/mL) did not reduce forskolin-induced elevation in cAMP in β-cells, whereas the α2-adrenergic agonist clonidine did (10 μM forskolin: 20.3 ± 1 nM cAMP; +512 ng/mL Cxcl14: 23.4 ± 1.5 nM; $p > 0.05$; +1 μM clonidine: 1.4 ± 0.2 nM, $p < 0.001$). However, Cxcl14 inhibited glucose uptake into islets (20 mM glucose: 100 ± 38.3%; +Cxcl14: 1, 10, 20, 40 and 80 ng/mL: 64.7 ± 38, 33.9 ± 20.5, 35.3 ± 16.4, 31.6 ± 14.4 and 18.2 ± 6.1%, $n = 6$, $p < 0.05$; 50 μM cytochalasin B: 12.3 ± 4.0%; $n = 6$, $p < 0.001$), inhibited ATP generation (20 mM glucose: 100 ± 24.7%; +Cxcl14: 1, 20, 40 and 80 ng/mL: 89.2 ± 16.8, 76.7 ± 23.4, 45.9 ± 8.6 and 35.5 ± 8.8%; 5 μM oligomycin A: 14.6 ± 3.8%; $n = 8$, $p < 0.001$) and it also reduced glucose-stimulated elevation in intracellular calcium. CXC-receptor mRNA expression profiling indicated that Cxcr4 and Cxcr7 are the most abundant family members in islets, and β-arrestin experiments demonstrated that these receptors are activated by the native ligand Cxcl12 (Cxcr4, % relative to basal: 100 ± 6.4%; +1 μM Cxcl12: 491.4 ± 37.2%. Cxcr7, % relative to basal: 100 ± 8.3%; +1 μM Cxcl12: 891.3 ± 53% $p < 0.001$). However, Cxcl14 did not promote β-arrestin recruitment at Cxcr4 or Cxcr7 and it did not antagonise Cxcl12 activation of these receptors.

Conclusion: Cxcl14 is expressed by islet δ-cells where it may have paracrine effects to inhibit GSIS through inhibition of glucose uptake, leading to reductions in intracellular ATP and calcium levels. It does not signal via the highly expressed islet Cxcr4 or Cxcr7 receptors. These observations, together with the previously reported association of Cxcl14 with obesity and glucose homeostasis, suggest that Cxcl14 down-regulation could be explored as a novel approach to treat type 2 diabetes.

Supported by: EFSD/Boehringer-Ingelheim Research Programme, RD Lawrence DUK

Disclosure: **P. Atanes:** Grants; This study was supported by grants from the EFSD/Boehringer-Ingelheim Research Programme and a Diabetes UK RD Lawrence Fellowship to Stefan Amisten (11/0004172).

413

Beta cell resident hypoxia inducible factor prolyl 4-hydroxylase PHD3 is involved in the regulation of glucose homeostasis

F. Cuozzo, D. Nasteska, A. Thakker, D.A. Tennant, D.J. Hodson;
University of Birmingham, Birmingham, UK.

Background and aims: The prolyl 4-hydroxylase PHD3 belongs to the α-ketoglutarate-dependent dioxygenase family of enzymes involved in oxygen-dependent regulation of cell phenotype. While its role in tumorigenesis and cancer growth has been extensively studied, scarce reports exist regarding the impact of PHD3 on insulin secretion. We have generated β-cell-specific PHD3 knockout mice ($\beta PHD3^{-/-}$) and investigated the phenotype under normal and diabetogenic diet conditions with a purpose of providing the first blueprint for PHD3-regulated glucose homeostasis.

Materials and methods: $\beta PHD3^{-/-}$ mice were generated by crossing Ins1Cre animals with those bearing a floxed *Egln3* gene (encoding for PHD3). Male and female $\beta PHD3^{-/-}$ mice and wild type (WT) littermates were kept on standard chow and/or high fat diet containing 60% fat (HFD). Gene and protein levels were detected by qPCR and western blot, respectively. Insulin secretion *in vitro* was measured by HTRF assay. Islet Ca^{2+}, ATP/ADP and cAMP dynamics were captured by high-speed spinning disk microscopy. Body weight was measured weekly. Glucose tolerance was assessed by intraperitoneal glucose tolerance test (IPGTT), with a glucose load of 1–2 g/kg body weight.

Results: $\beta PHD3^{-/-}$ mice exhibited reduced islet *Egln3* (0.55-fold *vs* WT, $p < 0.01$) and reduced PHD3 protein expression (0.6-fold *vs* WT, $p < 0.05$). When fed standard chow, $\beta PHD3^{-/-}$ mice had similar body weight to WT and showed no changes in glucose tolerance. Glucose- and fatty acid-stimulated insulin secretion, Ca^{2+} fluxes, as well as glucose-stimulated ATP and cAMP levels were all unaffected by PHD3 deletion. HFD induced similar weight gain in male $\beta PHD3^{-/-}$ and WT mice. Glucose levels during IPGTT were higher at 60 and 90 min after 4 weeks of HFD (60 min: 25.6 *vs*19.7 mmol/L, $\beta PHD3^{-/-}$ *vs* WT; 90 min: 20 *vs* 14.5 mmol/L, $\beta PHD3^{-/-}$ *vs* WT $p < 0.01$), and at 30 and 60 min after 8 weeks of HFD (30 min: 23.8 *vs* 20.2 mmol/L, $\beta PHD3^{-/-}$ *vs* WT; 60 min: 19.2 *vs* 15.8 mmol/L, $\beta PHD3^{-/-}$ *vs* WT $p < 0.05$). *In vitro* insulin secretion at 4 weeks of HFD was increased following stimulation with glucose (4.8 *vs* 2.6 ng/ml, $\beta PHD3^{-/-}$ *vs* WT; $p < 0.05$) and incretin mimetic (12 *vs* 7.2 ng/ml, $\beta PHD3^{-/-}$ *vs* WT; $p < 0.05$), although ATP/ADP rises were blunted ($\Delta F = 0.02$ *vs* 0.06 AU, $\beta PHD3^{-/-}$ *vs* WT; $p < 0.01$). However, by 8 weeks of HFD, glucose was unable to significantly stimulate insulin from isolated islets in $\beta PHD3^{-/-}$ animals (3 mM glucose: 8.8 vs 9 ng/ml, $\beta PHD3^{-/-}$ *vs* WT; 16.7 mM glucose: 14 *vs* 19.8 ng/ml, $\beta PHD3^{-/-}$ *vs* WT) and this was accompanied by defective glucose-stimulated Ca^{2+} fluxes ($\Delta F = 0.19$ *vs* 0.21 AU, $\beta PHD3^{-/-}$ *vs* WT; $p < 0.05$).

Conclusion: PHD3 expression in β-cells appears to be critical in preventing β-cell failure under metabolic stress, such as high fat diet. Elucidating the mechanisms by which PHD3 maintains insulin secretion and glucose homeostasis may be useful for designing new drug therapies for type 2 diabetes mellitus.

Supported by: MRC, ERC, Diabetes UK

Disclosure: **F. Cuozzo:** Grants; MRC, ERC, Diabetes UK.

414

Fetuin-A impairs islet differentiation and function via inhibition of TGFbeta-1 signalling

F. Gerst[1,2], A.-K. Fritz[3], E. Lorza Gil[1,2], G. Kaiser[1,2], E. Wolf[4], H.-U. Haering[1,2], S. Ullrich[1,2], E. Kemter[4];
[1]University of Tuebingen, IDM of the Helmholtz Center Munich, Tuebingen, [2]German Center for Diabetes Research (DZD e.V.), Tuebingen, [3]Department Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, Internal Medicine IV; University of Tuebingen, Tuebingen, [4]Molecular Animal Breeding and Biotechnology, Faculty of Veterinary Medicine; LMU Munich, Munich, Germany.

Background and aims: Fetuin-A is a foetal glycoprotein sustaining cell proliferation. Plasma concentration of fetuin-A declines towards the end of gestation and its expression is restricted to hepatocytes throughout adulthood. During obesity, fetuin-A is upregulated in the fatty liver. Previous observations suggest that fetuin-A impairs glucose-induced insulin secretion (GIIS) of isolated adult human islets. In contrast to the adult islets, the new born islets are largely glucose unresponsive and proliferative. Beta-cell differentiation is accompanied by activation of TGFβR signalling, a pathway sustaining also GIIS. This study aims to assess whether fetuin-A impairs islet cell differentiation and function via inhibition of TGFβR signalling.

Materials and methods: Freshly isolated, glucose-unresponsive pig neonatal islet cell clusters (NICCs) were maturated for 10 d in serum-free Ham's F10 medium containing 10 mM glucose, 50 μM IBMX, 2 mM L-glutamine, 10 mM nicotinamide, 1.6 mM $CaCl_2$, 0.6 mg/ml human serum

albumin (HSA) and supplemented for the last 4 d with 0.6 mg/ml fetuin-A or HSA (as control). Adult human islets obtained from the ECIT Centers were cultured for 1 h or 2 d in serum-free CRML1066 medium containing 5 mM glucose and supplemented with fetuin-A or HSA. Insulin secretion was assessed in static incubations. Gene expression was analysed by RT-PCR, protein expression by western blotting and their subcellular localisation by confocal microscopy.

Results: The maturation of NICCs was accompanied by increased expression of PDX1, insulin, glucagon and somatostatin. SMAD2/3 phosphorylation, the mRNA level of TGFBI, an extracellular matrix component which sustains beta-cell function, and p16/INK4a protein, a beta-cell maturation marker, were upregulated in the matured NICCs suggesting activation of TGFβR signalling. Moreover, PDX1 and p16/INK4a proteins accumulated in the nuclei upon maturation. Matured NICCs displayed a modest GIIS (1.35-fold of basal secretion) which was potentiated by palmitate (2-fold) and forskolin (3-fold). When maturation was carried out in the presence of fetuin-A, the NICCs acquired no glucose responsiveness. Forskolin-mediated potentiation of GIIS was compromised while palmitate still stimulated GIIS. The mRNA levels of PDX1, insulin and glucagon remained low. TGFβR signalling was inhibited in NICCs exposed to fetuin-A, as suggested by the reduced phosphorylation of SMAD2/3 and TGFBI mRNA level. Furthermore, the transcript of RanBP3L, a protein mediating the nuclear export of SMADs, was increased. In addition, fetuin-A counteracted the nuclear accumulation of PDX1 and p16/INK4a. In adult human islets, fetuin-A inhibited GIIS and reduced phosphorylation of SMAD2/3 and SMAD1/5/8 in a TLR4 independent manner.

Conclusion: These observations suggest that fetuin-A impairs beta-cell maturation, differentiation and function via inhibition of TGFβR signalling.

Supported by: DZD Grant 2018

Disclosure: **F. Gerst:** None.

415

Characterisation of a mechanotransductive signalling pathway in human islets of Langerhans: implications for beta cell fate

A. Galli[1], E.S. Di Cairano[1], E. Maffioli[2], E. Sogne[3], F. Bertuzzi[4], P. Milani[3], C. Lenardi[3], G. Tedeschi[2], C. Perego[1];
[1]Pharmacological and Biomolecular Sciences, University of Milan, Milan, [2]Veterinary medicine, University of Milan, Milan, [3]Physic, University of Milan, Milan, [4]Niguarda Cà Granda Hospital, Milan, Italy.

Background and aims: The interaction between cells and extracellular environment plays a pivotal role in the control of cell differentiation and fate, both in physiological and pathological conditions. Like other cells, β-cell behaviour is strongly influenced by extracellular matrix (ECM) interactions. While the involvement of the extracellular environment "chemistry" in this process has been greatly investigated, the contribution of the ECM physical properties has not been completely clarified. Taking advantage of the ability of our group to fabricate transition metal oxide nanostructured surfaces with multiscale controlled disorder as substrates to study the effect of nanoscale topography, aim of the proposed research was to characterize the mechanotransductive signalling complexes in human islets of Langerhans and verify their involvement in the regulation of β-cell fate.

Materials and methods: Cluster-assembled zirconia nanotopographies with specific roughness parameters were produced by Supersonic cluster beam deposition. Human islets were grown on these substrates for up to 15 days and the mechanotrasductive signalling complexes were isolated and characterized by proteomic analysis and super-resolution imaging techniques. β-cell function was assessed by measuring insulin secretion by ELISA assay under basal and stimulated conditions.

Results: β-cell viability and function was improved on nanostructured substrates as revealed by TUNEL assay and insulin secretion. Immunofluorescence analysis revealed modification of cell-substrate adhesion complexes, reorganization of the actin cytoskeleton and modification of the nuclear architecture. Proteomic changes were congruent with cell morphological and functional changes and showed that β-cells respond to environmental mechanical forces through a number of mechanosensors, including integrins and mechanosensitive ion channels (upregulation of Gene Ontology terms GO:0005925). Activation of mechanosensors in turn, caused the up-regulation of proteins important for actin polymerization (GO:0005856), nuclear architecture (GO:0031891) and import/export, thus modifying the program of gene transcription and cellular modeling.

Conclusion: Characterizing the mechanotrasductive signalling pathways may offer a unique possibility to identify potential targets of intervention in diabetes mellitus.

Disclosure: **A. Galli:** None.

416

The mechanism of impaired incretin responsiveness in the pancreatic islets of obese type 2 diabetes: a study of the ZFDM rat

N. Yokoi, T. Hayami, S. Hidaka, A. Kawabata, H. Takahashi, S. Seino;
Kobe University, Kobe, Japan.

Background and aims: The sensitivity of the pancreatic islets to the actions of the incretin hormones is often decreased in obese type 2 diabetes. However, the mechanism of impaired incretin responsiveness remains unknown. In this study, we tried to elucidate the mechanism of impaired incretin responsiveness in the pancreatic islets of an animal model of obese type 2 diabetes, the Zucker fatty diabetes mellitus (ZFDM) rat.

Materials and methods: Eight- and twelve-week-old male lean (*fa/+*) and fatty (*fa/fa*) rats were used. Pancreatic islets of *fa/fa* rats were further divided into non-large and large islets (diameter more than 300 μm). Histological analysis of the pancreas, insulin secretion experiment, transcriptome analysis, metabolome analysis, and western blotting of isolated islets were performed.

Results: In *fa/fa* male rats, the number of large islets increased with age. Incretin-induced insulin secretion was diminished in the large islets at 12 weeks of age. Expressions of glycolysis- and lactate production-related genes were increased while those of the TCA cycle- and the malate-aspartate shuttle-related genes were decreased in the large islets. Glycolysis-related metabolites and lactate were increased but glutamate production from glucose was decreased in the large islets. O-GlcNAcylation of proteins, which may compete with serine/threonine phosphorylation by PKA, was increased in the large islets.

Conclusion: An age-dependent increase of large islets exhibiting dysregulated glucose metabolism, defective glutamate production, and increased O-GlcNAcylation may cause impaired incretin responsiveness in obese type 2 diabetes.

Supported by: MEXT; AMED

Disclosure: **N. Yokoi:** None.

PS 020 Beta cell damage and protection

417

The selective serotonin reuptake inhibitor fluoxetine improves glucose homeostasis in mice and humans: effects on insulin secretion and functional beta cell mass

B. Liu[1], C.D. Moulton[2], I. Ruz-Maldonado[1], K. Toczyska[1], G.C. Huang[1], M.G. Zariwala[3], K. Ismail[2], P.M. Jones[1], S.J. Persaud[1];
[1]Department of Diabetes, Faculty of Life Sciences & Medicine, King's College London, London, [2]Department of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, [3]Faculty of Science & Technology, University of Westminster, London, UK.

Background and aims: We have previously reported that therapeutically relevant concentrations of fluoxetine (Prozac) are well tolerated by isolated β-cells and acute exposure to fluoxetine stimulated insulin secretion, increased β-cell proliferation and reduced apoptosis *in vitro*. The aim of the current study was to investigate the effect of fluoxetine on β-cell function and glucose homeostasis in obese mice and humans with impaired glucose homeostasis.

Materials and methods: Groups of 5 male ob/ob mice (56.0 ± 0.8 g) were administered 4 doses of fluoxetine (10 mg/kg body weight) or DMSO (vehicle) intraperitoneally over 2 weeks before being subjected to intraperitoneal glucose tolerance tests (2 g/kg glucose). BrdU (1 mg/ml) was provided in drinking water for 7 days prior to sacrifice and β-cell proliferation was identified in pancreas sections by immunohistochemistry using antibodies against BrdU and insulin. Glucose-dependent insulin secretion from islets isolated from these mice was assessed in perifusion and quantified by radioimmunoassay. The effects of 1 μM fluoxetine on insulin secretion from islets isolated from an obese (BMI = 36 kg/m^2) human donor was also determined by perifusion and radioimmunoassay. In a multi-ethnic primary care cohort with newly diagnosed type 2 diabetes (T2D), patients prescribed fluoxetine (n = 14) were compared with controls (n = 615) for 1-year changes in plasma insulin and HbA1c; linear regression analysis was used to adjust for potential confounders.

Results: Fluoxetine improved glucose tolerance in ob/ob mice (blood glucose concentrations, control vs fluoxetine; T = 0: 6.7 ± 0.8 mM glucose vs 6.5 ± 0.5; P > 0.5; T = 210 min: 36.3 ± 8.5 mM vs 16.5 ± 2.4; P < 0.05; n = 5), most likely a consequence of increased β-cell proliferation (BrdU positive β-cells/islet: 0.30 ± 0.17 vs 12.15 ± 2.88; n = 20; P < 0.001) and enhanced insulin secretion in response to 20 mM glucose (AUC, pg insulin/20 min: 304.8 ± 28.7 vs 464.7 ± 34.2; n = 4; P < 0.001). Acute exposure of obese donor islets to 1 μM fluoxetine resulted in rapid and reversible potentiation of glucose-stimulated insulin secretion (AUC: 20 mM glucose: 202 ± 23; +1 μM fluoxetine: 316 ± 25; n = 4; P < 0.05). Patients with newly diagnosed T2D who were prescribed fluoxetine for depression showed significant improvement in plasma insulin at one year compared to controls [β = 13.35 (1.84–23.85), p = 0.023] after adjustment for age, gender, ethnicity vascular risk factors and change in depressive symptoms. Fluoxetine treatment was also associated with non-significant reduction in HbA1c (fluoxetine: −0.54% [SD 2.02]; control: −0.05% [SD 1.24]).

Conclusion: These data support a role for fluoxetine in improving β-cell function in obese conditions and in patients with early T2D, and *in vitro* assessments in isolated human islets indicate that fluoxetine directly stimulates insulin secretion. Repurposing of fluoxetine thus represents a novel therapeutic strategy for the management of T2D.

Supported by: Diabetes UK, NIHR and the Royal College of Physicians of Edinburgh

Disclosure: **B. Liu:** None.

418

Prolactin protects beta cells against oxidative stress through HSPB1

L. Labriola[1], R.A.M. Wailemann[1], A.F. Dos Santos[1], V.M. Gomes[1], R.P. Silva[1], A. Laporte[2,3], F.C. Meotti[1], W.R. Terra[1], G. Palmisano[1], S. Lortz[2], L.F. Terra[1];
[1]University of Sao Paulo, Sao Paulo, Brazil, [2]Institute of Clinical Biochemistry, Hannover Medical School (MHH), Hannover, Germany, [3]Institute of Medical Biochemistry and Molecular Biology, University Medicine Greifswald, Greifswald, Germany.

Background and aims: Maintaining islet cell viability in vitro, although challenging, appears to be a strategy for increasing the outcome of pancreatic islet transplantation. We have shown that heat shock protein B1 (HSPB1) mediates prolactin (PRL) beta-cell inhibition of apoptosis. Since the role of HSPB1 in beta-cells is still unclear, we explored the molecular mechanisms by which HSPB1 mediates PRL-induced beta-cell cytoprotection.

Materials and methods: Wild type, HSPB1 silenced or overexpressing MIN6 cells were used as beta-cell models. Biochemical and cell biology parameters such as protein levels, oxidative stress quantification and cell viability were analysed by HPLC-mass spectrometry, western blotting, and fluorescent bioassays among other techniques.

Results: Lysates from PRL and/or cytokine-treated MIN6 beta-cells were subjected to HSPB1 immunoprecipitation. Of the 130 client proteins identified by mass spectrometry, 60 were interacting with HSPB1 under both situations whereas 49 were only detected in the presence of PRL and cytokines. Of note were oxidative stress resistance proteins such as MnSOD, CuZnSOD and PRDXs. We then investigate whether HSPB1-knocked down cells would show a different sensibility towards oxidative stress. Our results indicated not only that PRL was able to protect both control MIN6 cell lines against menadione-induced toxicity (EC50: 11.88 and 14.97 μM for control and PRL treated cells respectively), but also that this effect was mediated by HSPB1, since its silencing completely abrogated PRL's effect on cytoprotection (EC50: 11.61 and 11.91 μM for control and PRL, respectively). Using cells expressing cytosolic or mitochondrial variants of the D-amino acid oxidase (DAAO), we observed that HSPB1 was important mainly for the protection against ROS produced in mitochondria displaying an even greater (around 40%) decrease in the EC50 of MIN6-shHSPB1 cells when compared to that of control cells. HSPB1 silenced cells presented a higher mitochondria-targeted hydroethidine mean fluorescence signal than control cells (at 14 μM menadione. MIN6: 363.7%: MIN6-Sc: 347.7%; MIN6-shHSPB1 cells: 705.4%). HSPB1 overexpression led to opposite effects such as a significant increase of the EC50 of both menadione (16.0 μM vs. 14.49 μM for control cells) and H2O2 (32.58 μM vs. 26.2 μM for control cells) and also a reduced overall oxidative stress shown by DCF fluorescence. PRL treatment, HSPB1 silencing or overexpression did not change the expression of antioxidant enzymes; but influenced glutathione cell content reduction state (GSH/GSSG ratio was decreased in shHSPB1 cells by 50% compared to control cells) and glucose-6-phosphate dehydrogenase (G6PD) activity (MIN6-shHSPB1 cells displayed approximately 40% lower levels of G6PD activity).

Conclusion: We have shown that HSPB1 is important for pro-survival effects against ROS-induced beta-cell death. Altogether our results outline the importance of further studies investigating the importance of HSPB1 for beta-cell viability, since this could lead to the mitigation of beta-cell death through the up-regulation of an endogenous protective pathway.

Supported by: FAPESP, CNPq, EFSD, CAPES

Disclosure: **L. Labriola:** None.

419

A novel prolactin-receptor target in pancreatic beta cells

C. Huang[1], G. Makkar[1], V. Shrivastava[1], B.D. Kyle[2], A.P. Braun[2], F.C. Lynn[3];
[1]Biochemistry and Molecular Biology, University of Calgary, Calgary, [2]Physiology and Pharmacology, University of Calgary, Calgary, [3]Surgery, University of British Columbia, Vancouver, Canada.

Background and aims: Pancreatic islets adapt to the increase in insulin demand during pregnancy by upregulating insulin synthesis and secretion. One of the most highly up regulated genes in the islets during pregnancy is Lrrc55, an auxiliary protein, or γ-subunit of the large conductance, Ca^{2+}-activated (BK) channel. BK channel activity contributes to the regulation of insulin secretion; it also regulates apoptosis, as beta cells of BK channel-null mice have increased susceptibility to apoptosis. While BK channel activity is increased in the presence of Lrrc55, whether Lrrc55 participates in the regulation of insulin secretion and apoptosis in pancreatic islets is unknown. The aim of this study is to determine the role of Lrrc55 in pancreatic beta cells.
Materials and methods: Lrrc55 was overexpressed in INS-1 cells, MIN-6 cells, and isolated mouse islets using an adenovirus vector; adenoviral expression of GFP was used as a control. Beta cells were treated with 33mM glucose (HG) and 0.5 mM palmitate (PA) to induce cell death. Expression of pro-apoptotoc and pro-survival molecules of the ER signaling pathways were determined by qPCR or Western immunoblotting. Dead cells were measured by TUNEL. Intracellular calcium store was measured by Fura-4.
Results: Under non-pregnant condition, Lrrc55 expression is barely detectable in the pancreatic islets. During pregnancy, Lrrc55 expression was upregulated by >60-fold in islets isolated from the wild type mice but in heterozygous prolactin receptor null mice, it was only up regulated by 30-fold. Furthermore, this increase in Lrrc55 expression was only detected in islets and not in other tissues. Overexpression of Lrrc55 in beta cells protected them from glucolipotoxicity-induced apoptosis, accompanied by up regulation of pro-survival signals and down regulation of pro-apoptotic signals of the ER stress pathway. Expression of Lrrc55 prevented calcium depletion induced by glucolipotoxicity, which may contribute to its anti-apoptotic effect. Lastly, although Lrrc55 can facilitate BK channel activity, overexpression of Lrrc55 had minimal effect on glucose-stimulated insulin secretion, although it prevented the glucolipotoxicity-induced reduction in insulin synthesis.
Conclusion: Lrrc55 is a novel pro-survival factor that is up regulated in islets during pregnancy and it prevents conversion of adaptive unfolded protein response to unresolved ER stress and apoptosis in beta cells. Lrrc55 could be a potential therapeutic target in diabetes as it could reduce ER stress and promote beta-cell survival.
Supported by: CIHR, NSERC
Disclosure: **C. Huang:** None.

420

Impact of mitochondrial dysfunction on pancreatic islet cell composition in a mouse model of premature ageing

X. Yu[1], C. Arden[1], C. Chen[2], M. White[1], D. Turnbull[2], L. Greaves[2], M. Walker[1];
[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, [2]Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK.

Background and aims: Inherited mitochondrial DNA mutations can lead to maternally inherited diabetes and deafness (MIDD). There is evidence that mitochondrial mutations accumulate with age, and contribute to the ageing process. The $PolgA^{D257A}$ mutator mouse is a model of premature ageing due to the accelerated accumulation of mitochondrial DNA mutations, and has been previously shown to develop impaired insulin secretion with age. The aim of this project was to measure pancreatic islet cell mitochondrial protein expression and islet cell composition in aged PolgA mutator mice and age matched wild type controls.
Materials and methods: Quantitative quadruple immunofluorescence was used to detect the OXPHOS deficiency by measuring the expression of mitochondrial respiratory chain proteins. A triple immunofluorescence was applied to study the endocrine cell composition and endocrine function in pancreatic islets. Experiments were conducted in pancreas tissue from 44 week old PolgA mutator mice ($n = 5$) and their age-matched wild type controls ($n = 5$).
Results: We found evidence of mitochondrial complex I deficiency in islets from PolgA mice compared to their age-matched controls (Unpaired t test, $P < 0.05$). Triple immunofluorescence showed that the PolgA mutator mice had a lower β-cell percentage (mean ± SEM; 71.8 ± 4% vs 89.3 ± 4% $P < 0.05$) and a higher α-cell percentage (29.6 ± 4% vs 12.5 ± 5%; $P < 0.05$) compared to the wild type controls. However, there was no difference in islet size between the mutator and control mice. We also found that the level of insulin expression in the pancreatic β-cells was decreased in the PolgA mutator versus wild type control mice ($P < 0.05$).
Conclusion: This study shows that age-related mitochondrial dysfunction is associated with a change in islet cell composition towards more α-cells, fewer β-cells with decreased insulin expression. These changes are predicted to predispose to diabetes. Further work is required to determine whether the changes in islet cell composition are due to transdifferentiation driven by mitochondrial dysfunction.
Disclosure: **X. Yu:** None.

421

Pancreatic beta cell-specific deletion of CR6-interacting factor-1 (CRIF1) causes blunted first phase insulin secretion and altered islet morphology

H. Hong[1], K. Joung[2], Y. Kim[3], J. Chang[1], J.-H. Park[4], H. Kim[2], M. Shong[1,2];
[1]Medical Science, Chungnam National University School of Medicine, Daejeon, Republic of Korea, [2]Internal Medicine, Chungnam National University Hospital, Daejeon, Republic of Korea, [3]University of Colorado Denver Anschutz Medical Campus Barbara Davis Center for Diabetes, Aurora, USA, [4]Physiology, Keimyung University School of Medicine, Daegu, Republic of Korea.

Background and aims: A common factor in the etiology of type 2 diabetes mellitus (T2DM) is insufficient pancreatic beta cell function to meet peripheral insulin demand. The emphasis on pancreatic beta cells is reinforced by genome-wide association studies (GWAS) of T2DM which show that the beta cell is the main culprit in T2DM. The basic function of pancreatic beta cells is to regulate glucose homeostasis by secreting insulin. Mitochondrial function in beta cells plays an important role in controlling insulin secretion and beta cell mass. However, the mechanisms underlying progressive beta cell failure caused by mitochondrial OxPhos dysfunction are largely unknown. To investigate whether mitochondrial OxPhos dysfunction gradually leads to diabetes through progressive dysfunction of beta cells, we developed knockout mice with reduced mitochondrial OxPhos function by deleting *Crif1*, a critical protein for translation of OxPhos polypeptides within the mitochondria.
Materials and methods: The study has been carried out along the "Principles of laboratory animal care" and according to the national law, if applicable. Mice bearing islet beta cell-specific mitochondrial dysfunction were developed by breeding CRIF1 flox/flox mice with RIP2-cre mice. Metabolic parameters such as body weight and glucose tolerance were measured. The mitochondrial oxygen consumption rate (OCR) was measured using a Seahorse XF-24 extracellular flux analyzer for determining mitochondrial function. The GSIS (glucose stimulated insulin secretion) assay perifusion experiment was used to determine islet function. Also, Histomorphological analysis of islets was performed to examine the change of islet structure and composition.
Results: Heterozygous *Crif1*-deficient mice ($Crif1^{beta+/-}$) showed no difference in body weight or glucose tolerance compared to control mice ($Crif1^{beta+/+}$). In ex-vivo islets, basal and maximal respiratory capacity measured by OCR was not different between the two groups. However, glucose-induced OCR was significantly lower in $Crif1^{beta+/-}$ mice than $Crif1^{beta+/+}$ mice at 11 weeks. Induction of first phase insulin secretion by raising glucose from basal (5 mmol/l) to 11 mmol/l was blunted in

Crif1$^{beta+/-}$ mice compared to *Crif1*$^{beta+/+}$ mice at 14 and 22 weeks. In *Crif1*$^{beta+/-}$ mice at 18 and 22 weeks, islet area was enlarged, resulting from increased beta cell proliferation. In addition, islets of *Crif1*$^{beta+/-}$ mice had an increased alpha to beta cell ratio, with alpha cells located within the central portion of islets. We propose that beta cell dysfunction may increase alpha cell number through increased alpha cell proliferation or trans-differentiation from beta cells.

Conclusion: Beta cell specific Crif1 haploinsufficiency resulted in defect of first phase insulin secretion, and caused islet cell composition change as well as proliferation of beta cell for a compensation to maintain metabolic homeostasis. These results suggest that mitochondrial OxPhos function of beta cell has roles for cell composition of islet as well as insulin secretion

Disclosure: **H. Hong:** None.

422

The KINGS Ins2$^{+/G32S}$ mouse: a novel model of diabetes

A.L.F. Austin, C. Gentry, D. Andersson, S. Sharp, S. Bevan, P.M. Jones, A.J.F. King;
King's College London, London, UK.

Background and aims: Spontaneous hyperglycaemia was discovered in male mice in a C57Bl/6 colony at King's College London (KCL). Through gene screening, a spontaneous polymorphism of the *Ins2* gene (*Ins2*$^{+/G32S}$) was found with a substitution of glycine to serine at position 32 of the B chain of the preproinsulin molecule. We have named this mouse the **K**CL **in**sulin **G**32**S** (KINGS mouse). The human heterozygous variant of this mutation causes neonatal diabetes. To fully establish the phenotype of these mice, animals were monitored from weaning and assessed at different ages to determine onset and extent of hyperglycaemia, glucose tolerance, islet function and islet morphology.

Materials and methods: Random blood glucose concentrations were measured from weaning at 3 weeks to 20 weeks of age and animals with blood glucose above 16.7mmol/l deemed hyperglycaemic. Intraperitoneal glucose tolerance tests were performed at 4 weeks, 10 weeks and 20 weeks. Islet function was measured in isolated islets from 10 week old mice by static incubation. Islet morphology was investigated at 10 weeks using transmission electron microscopy to define islet ultrastructure.

Results: *Ins2*$^{+/G32S}$ males showed onset of hyperglycaemia at 30 ± 1.5 days and by 10 weeks blood glucose concentrations were 26.7 ± 0.9 mmol/l vs 9.4 ± 0.5 mmol/l in wild type littermates ($p < 0.001$, t-test, $n = 9$–11). Blood glucose levels in female *Ins2*$^{+/G32S}$ mice at 10 weeks were elevated but not hyperglycaemic (12.3 ± 0.8 mmol/l vs 8.9 ± 0.5 mmol/l in wild type littermates ($p < 0.007$, t-test, $n = 9$–10). Area under the curve from glucose tolerance tests showed that *Ins2*$^{+/G32S}$ males have impaired glucose tolerance at 4, 10 and 20 weeks compared to wild type controls. This progressively worsened from 4 to 10 weeks and from 10 to 20 weeks (4 weeks = 2503 ± 80 mmol/l/120 mins; 10 weeks = 3318 ± 167 mmol/l/120 mins; 20 weeks = 4022 ± 216 mmol/l/120 mins; $p = 0.022$ (4 vs 10 weeks), $p = 0.02$ (10 vs 20 weeks); $n = 5$–7, One-way ANOVA, Holm-Sidak post-hoc). Wild type mice showed no significant change over time with an average area under the curve of 1869 mmol/l/120 mins. Female *Ins2*$^{+/G32S}$ mice were glucose intolerant by 4 weeks (area under the curve = 2483 ± 145 mmol/l/120 mins vs 1438 ± 41 mmol/l/120 mins in wild types, $p < 0.001$) which did not deteriorate over time. *Ins2*$^{+/G32S}$ males at 10 weeks had a 95% reduction in glucose stimulated (20 mmol/l) insulin secretion (0.04 ± 0.01 ng/islet/h vs wild type: 0.86 ± 0.16 ng/islet/h; $p < 0.001$, $n = 5$) and a 97% reduction in insulin content (1.19 ± 0.15 ng vs wild type: 37.3 ± 4.9 ng; $p < 0.001$, $n = 5$). *Ins2*$^{+/G32S}$females at 10 weeks had a 63% reduction in glucose stimulated (20 mmol/l) insulin secretion (0.205 ± 0.014 ng/islet/h vs wild type = 0.552 ± 0.07 ng/islet/h; $p < 0.001$, $n = 5$) and a 69% reduction in insulin content (6.1 ± 0.6 ng vs wild type = 19.4 ± 1.5 ng; $p < 0.001$, $n = 5$). Transmission electron microscopy revealed ultrastructure disturbances in both genders with the *Ins2*$^{+/G32S}$ males in particular showing a depletion of insulin granules within the beta cells. They also presented with swollen mitochondria and a reduction in mitochondrial cristae, indicative of endoplasmic reticulum stress.

Conclusion: KINGS *Ins2*$^{+/G32S}$ mice have impaired glucose homeostasis with the male mice showing a more severe and progressive phenotype. In particular these mice show signs of impaired islet function and morphology as well as signs of endoplasmic reticulum stress. These mice represent a novel preclinical model of human diabetes with enhanced translational validity.

Disclosure: **A.L.F. Austin:** None.

423

Overexpression of eukaryotic translation initiation factor 2A (eIF2A) in pancreatic beta cells attenuates diabetes progression in Akita mice

E. Panzhinskiy[1], G. Soukhatcheva[2], S. Skovsø[1], D.A. Dionne[1], J.S. Wildi[1], S. Marcil[1], X. Hu[1], C.B. Verchere[2], E. Jan[3], J.D. Johnson[1];
[1]Cellular and Physiological Sciences, University of British Columbia, Vancouver, [2]Surgery, University of British Columbia, Vancouver, [3]Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.

Background and aims: Endoplasmic reticulum (ER) is a key mechanism mediating beta-cell apoptosis in diabetes. Previously, we demonstrated that overexpression of an alternative translation initiation factor eIF2A, that can initiate translation despite inhibition of protein synthesis during ER stress, protects beta cells *in vitro* from ER stress-induced apoptosis. Therefore, we investigated the protective mechanism of eIF2A in beta cells *in vivo* using Akita mice, which carry a mutant *Ins2*allele that produces an insulin protein which cannot fold properly, leading to spontaneous diabetes due to ER stress-induced apoptosis.

Materials and methods: For beta-cell specific overexpression,we designed adeno-associated virus 6 (AAV6), encoding either eIF2A-GFP or control GFP and driven by an insulin promoter. 1.5 x 10^11 viral particles were injected into the pancreatic duct of 6-week old *Ins2*$^{Akita/WT}$ female mice randomized into two groups. Two independent cohorts of 5–6 mice per group were used. Body weight and 4h fasting blood glucose levels were monitored weekly. Glucose tolerance and glucose-stimulated insulin secretion were assessed 3 weeks after AAV injection. Plasma insulin and proinsulin levels were measured using ELISA. Pancreatic islets or perfused pancreas sections were collected for RNA or immunofluorescent staining 4 weeks post AAV injection.

Results: As expected, *Ins2*$^{Akita/WT}$mice given GFP-control AAV6 showed increased fasting blood glucose levels with age, but this increase was attenuated by eIF2A overexpression in beta cells. *Ins2*$^{Akita/WT}$mice with eIF2A overexpression ($n = 10$) had lower fasting blood glucose levels compared to GFP control mice ($n = 11$) at 2 weeks (12.1 ± 1.1 vs 16.8 ± 0.7 mmol/l, $p = 0.0017$) and 3 weeks (11.5 ± 1.0 vs 17.0 ± 0.8 mmol/l, $p = 0.0005$) after AAV6 ductal injections. Beta-cell specific overexpression of eIF2A had no effect on body weight gain at any time point when compared to GFP controls. Three weeks after viral injection, *Ins2*$^{Akita/WT}$mice overexpressing beta-cell specific eIF2A showed significantly improved glucose tolerance when compared with controls transduced with AAV6-GFP alone (AUC 1631 ± 140 vs 2222 ± 85 respectively, $n = 10$–11, $p = 0.0067$). Overexpression of eIF2A in *Ins2*$^{Akita/WT}$ was associated with increased insulin secretion at 15 min post glucose challenge (0.73 ± 0.09 vs 0.47 ± 0.07 ng/ml, $n = 4$, $p = 0.04$) in comparison to GFP overexpressing *Ins2*$^{Akita/WT}$mice. In the fed state, no significant difference in plasma insulin or proinsulin levels were detected between eIF2A-GFP and control GFP groups. qPCR analysis revealed 35 ± 12% ($n = 4$, $p = 0.04$) decrease in mRNA levels of pro-apoptotic ER stress marker CHOP in pancreatic islets isolated from *Ins2*$^{Akita/WT}$ mice with eIF2A overexpression compared to GFP control. Furthermore, we observed 22.7 ± 8% ($n = 5$) decrease in intensity of immunofluorescent staining for ER stress marker BiP in pancreatic islets of *Ins2*$^{Akita/WT}$mice overexpressing beta-cell specific eIF2A compared to controls, despite no difference in beta-cell area between groups.

Conclusion: We conclude that overexpression of eIF2A in pancreatic beta cells preserves beta cell function in *Ins2*$^{Akita/WT}$ mice.
Supported by: CDA Operating Grant, JDRF Postdoctoral Fellowship
Disclosure: **E. Panzhinskiy:** None.

424

Metallothionein 1 inhibits glucose-stimulated insulin secretion and is differentially regulated in conditions of beta cell compensation and failure

M. Bensellam[1], Y.-C. Shi[2], J. Chan[2], D.R. Laybutt[2], J.-C. Jonas[1];
[1]IREC, EDIN, Université catholique de Louvain, Brussels, Belgium, [2]Garvan Institute of Medical Research, Sydney, Australia.

Background and aims: The mechanisms responsible for β cell compensation in obesity and for β cell failure in type 2 diabetes (T2D) are poorly defined. Metallothioneins play a role in both Zn^{2+} homeostasis and the regulation of cellular redox state. The mRNA levels of several metallothionein genes are upregulated in islets from subjects with T2D, but their role in β cells is not clear. Here we examined: 1) the temporal changes of islet *Mt1* and *Mt2* gene expression in models of β cell compensation and failure, and 2) the role of *Mt1* and *Mt2* in β cell function and glucose homeostasis.
Materials and methods: *Mt1* and *Mt2* expression was assessed in islets from control lean (chow diet) and diet-induced obese (DIO) mice (8 weeks high fat diet), and prediabetic (6-week-old) and diabetic (16-week-old) *db/db* mice and age-matched *db/+* (control) mice. *Mt1-Mt2* double knockout (KO) mice, *Mt1* overexpressing transgenic mice (Tg-*Mt1*) and corresponding control mice were studied. *Mt1* and *Mt2* were inhibited in MIN6 cells by small interfering RNAs. mRNA levels were assessed by real-time RT-PCR, plasma insulin and islet metallothionein levels by ELISA, glucose tolerance by *i.p.* glucose tolerance tests (*ip*GTT) and fasting-1h refeeding tests, insulin secretion by RIA, cytosolic free Ca^{2+} with Fura-2 LR, NAD(P)H by autofluorescence and cytosolic thiol redox state using roGFP1 ratiometric thiol redox probe.
Results: Increased plasma insulin levels (β cell compensation) correlated with marked downregulation of *Mt1* and *Mt2* mRNA levels in islets of DIO mice (*Mt1*: ~4-fold, $p < 0.01$ and *Mt2*: ~4.5-fold, $p < 0.05$), and prediabetic *db/db* mice (both by ~2-fold, $p < 0.01$). These findings were confirmed in β cells of DIO mice (β cell-specific translating ribosome affinity purification model). In contrast, β cell failure in islets from diabetic *db/db* mice correlated with a tendency for increased *Mt1* (~1.3-fold) and significantly upregulated *Mt2* (~1.6-fold, $p < 0.05$) mRNA levels. *Ex vivo* treatment of islets for 18–48h in high glucose (10–30 mM *vs.* 2–5 mM) strongly downregulated *Mt1* and *Mt2* mRNA and protein levels in parallel with increased insulin secretion. Interestingly, KO mice displayed markedly improved glucose tolerance during *ip*GTT ($p < 0.01$) and fasting-refeeding tests ($p < 0.01$), in association with increased plasma insulin levels (30 min following *ip*GTT, $p < 0.05$). Glucose-stimulated insulin secretion (GSIS) was potentiated in islets isolated from KO mice *vs.* control islets while insulin content was unchanged. In MIN6 cells, knockdown of *Mt1*, but not *Mt2*, potentiated GSIS by ~1.8-fold ($p < 0.01$). The potentiation of GSIS in KO islets occurred despite similar rises in intracellular Ca^{2+} and NAD(P)H levels, and the mRNA levels of β cell enriched genes preproinsulin, *Pdx1*, *Glut2* and *Pc* and stress response genes *Hmox1*, *Hspa5* and *Ddit3* were unchanged. Nevertheless, basal and acute H_2O_2-induced cytosolic roGFP1 oxidation was slightly lower in KO islets. On the other hand, overexpression of *Mt1* in islets from Tg-*Mt1* inhibited GSIS by ~1.5-fold ($p < 0.01$). Moreover, treatment of control islets with $ZnCl_2$, a potent inducer of metallothioneins, reduced GSIS. This effect was absent in KO islets.
Conclusion: We identified *Mt1* as a novel negative regulator of GSIS in mouse β cells. Our studies suggest a role for *Mt1* downregulation in β cell compensation in obesity, and for *Mt1* upregulation in β cell failure in T2D.
Supported by: MOVE-in Louvain fellowship (MB), ARC, NHMRC (DRL), SFD-2016 (JCJ)
Disclosure: **M. Bensellam:** None.

PS 021 Clinical and experimental immunology in type 1 diabetes

425

Older age of diagnosis is the major feature of persistent long term endogenous insulin secretion in type 1 diabetes

R.A. Oram[1,2], T.J. McDonald[1,3], S. Sabbah[4], S. Bloem[5], B. Shields[1], A. Hill[1], R. Bolt[1], S. Begom[4], L. Khatri[4], T. Tree[4], B.O. Roep[5,6], A.T. Hattersley[1], The TIGI Study Group;
[1]NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter, UK, [2]Academic Kidney Unit, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK, [3]Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK, [4]Immunobiology, King's College London, London, UK, [5]Leiden University Medical Centre, Leiden, Netherlands, [6]Department of Diabetes Immunology, City of Hope, Duarte, USA.

Background and aims: The majority of people with long term (>5 years) type 1 diabetes (T1D) have a small number of functioning beta-cells, and a small proportion have relatively high C-peptide despite longstanding T1D. The clinical correlates and mechanisms for persistent beta cell function are not known. We aimed to investigate the mechanisms of persistent beta cell function by studying the clinical, genetic, serological and immune associations of persistent endogenous insulin in the TIGI study.
Materials and methods: We identified T1D patients in the top and bottom 20% of C-peptide secretion for their duration of diabetes, assessed using a post meal urine C-peptide creatinine ratio(UCPCR) in a cross sectional regional study of 1005 patients. A median of 3 years later we confirmed their C-peptide status using a MMTT (90-minute serum C-peptide >40 pmol/L (CpHi) or undetectable (<3 pmol/L, CpLo). We measured GAD, IA-2 and ZnT8 autoantibodies and T1D genetic risk score (T1D GRS). Detailed immunophenotying of cryopreserved PBMC was performed including assessment of circulating leucocyte subsets, islet specific CD4 and CD8 T cells, and CD4 Treg.
Results: We investigated 48 CpHi and 65 CpLo with markedly different 90 min C peptide (median(IQR) 144(92,301) v <3(<3,<3)pmol/L, $p < 0.0001$), despite similar duration (12.2(7.5,22.0) v 10.9(8.2,15.4)y $p = 0.4$). Age of diagnosis was older in CpHi (16(13,22) v 6(3,10)y $p < 0.0001$). HbA1c was similar (69.5(63.0,81.0) v 69(57.0,79.0) mmol/mol $p = 0.4$) but insulin doses lower (0.69(0.55,0.96) v 0.83(0.74,1.01)$p = 0.003$)u/kg/24 hr. CpHi had more autoantibody positive (83 v 63% ≥1 autoantibody positive, $p = 0.025$) but no difference in T1D GRS (mean(SD) 0.274(0.025) v 0.275(0.026), $P = 0.9$), HLA DR3/DR4 status ($p = 0.7$) or individual proportion of T1D associated loci (30 individual loci all $p > 0.10$). Following age adjustment, there was no difference in total frequencies of circulating leucocyte populations, FOXP3 Treg function or the total frequency of autoreactive CD8 T cells with specificity for a range of islet antigens. Proliferation of CD4 T cells in response to islet antigens GAD65 or Proinsulin, was similar, however, the quality of response differed, with individuals from the CpHi group producing a higher level of the anti-inflammatory cytokine IL-10 in response to proinsulin (12.0(2,23) v 4.9l(2,11)mg/mL, $p = 0.01$).
Conclusion: A persistently high C-peptide in long standing T1D is a feature of an older age of diagnosis. It is associated with a higher proportion of islet autoantibodies and little evidence of increased genetic susceptibility or a less severe T cell mediated autoimmunity apart from an enhanced signature of islet specific immune regulation. The strong association of age of diagnosis and long term endogenous insulin secretion allows extrapolation from studies at diagnosis supporting a different immune phenotype in the islets at diagnosis.
Supported by: JDRF, NIHR, Diabetes UK, Wellcome Trust
Disclosure: **R.A. Oram:** None.

426

Repeat BCG vaccination creates lasting HbA_{1c} reductions in adult subjects with longstanding type 1 diabetes

D. Faustman[1], W.M. Kühtreiber[1], L. Tran[1], M. Dybala[1], S. Plager[1], S. Janes[1], A. Defusco[1], H. Zheng[2];
[1]Massachusetts General Hospital/Harvard Medical School, Boston, [2]Massachusetts General Hospital, Boston, USA.

Background and aims: The bacillus Calmette-Guerin (BCG) vaccine, originally developed for tuberculosis, is being trialed globally for new immune indications including allergy, autoimmunity and infection. A randomized, placebo-controlled, Phase I study of adult subjects with longstanding type 1 diabetes (T1D) who received the BCG vaccine (2 vaccinations 4 weeks apart) previously revealed potential disease-modulating, but not clinical, effects of the vaccine with 20 weeks of follow up (i.e., death of autoreactive T cells, transient and modest restoration of insulin secretion, induction of regulatory T cells [Tregs]). Here we report long-term follow up of subsequent study groups, including original Phase I trial subjects at year 08 and additional subjects up to year 05.
Materials and methods: This analysis includes data on 282 human research participants in both *in vivo* BCG vaccine clinical trial studies (n = 52) and *in vitro* mechanistic studies (n = 230). Of these research subjects, 211 had T1D and 71 were non-diabetic control subjects. Adults subjects with T1D were followed for 8 years (Phase I trial subjects) or up to 5 years (additional subjects) after BCG vaccinations. All subjects with T1D had disease >10 years duration without complications at enrollment. Mechanistic studies of RNAseq, metabolomics and epigenetics were performed in parallel to track the systemic and mechanistic effects of BCG vaccinations.
Results: Starting after year 03 of follow up, only BCG vaccinated subjects had lowered HbA1c for 1 year (Year 05 data: BCG-treated HbA1c 6.18 ± 0.34 [n = 9], placebo 7.07 ± 0.41 [n = 3], reference subjects with type 1 diabetes 7.22 ± 0.17 [n = 34, p = 0.02]). Follow-up of 6 Phase I trial subjects who have been followed for a total of 8 years, 4 years after the first documented lowering of HbA1c, confirms the ability of repeat BCG vaccination to maintain lowered HbA1c levels without hypoglycemia in long-term disease (BCG-treated HbA1c 6.65 ± 0.26 vs placebo 7.22 ± 0.38, p = 0.0002) for a total of 5 continuous years. For all BCG-treated subjects, the stable reductions in HbA1c were not associated with hypoglycemia. BCG-treated subjects had no change in their enrollment use of insulin pumps and none utilized a CGM device. The impact of BCG on blood sugars appeared to be driven by a novel systemic and blood sugar lowering mechanism in diabetes. We observed a systemic shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis, a state of high glucose utilization, and major epigenetic effects on the immune system related to Treg tolerance.
Conclusion: Repeat BCG vaccination in this trial was associated with stable and long-term lowering of HbA1c without hypoglycemia for over 5 years after an onset delay. The apparent stable and long-lasting impact of BCG on blood sugars in humans with T1D appears to be the result of a novel mechanism, as documented with metabolomics, mRNAseq, and epigenetic methods; namely, a systemic shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis. BCG via epigenetics resets Treg genes for genetic reprogramming of tolerance. The identification of a novel mechanism for significant blood sugar lowering with BCG opens the door for future trials in both type 1 and 2 diabetes with a safe, novel and affordable approach.
Disclosure: **D. Faustman:** None.

427

GAD65 autoantibodies are associated with incident diabetes in mid-life: the EPIC-InterAct study

O. Rolandsson[1], C.S. Hampe[2], S.J. Sharp[3], C. Langenberg[3], N. Wareham[3], EPIC-Interact Group;
[1]Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden, [2]Department of Medicine, University of Washington, Seattle, USA, [3]MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge School of Clinical Medicine, Cambridge, UK.

Background and aims: Type 1 diabetes (T1D), is characterized by autoimmune destruction of beta cells. The autoimmune response manifests itself in T cell reactivity and autoantibody responses directed against at least four beta-cell autoantigens including the 65kDa isoform of glutamic acid decarboxylase (GAD65). Although the pathogenesis of Type 2 diabetes (T2D) is different to that of T1D, there is some overlap as up to 40% of T2D patients demonstrate an autoimmune element revealed by the presence of autoantibodies, predominantly directed against GAD65. As is the case for T1D, it has been suggested that the presence of GAD65Ab preceeds the development of T2D, albeit with a lower prevalence. Susceptibility for development of GAD65Ab and T1D is at least in part mediated by risk alleles located within the HLA region on chromosome 6. However, the association between T2D-associated genes and autoantibody positive diabetes in adults remains to be established. Thus, our aim was to assess the association between GAD65Ab, genetic risk scores for T1D and T2D, and the development of diabetes.
Materials and methods: We investigated the associations in adults in EPIC-InterAct, a case-cohort study in 8 countries nested in the European Prospective Investigation into Cancer and Nutrition cohort (n = 340,234). GAD65Ab were analysed at baseline by radioligand binding assay in a random subcohort (n = 15,802) and in all incident cases ascertained and verified as T2D (n = 11,981). T1D and T2D genetic risk scores (GRS) were calculated. Associations were estimated using Prentice-weighted Cox regression (GAD65Ab/incident diabetes) and logistic regression (genetic risk/GAD65Ab positivity); all models accounted for country.
Results: GAD65Ab positivity at baseline was associated with development of diabetes (median follow-up: 11.7 years) (HR GAD65Ab positive vs negative 1.78, 95% CI 1.43–2.20) after adjustment for sex, center, physical activity, smoking status and education. T1D-GRS, but not T2D-GRS, was associated with GAD65Ab positivity in both the subcohort (OR 1.24 per SD 95% CI 1.03–1.50), and incident diabetes cases (OR 1.97 per SD 95% CI 1.72–2.26) when adjusted for sex and age. Of the five SNPs in the T1D GRS that are associated with HLA, three were associated with GAD65Ab positivity in the subcohort after adjustment for sex and age; the odds ratios for GAD65Ab positivity were 4.07 (95% CI 2.01–8.26) for DR3/DR3, 2.42 (95% CI 1.32–4.43) for DR3/DR4 - DQ8, and 3.93 (95% CI 1.78–8.68) for DR4-DQ8/DR4-DQ8. There was no overall association between T1D GRS and incident diabetes (HR 1.02 per SD T1D GRS, 95% CI 0.99–1.06) when adjusted for age, sex, physical activity, smoking status, education, and BMI. Because of the relationship between T1D GRS and antibody status, we stratified the analysis by GAD65Ab status. In GAD65Ab positive individuals, there was a significant association between T1D GRS and incident diabetes (HR 2.42 per SD (95% CI 1.84–3.21)), which was not evident in GAD65Ab negative individuals (HR 1.00 per SD T1D GRS, 95% CI 0.97–1.04).
Conclusion: Our findings suggest that a sub-group of individuals who present with incident diabetes in mid-life have an underlying autoimmune aetiology.
Supported by: EU FP6 program, Umeå University, National Institutes of Health
Disclosure: **O. Rolandsson:** None.

428

Exogenous IL-33 prevents diabetes induction in mice

N. Jovicic[1], I. Petrovic[2], S. Pavlovic[3], B. Ljujic[4], M. Miletic Kovacevic[1], N. Pejnovic[2], N. Arsenijevic[5], M.L. Lukic[5];
[1]Department of Histology and embryology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, [2]Department of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, [3]Department of Microbiology and immunology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, [4]Department of

Genetics, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, [5]Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia.

Background and aims: Type 1 diabetes is an autoimmune disease caused by the immune-mediated destruction of pancreatic β-cells. Prevention of type 1 diabetes requires early intervention in the autoimmune process against beta-cells of the pancreatic islets of Langerhans, which is believed to result from disordered immunoregulation. $CD4^+Foxp3^+$ regulatory T cells (Tregs) participate as one of the most important cell types in limiting the autoimmune process. We have previously shown that IL-33R (ST2) deletion enhanced susceptibility to multiple low dose streptozotocin (MLD-STZ) induced diabetes. The aim of this study was to investigate the preventive and therapeutic effect of IL-33 in MLD-STZ induced diabetes and to delineate the mechanisms of its influence on autoimmune attack.
Materials and methods: For the induction of diabetes C57BL/6 mice were treated with five doses of 40 mg/kg STZ, and 0.4 μg rIL-33 was administrated per mouse, four times, every second day from the day of disease induction. Glycemia, glycosuria and HbA1c levels were measured after diabetes induction and histological and immunohistochemical parametars in pancreatic islets were evaluated on day 28. Cellular make up of the pancreatic lymph nodes and islets were evaluated by flow cytometry.
Results: IL-33 was given simultaneously with the application of STZ and completely prevented the development of hyperglycemia, glycosuria and attenuated islet mononuclear cells infiltration. IL-33 treatment enhanced the bias toward Th2 immune response and increased the frequency and number of $ST2^+$ Tregs. This was accompanied by higher number of IL-13 and IL-5 producing $CD4^+$ T cells and increased presence of $ST2^+Foxp3^+$ regulatory T cells (Tregs) in pancreatic lymph nodes and islets. Using IL-33 also promotes islet infiltration with M2 macrophages.
Conclusion: We provide the first evidence that exogenous IL-33 completely prevents the development of T cell mediated inflammation of pancreatic islets and consecutive development of diabetes in C57BL/6 mice.
Supported by: Faculty of Medical Sciences, University of Kragujevac
Disclosure: **N. Jovicic:** None.

429

Immunomodulatory therapies with anti-IL-6 and anti-IL-17 combined with anti-TCR to regain normoglycaemia in the LEW.1AR1-iddm rat as a model for human type 1 diabetes

A. Joerns[1], H. Teraoku[2], T. Yoshimoto[2], D. Wedekind[3], S. Lenzen[2];
[1]Institute of Clinical Biochemistry, Hannover Medical School, Hannover, [2]Institute of Experimental Diabetes Research, Hannover Medical School, Hannover, [3]Institute of Laboratory Animal Science, Hannover Medical School, Hannover, Germany.

Background and aims: In other autoimmune diseases, such as psoriasis and rheumatoid arthritis, antibodies against IL-6 and IL-17 showed very promising therapy success. Combination therapies with both antibodies alone or combined with anti-TCR, a T cell antibody, have not yet been performed in type 1 diabetes mellitus (T1DM). The aim of the study was to elucidate the protection potential of these antibodies for the survival of the remaining beta cells from autoimmune destruction after onset of diabetes. The IDDM (LEW.1AR1-*iddm*) rat, a model of human T1DM, was used for these studies. In this model other combination therapies have been successfully evaluated.
Materials and methods: Animals were treated with anti-IL-17 (0.1 mg/kg b. wt.), anti-IL-6 (0.01 mg/kg b. wt.) alone or in combination with anti-TCR (0.5 mg/kg b. wt.) in a double or triple fashion consecutively over 5 days immediately after diabetes manifestation. Besides biochemical parameters changes in the pancreas were analysed quantitatively by biopsies at the time point of diabetes manifestation, at the end of therapy, and 60 days after the end of therapy for beta cell survival and immune cell infiltration on the gene and protein expression level.
Results: Prevention therapies in combination of anti-TCR with anti-IL-17 or/and anti-IL-6 starting immediately after disease manifestation reversed diabetes to normoglycaemia until 60 days without therapy. The therapy effectiveness in the triple combination was successful in the widest range of blood glucose concentrations between 8–18 mmol/l, followed by the combination with anti-IL-17 and thereafter with anti-IL-6. Monotherapies with both antibodies showed no therapeutic effect. Thereby the C-peptide concentrations increased from 1/3 of the normal control values of around 1000 pg/ml, to 2/3 in the double and nearly to the control values in the triple combination treated IDDM rats. In parallel, beta cell mass in the pancreas was nearly doubled after combination with anti-IL-6 in comparison to the diabetic control (4.38 ± 0.19 vs. 2.21 ± 0.30) and reached values close to normal in the double and triple combination with anti-IL-17 (double 5.41 ± 0.18; triple 5.50 ± 0.17 vs. 6.25 ± 0.20). The beta cell proliferation rate showed a fourfold increase in the double combination and a twofold increase in the triple combination with anti-IL-17, whereas the beta cell apoptosis rate was markedly reduced only in the combination with anti-IL-6 alone or additionally with anti-IL-17. Severe islet immune cell infiltration in the pancreas was abolished after all combination therapies in normoglycaemic IDDM rats. Residual infiltrating CD68 macrophages were observed in the islet periphery, whereas CD8 T cells and γ, δ T cells were only found in the double combination with anti-IL-17.
Conclusion: Anti-IL-6 reduced islet immune cell infiltration and anti-IL-17 possessed the strongest beta cell proliferation potential. Combining both anti-inflammatory cytokine antibodies with anti-TCR in the triple therapy revealed an optimal effect with a regain of stable normoglycaemia.
Supported by: DFG (German Research Foundation)
Disclosure: **A. Joerns:** None.

430

Gut microbiome signatures in LEW.1AR1-iddm rats during time course of beta cell autoimmunity

T. Schoeppe[1], J.-H. Sachs[1], B. Kreikemeyer[2], D.P.R. Herlemann[3], M. Tiedge[1];
[1]University Medical Center Rostock, Institute of Medical Biochemistry and Molecularbiology, Rostock, [2]University Medical Center Rostock, Institute of Medical Microbiology, Virology and Hygiene, Rostock, [3]Leibniz-Institute for Baltic Sea Research Warnemünde, Rostock, Germany.

Background and aims: The gut microbiome plays an important role in the pathogenesis of type 1 diabetes mellitus. It is still unknown how the gut microbiome affects the time course of beta cell autoimmunity from islet infiltration to overt diabetes. In this study we analyzed changes of the gut microbiome in LEW.1AR1-*iddm* rats during the process from islet infiltration (day 40) to complete beta cell loss and diabetes manifestation (day 60–70).
Materials and methods: Stool samples were collected from normoglycaemic (blood glucose <7.8 mmol/l) LEW.1AR1-*iddm* ($n = 68$) and diabetes resistant LEW.1AR1 ($n = 20$) rats at day 40, 50, 60 after birth and at the timepoint of diabetes manifestation. Total chromosomal DNA was isolated from ~200 mg frozen stool samples using the Qiagen QIAamp DNA stool extraction kit. The 16S rRNA genes were amplified on the V3 - V4 regions and sequenced on using the paired end Illumina MiSeq platfrom. The taxonomic classification of resulting sequences were performed and quality controlled using QIIME and SILVA_NGS.
Results: The analysis showed a different development of gut mirobiome communities in LEW.1AR1-*iddm* and LEW.1AR1 rats. While microbial diversity increased in both strains between day 40 and day 60, the diversity was significantly lower in LEW.1AR1-*iddm* rats than in LEW.1AR1 controls. In LEW.1AR1-*iddm* rats we also observed increased abundancies of Firmicutes, *Prevotella* and *Clostridium*. Heatmap analysis were used to rank most abundant bacteria species and confirmed a lower microbial diversity in LEW.1AR1-*iddm* rats with high abundancy of

*Lachnospiraceae*and *Clostridia*, which were formerly described as a trigger for inflammatory gastrointestinal diseases.
Conclusion: Microbial species with proinflammatory characteristics were increased at the beginning of islet infiltration in diabetes susceptible LEW.1AR1-*iddm* strain. These proinflammatory species constanly decreased up to the timepoint of diabetes manifestation. The data may have several implications for gut microbiome analysis in humans: (1) Microbiome analysis has a higher prognostic value in young individuals with high family risk of T1D and low autoantibody titres. (2) Age-dependent changes of microbial diversity and abundancies require repeated analyses of stool samples. (3) Probiotic interventions should start early in life before detection of autoantibodies.
Supported by: DDG
Disclosure: **T. Schoeppe:** Grants; DDG.

431

HERV-W-Env is involved in type 1 diabetes pathogenesis: new insights from mouse models

S. Levet[1], J. Medina[1], J. Joanou[1], N. Queruel[1], J. Pierquin[1], H. Perron[1,2]; [1]Geneuro Innovation, Lyon, France, [2]Geneuro, Plan-les-Ouates, Switzerland.

Background and aims: Human endogenous retroviruses (HERVs), known to represent 8% of the human genome, have been associated with several autoimmune diseases. In particular, the envelope protein of HERV-W family (HERV-W-Env), which has been involved in the pathogenesis of Multiple Sclerosis (MS), displays pro-inflammatory and autoimmune properties. This has initially been demonstrated in an MS context, but it subsequently turned out to be relevant for Type 1 Diabetes (T1D). We recently observed that HERV-W-Env protein and RNA are detected respectively in sera and PBMC of more than 50% of T1D patients. We demonstrated that this pathogenic protein is expressed by acinar cells in human T1D pancreas, and is associated with the recruitment of macrophages within the pancreas of these patients. HERV-W-Env also displays direct pathogenic properties, as it inhibits insulin secretion by human Langerhans islets.
Materials and methods: Two transgenic mouse models in which HERV-W-Env transgene is expressed under the control of HERV LTR and CAG promoter have been developed. In a first model, these transgenic mice have been generated in a C57Bl6/J background and have been challenged with 5 multiple low-dose streptozotocin (STZ, 40 mg/kg). In a second model, transgenic mice are currently backcrossed in a NOD/ShiLtJ background. Glycemia, insulinemia and pancreas histology are studied in both models.
Results: HERV-W-Env transgenic mice in the C57Bl6/J background were challenged by repeated STZ injections. We observed that transgenic mice are more susceptible to STZ-induced diabetes as they developed a more severe hyperglycemia ($P < 0.01$) and hypoinsulinemia ($P < 0.01$) than wild-type C57Bl6/J mice. These observations are consistent with endocrine pancreatic damage observed in HERV-W-Env-STZ transgenic mice which developed more severe insulitis ($P < 0.0001$) than wild-type-STZ mice. Interestingly, HERV-W-Env transgenic mice also displayed huge abnormalities in their exocrine pancreas, consisting in immune ($P < 0.0001$) and fatty infiltrates ($P < 0.0001$), which are not modified by STZ injections. These pre-existing exocrine abnormalities observed in HERV-W-Env transgenic mice could explain the pancreatic susceptibility to environmental insults, such as the ones caused by STZ. In a second model, transgenic mice are currently backcrossed in a NOD/ShiLtJ background. Preliminary results revealed that NOD transgenic males start to develop a hyperglycemia as soon as 4 weeks old compared to their controls littermates.
Conclusion: Early results from these transgenic mouse models support a role for HERV-W-Env in human T1D pathogenesis, in a sub-group of patients expressing this pathogenic protein. They provide additional rationale for the ongoing phase IIa clinical trial, which is designed to neutralize HERV-W-Env in T1D patients using a monoclonal antibody named GNbAC1. Six months interim results of this new therapeutic approach will be available 3rd quarter 2018.
Clinical Trial Registration Number: NCT03179423
Supported by: Geneuro
Disclosure: **S. Levet:** Employment/Consultancy; Geneuro Innovation.

432

EMC-D virus-induced diabetes in DBA/2 mice

K. Mine[1], S. Nagafuchi[2], H. Takahashi[2], Y. Yoshikai[1], H. Mori[2], Y. Matsuta[2], K. Anzai[2];
[1]Division of Host Defense, Medical Institute of Bioregulation, Kyushu University, Fukuoka, [2]Division of Metabolism and Endocrinology, Department of Internal Medicine, Saga University, Saga, Japan.

Background and aims: Human type 1 diabetes (T1D) research has suggested the close link between viral infections and T1D. In experimental animal model, D variant of encephalomyocarditis virus (EMC-D) induces T1D in male SJL, SWR, and DBA/2 mice within five days after infection. It was reported that a single autosomal-recessive gene, which is inherited in a Mendelian manner, controls susceptibility to EMC-D virus-induced diabetes (VID)(Nature, 1978). The natural susceptible gene(s) had been unknown until we reported that natural mutations of tyrosine kinase 2 (tyk2) gene determined susceptibility to EMC-D VID in SJL and SWR mice (Nat commun, 2015). It was also revealed that DBA/2 mice that lack tyk2 gene mutations should have other VID susceptibility gene(s). In this study, we assessed the pathogenesis of EMC-D VID in DBA/2 mice to explore the role of susceptibility gene(s) in DBA/2 mice.
Materials and methods: To analyze the mechanisms to develop EMC-D VID in mice, we carried out intraperitoneal challenge with 1.0×10^3 PFU of EMC-D virus to VID-resistant C57BL/6(B6) and DBA/2 male mice. Immunohistochemical analysis, islet isolation, and cell death assay, were performed.
Results: The levels of serum type 1 interferon (IFN) in virus infected DBA/2 mice were comparable to that of resistant-strain B6 mice, while virus titer in pancreas of DBA/2 mice was significantly higher than that of B6 mice at three to five days after infection. High dose of type 1 IFN transfer did not alter the outcome of EMC-D VID in DBA/2 mice indicating that increased levels of type 1 IFN did not possess biological significance to resist against VID in DBA/2 mice. Histopathological analysis showed that both B6 and DBA/2 mice had mild CD45-positive cell-infiltration around the islets at three days after infection, while insulin-negative areas were widely observed in DBA/2-islets. These observations indicated that islets of DBA/2 mice were destroyed by EMC-D virus before immune cell-infiltration. At five days after infection, B6 mice had CD45-positive cell-infiltration around, but not into the islets. In contrast, DBA/2 mice developed severe CD45-positive cell-infiltration in the islets concomitant with extensive destruction of the islets. Mouse embryonic fibroblasts (MEF) showed comparable anti-viral responses between DBA/2 and B6 mice at 100 U/ml IFN-β stimuli. On the other hand, the virus-induced β-cell death was significantly increased in DBA/2 β-cells than B6 β-cells by the stimulation with 100 U/ml IFN-β. Annexin V, a marker of apoptosis, positive β-cells were significantly increased in DBA/2 β-cells. In addition, caspase3, another marker of apoptosis, positive cells were noticeably detected in DBA/2-islets at five days after infection. These observation suggested that reduced β-cell defense against virus infection in DBA/2 mice determined susceptibility to EMC-D VID.
Conclusion: These observations suggested that virus-induced islet cell lysis is the first step of islets destruction and enhanced by inflammatory cell-infiltration in the islets. The weakness of antiviral responses and of type 1 IFN dependent reactivity within three days after infection may determine susceptibility to EMC-D VID in DBA/2 mice. Thus, it was suggested that gene(s) associated with early anti-viral responses or cell-survival associated genes in β-cells may involve EMC-D VID susceptibility gene(s).
Supported by: A grant from the Ministry of SCS of Japan and Japan IDDM Network.
Disclosure: **K. Mine:** None.

PS 022 Exercise is good for you

433

Effectiveness and applicability of an aggressive management of insulin therapy and CHO-integration strategy during prolonged endurance competition in athletes with type 1 diabetes

E. Gamarra[1], A. Benso[1], L. Nollino[2], M. Miccio[3], C. Agnoli[4], M. Sudano[5], M. Vasta[5];
[1]Medical Science, Endocrinology, Diabetology and Metabolic Unit, University of Turin, Turin, [2]UOC Malattie Endocrine, del Ricambio e della Nutrizione, AULSS 2 della Marca Trevigiana, Treviso, Italy, [3]freelance Nutritionist, Garda (Vr), [4]Fellow Athlete with Type 1 Diabetes and former President of Italian Non-Profit Organization "Diabetenolimits", Garda (Vr), [5]Diabetes and Endocrinology Unit AV1, General Hospital, Urbino, Italy.

Background and aims: General recommendations for T1DM athletes are usually focused on reducing insulin dose and/or defensive carbohydrates (CHO) eating to prevent hypoglycemia, independently of duration and intensity of the activity and fitness and nutritional status of the athlete. On the other hand, high CHO supplementation is strongly suggested in order to maximize performance in healthy endurance athletes. Aim of the present study was to investigate the effectiveness and applicability of an aggressive management of insulin therapy and CHO integration strategy, focused on maximizing the performance, during prolonged endurance race in T1DM athletes.

Materials and methods: 8 T1DM amateur athletes experienced in endurance sports (6 on MDI, 2 on CSII) participated to a trail running competition of approximately 3 hours, performing 3 or 4 laps of a 6 km muddy track, average 250 m d+ in rainy and windy weather conditions. Diabetes duration ranged from 9 to 37 yrs (mean 20 yrs), A1c values from 6.7 to 7.8% (mean 7.3%) and total insulin requirement from 0.26 to 0.59 U/kg/die (mean 0.46 U/Kg/die). Athletes were asked not to reduce their insulin dose profile before and during the race and, at the same time, to maintain an aggressive CHO approach, accordingly to their experience and medical staff advices. Clinical and athletic evaluations were performed before the race and at the end of each lap. Glucose values were evaluated with fingersticks (Abbott®).

Results: Mean capillary glycaemic value was 201 ± 31 mg/dl (range 161–242) before the first lap and 118 ± 51 mg/dl (range 78–217) at the end of the last lap, showing a trend toward decrease (−83 mg/dl vs baseline), in front of constant mean CHO integration (57 ± 19 g/h or 0.825 ± 0.24 g/kg/h, range 30–83 g/h or 0.42–1.18 g/kg/h), that was slightly less than recommendations for this kind of effort (>60–90 g/h). No cases of severe hypo- or hyperglycemia occurred (min 62 mg/dl, max 242 mg/dl). None of the athletes, both in MDI and CSII, modified his basal insulin profile, neither the bolus and insulin/CHO ratio was varied with respect to CHO supplementation during the race. Heart rate (maintained in zone 2–3 for most of the time and in zone 4 only for very short time) and mean lactate levels (3.5 ± 1.7 mmol/l, range 1.7–9.6) indicated that the effort of the athletes was mainly supported by aerobic metabolism.

Conclusion: The T1DM athletes evaluated safely and successfully completed a prolonged trail running endurance competition, following an aggressive management of insulin therapy and CHO integration strategy. The present data support the hypothesis that, differently from general sports recommendations in T1DM, essentially focused on avoiding hypoglycemia and severe hyperglycemia, in case of endurance T1DM athletes, the combination of exercise physiology, duration and intensity of the activity, fitness and nutritional status of the athlete should carefully be kept in consideration in the management of insulin therapy and CHO supplementation, in order to maximize performance.

Disclosure: **E. Gamarra:** None.

434

Assessment of glycaemic changes and parameterisation of physical exercise during football matches in adolescents with type 1 diabetes

A. Gawrecki[1], A. Michalak[2], S. Gałczyński[3], J. Nowaczyk[4], I. Dachowska[2], M. Szymańska[2], B. Dulińska[2], D. Zozulińska-Ziółkiewicz[1], A. Szadkowska[2];
[1]Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poznań, [2]Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Lodz, [3]The Academic Laboratory of Movement and Human Physical Performance DynamoLab, Medical University of Lodz, Lodz, [4]Department of Paediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland.

Background and aims: Maintaining normoglycemia throughout football matches is difficult for young players with type 1 diabetes mellitus (T1DM). Moreover, it is not clear whether two similar games elicit the same glycemic response in a particular player. The aim of this study was to parametrize physical activity and glycemic variability of adolescents with T1DM during two football matches.

Materials and methods: During summer camp for adolescents with T1DM, two football matches (each lasting 80 minutes +10-minute break) were organized for two 9-players adolescent teams (mean age 14.9 ± 1.4 years old, diabetes duration 7.2 ± 3.9 years, HbA1c 7.1 ± 0.6% [54 ± 0.32 mmol/mol]). The meetings were separated by a 4-day rest period. During the matches, players wore chest straps heart rate (HR) monitors coupled with GPS, which allowed for continuous tracking of their position and movement. To assess glycemic and metabolic response to exercise, lactate and blood glucose (BG) in capillary blood were measured at rest, after the first half and at the end of each match. Moreover, some players used continuous glucose monitoring (CGM) systems which allowed to measure glucose level every 5 or 15 minutes, depending on the CGM device.

Results: Mean BG before and after each match were as follows - match: no.1: 139 ± 61 mg/dl, 151 ± 95 mg/dl; no.2: 164 ± 80 mg/dl and 131 ± 78 mg/dl. No significant difference in BG between the matches ($p = 0.32$) or during each match ($p = 0.5$) was noted, changes between matches were also similar ($p = 0.9$). Hypoglycemia <70 mg/dl was observed in four players during the first match, two of them experienced glycemia <54 mg/dl. Similar proportions were noted for the second meeting (<70 mg/dl in four players, <54 mg/dl in one). No episode of glycemia <54 mg/dl lasted ≥15 min in CGM was observed. Recorded HRs (match no. 1: 77 ± 7% of maximum HR for a given age; match no. 2: 76 ± 8%) revealed a mixed aerobic-anaerobic character of exercise and were similar between the meetings ($p = 0.58$). During both matches, significant rise in capillary blood lactate was observed (match: no.1: 2 ± 0.6 do 6 ± 4.9; no.2: 1.9 ± 07 do 4 ± 2, $p = 0.005$), similar during both events ($p = 0.46$) although with tendency toward higher concentrations in match no 1 ($p = 0.051$). Mean distance covered by the players was comparable in both matches (match no.1: 6.1 ± 1 km, no.2: 6.2 ± 1.4 km, $p = 0.89$). No significant differences were noted regarding velocities and accelerations reached by each player during both matches.

Conclusion: HR monitoring coupled with GPS-based tracking can effectively parametrize physical activity during a football match. Under similar exercise workload particular participants displayed comparable changes in glycemia which gives hope for creating uniform guidelines for young football players with T1DM.

Disclosure: **A. Gawrecki:** None.

435

Variability and reproducibility in the rise in blood glucose levels in response to high intensity interval training (HIIT) in type 1 diabetes: the FIT reproducibility study

M.C. Riddell[1], R. Pooni[1], R.E. Brown[2], L. Yavelberg[1], Z. Li[3], C. Kollman[3], R. Aronson[2];

[1]School of Kinesiology and Health Science, York University, Toronto, Canada, [2]LMC Diabetes & Endocrinology, Toronto, Canada, [3]Jaeb Center for Health Research, Tampa, USA.

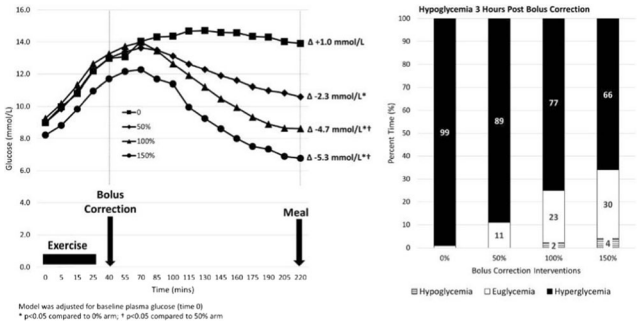
Background and aims: Hyperglycemia can occur when individuals with type 1 diabetes (T1D) perform high intensity interval training (HIIT). However, the reproducibility of the glycemic response to a HIIT session has not been definitively tested. The objective of this study was to investigate the reproducibility of the glycemic response to a HIIT session in physically active patients with T1D.
Materials and methods: Seventeen patients with T1D, all using insulin glargine 300 U/mL as basal insulin, were asked to perform four separate in clinic HIIT sessions in an overnight fasted state. HIIT consisted of two bouts of cycling at 90% peak power, separated by a series of 'CrossFit'-type activities, spanned over a 25 min period (~75–95% of maximal heart rate). Plasma glucose (YSI) was measured pre-exercise (−10 min) and at 5- and 15-min into the HIIT session, as well as at 5- and 15-min in recovery.
Results: A total of 64 HIIT sessions were compiled. Pre-exercise blood glucose levels were similar among the four HIIT visits (8.8 ± 1.0 mmol/L, mean SD), as were the rise in glucose levels in response to HIIT (+3.9 ± 1.6; +3.8 ± 1.8; +3.9 ± 2.3; +3.9 ± 1.5 mmol/L, in visits 1-4, respectively). In almost all occasions (63 of 64 sessions), HIIT produced a rise in glycemia, but the inter-individual responses did vary, ranging from −0.3 to +9.0 mmol/L. The change in glucose during HIIT was not influenced by the baseline glucose concentration and was predictable within an individual based on the measured response in visit 1 (composite correlation with post-exercise glucose rise among the four visits was 0.56 [0.33–0.79, 95% CI]).
Conclusion: Following HIIT, there appears to be a consistent increase from the pre-exercise glucose concentration in patients living with T1D and the degree of response is moderately reproducible within a given patient. Individualized insulin correction strategies, which take into account the rise in glucose observed and the patient's sensitivity to insulin, may be helpful in restoring glucose control after HIIT in patients living with T1D.
Clinical Trial Registration Number: NCT03057470
Supported by: Sanofi
Disclosure: **M.C. Riddell:** Grants; Sanofi.

436

Optimal insulin correction factor (ICF) for post-exercise hyperglycaemia following high intensity training in adults with type 1 diabetes: the FIT Study

R. Aronson[1], R.E. Brown[1], M.C. Riddell[2];
[1]LMC Diabetes & Endocrinology, Toronto, [2]School of Kinesiology and Health Science, York University, Toronto, Canada.

Background and aims: The phenomenon of post-exercise hyperglycemia following high-intensity training (HIT) in patients with type 1 diabetes (T1D) has led to debate of corrective therapy options but has not been definitively investigated to date. The aim of this study was to investigate the optimal bolus insulin correction factor to correct for post HIT exercise in physically active patients with T1D.
Materials and methods: The FIT study was a randomized, cross-over investigation of 4 post-HIT bolus insulin correction strategies in patients with T1D. Patients with T1D ($n = 17$) using multi-daily injections (MDI) were enrolled and underwent an 8-week insulin optimization period using insulin glargine 300 U/mL as their basal insulin. On 4 separate days, each subject performed 25 minutes of structured HIT in the morning. If hyperglycemia (>8.0 mmol/L) resulted, subjects received a bolus insulin correction 15 minutes post-HIT, based on their own ICF, adjusted by one of 4 commonly used multipliers: 0%, 50%, 100%, or 150%.
Results: At 180 minutes following bolus correction, change in plasma glucose (PG) was greatest in both the 100% (−4.7 mmol/L) and 150% (−5.3 mmol/L) ICF arms, vs 50% (−2.3 mmol/L, $p < 0.05$) and 0% (+1.0 mmol/L, $p < 0.05$) (Figure). Percent time in euglycemia (4.0–8.0 mmol/L) progressively increased with each increasing correction factor. Both the 100% and 150% ICF arms spent significantly less time in hyperglycemia (>8.0 mmol/L), and significantly more time in euglycemia, compared to both the 0% and 50% ICF arms ($p < 0.05$). Hypoglycemia was rare and only seen in the 100% arm (percent time 1.9%) and 150% arm (percent time 4.4%).
Conclusion: In correction of post-exercise hyperglycemia following HIT in patients with T1D, correction based on a patient's usual ICF is safe and effective. Optimal PG reduction, with very little hypoglycemia, occurred in the 100% ICF correction arm.



Clinical Trial Registration Number: NCT03057470
Supported by: Sanofi
Disclosure: **R. Aronson:** Grants; Sanofi.

437

Aerobic exercise training improves hepatic insulin sensitivity but lowers splanchnic glucose uptake in obese type 2 diabetic humans

J.J. Winnick[1], J.M. Gregory[2];
[1]Department of Internal Medicine, University of Cincinnati, Cincinnati, [2]Pediatric Endocrinology and Diabetes, Vanderbilt University, Nashville, USA.

Background and aims: Exercise improves hepatic insulin sensitivity in people with type 2 diabetes (T2D); manifest by diminished endogenous glucose production (EGP) during the fasting state and improved suppression of EGP in response to hyperinsulinemia. Hepatic insulin action is also known to augment splanchnic glucose uptake (SGU) in response to an oral glucose challenge, although the effect of exercise-induced gains in hepatic insulin sensitivity on SGU remain unclear.
Materials and methods: Obese humans with T2D were recruited to undergo 15 ± 1 weeks (mean ± sem) of aerobic exercise training (AEX; $n = 6$; 70% VO2 max; 4–5 days/week; 50 min per session) or remain sedentary for 15 ± 2 weeks (SED; $n = 5$). Prior to intervention, each subject underwent an isoglycemic/hyperinsulinemic clamp (ISO-clamp) to assess basal EGP and hepatic insulin sensitivity at insulin infusion rates of 20 and 40 mU/m^2/min. Each subject also underwent a 75 g oral glucose load clamp (OGL-clamp) to assess SGU. After the intervention, each subject underwent both the ISO-clamp and OGL-clamp a second time, thereby allowing us to assess pre-post changes in hepatic glucose metabolism.
Results: Age, BMI, body fat, HbA_{1C} and VO_2 max were similar in both groups at baseline. In response to the intervention, HbA_{1C} remained unchanged in SED but was reduced from 7.5 ± 0.4 to 7.1 ± 0.2% in AEX ($p < 0.05$). During the basal (i.e., fasting) period of the pre-intervention ISO-clamp, plasma glucose (151 ± 12 mg/dl), insulin (22 ± 2 uU/ml) and EGP (2.2 ± 0.2 mg/kg/min) were similar in both groups, and these values did not change in response to the 15 week intervention in either group.

Likewise, during the 20- and 40-mU/m^2/min insulin infusion periods of all ISO-clamp studies, glucose continued to be clamped at isoglycemia and insulin was also similar among groups at each time point (51 ± 3 and 92 ± 6 uU/ml, respectively). The hyperinsulinemia during the low-insulin period of the pre-intervention study suppressed EGP similarly in SED and AEX (70 ± 5%) and also suppressed EGP by 71 ± 2% in SED during the post-intervention clamp. However, EGP suppression was increased to 80 ± 6% in AEX during the post-intervention clamp ($p < 0.05$). Likewise, while the pre-intervention suppression of EGP during the high-insulin period was indistinguishable between SED and AEX, and similar to what was observed during the post-intervention ISO-clamp in SED, it was also greater in AEX post-intervention ($p < 0.05$). During the OGL-clamp, no differences in hepatic glucose metabolism were observed between groups or over time during the euglycemic/ hyperinsulinemic lead-in period, with average plasma glucose and insulin levels of 96 ± 1 mg/dl and 270 ± 9 uU/ml, respectively. This hormonal milieu, as expected, completely suppressed EGP in each group both before and after the intervention period (0.04 ± 0.09 mg/kg/min). The plasma glucose responses to the 75 g oral glucose challenge were similar between groups at baseline and did not change over time, while insulin levels remained elevated and similar over time in both groups. In response to this challenge, pre-post SGU increased by 22 ± 8% in SED, which was in marked contrast with AEX, which exhibited a 30 ± 20% decrease ($p < 0.05$).

Conclusion: Despite improved hepatic insulin action during the fasted state, aerobic exercise training reduces SGU in response to an oral glucose challenge.

Supported by: DK-093799

Disclosure: **J.J. Winnick:** Grants; DK-106364.

438

Plasma branched-chain amino acids predict change in insulin sensitivity after exercise training

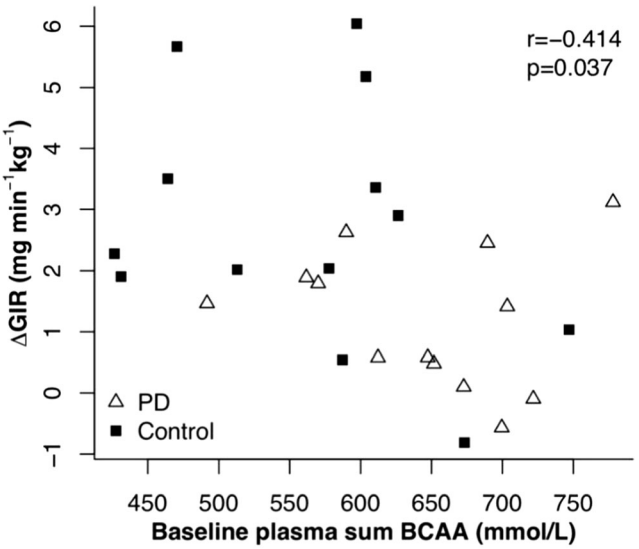
S. Lee[1,2], T.M. Langleite[1], H. Refsum[1], H.L. Gulseth[2], C.A. Drevon[1], K.I. Birkeland[2,1];
[1]University of Oslo, Oslo, [2]Oslo University Hospital, Oslo, Norway.

Background and aims: Insulin resistance (IR) is a hallmark of type 2 diabetes mellitus (T2DM), and it is related to physical inactivity and dietary energy surplus. Recently, branched-chain amino acids (BCAAs) were implicated in IR and T2DM. Physical exercise improves insulin sensitivity perhaps via effects on BCAAs. Our aim was to investigate relationships between BCAAs and IR in dys- ($n = 13$) and normoglycemic ($n = 13$) men during different modalities of physical exercise.

Materials and methods: Insulin sensitivity was estimated as the glucose infusion rate (GIR) during a euglycemic-hyperinsulinemic-clamp, skeletal muscle (SkM) and adipose tissue (AT) transcriptomics by mRNA-sequencing, liver fat by magnetic resonance spectrometry, plasma BCAAs by HPLC, in addition to VO_2max and %-mitochondrial volume in SkM. Tissue samples were obtained at rest, directly after and 2 h after a bicycle ergometer challenge of 70% VO_2max before as well as after 12 w of combined endurance- and strength training intervention. GIR and VO_2max were measured before and after the intervention.

Results: GIR, VO_2max and both AT and SkM BCAA catabolism (based on transcriptomics) were lower, whereas liver fat and plasma BCAAs were higher in dys- vs. normoglycemic men at baseline. GIR, liver fat, %-mitochondrial volume in SkM, both AT and SkM BCAA catabolism, and VO_2max improved similarly for both groups after 12 w exercise intervention. Baseline plasma BCAAs concentration correlated with improvements in GIR and VO_2max after 12 w exercise, independent of several baseline covariates such as age, group, and BMI. However, no net change in plasma concentration of BCAAs was observed after 12 w of exercise. Whereas changes in liver fat and AT BCAA catabolism correlated with reduced plasma concentration of BCAAs, the opposite was observed for plasma creatine kinase (representing muscle micro injury).

Conclusion: BCAAs metabolism seems dysregulated in men with dysglycemia, and baseline plasma BCAAs predicted change in GIR after 12 w exercise training. Although GIR improved after 12 w of exercise, no net change was observed in plasma BCAAs, perhaps because exercise influence several processes with opposite effects on plasma BCAAs.



Baseline plasma BCAAs concentrations correlated with change in insulin sensitivity after 12 w of exercise. Spearman's correlation was performed. PD, prediabetes; GIR, glucose infusion rate; BCAA, branched-chain amino acids represent the molar sum of leucine, iso-leucine and valine.

Clinical Trial Registration Number: NCT01803568

Disclosure: **S. Lee:** None.

439

Resistance training improves neuromuscular health in the elderly with type 2 diabetes: a randomised clinical trial

C.E. Botton[1], R.S. Pinto[2], L. Helal[3], B.D. Schaan[4], D. Umpierre[3];
[1]Health Technology Assessment Institute, Federal University of Rio Grande do Sul, Porto Alegre, [2]Graduate Program in Movement Human Sciences, Federal University of Rio Grande do Sul, Porto Alegre, [3]Graduate Program in Cardiology and Cardiovascular Sciences, Federal University of Rio Grande do Sul, Porto Alegre, [4]Graduate Program in Endocrinology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Background and aims: Elderly with type 2 diabetes mellitus (T2DM) have a 3-fold increased risk to physical disability as well as reduced muscle mass and strength compared with healthy individuals. Resistance training may lead to functional and muscular benefits. Thus, we aimed to assess the efficacy of resistance training in neuromuscular parameters in this population.

Materials and methods: This study is a 3-month randomized controlled clinical trial. Forty-four elderly (69.7 ± 6.9; 26 women) were randomly allocated (1:1), stratified by sex to either (1) a 12-week resistance training program (3 times a week), or (2) an active control group with stretching classes (once a week). Variables were assessed at baseline and after 12 weeks, as follows: quadriceps muscle thickness and muscle quality (assessed by ultrassonography), maximal strength (assessed by knee extension) and $HbA1_c$. Generalized

estimating equations were used to analyses based on intention-to-treat and *per protocol* (sessions adherence ≥70%) approaches.
Results: No differences were found between intervention and control groups at baseline; rectus femoris muscle quality after 12 weeks was also similar among groups ($P = 0.37$). Maximal strength and quadriceps muscle thickness increased in the resistance training group ($P < 0.001$) (Table 1). Lastly, the $HbA1_c$ levels did not significantly change for intervention (7.1 ± 1.0 to 6.8 ± 0.6) and control groups (7.2 ± 1.2 to 7.3 ± 0.2).
Conclusion: A 12-week resistance training program was efficacious to counteract the maximal muscle strength and thickness impairments in elderly with T2DM, although no improvement in metabolic control and muscle quality were found.

Table 1. Neuromuscular variables at baseline and after 12 weeks of intervention.

Intention-to-treat analysis							
	Resistance training group (n=22)			Control group (n=22)			
Variables	Baseline	3 months	Difference (CI 95%)	Baseline	3 months	Difference (CI 95%)	*P* value (group*time)
RF echo-intensity (a.u.)	67.3±14.2	61.9±13.6	-2.7 (-7.8 to 2.3)	70.8±13.3	70.0±13.6	0.8 (-5.2 to 7.0)	0.375
KE maximal strength (kg)	32.0±11.5	38.6±12.6*	4.6 (2.3 to 6.9)	29.4±10.6	30.8±11.2	0.5 (-0.7 to 1.9)	0.002
QMT (mm)	68.9±14.1	81.2±11.1	7.1 (5.0 to 9.3)*	71.7±16.0	74.0±17.5	0.5 (-0.9 to 2.1)	<0.001
Per protocol analysis							
	Resistance training group (n=13)			Control group (n=13)			
RF echo-intensity (a.u.)	62.7±14.2	62.1±13.6	-0.6 (-5.6 to 4.2)	69.2±15.1	68.9±14.2	-0.6 (-7.9 to 6.7)	0.988
KE maximal strength (kg)	35.8±10.0	41.3±11.0*	5.5 (3.4 to 7.6)	28.1±9.6	28.1 ±10.1	0.0 (-1.3 to 1.3)	<0.001
QMT (mm)	73.5±13.5	80.3±11.7*	6.8 (4.4 to 9.2)	70.1±17.8	70.8 ±17.8	0.7 (-1.0 to 2.4)	<0.001

RF= *rectus femoris* muscle; KE= knee extension; QMT= quadriceps muscle thickness; **P*<0.05*vs.* baseline.

Clinical Trial Registration Number: NCT02548000
Supported by: The study received support of CAPES, CNPq and FIPE
Disclosure: **C.E. Botton:** Grants; Fundo de Apoio a Pesquisa do Hospital de Clínicas de Porto Alegre, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico.

440

Metabolic response of skeletal muscle tissue to three-month exercise intervention in sedentary non-diabetic men

L. Szczerbinski[1], E. Siewiec[1], A. Citko[2], U. Puchta[1], J. Zapolska[3], M. Gorska[1], A. Kretowski[1,2];
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, [2]Clinical Research Centre, Medical University of Bialystok, Bialystok, [3]Department of Dietetics and Clinical Nutrition, Medical University of Bialystok, Bialystok, Poland.

Background and aims: Physical activity is a well-established tool in prevention and treatment of Type 2 Diabetes. Exercise-mediated remodelling of skeletal muscle is associated with improved metabolic health, but unfortunately its mechanisms are still poorly understood. The rapid development of "omics" technologies, including metabolomics, has introduced new opportunities for mapping molecular adaptations to exercise and indicating novel pathways involved in that process. The aim of the study was to assess changes in skeletal muscle metabolome under three-month exercise intervention.
Materials and methods: We performed global untargeted metabolomics by GC-TOF MS, HILIC-QTOF MS/MS and lipidomics by CSH-QTOF MS/MS to evaluate changes in skeletal muscle metabolism under three-month, highly supervised exercise intervention. Skeletal muscle samples were obtained from 37 sedentary, non-diabetic men (mean ± SD age: 47.51 ± 6.97 yrs; BMI: 30.01 ± 6.97 kg/m^2; fasting plasma glucose: 105.57 ± 13.89 mg/dl) before and after three months of exercise intervention, consisting of mixed trainings with endurance and strength exercises three times per week. Exercise intervention was highly supervised, monitored by MyWellness system (Technogym, Italy). To assess the effect of exercise on metabolites we compared metabolites concentrations before and after the intervention, summarizing metabolome-wide responses to exercise with Partial Least Squares Discriminant Analysis (PLS-DA) and Principal Component Analysis (PCA). To define significantly altered chemical classes, we performed chemical enrichment analysis using ChemRICH, which classifies metabolites by structural similarity.
Results: From a total of 663 detected metabolites (120 for GC, 426 for Lipidomics and 117 for HILIC), we found that 78 compounds were significantly altered in skeletal muscle tissue following exercise intervention. Significantly altered metabolites included fatty acids, amino acids, carnitines, complex lipids, and energy metabolites. The enrichment analysis identified eight significantly altered metabolite clusters: acidic amino acids, saturated fatty acids, and unsaturated triglycerides significantly increased and phospholipid ethers significantly decreased. Phosphatidylethanolamines were significantly altered with most species increased and one decreased. Unsaturated phosphatidylcholines were significantly altered with most species decreased and two increased, while carnitines and purines were also significantly altered with equal numbers of increased and decreased metabolites.
Conclusion: In summary, in this study we found significant global alterations in the human skeletal muscle metabolome following three-month exercise intervention. The largest observed changes in metabolite levels were observed in lipids species, especially in glycerophospholipids and glycerolipids. Further studies are required to determine how these changes impact exercise-induced health benefits.
Supported by: MNiSW/2016/DIR/200/NN and MNiSW/2014/DIR/383/GPII
Disclosure: **L. Szczerbinski:** None.

441

High intensity interval training improves insulin sensitivity in individuals with prediabetes

P. Mensberg[1], C. Frandsen[1], E. Espersen[1], T. Leineweber[1], H. Storgaard[1], K.S. Schlawitz[2], T.H.D. Petersen[2], J.N. Poulsen[2], F. Sørensen[3], J.L. Forman[3], F.K. Knop[1,4], T. Vilsbøll[1,4];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Hellerup, [2]Center for Prevention and Health, Gentofte Municipality, Gentofte, [3]Department of Public Health, Section of Biostatistics, Copenhagen, [4]Department of Clinical Medicine, Faculty of Health and Medical Sciences, Copenhagen, Denmark.

Background and aims: Lack of physical activity in the general population remains a major health issue. Recently, extremely low volume, high intensity interval training (HIIT), as low as 3 min per week, was demonstrated to improve insulin sensitivity and glycaemic control in young healthy individuals. We evaluated the effects of 12 weeks HIIT vs. no training on insulin sensitivity in individuals with prediabetes.
Materials and methods: Seventy sedentary obese middle-aged individuals with prediabetes (women $n = 36$; age: 60.8 ± 11.3 years (mean ± SD); BMI: 31.6 ± 4.4 kg/m^2; fasting plasma glucose (FPG): 6.6 ± 0.8 mmol/l; HbA1c: 39.0 ± 4.3 mmol/mol) were randomised to one of four groups: 1) HIIT (3 × 20 second's cycle sprint 3 times per week, 2) HIIT+walking (HIIT plus >10,000 steps per day), 3) walking (>10,000 steps per day) or 4) no exercise (control group). At baseline and after intervention insulin sensitivity indices were assessed during an OGTT and peak oxygen uptake during an incremental exercise test.
Results: Increased muscle insulin sensitivity (Cederholm index) was observed in the HIIT and HIIT+walking group vs. the control group (Table 1). Muscle insulin sensitivity remained unchanged in the walking only group. Whole body insulin sensitivity (Matsuda index) was unchanged with HIIT, but improved significantly with HIIT+walking and walking only, compared to the control group. Peak oxygen uptake increased from baseline to end of trial in the two HIIT groups and in the HIIT only group, peak oxygen at end of trial was higher compared to the control group (Table 1).

Conclusion: In individuals with prediabetes twelve weeks of HIIT as well as HIIT plus 10,000 daily steps significantly improved muscle insulin sensitivity and the latter also resulted in significantly greater whole body insulin sensitivity compared to no training. HIIT may constitute a time-efficient way to improve insulin sensitivity in individuals with prediabetes.

Table 1

	HIIT n=13		HIIT+walking n=14		Walking n=15		Control n=15	
	Baseline n=13	Change from baseline	Baseline n=14	Change from baseline	Baseline n=15	Change from baseline	Baseline n=15	Change from baseline
Cederholm index	29.2 [25.7;38.5]	4.3*,† [2.1;8.1]	36.8 [31.1;42.2]	1.7*,† [-1.8;12.4]	31.3 [25.1;37.1]	2.3 [-2.9;5.8]	26.0 [22.0;36.7]	-1.8 [-5.5;2.6]
Matsuda index	1.6 [1.0;2.5]	0.1 [-0.03;0.5]	2.6 [1.2;2.9]	0.2*,† [-1.2;0.8]	1.0 [0.6;1.5]	0.8*,† [0.5;1.5]	1.6 [1.4;2.0]	-0.2 [-0.5;0.2]
VO_{2peak} (ml/kg/min)	24.0±4.5	3.4±3.2*,†	25.5±6.6	1.8±2.6*	23.4±6.0	0.5±2.4	24.0±4.8	0.9±2.1

Data are presented as mean±SD or as median±IQR (when not normally distributed). Single asterisk (*) indicates significant difference (adjusted $p<0.2$) within the group. Dagger (†) indicates significant difference (adjusted $p<0.2$) compared to control group. VO_{2peak}: maximal oxygen uptake.

Clinical Trial Registration Number: NCT02212665
Disclosure: **P. Mensberg:** None.

PS 023 In gut we trust

442

Ghrelin in rat pancreatic islets decreases islet blood flow and impairs insulin secretion

C.J. Drott[1], P. Franzén[1], P.O. Carlsson[1,2];
[1]Medical Cell Biology, Uppsala University, Uppsala, [2]Medical Sciences, Uppsala, Sweden.

Background and aims: The peptide ghrelin is mainly produced in some of the epithelial cells in the stomach, but also, during starvation, by the ε-cells in the endocrine pancreas. Ghrelin, as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R1α), exerts a variety of metabolic functions including stimulation of appetite and weight gain. Its complete role is not yet fully understood, including whether it has any vascular functions. The present study evaluated if ghrelin may affect pancreatic and islet blood flow, and insulin secretion.
Materials and methods: Ghrelin and the GHS-R1α receptor antagonist GHRP-6 were injected intravenously in rats followed by blood flow measurements using a microsphere technique. The physiological effect exerted by ghrelin and GHRP-6 in vivo on glucose homeostasis was evaluated by intravenous glucose tolerance test and intraperitoneal insulin tolerance test, and in vitro through glucose-stimulated insulin release experiments.
Results: Ghrelin decreased islet blood flow (50.5 ± 4.4 (control) vs. 34.8 ± 4.2 (ghrelin) μl × min^{-1} × g $pancreas^{-1}$; $P < 0.05$), while GHRP-6 in fasted, but not fed, rats selectively increased islet blood flow fourfold (fed rats 50.5 ± 4.4 (control) vs. 52.3 ± 4.8 (GHRP-6); fasted rats 35.5 ± 6.7 (control) vs. 203 ± 38 μl × min^{-1} × g $pancreas^{-1}$(GHRP-6); $P < 0.05$). GHS-R1α was identified not only on glucagon producing cells, but also in the islet arterioles through PCR and immunohistochemistry. GHRP-6 in fasted rats, only, also improved the peak insulin response to glucose *in vivo* (235 ± 56 pmol/l(control) vs. 534 ± 108 pmol/l (GHRP-6); $P < 0.05$), thereby substantially blunting the hyperglycemia. GHRP-6 doubled glucose stimulated insulin release *in vitro* of both islets obtained from fed rats (35.7 ± 3.9 (control) vs. 64.8 ± 4.7 pmol/l (GHRP-6); $P < 0.05$) and fasted rats (35.9 ± 5.0 (control) vs. 66.2 ± 6.7 pmol/l (GHRP-6); $P < 0.05$).
Conclusion: Our results indicate a novel role for endogenous ghrelin as a local vasoconstrictor in the islets during fasting, thereby restricting the insulin response to hyperglycemia. This is to the best of our knowledge the first report that shows this physiological mechanism to restrict insulin delivery from the islets by acting on the vasculature.
Supported by: SE Research Council, EXODIAB, SE Child Diab Fund, Novo Nordisk Found
Disclosure: **C.J. Drott:** None.

443

C-terminal plasma degradation of PYY(1-36) and PYY(3-36) severely curtails effects on insulin secretion, beta cell mass and satiety

P.R. Flatt, R.A. Lafferty, N. Irwin;
Ulster University, Coleraine, UK.

Background and aims: Peptide YY (PYY) is known to exist in two major circulating forms, PYY(1-36) and PYY(3-36). PYY(3-36), generated by the action of DPP-4, has well documented anorectic actions with possible therapeutic implications for obesity, whereas the importance of PYY(1-36) for the regulation of pancreatic beta-cell survival has been described recently. Further to this, enzymatic C-terminal truncation of PYY related peptides has been reported, but the physiological impact of this C-terminal processing remains uncertain. The present study has therefore characterised plasma C-terminal degradation products of PYY(1-36) and PYY(3-36), and evaluated their effects on function, proliferation and survival of beta-cells as well as feeding behaviour and glucose homeostasis.

Materials and methods: PYY(1-36) and PYY (3-36) were incubated with murine plasma (4 h, $n = 4$) and evaluated by HPLC/MS to assess enzyme stability. BRIN-BD11 beta-cells ($n = 8$) were used to evaluate the acute (20 min) effects on insulin release (10^{-12}–10^{-6} M) of PYY(1-36), PYY(3-36) plus their related C-terminal degradation products. Actions of PYY peptides (10^{-8} and 10^{-6} M) on beta-cell proliferation, by Ki-67 antibody staining, and protection against cytokine-induced (IL-1β 100 U/ml, IFN-γ 20 U/ml, TNF-α 200 U/ml) apoptosis, by TUNEL assay, were examined in clonal rodent BRIN BD11 and human 1.1B4 cells ($n = 4$). Acute effects of the peptides (25 nmol/kg; i.p.) on food intake, glucose and insulin concentrations were evaluated in overnight fasted (12 h) mice ($n = 8$). In addition, the impact of the ACE inhibitor captopril (50 mg/kg, i.p.) on PYY(3-36) induced appetite suppression was also assessed in mice ($n = 8$).
Results: C-terminal degradation products, PYY(1-34) and PYY(3-34), were detected by HPLC and mass spectrometry analyses following incubation of PYY(1-36) and PYY(3-36) in murine plasma. PYY(1-36) and PYY(3-36) inhibited ($P < 0.05$–$P < 0.001$) glucose-stimulated insulin secretion (GSIS) from BRIN-BD11 beta-cells, whereas PYY(1-34) and PYY(3-34) had no effect on GSIS. All peptides examined, namely PYY(1-36), PYY(3-36), PYY(1-34) and PYY(3-34), lacked effects on glucose tolerance or glucose-induced insulin release. However, both PYY(1-36) and PYY(3-36) significantly ($P < 0.05$–$P < 0.001$) enhanced proliferation of BRIN BD11 and 1.1B4 beta-cells, and also fully protected ($P < 0.01$–$P < 0.001$) these cells against cytokine-induced apoptosis. The C-terminal degradation products, PYY(1-34) and PYY(3-34), were entirely ineffective in this regard. As expected, PYY(3-36) induced clear acute reductions ($P < 0.05$–$P < 0.01$) of food intake in mice, but these effects were eliminated by removal of the C-terminal dipeptide from PYY(3-36). Interestingly, captopril significantly ($P < 0.05$) augmented the appetite suppressive actions of PYY(3-36).
Conclusion: PYY is an enteroendocrine derived peptide hormone with an important role in metabolism linked to regulation of energy expenditure and pancreatic beta-cell survival. The impact of C-terminal degradation of both PYY(1-36) and PYY(3-34) on receptor interaction and subsequent bioactive profile at islet and hypothalamic sites of action needs further consideration, as it appears to dramatically diminish biological activity.
Supported by: DEL NI
Disclosure: **P.R. Flatt:** None.

444

A novel exendin-4/gastrin/xenin-8-Gln fusion peptide, in combination with a stable GIP agonist, substantially improves metabolic control in high fat fed mice

N. Irwin, A. Hasib, M.T. Ng, P.R. Flatt, V.A. Gault;
Ulster University, Coleraine, UK.

Background and aims: Enteroendocrine derived hormones such as gastrin, glucagon-like-peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and xenin are known to exert complementary beneficial metabolic effects in diabetes. The present study characterised a novel fusion peptide, exendin-4/gastrin/xenin-8-Gln, and evaluated therapeutic utility in combination with the GIP receptor agonist, ($DAla^2$)GIP, in high fat fed mice.
Materials and methods: Exendin-4/gastrin/xenin-8-Gln was synthesised by coupling residues 1–28 of exendin-4, with gastrin-6 and xenin-8-Gln, using 8-amino-3,6-dioxaoctanoic acid linker molecules. The peptide was incubated with murine plasma ($n = 4$) to assess enzyme stability. BRIN-BD11 cells were used to evaluate insulinotropic activity of exendin-4/gastrin/xenin-8-Gln (10^{-12}-10^{-6} M), with GLP-1, neurotensin, and CCK-B receptor antagonists employed to ascertain insulin secretory receptor balance. Acute effects of the fusion peptide on food intake, glucose and insulin concentrations were examined in lean mice ($n = 8$). High fat fed (HFF) mice ($n = 8$) were used to assess chronic effects of exendin-4/gastrin/xenin-8-Gln alone, and in combination with ($DAla^2$)GIP, (each peptide at 25 nmol/kg; *ip*) using a twice-daily injection regimen for 21 days. Body weight, glucose and insulin concentrations were measured every 3 days. Oral glucose tolerance (18 mmol/kg), metabolic response to GIP (25 nmol/kg; *ip*) and insulin sensitivity (10 U/kg; *ip*) were determined at the end of the study. Plasma lipid, glucagon and amylase activity were also assessed on day 21.
Results: Exendin-4/gastrin/xenin-8-Gln was enzyme resistant and enhanced ($P < 0.001$) insulin secretion from BRIN-BD11 cells, with GLP-1 and neurotensin receptor pathways being important. Acute injection of exendin-4/gastrin/xenin-8-Gln in combination with glucose significantly ($P < 0.001$) lowered glucose and increased insulin concentrations in mice, with antihyperglycaemic effects evident ($P < 0.001$) 8 h post-injection. Exendin-4/gastrin/xenin-8-Gln also induced significant ($P < 0.001$) appetite suppressive effects. Administration of exendin-4/gastrin/xenin-8-Gln alone, or in combination with ($DAla^2$)GIP, twice daily for 21 days in HFF mice, reduced ($P < 0.01$) percentage body fat compared to saline controls. The treatment regimens significantly ($P < 0.05$–$P < 0.001$) decreased circulating glucose and increased insulin concentrations, with no impact on glucagon levels or amylase activity. Exendin-4/gastrin/xenin-8-Gln in combination with ($DAla^2$)GIP also reduced ($P < 0.05$) LDL-cholesterol levels. In addition, the combined treatment group presented with clear improvements in glucose tolerance, which was superior to either treatment alone. Similarly, GIP-induced reductions in blood glucose and elevations of insulin were enhanced ($P < 0.05$–$P < 0.01$) by treatment with exendin-4/gastrin/xenin-8-Gln in combination with ($DAla^2$)GIP. All treatment groups had superior ($P < 0.05$–$P < 0.001$) glucose-lowering actions in response to exogenous insulin administration.
Conclusion: Exendin-4/gastrin/xenin-8-Gln is a fusion peptide with clear antidiabetic potential. Efficacy was improved through concurrent administration of a stable GIP molecule, adding support to the promise of multi-targeting peptides for the treatment of diabetes.
Supported by: EFSD/Sanofi; Invest NI & DEL NI
Disclosure: **N. Irwin:** Grants; EFSD/Sanofi grant, Department of Education and Learning, Northern Ireland, Invest Northern Ireland.

445

Differential efficacy of a GLP-1R/GCGR dual agonist versus a GLP-1R agonist in diet-induced obese mice

M.P. Valdecantos[1], L. Ruiz[1], M.J. Obregon[1], A. Dos Santos[2], M.A. Bednarek[2], J. Grimsby[2], C. Rondinone[2], A.M. Valverde[1];
[1]Metabolismo y Señalización Celular, Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, Spain, [2]MedImmune Inc, Gaithersburg, USA.

Background and aims: The increasing incidence of obesity and type 2 diabetes worldwide has prompted the need for new therapies. In this study we have designed a pharmacological intervention protocol with a GLP-1R or dual-acting GLP-1R/ GCGR agonist aimed to investigate their differential effects and the mechanism of action involved in preventing diet-induced obesity in mice.
Materials and methods: Eight week-old male C57BL/6 mice were fed chow or HFD for 10 weeks. HFD-fed mice were subsequently s.c. injected every 2 days with vehicle or a GLP-1R agonist (10 nmol/kg) or G49, a GLP-1R/GCGR dual agonist, (4 mg/kg) for 6 weeks. Another group of animals were administered a single dose of G49 (4 mg/kg). Parameters that assess obesity, BAT activation, lipolysis, glucose homeostasis and insulin sensitivity were analyzed at 6 weeks of treatment and after 6, 12, 24 and 48 hours post G49 treatment.
Results: After 6 weeks of treatment, body weight loss was greater in mice injected with G49 (20.05% ± 5.94) compared to animals treated with the GLP-1R agonist (6.61% ± 5.29; $p < 0.001$). Indirect calorimetry revealed an increase in energy expenditure in G49 treated animals, but not in mice injected with the GLP-1R agonist in both the dark (16.7 ± 1.57 vs 13.9 ± 0.63 Kcal/h/Kg) and light cycles (14.6 ± 1.4 vs 11.8 ± 0.26 Kcal/h/Kg) ($p < 0.001$). G49 was more effective than the GLP-1R agonist in increasing mRNA levels of BAT activation-related genes, as well as in the induction of browning-related genes in iWAT ($p < 0.05$–$p < 0.001$). Furthermore, G49 increased BAT type II deiodinase (Dio2) mRNA and DIO2 activity, T3 and T4 concentration, effects not observed after treatment with GLP-1R agonist ($p < 0.001$). Moreover, a single dose of G49 reduced body weight at 6 h (4.6

± 1.4% of the initial BW) concomitant with a reduction in eWAT ($p < 0.001$) and iWAT ($p < 0.001$) depots and a transient elevation in plasma free fatty acids (0.81 ± 0.09 vs 0.59 ± 0.06 mmol/L in vehicle-treated mice) relative to vehicle treatment. Lipolysis was stimulated in ex vivo eWAT explants isolated from mice treated with a single dose of G49. The analysis of BAT revealed an increase in UCP-1 immunostaining and protein levels at 48 h post-single injection, reflecting rapid BAT activation. Treatment with G49 for 4 h induced lipolysis in both differentiated 3T3L1 and brown adipocytes (BA) ($p < 0.01$). Gene expression analysis of BA treated with G49 for 16 h showed an increase in *Ucp1*, mitochondrial biogenesis and beta oxidation-related genes ($p < 0.05$–$p < 0.01$). Interestingly, G49 treatment during differentiation of BA increased protein levels of UCP1 and mitochondrial-biogenesis-related genes.
Conclusion: Our results strongly suggest a novel role of G49, an oxyntomodulin-like dual acting GLP-1R/GCGR agonist, in reducing obesity by increasing energy expenditure due to its effects in BAT and browning of WAT. Moreover, the results revealed an acute effect of G49 in inducing lipolysis in eWAT and activation of BAT with a similar pattern in white and brown adipocytes in culture. These data suggest that G49 might trigger a cross-talk between eWAT and BAT which is mediated by the release of FFA which are fuels for BAT thermogenesis
Supported by: SAF2015-65267-R (MINECO-FEDER, Spain); MedImmune (MA-416191)
Disclosure: **M.P. Valdecantos:** None.

446

Increasing the cellular populations secreting incretins improves glucose homeostasis and beta cell regeneration

M. Terasaki[1,2], C.L. Mattsson[1], K.-C. Liu[1], J. Charbord[1], Q. Zhou[3], T. Hirano[2], O. Andersson[1];
[1]Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden, [2]Diabetes, Metabolism and Endocrinology, Showa University School of Medicine, Tokyo, Japan, [3]Stem Cell and Regenerative Biology, Harvard University, Cambridge, USA.

Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors are widely used in patients with type 2 diabetes. However, their use could lead to tonic supraphysiological levels of incretins. We speculated that it might be advantageous to expand the cellular populations secreting GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), such that one has the capacity to secrete more incretins upon food ingestion, instead of constantly having increased levels of circulating incretins.
Materials and methods: Using a transgenic zebrafish model, we performed an *in vivo* screen of 1,300 small molecules for stimulators of GIP expression by measuring luciferase activities. We then evaluated whether the hit-compounds can increase the number of GIP-expressing K-cells in the intestine by using immunohistochemistry, and subsequently examined whether the hits also increase L-cell numbers. The glucose levels and related gene expression levels were determined by a glucose assay kit and by SYBR-based RT-PCR, respectively. We also evaluated the effect of the hit-compounds on pancreatic β-cells in transgenic zebrafish with or without β-cell ablation using metronidazole. Moreover, we examined whether the effects were conserved to the mouse, using wild-type (C57BL/6J) mice and diabetic *db/db* (C57BLKS/J Iar- +*Leprdb*/+*Leprdb*) mice, which were administered with the hit-compounds *in vivo.*
Results: We identified small molecules that converge on a fatty acid signaling pathway with a common downstream transcriptional regulator, to increase the number of incretin-expressing cells originating from Neurog3-expressing enteroendocrine progenitors. This did not only lead to an increased number of incretin-expressing cells, but also to reduced glucose levels and promoted β-cell regeneration by increasing β-cell proliferation, effects that were accompanied with increased insulin mRNA levels. Consistent with these findings, our identified compounds increased incretin-expressing cell numbers in the mouse intestine with augmented plasma levels of GIP and GLP-1, and improved oral glucose tolerance tests and HbA1c levels in diabetic *db/db* mice.
Conclusion: Our identified compounds increased incretins-expressing cell numbers in both zebrafish and mouse intestine with improved glucose homeostasis *in vivo*, indicating the effect of the hits is conserved across species.
Supported by: EFSD/JDS Reciprocal Travel Research Fellowships
Disclosure: **M. Terasaki:** None.

447

Transcriptional factor pancreatic duodenal homeobox-1 (Pdx1) is involved in age-related glucose-dependent insulinotropic polypeptide (GIP) hypersecretion in mice

E. Ikeguchi[1], N. Harada[1], Y. Kanemaru[1], A. Sankoda[1], S. Yamane[1], K. Iwasaki[1], M. Imajo[2], Y. Murata[1], K. Suzuki[1], E. Joo[1], N. Inagaki[1];
[1]Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, [2]Laboratory of Bioimaging and Cell Signaling, Graduate School of Biostudies, Kyoto University, Kyoto, Japan.

Background and aims: Fat accumulation with aging is a serious problem. Glucose-dependent insulinotropic polypeptide (GIP) is an incretin secreted from enteroendocrine K cells in response to glucose and fat ingestion. GIP potentiates insulin secretion through the GIP receptor (GIPR) expressed in pancreatic β-cells. GIP plays an important role in maintaining blood glucose levels by inducing hypersecretion of insulin in high-fat diet (HFD)-induced obesity. GIPR expressed in adipose tissue is involved in HFD-induced insulin resistance. Thus, GIP is a key hormone for fat accumulation. GIPR-knockout mice show reduced fat mass and improved insulin sensitivity associated with aging. Therefore, GIP could be involved in fat accumulation and insulin resistance with aging. However, age-related changes of GIP secretion remain unclear. The present study aimed to elucidate age-related changes of GIP secretion and K cells under normal diet condition using GIP reporter mice.
Materials and methods: Male GIP reporter (GIP-GFP knock-in heterozygous) mice were divided into two groups: 3–4 months old (young) mice and 1 year old (aged) mice. Their body composition, GIP secretion, and insulin tolerance were evaluated. Immunohistochemical and flow cytometry analyses were performed to assess K cell number. The expression levels of GIP mRNA and transcriptional factors in sorted K cells were measured. A target gene expression in small intestine was suppressed by intestine-specific gene transfer (iGT). K cell number, GIP mRNA expression and content in small intestine, and GIP secretion were estimated after the suppression of the target gene by the introduction of siRNA.
Results: Body weight of aged mice was significantly higher than that of young mice. Aged mice accumulated more body fat, and blood glucose reduction was significantly less in aged mice during insulin tolerance test, indicating that aged mice exhibit the phenotype of fat accumulation and insulin resistance. Aged mice showed hypersecretion of GIP and insulin during oral glucose tolerance test and under free feeding, while blood glucose levels did not differ between the two groups. K cell number was increased in small intestine of aged mice. In aged mice, the mRNA expression levels of GIP and transcriptional factor pancreatic and duodenal homeobox-1 (Pdx1) were increased in sorted K cells. K cell number, GIP mRNA expression and GIP content in small intestine were decreased in the mice after posteriori suppression of Pdx1 using iGT, resulting in reduced GIP secretion after glucose ingestion.
Conclusion: Pdx1 increases K cell number and GIP mRNA expression in small intestine of aged mice, which results in GIP hypersecretion with aging.
Disclosure: **E. Ikeguchi:** None.

448

GLP-1 releaser D-allulose effectively and glucose-dependently corrects hyperglycaemia

T. Yada[1], M. Sendo[2], M. Yoshino[2], Y. Yamada[3], D.J. Drucker[4], M. Tokuda[5], Y. Iwasaki[6];
[1]Integrative Physiology, Kansai Electric Power Medical Research Institute, Kobe, Japan, [2]Physiology, Jichi Medical University, Shimotsuke, Japan, [3]Endocrinology, Diabetes and Geriatric Medicine, Akita University, Akita, Japan, [4]Lunenfeld Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, Canada, [5]Cell Physiology, Kagawa University, Kagawa, Japan, [6]Animal Science, Kyoto Prefectural University, Kyoto, Japan.

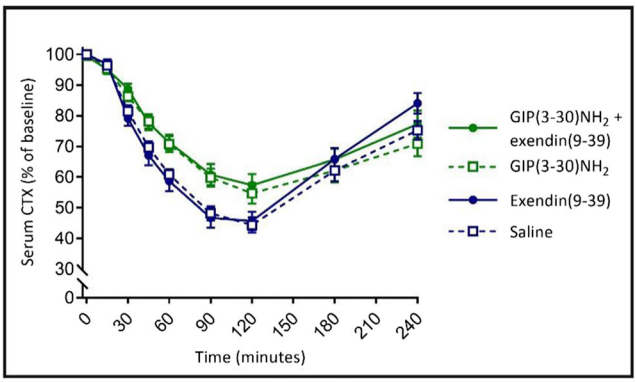
Background and aims: We previously reported that oral administration of D-allulose (Allu), a rare sugar with sweetness but not calorie, induced release of GLP-1 and promoted glucose tolerance in normal B6J mice and those fed high fat diet (HFD). The Allu-induced promotion of glucose tolerance was blunted in GLP-1R deficient mice and in mice receiving vagal afferent denervation. Allu enhanced both initial insulin secretion in glucose tolerance test and insulin action. Type 2 diabetes is featured with elevation of casual blood glucose (cBG) as well as postprandial blood glucose. This study aimed (1) to clarify the effect of Allu on cBG levels, and its dependency on glycemic levels or healthy vs diabetic states. Moreover, Allu serves as GLP-1 releaser, providing a novel category of incretin medicine. Hence it is of relevance to compare the effects of Allu and conventional inctretin medicine. This study aimed (2) to compare Allu and GLP-1R agonist, exentine-4 (Ex4), in the efficacy of lowering cBG and potential of causing hypoglycemia in normoglycemic and hyperglycemic mice.
Materials and methods: Lean B6J mice with body weight (BW;25 g), obese B6J mice fed HFD for 80–90 days (40 g), and obese db/db mice (45 g) were used. Male mice ($n = 5–6$ for each experiment) were mildly fasted for 4 hours from 9:00 to 13:00. Allu (1 g/kg) (Matsutani Chem.) or saline was per oral (po) administered and blood glucose was measured at 0, 1, 2 and 3 hr. GLP-1R agonist Ex4 (Abcam) was subcutaneously (sc) administered at 0.5 nmol/kg, a dose that suppressed food intake. Statistical analysis was performed by one-way or two-way ANOVA followed by Dunnett's, Tukey's or Bonferroni's post hoc test. $P < 0.05$ was considered significant. All data were expressed as means ± SEM.
Results: (1) In B6J mice with cBG levels around 100 mg/dL, po Allu failed to significantly alter cBG at 1–3 hr after Allu administration. In contrast, Allu reduced cBG from 200 to 150 mg/dL (ΔcBG;50) at 1–3 hr in HFD fed diabetic B6J mice, and from 400 to 220 mg/dL (ΔcBG;180) at 1–5 hr in db/db mice. (2) In B6J mice, Ex4 significantly reduced cBG from 110 to 65 mg/dL (ΔcBG;45) at 1 hr. In HFD fed B6J mice, Ex4 significantly reduced cBG from 185 to 160 mg/dL (ΔcBG;25) only at 1 hr, and in db/db mice it reduced blood glucose from 400 to 300 mg/dL (ΔcBG;100) only at 1 hr.
Conclusion: Allu exhibited greater cBG-lowering effect at higher cBG levels, and no effect at normal glucose levels. This result indicates glucose-dependent ability of Allu to attenuate hyperglycemia. In contrast, Ex4 induced mild hypoglycemia in normal B6J mice and ameliorated hyperglycemia only transiently in HFD fed B6J and db/db mice. The results demonstrate that Allu ameliorates hyperglycemia with greater efficacy and longer-lastingly than Ex4 in type 2 diabetic mice. Oral Allu, as a GLP-1 releaser, may provide a novel category of incretin-based medicine with high efficacy and safety to correct hyperglycemia.
Supported by: Research grant from Matsutani Chem Co.
Disclosure: **T. Yada:** Grants; Research Grant from Matsutani Chemical Co.

449

The role of the incretins in the postprandial bone remodelling

M.M. Helsted[1,2], L.S. Gasbjerg[1,2], A.R. Lanng[1], S. Stensen[1], B. Hartmann[2], M.B. Christensen[3,4], J.J. Holst[2], T. Vilsbøll[1,2], M.M. Rosenkilde[2], F.K. Knop[1,2];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Hellerup, [2]Department of Biomedical Sciences and Novo Nordisk Center for Metabolic Research, University of Copenhagen, Copenhagen, [3]Department of Clinical Pharmacology, Bispebjerg Hospital, Copenhagen, [4]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark.

Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is, like glucagon-like peptide 1 (GLP-1), an incretin hormone and thus potentiates glucose-induced insulin secretion in healthy subjects. Furthermore, GIP is known to affect bone metabolism and has been suggested as an important mediator in an entero-osseous axis. We used a novel high-affinity competitive GIP receptor antagonist, GIP(3-30)NH_2, and the competitive GLP-1 receptor antagonist exendin(9-39) to examine the contributions of the endogenous GIP and GLP-1, respectively, to postprandial suppression of bone resorption, measured by carboxy-terminal collagen crosslinks (CTX), and postprandial bone formation, measured procollagen type 1 amino-terminal propeptide (P1NP), in healthy subjects.
Materials and methods: In two randomised and double-blinded cross-over sub-studies, the separate and combined impact of endogenous GIP and GLP-1 was investigated. In sub-study 1, 18 healthy men (age 20–70 years, body mass index (BMI) 22–34 kg/m^2) received four oral glucose tolerance tests (OGTT). In sub-study 2, 12 healthy men (age 19–65 years, BMI 20–25 kg/m^2) received four liquid mixed meal tests. In both studies, subjects received combinations of infusions of GIP(3-30)NH_2 (800 pmol/kg/min), exendin(9-39) (20 min of 1000 pmol/kg/min, then 450 pmol/kg/min), and/or matching volumes of saline. Thus, subjects received: A) GIP(3-30)NH_2 + exendin(9-39), B) GIP(3-30)NH_2 + saline, C) exendin (9-39) + saline; D) saline + saline. The antagonist concentrations were chosen based on respective inhibitory potencies in vitro. We measured serum levels of CTX, P1NP, and parathyroid hormone (PTH).
Results: CTX decreased on all study days. Significant differences in baseline-subtracted area under the curve (bsAUC) between day B1 and D1 ($p = 0.009$), A1 and D1 ($p = 0.012$), B2 and C2 ($p = 0.020$) and B2 and D2 ($p = 0.030$) were evident. During infusion with GIP(3-30)NH_2, CTX decreased significantly less than during placebo infusion (42 ± 3.7% (B1) vs 56 ± 2.5% (D1) and 52 ± 2.8% (B2) vs 63 ± 3.5% (D2)) (Figure shows results from sub-study 1). There was no effect of exendin(9-39) (A1 vs B1, C1 vs D1, A2 vs B2, and C2 vs D2). In both sub-studies, the interventions did not affect P1NP or PTH.
Conclusion: Endogenous GIP contributes significantly to postprandial suppression of bone resorption in humans whereas an effect of endogenous GLP-1 could not be demonstrated using exendin 9-39.



Sub-study 1. Serum CTX levels in percent of baseline during OGTT in healthy male subjects (n=18). Infusions of GIP(3-30)NH_2 + exendin(9-39), GIP(3-30)NH_2, exendin (9-39), and saline.

Clinical Trial Registration Number: NCT03133741
Supported by: Novo Nordisk Foundation
Disclosure: **M.M. Helsted:** Grants; Novo Nordisk Foundation.

PS 024 Following the signal inside muscle or fat

450

The thromboxane A2 receptor modulates glucose metabolism in skeletal muscle

A.M. Abdelmoez[1], A. Krook[1], J.R. Zierath[2], N.J. Pillon[1];
[1]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, [2]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

Background and aims: Obesity and type-2 diabetes are associated with chronic low grade inflammation. Activation of immune responses and infiltration of immune cells in the adipose tissue, liver and skeletal muscle contribute to the development of insulin resistance. Skeletal muscle is the major contributor to post-prandial regulation of glucose levels and therefore a key player in the development of type 2 diabetes. However, inflammatory responses occurring within skeletal muscles that affect myocytes metabolic responses are poorly characterized.
The thromboxane A2 receptor (TBXA2R) is a G-protein coupled receptor present at the cell surface of platelets but we also discovered that it is expressed in skeletal muscle cells. In addition, phosphoproteomic data has shown increased TBXA2R phosphorylation in skeletal muscle after an acute exercise bout. TBXA2R responds to prostaglandins and thromboxane, compounds known to activate inflammation, but its role in skeletal muscles metabolic responses has not been studied. We hypothesize here that TBXA2R affects skeletal muscle cells glucose metabolism, as part of skeletal muscle cells response to exercise.
Materials and methods: L6 rat skeletal muscle cells and primary human myoblasts were grown and differentiated into myotubes. Cells were incubated with the thromboxane A2 receptor agonists U46619 or I-BOP for four hours, then incubated with/without insulin for 20–30 minutes before adding ^{3}H- or ^{14}C-labeled glucose for 15 or 90 minutes for the glucose uptake and glucose incorporation into glycogen (glycogen synthesis) assays respectively. Proteins were extracted, and glucose and glycogen metabolism, insulin signalling, and inflammatory pathways were investigated.
Results: Incubation with the thromboxane A2 agonists significantly increased basal and insulin-stimulated glucose uptake and glycogen synthesis in L6 and human myotubes. Human cells were more responsive than rat L6 cells, which corresponds with higher expression of the TBXA2R receptor in human cells. Preliminary data showed that activation of TBXA2R triggered an increase in protein kinase C (PKC) phosphorylation as well as a large decrease of the inhibitory phosphorylation of glycogen synthase (GS), which corresponds with the increase in glycogen synthesis. Activation of TBXA2R did not increase phosphorylation of MAPK pathways (p38 and JNK), while the phosphorylation of p65/NFκB (nuclear factor κB) was decreased. Phosphorylation of Akt and AS160 (Akt substrate of 160 kDa) was unaffected, which indicates that the metabolic effects of TBXA2R were independent from insulin signaling pathway.
Conclusion: Our data suggests that TBXA2R modulates glucose metabolism in skeletal muscle, and mapping of its signaling network will likely identify novel targets to improve insulin-independent glucose uptake. TBXA2R modulation in skeletal muscle may represent an interesting approach for the improvement of glucose control in metabolic diseases.
Disclosure: **A.M. Abdelmoez:** None.

451

Mechanical stretch-induced extracellular vesicles improved insulin-stimulated glucose uptake in myotubes and adipocytes

T. Saito[1,2], Y. Shimoda[1], T. Shimizu[1], J. Okada[1], R. Shibusawa[1], Y. Kasai[1], A. Osaki[1], E. Yamada[1], S. Okada[1], M. Yamada[1];
[1]Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, [2]Center for Medical Education, Gunma University Graduate School of Medicine, Maebashi, Japan.

Background and aims: Recently, extracellular vesicles (EVs) such as exosomes and microvesicles are indicated the possibility of a signal transmitter in intercellular network, because they include DNA, miRNAs and proteins. We have reported that mechanical stretch improved insulin-stimulated glucose uptake through the insulin-independent signaling mechanism in C2C12 myotubes. We hypothesized that EVs secreted by mechanical stretch affected on glucose uptake mechanism and improved insulin sensitivity by the means of paracrine or autocrine systems. Therefore, this study aimed to investigate the functions of EVs derived from mechanical stretched myotubes on insulin-stimulated glucose uptake in vitro.
Materials and methods: C2C12 myoblasts were grown on an elastic silicone chamber and induced differentiation. Differentiated C2C12 myotubes were stimulated by cyclic uniaxial stretch (10% of initial length, 10 cycle/min) for 5 hours. EVs were isolated from supernatants with Mag-capture isolation kit and co-incubated with well differentiated C2C12 myotubes or 3T3L1 adipocytes before insulin stimulation. The cells were stimulated with 10nM insulin for 10 minutes and then glucose uptake experiment was performed. The amount of 2-deoxyglucose uptake was measured by enzymatic assay. And the signaling pathway was examined by western-blotting.
Results: EVs were isolated in basal and stretched condition and the protein amount of EVs was not seen any differences between them by protein assay. These EVs expressed exosomes biomarker, CD81 equally in each condition. EVs from mechanical stretch statistically increased insulin-stimulated glucose uptake by 22% and Akt phosphorylation in C2C12 myotubes. Interestingly, this ameliorating of insulin sensitivity was observed in 3T3L1 adipocytes by stretch-induced EVs from C2C12 myotubes. Glucose transporter 4 (Glut4) expression was elevated in both C2C12 myotubes and 3T3L1 adipocytes co-incubated with stretch-induced EVs.
Conclusion: These results suggest that mechanical-stretch induced extracellular vesicles from muscle cells have the potential for improvement insulin sensitivity of muscle cells and adipocytes and the target for treatment of diabetes.
Supported by: KAKENHI
Disclosure: **T. Saito:** None.

452

Caveolin and clathrin mediate insulin receptor internalisation and are associated with high-insulin induced insulin resistance in muscle cells

H. Cen[1], L. Abraham[2], M. Gold[2], J.D. Johnson[1];
[1]Department of Cellular and Physiological Science, University of British Columbia, Vancouver, [2]Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada.

Background and aims: InsR internalization upon insulin binding enables the InsR signaling from endosomal compartments and is a possible mechanism to selectively activate different downstream signaling pathways. InsR endocytosis through either clathrin- or caveolin-mediated pathways have been observed in different cell lines, but the specific internalization routes and insulin dose-dependent dynamics of InsR have not been determined in skeletal muscle cells. Hyperinsulinemia thought to be a compensation for peripheral insulin resistance may be the cause. The mechanism by which high insulin exerts its deleterious effects on insulin signaling remains unclear. We are determining the InsR internalization routes and dynamics in muscle cells and the associated mechanisms of high insulin-induced insulin resistance.
Materials and methods: The interaction of InsR with Cav3 or clathrin in response to insulin were assessed by co-immunoprecipitation (Co-IP)

using skeletal muscles from mice injected with 1.5 U insulin. The localization and trafficking of inter-domain tagged InsR around cell membrane were imaged by TIRF microscopy. Diffusion coefficient was calculated from the single particle tracking of InsR domains to determine the dynamics of InsR. Differentiated C2C12 myotubes were treated with 200 nM insulin for ~16 hours to establish an *in vitro* cell model for high insulin-induced insulin resistance. Surface biotinylation assay, which allows the isolation of labeled surface or internalized proteins and their interacting proteins, was used to illustrate the distribution and internalization kinetics of InsR at normal and insulin resistance conditions.

Results: InsR had increasing interaction with Caveolin 3 (Cav3) at 5 and 10 min after insulin stimulation in mouse skeletal muscle, while clathrin had elevated interaction with InsR at 5 min, determined by Co-IP. Inter-domain tagged InsR co-localized with both caveolin and clathrin in C2C12 myoblasts. The presence of 2 nM insulin resulted in the higher diffusion coefficient of InsR, which suggested a positive effect of insulin on accelerating the movement of the insulin receptor. High insulin-treated C2C12 myotubes exhibited attenuated insulin signaling highlighted by moderated Akt and ERK phosphorylation. The baseline Akt phosphorylation was also decreased. The InsR level was significantly downregulated by ~50%, and the total Akt and ERK levels were unaltered. The surface InsRs were also decreased by ~40%, while the surface to total InsR ratio was unaffected. Interestingly, both Cav3 and AP2, the adaptor protein that binds to receptors and initiates clathrin-mediated endocytosis, were increased in high insulin-treated cells, while surface-bound Cav3 had no apparent difference. The internalization kinetics of InsR were not significantly affected by different insulin concentrations or high-insulin treatment.

Conclusion: InsR internalization may be mediated by both clathrin and caveolin is skeletal muscle cells. The mechanisms of high-insulin induced insulin resistance may involve the downregulation of both total and surface InsR, which is associated with upregulation of Cav3 and AP2. We are investigating the role of dual internalization routes on InsR signaling in normal and high-insulin induced insulin resistant cells.

Disclosure: **H. Cen:** None.

453
WITHDRAWN

454
Prdx6 reduced the risk of type 2 diabetes-associated sarcopenia by improving skeletal muscle cells differentiation

F. Pacifici[1], B. Capuani[1], F. Piermarini[1], D. Pastore[1], R. Arriga[1], A. Coppola[1], S. Rea[1], G. Donadel[1], A. Bellia[1], D. Della-Morte[1,2], D. Lauro[1]; [1]University of Rome Tor Vergata, Rome, [2]Università Telematica San Raffaele Roma, Rome, Italy.

Background and aims: Diabetes Mellitus (DM) is a group of metabolic disorders characterized by a state of hyperglycemia resulting from altered insulin secretion by pancreatic beta cell, insulin action (insulin resistance) or both. This pathological condition is frequently associated with muscle mass loss, a condition defined as sarcopenia, and with diabetic myopathy, represented by an impairment of the regenerative power of muscle fiber and by an altered differentiation of progenitor cells. Oxidative stress has been identified among the main causes of muscular decline typical of diabetic patients. We reported that Peroxiredoxin6 (Prdx6), a relatively new antioxidant enzyme belonging to the Peroxiredoxin's family, has a central role in controlling glucose homeostasis by exerting a potent antioxidant role. Therefore, in the present study, we aimed to investigate whether Prdx6 may be implicated in Type 2 DM (T2DM)-sarcopenia pathological link.

Materials and methods: Wild type (wt) and Prdx6 knockout ($Prdx6^{-/-}$) three-months old male mice were used for this study. Moreover, a murine myoblasts cell line (C2C7) stably silenced for Prdx6 ($Prdx6^{KD}$) was generated and compared with control scramble (scr) cells. Four limbs GRIP test was achieved in mice model to assess muscle strength. Gene expression of myogenic and atrophy-associated factors was evaluated by performing qRT-PCR on muscle. Protein extracts from $Prdx6^{KD}$ C2C7 cell line was processed by performing western blot analysis. Sera levels of biomarkers of sarcopenia were evaluated by Luminex assay.

Results: We observed significant reduced muscle strength in $Prdx6^{-/-}$ mice compared to control group by performing GRIP test, suggesting presence of sarcopenia in this mice model. In order to investigate possible influencers responsible for this phenomenon, we analyzed gene expressions of the main factors involved in the differentiation of myogenic muscle cells, such as MyoD and Myogenin. We observed significant decreased levels of both genes in $Prdx6^{-/-}$ mice compared to control group, suggesting an impairment of the regenerative potential of muscle fibers in these mice. Muscle atrophy was studied by evaluating the gene expression of MuRF1 and Atrogin-1 that finely regulate protein degradation at skeletal muscle level. According to our hypothesis, the expression levels of both enzymes were significantly increased in $Prdx6^{-/-}$ mice, confirming the presence of muscle atrophy. To further confirm sarcopenia, we evaluated sera levels of IGF-1, GDF-15 and TNF-alpha, a well-established markers of muscle loss. We observed a significant pathological modulation of these factors validating the presence of T2DM-sarcopenia associated noxious link. An *in vitro* cellular model of murine muscle cell $Prdx6^{KD}$ was used to confirm the reduction of myogenic factors. A significant decrease in proteins expression of MyoD and Myogenin, which could be regulated by specific muscle miRNA up-regulation, was evident.

Conclusion: Our study, innovatively, highlights a fundamental role of the antioxidant enzyme, Prdx6, in the mechanism of diabetes-associated sarcopenia, suggesting how Prdx6 can be considered a potential therapeutic target for restore muscle loss in these patients. Further studies are needed in order to understand molecular mechanisms underlying this phenomenon.

Supported by: Fondazione Roma NCDS-2013-00000331- Sarcopenia and Insulin Resistance

Disclosure: **F. Pacifici:** None.

455
The novel adipokine Wnt1-inducible signalling pathway protein-1 (WISP1) associates with insulin resistance and impairs insulin action

N. Rudovich[1,2], T. Hörbelt[3,4], C. Tacke[2,5], M. Markova[2,4], F. Van de Velde[6], M. Bekaert[6], Y. Van Nieuwenhove[7], H.G. Thoresen[8], O. Kuss[9,4], V. Lange[10,11], B. Lapauw[6], A. Schürmann[12,4], A.F. Pfeiffer[2,5], O. Pivovarova[2,4], D. Ouwens[3,4];
[1]Department of Internal Medicine, Spital Bülach, Bülach, Switzerland, [2]Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany, [3]Institute for Biochemistry and Pathobiochemistry, German Diabetes Center, Düsseldorf, Germany, [4]German Center for Diabetes Research (DZD), Muenchen-Neuherberg, Germany, [5]Department of Endocrinology, Diabetes and Nutrition, Charité University Medicine, Berlin, Germany, [6]Department of Endocrinology, Ghent University Hospital, Ghent, Belgium, [7]Department of Surgery, Ghent University Hospital, Ghent, Belgium, [8]Department of Pharmaceutical Biosciences, University of Oslo, Oslo, Norway, [9]Institute for Biometrics and Epidemiology, German Diabetes Center, Duesseldorf, Germany, [10]Center for Obesity and Metabolic Surgery, Berlin, Germany, [11]Helios Klinikum Berlin-Buch, Berlin, Germany, [12]Department of Experimental Diabetology, German Institute of Human Nutrition, Potsdam, Germany.

Background and aims: Wnt1-inducible signalling pathway protein-1 (WISP1) was recently identified as a pro-inflammatory adipokine. We examined whether WISP1 expression and circulating levels are altered in type 2 diabetes, and whether WISP1 affects insulin signalling in muscle cells and hepatocytes.
Materials and methods: Serum and visceral adipose tissue (VAT) biopsies for analysis of circulating WISP1 levels by ELISA and *WISP1* mRNA expression by qPCR were collected from normal-weight control men ($n = 33$), and obese men with ($n = 56$) and without type 2 diabetes ($n = 46$) undergoing surgery. Insulin signalling was analysed in primary human skeletal muscle cells (hSkMC) and murine AML12 hepatocytes following incubation with WISP1 and insulin by Western blotting. WISP1 effects on insulin-stimulated glycogen synthesis and gluconeogenesis were investigated in hSkMC cells and murine hepatocytes, respectively.
Results: Circulating WISP1 levels were higher in obese men independent of type 2 diabetes than in controls (70.8 (55.2–86.4) ng/l vs. 42.6 (28.5–56.6) ng/l; $p < 0.05$, respectively). VAT *WISP1* expression was 1.9-fold higher in morbidly obese men versus controls ($p < 0.05$). Circulating WISP1 levels were positively associated with blood glucose in the oral glucose tolerance test and circulating heme oxygenase-1 but negatively with adiponectin levels. In hSkMC cells and AML12 hepatocytes, recombinant WISP1 impaired insulin action by inhibiting the phosphorylation of insulin receptor, Akt, and its substrates glycogen synthase kinase 3β, FOXO1 and p70S6 kinase, as well as insulin-stimulated glycogen synthesis and suppression of gluconeogenic genes.
Conclusion: Circulating WISP1 and *WISP1* expression in VAT are increased in obesity independent of glycaemic status. Furthermore, WISP1 impaired insulin signalling in muscle and liver cells.
Supported by: German Centre for Diabetes Research and EFSD/AZ
Disclosure: **N. Rudovich:** Grants; European Foundation for the Study of Diabetes.

456

Plasma methylglyoxal is associated with insulin resistance and induces impairment of insulin-induced akt-phosphorylation in microvascular endothelial cells

T.A. Chimhanda[1], D.E.M. Maessen[1], N. Hanssen[1], M.K.C. Hasselink[2], P. Schrauwen[2], C.D.A. Stehouwer[1], C.G. Schalkwijk[1];
[1]Internal Medicine, Maastricht University Medical Center, Maastricht, [2]Human Biology, Maastricht University Medical Center, Maastricht, Netherlands.

Background and aims: Insulin resistance (IR) is a major mechanism in the pathological progression of diabetes, in which skeletal muscle cells and endothelial cells play key roles. The dicarbonyl methylglyoxal (MGO), the major reactive precursor in the formation of advanced glycation endproducts (AGEs), has been linked to the development of IR. However, the underlying mechanism through which MGO contributes to IR is unknown. Therefore, we investigated first in humans, whether increased plasma and muscular MGO levels were associated with insulin resistance. Next, we studied the effect of MGO on the insulin-signaling cascade *in vitro* in cultured muscle and microvascular endothelial cells.
Materials and methods: Plasma samples and biopsies of the vastus lateralis muscle from 11 insulin resistant individuals and 11 age-matched controls were obtained in the fasted state. Whole body insulin sensitivity was determined by the glucose infusion rate (GIR) during a hyperinsulinaemic euglycaemic clamp. The dicarbonyls MGO, glyoxal (GO) and 3-deoxyglucosone (3-DG), a panel of AGEs and insulin modifications were determined by UPLC-MS/MS. The effect of MGO and AGEs on insulin-induced phosphorylation of Akt was determined in mature cultured C2C12 myotubes and in microvascular endothelial cells (MVEC) by western blotting. Glyoxalase-1 activity was measured by spectrophotometry. Independent two-sample t-tests were used to compare data between healthy controls and insulin resistant individuals. One-way ANOVA was used for comparing the *in vitro* effects of MGO on Akt-phosphorylation and a $p < 0.05$ value was considered statistically significant.
Results: At baseline, IR individuals (GIR 54 ± 12 μmol/min/kg) showed significantly elevated plasma MGO (316 ± 10 nmol/L vs 457 ± 29; $p < 0.001$), GO (698 ± 48 nmol/L vs 1593 ± 175; $p < 0.001$) and 3-DG (1205 ± 36 nmol/L vs 2909+/187; $p < 0.001$), but not AGEs levels, as compared to age-matched healthy controls (GIR 179 ± 20 μmol/min/kg). In addition, intramuscular levels of the dicarbonyls, AGEs and the glyoxalase-1 activity were similar between the two groups. Extracellular supplementation of MGO-derived AGEs and MGO (up to 200 μM) to mature cultured C2C12 myotubes did not affect insulin-induced phosphorylation of Akt, while supplementation of MGO, but not MGO-derived AGEs, to MVECs inhibited significantly insulin-induced Akt-phosphorylation in a time and concentration-dependent manner up to ~30% at maximum. In addition, we found that MGO could directly modify insulin, accompanied by an impaired insulin-induced phosphorylation of Akt.
Conclusion: Overall, MGO contributes to impaired insulin sensitivity through direct modification of insulin and through modulation of the insulin-signaling cascade in endothelial cells but not muscle cells, highlighting MGO as a potential target in the treatment of insulin resistance.
Supported by: DFN and DHF
Disclosure: **T.A. Chimhanda:** None.

457

Effect of glycated albumin in the expression of glucose transporter GLUT4 in adipocytes: potential participation of transcription factor NF-kB

M.L.E. Michalani[1], M. Passarelli[2], U.F. Machado[1];
[1]Physiology and Biophysics, University of Sao Paulo, Sao Paulo, [2]University of Sao Paulo, Sao Paulo, Brazil.

Background and aims: The loss of glucose homeostasis, a feature of diabetes, is related to insulin resistance that leads to reduced peripheral glucose uptake. In this process, the insulin-sensitive glucose transporter isoform 4 (GLUT4) encoded by the *Slc2a4* gene, plays a decisive role. In fact, reduced levels of GLUT4, in insulin-sensitive territories, such as adipose and skeletal muscle tissues, contributes for hyperglycemia. Increasing circulating glucose levels results in increased production of advanced glycation end products (AGEs) which, by interacting with their receptor (RAGE), activate the nuclear factor NF-kappa-B (NFKB) pathway. NFKB has been well described to inhibit *Slc2a4* gene expression, contributing to a vicious circle of impaired glucose utilization by GLUT4-expressing tissues. Although AGEs are well studied in the pathogenesis of diabetes complications, little is known about their potential contribution to impair insulin-stimulated glucose uptake. Clarifying this mechanism, we may reveal a regulatory role for AGEs not only in the development of chronic complications of diabetes, but also in the loss of glycemic homeostasis.
Materials and methods: Differentiated 3T3-L1 adipocytes were treated with bovine serum albumin without modification or conjugated with glycolaldehyde, at different concentrations (0.4, 3.6 and 5.4 mg/mL) and periods (24 and 72 hours). Expression of the *Slc2a4*, *Nfkb1* and *Rela* mRNAs by RT-qPCR and GLUT4, p50 and p65 proteins by Western blotting were evaluated. The statistical test used was two-tailed unpaired Student's t test.

Results: In 3T3-L1 cells, glycated albumin in short term (24 hours) and low dose (0.4 mg/mL) increased *Slc2a4* mRNA (99%, $P < 0.001$) and GLUT4 protein (52%, $P < 0.05$) expression, however, in long term (72 hours) and high dose (5.4 mg/mL) it reduced the gene (57.5%, $P < 0.001$) and protein (37%, $P < 0.01$) expression. In addition, glycated albumin during 24 hours and at all three doses had a stimulatory effect on the expression of *Rela* (22%, $P < 0.05$; 28%, $P < 0.01$; 45%, $P < 0.001$) and *Nfkb1* (12%, $P < 0.05$; 30%, $P < 0.01$; 69%, $P < 0.001$) genes, indicating an increase in proinflammatory activity. This was accompanied by an increase in the nuclear content of p50 and p65 proteins, at long term and high dose (98%, $P < 0.05$; 29%, $P < 0.01$, respectively). **Conclusion:** The results indicate that glycated albumin, in a time/dose-dependent manner, can induce proinflammatory activity in the adipocyte, reducing *Slc2a4*/GLUT4 expression. This characterizes a hormetic effect of AGEs, which in chronic condition contributes to impair glycemic homeostasis.

Supported by: FAPESP (#2016/17002-4)

Disclosure: **M.L.E. Michalani:** None.

PS 025 Clocking in on exercise and nutrition

458

Skeletal muscle contraction protects against hyperglycaemia-induced insulin resistance and associated transcriptomic (dys) regulation

S. Carter, J.P. Barlow, T.P.J. Solomon;
University of Birmingham, Birmingham, UK.

Background and aims: Skeletal muscles represent a major site of glucose uptake. Insulin serves as a potent stimulus to increase glucose uptake to maintain normoglycaemia. Therefore, impairment in responsiveness to insulin contributes to increasing hyperglycaemia and, if untreated, the progression towards type 2 diabetes. Exercise enhances skeletal muscle insulin sensitivity and therefore serves as a key intervention to prevent type 2 diabetes. Dissecting the molecular mediators controlling this protective effect will aid understanding which could be targeted to prevent insulin resistance and associated metabolic abnormalities. Therefore, we set out to characterise the underlying transcriptional changes responsible for these exercise-mediated protective effects.

Materials and methods: Differentiated C2C12 myotubes were exposed to 24 hours of contractile activity (electrical pulse stimulation; EPS) or rest, followed by 0, 6, 18 or 24 hours of normo- (5 mM) or hyperglycaemic (25 mM) conditions. We evaluated the functional (basal and insulin-stimulated glucose uptake) and molecular (RNA sequencing followed by analysis of differential gene expression using NOISeq) responses to these conditions of rest *vs.* contraction and/or normo- *vs.* hyperglycaemia. Follow-up annotation and pathway enrichment analysis was conducted on differentially expressed genes. Data are presented as mean ± SEM.

Results: Data demonstrates the time-course for functional and molecular responses to different durations of a) hyperglycaemia (6, 18 and 24 hours) and b) rest following contraction cessation (0, 6, 18 and 24 hours). In non-EPS treated cells, 24 hours of hyperglycaemia significantly impaired insulin-stimulated glucose uptake *vs.* control (fold increase over basal: 1.00 ± 0.24 *vs.* 1.91 ± 0.20, $P < 0.05$), and this impairment coincides with differential expression of 115 transcripts (58 up-regulated; 57 down-regulated). Interestingly, prior skeletal muscle contraction ameliorated this hyperglycaemia-induced insulin resistance (fold increase over basal: 1.57 ± 0.34, $P > 0.05$ *vs.* control), and prevented the (dys) regulation of several transcripts otherwise induced by hyperglycaemia. In particular, prior contractile activity prevented the hyperglycaemia-induced (dys) regulation of several non-coding RNAs that were otherwise up-regulated (e.g. miR-27a, miR-125a and SNORD70) or down-regulated (e.g. miR-378a and miR-194-1) by 24 hours of hyperglycaemia.

Conclusion: The present study combines functional and transcriptomic profiling of hyperglycaemia and/or contraction treated skeletal muscle cells. Our data demonstrate that skeletal muscle contraction *per se* protected skeletal muscle cells against hyperglycaemia-induced insulin resistance, and that this protective effect coincides with contraction-mediated normalisation of several hyperglycaemia-responsive transcriptional changes. Our ongoing experiments will define possible causal roles for these novel contraction-responsive transcripts, and establish whether they can be targeted to modulate skeletal muscle insulin sensitivity in human skeletal muscle cells from individuals with widely varying insulin sensitivity.

Supported by: The Physiological Society

Disclosure: **S. Carter:** None.

459

Endurance exercise training in the fasted or fed state in male patients with type 2 diabetes: safe and equally effective

K. Verboven[1], I. Wens[1], B. Lapauw[2], P. Calders[2], D. Hansen[1];
[1]Rehabilitation Research Center (REVAL), Biomedical Research Unit (BIOMED), Hasselt University, Hasselt, [2]Department of Endocrinology, Ghent University Hospital, Ghent, Belgium.

Background and aims: Exercise training is a cornerstone in the care of patients with type 2 diabetes mellitus (T2DM). A possible role for nutritional state, either being in the fed or fasted state, is of particular interest during exercise training interventions in T2DM patients. The present study investigates the impact of endurance exercise training in the fasted versus the fed state on clinical outcome measures, glycemic control and skeletal muscle characteristics in male T2DM patients.
Materials and methods: Twenty male T2DM patients (age 60 ± 2 years, BMI 29.2 ± 0.8 kg/m², HbA1c 7.4 ± 0.3% (57 ± 4 mmol/mol)) participated in a randomized clinical trial in which individuals performed a supervised endurance exercise intervention for 12 weeks. Training sessions (including walking and cycling) were performed in the fasted state (FAST, $n = 10$) or after having a breakfast (FED, $n = 10$) (groups matched for age, BMI and glycated hemoglobin level). Before and after the exercise intervention, patients were evaluated for glycemic control, blood lipid profile, body composition and physical fitness and a fasting skeletal muscle biopsy was obtained for gene expression analyses.
Results: Exercise training intervention was well tolerated, with no difference between FAST and FED with respect to adherence (91 ± 1% vs. 94 ± 1%, respectively; $P = 0.280$) or mean Borg score (11 vs. 12, respectively; $P = 0.052$) and without any incident of hypoglycemia. In both FAST and FED, exercise training significantly decreased BMI ($P_{TIME} = 0.014$), body fat percentage ($P_{TIME} = 0.002$), whole-body fat mass ($P_{TIME} < 0.001$) and significantly improved VO_2peak ($P_{TIME} = 0.042$) and Wpeak/lean tissue mass ($P_{TIME} = 0.005$), without any interaction effects ($P_{TIME*GROUP} > 0.05$, respectively). Exercise training reduced respiratory exchange ratio during different levels of submaximal exercise (*i.e.* 20%, 40% and 60% VO_2peak) in both groups (for all levels $P_{TIME} < 0.050$; $P_{TIME*GROUP} > 0.050$). HDL significantly increased in both FAST and FED ($P_{TIME} = 0.028$; $P_{TIME*GROUP} = 0.435$). Glycated hemoglobin levels significantly decreased after exercise training ($P_{TIME} < 0.001$), with the greatest reduction (−0.36%) in the FED compared to the FAST group (−0.07%) ($P_{TIME*GROUP} = 0.004$). At skeletal muscle level, no interaction effects were observed for genes related to lipid metabolism or oxidative capacity ($P_{TIME*GROUP} > 0.05$).
Conclusion: Exercise training in the fasted or in the fed state were both safe and effective in improving glycemic control, body composition, physical fitness and HDL in male patients with T2DM.
Clinical Trial Registration Number: NTR4711
Supported by: Yvonne and Jacques Francois de Meurs grant (King Baudouin Fund)
Disclosure: **K. Verboven:** Grants; Yvonne and Jacques Francois de Meurs grant (King Baudouin Fund).

460

Time dependence of glucagon under hypoglycaemic conditions

C. Pedersen[1], G. Kraft[2], D. Laneve[2], M. Scott[2], M.N. Najdowski[2], B. Farmer[2], P. Williams[2], A.D. Cherrington[2], D.S. Edgerton[2];
[1]Novo Nordisk A/S, Maaloev, Denmark, [2]Vanderbilt University, Nashville, USA.

Background and aims: Glucagon's (GGN) effect on hepatic glucose production (HGP) is time dependent under hyperglycemic conditions. It is not known whether this is also the case under hypoglycemic conditions. This question was addressed using adrenalectomized animals to avoid the confounding effects of other counterregulatory hormones.
Materials and methods: Nine dogs were subjected to adrenalectomy and catheter placement in the femoral artery, portal vein and hepatic vein, as well as flow probes on the hepatic artery and portal vein. Mineralocorticoid therapy was initiated pre-surgery (desoxycorticosterone pivalate, 1.2 mg/kg) and after surgery hydrocortisone was administered (12.5 mg) twice daily for glucocorticoid maintenance. On the day of the study, basal infusions of epinephrine (7.25 ng/kg/min) and cortisol (0.6 μg/kg/min) were started. After the control period, somatostatin was infused to disable the endocrine pancreas, and insulin was infused in a leg vein (800 μU/kg/min). After 30 minutes, when plasma glucose started to fall, GGN was infused at a basal rate (1 ng/kg/min, BaGGN group, $n = 4$) or a rate 8 fold basal (8 ng/kg/min, HiGGN group, $n = 5$) for 4 hours. Studies were paired and glucose was infused to match the arterial glucose levels between groups.
Results: Epinephrine and cortisol were basal throughout the study in both groups. Insulin rose to ≈40 ± 3 μU/mL in both groups ($P > 0.05$), while GGN dropped to ≈21 ± 3 pg/mL when somatostatin started, then rose to 39 ± 3 and 227 ± 11 pg/mL in the BaGGN and HiGGN groups, respectively ($P < 0.05$). To maintain a glucose level of 41 mg/dL in both groups, the glucose infusion rate averaged 2.5 ± 0.3 and 0.5 ± 0.4 mg/kg/min in BaGGN and HiGGN, respectively ($P < 0.05$). Net hepatic glucose output (NHGO) was similar in both groups during the basal period and prior to GGN infusion. During GGN infusion NHGO rose to 5.6 ± 1.3 mg/kg/min by 15 min in the HiGGN group but stayed at 0.7 ± 0.2 mg/kg/min in the BaGGN group. NHGO fell in the HiGGN group ≈50% over 60 min and plateaued at 2.3 ± 0.2 mg/kg/min for the last 120 min of study, while NHGO in the BaGGN group remained at 0.4 ± 0.1 mg/kg/min (Figure). Importantly, the hypoglycemic induced rise in norepinephrine (334 ± 40 and 244 ± 19 pg/mL, $P = 0.08$) and plasma glycerol (225 ± 15 and 152 ± 6 μmol/L, $P = 0.05$) were significantly smaller when GGN was elevated despite plasma glucose being identical in the groups.
Conclusion: The effect of GGN on HGP during hypoglycemia is biphasic, characterized by an initial burst occurring over about 60 min followed by a reduced but sustained effect over the last 120 min of the experiment. Of note, the sympathetic nervous system response to the hypoglycemia was blunted when GGN was elevated, resulting in a decrease in lipolysis. This finding suggests that there is reciprocity between GGN and the sympathetic neural system such that when GGN is increased the sympathetic nervous system response to hypoglycemia is downregulated.

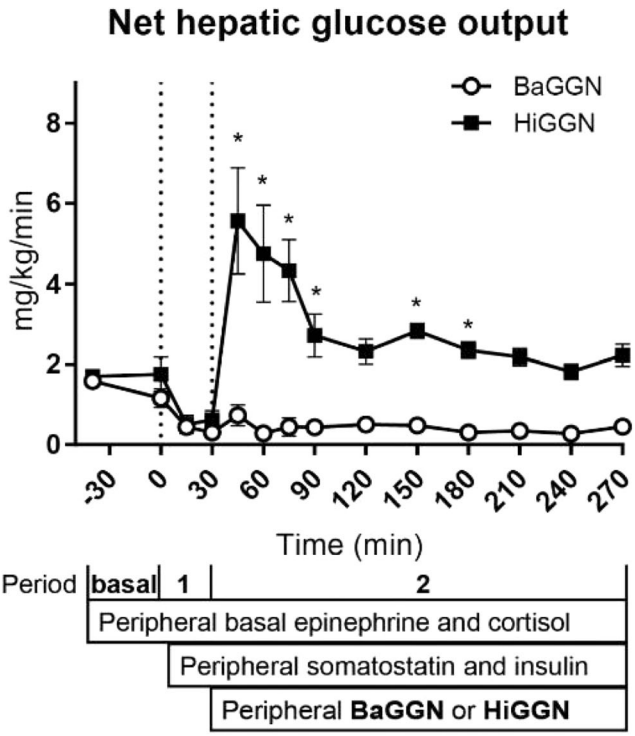


Figure: Net hepatic glucose output during a hyperinsulinemic hypoglycemic clamp in adrenalectomized dogs with basal (BaGGN n=4) or an 8 fold basal (HiGGN, n=5) infusions of glucagon. Values are mean ± SEM, *p < 0.05 compared to the BaGGN group.

Disclosure: **C. Pedersen:** None.

461

Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in men with type 2 diabetes

M. Savikj[1,2], B.M. Gabriel[1], P. Alm[1], J. Smith[1], K. Caidahl[3,4], M. Björnholm[1], T. Fritz[3,4], A. Krook[1], J.R. Zierath[1,3], H. Wallberg-Henriksson[1];
[1]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, [2]Faculty of Medicine, University of Oslo, Oslo, Norway, [3]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, [4]Department of Clinical Physiology, Karolinska University Hospital, Stockholm, Sweden.

Background and aims: Exercise is a recommended intervention for prevention and treatment of type 2 diabetes. Low-volume High Intensity Interval Training (HIIT) remodels skeletal muscle and the cardio-respiratory system to a similar or greater extent than continuous moderate-intensity training, with reduced time commitment and exercise volume. Even though perturbed circadian rhythms are associated with metabolic dysfunction, time of day at which most robust adaptions to exercise can be achieved is unknown. We compared the efficacy of morning and afternoon HIIT in lowering blood glucose levels in participants with type 2 diabetes.
Materials and methods: Eleven men with type 2 diabetes underwent a randomized cross-over trial with either 2 weeks morning or afternoon HIIT (3 bouts/week), followed by a 2-week washout period and a subsequent period of the opposing training regime. Continuous glucose monitoring (CGM) based blood glucose levels were recorded throughout the study and hourly time-point means were compared. Blood samples were collected pre-training and after both training regiments.
Results: During week 1 of training, afternoon HIIT reduced CGM-based glucose levels as compared to either pre-training (mean change 0.9 mmol/l, $p < 0.05$) or morning HIIT (1.1 mmol/l, $p < 0.05$) at 4 and 10 time-points throughout the day, respectively. Conversely, during week 1 of training, morning HIIT increased CGM-based glucose levels as compared with pre-training (1.2 mmol/l, $p < 0.05$) at 4 time-points throughout the day. During week 2 of training, afternoon HIIT reduced CGM-based glucose levels as compared to either pre-training (0.9 mmol/l, $p < 0.05$) or morning HIIT (0.9 mmol/l, $p < 0.05$) at 4 and 6 time-points throughout the day, respectively. Conversely, during week 2 of training, morning HIIT increased CGM-based glucose levels as compared with pre-training (0.9 mmol/l, $p < 0.05$) at 2 time-points throughout the day. The elevated glucose concentration with morning HIIT persisted even into the subsequent days after exercise. Additionally, plasma T_4 was decreased and TSH was increased following the training as compared with pre-training, suggesting a hormonal mechanism behind the differing responses, as these changes were potentiated following afternoon HIIT. Plasma triglycerides levels were elevated following morning HIIT as compared with pre-training, suggesting changes in fuel utilization with morning training.
Conclusion: This was a field-based study in 'free-living' individuals, thus the specific factors responsible for the differing blood glucose levels between morning and afternoon exercise remain to be elucidated. Afternoon HIIT is more efficacious than morning HIIT in controlling blood glucose levels in type 2 diabetic men whereas morning HIIT may even be deleterious for optimal blood glucose control. Our data highlight the importance of optimizing the timing of exercise bouts to improve glycaemic control in people with type 2 diabetes.
Supported by: Novo Nordisk, Wenner-Gren and Diabetes foundation, SRP Diabetes
Disclosure: **M. Savikj:** None.

462

Carbohydrates standard breakfast as a more physiological test of glucose tolerance than the OGTT in obese patients: glycaemic variability and insulin secretion indexes

I. Banu, A. Rezki, M. Fysekidis, S. Chiheb, E. Cosson, P. Valensi;
AP HP Jean Verdier Hospital, Department of Endocrinology-Diabetology-Nutrition, CRNH-IdF, CINFO, Bondy, France.

Background and aims: Glycemic variability (GV) may play a role in diabetic complications. We recently showed that GV is more marked in non-diabetic patients with elevated HbA1c level. The aim was here to examine the glycemic and insulinemic response profile to a standard breakfast including 75 g of carbohydrates and the relationship with GV in obese patients with different glycemic status (normal, NGT or impaired glucose tolerance, IGT, or type 2 diabetes, T2D).

Materials and methods: We included 82 obese patients without known dysglycemia we separated in 34 NGTs, 38 IGTs and 10 T2Ds according to oral glucose tolerance test (OGTT). During OGTT, the insulinogenic index (IGI = insulin T120-insulin T0)/(glucose T120-glucose T0) and the oral disposition index (ODI=IGI/insulin T0) were calculated. A "French" standard breakfast including 75 g of carbohydrates was given with plasma glucose and insulin measurements for 2 hours. The area under curve (AUC-glucose) was calculated for 2 hours after breakfast. GV was evaluated by calculating SD-glucose, CONGA, J index and MAGE from 24-h continuous glucose monitoring including the standard breakfast.
Results: IGTs and T2Ds were older than NGTs (51 ± 13 and 54 ± 12 *vs* 41 ± 13 years, $p = 0.002$). They had lower IGI and ODI, and higher HbA1c and GV indexes than NGTs ($p < 0.05$ to $p < 0.0001$). After the standard breakfast, they had higher AUC-glucose. In all patients, AUC-glucose correlated positively with HbA1c and GV indexes and negatively with ODI ($p < 0.02$ to $p < 0.0001$), independently of age and BMI.
Conclusion: In obese patients without known dysglycemia, the magnitude of post-prandial excursion (AUC-glucose) after the carbohydrates standard breakfast is in line with the severity of glycemic disorder identified by OGTT. AUC-glucose correlates positively with greater 24h glycemic variability and negatively with insulin secretion. This breakfast could be proposed as a more physiological test than OGTT.
Disclosure: **I. Banu:** None.

463

Fructose contributions to hepatic triglyceride synthesis in the presence of glucose during overnight feeding in mice

J. Jones[1,2], J. Silva[1], F. Martins[3], I. Viegas[1], C. Marques[1], M. Macedo[3,2];
[1]Center for Neurosciences, Cantanhede, [2]Portuguese Diabetes Association (APDP), Lisbon, [3]Chronic Diseases Research Center (CEDOC), Lisbon, Portugal.

Background and aims: Fructose is considered to be a potent lipogenic substrate and its increased consumption may be associated with soaring NAFLD incidence rates. However, its precise contribution to hepatic *de novo* lipogenesis (DNL) is not well characterized. Moreover, fructose is typically accompanied by equivalent amounts of glucose, which can also contribute to DNL. Current stable-isotope tracer methods quantify the fractional synthetic rate of triglyceride fatty acids and glycerol moieties from all precursors but cannot resolve contributions from individual substrates. We combined deuterated water (^2H_2O) and [U-^{13}C]fructose to measure the specific contribution of exogenous fructose to newly-synthesized triglyceride fatty acids and glycerol in the presence of an equivalent amount of exogenous glucose under natural feeding conditions.
Materials and methods: At the start of the dark period in a 12h/12h dark/light cycle, seven adult male C57/BL6 mice fed with standard chow were given an intraperitoneal injection of 99.9% ^2H_2O/0.9% NaCl to raise body water ^{2}H-enrichment to ~4%. The drinking water was supplemented with 5% ^2H_2O, 17.5% w/w unlabeled glucose and 17.5% w/w fructose enriched to 20% with [U-^{13}C]fructose. Animals were allowed to feed naturally overnight and then sacrificed at the end of the dark cycle. Livers were freeze-clamped and triglycerides were extracted and purified from other lipid species. Triglycerides were analyzed for ^{2}H and ^{13}C-

enrichment in the terminal fatty acid methyls and the glyceryl moiety by ^{2}H and ^{13}C NMR at 11.7T and 14.1T, respectively.
Results: ^{2}H-enrichment of the terminal fatty acid methyl hydrogens measured by ^{2}H NMR showed that the fraction of newly-synthesized triglyceride fatty acids from all lipogenic precursors was 18 ± 3% (mean ± S.E.). From the ^{13}C NMR analysis the contribution of exogenous fructose to total triglyceride fatty acids was found to be 5 ± 1% and its contribution to newly-synthesized fatty acids was calculated to be 27 ± 3%. For the triglyceride glycerol moiety, 29 ± 5% was newly synthesized and fructose contributed 77 ± 12% of this newly synthesized fraction.
Conclusion: The integration of $^{2}H_2O$ and ^{13}C-enriched substrates coupled to $^{2}H/^{13}C$-NMR analysis of triglyceride allows contributions of specific substrates to triglyceride fatty acid and glycerol synthesis to be determined. In the presence of equimolar amounts of glucose, fructose contributed modestly to hepatic triglyceride fatty acid synthesis but was the dominant precursor for synthesis of the glyceryl moiety.
Supported by: FCT PTDC/BIM-MET/4265/2014
Disclosure: **J. Jones:** None.

464

An essential role of ghrelin in circadian rhythmicity of voluntary exercise under constant darkness

Y. Tajiri[1], Y. Sakai[1], T. Sato[2], M. Kojima[2], M. Nomura[1], H. Mifune[1];
[1]Kurume University School of Medicine, Kurume, [2]Kurume University, Kurume, Japan.

Background and aims: We previously reported a diurnal rhythm of ghrelin with its peaks at the beginning and at the end of the dark period concomitant with an increase of voluntary exercise in the wild type (WT) mice. Those accelerations of exercise were severely attenuated in the ghrelin knockout (GKO) mice, suggesting the relevance of ghrelin surge to the motivation for voluntary exercise (52nd EASD annual meeting). Exercise is also known to be essential for the entrainment to circadian rhythmicity, separate of light-entrained oscillator, suggesting the crucial role of ghrelin as exercise-entrained oscillator. In the present study, we compared exercise patterns in the WT and GKO mice under constant darkness to evaluate the relevance of ghrelin to the entrainment of circadian clock independent of light on-off rhythmicity.
Materials and methods: Eight-week-old male WT (n = 6) and GKO (n = 10) mice were individually housed in cages with SW-15 running wheels under a 12 h light: 12 h dark cycle (L/D, light on 7:00–19:00) at a controlled ambient temperature and provided with food and water ad libitum until 13 weeks old. Then, both groups of mice were subjected to wheel running under constantly dark condition (D/D) for the next 60 days to measure the free-running period of voluntary exercise. At 21 weeks old, light was turned on at 7:00 and the L/D condition was resumed for the next 10 days. The number of revolutions was acquired every 15 minutes. The obtained data of actogram was subjected to Fast Fourier Transform to obtain the spectrum, and the period of the absolute value was calculated to obtain the periodogram using ClockLab Analysis Software Ver. 6.
Results: Body weight of WT and GKO mice were comparable at 8 and 22 weeks old. A marked increase of wheel-running activity was observed especially at the beginning of dark period in either WT or GKO mice under L/D condition. Under D/D condition, actograms of both groups showed free-running periods of less than 24 hours in terms of the onset of voluntary exercise. However, regarding the termination (offset) of exercise, clearly irregular rhythmicity and dispersed pattern was observed in the GKO mice in contrast to the WT mice (Figure). Analysis by periodgram revealed that a dominant free-running period was significantly shorter in the GKO mice compared to that in the WT mice (16.6 ± 1.5 vs. 22.7 ± 1.8 hours, means ± S.E. $P < 0.05$), in agreement with other various and irregular rhythmicity observed in GKO mice under D/D condition. Both WT and GKO mice were immediately re-entrained after the resumption of L/D condition.
Conclusion: Ghrelin in itself is not relevant to the maintenance of the exercise pattern under L/D condition because of the immediate re-entrainment in either WT or GKO mice. In contrast, under D/D condition ghrelin is quite essential for the maintenance of circadian rhythmicity of voluntary exercise, especially in the termination of it, suggesting a crucial role of this peptide as exercise-entrained oscillator independent of light-entrained rhythmicity.

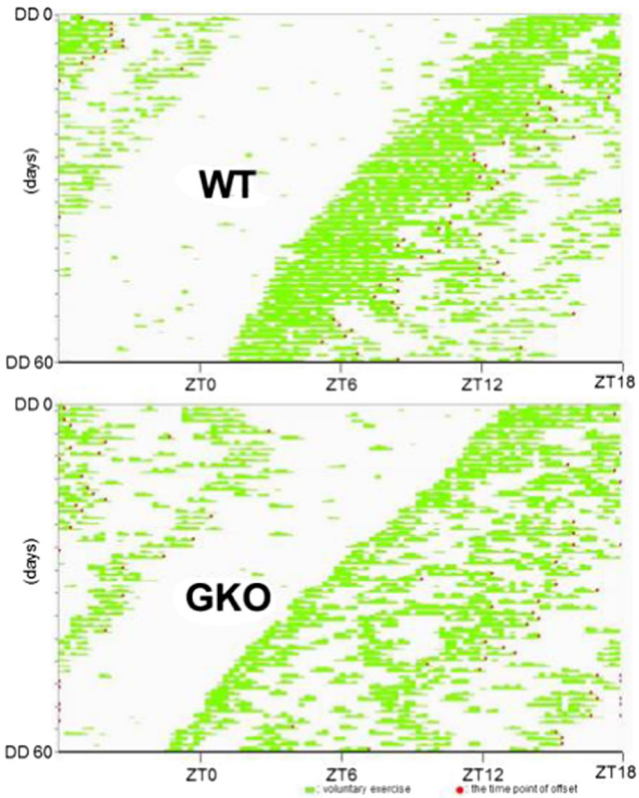


Figure. Actograms for voluntary exercise under constant darkness

Disclosure: **Y. Tajiri:** None.

465

Changes in mitochondrial morphology lead to disruption of circadian rhythms in skeletal muscle

L. Sardon Puig[1], B. Gabriel[1], N.J. Pillon[1], J. Smith[1], R. Sjögren[1], C. Cantó[2], A. Krook[1], J. Zierath[1];
[1]Karolinska Institutet, Stockholm, Sweden, [2]Nestlé Institute of Health Science, Lausanne, Switzerland.

Background and aims: Circadian rhythms modulate fundamental physiological processes and are mainly regulated by day-night cycles. However, most cells of the body also follow an endogenous clock. Disruption of circadian rhythms is associated with the development of metabolic disorders, but the pathways involved have not been fully elucidated. Mitochondrial morphology is directly linked to mitochondrial function, which include satisfying the metabolic demands of the cell and ensuring the removal of damaged organelles. For that reason, proper regulation of the fusion and fission process is required to maintain a functional reticular mitochondrial network. Mitofusin1 (Mfn1), Mitofusin2 (Mfn2) and Opa1 are key proteins in the outer and inner

mitochondrial membrane orchestrating fusion processes, and their ablation causes mitochondrial fragmentation that leads to impaired metabolism in skeletal muscle. Very little is known about the interactions between mitochondrial fragmentation and circadian rhythms and how metabolic challenges might regulate this interaction. We therefore hypothesized that impairment of mitochondrial fusion leads to the disruption of the muscle internal clock.

Materials and methods: Primary skeletal muscle cells from healthy (NGT) and type 2 diabetic (T2D) volunteers were grown and differentiated into myotubes. After synchronization by serum shock (50%FBS, 2h), cells were exposed to palmitate (0.4mM) and samples were collected every 6h up to 56h. RNA was extracted and used for RNA sequencing. Silencing of Mfn1, Mfn2 or Opa1 was performed to differentiated C2C12 and primary skeletal muscle cells using siRNA. Cells were synchronized and exposed to different nutrient challenges for up to 48h. Samples were collected every 6h for further analysis. RNA from tibialis anterior from Mfn2 muscle knock out (MKO) mice on low and high fat diet was extracted and clock gene expression analysed and compared to the expression of their littermates.

Results: Differential expression analysis comparing cells treated with palmitate and controls lead to the identification of pathways related to mitochondria and lipid metabolism suggesting a link between palmitate and mitochondrial fragmentation. Silencing of Mfn2 and Opa1 in C2C12 and primary human skeletal muscle cells lead to a disrupted rhythm of clock gene expression. Similar effect was observed in Mfn2 MKO mice which exhibited impaired clock gene expression compared to wildtype littermates. This effect was accentuated on a high fat diet and paralleled the effect of palmitate in muscle cells in vitro.

Conclusion: Our data suggest that nutritional challenges, such as palmitate, can induce mitochondrial dysfunctions that can lead to dysregulation of skeletal muscle internal clock and might provide a link between metabolic dysregulation in obesity and type 2 diabetes mellitus and impaired circadian rhythms.

Supported by: ITN - ChroMe

Disclosure: **L. Sardon Puig:** None.

PS 026 Healthy diet for a healthy rodent

466

Dietary methionine restriction protects from type 2 diabetes in NZO mice

T. Castaño-Martinez[1,2], W. Jonas[1,2], T. Laeger[1,2];
[1]Department of Experimental Diabetology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, [2]German Center for Diabetes Research (DZD), München-Neuherberg, Germany.

Background and aims: In general, diets low in protein or methionine have been shown to reduce the body weight by increasing energy expenditure and insulin sensitivity. Protein restriction prevented type 2 diabetes in diabetes-susceptible New Zealand obese (NZO) mice despite hyperphagia and increased fat mass. An elevated concentration of circulating FGF21 has been implicated as a potential underlying mechanism. Therefore, we tested in NZO mice whether a methionine restriction (MR) in a high-fat regimen protects against diabetes.

Materials and methods: After weaning, male NZO mice were placed on isocaloric high-fat diets (protein, 16 kcal%; carbohydrate, 52 kcal%; fat, 32 kcal%) that provided methionine at control (0.86%; CON) or low levels (0.17%; MR) for 9 weeks. Glucose homeostasis, energy expenditure, food intake and other metabolic endpoints were assessed. Six weeks after the diet switch, an oral glucose tolerance test (OGTT) was performed after a 6-hour period of fasting.

Results: Despite no differences in the ratio of body fat, lean mass, and in food intake between the groups, MR prevented the onset of hyperglycemia, whereas CON-fed mice exhibited a rise of blood glucose (blood glucose at week 9; mean ± SEM; CON = 15.5 ± 1.7 mM, MR = 7.4 ± 0.3 mM) which was associated with elevated plasma insulin concentrations (week 9; CON = 4.6 ± 1.3 μg/l, MR = 0.6 ± 0.1 μg/l). MR-fed mice did not improve glucose clearance during the OGTT but exhibited an increased insulin sensitivity (Matsuda index; CON = 16.8 ± 2.4, MR = 80.1 ± 14.6). At week 9, mice on MR showed an elevated hepatic FGF21 secretion (plasma FGF21; CON = 0.6 ± 0.1 ng/ml, MR = 8.7 ± 0.9 ng/ml), higher plasma adiponectin levels (CON = 23.0 ± 1.1 μg/ml, MR = 33.4 ± 2.0 μg/ml), and an increased energy expenditure (CON = 9.1 ± 0.5 kcal h^{-1} body weight kg^{-1}, MR = 12.5 ± 0.9 kcal h^{-1} body weight kg^{-1}) during both the dark and light periods not associated with an increase in locomotor activity. Interestingly, neither the final mass of subcutaneous white adipose tissue nor of brown adipose tissue was different between the groups. Final liver weight was lower in the MR-fed mice than in the CON-fed mice, due to decreased hepatic triacylglycerol and glycogen concentrations.

Conclusion: NZO mice are protected from hyperglycemia through methionine restriction, which elevates FGF21 secretion by the liver and increases adiponectin secretion by the adipose tissues. These effects might improve hepatic insulin sensitivity. A protective effect of methionine restriction is independent of food intake and adiposity.

Supported by: Deutsche Forschungsgemeinschaft (DFG): LA 3042/4-1

Disclosure: **T. Castaño-Martinez:** Grants; Deutsche Forschungsgemeinschaft: LA 3042/4-1.

467

Medium-chain triglyceride oil does not induce GIP secretion and induces less body weight and fat mass gain compared with long-chain triglyceride oil

Y. Murata, N. Harada, S. Yamane, K. Iwasaki, Y. Kanemaru, T. Harada, A. Sankoda, E. Ikeguchi, S. Kuwahara, E. Joo, H. Poudyal, N. Inagaki;
Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is an incretin secreted from enteroendocrine K-cells and potentiates

glucose-dependent insulin secretion from pancreatic β-cells. GIP plays an important role in maintaining postprandial blood glucose levels under long-chain triglyceride (LCT) diet-induced obesity. GIP also enhances LCT diet-induced obesity and insulin resistance. Medium-chain triglyceride (MCT) consists of medium chain fatty acids and long-term intake of MCT diet induces less body weight and fat mass gain than that of LCT diet. LCT oil and LCT diet strongly stimulate GIP secretion from K-cells but the effect of MCT oil and MCT diet on GIP secretion is unknown. In this study, we evaluated GIP secretion after MCT oil and MCT diet ingestion in wild-type (WT) mice and investigated the effect of long-term MCT diet-induced GIP secretion on body weight, fat mass, and glucose tolerance using WT and GIP-knockout (GIP KO) mice.
Materials and methods: Oral LCT (lard oil) and MCT oil tolerance tests were performed in WT mice and plasma total GIP levels were measured. 6-week old mice (WT and GIP KO mice) were divided into the three groups [Control fat (CF) diet (10% fat), LCT diet (45% fat), and MCT diet (45% fat)] and long-term food tolerance tests were performed. Non-fasting GIP levels and body weights were weekly measured during experiments. Oral glucose tolerance test (OGTT) and CT scan analysis were performed to evaluate glucose tolerance and fat mass, respectively.
Results: Single administration of LCT oil increased plasma GIP levels in WT mice but that of MCT oil did not. Long-term LCT diet feeding significantly increased non-fasting GIP levels compared with CF diet feeding in WT mice. There was no significant difference in GIP levels between MCT diet-fed and CF diet-fed WT mice. In GIP KO mice, non-fasting GIP levels were not detectable in the three diet groups. Body weight of LCT and MCT diet-fed WT mice was 47.0% and 10.7% higher than that of CF diet-fed WT mice after 24-week diet feeding, respectively. In GIP KO mice, body weight of LCT diet and MCT diet-fed mice was 28.1% and 7.5% higher than that of CF diet-fed mice, respectively. Visceral fat mass was 7.5 and 2.4-fold larger in LCT diet and MCT diet-fed WT mice than CF-diet fed WT mice, respectively. In GIP KO mice, the fat mass was 5.5-fold larger in LCT diet group than that in CF diet group but the mass was not significantly difference between MCT diet and CF diet groups. In WT mice, Area under the curve (AUC) of blood glucose (BG) during OGTT was not different among the three diet groups. AUC-insulin in LCT and MCT diet groups was higher compared with that in CF diet group. AUC-insulin in LCT diet group was the highest among the three diet groups. In GIP KO mice, AUC-insulin was significantly increased in LCT diet group than that in CF diet group but AUC-BG in LCT group was significantly higher than that in CF diet group. There was no statistical difference in AUC-BG and AUC-insulin between MCT diet and CF diet groups.
Conclusion: Long-term intake of LCT diet induced GIP hypersecretion but that of MCT diet did not. Therefore, GIP contributes to adiposity and preservation of postprandial glucose levels under long-term LCT diet feeding but the effect of GIP is smaller under MCT diet feeding.
Disclosure: **Y. Murata:** None.

468

High fat diet induces a blunted antihyperglycaemic effect of the neuropeptide 26RFa in mice

G. Prevost[1,2], J. Bons[2,1], A. Arabo[3], M. Picot[1], J. Maucotel[3], H. Berrahmoune[1,2], M. El Mehdi[1], S. Cherifi[1], M. Coeffier[4], J. Leprince[1], V. Brunel[5], P. Dechelotte[4], H. Lefebvre[1,2], Y. Anouar[1], N. Chartrel[1];
[1]Normandie Univ, UNIROUEN, INSERM U1239, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication (DC2N), Rouen, [2]Normandie Univ, UNIROUEN, Rouen University Hospital, Department of Endocrinology, Diabetes and Metabolic Diseases, Rouen, [3]Normandie Univ, UNIROUEN, Rouen, [4]Normandie Univ, UNIROUEN, INSERM U1073 "Nutrition, Inflammation and dysfunction of gut-brain axis", Rouen, [5]Normandie Univ, UNIROUEN, Rouen University Hospital, Department of Biochemistry, Rouen, France.

Background and aims: The neuropeptide 26RFa also referred to as QRFP (for pyroglutamilated RFamide peptide) is the latest member of the RFamide peptide family discovered. 26RFa was identified as the cognate ligands of the human orphan G-protein-coupled receptor, GPR103. 26RFa stimulates food intake via a modulation of the NPY/POMC network in the arcuate nucleus. Recently, we have also shown that 26RFa regulates glucose homeostasis by acting as an incretin. As type 2 diabetes is associated with a reduced incretin effect of the two other incretins GLP-1 and GIP, we have investigated in the present study whether an eventual dysfunction of the 26RFa incretin effect and/or production occurs in diabetic conditions.
Materials and methods: A model of high fat diet mice and human β cell line exposed to palmitate were used to analyze the 26RFA effect in diabetic conditions. Q-PCR and immunohistochemistry for GPR103 were also performed in the insulin target tissues. 26RFa blood level and tissue content were performed using a radioimmunoassay
Results: In high fat diet mice with diabetes , we first found a loss of the anti-hyperglycemic effect of 26RFa during IPGTT which is associated with a total loss of its insulinotropic activity. Moreover, we reported a marked reduction of the insulin-sensitive effect of the neuropeptide and during insulin tolererance test. Insulin secretion induced by the 26RFa neuropeptide is also completely blunted in human β cell line exposed to palmitate. Q-PCR and immunohistochemical experiments revealed a down-regulation of the 26RFa receptor in insulin target tissues of diabetic mice. Finally, our data show that the kinetic of release of 26RFa after an oral glucose challenge is profoundly altered in diabetic conditions both for plasma level and 26RFa intestine content.
Conclusion: the present data indicate that high fat diet in mice is associated with a significant reduction of the antihyperglycemic effect of the neuropeptide 26RFa.
Supported by: SFD
Disclosure: **G. Prevost:** None.

469

Murine lipid-induced hepatic insulin resistance is not pathway-selective

D.F. Vatner, L. Goedeke, X. Li, M.C. Petersen, J.C. Rogers, G. Casals, G.W. Cline, V.T. Samuel, G.I. Shulman;
Yale University School of Medicine, New Haven, USA.

Background and aims: Insulin suppresses hepatic glucose production and increases hepatic de novo lipogenesis (DNL). Paradoxically, hepatic DNL remains elevated in insulin-resistant subjects, leading to the hypothesis that hepatic insulin resistance is pathway-selective. Prior studies of DNL in hepatic insulin resistance are complicated by confounders, such as use of markedly unphysiologic animal models or comparison of animals on different diets and thus different availability of DNL precursors. We sought to determine the effect of lipid-induced hepatic insulin resistance *per se* on insulin-stimulated lipogenesis.
Materials and methods: We measured DNL using the deuterated water method in Insulin receptor T1150A knockin (*KI*) mice, a strain protected from diacylglycerol-mediated hepatic insulin resistance, and in littermate controls (WT). Diets were matched: 60% high fat diet (HFD) with 1% dextrose in drinking water.
Results: After two days of fat-feeding, before the development of significant insulin resistance, *KI* and WT mice displayed similar rates of DNL (*KI* 24.3% ± 3.6; WT 22.2% ± 3.5). After 9 days of HFD, when WT but not *KI* mice have developed hepatic insulin resistance, rates of DNL were reduced in WT mice but preserved in *KI* mice (*KI* 16.4% ± 2.4; WT 7.4% ± 1.3; $P < 0.01$). After 4 weeks of HFD, with the development of skeletal muscle resistance, rates of DNL increased in WT mice to reach those observed in *KI* mice (*KI* 19.3% ± 2.6; WT 15.9% ± 2.3). Consistent with the insulin-resistant phenotype, Sterol regulatory element-binding protein-1c (Srebp-1c) cleavage was reduced in the setting of hepatic insulin resistance in WT mice; this effect was attenuated in *KI* mice. The Srebp-regulated lipogenic proteins Fatty acid synthase and Stearoyl-CoA desaturase-1 were both reduced in 9d HFD fed livers regardless of

genotype; at 4 weeks, protein abundance remained suppressed in WT mice but was restored in *KI* mice. Pyruvate kinase L/R, a protein associated with DNL but not regulated by Srebp, was unaffected by HFD-induced hepatic insulin resistance.
Conclusion: Regulation of DNL is subject to lipid-induced hepatic insulin resistance, challenging the selective hepatic insulin resistance hypothesis. Increased DNL seen in insulin resistant subjects may be due to hyperinsulinemia and diversion of substrate from the insulin resistant periphery.
Supported by: NIH K23 DK-10287
Disclosure: **D.F. Vatner:** Grants; National Institutes of Health.

470

CD44 plays a key role in regulating high fat diet induced muscle insulin resistance

A. Hasib[1], C.K. Hennayake[1], D.P. Bracy[2], D.H. Wasserman[2], L. Kang[1]; [1]Division of Molecular and Clinical Medicine, School of medicine, University of Dundee, Dundee, UK, [2]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, USA.

Background and aims: Increased extracellular matrix (ECM) hyaluronan is associated with diet-induced insulin resistance (IR). Reduction of muscle hyaluronan by PEGPH20 (PEGylated human recombinant hyaluronidase-PH20) ameliorates IR in diet-induced obese mice. CD44, the main cell-surface hyaluronan receptor, is positively correlated with type 2 diabetes. This study determines the role of CD44 in skeletal muscle IR. The hypotheses that *1*) genetic deletion of CD44 improves diet induced skeletal muscle IR and *2*) improved IR by PEGPH20 treatment is dependent on the presence of CD44 were tested in the present study.
Materials and methods: Global CD44-deficient ($CD44^{-/-}$) mice and their wild-type littermates ($CD44^{+/+}$) were fed chow-diet or 60% high-fat-diet (HFD) for 16 weeks. HFD-fed $CD44^{-/-}$ mice were also treated with PEGPH20 (1 mg/kg) for 3 weeks to evaluate its CD44-dependent action. Insulin sensitivity was measured by hyperinsulinaemic-euglycaemic clamp.
Results: HFD feeding increased muscle CD44 protein expression in $CD44^{+/+}$ mice ($p = 0.001$). Male HFD-fed $CD44^{-/-}$ mice exhibited reduced diet-induced obesity, adiposity and fasting insulin levels compared to HFD-fed $CD44^{+/+}$ ($p < 0.05$,$p < 0.01$). During hyperinsulinaemic-euglycaemic clamp, glucose infusion rate (GIR), glucose disappearance rate (Rd) and glucose uptake in gastrocnemius (Rg) were similar between $CD44^{+/+}$ and $CD44^{-/-}$ mice, in both chow and HF-fed state. However, $CD44^{-/-}$ mice exhibited lower clamp insulin levels (6.8 ± 0.8 vs 4.6 ± 0.4, $p > 0.01$, chow-fed; 14.5 ± 1.8 vs 7.0 ± 1.0, $p > 0.001$, HFD-fed), suggesting that $CD44^{-/-}$ mice were responding to lower insulin for glucose disposal compared to $CD44^{+/+}$ mice. Therefore, the insulin sensitivity indices were normalized to insulin concentrations during clamp. After normalization, there was no notable change in whole body insulin sensitivity of chow-fed $CD44^{-/-}$ mice, however HFD-fed $CD44^{-/-}$ mice exhibited higher GIR {5.9 ± 1.2 vs 2.3 ± 0.3 [mg/kg/min/(ng/mL insulin)]; $p < 0.01$}, Rd {6.4 ± 1.0 vs 2.9 ± 0.3 [mg/kg/min/(ng/mL insulin)], $p < 0.01$} and gastrocnemius Rg {3.0 ± 0.8 vs 1.0 ± 0.2 [umol/100 g/min/(ng/mL insulin)]; $p < 0.05$} than HFD-fed $CD44^{+/+}$ mice, suggesting ameliorated systemic and muscle IR. Reduced muscle insulin resistance in HFD-fed $CD44^{-/-}$ mice was associated with increased gastrocnemius vascularization (CD31 protein expression: $1.8 \pm 0.0.2$ vs 1.1 ± 0.2, $p < 0.05$), rather than insulin signalling. PEGPH20 treatment in HFD-fed $CD44^{-/-}$ mice increased energy expenditure and decreased respiratory exchange ratio (RER) compared to vehicle-treated HFD-fed $CD44^{-/-}$ mice ($p < 0.05$), despite no changes in body weight, food intake, glucose tolerance, insulin tolerance or locomotor activity. The hyperinsulinaemic-euglycaemic clamp showed similar GIRs and muscle glucose uptake between vehicle- and PEGPH20-treated $CD44^{-/-}$ mice.
Conclusion: Global CD44 deletion ameliorates skeletal muscle IR possibly via increased vascularization and the beneficial effects of PEGPH20 are CD44-dependent. These results suggest a critical role of CD44 in promoting hyaluronan-mediated muscle IR, therefore representing a potential therapeutic target for diabetes.
Supported by: Diabetes UK, European Commission
Disclosure: **A. Hasib:** Grants; Diabetes UK, European Commission.

471

Long-term hypercaloric diet consumption exacerbates age-induced metabolic dysfunction: beneficial effects of CSN denervation

J.F. Sacramento, B.F. Melo, C.S. Prego, S.V. Conde;
CEDOC, NOVA Medical School|Faculdade de Ciências Médicas, Lisbon, Portugal.

Background and aims: We have previously described that carotid body (CB), a peripheral O_2 sensor, that controls energy homeostasis, is involved in the genesis of insulin resistance and glucose intolerance, since the abolishment of its activity restores these features in young animal models of prediabetes and type 2 diabetes. Knowing that ageing itself is associated with deregulation of glucose homeostasis, we have evaluated the long-term intake of hypercaloric diet on age-induced metabolic dysfunction. Additionally, the beneficial effects of carotid sinus nerve (CSN), the CB sensitive nerve, resection on metabolic dysfunction were evaluated. Finally, and since we have previously described that metabolic dysfunction is associated with CB overactivation, we tested CB function in hypercaloric aged animals.
Materials and methods: Two groups of Wistar rats (8–10 weeks) were used: the control group fed a sham diet and the high-fat high-sucrose (HFHSu) group fed a 60% lipid-rich diet with 35% sucrose in drinking water. The groups were randomly divided and submitted to 14, 25 and 44 weeks of respective diets. In this last group when the animals reached 1 year, they were submitted to CSN resection or to a sham surgery. Follow-up of the animals was made until 9 weeks post-surgery. Insulin sensitivity was evaluated through an insulin tolerance test and glucose tolerance by an OGTT. Fasting glycemia, insulinemia and ventilatory responses to hypoxia were also monitored. At a terminal experiment, blood pressure and sympathetic nervous system activity were evaluated and fat depots were analyzed. CB dysfunction was evaluated thought the morphometric analysis of tyrosine hydroxylase (TH) and GFAP in CBs.
Results: Age decreased insulin sensitivity in control animals by 59.11% (K_{ITT} CTL = 4.50 ± 0.36%glucose/min). HFHSu diet during 14, 25 and 44 weeks decreased insulin sensitivity by 55.98, 51.39 and 58.30%, respectively (K_{ITT} HFHSu before diet = 4.13 ± 0.19%glucose/min). CSN resection completely restored insulin sensitivity in control and HFHSu old animals, an effect that was maintained until 9 weeks after surgery. HFHSu diet decreased glucose tolerance, an effect that was exacerbated in HFHSu old animals (AUC HFHSu 14 weeks diet = 23803 ± 586 mg/dl*min; AUC HFHSu 44 weeks diet = 27449 ± 1265 mg/dl*min ($p < 0.05$)). In contrast, age did not alter glucose tolerance in control animals. CSN resection in HFHSu old animals decreased glucose intolerance by 13.23% (AUC HFHSu 9 weeks after CSN resection = 24392 ± 558 mg/dl*min). The increase in fat depots was proportional to the period of time that animals underwent HFHSu consumption. In contrast, in the control animals, only perienteric fat increased with age (CTL = 10.14 ± 0.76 g/kg; CTL Old = 19.82 ± 3.58 g/kg). CSN resection in HFHSu old animals did not modify fat depots. The expression of TH in the CB increased with HFHSu diet by 82.55% and, in contrast, was decreased in control old animals.
Conclusion: Ageing induces insulin resistance in rats and long-term hypercaloric consumption in old animals did not modify age-induced insulin resistance, while exacerbates glucose intolerance. Moreover, CSN resection in old animals restored insulin sensitivity and ameliorates glucose intolerance, effects that were not mediated by amelioration of fat deposition. Our results suggest that modulation of CB activity might be also important in age induced insulin resistance as well as in age-induced metabolic dysfunction exacerbated by diet.

Supported by: JFS and BFM with PhD grants: PD/BD/105890/2014 and PD/BD/128336/2017
Disclosure: **J.F. Sacramento:** None.

472

GIP-xenin hybrid peptide enhances the metabolic benefits of exenatide in high fat fed and db/db diabetic mice

V.A. Gault, A. Hasib, M. Ng, R.A. Perry, P.R. Flatt, N. Irwin;
Ulster University, Coleraine, UK.

Background and aims: Metabolic and body weight benefits of GLP-1 mimetics, such as exenatide, in type 2 diabetes are not as impressive as first predicted from preclinical studies. Thus, methods to augment antidiabetic effects of GLP-1 compounds are highly sought after and therapeutically relevant. In the present study we evaluated therapeutic utility of combined treatment with exenatide and a recently characterised GIP-xenin hybrid peptide, namely (DAla^2)GIP-xenin-8-Gln (GIP-XEN), in high fat fed (HFF) and *db/db* diabetic mice. This combined treatment approach should lead to positive modulation of both arms of the incretin axis with improved metabolic effects.
Materials and methods: Groups of mice ($n = 8$) received twice-daily injections (09:30 and 17:30 hr) of saline vehicle, GIP-XEN, exenatide or a combination of both peptides (each at 25 nmol/kg bw; *ip*) for 32 days in HFF mice and 30 days in *db/db* mice. Energy intake, body weight, circulating glucose and insulin concentrations were measured at regular intervals. At the end of each study, glucose tolerance (18 mmol/kg bw; *ip*), biological response to GIP (25 nmol/kg; *ip*) in combination with glucose and peripheral insulin sensitivity (10 U/kg in HFF mice and 30 U/kg in *db/db* mice; *ip*) were examined. In addition, pancreatic islets were isolated at the end of the treatment period in both HFF and *db/db* mice by collagenase digestion, and effects of various secretagogues on insulin secretion determined.
Results: Twice-daily administration of GIP-XEN, exenatide or combination of both peptides had no impact on energy intake or body weight in HFF mice, although all exenatide treated *db/db* mice had reduced ($P < 0.001$) energy intake compared to saline controls by the end of the study. Circulating glucose concentrations and HbA1c values were significantly ($P < 0.05$-$P < 0.001$) decreased and insulin levels increased by treatment with GIP-XEN in combination with exenatide in both HFF and *db/db* mice, compared to respective control mice. Oral glucose tolerance ($P < 0.05$) was also improved by combined GIP-XEN and exenatide treatment in both diabetic mouse models, and insulin sensitivity was substantially enhanced ($P < 0.001$) in HFF mice. Circulating and glucose-stimulated insulin concentrations were elevated ($P < 0.05$) in mice treated with the GIP-XEN and exenatide combination when compared to either treatment alone. Interestingly, GIP-mediated glucose-lowering effects were improved ($P < 0.05$) by treatment with GIP-XEN in combination with exenatide in HFF, but not *db/db* mice. None of the treatment interventions augmented GIP-induced insulin secretion in either mouse model. Pancreatic islets isolated from HFF mice did not display any differences in glucose-stimulated insulin secretion between control and treatment groups, however mice treated with GIP-XEN in combination with exenatide had augmented ($P < 0.01$–$P < 0.001$) insulin secretory responsiveness to Ca^{2+}, IBMX, KCl and PMA.
Conclusion: Prolonged twice-daily treatment with GIP-XEN and exenatide induced an impressive profile of beneficial metabolic effects in both HFF and *db/db* diabetic mice, including improvements of glucose tolerance, augmentation of the glucose-lowering action of GIP and overall beta-cell responsiveness. These data highlight the potential to enhance the antidiabetic properties of clinically approved GLP-1 mimetics, especially with hybrid peptides such as GIP-XEN that merits further clinical consideration.
Supported by: Invest Northern Ireland Proof of Concept Funding
Disclosure: **V.A. Gault:** None.

PS 027 Pinpointing pancreatic performance

473

Postnatal loss of pancreatic beta cell insulin receptor affects insulin secretion observed under long-term high-fat diet

A. Oakie[1,2], L. Zhou[1], S. Rivers[1], C. Cheung[1], J. Li[1,3], R. Wang[1,3];
[1]Children's Health Research Institute, London, [2]Department of Pathology and Laboratory Medicine, University of Western Ontario, London, [3]Department of Physiology and Pharmacology, University of Western Ontario, London, Canada.

Background and aims: The presence of insulin receptor (IR) on insulin-secreting beta cells is required for proliferation, survival, and up-regulation of insulin production, and its expression suggests an autocrine role in insulin signalling. Congenital beta cell -specific *IR* knockout (βIRKO) mouse studies have demonstrated the development of age-dependent glucose intolerance and decreased insulin secretion. However, it is not known if the loss of beta cell IR signalling postnatally interferes with insulin release. In this study, we examined the effects of reduced postnatal IR signalling on maintaining insulin release under normal and high-fat diet feeding.
Materials and methods: We utilized a tamoxifen-inducible *mouse insulin 1* promoter driven Cre-recombinase *IR* knockout mouse model (MIP-βIRKO) to determine the functional role of beta-cell IR in adult mice. Knockdown of IR was induced in postnatal male mice at 4 weeks of age, and mice were placed on either a chow diet or a high fat diet (HFD, 60% kcal) for an 18-week period at 6 weeks of age. Metabolic tests for glucose tolerance and insulin secretion were completed in both diet groups. Histological analyses of islets were performed to examine islet morphology and expression of beta cell transcription factors and proteins involved in glucose-induced insulin release (SNAREs). The INS-1 832/13 cell line was used to examine *in vitro* effects of insulin stimulation (100 nM for 1 hour) on SNARE proteins.
Results: Using western blot analysis, we demonstrated an approximately 50% reduction of IR protein level in isolated MIP-βIRKO islets in comparison to control groups. MIP-βIRKO mice that were fed chow diet for 18 weeks did not develop a change in glucose tolerance and had normal islet morphology when compared to control litter-mates. However, HFD MIP-βIRKO mice demonstrated significantly impaired glucose tolerance compared to HFD control mice. HFD MIP-βIRKO mice showed reduced insulin release with *in vivo* GSIS, and *ex vivo* GSIS of HFD MIP-βIRKO islets identified decreased insulin secretion at low (2.2 mM) glucose levels when compared to HFD control mice. Histological analyses showed no change in beta cell mass or proliferation between HFD control or MIP-βIRKO mice, yet decreased Glut2 and pAkt^{S473} levels were found in HFD MIP-βIRKO mice. The examination of the exocytotic SNARE proteins revealed that Munc18-1, Snap25, and Vamp2 were down-regulated in HFD MIP-βIRKO mouse islets. Preliminary INS-1 cell studies demonstrated that 100 nM insulin induced increased Snap25 with no change in other SNARE proteins.
Conclusion: The results from this study demonstrate that IR knockdown in adult beta cells affects insulin release under HFD stress due to the decreased expression of proteins involved in glucose-sensing and insulin exocytosis. This data indicates that beta cell autocrine IR signalling is required to maintain sufficient insulin secretion against hyperglycemic stress and that the IR-insulin signalling pathway may be directly linked to the regulation of exocytotic proteins.
Supported by: Canadian Institute of Health Research
Disclosure: **A. Oakie:** None.

474

Corticotropin-releasing hormone: a novel signal for regulating the islet adaptation to pregnancy

S.J.S. Simpson, L.I.F. Smith, T.G. Hill, P.M. Jones, J.E. Bowe; Department of Diabetes, King's College London, London, UK.

Background and aims: Corticotropin releasing hormone (CRH) is the neuroendocrine hormone released from the hypothalamus into the pituitary portal system to regulate adrenal glucocorticoid production via the pituitary. It is generally present at trace levels in the periphery. Despite this lack of CRH in the circulation, both CRH and CRH receptors are present on mouse and human islets. Furthermore, CRH treatment directly affects islet function and has been shown to improve islet transplantation outcomes. However the physiological relevance of CRH action on the islets is currently unclear. Whilst circulating CRH is generally low, during gestation maternal levels rise exponentially due to placental release, which correlates with the islet adaptation necessary to overcome maternal insulin resistance. This study therefore examined the effects of CRH as a potential signal involved in the islet adaptation to pregnancy.
Materials and methods: Expression of CRH and both CRH receptors (CRHR1/2) was confirmed in isolated CD1 mouse islets via qPCR. Effects of exogenous CRH (50 nM) on insulin secretion was assessed using both a dynamic perifusion system and static incubations, with insulin release measured by radioimmunoassay. Pregnant mice were implanted subcutaneously with osmotic minipumps containing saline or CRH receptor antagonist (α helical $CRH_{9\text{-}41}$) to test the effects of endogenous CRH during pregnancy. Intraperitoneal glucose tolerance tests (IPGTT) and insulin tolerance tests (IPITT) were performed in these animals at day G.16 and G.18 respectively. Statistical significance was determined by students T-test or two way ANOVA followed by Sidak's multiple comparisons as appropriate.
Results: Male and female islets express CRH, CRHR1 and CRHR2 mRNA (female CRHR1: 0.007 ± 0.003 vs CRHR2: 0.001 ± 0.001, male CRHR1: 0.003 ± 0.0004 vs CRHR2: 0.0008 ± 0.0004 mRNA expression relative to GAPDH). In perifusion experiments 50 nM CRH potentiated glucose stimulated insulin secretion (GSIS) in male and female islets by 264 ± 79% and 75 ± 22% respectively compared to 20 mM glucose alone, $p < 0.001$). In static incubations CRH caused a similar potentiation of GSIS (0.20 ± 0.04 vs 0.64 ± 0.15 ng $islet^{-1}$ h^{-1}; 20 mM glucose vs 20 mM glucose +50 nM CRH; $n = 7$ observations). The stimulatory effect of CRH was inhibited by the addition of both CRHR1 (Antalarmin) and CRHR2 (Astressin $_2$B) specific antagonists respectively (20 mM glucose +50 nM CRH: 0.64 ± 0.15; 20 mM glucose +50 nM CRH + 1 μM Antalarmin: 0.22 ± 0.03; 20 mM glucose +50 nM CRH + 1 μM $Astressin_2B$: 0.51 ± 0.01 ng $islet^{-1}$ h^{-1}; $n = 7$ observations). Pharmacological blockade of both CRHR1 and R2 *in vivo* during pregnancy resulted in significantly impaired glucose tolerance ($p = 0.0078$; control vs α-helical $CRH_{9\text{-}41}$; $n = 8$–9), which was most pronounced at 15 minutes post glucose (control: 12.98 ± 1.24 vs α-helical $CRH_{9\text{-}41}$: 16.83 ± 1.60 mM; $p = 0.0017$; $n = 8$–9). Insulin sensitivity was unaffected by α-helical $CRH_{9\text{-}41}$ treatment (AUC, control: 359 ± 21 vs α-helical $CRH_{9\text{-}41}$: 395 ± 21; $p = 0.237$; $n = 8$–9).
Conclusion: Both CRHR subtypes are present in mouse islets. Stimulation with CRH results in significant potentiation of GSIS, an effect which appears to be primarily mediated by CRHR1 although CRHR2 may also play a role. Pharmacological blockade of endogenous CRH, through inhibition of both CRH receptors, impairs glucose tolerance but not insulin sensitivity. These results are consistent with a novel physiological role for CRH in regulating the islet adaptation to pregnancy.
Supported by: KCL MRC DTP
Disclosure: **S.J.S. Simpson:** None.

475

Effect of sustained hyperglycaemia on insulin sensitivity and beta cell function in normal glucose tolerant subjects with and without family history of type 2 diabetes

D. Tripathy[1], A. Merovci[2], X. Chen[2], E. Maldonado-Corchado[2], R. DeFronzo[2];
[1]Medicine/Diabetes, South Texas Veteran's Health Care System, San Antonio, [2]Medicine/Diabetes, The University of Texas Health Science Center at San Antonio, San Antonio, USA.

Background and aims: Short-term hyperglycemia induces insulin resistance and beta cell dysfunction in rodents. However the effect of chronic physiological hyperglycemia on beta cell function in humans is not clear. The aim of the present study was to evaluate effect of a sustained (72 hr) physiologic increase in plasma glucose concentration on insulin secretion in healthy NGT individuals with (FH+) and without (FH-) a family history of T2DM.
Materials and methods: 20 NGT subjects: 12 without family history of T2DM (FH-), (9M/3F, age = 50 ± 4 yrs, BMI = 27 ± 1 kg/m^2) and 8 with FH (FH+) (4M/4F, Age = 48 ± 3 yrs, BMI = 26 ± 1 kg/m^2) participated in a OGTT and 2 step hyperglycemic (+125 and + 300 mg/dl) clamp followed by IV arginine (5 g) bolus clamp before and after a 72 hour glucose infusion to increase plasma glucose conc by ~50 mg/dl above baseline. The acute insulin response ($AIR_{0\text{-}10}$min), 2nd phase (SP) insulin secretion ($SPIS_{10\text{-}80}$ and $SPIS_{90\text{-}160}$ minutes) responses during each hyperglycemic step and following arginine (AIR_{Arg}) were assessed. Insulin sensitivity was assessed as the glucose infusion rate/steady state plasma insulin (M/I) during the hyperglycemic clamp.
Results: There was no difference in FPG, 2-h Glucose, fasting, and 2-h insulin concentrations between FH+ and FH- subjects. The FPG increased from 97 ± 4.5 to 140 ± 4.9 mg/dl during 72 hours of glucose infusion. The first phase insulin secretion (0–10 min) increased by 80% and 60% respectively in FH- and FH- subjects, while the second phase insulin secretion (10–80 min) increased by 3 fold (>300%) in both FH- and FH+ subjects respectively following chronic glucose infusion. The insulin sensitivity M/I declined in FH- (21 ± 5 to 8.1 ± 2.03, mg/kg.MU.l, $p = 0.02$) and in FH+ (16 ± 3 to 7 ± 1, mg/kg.MU.l, $p = 0.03$) following chronic glucose infusion. The insulin secretion/insulin resistance (disposition index, $ACR_{0\text{-}10}$ X M/I) did not significantly change in FH+ or FH- subjects before and after sustained hyperglycemia. However the disposition index ($SPIR_{90\text{-}160}$ X M/I), was reduced in FH- (9591 ± 1941 to 4198 ± 938, $p = 0.02$) and FH+ (6775 ± 1280 vs 2397 ± 640, $p = 0.01$) following chronic glucose infusion.
Conclusion: These results demonstrate that sustained physiologic hyperglycemia (i) impairs insulin sensitivity, (ii) increases absolute insulin secretion (iii) only impairs second phase insulin secretion. This suggests that healthy individuals are able to compensate for insulin resistance induced by short-term glucotoxicity.
Supported by: NIH
Disclosure: **D. Tripathy:** None.

476

The increment of noradrenergic fibers correlates with the density of dedifferentiated beta cells and impairs beta cell function in humans

F. Cinti[1], T. Mezza[1], I. Severi[2], M. Suleiman[3], C. Cefalo[1], G. Sorice[1], S. Moffa[1], F. Impronta[1], S. Alfieri[4], A. Pontecorvi[1], L. Marselli[3], S. Cinti[2], P. Marchetti[3], A. Giaccari[1], D. Accili[5];
[1]Center for Endocrine and Metabolic Diseases, Fondazione Policlinico Universitario A.Gemelli, Catholic University of Sacred Heart, Rome, Italy, [2]Department of Experimental and Clinical Medicine, Center of Obesity, University of Ancona (Politecnica delle Marche), Ancona, Italy, [3]Department of Experimental and Clinical Medicine, University of Pisa,, Pisa, Italy, [4]Digestive Surgery, Fondazione Policlinico Universitario A.Gemelli, Catholic University of Sacred Heart, Rome, Italy, Rome, Italy, [5]Department of Medicine, Naomi Berrie Diabetes Center, Columbia University, New York City, USA.

Background and aims: β cell dedifferentiation has recently been indicated as the main mechanism responsible for the functional "disappearance" of β cells from the islets of diabetic individuals. Given the results obtained in a mouse model of type 2 diabetes (T2D), which found a

significant increase in the density of noradrenergic fibers in diabetic compared to non-diabetic mice, we attempted to replicate the study in humans, examining the possible involvement of noradrenergic fibers in the process of dedifferentiation.

Materials and methods: We analyzed human pancreas samples from organ donors (8 non diabetic and 9 with type 2 diabetes) and 11 patients undergoing pancreatoduodenectomy, who previously underwent an Oral Glucose Tolerance Test [Normal Glucose Tolerance - NGT (5), Impaired Glucose Tolerance - IGT (3) and T2D (3)] and a 2h hyperglycemic clamp. The dedifferentiation score was calculated as the percentage of synaptophysin positive cells negative for the four major pancreatic hormones. Tyrosine hydroxylase (TH) was used as a marker for the evaluation of noradrenergic fiber expression. Transmission electron microscopy was performed to localize fibers inside the islet. β cell glucose sensitivity (GS) was calculated as the ratio of insulin secretion and glucose increments, during the hyperglycemic clamp.

Results: The islets of diabetic subjects were about 3 times more innervated than controls (0.32 ± 0.12 vs. 0.90 ± 0.21 n.fibersTH+/islet; $p < 0.01$), with a stepwise increase from NGT-IGT to T2D; the increase of these fibers correlated positively with the dedifferentiation score ($p < 0.001$; $r = 0.69$). Moreover, the increase of noradrenergic fibers negatively correlated with GS, supporting the possibility of their functional role ($p = 0.018$, $R = -0.84$).

Conclusion: In conclusion, our data show an increase in the number of sympathetic nerve fibers, potentially able to transmit inhibitory signals on insulin secretion in the islets of diabetic subjects. The important correlation with the glucose sensitivity and dedifferentiation score suggests a significant role of noradrenergic fibers in the dedifferentiation process and β cell dysfunction, introducing the possibility of new future strategies for the preservation of cellular mass/secretion and, therefore, for the prevention and/or treatment of diabetes.

Disclosure: **F. Cinti:** None.

477

The role and mechanism of TXNIP in aging related proliferation and secretion dysfunction of pancreatic beta cells

Y. Li, D. Liu, G. Yang;
Department of Endocrinology, The Second Affiliated Hospital Chongqing Medical University, Chongqing, China.

Background and aims: Thoredoxin interacting protein (TXNIP) is the interacting protein of Thioredoxin, that can reduce insulin secretion of pancreatic β cells. Thioredoxin was confirmed as an anti-aging gene, but there is little known about the relationship between TXNIP and pancreatic β cells aging. The present study was designed to explore it.

Materials and methods: Three aged-periods (youth, mid-aged, elderly) human normal pancreas tissue and serum were collected, the expression of TXNIP protein was measured by Immunofluorescence and ELISA. 4-week-old C57BL/6J mice($n = 48$) and TXNIP$^{-/-}$ mice($n = 48$) were randomly assigned into 2 groups respectively, fed on a normal chow diet(NC) or a high fat diet(HFD) separately, each group was fed for four periods (3 months, 9 months, 12 months, 18 months). At each period of all groups , GTT, ITT, serum insulin and basic physiological metabolic indexes were measured; the mass of pancreatic β-cell was counted; the expression of TXNIP and aging marker protein (p21, p16) of pancreatic β cells were measured by Immunofluorescence and Western blot. Proliferation capacity of pancreatic β-cell was assessed by ki67 expression in insulin+ cells from 3 months mice. Ultrastructural changes of pancreatic β-cell were visualized and compared by electron microscopy in 12 months mice. Meanwhile, primary pancreatic β-cell were isolated and cultured from 3 and 18 months mice. Ex vivo glucose-insulin secretion level of primary pancreatic β-cell incubated in medium containing 2.8mM or 16.7mM glucose was measured by ELISA. The expression of TXNIP, p21, p16 of primary pancreatic β-cell were also measured by Immunofluorescence and Western blot.

Results: The expression of TXNIP protein was gradually increased with aging in the human normal pancreatic β-cells and serum. Compared with the age-diet-matched C57 groups, the FBG was lower, the insulin secretory function was better, and the insulin resistance was milder, among each TXNIP$^{-/-}$ groups, significantly. The serum TC,LDL were lower, insulin and HDL were higher in TXNIP$^{-/-}$ groups, compared with the age-diet-matched C57 mice. Among 3,9,12,18 months period in C57 NC/HFD groups, the mass of pancreatic β-cell was down-regulated and the protein expression of TXNIP, p16, p21of pancreatic β-cells were up-regulated with aging. Compared with the age-matched C57 NC group, the mass of pancreatic β-cell was down-regulated and the expression of TXNIP was up-regulated in 9, 12, 18 months C57 HFD groups. The mass of pancreatic β-cell were larger and the expression of protein p16, p21 were lower obviously in 9, 12, 18 months TXNIP$^{-/-}$ groups than those in the age-diet-matched C57 mice. Meanwhile, proliferation capacity of pancreatic β-cell was better in 3-month-old TXNIP$^{-/-}$ group than that in the age-diet-matched C57 groups. Mitochondrial and the endoplasmic reticulum became more swell, mitochondria decreased more seriously, in 12-month-old C57 HFD group as compared with 12-month-old TXNIP$^{-/-}$ HFD group. In TXNIP$^{-/-}$ group, ex vivo glucose-insulin secretion level was better and the protein expression of p16, p21 were lower in 18-months-old primary pancreatic β-cell, as compared with the age-diet-matched C57 group.

Conclusion: TXNIP could be a pro-aging gene of pancreatic beta cells, aggravates the aging-induced proliferation and secretion dis-function of pancreatic beta cells, meanwhile accelerates metabolic disorders.

Supported by: NSFC (81370467)

Disclosure: **Y. Li:** None.

478

Mitochondrial respiratory chain complex IV dysfunction and beta cell failure is associated with a novel mutation in the mitochondrial assembly factor NDUFAF5

S. Weksler-Zangen[1], A. Kogot-Levin[1], G. Aharon Hananel[1], E. Gurgul-Convey[2], I. Raz[1], A. Saada[3];
[1]Diabetes Unit, Hadassah University Hospital, Jerusalem, Israel, [2]Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany, [3]Monique and Jaques Roboh Department of Genetic Research and of Genetic and Metabolic diseases, Hadassah University Hospital, Jerusalem, Israel.

Background and aims: Mitochondrial respiratory chain (MRC) function is a major determinant of insulin secretion from pancreatic β-cells. The Cohen diabetic sensitive (CDs) rat is a unique model of cytochrome c oxidase (COX, MRC, complex-IV) deficiency, developing hyperglycemia when fed a diabetogenic high sucrose, copper-deficient diet (DD). Copper is a key-element for the catalytic activity of complex-IV. Yet, the precise mechanisms leading to mitochondrial dysfunction are unknown. A mutation in NDUFAF5, the gene encoding MRC complex I assembly factor 5 was associated with MRC complex I & IV deficiencies in Leigh-syndrome patients. However, the role of NDUFAF5 in β-cell function is not yet defined. Aim: We examined the role of COX and NDUFAF5 in β-cell failure.

Materials and methods: Blood glucose and insulin concentrations were measured before and during oral glucose tolerance tests performed at different periods on DD or copper supplemented DD. Islets complex I and complex IV activity were measured spectrophotometrically. Whole genome sequencing was performed in blood-DNA using Illumina HiSeq2500 followed by in-silico variant-analysis. The effect of NDUFAF5 gene silencing (siRNA) on glucose stimulated insulin secretion (GSIS) was assessed in rat (INS1E) and human (EndoCβH1) β cells.

Results: Complex IV & I activity were significantly reduced in CDs rat islets as compared to control rat islets ($P < 0.01$). A highly significant positive correlation between COX activity and GSIS and an inverse correlation with blood-glucose-levels ($R^2 = 0.984$ and $R^2 = -0.915$, $P <$

0.0001 respectively) were found in islets of CDs rats fed DD or copper supplemented-DD. Whole-genome-sequencing identified a *novel* homozygous missense variant, p.P318L (c.C1002T), in the NDUFAF5 gene predicted to be pathogenic by in-silico analysis. NDUFAF5 protein levels were significantly decreased in CDs rat islets vs. control rat islets. siRNA suppression of NDUFAF5 in rat INS1E and human EndoCβH1 β-cells reduced GSIS by ~20%.

Conclusion: We demonstrate a tight correlation between impaired mitochondrial function and β-cell dysfunction in CDs rats. NDUFAF5 may play an important role in the CDs mitochondrial defect and genetic susceptibility to develop diabetes. Our data emphasize a crucial role for mitochondria defect in the pathogenetic processes culminating in type 2 diabetes.

Supported by: Cohen fund, Ministry for Science and Culfure of Lower Saxony

Disclosure: **S. Weksler-Zangen:** None.

479

The increase in calcium by arginine, but not by strong potassium depolarisation is different between pancreatic alpha and beta cells

E.-H. Frueh, I. Rustenbeck;
University of Braunschweig, Braunschweig, Germany.

Background and aims: Pancreatic alpha-cells release their peptide hormone by depolarization-triggered exocytosis. Since alpha-cells express glucokinase and K_{ATP} channels, one might expect that the increase but not the decrease of glucose stimulates glucagon secretion. To explain the inverse glucose dependence of glucagon secretion it has been suggested that the alpha cell Ca^{2+} channels desensitize at strong depolarization, thus abrogating stimulated Ca2+ influx. Here, we have compared the effect of depolarizing stimuli on alpha- and beta-cells.

Materials and methods: Insulin and glucagon were determined by ELISA (Mercodia) from the fractionated efflux of batch-perifused NMRI mouse islets. Alpha-cells were isolated from such islets by incubation with alloxan for 10 min. Thereafter, these islets were dissociated by incubation in Ca2+ free solution. The surviving cells were cultured for 24 h in RPMI 1640 with 5 mM glucose. The same procedure without alloxan treatment was used to isolate beta cells. The cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by microfluorometry of Fluo-4. The plasma membrane potential was measured by patch-clamping using the perforated patch mode.

Results: Decreasing the glucose concentration from 10 to 1 mM led to a transient increase of glucagon secretion before the insulin secretion was diminished. Raising subsequently the glucose concentration from 1 to 30 mM increased the insulin secretion before the glucagon secretion was diminished. 1 mM glucose is thus a glucose concentration which is compatible with stimulated glucagon secretion. In the presence of 1 mM glucose all of the non-beta cells (survivors of alloxan treatment) reacted to 20 mM arginine by increasing $[Ca^{2+}]_i$, but only 50% did so in response to 1 mM glutamate or 1 mM glutamine. All cells which responded to glutamate also increased $[Ca^{2+}]_i$, in response to KCl. The strongly depolarizing concentration of 40 mM KCl was more effective than the moderately depolarizing concentration of 15 mM KCl. The response pattern caused by 40 mM KCl showed the typical fast increase followed by a decrease to moderately elevated plateau. 20 mM arginine caused a similarly strong, but more sustained elevation of $[Ca^{2+}]_i$, clearly higher than the beta-cell response to arginine. All non-beta cells failed to increase $[Ca^{2+}]_i$ in response to a maximally effective concentration of tolbutamide (500 μM). 15 mM KCl depolarized alpha-cells (defined as those, which survived alloxan treatment and responded to arginine and glutamate) by 14 mV, 40 mM KCl depolarized them by 34 mV. With beta-cells the depolarizing effect of 15 mM KCl was 21 mV and that of 40 mM KCl was 40 mV.

Conclusion: The clear concentration dependence of the KCl-induced $[Ca^{2+}]_i$, increase argues against a desensitization of Ca2+ influx as the underlying mechanism of the inverse glucose dependence of glucagon secretion. This view is supported by the sustained elevation of $[Ca^{2+}]_i$, in response to 20 mM arginine.

Disclosure: **E. Frueh:** None.

480

Endocytosis occurs right after fusion pore open and plays crucial roles in granule collapse

W. Ma[1], J. Tong[1], J. Chang[2], P. Thorn[1];
[1]Charles Perkins Centre, University of Sydney, Sydney, [2]University of Queensland, Brisbane, Australia.

Background and aims: Exocytosis is the process of granule fusion and release of granule content. Current models propose that, coincident with loss of content, the granule collapses into the cell membrane. This is then followed by the recruitment of endocytic machinery, such as clathrin, to the cell membrane and the endocytic recovery of the granule membrane and membrane proteins. Recent studies question this temporal sequence with evidence that endocytosis occurs much more rapidly after exocytosis than has been thought. However, the time course of recruitment of endocytic machinery and their roles remain unknown. Our study aims to find out when the endocytic machinery is recruited to the fused granule and whether they play any role in the granule fusion process.

Materials and methods: 2-Photon microscopy was used to image the exocytosis of insulin granules in β-cells. SRB extracellular dye was used to label fused granules. Primary β-cells were prepared from Lifeact-GFP mice to study the actin remodeling during granule fusion. Primary β-cells from WT mice were infected with Proinsulin-GFP adenovirus to study the content release during secretion. Stable Min6 cell-lines were generated by infecting with lentivirus expressing Clathrin-GFP, Arp3-GFP and Dynamin-GFP to trace the corresponding proteins' translocalization. Drugs including Arp3 inhibitor CK666 and CK689 (control); Clathrin inhibitor Pitstop2 and Dynamin inhibitor Dyngo 4a were utilized to study the effect on granule fusion dynamics.

Results: In our study of pancreatic β-cells, it is observed during glucose stimulated insulin secretion, that insulin granules are emptied immediately after the fusion pore open while the granule still holds its structure. Actin remodeling was observed by 2-photon microscopy on the site of fusion. Confocal microscopy showed that actin was coated around the fusing granule. Clathrin, Arp2/3, then Dynamin are recruited to the site of fusion immediately after the opening of the fusion pore. This process drives actin polymerization at the site of fusion. Inhibition of Clathrin, Arp2/3 and dynamin with drugs significantly altered the dynamics of granule fusion and affected actin remodeling. Blocking Arp2/3 by CK666 abolished the actin coating and greatly slowed down or paused the progress of granule collapse.

Conclusion: Our data shows that Insulin was released immediately after the pore opened, which indicates decrease in granule volume is not necessary for loss of content. Endocytic machinery recruit to the site of fusion right after the fusion starts. Interference with the endocytic proteins' function significantly altered the granule fusion dynamics. This results indicates that the vesicle components and membrane start to pinch off during the fusion process by endocytosis, which is responsible for the granule collapse.

Supported by: NHMRC, SREI Sydney University, Diabetes Australia, JDRF

Disclosure: **W. Ma:** None.

PS 028 Balancing the books: insulin delivery and clearance

481

Does and to what extent insulin decides the fasting glucose levels?

M. Watve, M. Diwekar, S. Kulkarni, A. Ojha;
Biology, Indian Institute of Science Education and Research, Pune, Pune, India.

Background and aims: The fasting steady state level of glucose is assumed to be mainly determined by insulin signaling although other insulin independent neuronal and hormonal mechanisms are known to regulate glucose. This assumption has not been critically evaluated and the relative role of insulin dependent and independent mechanisms in determining fasting glucose remains unknown. We use multiple approaches to examine the role of insulin in determining fasting plasma glucose.
Materials and methods: We examine whether glucose and insulin levels affect each other in a fasting steady state using multiple approaches including (i) a series of mathematical models with classical and alternative assumptions and their steady state solutions (ii) experimental results of tissue specific insulin receptor knock-outs in rodents (ii) insulin suppression and insulin raising experiments in rodents and humans (iv) human population data on glucose and insulin during fasting and glucose tolerance curve in type 2 diabetic and non-diabetic subjects. (v) A network model incorporating insulin dependent and independent mechanisms and their regulators. (vi) Re-examining classical evidence for insulin mediated regulation of fasting glucose.
Results: (i) In mathematical modeling the set of assumptions that allow insulin signaling to determine fasting glucose levels, does not allow an insulin resistant hyperinsulinemic, normoglycemic state. The classical insulin centric model and clinical picture of type 2 diabetes and prediabetes are mutually incompatible. (ii) Muscle, fat, beta cell and liver specific insulin receptor knockouts have failed to give compensatory hyperinsulinemic normoglycemic state and long lasting fasting hyperglycemic state (iii) In all insulin suppression experiments in rodents as well as humans, the apparent insulin sensitivity increased after insulin suppression and fasting glucose remained normal. Disabling insulin degrading enzyme increased fasting insulin levels but did not decrease fasting glucose. (iv) Fasting glucose and insulin are poorly correlated in human data and their relationship is not explained by the classical pathway. (v) Network model reveals that insulin signaling is not central to glucose homeostasis and type 2 diabetic state. Targeting insulin signaling does not result in a stable non-diabetic state. This result is compatible with the failure of reversing diabetes with a focus on insulin signaling. (vi) The classical evidence for insulin mediated regulation of fasting glucose has alternative interpretations that have not been eliminated.
Conclusion: All evidence converges to the conclusion that although alteration in insulin levels or insulin sensitivity alters the glucose tolerance curve, it does not alter the fasting steady state levels of plasma glucose. There is no evidence that the fasting glucose levels are determined by insulin signaling and the classical pathway of glucose homeostasis needs to be re-examined. The fasting glucose level is likely to be mainly determined by insulin independent glucose regulation mechanisms.
Disclosure: **M. Watve:** None.

482

Insulin clearance is modulated by insulin sensitivity independently of hypersecretion

R. Bizzotto[1], A. Natali[2], A. Gastaldelli[3], R.A. De Fronzo[4], S. Arslanian[5], E. Ferrannini[3], A. Mari[1];
[1]CNR Institute of Neuroscience, Padova, Italy, [2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [3]CNR Institute of Clinical Physiology, Pisa, Italy, [4]Department of Medicine, University of Texas Health Science Center, San Antonio, USA, [5]Division of Weight Management, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, USA.

Background and aims: The study of the determinants of endogenous insulin clearance (EIC) is complicated by the phenomenon of saturation, which, if neglected, leads to overestimating the associations between reduced insulin clearance and conditions of elevated insulin secretion rates (ISR) such as insulin resistance and obesity. To overcome this problem, we have developed a novel physiologically-based mathematical model of insulin kinetics able to describe EIC over a wide range of insulin concentrations obtained by glucose-stimulated ISR and exogenous insulin infusion.
Materials and methods: To develop the model we used data from multiple studies: a) intravenous glucose infusion with different patterns (7 different studies, $N = 211$ subjects); b) one- or two-step hyper insulinaemic euglycaemic clamp with different insulin infusion rates (3 studies, $N = 1685$); c) hyper glycaemic and euglycaemic clamp tests in the same subject (1 study, $N = 126$). Subjects were non-diabetic (ND, $N = 1519$) or had type 2 diabetes (T2D, $N = 300$), had a wide range of age (10–76 y) and BMI (17–64 kg/m^2) and were multiracial. Insulin kinetics was described by a circulatory model in which fractional insulin extraction from plasma is saturable in the liver whilst is constant in extra-hepatic organs. ISR was estimated from C-peptide by deconvolution. Standardized EIC, i.e. not dependent on the subject's insulin levels, was estimated for ISR equal to 100 and 400 pmol min^{-1} m^{-2} (EIC_{100} and EIC_{400}), aiming at representing both fasting and fed ISR, respectively. In the euglycaemic clamp studies ($N = 1879$), insulin sensitivity was measured as the M/I index.
Results: The model predicted actual plasma insulin concentrations accurately, with homogeneous parameters across the different studies. EIC decreased by 28.0 ± 0.3% when ISR varied from 100 to 400 pmol min^{-1} m^{-2}. In a multiple regression model of EIC_{100} including M/I, T2D, BMI, age, sex, race and study, independent significant predictors were: T2D (standardized $\beta = 0.15$ T2D *vs* NGT), sex ($\beta = 0.04$ males *vs* females), race ($\beta = 0.08$ white *vs* black), and M/I ($\beta = 0.23$) ($r^2 = 0.35$). M/I was the most important predictor of EIC_{100} ($\Delta r^2 = 0.15$). EIC_{100} in the 1st and 3rd M/I quartiles was 0.95 and 1.17 L min^{-1} m^{-2}. Similar results were obtained for EIC_{400}. An increase in ISR from 60 to 100 pmol min^{-1} m^{-2} (representing fasting ISR in insulin-sensitive and insulin-resistant subjects) was due to a 4% EIC reduction caused by saturation and to a 28% reduction produced by insulin resistance. In a subset of ND subjects in whom fasting AST, ALT, GGT, HDL, LDL, cholesterol, triglycerides, and FFA were available ($N = 1053$), additional independent predictors of EIC_{100} were FFA ($\beta = 0.08$), AST ($\beta = -0.11$) and GGT ($\beta = 0.08$), though with an overall modest impact ($\Delta r^2 = 0.02$).
Conclusion: The direct relationship of EIC with insulin sensitivity is primary and not an indirect effect of saturation. Insulin sensitivity is the most important determinant of EIC. T2D, sex, race, FFA, AST and GGT, but not BMI, are correlates of EIC independent of insulin sensitivity, although their quantitative contribution is modest.
Supported by: İMİ-JŲ 115156 ĐĐMǿŘě
Disclosure: **R. Bizzotto:** None.

483

Pharmacological characterisation of an ultra-long acting once-weekly insulin-Fc fusion with continuous glucose monitoring

C.D. Church[1], V. Howard[2], S. Oldham[2], R. Gaddipati[2], K. Mather[2], A. Rossi[1], G. Browne[3], L. Liang[1], J. Naylor[1], J. Dhillon[3], D. Hornigold[1], J. Trevaskis[2], D. Baker[1], C.J. Rhodes[2], A. Buchanan[3];
[1]Cardiovascular, renal and metabolism (CVRM), MedImmune, Cambridge, UK, [2]Cardiovascular, renal and metabolism (CVRM), MedImmune LLC, Gaithersburg, USA, [3]Antibody Discovery and Protein Engineering (ADPE), MedImmune, Cambridge, UK.

Background and aims: Basal insulin analogues have been designed and produced to mimic endogenous insulin secretion. Current basal insulin therapy for type 2 diabetes mellitus support daily dosing for patients with a focus on minimising nocturnal hypoglycaemia events. However, daily injections result in poor patient compliance. Therefore, an insulin analogue with a longer action time with less frequent administration could improve patient compliance and thus glycaemic control. Aim: Develop a once-weekly insulin analogue for the control of hyperglycaemia in patients with type 2 diabetes mellitus who require basal insulin.
Materials and methods: To achieve extended insulin action pharmacology, a panel of recombinant native single chain insulin molecules were fused to fragment crystallizable region (Fc) consisting of B chain to A chain-linker variants fused to Fc. Insulin-Fc variants were profiled in human and rodent insulin action assays including insulin receptor signalling, phosphorylated AKT (Ser473) in hepatocytes and glucose uptake in adipocytes *in vitro*. Additionally, blood glucose was monitored over one week in rodent models of obesity and hyperglycaemia (high-fat diet-induced obesity, *db*/*db*and Zucker diabetic fatty ZDF rats) following a single subcutaneous injection of Insulin-Fc variants (10, 30, 60 or 90 nmol/kg). We utilised continuous glucose monitoring (CGM) telemetry to profile the day-night control of blood glucose in ZDF rats dosed with Insulin-Fc variants.
Results: Insulin-Fc variants were designed and produced in CHO cells. A panel of Insulin-Fc variants showed a wide range of potencies for insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1R) in a luminescence linked reporter assay, as compared to endogenous insulin. Further characterisation of Insulin-Fc variants in primary mouse, rat and human hepatocytes for phosphorylated AKT (Ser473) signalling demonstrated several variants with an EC_{50} potency within 100-fold of endogenous insulin. Glucose uptake in 3T3L1 adipocytes confirmed the potency of Insulin-Fc variants (EC_{50} within 100-fold of endogenous insulin). Two Insulin-Fc molecules were subsequently characterised in *db*/*db* and high-fat diet-induced obesity (DIO) mouse models and this revealed greater than 50% blood glucose lowering (AUC, $P < 0.05$) for 5 days following administration of a single dose of Insulin-Fc. High resolution blood glucose profiling using CGM telemetry in ZDF rats showed enhanced glucose control in both day and night periods (AUC, $P < 0.05$) up to 10 days following a single-dose (90 nmol/kg) of Insulin-Fc. Chronic repeat-dosing of an Insulin-Fc variant every 10 days for 5 weeks demonstrated improved glycaemic control (−22% HbA1c from baseline with 60 nmol/kg, $P < 0.05$).
Conclusion: We report a novel basal insulin analogue with fusion of insulin to Fc that exhibits extended pharmacokinetic and pharmacodynamic profiles in rodent models of hyperglycaemia, consistent with once-weekly administration in humans. High resolution continuous glucose monitoring demonstrates enhanced glycaemic control in ZDF rats during both day and night periods with Insulin-Fc variants.
Disclosure: **C.D. Church:** Employment/Consultancy; MedImmune. Stock/Shareholding; MedImmune.

484

Association between a metabolic syndrome severity score and all-cause mortality in type 1 diabetes: a 10-year follow-up study

R. Miccoli, M. Garofolo, R. Giannarelli, F. Campi, D. Lucchesi, L. Giusti, V. Sancho Bornez, A. Dardano, G. Daniele, S. Del Prato, G. Penno;
Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

Background and aims: The prevalence of distinctive traits of the Metabolic Syndrome (MetS) is increasing among individuals with type 1 diabetes mellitus (T1DM). We have therefore determined in a longitudinal study the association between a "MetS severity score" (MetS score; courtesy of Dr. Mark DeBoer) and all-cause mortality in T1DM.
Materials and methods: We consecutively recruited 774 T1DM individuals (age 40.2 ± 11.7 years; diabetes duration (DD) 19.4 ± 12.2 years; BMI 24.8 ± 3.6 kg/m^2; HbA1c 7.8 ± 1.2%) for whom all-cause mortality was assessed over a 10.6 ± 2.6 year follow-up. MetS score was based on waist circumference (WC), systolic blood pressure (sBP), HDL-cholesterol and triglycerides with exclusion of blood glucose. Hazard ratios (HRs) for MetS score stratified by quartiles and all-cause mortality were determined by unadjusted and adjusted Cox regression.
Results: Mean MetS score was −0.50 ± 0.67 (mean ± SD; median −0.58; IQR −0.96 to −0.15). After stratification by quartiles, compared to Q1, Q2–4 showed a progressive increase of age, BMI, diastolic blood pressure, fasting glucose, HbA1c, total-, LDL- and nonHDL-cholesterol, uric acid and fibrinogen ($p < 0.0001$). WC, sBP and triglycerides, and ACR also increased, while HDL and eGFR (CKD-EPI) decreased ($p = 0.006$). A progressive increase was found in percentage of males ($p = 0.061$), CV events (1.0%, 4.6%, 6.2% and 9.3%; $p = 0.003$), eGFR <60 ml/min/1.73 m^2 ($p = 0.038$), micro- or macroalbuminuria, ($p = 0.007$), and retinopathy ($p = 0.003$) as well as use of BP-lowering agents, RAS-blockers, lipid-lowering and anti-platelet drugs ($p < 0.0001$). A total of 52 deaths occurred during the 8,184 person-years of follow-up (6.7%; 6.36 × 1000 person-years). Death rate increased with MetS score: Q1 2.1%; Q2 5.7% (HR 3.10, 95%CI 0.99–9.75, $p = 0.053$); Q3 5.7% (2.78, 0.88–8.73, $p = 0.080$); Q4 13.5% (7.02, 2.45–20.12, $p < 0.0001$; K-M, Log Rank 21.46, $p < 0.0001$). Adjusting for age (HR 1.08, 95%CI 1.06–1.11, $p < 0.0001$) and sex (M, 1.737, 0.98–3.08, $p = 0.059$), HRs vs Q1 ($p = 0.020$) were: Q2 2.59 (95%CI 0.82–8.15, $p = 0.104$); Q3 1.94 (0.61–6.12, $p = 0.258$); Q4 4.24 (1.46–12.31; $p = 0.008$). Adjusting for age ($p < 0.0001$), sex ($p = 0.022$), DD, BMI, HbA1c, LDL-cholesterol, CVD, retinopathy, eGFR ($p = 0.013$), ACR ($p < 0.001$), and prior CV events, HRs vs Q1 ($p = 0.047$) were: Q2 3.36 (95%CI 1.05–10.76, $p = 0.041$); Q3 2.21 (95%CI 0.69–7.08, $p = 0.352$); Q4 4.05 (95%CI 1.39–11.75; $p = 0.01$). In a fully adjusted model that adds also variables included in the equation (i.e., WC, sBP, triglycerides and HDL), age, sex, eGFR and ACR were independent predictor of death, while HRs of MetS score quartiles ($p = 0.056$) were: Q2 3.18 (95%CI 0.99–10.19, $p = 0.051$); Q3 2.11 (95%CI 0.66–6.75, $p = 0.211$); Q4 3.87 (95%CI 1.33–11.23; $p = 0.013$).
Conclusion: Our results suggest that the MetS score may predict all-cause mortality independent of both other cardiovascular risk factors as well as of the single components of MetS. However, larger studies or meta-analytic approaches combining multiple cohorts will be necessary to confirm this initial observation.
Supported by: **R. Miccoli:** None.

485

Estimation of glucose disposal rate in type 1 diabetes using clinical and research biomarkers

A.J. Jenkins[1,2], A.S. Januszewski[1,2], N. Sachithanandan[2,3], G. Ward[2,3], C. Karschimkus[2], D.N. O'Neal[1,2];
[1]NHMRC Clinical Trials Centre, University of Sydney, Camperdown, Australia, [2]Department of Medicine, University of Melbourne, Fitzroy VIC, Australia, [3]Department of Endocrinology, St Vincent's Hospital, Fitzroy VIC, Australia.

Background and aims: In Type 1 diabetes Insulin resistance is a novel risk factor for vascular complications. The gold-standard for assessing insulin sensitivity is the glucose disposal rate (GDR) measured by euglycemic hyperinsulinemic clamp, a research tool. Based on clamp studies ($n = 24$), GDR range 3.8–13.4 mg/kg/min, Williams et al developed an equation to estimate GDR (eGDR) based on clinical factors (hypertension, waist/hip ratio and HbA1c), which has been associated with and predictive of complications. **Aim:** To develop a panel of clinical ± research biochemical biomarker equations which correlate with clamp study quantified GDR (and other insulin sensitivity-related measures) in T1D adults.
Materials and methods: An euglycemic, hyperinsulinemic clamp was performed in 28 T1D adults (age (mean ± SD) 43 ± 22 yrs, T1D duration 21 ± 11 yrs, HbA1c 7.7 ± 1.4%; 9 with microvascular complications)

GDR, M/I (mean glucose infusion rate over 60 min from insulin infusion start/mean plasma insulin concentration during the same period) and Log10 M/I, all measured during the last 60 min of the clamp were dependent variables. Thirty-eight biomarkers (demographics, clinical variables (e.g. HbA1c, lipids) and research biomarkers (of inflammation, adipokines, and oxidative stress) were independent variables. Exhaustive search was used to select best performing models with up to 5 variables (based on R^2) and coefficients were calculated using multiple regression. eGDR, eM/I and eLog M/I were calculated using: 1) all variables, 2) only demographics and routine chemistry; 3) only research biomarkers; 4) only research biomarkers and demographics.

Results: GDR, M/I and Log10 M/I mean, range were 7.06 mg/kg/min and 1.87–14.05 mg/kg/min, 0.014 and 0.002–0.037, −1.95 and −2.70–−1.43 respectively. Levels of all three parameters calculated in the last 30, 60 and 90 min of the clamp correlated with each other $r = 0.99$, $p < 0.0001$. The best eLog10 M/I models accounted for up to 68% of Log10 M/I variability. The best eLog10 M/I model derived from demographic and clinical parameters included (standardized beta): gender (0.77), age (0.34), HDL-C (0.77), pulse pressure (−0.39) and waist/hip ratio (−0.27). The best eLog10 M/I model derived from research biomarkers included CRP (−0.39), total adiponectin (0.63), leptin (−0.39) and IL-6 (0.38).

Conclusion: Estimating GDR using simple, routinely available parameters is feasible and provide alternates to assess insulin resistance in T1D in research and potentially clinical settings.

Table 1: R^2 and p values for 4 models predicting GDR, M/I and Log10 M/I

Model	Models estimating measured clamp parameters using 4 or 5 parameters	GDR	M/I	Log10 M/I
1	All variables	0.51, p<0.002	0.59, p<0.0003	0.68, p<0.00002
2	Clinical chemistry + demographics	0.55, p<0.002	0.61, p<0.0005	0.67, p<0.00009
3	Research biomarkers	0.44, p<0.008	0.58, p<0.0003	0.68, p<0.00002
4	Research biomarkers + demographics	0.44, p<0.008	0.55, p<0.0008	0.68, p<0.00002

Disclosure: **A.J. Jenkins:** None.

486

The higher insulin sensitivity the higher exercise capacity in patients with type 1 diabetes

A. Uruska, P. Niedzwiecki, D. Naskret, O. Rozanska, A. Cieluch, D. Powezki, M. Chudzinski, D. Zozulinska-Ziolkiewicz;
Department of Internal Medicine and Diabetology, Poznan University of Medical Science, Poznan, Poland.

Background and aims: Insulin resistance (IR) is an important clinical problem in type 1 diabetes. Decreased insulin sensitivity is a risk factor for the development of chronic diabetic complications and influences metabolic management. It has a direct effect on the function of the heart and blood vessels in this group of patients. Thus IR could be connected with physical exercise capacity in type 1 diabetes. On the other hand, physical activity has an extremely beneficial influence on the prognosis of patients with type 1 diabetes. This study aimed to evaluate the influence of insulin resistance on physical exercise capacity in patients with type 1 diabetes.

Materials and methods: The study included 42 men with type 1 diabetes, the age was 32 years (IQR 28–36), with a duration of diabetes of 5 years (IQR 2–13), HbA1c 7.6% (IQR 6.5–8.4), daily insulin dose: 0.43 U/kg/day (IQR 0.31–0.57) and BMI 23.5 kg/m^2 (IQR 21.6–26.2). The study excluded patients with hypertension, chronic complications of diabetes, acute inflammation, with episodes of severe hypoglycemia or DKA in one month before the test. Insulin sensitivity was assessed using a normoglycemic-hyperinsulinemic clamp with measurement of glucose disposal rate (GDR). The clamp was performed 48–56 hours before exercise test. During cardiopulmonary exercise test on a cycle ergometer, rate of oxygen consumption was measured and maximal oxygen uptake (VO2max) was assessed.

Results: There was no statistically significant correlation between VO2max (ml/min/kg) with diabetes duration (Rs −0.32, $p = 0.08$), daily insulin dose (Rs −0.22, $p = 0.23$) and HbA1c level (Rs −0.34, $p = 0.07$). VO2max was negatively correlated with BMI (Rs −0.47, $p = 0.008$). Moreover, significant positive correlation of GDR and VO2max was observed (Rs 0.78, $p = 0.01$).

Conclusion: There is a link between insulin resistance and physical exercise capacity in type 1 diabetes. Higher insulin sensitivity is correlated with higherVO2max in patients with type 1 diabetes.

Supported by: PUMS Grant for Young Scientists

Disclosure: **A. Uruska:** None.

487

Impaired subcutaneous insulin delivery in type 2 diabetes patients

E. Fryk[1], J. Olausson[2], L. Strindberg[1], B. Becattini[1], K. Mossberg[1], M. Schmelz[3], H. Brogren[1], G. Solinas[1], P.-A. Jansson[1];
[1]Molecular and clinical medicine, Sahlgrenska University Hospital, Göteborg, Sweden, [2]Department of Clinical Microbiology, Sahlgrenska University Hospital, Göteborg, Sweden, [3]Department of Experimental Pain Research Mannheim, Medical Faculty, Heidelberg, Germany.

Background and aims: Previous studies showed a delayed transendothelial transport (TET) of insulin in insulin-resistant participants in agreement with an *endothelial barrier for insulin* in skeletal muscle. We hypothesized that an impaired TET of insulin is also present in adipose tissue and affects kinetics of insulin's antilipolytic effect in a study of lean, obese and type 2 diabetes (T2D) participants.

Materials and methods: Nine T2D patients (Age: 59 ± 4 years, BMI: 34 ± 2 kg/m^2, fP-Glucose: 8.3 ± 1.7 mmol/l, fS-Insulin: 10.3 ± 5.3 mU/l) were matched for age and BMI with 9 obese (Age: 62 ± 5, BMI: 32 ± 2, fP-Glucose: 5.8 ± 0.6, fS-Insulin: 10.1 ± 3.5), and for age with nine lean participants (Age: 61 ± 5, BMI: 23 ± 2, fP-Glucose: 5.3 ± 0.5, fS-Insulin: 3.6 ± 1.2). Participants underwent a 75 g oral glucose tolerance test (OGTT) after fasting overnight and they were sampled for arterialized venous blood (plasma free fatty acids (P-FFA) and subcutaneous interstitial fluid with the microdialysis technique (interstitial (I)-insulin and I-glycerol) for 3 hours. Xenon-clearance was used for assessment of adipose tissue blood flow (ATBF). Insulin appearance and insulin delivery index (IDI) were calculated during the first hour after glucose ingestion and the ratio between interstitial and circulating insulin (I/C-ratio) was calculated 60 min after the OGTT.

Results: Glucose and insulin levels were increased after 60 min (15.6 ± 2.9, 10.8 ± 3.1 and 10.9 ± 1.2; 33.5 ± 12.1, 49.4 ± 33.5 and 34.7 ± 10.9) in T2D, obese and lean subjects, respectively. During the first 60 min insulin delivery was lower in T2D compared with lean ($p < 0.05$) but not obese subjects (p = NS). Subcutaneous insulin appearance correlated with maximum suppression of interstitial glycerol ($r_s = 0.5$, $p < 0.05$, $n = 22$) and P-FFA ($r_s = 0.5$, $p < 0.01$, $n = 27$) during the OGTT. However, ATBF AUC_{180} did not correlate with insulin delivery. The I/C-ratio at 60 min (0.56 ± 0.22, 0.45 ± 0.35 and 0.34 ± 0.08) was lower in T2D than in lean ($p < 0.05$) but not obese subjects (p = NS). Correlations were determined with Spearman rank and comparisons between the groups were assessed with Mann-Whitney *U* test.

Conclusion:: There is a significantly decreased subcutaneous transendothelial delivery of insulin in type 2 diabetes compared with lean but not obese participants.

Supported by: Swedish Research Council, Swedish Diabetes Foundation

Disclosure: **E. Fryk:** None.

PS 029 Visit to the diabetes zoo: novel animal models

488

T63 displays beneficial effects on various tissues in animal models of diabetes via pleiotropic protective actions

V. Chavanelle[1], Y.F. Otero[1], P. Sirvent[1], P.D. Cani[2], S.L. Peltier[1];
[1]Valbiotis, La Rochelle, France, [2]Louvain Drug Research Institute, Louvain, Belgium.

Background and aims: Nowadays, about 60 million people are diagnosed with type 2 diabetes (T2D) in Europe and its prevalence is increasing, mostly due to overweight and obesity. Prediabetes precedes T2D and is reversible although 70% of individuals with prediabetes will eventually develop T2D if no intervention is made. Additional strategies are needed to manage prediabetes. The pathophysiology of prediabetes is complex and involves impairments in many organs. It has been previously shown that T63, an active principle ingredient candidate for managing prediabetes, was effective to prevent glucose homeostasis impairments in rodent models of T2D. In an open phase I/II clinical study, T63 also improved glucose and insulin responses to a carbohydrate challenge in healthy subjects. This study provides new data on 2 different animal models of T2D and prediabetes that show an action of T63 on various organs, suggesting a pleiotropic effect of the candidate and bringing new leads for understanding the mechanisms involved.
Materials and methods: All experiments were carried out along the "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985) and approved by the local ethics committee (C2EA-02). Forty db/db mice (aged 5 weeks) were fed for 6 weeks either a control diet or the same diet supplemented with 2.7% T63. Thirty-six male C57BL6 mice (aged 5 weeks) were randomised into 3 groups. Control group was fed a control low-fat diet. High Fat (HF) group was fed a HF diet (36% fat). HF-T63 group was fed a HF diet supplemented with 2.7% T63. Total duration of the HF study was 16 weeks.
Results: In db/db mice, T63 lowered fasting glycaemia and delayed the defect of insulin secretion, one of the hallmarks of T2D. In HF-fed mice, T63 improved an insulin-sensitivity index following an oral glucose test. In the inguinal fat, HF diet impaired the activation of the insulin pathway following an acute injection of insulin. Interestingly, T63 restored the activation of Akt in this tissue (Insulin stimulated phosphorylation ratio 6.14-fold higher than vehicle vs. 1.01-fold in HF group, $p < 0.01$). Additionally, hepatic triglyceride content, known as a powerful inductor of liver insulin resistance, was reduced in HF-T63 group, compared to HF (−40%, $p < 0.001$). T63 administration increased liver gene expression of FGF21, which has been involved in the regulation of many metabolic pathways. T63 also showed preventive effects on HF diet-induced gut microbiota dysbiosis. Specifically, the relative abundance of *Parasutterella* and *Barnesiella*, two bacteria previously linked with obesity and insulin sensitivity, was increased in HF-T63 group compared to HF (1.55% vs. 0.33%, $p < 0.001$ and 9.36% vs. 3.12%, $p < 0.001$).
Conclusion: In conclusion, T63 improved glucose metabolism in 2 mouse models of prediabetes and T2D via actions on various tissues. The evolution of the physiopathology of T2D is complex and involves many organs. The pleiotropic effects of T63 make it a promising intervention strategy to prevent T2D development. The candidate is currently under investigation in an international phase II clinical trial on prediabetic subjects.
Disclosure: **V. Chavanelle:** None.

489

Liver-specific nicotinamide N-methyltransferase deficiency protects mice from body weight gain and improves insulin sensitivity under standard diet

S. Brachs[1,2], J. Polack[1,2], M. Brachs[1], R. Elvert[3], F. Bärenz[3], S. Ruf[3], M. Kabiri[3], A. Kannt[3,4], J. Spranger[1,2];
[1]Department of Endocrinology, Diabetes and Nutrition, Charité - Universitätsmedizin Berlin, Berlin, [2]DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, [3]Sanofi Research and Development, Frankfurt am Main, [4]Institute of Experimental and Clinical Pharmacology and Toxicology, Heidelberg University, Mannheim, Germany.

Background and aims: Experimental evidence reveals a correlation between Nicotinamide N-methyltransferase (NNMT) activity and obesity and related metabolic disease. Using a whole-body *Nnmt* knockout (KO) mouse model, we recently investigated the effects of *Nnmt* deletion on energy metabolism and the development of obesity under different diets. In humans, *NNMT* expression is highest in liver followed by adipose tissue (AT). Contrary to mice, *Nnmt* expression is high in liver but appears to be exceeded at least tenfold in AT. Therefore, we generated AT-specific as well as liver-specific *Nnmt* KO mice to investigate and dissect the metabolic role of NNMT.
Materials and methods: A conditional $Nnmt^{fl/fl}$ mouse line was bred to both a FABP-Cre and an Alb-Cre deleter line to generate an AT-specific ($ANNMT^{-/-}$) and a liver-specific NNMT KO ($LNNMT^{-/-}$), respectively. Body composition, metabolic parameters, glucose homeostasis and insulin sensitivity were assessed in $ANNMT^{-/-}$ and $LNNMT^{-/-}$ compared to control mice (WT) feeding either a standard diet (SD) or a high-fat diet (HFD). In all cohorts, we measured 1-methylnicotinamide (MNAM) concentrations, performed ipGTTs and conducted hyperinsulinemic-euglycemic (HE) clamps.
Results: Analyzing $ANNMT^{-/-}$ mice, we did not observe any impact of the AT-specific *Nnmt* deletion on metabolism. $ANNMT^{-/-}$ mice showed similar weight gain, body composition, glucose tolerance and insulin sensitivity, however, had a strongly reduced *Nnmt* expression and MNAM levels in AT. No such differences were found in liver of $ANNMT^{-/-}$ mice. In contrast, $LNNMT^{-/-}$ mice showed virtually no *Nnmt* expression in liver and low hepatic MNAM levels whereas plasma MNAM was similar compared to WT and slightly higher in AT. Regardless of feeding SD or HFD, weight gain and especially body fat content did not increase in $LNNMT^{-/-}$ mice and they, consequently, displayed lower plasma insulin levels. Furthermore, liver weights of $LNNMT^{-/-}$ mice were significantly reduced compared to WT. Despite of their lean phenotype, no improvements of acute glucose handling were revealed performing ipGTTs with mice on SD or HFD. However analyzing insulin sensitivity, $LNNMT^{-/-}$ mice fed a SD exhibit improved glucose infusion rates (GIR) and an enhanced glucose uptake (GU) during a HE clamp. This was not maintained in $LNNMT^{-/-}$ mice on HFD. They showed a low GIR, a suppression of endogenous glucose production about 50% and a reduced GU similar as WT mice.
Conclusion: In summary, we did not reveal a metabolic impact of AT-specific *Nnmt* deletion whereas we observed a strong improvement of body composition in $LNNMT^{-/-}$ mice under SD and HFD showing a reduced fat mass and less body weight gain. Nevertheless, no effect of liver-specific *Nnmt* deletion was found on glucose handling. However, insulin sensitivity was significantly elevated in $LNNMT^{-/-}$ on SD but this effect was not preserved in $LNNMT^{-/-}$ mice fed a HFD.
Supported by: Joint lab between Charité and Sanofi
Disclosure: **S. Brachs:** Other; Research support by a joint lab between Charité and Sanofi.

490

Renal denervation stimulates hepatic glucose uptake in dogs

L.M. Moore, D.S. Edgerton, E. Allen, B. Farmer, P. Williams, A.D. Cherrington, G. Kraft;
Vanderbilt University, Nashville, USA.

Background and aims: Renal denervation (RDN) has generated considerable interest as a method to treat drug-resistant hypertension. Recent data showed that RDN can improve hepatic insulin sensitivity; however

the mechanism remains unknown. The portal signal, generated during the absorption of glucose into the portal vein, is a major determinant of hepatic glucose uptake (HGU) and it has been shown to result from a decrease in the sympathetic tone to the liver. The objective of this study was to investigate whether renal denervation would reduce hepatic sympathetic tone, thereby preventing the stimulating effect of portal glucose delivery on HGU.

Materials and methods: Dogs underwent bilateral surgical renal sympathetic denervation ($n = 5$). Renal denervation was confirmed after the study by the nearly complete loss of NE content in both kidneys (Table). The study consisted of a 3 hour hyperinsulinemic hyperglycemic pancreatic clamp. In the first 90 mins (Period 1), all glucose was infused into a peripheral vein; during the last 90 mins (Period 2), glucose was also infused intra-portally (4 mg/kg/min) to establish the portal glucose signal, and glucose was still infused into a leg vein in order to equalize the hepatic glucose load in both periods.

Results: Arterial insulin (21 ± 3 and 20 ± 4 μU/mL) and glucagon (24 ± 4 and 23 ± 2 pg/mL) levels were not different in the 2 periods. During Period 1 the glucose concentration was maintained at 221 ± 5 mg/dL while during Period 2 it was 200 ± 4 mg/dL. The liver glucose loads were matched between periods (42 ± 5 and 43 ± 4 mg/kg/min), as designed. As previously reported, RDN surgery was associated with an increased liver glucose uptake (3.6 ± 0.4 mg/kg/min during Period 1 compared to values of ≈2 mg/kg/min under the same conditions in non-RDN animals). Notably, there was no increase in net hepatic glucose uptake from Period 1 to Period 2 (3.5 ± 0.7 mg/kg/min) in the RDN animals. This value was not different from that seen in non-RDN animals exposed to the portal glucose signal. Interestingly renal denervation was associated with a marked decrease in liver NE content (−85%, see table).

Conclusion: Reduced hepatic sympathetic input appears to be the mechanism by which renal denervation increases HGU. The notion that RDN and the hepatic portal vein glucose signal act via the same mechanism is supported by the fact that the latter had no effect on HGU in dogs that had undergone RDN.

Norepinephrine content (ng/g of tissue)	Control (n=6)	Renal denervation (n=5)	% decrease	P value
Right Kidney	914±132	123±90	87	<0.001
Left Kidney	958±157	33±18	97	<0.001
Liver	245±18	36±19	85	<0.001

Table: Norepinephrine content (in ng/g of tissue) in organs of sham operated control (previously presented) and renal denervated animals collected at the end of study. Values are the average of 2 biopsies of the cortex for the kidneys and 2 lobes of the liver

Supported by: NIH
Disclosure: **L.M. Moore:** None.

491

A novel preclinical model to define the window between plasma glucose lowering versus water retention in the gut during SGLT1 inhibition

M. Fritsch Fredin[1], M. Sundqvist[2], H. Peilot Sjögren[3], C. Kankkonen[3], M. Persson[2], M. Strimfors[1], D. Lindén[1], T. Rydén-Bergsten[4], D. Karlsson[1];
[1]Bioscience, AstraZeneca R&D Molndal, Molndal, [2]DMPK, AstraZeneca R&D Molndal, Molndal, [3]Discovery Sciences, AstraZeneca R&D Molndal, Molndal, [4]AstraZeneca R&D Molndal, Molndal, Sweden.

Background and aims: Sodium Glucose co-Transporter 1 (SGLT1) inhibition improves glucose control at the expense of increasing the risk for developing diarrhoea due to glucose-driven gut water accumulation. Since rodents are resistant to SGLT1 inhibition-induced diarrhoea, it has been difficult to develop a preclinical model that predicts the therapeutic window for SGLT1 inhibitors in man. The aim of this work was to first develop a surrogate rat model for diarrhoea based on water retention in the gut. SGLT1 inhibitors with clinical data (LIK066, canagliflozin and sotagliflozin), were used to build the water retention model. The model was then used to explore the therapeutic window for a gut-restricted SGLT1 inhibitor (mizagliflozin).

Materials and methods: Fasted Sprague-Dawley rats were gavaged with 2g/kg glucose and increasing doses of mizagliflozin, LIK066, canagliflozin or sotagliflozin ($n \geq 3$/dose group and $n = 16$ vehicle). One hour later, animals were euthanized and the water content in the small intestine and jejunual mucosal drug exposure were quantified. Another set of fasted rats underwent an oral glucose tolerance test (OGTT) using 2 g/kg glucose containing 7400 Bq/μl [^{14}C(U)]-D-glucose tracer. Blood samples were collected before and up to 240 minutes post gavage for the analyses of plasma ^{14}C-glucose, glucose, insulin and drug exposure ($n = 3$–6). *In vitro* potencies of the SGLT1 inhibitors were assessed by their ability to reduce the transport of the glucose analogue, Methyl α-D-glucopyranoside ([^{14}C]-AMG) in HEK293S cells overexpressing SGLT1. The assay was run at physiological glucose concentration (5mM) and in the absence of serum.

Results: Water retention in the small intestine was dose-dependently increased for all compounds tested. By modelling the free jejunal SGLT1 inhibitor exposure level, water retention occurred at exposure levels 1–3 fold above compound IC_{50}. During the OGTT, mizagliflozin dose-dependently decreased ^{14}C-glucose appearance in the plasma ($AUC_{0\text{-}120min}$ for mizagliflozin; 0.15 μmol/kg AUC = 666 ± 100*, 0.5 μmol/kg AUC = 448 ± 87*, 1.5 μmol/kg AUC = 227 ± 79* *versus* vehicle AUC = 831 ± 77, *$p < 0.05$). Plasma glucose and insulin were dose-dependently decreased (glucose for mizagliflozin; 0.15 μmol/kg AUC = 361 ± 68*, 0.5 μmol/kg AUC = 240 ± 42*, 1.5 μmol/kg AUC = 127 ± 58* *versus* vehicle AUC = 462 ± 57 and insulin for mizagliflozin; 0.15 μmol/kg, AUC = 13669 ± 5282, 0.5 μmol/kg AUC = 7771 ± 1431*, 1.5 μmol/kg AUC = 4147 ± 3013* *versus* vehicle AUC = 22862 ± 6846, *$p < 0.05$). *In vivo* IC_{50} for plasma glucose lowering of mizagliflozin occurred at jejunal concentrations of 1.2 μM (95% CI: 0.9−1.3 μM) while water retention IC_{50} was 2.5 μM (95% CI: 1.8–3.2 μM), thus approximately at 2-fold higher gut exposure compared to the glucose lowering.

Conclusion: We have developed a new rat model that uses water retention in the gut as a surrogate marker for diarrhoea. We demonstrate a 2-fold separation between glucose lowering and water retention for a gut-restricted SGLT1 inhibitor, mizagliflozin. This new water retention model can help to assess the risk for diarrhoea using SGLT1 inhibition before entering clinical trials. Whether the model can be used for other osmotic mechanisms in the gut needs to be explored.
Disclosure: **M. Fritsch Fredin:** None.

492

Effect of hyperamylinaemia on expression of genes involved in metabolic hormone signalling in the brain

T. Nie, S. Zhang, G. Vazhoor Amarsingh, H. Liu, G.J.S. Cooper;
School of Biological Sciences, The University of Auckland, Auckland, New Zealand.

Background and aims: Amylin is postulated to be involved in the development of Type 2 diabetes (T2D), as human amylin forms pancreatic amyloid in diabetic patients. Mice expressing human amylin have been developed to study this. But β-cell loss limits the expression of amylin and insulin. Thus, these mice can't be used to investigate chronic hyperamylinaemia nor the insulin resistant stage of T2D. Our group has developed transgenic mice which overexpress a nonamyloidogenic

variant with three proline substitutions at residues 25, 28 and 29, called the Line 44 model. These mice develop obesity, hyperglycaemia, hyperamylinaemia and hyperinsulinaemia. Homozygous (HOM) mice show more severe hyperglycaemia than hemizygous (HEM) mice. We aimed to examine how the expression of genes involved in amylin, insulin and leptin signalling in the brain was affected by hyperamylinaemia in this model at different disease stages.

Materials and methods: Brain samples were taken from HEM, HOM and nontransgenic (NON) mice at 100 days (prediabetic) and diabetic onset (three consecutive blood glucose measurements >11 mmol/l). The brain was split into four parts: the hindbrain, midbrain, left and right cortices. We used molecular probes to measure the expression of 42 genes in HEM and HOM mice and compared this to NON controls. $n = 5$ NON, 3 HEM, 4 HOM (prediabetic), and 6 NON, 6 HEM, 6 HOM (diabetic).

Results: Statistical analysis was performed with the NanoStringDiff package in R. We applied a false discovery rate of 5% and found several genes with significantly altered expression. We examined the fold change (FC, shown as $\log_2$) to determine their likely biological impact.

Notable genes included: *Cart* is an appetite suppressor normally increased by insulin and leptin. We found it decreased in the prediabetic hindbrain (FC = −1.51 in HEM, −0.64 in HOM) and the diabetic cortices (Left: FC = −1.37 in HEM, −0.99 in HOM. Right: FC = −1.06 in HEM, not significant in HOM). *c-fos* is a common marker of amylin signalling. Expression was decreased in the prediabetic hindbrain (FC = −0.96 in HEM, −1.61 in HOM). Interestingly, it was increased in HEM but decreased in HOM in both cortices at diabetic onset (Left: FC = 1.18 in HEM, −0.65 in HOM. Right: FC = 1.12 in HEM, −0.77 in HOM). *Pomc* is another appetite suppressor increased by insulin and leptin signalling. In the hindbrain, expression was reduced at 100 days (FC = −2.08 in HEM, −0.84 in HOM) but increased in HOM when diabetic (FC = 1.87). *Socs3*, a well-known inhibitor of leptin signalling, was significantly differentially expressed in HOM mice only. It was decreased in the prediabetic hindbrain (FC = −1.01). However, *Socs3* was increased in all other regions at both time points (Prediabetic: FC = 2.06 in midbrain, 1.66 in left cortex, 2.00 in right cortex. Diabetic: FC = 1.78 in midbrain, 1.27 in left cortex, 1.16 in right cortex).

Conclusion: Hyperamylinaemia altered the brain expression of a number of genes in the signalling pathways of amylin, insulin and leptin. These changes may have contributed to resistance to these hormones, leading to the obese and diabetic phenotype of the Line 44 model. Reduced hindbrain *c-fos* suggested the presence of amylin resistance before the onset of diabetes. Lowered *Cart* and *Pomc* expression indicated resistance to insulin and/or leptin which may have led to hyperphagia and obesity. Elevated *Socs3* levels in HOM mice suggested downregulation of leptin signalling which may have contributed to the more severe diabetic phenotype seen in HOM mice.

Supported by: MBIE, University of Auckland Doctoral Scholarship

Disclosure: **T. Nie:** None.

493

Evaluation of GPR120 regulation of glucose homeostasis and incretin secretion using intestinal cell lines and incretin receptor knockout mice

A.M. McKillop[1], B.M. Moran[2], M.G. Miskelly[1], P.R. Flatt[1];
[1]School of Biomedical Sciences, University of Ulster, Coleraine, UK, [2]Department of Biopharmaceutical and Medical Science, Galway-Mayo Institute of Technology, Galway, Ireland.

Background and aims: GPR120 is a rhodopsin-like GPCR that has a high affinity for long chain saturated C14-18 fatty acids and unsaturated C16-22 fatty acids. Stimulation of GPR120 by free fatty acids results in elevation of [Ca2+]i and activation of the ERK cascade suggesting interactions with the Gαq family of G proteins. The aims of this study were to assess the role of the GPR120 agonist GW-9508 in glucose homeostasis and incretin regulation from enteroendocrine L- and K-cells in diabetic and incretin receptor knockout mice, and determine the cellular localisation of GPR120 in mouse intestinal tissue and clonal GLP-1 and GIP cell lines.

Materials and methods: Cellular localisation of GPR120 was determined by double staining immunohistochemistry in intestinal tissue from NIH Swiss mice, in GLP-1 secreting GLUT-ag cells and GIP secreting pGIP/neo STC-1 cells. Anti-hyperglycaemic, insulinotropic and incretin secreting properties of GW-9508 (0.1 μmol/kg body weight) were explored with oral and intraperitoneal glucose (18 mmol/kg body weight) tolerance tests (GTT) in lean and high fat fed (HFF) diabetic mice. *In vivo* studies were also carried out in age matched GLP-1 receptor knockout, GIP receptor knockout and wild-type C57/BL6 mice.

Results: Compared to intraperitoneal injection, oral administration of GW-9508 (0.1 μmol/kg body weight) together with glucose reduced the glycaemic excursion by 22–31% ($p < 0.05$–$p < 0.01$) and enhanced glucose-induced insulin release by 30% ($p < 0.01$), compared to glucose control. GW-9508 increased total GLP-1 release by 44% ($p < 0.01$) after 15 min and stimulated total GIP release by 47% ($p < 0.001$) after 15 min. In high fat fed diabetic mice, orally administered GW-9508 lowered plasma glucose by 17–27% ($p < 0.05$–$p < 0.01$) and augmented insulin release by 22–39% ($p < 0.05$–$p < 0.001$). GIP (1-42) and GLP-1 (7-36) (25 nmol/kg body weight) administered orally with glucose had no effect in GIP and GLP-1 knockout mice, respectively; the glucose lowering and insulinotropic effects of GW-9508 were abolished. GW-9508 lowered the glycaemic excursion by 14% ($p < 0.05$) and increased insulin release by 24% ($p < 0.01$) in C57/BL6 wild-type mice. GW-9508 increased circulating total GLP-1 release by 39–44% ($p < 0.01$) and total GIP by 37–47% ($p < 0.01$–$p < 0.001$) after 15 and 30 min of GW-9508 administration in wild-type mice. Immunocytochemistry demonstrated co-localisation of GPR120 expression and incretin hormones in mouse enteroendocrine L- and K-cells, GLP-1 secreting GLUTag cells and GIP secreting pGIP/Neo STC-1 cells.

Conclusion: GPR120 is expressed on intestinal L- and K-cells and stimulated GLP-1/GIP secretion plays an integral role in mediating enhanced insulin secretion and improved glucose tolerance, suggesting development of potent and selective GPR120 agonists as a therapeutic approach for diabetes.

Disclosure: **A.M. McKillop:** None.

494

Characterisation of a new mouse model for gestational diabetes

K. Grupe, F. Dannehl, M. Planteur, S. Scherneck;
Technical University of Braunschweig, Braunschweig, Germany.

Background and aims: Gestational diabetes (GDM) is a complex metabolic disease in which both environmental factors and a genetic predisposition are involved. Patients show impaired glucose tolerance in pregnancy and have a higher risk to develop type 2 diabetes in later life. However, adequate models to study the pathomechanisms and the interaction of genetic and lifestyle factors are still lacking. Female New Zealand obese (NZO) mice are characterized by a polygenic metabolic disorder showing increased body weight and insulin resistance on the one hand, but do not develop manifest diabetes on the other hand. The aim of this study is to investigate the capability of NZO mice as a model for the human disease.

Materials and methods: In NZO and NMRI (control) mice oral glucose tolerance tests (oGTT; 2 mg glucose/g body weight) were performed both preconceptional and on day 14 of gestation. Blood glucose and insulin levels were measured within a time course between 0 (basal) and 240 minutes after glucose administration. At both aforementioned times (i.e. preconceptional and on day 14 of gestation), pancreata were histologically assessed, particularly the proliferative response of the islets of Langerhans (Ki67-positive islet cells) as a consequence of the increased insulin demand due to pregnancy.

Results: Compared to NMRI control mice the NZO strain showed no hyperglycaemia neither prior to conception (141 vs 126 mg/dl, n.s.) nor at day 14 of gestation (123 vs 114 mg/dl, n.s.). However, the oGTT revealed impaired glucose tolerance within the NZO mouse strain at both times that resulted in an increased AUC of 40%. Preconceptional insulin values were higher in the NZO mice, whereas the levels did not differ between the two strains on day 14 of gestation. The islets of both strains had intact immunoreactivity for insulin and showed no differences in their proliferation prior to conception (Ki67-positive nuclei 3.1% vs 2.4%, n.s.). On day 14 of gestation islet cells of NZO mice showed significantly lower proliferation in comparison to the NMRI control strain (3.7% vs 7.2%, $p < 0.01$).

Conclusion: Having impaired glucose tolerance and a decreased compensatory response of the islets of Langerhans during pregnancy, NZO mice show important characteristics of GDM. In addition, preconceptionally elevated insulin levels and peripheral insulin resistance indicate the occurrence of prediabetes within the NZO strain. In conclusion, the NZO mouse provides a suitable model to study the human disease.

Supported by: DDG

Disclosure: **K. Grupe:** None.

495

Insights in the role of a fatty acid oxidation enzyme in insulin secretion: rare genetic variants and a new murine model of SCHAD deficiency

K. Velasco[1], J. Lüdeke[1], H. Hovland[1], Å. Ottesen[1], M. Choi[1], L.M. Myklebust[2], K. Fjeld[3], I. Aukrust[3], T. Arnesen[2], P. Njølstad[4], R.N. Kulkarni[5], A. Molven[1];
[1]Department of Clinical Medicine, University of Bergen, Bergen, Norway, [2]Department of Biomedicine, University of Bergen, Bergen, Norway, [3]Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway, [4]Department of Clinical Science, University of Bergen, Bergen, Norway, [5]Joslin Diabetes Center, Boston, USA.

Background and aims: Congenital Hyperinsulinism of Infancy (CHI) is a group of inherited disorders characterized by persistent hypoglycemia and hyperinsulinemia. CHI is caused by mutations in genes that affect regulation of insulin secretion in β-cells. One such gene is *HADH*, which encodes short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD), a ubiquitously expressed mitochondrial enzyme of the fatty acid oxidation pathway. Literature data suggest that SCHAD-CHI is caused by specific deficiency of this protein in β-cells and that rare SCHAD variants may protect against diabetes. This study was undertaken to investigate how SCHAD is involved in regulation of insulin secretion.

Materials and methods: Sixteen rare amino acid substitutions present in human populations were characterized in terms of subcellular localization, protein stability and enzymatic activity by cloning into bacterial/eukaryotic expression vectors. To avoid that endogenous SCHAD protein obscured functional assessment, a SCHAD-negative HEK293 cell line was constructed using CRISPR technology. A β-cell-specific SCHAD knock-out mouse (B-SCHADKO) was constructed by crossing *Hadh*-floxed animals with mice expressing Cre under the regulation of the *Ins1* promoter.

Results: Intracellular targeting to the mitochondria was unaffected for all variants. Four variants (G34R, I184F, P258L, G303S; all previously linked to CHI) exhibited very low protein levels when expressed in eukaryotic cells, most likely due to a posttranslational quality control mechanism as they could be stably expressed in a cell-free system. A one way ANOVA showed that there were significant differences among the enzymatic activity of the variants [$F(11,24) = 194.1$, $p < 0.001$]. A post-hoc Dunnett's test showed that the pathogenic variants H170R, P258L and G303S (respectively 7.33 ± 2.3, 9.7 ± 0.4, 0.8 ± 1.3 μmol/min/mg) have significantly lower enzymatic activity than the WT (180.6 ± 2.9 μmol/min/mg) at $p < 0.001$, while the P215T (211.34 ± 4.6 μmol/min/mg) variant has significantly higher activity than the WT at $p = 0.10$. The remaining variants had similar properties as wild-type SCHAD protein. Both male and female B-SCHADKO mice exhibited significantly reduced plasma glucose in the random fed state (♂ 8.7 ± 0.8 vs. 6.3 ± 1.5 mmol/L, $p = 0.0016$; ♀ 7.9 ± 0.4 vs. 5.3 ± 1.1 mmol/L, $p < 0.0001$) and after overnight fasting (♂ 4.5 ± 0.7 vs. 3.1 ± 0.7 mmol/L, $p < 0.0002$; ♀ 3.4 ± 0.4 vs. 2.3 ± 0.2 mmol/L, $p < 0.0001$). Insulin and tolerance tests showed that GSIS and overall glucose homeostasis were not significantly different in B-SCHADKO and control mice.

Conclusion: Disease-associated SCHAD variants, but not the tested rare population variants showed altered functional properties. The hypoglycemic phenotype of SCHAD deficiency is beta-cell autonomous, but cannot be explained simply by increased insulin secretion.

Supported by: Novo Nordisk Foundation and Research Council of Norway

Disclosure: **K. Velasco:** None.

496

A new implantable insulin-delivery device designed for extraperitoneal space, an alternative site for insulin delivery

J. Magisson[1], A. Sassi[1], A. Kobalyan[1], C.-T. Burcez[1], R. Bouaoun[1], K. Gouget Kaufmann[1], M. Vix[2], N. Jeandidier[3], S. Sigrist[1];
[1]Defymed S.A.S, Strasbourg, [2]IRCAD, Strasbourg, France, [3]Department of Endocrinology, Diabetes and Metabolic Diseases, Strasbourg, France.

Background and aims: Glycaemic instability and hypoglycaemia are major problems for late stage diabetic patients and are related to non-physiologic subcutaneous (s. c.) administration of insulin. Intraperitoneal (i. p.) delivery with implantable pumps or catheter-based devices enables first hepatic pass of insulin via portal absorption and is clinically relevant. However, it faces limitations such as insulin stability, high infection rate, catheter obstruction. Consequently, a new implantable device, ExOlin®, was developed. It consists in a flat pouch made of porous membranes with a catheter and an s. c. injection port for easy administration of insulin. To avoid peritoneal inflammation and tissue adhesions, this device is placed in the extraperitoneal space located between parietal peritoneum and fascia of abdominal muscles. In this study, kinetics of insulin delivery in extraperitoneal was compared to s. c. and i. p. routes.

Materials and methods: Plasma insulin levels in portal and peripheral veins assessed by ELISA assay were compared in extraperitoneal, s. c. and i. p. 5, 15, 30, 60 min after administration in healthy rats and 5 and 30 min after administration in swine (2U of rapid Human insulin/rat, 40U of rapid Human insulin analog/swine), In swine, injection was performed into ExOlin®. Kinetics of insulin delivery through ExOlin® device was tested in diabetic rats implanted in i.p. or extraperitoneal by measurement of glycaemia during 150 min after injection of 2U of rapid Human insulin.

Results: In rats, portal insulin levels were different between s. c., i. p. and extraperitoneal routes at 5 min post injection (i. p.: 2557 ± 661 mU/L $n = 9$; extraperitoneal: 1577 ± 887 mU/L $n = 10$ vs s. c.: 451 ± 102 mU/L $n = 9$) with higher portal insulin level for i. p. compared to s. c. ($p < 0.01$). Then, a progressive increase was observed for s. c. group showing higher levels of insulin compared to extraperitoneal at 60 min (s. c. 2262 ± 459 mU/L $n = 8$ vs extraperitoneal 695 ± 196 mU/L $n = 9$; $p < 0,05$). In parallel, levels of insulin in caudal vein peaked at 15 min for i. p. and extraperitoneal sites, while it raised rapidly for s. c. group until 30 min post-injection and stabilized at 60 min with levels significantly higher than i. p. or extraperitoneal groups (at 60 min: s. c. 1019 ± 193 mU/L $n = 8$; i. p. 269 ± 58 mU/L $n = 8$ extraperitoneal 463 ± 85 mU/L $n = 10$; $p < 0,01$ s. c. vs i. p. and extraperitoneal). In swine with ExOlin®, higher insulin levels in portal vein compared to ear vein were measured 5 min post-injection (112 ± 37 mU/L in portal $n = 4$; 57 ± 32 mU/L in ear vein $n = 4$). In diabetic rats, injection of Human insulin in ExOlin® in i. p. or extraperitoneal induced a decrease of glycaemia comparable to the one obtained with a direct injection.

Conclusion: In this study, we demonstrated in small and large animal models that extraperitoneal site is promising for insulin delivery due to kinetics and portal absorption comparable to i. p. route. We showed that a membrane-based insulin delivery device does not alter portal absorption kinetics. Combination of both device and delivery site constitutes a potent alternative to i. p. route in brittle and hypoglycaemia prone type 1 diabetes.
Disclosure: **J. Magisson:** None.

PS 030 Keeping it in the family: glucagon, GIP, GLP1, GLP2

497

Proteomics of secreted glucagon reveals heterogeneous complexes as novel mediators of alpha cell function

F. Asadi[1], S. Dhanvantari[2,3];
[1]Pathology, The University of Western Ontario, London, [2]Pathology and Medical Biophysics, The University of Western Ontario, London, [3]Lawson Health Research Institute, London, Canada.

Background and aims: Targeting glucagon secretion from pancreatic alpha cells has been proposed as a therapeutic strategy for the treatment of diabetes. To identify possible modulators of glucagon secretion, we are using a proteomics approach that targets glucagon within secretory granules and secreted glucagon-immunoreactive complexes. Our ongoing work has identified a novel and dynamic network of proteins associated with glucagon within secretory granules in αTC1-6 cells. The aims of the present study are *i)* to investigate the potential roles of these proteins in modulating glucagon secretion; and *ii)* to investigate if glucagon remains associated with secretory granule proteins after secretion.
Materials and methods: Protein complexes in isolated secretory granule fractions from αTC1-6 cells were pulled down with Fc-glucagon, and proteins were identified using LC-MS/MS. Functional readouts of select proteins were conducted through siRNA-mediated silencing in αTC1-6 cells and measurement of glucagon secretion by ELISA. To investigate the association of secretory granule proteins with glucagon after secretion, cells were cultured in DMEM without glutamine, sodium pyruvate and FBS for 24h. Media were removed and extracted in acid/ethanol (95% ethanol containing 0.18 M HCl) and separated into soluble and insoluble fractions by centrifugation. The acid/ethanol-soluble fraction was used to identify glucagon-immunoreactive complexes by immunoblotting, and to identify associated proteins within these complexes by LC-MS/MS of silver nitrate-stained bands. Values were compared among groups using 1-way ANOVA ($\alpha = 0.05$).
Results: Several novel proteins were identified within secretory granules that interact with glucagon: peroxiredoxin-2, malate dehydrogenase cytoplasmic, aconitate hydratase mitochondrial, 14-3-3 protein zeta/delta, ELKS/Rab6-interacting/CAST family member 1 (ERC1), tubulin alpha-1B, ATP synthase subunit alpha mitochondrial, histone H4, Sodium/potassium-transporting ATPase subunit gamma, protein disulfide-isomerase. Glucagon secretion was increased ($p < 0.001$, $n = 3$) upon siRNA silencing of ERC1. Glucagon secretion was decreased after knock-down of 14-3-3 zeta/delta, malate dehydrogenase cytoplasmic, Sodium/potassium-transporting ATPase subunit gamma, protein disulfide-isomerase ($p < 0.001$, $n = 3$), peroxiredoxin-2, ATP synthase subunit alpha mitochondrial, and histone H4 ($p < 0.01$, $n = 3$) and tubulin alpha-1B ($p < 0.05$, $n = 3$). Immunoblotting of both crude and acid-EtOH-extracted media revealed two glucagon-immunoreactive bands at 10 kDa and 22 kDa. Proteomic analysis of both bands revealed the presence of glucagon and secretory/regulatory proteins, such as chromogranin A, secretogranins and carboxypeptidase E. Most notably, 14-3-3 zeta/delta was associated with the 22 kDa band, and siRNA silencing of 14-3-3 zeta/delta reduced the density of this band ($p < 0.05$, $n = 6$).
Conclusion: We have discovered a novel network of secretory granule proteins that interact with glucagon to regulate its secretion. Our results also indicate that glucagon is secreted as heterogenous complexes of secretory proteins. In particular, 14-3-3zeta/delta is associated with glucagon within secretory granules and after secretion, and may be a novel player in the regulation of glucagon secretion.
Supported by: NSERC, OGS, Dean's Award
Disclosure: **F. Asadi:** Grants; The Natural Sciences and Engineering Research Council of Canada, The Ontario Graduate Scholarship. Other; Dean's Scholarship for Graduate Research.

498

Pancreatic alpha cells respond to glucose with a change in the frequency of intracellular calcium oscillations

J.A. Kellard, J.G. Knudsen, L. Briant, P. Rorsman;
Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

Background and aims: Glucagon, secreted from pancreatic α-cells during hypoglycaemia, elevates blood glucose by increasing hepatic glucose output. α-cells exhibit glucose-dependent intracellular Ca^{2+} oscillations, which drive glucagon secretion. Understanding how glucose influences Ca^{2+}oscillations is important, as glucagon secretion becomes defective in diabetes. However, the precise relationship between oscillations in Ca^{2+} and glucose in α-cells remains unknown. Studies have reported that glucose increases, decreases or does not affect Ca^{2+} activity in α-cells. These discrepancies may be due to differences in methodology and/or accuracy of α-cell identification. By applying new, and more reliable, methodology to α-cell identification we aim to increase our understanding of the relationship between glucose and Ca^{2+} oscillations in α-cells under different conditions. Diabetes and obesity are known to be inextricably linked. In order to explore whether this is (in part) due to α-cell dysfunction induced by elevated circulating free fats acids (FFA) we investigated Ca^{2+} responses following prolonged exposure to elevated FFA.
Materials and methods: Mice expressing a genetically-encoded Ca^{2+} indicator (GCaMP) specifically in α-cells (using a Cre-Lox system under control of the glucagon promotor) were generated and validated by staining and FACS followed by quantification of mRNA expression. Ca^{2+} responses to glucose were imaged using confocal microscopy in freshly isolated islets and in islets incubated in elevated (0.72 mM) FFA for 48h.
Results: 76% of $GCaMP^{+}$ cells co-stained for glucagon ($n = 4$ mice). Cells FACS sorted for fluorescent marker expression had 20 fold higher glucagon mRNA content than cells negative cells for the fluorescent marker. Ca^{2+} oscillation frequency in freshly isolated islets was higher at 1 mM glucose (0.97 ± 0.1/min) than at both, 6 mM (0.53 ± 0.08/min; $p = 0.0008$) and 15 mM glucose (0.47 ± 0.06/min; $p < 0.0001$; $n = 144$ cells from 6 mice). Ca^{2+} oscillation amplitude was unaffected by changes in glucose ($p = 0.7774$). In FFA incubated islets, Ca^{2+} oscillation frequency at 6 mM glucose (0.65 ± 0.1/min) was greater than at both, 1 mM (0.33 ± 0.06/min; $p = 0.035$) and 15 mM glucose (0.35 ± 0.08/min; $p = 0.0161$; $n = 21$ cells from 3 mice).
Conclusion: These data validate GCaMP as a reliable tool for α-cell identification and Ca^{2+} monitoring. Glucose influenced the frequency but not amplitude of Ca^{2+} oscillations. This glucose-dependency was altered following high-fat incubation. We will next seek to investigate these findings using Ca^{2+} imaging in a high fat diet fed α-cell GCaMP mice.
Supported by: Wellcome Trust
Disclosure: **J.A. Kellard:** None.

499

Loss of melatonin receptor 1 in mouse pancreatic islets results in dysregulated glucagon secretion in vitro

C.L. Lyons, E. Cowan, D.G. Abellán, C.L.F. Nagorny, H. Mulder, M. Fex;
Unit of Molecular Metabolism, Department of Clinical Sciences, Lund University Diabetes Centre, Lund University, Malmö, Sweden.

Background and aims: Disturbances of circadian rhythm, regulated by the hormone melatonin, is associated with an increased risk of developing type 2 diabetes (T2D). In fact, genome-wide association studies (GWAS) have identified a single nucleotide polymorphism in the melatonin receptor 1B (MTNR1B/Mt2) which results in reduced insulin secretion and future risk of T2D in risk allele carriers. Nevertheless, there is still a lack of knowledge regarding the melatonin receptor 1A (MTNR1A/Mt1) and regulation of glucose homeostasis. Due to its location on the pancreatic alpha cells, this receptor may be involved in regulation of glucagon secretion. Here, the aim was to investigate the islet cell-specific effects of melatonin, with a focus on Mt1, to delineate the missing link between increased melatonin action and impaired islet function.
Materials and methods: Wildtype (WT) and *Mt1* knock out ($Mt1^{-/-}$) mice, on a C3H/He background, were maintained at a 12h light/dark cycle, and fed *ad libitum*. Intravenous glucose tests (IVGTTs) were performed in 12-week-old sedated mice ($n = 10$–16 mice). D-glucose (1 g/kg) was injected intravenously and plasma glucose and insulin levels measured by ELISA. Islets were isolated from collagenase-digested pancreas (12–18 wk), handpicked and incubated with 1 mM, 2.8 mM, 6 mM and 16.7 mM glucose ±100 nm melatonin, or 10 mM arginine for 1 h after a 1 h starvation with buffer containing 2.8 mM glucose ($n = 6$–12 mice). Release of insulin and glucagon *in vitro* was measured by ELISA. The studies were approved by the animal ethics committee at Lund University.
Results: Loss of *Mt1* results in enhanced insulin secretion ($^{**}p < 0.01$) and reduced glucose clearance ($^{**}p < 0.01$) *in vivo*, following a glucose challenge, compared to WT littermates. Male $Mt1^{-/-}$ mice weighed significantly less than WT mice ($^{**}p < 0.01$), but both males and females were used in the IVGTTs. In contrast, when the islets were stimulated *in vitro* with low (2.8 mM) and high glucose (16.7 mM), there was no difference in insulin secretion between the genotypes. Furthermore, stimulation with increasing glucose concentrations reduced glucagon secretion in WT islets but caused a significant increase of glucagon release in $Mt1^{-/-}$ islets at 6mM glucose ($^{*}p < 0.05$) and a 1.7-fold increase at 16.7 mM glucose ($^{**}p < 0.01$). Strikingly, addition of arginine, a depolarizing agent, in combination with high glucose, normalized glucagon secretion in the $Mt1^{-/-}$ islets, suggesting that loss of Mt1 signaling impairs plasma membrane depolarization. Incubation of $Mt1^{-/-}$ islets with melatonin stimulated insulin secretion ($^{*}p < 0.05$) at high glucose but did not affect glucagon secretion.
Conclusion: Loss of functional Mt1 signalling can influence whole body metabolism, and *in vitro* glucagon and insulin secretion. Additional studies will delineate the dose response relationship between melatonin, insulin and glucagon *in vitro*, and utilize fluorescently labelled $Mt1^{-/-}$ alpha cells for *in vivo* analysis, to determine the mechanism of action of melatonin. In summary, melatonin plays a role in metabolic dysfunction and islet cell function. Elucidation of these pathways may aid in the development of therapeutics to combat T2D.
Supported by: VR, GKF
Disclosure: **C.L. Lyons:** None.

500

Bone protective effect of a novel long-acting GLP-1/GIP/Glucagon triple agonist (HM15211) in an animal model

S. Lee, Y.-Y. Kim, J. Lee, S.-H. Lee, Y. Kim, I. Choi, S. Kim;
Hanmi Pharmaceutical Co. Ltd, Seoul, Republic of Korea.

Background and aims: Severe weight loss is often associated with reduction of bone mineral density (BMD) and an imbalance between bone formation and reabsorption in obese people. As a consequence, there can be an increased risk of bone fractures with body weight loss. Several studies have proposed that the gut hormones, GIP and GLP-1, might be modulators of bone growth and remodeling. HM15211 is a novel long-acting GLP-1/GIP/Glucagon agonist that is being developed for the treatment of obesity. In this study, we investigated whether treatment with HM15211 prevents bone loss under a severe weight loss condition, and the underlying mechanism of action.
Materials and methods: Diet induced obesity (DIO) osteoporosis rat model was induced by surgical ovariectomy and fed 60% kcal fat diet (cat# D12492, Research diet Inc., USA) to immatured 5 weeks old female sprague dawley (SD) rats for 2 months. Plasma levels of decarboxylated osteocalcin and P1NP (procollagen type 1 pro-peptide) were measured by rat GLU-osteocalcon high sensitive EIA kit and rat P1NP ELISA kit. Bone mineral density (BMD) of femur bones were monitored using a high resolution in vivo micro-CT system. Bone protection related gene

expression was analyzed in mouse osteoblast cell (MC3T3-E1) following chronic treatment of HM15211

Results: *In vitro* study, to elucidate the underlying molecular mechanism, related marker gene expression was investigated using the mouse osteoblast cell. In line with the bone protective effect in vivo, HM15211 led to significant increases in type1 collagen-α1 and carboxylated osteocalcin expression, which were blunted by inhibition of GIPR-mediated signaling. In vivo study, After 4 weeks subcutaneous treatment of HM15211 (120 μg/kg/Q3D), lower levels of serum decarboxylated osteocalcin (42.2 ng/mL) and higher serum P1NP (procollagen type I pro-peptide, 53.2 ng/mL) were observed compared with those of vehicle- (156.4 ng/mL for osteocalcin, 28.6 ng/mL for PINP) and liraglutide-treated groups (94 μg/kg/BID, 120.6 ng/mL for osteocalcin, 29.4 ng/mL for PINP). Furthermore, HM15211 showed comparable BMD of femur bones and lumbar spine with vehicle group while weight loss was greater (−26.0% vs. vehicle) compared to liraglutide (−11.5% vs. vehicle).

Conclusion: These results of animal study suggest that treatment with HM15211 effectively prevents bone loss even after potent body weight loss in high fat dieted ovariectomized obese rats. In conclusion, these results suggest that HM15211 might provide potent weight loss without the otherwise inevitable bone loss.

Disclosure: **S. Lee:** None.

501

Separate effects of glucagon like peptide-2 (GLP-2) and glucose-dependent insulinotropic polypeptide (GIP) on bone remodelling

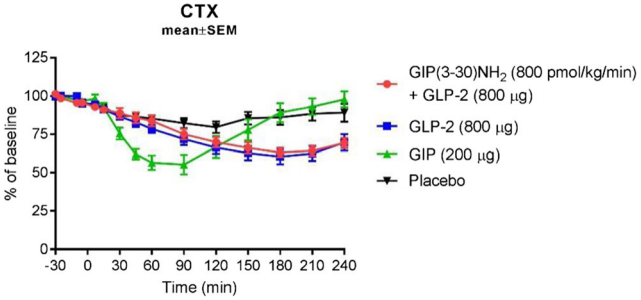
K. Skov-Jeppesen[1,2], M. Svane[3,2], C. Martinussen[3], M. Gabe[1], L. Gasbjerg[1,2], S. Madsbad[3], J. Holst[1,2], M. Rosenkilde[1,2], B. Hartmann[1,2]; [1]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, [2]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, [3]Department of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark.

Background and aims: Glucagon like peptide-2 (GLP-2) and glucose-dependent insulinotropic polypeptide (GIP) both reduce bone resorption in humans. The GIP receptor (GIPR) is expressed on osteoclasts, osteoblasts, and osteocytes indicating a direct effect of GIP on bone remodelling. The GLP-2 receptor (GLP-2R), on the other hand, has not been identified on bone cells and the mechanism underlying the antiresorptive effect of GLP-2 remains unknown. Based on unpublished *in vitro* studies showing that GLP-2 acts as an agonist on the GIPR, we speculated whether the effect of GLP-2 on bone remodelling is mediated through the GIPR. We investigated this by antagonizing the GIPR using our newly developed selective GIPR-antagonist GIP(3-30)NH_2.

Materials and methods: The study was a randomized, placebo-controlled, single-blinded, crossover study with four study days each consisting of a continuous intravenous infusion from time = −20 min to time = 240 min (GIP(3-30)NH_2 800 pmol/kg/min or saline) and a subcutaneous injection at time = 0 min (GLP-2 (800 ug), GIP(1-42) (200 ug), or saline) in the following combinations: GIP(3-30)NH_2+GLP-2; GLP-2 alone; GIP(1-42) alone; and placebo (saline). Our main outcome was C-telopeptide of type 1 collagen (CTX) as a marker of bone resorption and N-terminal propeptide of type 1 collagen (P1NP) as a marker of bone formation.

Results: Eight healthy, non-smoking, caucasian men with a median age of 27 years (range 20–34 years) and a median body mass index of 22.6 kg/m^2 (range 21.1–23.9 kg/m^2) were included in the study. CTX significantly decreased for both GIP(1-42) and GLP-2 injections reaching a nadir of 55.3 ± 6.3% (mean ± SEM) of baseline (time = 90 min) for GIP(1-42) and 60.5 ± 5.0% (time = 180 min) for GLP-2 compared with 85.4 ± 4.2% (time = 180) for placebo. Co-administration of GLP-2 with GIP(3-30)NH_2 did not significant change the effect of GLP-2 as a nadir of 63.2 ± 3.1% was reached after 180 min ($p = 0.95$). GIP(1-42) increased P1NP to 115.1 ± 2.2% of baseline (time = 30 min), significantly more than placebo. This increase was not observed for GLP-2 alone or in combination with GIP(3-30)NH_2, as both situations significantly decreased P1NP compared with placebo, but did not differ from each other (91.3 ± 1.1% and 88.1 ± 3.0%, $p = 0.50$).

Conclusion: We found that the GLP-2 induced reduction in bone resorption (CTX) was not antagonized by our selective GIPR antagonist, GIP(3-30)NH_2, and we conclude that the antiresorptive effect of GLP-2 is independent of the GIPR in humans. Importantly, we found that subcutaneous injected GIP(1-42) reduced bone resorption (CTX) and also, in contrast to GLP-2, increased bone formation (P1NP) in healthy people indicating an uncoupling effect of GIP.



Clinical Trial Registration Number: H-16047626

Supported by: Novo Nordic Foundation

Disclosure: **K. Skov-Jeppesen:** None.

502

Direct effects of glucagon on human adipose tissue metabolism

M.J. Pereira, G.J. Boersma, P.G. Kamble, P. Lundkvist, K. Almby, J.W. Eriksson;
Uppsala University, Uppsala, Sweden.

Background and aims: Glucagon acts as a counter-regulatory hormone to insulin and is therefore essential for glucose regulation. The main physiological role of glucagon is to stimulate hepatic glucose output, for example during hypoglycemia. In type 2 diabetes, fasting blood glucagon levels are elevated and contribute to hyperglycemia. In *in vitro* experiments, glucagon has been shown to increase lipolysis in human adipose tissue, however, *in vivo* experiments using glucagon infusion does not give consistent results. In addition, discordant data exist regarding the impact of glucagon on adipocyte glucose metabolism. Therefore, we aimed to study the action of glucagon on human adipocyte glucose and lipid metabolism.

Materials and methods: Abdominal subcutaneous adipose tissue was obtained with needle biopsies from healthy volunteers (6M/7F, 22–56 yrs, BMI: 22.0–34.4 kg/m^2). Adipocytes were isolated with collagenase and pre-incubated without (control) or with glucagon (0.01–100 nM) and w/wo insulin for 10–15 min. Then, 14C-glucose uptake was measured during 45 min and lipolysis was assessed with or without the beta-adrenergic agonist, isoproterenol, by measuring the glycerol release into the medium during 2 h.

Results: Glucagon dose-dependently (0.01–100 nM) increased basal and insulin-stimulated (25 and 1000 μM) glucose uptake in adipocytes by about 2-fold and 1.7-fold ($p < 0.05$), respectively, with the maximum effect observed at 10 nM (a supraphysiological level). The increase in both basal and insulin-stimulated glucose uptake was already significant with a pathophysiological concentration of glucagon: 0.1 nM and 1.3-fold increase ($p < 0.05$), compared with basal. When adipocytes were treated with glucagon 10 nM and insulin (25 or 1000 μU/mL) together, the rise in glucose uptake was additive ($p < 0.05$), suggesting independent mechanisms of

action. In addition, treatment with glucagon did not modify the adipocyte sensitivity to insulin on glucose uptake. Treatment of adipocytes with glucagon dose-dependently increased basal and isoproterenol-stimulated lipolysis up to 3.7- and 2.0-fold ($p < 0.05$) at 1 nM, respectively, compared to control. However, treatment of adipocytes with glucagon did not affect the ability of insulin to inhibit isoproterenol-stimulated lipolysis.

Conclusion: Glucagon at high concentrations can paradoxically increase glucose uptake in human adipocytes, which would promote lowering of circulating glucose. Conversely, lipolysis was increased. Potentially, these specific actions of glucagon in adipose tissue may favor fatty acid over glucose availability as energy substrates in other tissues like muscle and liver.

Supported by: Exodiab, ALF, SSMF, DF

Disclosure: **M.J. Pereira:** None.

503

Postprandial effects of individual and combined GIP and GLP-1 receptor antagonism in healthy subjects

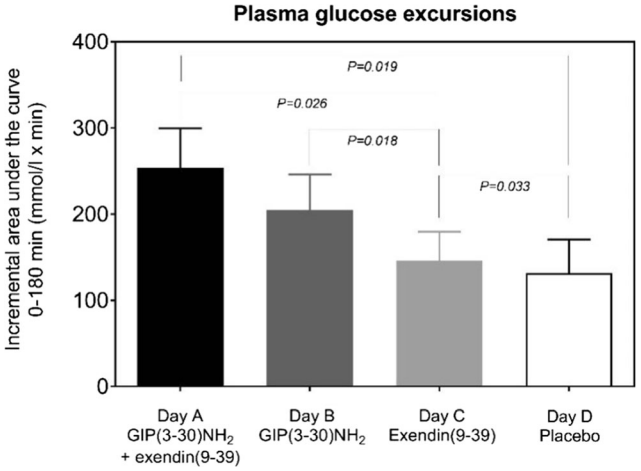
L.S. Gasbjerg[1,2], M.M. Helsted[3,2], A.H. Sparre-Ulrich[1], A.R. Lanng[2], S. Stensen[2], M.H. Jakobsen[3], B. Hartmann[1], M.B. Christensen[4,5], J.J. Holst[1], T. Vilsbøll[2,4], M.M. Rosenkilde[1], F.K. Knop[2,6];
[1]Department of Biomedical Sciences and Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, [2]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Hellerup, [3]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, [4]Department of Clinical Medicine, University of Copenhagen, Copenhagen, [5]Department of Clinical Pharmacology, Bispebjerg Hospital, Copenhagen, [6]Department of Clinical Medicine and Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark.

Background and aims: The gut-derived incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are known for insulinotropic and glucose-lowering effects. However, their individual roles in postprandial glucose homeostasis are unknown. In this study, we infused the novel, selective GIP receptor antagonist GIP(3-30)NH_2 and the GLP-1 receptor antagonist exendin(9-39) during a meal to determine the roles of endogenous GIP and GLP-1 on postprandial glucose metabolism.

Materials and methods: On four separate days, 12 healthy men (age 19–65 years, body mass index 20–25 kg/m^2) underwent a 270-minute liquid mixed meal test (1894 kJ: 49.3% carbohydrates, 5.9% protein, 34.8% fat) with randomised and double-blinded infusions (from −20 to 270 min) of GIP(3-30)NH_2 (800 pmol/kg/min) + exendin(9-39) (20 min of 1000 pmol/kg/min, thereafter 450 pmol/kg/min) (A), GIP(3-30)NH_2 (B), exendin(9-39) (C) and saline (D). The antagonist infusion rates were chosen based on their inhibitory potencies in vitro.

Results: On Day A, B and C, glucose excursions were significantly increased by 85 ± 29%, 55 ± 20% and 15 ± 16% (mean ± SEM), respectively, compared to D. Day A and B excursions were significantly higher than C (Fig. 1). Glucagon levels at 60 min differed between Day A and B (11.5 ± 1.2 vs. 7.5 ± 0.6 pmol/l (mean ± SEM), $P = 0.01$), and Day B and C (7.5 ± 0.6 vs. 12.9 ± 1.5 pmol/l, $P = 0.008$). GLP-1 was higher on Day A ($P = 0.01$) and C ($P = 0.02$) than D. No significant effects on insulin, C-peptide, gastric emptying (paracetamol absorption test) or GIP were observed.

Conclusion: Following a liquid mixed meal, endogenous GIP affects postprandial plasma glucose excursions more than GLP-1, and GIP and GLP-1 additively lower plasma glucose. Furthermore, opposite effects of GIP and GLP-1 on postprandial glucagon concentrations were observed.



Clinical Trial Registration Number: NCT03013296
Supported by: Novo Nordisk Foundation

Disclosure: **L.S. Gasbjerg:** Grants; Novo Nordisk Foundation. Stock/Shareholding; Antag Therapeutics Aps.

504

Individual and combined glucose-lowering effects of glucagon receptor antagonism and dipeptidyl peptidase-4 inhibition

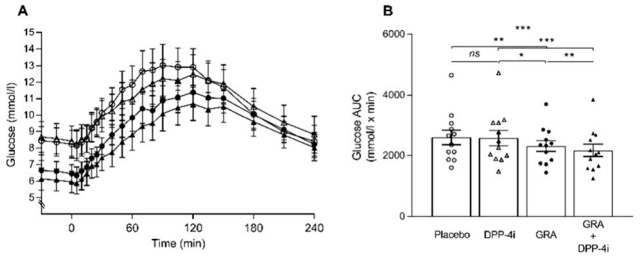
H. Maagensen[1], S. Haedersdal[1], A. Lund[1], E. Nielsen-Hannerup[1], J. Holst[2], F. Knop[1,2], T. Vilsbøll[3,4];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Hellerup, [2]Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, [3]Steno Diabetes Center Copenhagen, Gentofte, [4]Department of Clinical Medicine, Faculty of of Health and Medical Sciences, Copenhagen University, Copenhagen, Denmark.

Background and aims: Type 2 diabetes is characterised by absolute or relative hypoinsulinaemia and hyperglucagonaemia. Dipeptidyl peptidase 4 inhibitors (DPP-4i) augment insulin secretion and decrease glucagon secretion, but if glucagon is removed from the metabolic equation, the effect of DPP-4i is unknown.

Materials and methods: In a randomised, placebo-controlled, double-dummy, double-blinded, cross-over study, patients with type 2 diabetes ($n = 12$, age [mean (SD)]: 60.9 (7.8) years, BMI 34.6 (7.1) kg/m^2; HbA1c 50.3 (10.5) mmol/mol) underwent four 4-hour liquid mixed meal tests preceded by single-dose administration of 1) placebo, 2) DPP-4i (5 mg linagliptin) 2 hours before the meal, 3) glucagon receptor antagonist (GRA) (300 mg LY2409021) 10 hours before the meal, and 4) GRA + DPP-4i. Indirect calorimetry, plasma glucose, C-peptide, glucagon and paracetamol (paracetamol absorption test) was measured. Stable isotopes were infused for glucose and glycerol turnover and endogenous glucose production.

Results: Compared to placebo, fasting plasma glucose was lowered by GRA but not DPP-4i. Adding DPP-4i to GRA did not lower fasting plasma glucose further. Compared to placebo, DPP-4i increased insulin responses to the meal (C-peptide AUC) ($p < 0.01$), but did not result in a difference in plasma glucose excursions (Figure). GRA alone lowered glucose AUC, and the combination of GRA and DPP4i lowered glucose AUC further.

Conclusion: The combination of DPP-4i and GRA has additive effects on postprandial glucose excursions. This seems to be driven by the efficient reduction in fasting plasma glucose by GRA combined with additional reduction in postprandial glucose excursions by DPP-4i.



Plasma glucose excursions (A) and AUC (B) following a mixed meal tolerance test preceded by single doses of 1) placebo (open circles), 2) dipeptidyl peptidase 4 inhibitor (DPP-4i) (open triangles), 3) glucagon receptor antagonist (GRA) (filled circles) and 4) GRA+DPP-4i (filled triangles). Data are mean ± SEM. *** $p < 0.001$ ** $p < 0.01$, * $p < 0.05$, ns $p > 0.05$.

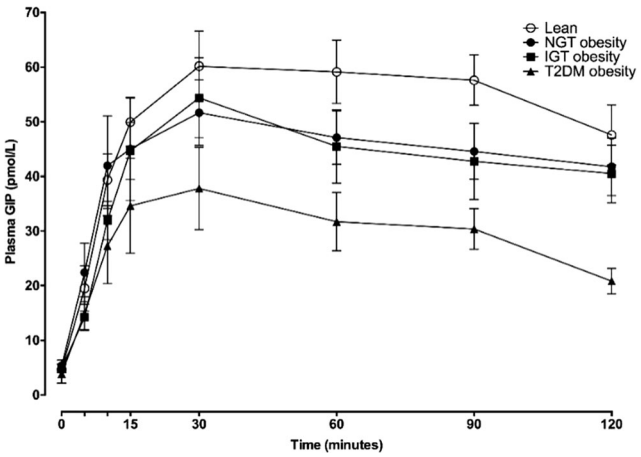
Clinical Trial Registration Number: NCT02792400
Supported by: The Danish Diabetes Association supported by the Novo Nordisk Foundation
Disclosure: **H. Maagensen:** None.

505

The initial rise in GIP secretion during OGTT correlates with the initial suppression of glucagon secretion in adolescents with obesity and type 2 diabetes

H. Kristinsson[1], H. Manell[1], M. Dahlbom[2], J. Presto[3], C. Gäredal[3], H. Ritzén[3], M. Vilhelmsson[3], E. Kilstedt[3], F. Johnson[3], H. Stenberg[3], A. Forslund[2], P. Bergsten[1];
[1]Uppsala University, Uppsala, [2]Akademiska Sjukhuset, Uppsala, [3]Mercodia, Uppsala, Sweden.

Background and aims: We have previously reported that glucagon levels at fasting and during OGTT are increased in obese adolescents as glucose intolerance develops. Specifically, we observed that initial suppression of glucagon during OGTT is absent in obese children with prediabetes or overt T2DM. Obese adults have increased incretin GIP levels, which are further elevated in subjects with T2DM. These observations have been interpreted as increased GIP levels may be the reason behind increased glucagon secretion in obese adults. We hypothesized that elevated glucagon levels at fasting and during OGTT in obese adolescents with or without T2DM were associated with increased levels of GIP.
Materials and methods: GIP and glucagon in plasma samples from adolescents with obesity and normal glucose tolerance (NGT = 12), impaired glucose tolerance (IGT = 19) or type 2 diabetes mellitus (T2DM = 4) and lean (n = 17) 10–17 year old adolescents were measured from plasma samples collected at fasting and during OGTT at −5, 5, 10, 15, 30, 60, 90 and 120 minutes by ELISA (Mercodia AB, Uppsala, Sweden). The GIP ELISA measures total GIP levels (GIP 1–42 and the inactivated part GIP 3–42). Ethical approval was obtained from the Uppsala Ethical Review Board.
Results: Fasting GIP levels (mean ± SEM) were similar between lean adolescents (4.7 ± 0.6 pM) and obese with NGT (5.4 ± 1.0 pM), IGT (4.9 ± 0.8 pM) or T2DM (3.9 ± 1.7 pM). In contrast, corresponding fasting glucagon concentrations varied greatly between the groups and were lowest for lean adolescents (7.8 ± 0.5 pM) followed by obese NGT (11.1 ± 1.0 pM), IGT (13.8 ± 1.1 pM) and highest for T2DM (18.4 ± 1.5 pM). During OGTT the initial rise in GIP secretion, (AUC_{10}), was highest in obese NGT (177 pmol/L*min) followed by lean controls (161 pmol/L*min), obese IGT (115 pmol/L*min) and T2DM (114 pmol/L*min) (Fig 1). The initial suppression in glucagon secretion was most accentuated in lean with AUC_{10} of −3.3 pmol/L*min followed by −1.1 pmol/L*min in obese NGT while in obese IGT and obese T2DM there was a rise in glucagon secretion of 3.9 and 30.6 pmol/L*min, respectively. GIP secretion throughout the OGTT was highest in lean adolescents with AUC_{120} of 5812 pmol/L*min, followed by obese NGT (4683 pmol/L*min), IGT (4586 pmol/L*min) and obese T2DM adolescents (3128 pmol/L*min). Lean adolescents had the lowest amount of glucagon secreted during the OGTT and T2DM the highest.
Conclusion: In lean and obese adolescents there is no correlation between plasma GIP and glucagon levels at fasting. In contrast, during OGTT reduced initial GIP secretion response is associated with lack of glucagon suppression. We conclude that, firstly, fasting hyperglucagonemia in obese adolescents developing T2DM does not seem to be driven by GIP secretion and, secondly, that accentuated initial GIP secretion during OGTT may reduce glucagon levels.



Supported by: FP7/2007–2013)-279153 (Beta-JUDO).
Disclosure: **H. Kristinsson:** None.

PS 031 Slimming down: with or without surgery

506

Glucagon and gastrointestinal hormones changes after Roux-en-Y gastric bypass: an IMI DIRECT study

J. Gassenhuber[1], F. Frau[1], V. Raverdy[2], F. Baerenz[1], M. Gebauer[1], F. Pattou[2];
[1]Sanofi, Frankfurt, Germany, [2]CHU Lille, University of Lille, Inserm U1190, Lille, France.

Background and aims: Type 2 diabetes (T2D) and associated obesity have reached epidemic proportions worldwide. The most effective weight loss treatment for obesity is bariatric surgery and has been proven superior to medical treatments. The mechanisms behind long lasting weight reduction and T2D remission after surgery are still unclear. It has been hypothesized that changes of circulating levels of gut hormones, in particular GLP1, are essential for this outcome. Several studies have already measured hormone levels after Roux-en-Y Gastric Bypass (RYGB), but often with a small sample size and, therefore, uncertainty on the changes still exist.
Materials and methods: We analyzed the changes of active/total GLP-1, GIP, PYY, Ghrelin and Glucagon in 39 obese subjects, from the IMI-DIRECT consortium, before (M0) and 3 months (M3) after RYGB. Mixed meal tolerance tests (MMTT) were performed after an overnight fast. Blood samples were taken at fasting and 15, 30, 60, 90, 120 and 180 minutes after ingestion of a standardized meal. In addition, pre- and post-prandial amino acid serum levels were quantified for 9 patients at fasting (T0) and 15 minutes (T15) after the mixed meal.
Results: We detected strong postprandial changes in several incretin hormones after gastric bypass. In particular, we showed a high increase in glucagon levels during the MMTT. Before surgery, the increase was 29 pg/ml (T0 = 53 ± 25 pg/ml to T15 = 82 ± 54 pg/ml), but after surgery we saw an increase of 208 pg/ml (T0 = 32 ± 17 pg/ml to T15 = 240 ± 104 pg/ml). The difference in the AUC for Glucagon for the MMTT was highly significant $p = 1.32E-06$. The sum of all amino acids in serum was similar before and after surgery at baseline, but the increase of serum amino acids at T15 was ~3 times higher after surgery. For instance the increase in serum Arginine during MMTT at T15 before surgery was 17 μmol, (M0T0 = 64 ± 15 μmol ≥ M0T15 = 81 ± 20 μmol) but after RYGB it increased to ~58 μmol (M3T0 = 77 ± 37 μmol ≥ M3T15 = 135 ± 54 μmol).
Conclusion: This result might be the missing piece for the explanation of mechanism behind diabetes remission and huge weight loss after surgery. In fact, the known glucagon effect of reducing appetite and increasing energy expenditure should be synergistic to the well described GLP1 effects. The strong increase of serum amino acids might be the driver of increased glucagon secretion after RYGB.
Clinical Trial Registration Number: NCT01129297
Supported by: IMI DIRECT
Disclosure: **J. Gassenhuber:** None.

507

Increased GLP-1 levels after bariatric surgery are not explainable by adaptations to enteroendocrine cells or the intestinal peptidome

P. Larraufie, A.K. McGavigan, R.G. Kay, F. Reimann, F.M. Gribble;
Institute of Metabolic Science, Cambridge, UK.

Background and aims: Bariatric surgery is a treatment of choice for obesity due to its long term effectiveness and association with diabetes remission. Post-prandial plasma levels of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are dramatically increased after surgery, and likely contribute to the beneficial metabolic effects due to their multiple actions including enhanced insulin secretion and reduced food intake. The aim of this study was to investigate whether transcriptomic changes in enteroendocrine cells (EECs) or alterations in the intestinal tissue peptidome contribute to the post-surgical changes in plasma gut hormone profiles.
Materials and methods: Vertical Sleeve Gastrectomy (VSG) was performed on lean NeuroD1-Cre/YFP mice expressing a fluorescent reporter in EECs. Glucose gavage was performed 6 weeks after surgery on VSG-operated, weight-matched and sham operated mice, for plasma hormone and glucose measurements. EECs were purified by flow cytometry from the upper and lower small intestine and colon, and analysed by RNA sequencing. Mucosal extracts from sequential 5cm segments along the length of the gastrointestinal tract of fasted mice were analysed by mass-spectrometry (LC-MS/MS) to identify and quantify peptide hormones.
Results: VSG-operated mice lost more weight than sham controls during the first week after surgery. Mirroring the changes seen after bariatric surgery in humans, the VSG group had increased plasma GLP-1 and insulin levels after an oral glucose challenge, and more rapid glucose clearance. LC-MS/MS analysis identified and quantified a full range of processed gut hormones in tissue extracts, including GIP, GLP-1/2, oxyntomodulin, PYY, neurotensin, SST14/28 and secretin. The tissue peptide profile of the longitudinal gut axis was not different after VSG compared with sham or weight-matched controls. RNAseq analysis of purified EECs demonstrated representation of a range of EEC types, including cells producing GLP-1, CCK, GIP, PYY or serotonin. Principal component analysis revealed strong clustering of the EEC samples based on their tissue of origin, but no differences attributable to the surgery group. Only a few genes were differently expressed between the different surgical conditions, with a significant overrepresentation of genes involved in metabolite and small molecule transport and metabolism.
Conclusion: VSG did not alter the EEC transcriptome or tissue peptidome from different regions of the intestinal tract, leading us to conclude that adaptations to EEC identity or local intestinal hormone biosynthesis do not underlie altered gut hormone levels after surgery. Instead, the dramatically increased post-prandial plasma GLP-1 and PYY profiles observed after bariatric surgery are likely explained by other physiological modifications such as differences in intestinal nutrient delivery, digestion and absorption.
Supported by: ECG-Sfe
Disclosure: **P. Larraufie:** None.

508

The effect of Roux-en-Y gastric bypass surgery on the gut mucosal gene expression profile and circulating gut hormones

M.M. Christensen[1], T. Jorsal[1], B. Mortensen[1], E. Wandall[2], E. Langholz[2], S. Friis[2], D. Worm[3], R.K. Støving[4], A. Aldries[5], C.B. Juhl[5], J. Rehfeld[6], N. Vrang[7], J. Jelsing[7], T. Vilsbøll[1], F.K. Knop[1];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, University of Copenhagen, Copenhagen, Denmark, [2]Endoscopic Unit, Herlev-Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark, [3]Department of Medicine, Amager Hospital, University of Copenhagen, Copenhagen, Denmark, [4]Elite Research Center for Medical Endocrinology & Center for Eating Disorders, Odense University Hospital, Odense, Denmark, [5]Surgical Unit, Sydvestjysk Sygehus, Esbjerg, Denmark, [6]Department of Clinical Chemistry, Centre of Diagnostic Investigations, Rigshospitalet, Copenhagen, Denmark, [7]Gubra, Hørsholm, Denmark.

Background and aims: The weight loss and improved glucose homeostasis observed after Roux-en-Y gastric bypass (RYGB) surgery is associated with changes in circulating concentrations of gastrointestinal peptide hormones such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin. In order to potentially discover unrecognized RYGB-induced alterations in the expression of gut hormones, we aimed to investigate the entire mRNA gene expression profile encoding signalling peptides in small intestinal biopsies obtained before and after surgery.

Materials and methods: Twenty morbidly obese individuals referred to RYGB (sex: 5/15 (male/female); glycaemic status: 3/17 (type 2 diabetes/ normal glucose tolerance); age [median (ranges)]: 47 (29;56) years; body weight 123.1 (100.9;173.0) kg; BMI: 43.4 (35.5;52.2) kg/m^2) underwent upper enteroscopy with mucosal biopsy retrieval and liquid mixed meal tolerance tests before and after surgery. Next-generation full mRNA sequencing of biopsies was performed. Available assays for measurements of circulating peptide hormones corresponding to the differentially regulated genes were applied.

Results: Global gene expression analysis of mucosal biopsies identified sixteen robustly expressed genes (reads per kilobase million >1) encoding enteroendocrine and neuroendocrine peptide hormones (*ADM*, *ADM2*, *CCK*, *EDN2*, *EDN3*, *GCG*, *GHRL*, *GIP*, *GUCA2A*, *GUCA2B*, *MLN*, *NPY*, *NTS*,*OXT*, *PYY* and *SST*) with significantly altered mRNA expression after RYGB (false discovery rate adjusted-p value <0.1). Corresponding changes in postprandial plasma hormone responses were seen for GLP-1, glucagon, neurotensin and ghrelin.

Conclusion: These findings show that RYGB surgery affects the transcription of a range of genes encoding signalling peptides in the small intestine, indicating that the beneficial metabolic effects of RYGB rely on a complex modulation of enteroendocrine and neuroendocrine factors in the gut.

Clinical Trial Registration Number: NCT03093298
Supported by: Sanofi aventis
Disclosure: **M.M. Christensen:** None.

509

Post-prandial hypoglycaemia after Roux en-Y gastric bypass: Could Neuromedin U play a role in the pathogenesis?

D. Bottazzo[1], D. Tricò[1], D. Guarino[2], S. Baldi[2], M. Anselmino[3], S. Taddei[1], E. Ferrannini[4], M. Nannipieri[1];
[1]Clinical and Experimental Medicine, University of Pisa, Pisa, [2]Clinical and Experimental Medicine, CNR Institute of Clinical Physiology and University of Pisa, Pisa, [3]Bariatric Surgery Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, [4]CNR Institute of Clinical Physiology, Pisa, Italy.

Background and aims: Neuromedin U (NMU), a multifunctional neuropeptide, is produced in the human foregut and suppresses insulin secretion from pancreatic beta cells, suggesting that NMU may play a role in regulating glucose metabolism. Post-prandial hypoglycaemia (PPHG), a well-recognised complication of bariatric surgery, is induced by an early inappropriate insulin secretion, in part dependent on a lack of inhibition of insulin secretion. We evaluated the role of NMU in the pathogenesis of PPHG in subjects treated by Roux en-Y gastric bypass (RYGB).

Materials and methods: Nineteen morbidly obese, non-diabetic subjects, treated by RYGB, who developed typical hypoglycaemic symptoms in the post-prandial state under everyday life conditions, received a 5-hr OGTT 18–24 months after surgery. PPHG was defined as a plasma glucose ≤3.3 mmol/L during the OGTT in the presence of typical hypoglycaemic symptoms. A group of 15 subjects treated by RYGB 24 months previously but negative for hypoglycaemic symptoms, served as control group. Insulin sensitivity was assessed by the OGIS index and beta cell function by modelling analysis of the C-peptide response to the OGTT. Plasma NMU concentrations were measured by an ELISA method at fasting and during the OGTT time corresponding to the individual glucose nadir.

Results: After surgery, BMI was lower in PPHG than No-PPHG (29.2 ± 4.0 *vs* 32.8 ± 5.7 kg/m^2, $p = 0.045$). Fasting and mean plasma glucose and fasting insulin concentrations and secretion rates were similar in both groups. As expected, the glucose nadir was significantly lower in PPHG subjects (2.7 ± 0.5 *vs* 4.0 ± 0.3 mmol/L, $p < 0.0001$). Mean insulin concentrations and insulin secretion rates during OGTT were higher in PPHG than in No-PPHG ($p = 0.05$). The area under the insulin curve in the 1st hour was higher in PPHG than in No-PPHG (58.1 ± 28.2 *vs* 40.3 ± 20.1 pmol L^{-1}·min, $p = 0.05$). Peripheral insulin sensitivity was similar in both groups, whereas beta cell glucose sensitivity was higher in PPHG than No-PPHG (125 ± 64 *vs* 74 ± 49 pmol min^{-1} m^{-2} mM^{-1}, $p = 0.02$). Fasting plasma NMU concentrations were similar in PPHG and No-PPHG (686 ± 304 *vs* 538 ± 309 pg/ml), whereas NMU concentrations at the time of glucose nadir were lower in PPHG compared to No-PPHG (620 ± 369 *vs* 929 ± 453 pg/ml, $p = 0.03$). There was a strong positive correlation between fasting and NMU nadir concentrations ($r = 0.85$, $p < 0.0001$), while NMU at glucose nadir was inversely correlated with Ins AUC-60 ($r = 0.31$, $p = 0.04$).

Conclusion: Individuals with spontaneous post-prandial hypoglycaemia after RYGB have lower NMU concentrations at the glucose nadir during an OGTT. The inverse relation of NMU at glucose nadir and 1st-hour insulin secretion is compatible with a defective response of NMU to suppress glucose-stimulated insulin secretion and to prevent hypoglycaemia.

Supported by: EMIF grant (IMI JU GA 115372-2). EMIF JU-GRANT
Disclosure: **D. Bottazzo:** None.

510

Different nutrient handling and gut hormone response after gastric bypass and sleeve gastrectomy

M.S. Svane[1,2], K.N. Bojsen-Møller[1,2], C. Martinussen[1,2], C. Dirksen[1], S. Reitelseder[3], L. Holm[3,4], V.B. Kristiansen[5], J.J. Holst[2], S. Madsbad[1,2];
[1]Dept. of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark, [2]NNF Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark, [3]Institute of Sports Medicine, Bispebjerg Hospital, Copenhagen, Denmark, [4]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK, [5]Dept. of Surgical Gastroenterology, Hvidovre Hospital, Hvidovre, Denmark.

Background and aims: Sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB) induce comparable weight loss and improvements in glycemic control despite marked differences in postoperative gastrointestinal rearrangements. Accelerated transit of nutrients to the small intestine and exaggerated secretion of GLP-1 are important for the beneficial effects of RYGB, whereas the responsible mechanisms behind SG are less investigated. We hypothesized that absorption of glucose and protein is accelerated after both SG and RYGB compared with unoperated controls (C) and compared absorption of nutrients and gastro-entero-pancreatic hormone profiles between groups.

Materials and methods: 12 SG and 12 RYGB operated and 12 C matched on age, sex, BMI, and post-operative weight loss were investigated. Absorption rates of ingested glucose and phenylalanine (phe) originating from ingested casein protein and protein metabolism were determined via double tracer technique with primed-continuous iv infusions of [6,6-D_2]glucose, L-[ring-D_5]phe, L-[ring-3,5-D_2]tyrosine and [$^{15}N_2$]urea combined with a 6 hour liquid mixed meal test (400 kcal, 50E% carb, 35E% fat, 15E% prot) containing [$^{13}C_6$] glucose and intrinsically [^{15}N] phe labelled casein.

Results: Peak rate of appearance (R_a) of oral glucose was higher after RYGB and SG compared with C (RYGB: 36 ± 1 μmol/kg FFM/min, SG: 27 ± 1, C 22 ± 1; $p < 0.05$ for all comparisons) and peak R_a of phe was higher after RYGB, but similar in SG and C (RYGB 0.37 ± 0.04, SG 0.23 ± 0.02, C 0.17 ± 0.01, $p < 0.01$ for RYGB vs SG and C, $p = 0.22$ for SG vs C). In addition, initial oral recovery of ingested phe was greatly accelerated after RYGB vs SG and C (60 min oral recovery: RYGB: 30 ± 2%, SG: 13 ± 3, C: 4 ± 0.5, $p < 0.01$ for all comparisons), but the total 6 hour oral recovery did not differ (RYGB: 67% ± 3, SG: 70 ± 4, C: 67 ± 4, $p = 0.6$). Likewise, net protein synthesis was more enhanced within the first postprandial hour after RYGB ($p < 0.01$ vs. SG and C), whereas total 6 hours net protein balance and urea turnover did not differ between groups. The rapid absorption of glucose and protein after RYGB was associated with higher but more transient excursions of plasma glucose

and amino acids followed by increased secretion of glugacon-like peptide 1 (GLP-1), insulin and peptide YY (PYY) exclusively after RYGB. In contrast, ghrelin concentration was lower and glucose-dependent insulinotropic polypeptide (GIP) was higher after SG compared with RYGB.

Conclusion: Postprandial nutrient absorption and gastro-entero-pancreatic hormone secretions differed after RYGB and SG. RYGB was characterized by accelerated glucose and protein absorption, followed by exaggerated glucose excursions and early stimulation of whole-body protein synthesis. In contrast, glucose absorption and protein turnover after SG was modestly accelerated and largely resembled that of controls. GLP-1 and PYY were increased especially after RYGB, whereas ghrelin was lower after SG. Hence, different mechanisms may underlie improved glycemic control and weight loss after these two surgical procedures.

Clinical Trial Registration Number: NCT03046186
Supported by: European Research Council (ERC) grant No 695069-BYPASSWITHOUTSURGERY
Disclosure: **M.S. Svane:** Grants; European Research Council under Horizon 2020, Capital Region.

511

The different mechanisms of action of GIP and GLP-1 explain their different efficacy as therapeutic agents in type 2 diabetes

E. Grespan[1], T. Giorgino[1], A. Natali[2], E. Ferrannini[3], A. Mari[1];
[1]CNR Institute of Neuroscience, Padua, [2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, [3]CNR Institute of Clinical Physiology, Pisa, Italy.

Background and aims: The reduced incretin effect in type 2 diabetes (T2D) represents an important cause of postprandial hyperglycaemia, but the different pharmacologic efficacy of its major players, GLP-1 and GIP, remains unexplained. At cellular level the mechanisms activated by the two hormones and the defects of T2D are still poorly understood. In this study, we have extended a recently developed mathematical model of the β-cell to 1) investigate the role of incretins at the cellular level on Ca^{2+} signalling and on the glucose mediated amplifying pathway (AP); 2) characterise incretin action *in vivo* in subjects with normal glucose tolerance (NGT) or T2D; 4) provide an explanation for the different insulinotropic activity of GIP and GLP-1 in T2D subjects.

Materials and methods: We used *in vivo* data from: A) two studies with constant infusions of GIP or GLP-1 at basal glucose; B) four hyperglycaemic clamp studies with boluses or constant infusion of GIP or GLP-1; C) a graded glucose infusion test with constant infusion of GLP-1; D) two OGTT or isoglycaemic intravenous glucose infusion studies with GIP or GLP-1 infusion. In the β-cell model, we hypothesize that GIP and GLP-1 increase insulin secretion rate (ISR) by a transient increase in Ca^{2+} levels (the first 10–20 min after the incretin stimulus) and by potentiating the AP; the $Ca^{2+}$and glucose-dependent refilling function representing the AP is multiplied by a time-dependent factor (K_{incr}); $K_{incr} = 1$ without and $K_{incr} > 1$ with incretin stimulation.

Results: A transient Ca^{2+} increase is necessary to reproduce the transient ISR increase observed with GIP infusion at basal glucose in NGT subjects (Study A). This mechanism also accounts for the increase in early ISR during the OGTT (Study D). The amplification of the refilling function through the factor K_{incr} accounts for the sustained ISR potentiation in all studies. The estimated effect on transient Ca^{2+} increase was similar for GIP and GLP-1 and was preserved in T2D compared to NGT. In contrast, the effects of GIP and GLP-1 on K_{incr} had markedly different patterns: K_{incr}increased linearly with GLP-1 over a wide dose range, while with GIP K_{incr} reached a plateau already at low GIP concentrations (Figure). K_{incr} sensitivity to GLP-1 was reduced by ~30% in T2D subjects compared to NGT, while for GIP the maximal K_{incr} was reduced by ~50%.

Conclusion: By modelling a variety of *in vivo* protocols, the following cellular mechanisms of incretins emerge: 1) a transient rise in intracellular Ca^{2+}, which underlies the early effects of incretins; and 2) a potentiation of the AP, which mediates the sustained ISR. Our analysis suggests that in T2D the incretin effect on Ca^{2+} is preserved while the amplification of the AP is impaired, though not abolished. Finally, we found that saturation of GIP effects, more than impaired sensitivity, underlies the lack of insulinotropic activity of pharmacological doses of GIP in T2D.

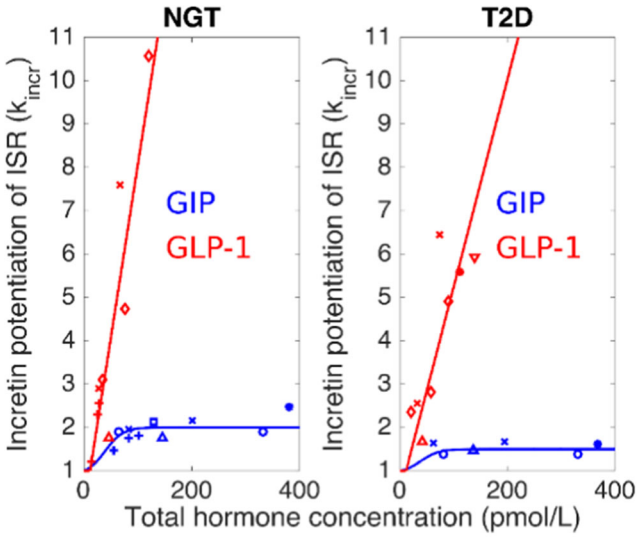


Figure: Relationship between K_{incr} and total GIP (blue lines) or GLP-1 (red lines) concentration. The analysis reveals saturation of the effect of GIP but not of GLP-1. Markers represent the different studies.

Disclosure: **E. Grespan:** None.

512

Remission of type 2 diabetes: underlying mechanisms revealed by the Diabetes Remission Clinical Trial (DiRECT)

R. Taylor[1], A. Al-Mrabeh[1], S. Zhyzhneuskaya[1], C. Peters[1], A.C. Barnes[2], B.S. Aribisala[3], K.G. Hollingsworth[1], N. Sattar[4], M.E.J. Lean[5];
[1]Magnetic Resonance Center, Newcastle upon Tyne, [2]Human Nutrition Research Group, Newcastle University, Newcastle upon Tyne, [3]Magnetic Resonance Center, Newcastle University, Newcastle upon Tyne, [4]Institute of Cardiovascular and Medical Science, University of Glasgow, Glasgow, [5]Human Nutrition, School of Medicine, University of Glasgow, Glasgow, UK.

Background and aims: DiRECT is a prospective, randomised study of type 2 diabetes (<6 years duration) which demonstrated 46% of participants had normal glucose control off all medication at 12 months. It is important to understand the mechanisms underlying this reversal.

Materials and methods: Weight loss was induced using a liquid diet replacement (825–853 kcal/day) for 3–5 months with withdrawal of all anti-diabetic drugs on day 1. Liver and pancreas fat was quantified by 3-point Dixon MRI. Hepatic very low density lipoprotein triglyceride (VLDL1-TG) metabolism and first phase insulin secretion, were measured before and after weight loss. Those with HbA1c <48 mmol/mol (<6.5%) and fasting plasma glucose <7.0 mmol/l after weight loss (n = 40; responders) were compared with non-responders (n = 18).

Results: At baseline, the two groups were similar in age (53.0 ± 1.2 vs. 53.3 ± 1.9 years) and BMI (34.9 ± 0.7 vs. 35.7 ± 1.2 kg/m^2) but not duration of diabetes (2.7 ± 0.3 vs. 3.8 ± 0.4 years, p = 0.04). HbA1c was 57.5 ± 1.7 vs. 62.5 ± 2.1 mmol/mol respectively; p < 0.07). During the weight loss phase, weight decreased in responders (100.6 ± 2.6 to 84.4 ± 2.1 kg;

$p < 0.0001$; $n = 40$) and in non-responders (102.1 ± 4.4 to 88.7 ± 4.4 kg; $p < 0.0001$; $n = 18$. Responders had a non-significantly lower fasting plasma glucose than non-responders (8.3 ± 0.4 vs. 9.3 ± 0.7 mmol/l; $p = 0.18$). Decreases occurred in liver fat in both responders and non-responders (16.7 ± 1.5 to 3.3 ± 0.7%, $p < 0.0001$; and 14.5 ± 2.6 to 2.6 ± 0.5%; $p < 0.0001$). In responders, VLDL1-TG production decreased after weight loss (560.7 ± 30.9 to 413.6 ± 25.8 mg/kg/day, $p < 0.0001$). In non-responders, there was a non-significant fall (581.1 ± 52.1 to 521.8 ± 41.9 mg/kg/day; $p = 0.28$) although the difference was not significantly different between responders and non-responders (−147.2 ± 33.8 vs. −59.2 ± 52.7; $p = 0.17$). Total plasma triglyceride (largely chylomicrons plus VLDL1-TG) fell similarly in responders and non-responders after intervention (1.84 ± 0.13 to 1.30 ± 0.13 mmol/l, $p < 0.0001$ and 1.91 ± 0.25 to 1.24 ± 0.14 mmol/l respectively, $p = 0.002$). Pancreas fat content fell similarly in responders and non-responders (8.7 ± 0.4 to 7.8 ± 0.4%; $p < 0.0001$ vs. 7.9 ± 0.6 to 7.1 ± 0.4%; $p = 0.004$; $p = 0.25$). First phase insulin secretion increased in responders after weight loss from 0.04[−0.05–0.32] to 0.11 [0.0005–0.51] nmol/min/m^2 ($p < 0.0001$), whereas no change was observed in the non-responders (0.02[−0.07–0.13] to 0.01[−0.04–0.05] nmol/min/m^2; $p = 0.59$).

Conclusion: Failure of restoration of first phase insulin secretion, but not differential lipid or hepatic responses, characterises non-responders. De-differentiation of beta cells in early type 2 diabetes is not reversible in all, despite removal of the metabolic stress. Such individuals are characterised by longer duration of diabetes even in the first 6 years of type 2 diabetes.

Supported by: Diabetes UK Award number 13/0004691

Disclosure: **R. Taylor:** Grants; Grant from Diabetes UK (Award number 13/0004691). Non-financial support; Low calorie liquid diet product donated by Cambridge Weight Plan.

513

Ethnic differences in hepatic and adipose tissue insulin sensitivity in normal glucose tolerant black west African and white European men

O. Bello[1], M. Ladwa[1], C.S. Marathe[1], F. Shojee-Moradie[2], N. Jackson[2], J.L. Peacock[3], A.M. Umpleby[2], S.A. Amiel[1], L.M. Goff[1];
[1]Division of Diabetes & Nutritional Sciences, King's College London, London, [2]Diabetes and Metabolic Medicine, University of Surrey, Guildford, [3]School of Population Health and Environmental Sciences, King's College London, London, UK.

Background and aims: There is a disproportionately high prevalence of type 2 diabetes (T2D) in populations of black ethnicity; greater insulin resistance has been extensively reported however little is known about distinctions in tissue-specific insulin sensitivity. We aimed to explore ethnic differences in insulin sensitivity of peripheral glucose disposal, hepatic glucose output and adipose tissue lipolysis using tracer techniques, in healthy black west African (BWA) and white European (WE) men.

Materials and methods: Twenty-one BWA and 19 WE normal glucose tolerant men underwent a 2-step hyperinsulinemic-euglycemic clamp (insulin dose 10 mU/m^2 BSA/min and 40 mU/m^2 BSA/min) with stable [6,6 $^2H_2$]-glucose and [$^2H_5$]-glycerol isotope infusions. We assessed peripheral glucose disposal as peripheral insulin sensitivity (IS_{PERI}) using the percentage change in rate of glucose disappearance in the high dose insulin infusion, hepatic glucose output as hepatic insulin sensitivity (IS_{HEP}) and adipose tissue lipolysis as adipose tissue insulin sensitivity (IS_{ADI}) using percentage suppression of glucose and glycerol appearance in the low dose insulin infusion, respectively. Participant characteristics and clamp derived measures were assessed for normality and compared using independent samples t-test and Mann Whitney *U* test; data are presented as mean ± SD, geometric mean (95% CI) or median (interquartile range).

Results: Participants were matched for age (BWA 25 (18) *vs* WE 28 (30) years, $p = 0.49$) and BMI (BWA 26.8 ± 3.6 *vs* WE 25.5 ± 4.0 kg/m^2, $p = 0.29$). There were no ethnic differences in waist circumference (BWA 87.5 (83.4, 91.8) *vs* WE 89.3 (84.1, 94.9) cm, $p = 0.57$), body fat percentage (BWA 20.7 ± 6.2 *vs* WE 19.4 ± 6.2%, $p = 0.52$), cholesterol (BWA 4.26 (3.85, 4.73) *vs* WE 4.53 (4.05, 5.05) mmol/l, $p = 0.41$) or fasting glucose (BWA 5.12 ± 0.46 *vs* 5.19 ± 0.41 mmol/l, $p = 0.62$), however fasting triglycerides were significantly lower in BWA men (BWA 0.67 (0.59, 0.77) *vs* WE 0.88 (0.73, 1.05) mmol/l, $p = 0.02$). We observed no ethnic differences in peripheral insulin sensitivity (IS_{PERI}; BWA 324.4 ± 121.6 *vs* WE 350.7 ± 105.3%, $p = 0.56$). However, BWA exhibited significantly lower hepatic (IS_{HEP}; BWA 65.3 (14.0) *vs* WE 75.7 (9.2) %, $p < 0.01$) and adipose tissue insulin sensitivity (IS_{ADI}; BWA 20.4 ± 20.4 *vs* WE 63.7 ± 12.8%, $p < 0.01$) compared to WE.

Conclusion: In this preliminary analysis comparing normal glucose tolerant men of BWA and WE ethnicity, matched for age and whole body adiposity, we have observed ethnic differences in insulin sensitivity of hepatic glucose output and adipose tissue lipolysis despite similar peripheral glucose disposal. Our findings suggest there may be ethnic distinctions in health which may contribute to the pathophysiology of type 2 diabetes by which the BWA population experience greater insulin resistance of the liver and adipose tissue.

Supported by: Funded by Diabetes UK

Disclosure: **O. Bello:** None.

PS 032 Novel biomarkers

514

Metabolomics reveal changes in plasma concentrations of 3-hydroxybutyric acid, citric acid, oleic acid and proline in gestational diabetes

N.J. Johansen[1], M. Demant[1], T. Suvitaival[1], K. Trost[1], L. Ahonen[1], L. Bonde[1], J.A. Svare[2], C.L. Quigley[1], T. Vilsbøll[1,3], F.K. Knop[1,3];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]2Department of Gynecology and Obstetrics, Herlev Hospital, [3]Department of Clinical Medicine, University of Copenhagen, Denmark.

Background and aims: The reversible nature of gestational diabetes mellitus (GDM) provides a unique possibility to describe diabetic *vs* normal glucose homeostasis in the same subject. With a target-discovery focus, we applied comprehensive metabolomics to provide a detailed here-and-now fingerprint of the overall metabolic state in pregnant third trimester (TT) women with GDM and normal glucose tolerance (NGT), respectively, and again post-partum (PP).
Materials and methods: Fasting and postprandial (75 minutes from ingestion of a standardised mixed meal) plasma samples obtained from 9 women with GDM (age: 31 ± 6 years; BMI: 31.6 ± 6.4 kg/m^2) and 6 pregnant women with NGT (age: 28 ± 3 years; BMI: 29.7 ± 5.4 kg/m^2) during TT and 3–4 months PP (at which point all women with GDM had re-established NGT) were analysed using two-dimensional gas chromatography with time-of-flight mass spectrometry.
Results: During TT, fasting 3-hydroxybutyric acid and citric acid were elevated in the GDM *vs* NGT group ($p \leq 0.0060$) with normalisation of both metabolites in the GDM group after birth and no between-group differences during PP ($p \geq 0.77$). Fasting malic acid and oleic acid decreased significantly in the GDM group following delivery ($p < 0.010$), but no between-group differences were observed during TT or PP. In the GDM group, postprandial malic acid levels increased PP *vs* TT ($p = 0.030$). We observed a trending higher fasting proline in the GDM group *vs* the NGT group and significant increases in fasting proline in both groups following delivery ($p \leq 0.014$).
Conclusion: Our results provide insights into the effect of pregnancy and GDM on plasma metabolites and suggest that 3-hydroxybutyric acid, citric acid, malic acid, oleic acid and proline may play hitherto underestimated roles in the pathophysiology of GDM.
Disclosure: **N.J. Johansen:** None.

515

A targeted proteomic profile of prevalent diabetes in a population-based sample

K. Beijer[1], J. Sundström[1], J. Ärnlöv[2], T. Fall[1], E. Ingelsson[3], L. Lind[1];
[1]Medical Sciences, Uppsala University, Uppsala, Sweden, [2]Karolinska Institute, Stockholm, Sweden, [3]Stanford University, Palo Alto, USA.

Background and aims: In genome-wide association studies (GWAS), a great number of genes linked to diabetes have been identified. The aim of the present study, was to investigate if circulating levels of a large number of preselected proteins were associated with diabetes.
Materials and methods: In 2,467 subjects in the population-based EpiHealth study (ages 45–75 years, 50% females), 249 proteins were analyzed by the proximity extension assay (PEA) technique. Diabetes was defined as taking antidiabetic treatment or having a fasting glucose of >7.0 mmol/l. Two thirds of the cohort was used for the discovery analysis, and the rest of the sample was used for validation.
Results: Following adjustment for age, sex, BMI, education, physical activity, smoking and alcohol intake, 68 proteins were significantly related to prevalent diabetes in the discovery analysis using a false discover rate (FDR) of <5%. Twenty-nine of those proteins, could be validated (FDR <5%). The ten top findings were (in descending order); Cathepsin D ($p < 9.56*10^{-6}$), Retinal dehydrogenase 1, Alpha-L-iduronidase, Hydroxyacid oxidase 1, Galectin-4, Growth/differentiation factor 15, Lipoprotein lipase, Interleukin-1 receptor antagonist protein, Cathepsin O, Sialic acid-binding Ig-like lectin 7 ($p = 0.0011$). The 29 proteins represent several physiological pathways.
Conclusion: Using a discovery/validation approach within a population-based sample, 29 proteins were identified as being linked to prevalent diabetes in the cross-sectional setting. These proteins are involved in several physiological pathways. Future prospective studies are needed to investigate the importance of these findings.
Disclosure: **K. Beijer:** None.

516

Linagliptin treatment is associated with improved cobalamin (vitamin B_{12}) storage in mice and potentially in humans

H. Tammen[1], M. Kömhoff[2], M. Mark[3], B. Hocher[4], D. Delic[3], R. Hess[1], M. von Eynatten[5], T. Klein[3];
[1]PXBioVisioN GmbH, Hannover, [2]Department of Pediatrics, University Marburg, Marburg, [3]Boehringer Ingelheim GmbH & Co. KG, Biberach, [4]Institute of Nutritional Sciences, University of Potsdam, Potsdam, [5]Boehringer Ingelheim International GmbH, Ingelheim, Germany.

Background and aims: Linagliptin (LINA) is a dipeptidyl peptidase 4 inhibitor indicated for the treatment of type 2 diabetes (T2D). We utilized specimens from an experimental animal model (5/6 nephrectomized mice) to explain nephron- and cardio-protective effects of LINA. Kidney, heart and liver samples were analyzed using multiplex mass spectrometry methods. The results were validated in human plasma and urine specimens using ELISA assays.
Materials and methods: Analysis of mice tissue samples (kidney $n = 38$, liver $n = 12$, heart $n = 38$) was performed by reversed-phase liquid chromatography coupled with matrix-assisted laser desorption ionisation mass spectrometry. Assessments of methylmalonic acid (MMA), as a marker of cobalamin homeostasis, were performed in human urine and plasma samples using a commercially available competitive ELISA assay.
Results: As expected, LINA was associated with changes in expression and/or lytic processing of proteins and peptides. Surprisingly, LINA treatment resulted in a significant increase in intracellular levels of cobalamin (approx. 5-fold [heart, liver], up to 20-fold [kidney]; all $p < 0.001$, ANOVA). To determine if increased cobalamin storage with LINA treatment may also occur in humans, we measured MMA in patients' plasma ($n = 301$; 150 placebo and 151 LINA) and urine ($n = 245$; 125 placebo and 120 LINA) samples at two timepoints (V3 and V7; 6 months apart) from the previously completed MARLINA-T2D trial (24 weeks' treatment with LINA or placebo in T2D patients with early stage kidney disease). MMA is regarded as a surrogate marker of cobalamin supply and elevated MMA levels are indicative of a functional cobalamin deficit. MMA levels are primarily determined by cobalamin supply, i.e. an intervention with LINA cannot improve the cobalamin status if an insufficient supply is present. In contrast to the standard diet in the animal model applied, cobalamin supply is more prone to variations in humans. Consequently, analysis of measured MMA levels from the clinical study focused on the outcome with LINA at V7 in patients with good cobalamin supply at baseline (i.e. low MMA plasma levels at V3). Our results show: 1) Applying a threshold of 140 nmol/L at V3 (baseline), LINA treatment for 24 weeks was associated with a significant reduction in MMA levels at V7 ($p = 0.02$, $n = 94$, Kruskal-Wallis). 2) In this patient subset we found a strong correlation (Pearson's $r = 0.95$) between MMA levels at V3 and reduced levels of MMA at V7 with LINA. This correlation was evident both in plasma and urine.
Conclusion: Our observations suggest that, in humans, a mechanism congruent to the one in mice potentially exists, indicating that LINA treatment might improve cobalamin homeostasis. This novel treatment effect of LINA could be of particular clinical relevance in patient populations with a higher prevalence of cobalamin deficiency, such as older

patients, Asian patients or patients on metformin. Validation of our findings in larger cohorts over longer treatment periods, alongside assessments of additional biomarkers of the cobalamin status (e.g. transcobalamin II), and quantification of the potential clinical implications, merit further research.

Clinical Trial Registration Number: NCT01792518

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **H. Tammen:** Non-financial support; Boehringer Ingelheim.

517

Insulin-like Growth Factor-1 (IGF-1) regulates the levels of serum uric acid through the urate transporters

A. Fuoco, G.C. Mannino, R. Spiga, C. Di Fatta, E. Mancuso, C. Averta, F. Andreozzi, G. Sesti;
Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Catanzaro, Italy.

Background and aims: Increasing evidence suggests that low plasma IGF-1 levels are associated with reduced insulin-sensitivity, obesity, metabolic syndrome, and predict development of both Type2 Diabetes Mellitus and cardiovascular diseases. We have recently demonstrated a significant inverse correlation between plasma levels of IGF-1 and uric acid (UA). Previous studies have reported an association between the polymorphism rs35767G/A, located in the promoter of *IGF-1* gene, and the circulating concentrations of this hormone. In this study, we assessed the association of rs35767 G/A with UA concentration in serum and urine, in a cohort of adult Whites. We also investigated IGF-1 ability to modulate the expression of transporters involved in reabsorption and secretion of UA in the kidney.

Materials and methods: The study group comprised 2794 adult Whites. 24-hour urinary uric acid concentration was available for 229 subjects. rs35767 polymorphism was screened using TaqMan genotyping assays. HEK293 (human embryonic kidney-293) cell line was treated with IGF-1 (1, 5, 10, 50 nM) for 24-hours, and differences in the expression of urate transporters were evaluated via Western Blot and real time rtPCR.

Results: Individuals carrying the IGF-1-raising allele (rs35767T) exhibited significantly lower levels of serum urate according to both additive and recessive models, after correction for gender, age, BMI, glucose tolerance, glomerular filtration rate, and anti-hypertensive treatment. TT genotype carriers displayed higher uricosuria as compared with C allele carriers, after adjusting for confounders. Exposure of HEK293 cells to IGF-1 resulted in a dose-dependent increase ($p < 0.01$) of UA transporters deputed to UA excretion (MRP4, NPT1 and BCRP), and reduction of GLUT9 expression, the major mediator of UA reabsorption, both at mRNA and protein level ($p < 0.05$).

Conclusion: We observed a significant association between the functional polymorphism rs35767 near *IGF-1* with serum urate concentrations and we provide a mechanistic explanation supporting a causal role for IGF-1 in the regulation of UA homeostasis.

Disclosure: **A. Fuoco:** None.

518

Genetic regulation of pigment epithelium-derived factor (PEDF), a multifunctional anti-tumour factor: an exome-chip association analysis in subjects with type 2 diabetes

C.Y.Y. Cheung, P.C.H. Lee, C.S. Tang, A. Xu, C.H.Y. Fong, K.K.K. Ng, K.H.M. Kwok, W.-S. Chow, Y.-C. Woo, M.M.A. Yuen, K.K.B. Tan, T.-H. Lam, H.-F. Tse, P.-C. Sham, K.S.L. Lam;
The University of Hong Kong, Hong Kong, Hong Kong.

Background and aims: Diabetes is associated with an increased risk of cancers. The anti-tumour effects of pigment epithelium-derived factor (PEDF) have been extensively reported. However, the association of circulating PEDF level with cancer remains controversial and little is known of its genetic regulation. No genome-wide or exome-wide association studies on circulating PEDF level have been published to date. This study aimed to identify the genetic determinants influencing circulating PEDF level and evaluate the relationship between circulating PEDF level and cancer in subjects with type 2 diabetes (T2DM).

Materials and methods: An exome-chip association study evaluating the genetic determinants of circulating PEDF level was conducted in 5385 Chinese subjects with T2DM. The discovery stage involved 2936 subjects, followed by a replication analysis in 2449 independent subjects with T2DM who had not been genotyped with the exome-chip. A case-control study was then conducted to examine the relationship between serum PEDF level and history of cancer in 421 cases and 4964 controls within the same cohort.

Results: All genome-wide significant association signals were detected at chromosome 17p13.3, a region which has been implicated in multiple human cancers. The strongest association with circulating PEDF level was detected at a missense variant of *SERPINF1*, the gene which encodes the PEDF protein ($P_{combined} = 2.06 \times 10^{-57}$; β[SE]: −0.33[0.02]). Two missense variants of *SMYD4* ($P_{combined} = 7.56 \times 10^{-25}$; β[SE]: 0.21[0.02]) and *SERPINF2* ($P_{combined} = 8.22 \times 10^{-10}$; β[SE]: −0.15[0.02]) showed novel associations at genome-wide significance. Elevated serum PEDF level was found to be independently associated with history of cancer ($P = 1.35 \times 10^{-3}$; OR[95%CI]: 1.92[1.29–2.87] per ug/ml), after adjustment for age, gender, body mass index and duration of diabetes.

Conclusion: We identified three missense variants of *SERPINF1*, *SMYD4* and *SERPINF2* significantly associated with circulating PEDF level in an exome-chip association study. The observed elevated circulating level of PEDF in T2DM subjects with a history of cancer might represent a counter-regulatory response, or a compensatory mechanism. Our data provided novel insight into the genetic regulation of PEDF. The findings from this study may stimulate further prospective investigations into the use of circulating PEDF level and its associated genetic variants as potential biomarkers for cancer progression and development, especially in individuals with T2DM.

Supported by: The Hong Kong Research Grant Council: Theme Based Research Scheme (T12-705)

Disclosure: **C.Y.Y. Cheung:** None.

519

Expression of lymphocytes phenotypic markers in patients with diabetic foot syndrome and healing rate of ulcerative defects

M. Mashkova[1], T. Mokhort[1], V. Goranov[1], L. Chvatova[1], O. Symantovich[1], I. Pukita[2], E. Yurenya[2], A. Shyshko[1];
[1]Belarusian State Medical University, Minsk, [2]City Endocrinology Center, Minsk, Belarus.

Background and aims: to study the relationship between lymphocytes phenotypic markers expression in patients with diabetic foot syndrome (DFS) with a healing rate of ulcerative defect.

Materials and methods: 13 patients with DFS (DFS group) and 13 diabetic patients without foot ulcers (control group) (both groups comparable in age and other clinical and general laboratory characteristics, ongoing treatment, offloading mode) were invoved in the study. All DFS patients had chronic non-infected foot ulcers Wagner 2. Ulcer area was calculated at the first visit and in 8 weeks. Peripheral blood lymphocytes phenotyping was performed in both groups.

Results: Patients with diabetic foot ulcers,in comparison with diabetic controls, had significantly higher percentages of B lymphocytes (CD19+) ($p < 0.05$) and CD4+ T cells ($p < 0.05$), but no significant difference for

CD8+ T cells. Percentage of CD 95+ cells was significantly reduced in DFS group ($p < 0.05$) in comparison with control group (table 1). In correlation analysis significant positive correlation was revealed between CD95+ marker expression and wound healing rate in DFS group ($r = 0.5112$, $p < 0.05$).

Conclusion: Our study shows that the alteration of reparative processes in diabetic ulcers is accompanied by a change in the receptor sensitivity of lymphocytes (CD95), and hence their ability to Fas-mediated apoptosis.

Table 1. – Lymphocyte markers in control DFS and control groups

CD marker	Control group*	DFS group*
CD3+,%	56,2 [55,1-57,8]	74,2 [68,3-80,1]**
CD4+,%	41,3 [40,2-42,5]	56,8 [48,3-65,8]**
CD8+,%	27,0 [26,4-27,8]	26,7 [23,5-28,7]
CD19+,%	9,3 [8,7-10,1]	13,9 []11,3-17,1]**
CD95+,%	6,7 [5,4-8,1]	3,8 [2,3-5,9]**

* The data are presented as Me [25-75].
** p< 0,05 vs control group.

Disclosure: **M. Mashkova:** None.

PS 033 Inflammation, adipose tissue and obesity: human studies

520

A lower level of intestinal inflammation markers is associated with a higher insulin resistance in patients with morbid obesity

A. Ho Plágaro[1], C. Gutiérrez Repiso[1], C. Santiago Fernández[1], S. García Serrano[2], L. Garrido Sánchez[1], S. Valdés[2], M. Gonzalo[2], G. Alcaín Martínez[1], F. Martín Reyes[1], A. Rodríguez Cañete[2], F. Moreno Ruiz[2], C. Montiel Casado[2], E. García Fuentes[1];
[1]Hospital Universitario Virgen de la Victoria/Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, [2]Hospital Regional Universitario/Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain.

Background and aims: Inflammation in different tissues, such as adipose tissue, has been typically associated with the development of metabolic diseases such as obesity and insulin resistance. However little is known about the inflammation at intestinal level. The aim of this study is to evaluate the expression of different inflammatory proteins in duodenal biopsies from morbidly obese subjects and its relationships with insulin resistance.

Materials and methods: The study was undertaken in 40 morbidly obese subjects and in 12 non-obese non-diabetic control subjects. Morbidly obese subjects were classified in subjects with low homeostasis model assessment of insulin resistance (HOMA-IR) value (MO-low-IR), subjects with high HOMA-IR value (MO-high-IR) (both groups without treatment for T2D) and subjects with T2D who were only receiving metformin treatment (MO-metf-T2D). A duodenum biopsy was collected by endoscopy in both morbidly obese subjects and control subjects, and inflammatory protein levels were analyzed by ProcartaPlex Immunoassays.

Results: In the control group we found a lower level of IL-2, IL-4, IL-6, IL-9, IL-13, IL-18 and IL-27 with respect to MO-low-IR group ($p < 0.05$, respectively), a lower level of IL-4 and IL-9 with respect to MO-high-IR group ($p < 0.05$, respectively), and a lower level of IL-4, IL-9 and IL-18 with respect to MO-metf-T2D group ($p < 0.05$, respectively). In the MO-low-IR group we found a higher level of IL-4, IL-6, IL-13 and IL-27 with respect to MO-high-IR group ($p < 0.05$, respectively), and a higher level of IL-13 with respect to MO-metf-T2D group ($p < 0.05$). No significant differences were found between MO-high-IR and MO-metf-T2D group.

Conclusion: Morbidly obese subjects have higher levels of pro/anti-inflammatory cytokines than control subjects. MO-high-IR group has lower levels of cytokines related to Th1 and Th2 responses than MO-low-IR.

Supported by: ISCIII (PI15-01845), FEDER funds ("A way to make Europe"), FPU (FPU14/01972)

Disclosure: **A. Ho Plágaro:** None.

521

Omentin-induced secretion of proteins by primary human adipocytes and stimulation of the innate immune system

C. Niersmann[1,2], S.M. Hauck[2,3], J.M. Kannenberg[1,2], K. Röhrig[1,2], C. von Toerne[3], M. Roden[1,4], C. Herder[1,2], M. Carstensen-Kirberg[1,2];
[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, [2]German Center for Diabetes Research, München-Neuherberg, [3]Research Unit Protein Science, Helmoltz Zentrum München, German Research Center for Environmental Health, München-Neuherberg, [4]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Background and aims: Omentin was initially identified as anti-inflammatory adipokine with insulin-sensitizing and atheroprotective

properties. In contrast, we recently found that omentin induces the release of 29 pro-inflammatory chemokines and cytokines in primary human adipocytes which might be mediated by the activation of the inflammatory NFκB, p38 and ERK pathways. However, details on the underlying mechanism of omentin are currently unclear. Therefore, the proposed project aimed to investigate the impact of omentin on the secretion of proteins by primary human adipocytes and based on these results to identify the signaling pathways in which the omentin-regulated proteins are overrepresented.

Materials and methods: Primary human adipocytes from five non-diabetic donors were treated without or with 500 ng/ml or 2000 ng/ml omentin for 24h. The secretome of adipocytes was analyzed using LC-MSMS-based proteomics analysis. Differences in protein secretion between omentin-treated and untreated human adipocytes were assessed using the paired t-test. In addition, we analyzed the overrepresentation of proteins that were regulated by 2000 ng/ml omentin in canonical pathways using the Ingenuity Pathway Analysis software.

Results: In the supernatants of the adipocytes 3493 proteins were detectable. Of these proteins, 140 proteins were differentially regulated by both omentin concentrations compared to untreated adipocytes ($p < 0.05$). Omentin-regulated proteins were overrepresented in seven canonical pathways ($p < 0.05$). These signaling pathways include the complement system (e.g. C3: +215%, C1R: +70%), the inhibition of matrix metalloproteases (e.g. MMP2: +78%, TIMP1: +166%) and the acute phase response signaling (e.g. CFB: +3423%, SERPINA3 + 140%) ($p < 0.001$). Most of the potential upstream regulators are pro-inflammatory cytokines (e.g. IL-1β, TNF, IFNγ) (all $p < 0.05$) that are involved in various immune responses and inflammatory processes. Another group of potential activators are complexes of inflammatory pathways (e.g. NFκB) ($p < 0.001$). TNFAIP6 is the most regulated protein (+13940%) ($p < 0.01$) and is known as negative regulator of inflammatory processes.

Conclusion: Our findings suggest that omentin regulates the release of proteins by human adipocytes via processes that are involved in the innate immune system. Thereby, our data give evidence for the activation of the inflammatory NFκB signaling pathway. The upregulation of TNFAIP6 might represent an endogenous counterregulation of the pro-inflammatory effects of omentin in human adipocytes.

Supported by: DZD

Disclosure: **C. Niersmann:** None.

522

The impact of inflammation on alternative splicing at PPARG locus in hypertrophic obesity

S. Cataldi[1], M. Aprile[1], P. Italiani[2], A. Ciccodicola[1], V. Costa[1];
[1]Institute of Genetics and Biophysics "A. Buzzati-Traverso" (IGB-CNR), National Research Council, Naples, [2]Institute of Protein Biochemistry (IBP-CNR), National Research Council, Naples, Italy.

Background and aims: PPARγ is the master transcription factor controlling the adipocyte differentiation. Moreover, it regulates the expression of genes responsible of lipid metabolism, glucose and insulin homeostasis. During hypertrophic obesity, characterized by chronic inflammation, the overall expression and activity of PPARγ is compromised in adipose tissue determining defective adipogenesis and insulin resistance. Interestingly, our group identified dominant negative PPARγ isoforms (DNIs), highly expressed in human and mouse adipose tissue and able to affect PPARγ target genes involved in lipid and glucose homeostasis. Here we aim to analyze the impact of inflammation and the expression of PPARγ canonical and DNIs in hypetrophic adipocytes *in vitro* model.

Materials and methods: h*TERT*-immortalized adipose-derived mesenchymal stem cells (AdMSCs) were used as *in vitro* model of adipocyte differentiation. AdMSCs were treated with two differentiation mix containing insulin, rosiglitazone and other adipogenic *stimuli* for 20 days. Then, different experimental conditions (e.g. prolonged differentiation time and free fatty acid mix) were used to induce hypertrophy in mature adipocytes. Lipid accumulation was evaluated by Oil red O staining and densitometric measurement. Undifferentiated AdMSCs were treated for 24hrs with conditioned media of human monocyte-derived macrophages (LPS-stimulated), hypertrophic adipocytes, human cancer cell lines (including MCF7 and MDA) and human recombinant proteins of proinflammatory cytokines and chemokines. Expression analyses were performed by qPCRs and data were reported as mean values of at least three biological replicates and analyzed by paired Student t test. *P* value <0.05 was considered statistically significant.

Results: Human hypertrophic adipocytes generated *in vitro* from AdMSCs showed a significant increase of lipid droplet size and lipid accumulation compared to terminally differentiated mature adipocytes. The expression of PPARγ-target genes, including *GLUT4*, *IRS2* and *ADIPOQ*, was significantly impaired in hypertrophic cells *vs* mature adipocytes. Notably, these cells showed an increased expression ratio of DNIs/canonical isoforms compared to mature ones. Furthermore, we assessed the effects of several pro-inflammatory *stimuli* on undifferentiated AdMSCs - including conditioned media of human macrophages, hypertrophic adipocytes and cancer cell lines, as well as human recombinant cytokines and chemokines (e.g. IL8, TNFα, IL1β, IL6) - detecting an increased ratio of DNIs/canonical isoforms, similarly to hypertrophic adipocytes. These results suggest that inflammatory *milieu* in hypertrophic adipose tissue could alter the relative expression of canonical and dominant negative PPARγ isoforms, in turn compromising adipocyte differentiation and insulin-sensitivity.

Conclusion: For the first time we report the expression of PPARγ DNIs in hypertrophic adipocytes. Our data reveal a significant shift of DNIs/canonical PPARγ levels in these cells and a concomitant reduced expression of PPARγ-target genes, in line with the global impairment of PPARγ activity in the obese state. Additionally, our data suggest that this alteration is due to pro-inflammatory *stimuli* and cells which are hallmark of insulin-resistant adipose tissue in hypertrophic obesity.

Disclosure: **S. Cataldi:** None.

523

Resistin inhibits neuronal autophagy through Toll Like Receptor (TLR) 4

Y. Benomar, J. Miao, S. Al Rifai, G. Poizat, L. Riffault, D. Crepin, M. Taouis;
Molecular Neuroendocrinology of food intake, Paris-Saclay Institute of Neuroscience UMR9197, University Paris Sud, Orsay, France.

Background and aims: Autophagy is known to play a crucial role in the maintenance of cellular energy homeostasis and was identified as a non-selective degradation pathway induced when cells are energy-deprived. Autophagy also occurs in non-starved cells by participating in cellular inflammatory responses through mainly eliminating injured and aged mitochondria that constitute an important source of reactive oxygen species implicated in cellular inflammation. We have previously reported that resistin, through the activation of hypothalamic TLR4 receptor, induces whole body insulin resistance and promotes neuronal inflammation, but however, whether a link exists between resistin and autophagy in neurons is unknown. In the present study, we hypothesized that resistin-induced neuroinflammation could be attributed, at least partially, to a defect or deregulation of autophagy process in neuronal cells.

Materials and methods: Using human neuroblastoma cell line SH-SY5Y cells, we analysed the impact of serum deprivation and resistin treatment, for different period of times, on key cellular markers of autophagy, including ATG7, Beclin1, LC3I/II and p62, this was assessed by immunohistochemistry, western blotting and RTqPCR analyses. The role of TLR4 on neuronal autophagy induced by resistin, was also assessed in TLR4-depleted SH-SY5Y cells. Additionally, resistin-dependent neuronal autophagy was evaluated in vivo, in the hypothalami of wild type and TLR4 knockout mice treated with or without resistin through ICV route.

Results: As expected, we show that serum-starvation increases autophagy in human SH-SY5Y neuroblastoma cell line as evidenced by increased expression of LC31/II and ATG7 and downregulation of p62 considered as key autophagy markers. Interestingly, we report that resistin inhibits starvation-induced neuronal autophagy, and this effect is exacerbated in the presence of NH4Cl, an inhibitor of autosomal degradation. Using siTLR4 approach, we also demonstrate that resistin impairs autophagy through TLR4. Furthermore, we decipher the signaling pathways involved in resistin action and demonstrate that resistin inhibits AMPK phosphorylation and increases Akt/mTOR phosphorylation contrasting with autophagy impact on these signalling pathways. Finally, we validated the impact of resistin in mice and showed, in the hypothalamus, that the inhibitory effect of resistin towards autophagy markers is completely abolished in TLR4 knockout mice.
Conclusion: Altogether, these findings reveal resisitn/TLR4 as a new regulatory pathway of neuronal autophagy and contribute to the understanding of the underlying mechanisms involved in the impairment of neuronal autophagy.
Disclosure: **Y. Benomar:** None.

524

Obesity is associated with a more inflammatory phenotype of macrophages in human pancreatic islets

W. He, K. Maedler;
University of Bremen, Bremen, Germany.

Background and aims: Obesity is a major risk factor for the development of type 2 diabetes (T2D). A chronic, low-grade, sterile inflammation is present in obesity; tissue macrophages contribute to such inflammation, leading to persistent insulin resistance and β-cell failure. Changes in macrophage polarization are tightly associated to macrophage function and are involved in many diseases. In this current research, we aimed to characterize potential obesity-related changes in macrophage polarization markers in human pancreatic islets.
Materials and methods: To establish the relationship between obesity and islet macrophage markers, expression of macrophage polarization markers *ITGAX (CD11c)*, *NOS2 (iNOS)* and *IL1b* as M1 macrophage-associated genes, *CD163*, *IL10* and *TGFb* as M2 macrophage-associated genes and *CD68* as general macrophage marker was analyzed by qRT-PCR in isolated human pancreatic islets from obese (BMI >30) and non-obese (BMI <30) donors as well as in human islets following depletion of islet resident macrophages by treatment of clodronate liposomes.
Results: Islets from obese donors expressed significantly more *NOS2* (2.7-fold) than from non-obese donors, while general macrophage marker *CD68* and M2 macrophage markers *CD163* and *IL10* were not significantly changed between obese and non-obese donors. After depletion of islet resident macrophages by clodronate treatment, expression of *ITGAX*, *IL1b*, *IL10* and *CD163* was mostly deprived in human islets, indicating macrophage-dependency of these genes. In contrast, *TGFb* expression was unchanged, suggesting a macrophage-independent source in human islets. *NOS2*expression was not affected by clodronate treatment in islets from non-obese donors, but reduced in islets from obese donors, which suggests an obesity-induced iNOS expression in macrophages of human islets.
Conclusion: Our results indicate that CD11c, IL-1β, IL-10 and CD163 in human islets are associated with islet macrophages. The increased islet macrophage *NOS2* expression in obese individuals suggests that obesity is associated with a switch to an M1-like inflammatory phenotype in islet macrophages, which may further contribute to obesity-induced islet inflammation and finally β-cell failure.
Supported by: German Research Foundation (DFG)
Disclosure: **W. He:** None.

525

Human obesity alters circadian clock function through NF-κB activation

E. Maury[1], L. Noel[1], B. Navez[2], S.M. Brichard[1];
[1]Endocrinology, Diabetes and Nutrition Unit, Institute of Experimental and Clinical Research, Faculty of Medicine, Catholic University of Louvain, Brussels, [2]Digestive Surgery Unit, Medical Sector, Catholic University of Louvain, Brussels, Belgium.

Background and aims: Nutritional environment affects the transcription-translation feedback oscillators of the circadian clock that maintain metabolic homeostasis. These loops are composed of activators (BMAL1 and CLOCK) that induce the transcription of repressors, the most important being PERIOD 2 (PER2). PER2 inhibits the forward limb and this process generates a rhythm of ~24 hr. Experimental genetic models have provided evidence that many complications of obesity are mediated through metabolic inflammation induced by activation of the transcription factor NF-κB. Here, we investigated the link between clock disruption and NF-κB mediated inflammation in human obesity.
Materials and methods: Human omental adipose tissue was obtained from 5 obese and 5 non-obese age-matched patients (body mass index: ~50 *vs.* 25 kg/m^2) undergoing abdominal surgery. Using fluorescence-activated cell sorting analysis, omental preadipocytes obtained from the stromal vascular fractions were identified as PDGFRα$^+$, CD31$^-$, CD45$^-$ cells. Preadipocytes were infected with *Per2-dLuciferase*-expressing lentivirus (a rapidly-degradable form of luciferase driven by the *Per2* gene promoter), synchronized and bioluminescence was recorded continuously for 5–7 days. Binding of the p65 subunit of NF-κB and BMAL1 to *PER2* promoter was measured by chromatin immunoprecipitation (ChIP). ChIP of RNA polymerase II was also performed to explore the rhythms in nascent transcription. Additionally, ChIP were conducted on *CCL2*, a chemokine involved in the pathogenesis of type 2 diabetes. In some experiments, NF-κB inhibition was achieved either by pharmacological or gene silencing approaches, restoring NF-κB activity of obese subjects to a non-obese state.
Results: By measuring *Per2-dLuciferase* bioluminescence, we described endogenous cell-autonomous human adipose tissue oscillators, independently of food intake and nutrient signals. We found a period lengthening of *Per2-dLuciferase* oscillations in obese preadipocytes (23.33 ± 0.11 hr *vs.* 22.53 ± 0.27 hr, $p < 0.05$), reflecting an altered function of the molecular clock. ChIP assays revealed that p65 binding to *PER2* is enhanced by almost 10 times in obese preadipocytes ($p < 0.05$) while binding of BMAL1 and RNA polymerase II are significantly decreased ($p < 0.05$) consistently with the reduced *PER2* expression observed. Increased NF-κB activity was also associated with altered BMAL1 occupancy on *CCL2* promoter and a ~10 fold increased recruitment of RNA polymerase II ($p < 0.05$), consistently with increased CCL2 expression and secretion. NF-κB inhibition in obese preadipocytes restored BMAL1 binding and clock function to a non-obese level.
Conclusion: Collectively, our findings demonstrate that obesity alters clock function through NF-κB signaling in human omental adipose tissue. Our results suggest that the disruption of these oscillators in obesity leads to abnormal chemokine production and may contribute to metabolic disorders.
Supported by: ATTRACT Innoviris (ref. 2016-BB2B-4)
Disclosure: **E. Maury:** None.

526

Complement C3 and C4, but not their regulators or activated products, are associated with metabolic syndrome: the CODAM study

Y. Xin, E. Hertle, C.J.H. van der Kallen, C.G. Schalkwijk, C.D.A. Stehouwer, M.M.J. van Greevenbroek;
Internal Medicine, Maastricht University Medical Centre and CARIM School for Cardiovascular Diseases, Maastricht, Netherlands.

Background and aims: Metabolic syndrome is identified as a precursor for advanced metabolic diseases, such as type 2 diabetes and cardiovascular disease. Obesity is one of the initial events in the pathological processes that define metabolic syndrome. Plasma complement concentrations are higher in people with obesity and may contribute to adipose tissue dysfunction and development of the metabolic syndrome. We investigated the associations of components of the alternative (C3, C3a, Bb, factor D [FD], factor H [FH], properdin], and the classical complement pathways (C4, C1q, C1-inhibitor [C1-INH]) with prevalent and incident metabolic syndrome in a cohort with moderately increased risk of cardiometabolic disease.
Materials and methods: The study cohort comprised 574 participants (61% men, age 59.6 ± 7.0 years) at baseline and 489 participants after 7-year follow-up. Linear regression models were used to evaluate the associations between complement concentrations and metabolic syndrome components (i.e. triglycerides, HDL-cholesterol, glucose, systolic and diastolic blood pressure, waist circumference) at baseline. Multiple logistic regression analyses were used to investigate associations of baseline plasma complement concentrations (standardized values) with (1) prevalent metabolic syndrome, and (2) incident metabolic syndrome in those without metabolic syndrome at baseline (n = 189). Additional analyses were done to investigate the possible driver(s) and underlying mechanisms of the prospective associations.
Results: In linear regression analyses, all complement factors, except for factor Bb, were significantly, and in an adverse direction, associated with one or more individual components of metabolic syndrome. In logistic regression analyses, C3 was higher in those with metabolic syndrome compared to those without (odds ratio (OR) = 3.60 [95% confidence interval: 2.73; 4.75]). Similar associations were observed for C3a (OR = 1.25 [1.03; 1.52]), FH (OR = 2.93 [2.24; 3.83]), properdin (OR = 1.88 [1.50; 2.34]), and C4 (OR = 1.39 [1.13; 1.69]), but not for Bb, C1q or C1-INH. Only C3 (OR = 1.48 [1.02; 2.14]) and C4 (OR = 1.95 [1.32; 2.88]) were associated with incident metabolic syndrome (n = 40 cases). After additional adjustment for baseline levels of components of the metabolic syndrome, this prospective association was substantially attenuated for C3, but not for C4.
Conclusion: Our results show significant cross-sectional associations of several complement components with the prevalence of metabolic syndrome. Among those, only C3 and C4 were found to be involved in the development of metabolic syndrome, potentially via different underlying mechanisms. Further studies are needed to better understand the etiological pathways that underlie the relationship between complement and the metabolic syndrome.
Disclosure: **Y. Xin:** None.

527

Circulating WISP1/CCN4 is associated with body fat distribution and adipose tissue dysfunction in subjects with type 2 diabetes

V.V. Klimontov[1], D.M. Bulumbaeva[1], A.P. Lykov[1], O.N. Fazullina[1], N.P. Bgatova[1], A.F.H. Pfeiffer[2,3], O. Pivovarova[2,4], N. Rudovich[2,5];
[1]Research Institute of Clinical and Experimental Lymphology – Branch of the Institute of Cytology and Genetics, Novosibirsk, Russian Federation, [2]German Center for Diabetes Research (ZDZ), Muenchen-Neuherberg, Germany, [3]Charité University Medicine, Berlin, Germany, [4]German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany, [5]Spital Bülach, Bülach, Switzerland.

Background and aims: Wnt1-inducible signaling pathway protein 1 (WISP1), also known as CCN4, is a member of the CCN family of secreted, extracellular matrix associated signaling proteins. Recently WISP1 was validated as a novel adipokine that may play a role in linking obesity to inflammation and insulin resistance. The data on WISP1 in diabetes are scarce. The aim of our study was to assess the relationships between the circulating WISP1 and the levels of other adipokines, inflammatory markers, fat mass and body fat distribution, and parameters of glycemic control in type 2 diabetic subjects.
Materials and methods: We observed 156 patients, 45 M/111 F, from 41 to 80 years of age (median 61 years), including 102 subjects with obesity. Twenty four non-obese non-diabetic subjects, matched by age and sex, were acted as control. The levels of WISP1, high-sensitivity C-reactive protein (hsCRP), alpha1-acid glycoprotein, and macrophage inflammatory protein 1alpha (MIP-1alpha) were measured in the fasting serum by ELISA. Serum concentrations of leptin, resistin, visfatin, adipsin, adiponectin, IL-6, IL-8, IL-18 and TNF-alpha were determined by Multiplex analysis. The fat mass and distribution was assessed by DEXA. Glucose variability (GV) parameters: Mean Amplitude of Glucose Excursions, Continuous Overlapping Net Glycemic Action, High Blood Glucose Index, and Low Blood Glucose Index were derived from continuous glucose monitoring. The mean diameter of adipocytes was estimated in the samples of subcutaneous adipose tissue in 25 patients.
Results: Patients with diabetes, as compared to control, had significantly higher levels of WISP1 (p = 0.02), leptin (p = 0.005), resistin (p < 0.0001), adipsin (p < 0.0001), visfatin (p = 0.0003), hsCRP (p < 0.0001), AGP (p < 0.0001), MIP-1alpha (p = 0.006) and IL-6 (p = 0.01). Other investigated molecules did not shown significant differences. Serum WISP1 levels demonstrated positive correlation with percentage of fat mass in central abdominal area (r = 0.46, p < 0.001). No associations with BMI, total fat mass and mean adipocyte diameter were found. The concentrations of WISP1 demonstrated positive correlations with resistin, visfatin and MIP-1alpha levels (r = 0.36, r = 0.28 and r = 0.47 respectively, all p < 0.01), but it did not correlated with other inflammatory markers, HbA1c and estimated GV parameters. In a multiple regression analysis percentage of fat mass in central abdominal area was the only reliable predictor of serum WISP1 levels (beta = 0.393, p = 0.04).
Conclusion: In subjects with type 2 diabetes serum levels of circulating WISP1 are associated with body fat distribution and adipose tissue dysfunction. The relationships between WISP1 and inflammation need further investigations.
Disclosure: **V.V. Klimontov:** None.

PS 034 Animal models of type 2 diabetes and obesity

528

Genetic deletion of RAGE in db/db mice interferes in the liver with other AGE-receptors and AGE-detoxifying systems sustaining lipogenesis and inflammation

R. Mastrocola[1,2], A.S. Cento[1], D. Collotta[3], F. Chiazza[3], F. Barutta[4], J. Scheijen[2], M. Aragno[1], K. Gaens[2,5], M. Collino[3], C.G. Schalkwijk[2,5], K. Wouters[2];
[1]Dept. of Clinical and Biological Sciences, University of Turin, Turin, Italy, [2]Dept. of Internal Medicine, University of Maastricht, Maastricht, Netherlands, [3]Dept. of Drug Science and Technology, University of Turin, Turin, Italy, [4]Dept. of Medical Sciences, University of Turin, Turin, Italy, [5]CARIM School for Cardiovascular Diseases, University of Maastricht, Maastricht, Netherlands.

Background and aims: Advanced glycation end products (AGEs) are toxic compounds involved in the onset of insulin resistance in obesity. In particular, AGEs are preferentially trapped by adipose tissue through the binding with the AGE-receptor RAGE, leading to the activation of proinflammatory signalling in adipocytes that can interfere with peripheral insulin sensitivity. The genetically-induced deletion of RAGE in leptin receptor deleted (db/db) mice, a model of type 2 diabetes/obesity, is reported to prevent AGEs trapping in adipocytes, paralleled by increased circulating levels of AGEs, and reduced adipose tissue inflammation and insulin resistance. Since this might increase the exposition to AGEs of highly perfused organs, such as the liver, we aimed to analyze whether the deletion of RAGE affected hepatic AGEs accumulation and detoxification in the liver of obese/diabetic animals.
Materials and methods: At 13 weeks of age, wild-type C57, db/db and db/db RAGE-/- mice were sacrificed, plasma was collected and liver was removed. Gene and protein expression of AGEs receptors and detoxifying systems were analyzed in parallel to hepatic AGEs content, activation of lipogenesis, and markers of inflammation.
Results: The deletion of RAGE in the liver of db/db mice was associated with decreased expression of AGE-receptor-1 ($P < 0.05$ vs. db/db) and reduced expression and activity of glyoxalase-1 ($P < 0.01$ vs. db/db), two major AGEs detoxifying systems, and increased galectin-3 expression ($P < 0.05$ vs. db/db), another AGEs-receptor. The latter may be a compensatory response to remove plasma AGEs. Thus, despite the lacking of RAGE, high levels of intrahepatic AGEs were maintained in db/db RAGE-/- mice, due to either the trapping exerted by galectin-3 and/or the reduced potential of detoxifying systems. These alterations were also associated to persistent activation of the SREBP1c lipogenic pathway ($P < 0.001$ vs. C57), and the proinflammatory NLRP3 signaling pathway ($P < 0.05$ vs. C57), that were not prevented by RAGE deletion compared to db/db mice.
Conclusion: RAGE deletion in the liver of an animal model of type 2 diabetes influences other AGE-receptors and AGE-detoxifying systems. In particular, the increase in galectin-3 in db/db RAGE-/- mice liver might be responsible for the sustained hepatosteatosis and inflammation. This complex mechanism of control should be taken into account when investigating on the pathogenic contribution of AGEs to hepatic obesity/diabetes complications.
Supported by: EMBO short-term fellowship
Disclosure: **R. Mastrocola:** None.

529

Constitutive Androstane Receptor deficiency leads to sexually dimorphic metabolic disorders in aging

C. Lukowicz[1], M. Régnier[1], F. Lasserre[1], A. Polizzi[1], A. Montagner[2], S. Smati[1], Y. Lippi[1], F. Lenfant[1], V. Theodorou[1], L. Gamet-Payrastre[1], S. Ellero-Simatos[1], P. Gourdy[2], N. Loiseau[1], H. Guillou[1], L. Mselli-Lakhal[1];
[1]INRA ToxAlim, Toulouse, [2]INSERM I2MC, Toulouse, France.

Background and aims: The Constitutive Androstane Receptor (CAR or NR1I3) is a key transcription factor regulating the expression of xenobiotic metabolizing enzymes. It is highly active in the liver and plays an important role in the protection of the organism against exogenous but also endogenous toxic molecules such as bile acid and bilirubin. It is also involved in the catabolism of thyroid and steroid hormones. Therefore CAR contributes both to detoxication and to the energy homeostasis. Its contribution to the regulation of metabolism remains misunderstood and has been mostly studied in males. However many aspects of liver function are gender-dependent. In this work, we aimed at investigating whether sex-steroid hormones may influence CAR-dependent signaling.
Materials and methods: We followed male and female C57BL6/J (WT) or CAR knockout mice (CAR-/-) fed with a standard chow diet for 16 months and assessed different metabolic parameters such as body weight, glucose and insulin tolerance over 1 year. We collected the livers and other tissue samples and analyzed them through biochemical, histological, and transcriptomic approaches.
Results: CAR-/- males become obese in aging (body weight gain +11.02 g compared to WT). This obesity is associated with a glucose and insulin intolerance as well as hyperglycemia and hyperinsulinemia. CAR-/- males also develop a dyslipidemia and an important steatosis, with significant hepatocyte cytolysis. CAR -/- females show a different metabolic profile with a slight overweight, a better glucose tolerance and no dyslipidemia and no steatosis. Liver transcriptomic analyzes performed in both gender revealed a sexually dimorphic profile of CAR-dependent functions with distinct regulation of gene involved in steroid hormone catabolism between male and female CAR-/- (*Cyp17a1*, *Cyp21a1*, *Srd5a1*, *Srd5a3*). Ovariectomy of female CAR-/- mice led them to develop the same metabolic disorders as observed in males, namely obesity, glucose intolerance, fasted hyperglycemia and hepatic steatosis.
Conclusion: This study highlights a gender-dependent impact of CAR on metabolism. The absence of CAR promotes metabolic disorders in males, potentially due to high levels of corticosterone. In contrast, females are protected from these diseases through a process requiring sexual steroid hormones. This protective mechanism offers interesting possibilities for the treatment of metabolic syndrome.
Supported by: Région Occitanie ANR
Disclosure: **C. Lukowicz:** None.

530

Inhibition of MG53 E3 ligase activity as a possible new target in the treatment of type 2 diabetes

J. Reinke[1], T. Sadowski[2], K. Breitschopf[3];
[1]R&D Diabetes Division, GI Endocrinology & Obesity, Sanofi-Aventis Deutschland GmbH, Frankfurt, [2]R&D Diabetes Division, Comorbidities and Complications, Sanofi-Aventis Deutschland GmbH, Frankfurt, [3]Global Research Project Management, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany.

Background and aims: Mitsugumin 53 (MG53) belongs to the muscle-specific tripartite motif-containing (TRIM) family and is also known as TRIM72. In mice MG53 is expressed in skeletal muscle and heart, and plays a dual role as an E3 ligase and as a cellular membrane repair protein. The E3 ligase function of MG53 was proposed to target both insulin receptor (IR) and insulin receptor substrate 1 (IRS1) for ubiquitin-dependent degradation. MG53 deficiency protects mice against high fat diet (HFD)-induced metabolic syndrome, but also leads to progressive skeletal myopathy. The aim of this study were to develop a better understanding of the MG53 ligase function in the setting of T2D, and to demonstrate that selectively inhibiting the ligase activity of MG53 is appropriate for developing insulin sensitizing anti-diabetic drugs.

Materials and methods: To address our aims, we generated a transgenic mouse carrying a C14S substitution in the MG53 gene (MG53-KI mouse). This mutation has been described to specifically inactivate the E3 ligase function, while all other functions of MG53 are maintained. Investigation and characterization of the MG53-KI mice compared to their littermate-control wildtype mice (WT) under chow (ND) and high fat diet (HFD) conditions, including gene and protein expression (qRT-PCR, western blot), ipGTT and insulin signaling pathway analysis, were performed.
Results: Mice specifically lacking MG53 ligase function (MG53-KI) and WT mice showed the same *TRIM72* mRNA expression level in skeletal muscle. However, MG53 relative protein expression was, independent of the diet, reduced by more than 95% (ND: WT ($n = 5$) vs. MG53-KI ($n = 6$), $p < 0.001$; HFD: WT ($n = 6$) vs. MG53-KI ($n = 7$), $p < 0.001$) in MG53-KI mice compared to WT mice. Body weight and body composition analysis performed after MG53-KI and WT mice were placed for 22 weeks on ND or HFD exhibited no differences. An ipGTT performed in WT or MG53-KI mice placed on HFD for 10 weeks revealed a slightly decreased glucose tolerance in MG53-KI animals (Glucose AUC: WT ($n = 6$) vs. MG53-KI ($n = 7$), $p < 0.05$). Insulin signaling pathway activity in skeletal muscle was not altered as shown by similar AKT and GSK-3 α/β phosphorylation.
Conclusion: The newly generated MG53-KI mouse showed normal *TRIM72* mRNA expression level, but resembled the MG53 KO mouse in respect to MG53 protein expression. However, published data demonstrating that MG53 deficiency protects mice against HFD-induced metabolic syndrome was not reproduced. Our data does not support a role for MG53 on metabolic parameters in the setting of T2D. The reason for reduced MG53 protein levels in the MG53-KI mouse is currently unknown and requires further study.
Disclosure: **J. Reinke:** None.

531

Interindividual susceptibility to type 2 diabetes in mice: hepatic transcriptome and DNA methylome profiling

S. Saussenthaler[1,2], M. Ouni[1,2], M. Jähnert[1,2], P. Huypens[2,3], J. Beckers[2,3], A. Schürmann[1,2];
[1]German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke, [2]German Center for Diabetes Research (DZD), Neuherberg, [3]Helmholtz Center Munich (HMGU), Neuherberg, Germany.

Background and aims: The development of type 2 diabetes (T2D) is driven by genetic as well as life style factors. However, genetically identical mice maintained on a high-fat diet exhibit a broad variation in T2D onset. The aim of this study was to identify perturbations in the hepatic transcriptome and DNA methylome prior to the onset of T2D and to examine whether these DNA methylation differences are sufficient for the prediction of the disease.
Materials and methods: Female New Zealand Obese mice were classified into diabetes-resistant (DR) and diabetes-prone (DP) cohorts based on their liver fat content combined with early blood glucose concentrations at 10 weeks of age. This prediction allowed the isolation of metabolically relevant tissues, including the liver, several weeks before T2D onset. Liver transcriptome and DNA methylome were analyzed using RNA sequencing and whole genome bisulfite sequencing, respectively ($n = 6$ per group). Pathway enrichment analysis was conducted with DAVID 6.7 in order to determine biological processes and signaling pathways that are dysregulated prior to the manifestation of T2D.
Results: Liver transcriptome analysis uncovered 1372 differentially expressed transcripts ($p < 0.05$; FPKM >1) between DR and DP mice. These transcripts were enriched in metabolic processes, such as fatty acid metabolism and citrate cycle. Additionally, whole genome bisulfite sequencing led to the identification of 455,782 differentially methylated CpG sites between both groups ($p < 0.05$; minimal 24 read counts per group). The integration of both datasets as well as implementation of stringent filtering criteria including significant Pearson correlation of CpG methylation and corresponding mRNA expression ($p < 0.05$) led to the identification of 151 CpG sites that are highly conserved between mouse and human and related to 112 transcripts. Identified transcripts showed significant enrichment in insulin, VEGF and mTOR signaling as the most relevant hepatic alterations preceding T2D.
Conclusion: In the liver, several genes involved in insulin, VEGF and mTOR signaling are affected by DNA methylation. Additionally, the most promising candidates will be analyzed in blood cells in order to evaluate them as T2D biomarker in humans.
Supported by: 82DZD00302
Disclosure: **S. Saussenthaler:** None.

532

Two immune-related GTPases prevent from hepatic fat accumulation by inducing autophagy

K. Schwerbel[1,2], A. Kamitz[1,2], M. Jähnert[1,2], P. Gottmann[1,2], F. Schumacher[3], B. Kleuser[3], T. Haltenhof[4], F. Heyd[4], M. Roden[5,2], A. Chadt[5,2], H. Al-Hasani[5,2], W. Jonas[1,2], H. Vogel[1,2], A. Schürmann[1,2];
[1]German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke, [2]German Center for Diabetes Research (DZD), Neuherberg, [3]University of Potsdam, Potsdam, [4]Freie Universität Berlin, Berlin, [5]German Diabetes Center (DDZ), Düsseldorf, Germany.

Background and aims: The cause of non-alcoholic fatty liver disease (NAFLD) is multifactorial including genetic and environmental factors. However, the genetic basis of this disease still remains incompletely defined. In a backcross population of New Zealand obese (NZO) and C57BL/6J (B6) mice, a major quantitative trait locus (QTL) designated *Ltg/NZO* for increased liver triglycerides was identified on chromosome 18. Two genes coding for immune-related GTPases appear to be the most likely genes responsible for the effect of *Ltg/NZO*. The aim of the study was to characterize the role and function of both GTPases.
Materials and methods: Recombinant congenic mice carrying 5.3 Mbp of *Ltg/NZO* were fed a high-fat diet and metabolically characterized in respect to their hepatic insulin sensitivity, autophagic capacity and lipid profile. Bioinformatics analysis and Electrophoretic Mobility Shift Assay (EMSA) were performed to elucidate the genetic cause for the differential expression pattern.
Results: NZO-allele carriers ($Ltg/NZO^{N/N}$) showed 2-fold higher liver triglyceride concentration than B6-allele carriers ($Ltg/NZO^{N/B}$) due to a reduced induction of autophagy in the liver. Furthermore, $Ltg/NZO^{N/N}$ revealed impaired hepatic insulin sensitivity in line with higher diacylglycerol levels. Haplotype mapping and expression studies identified two immune-related GTPases, *Ifgga2 and Ifgga4*, as most likely candidates of *Ltg/NZO*. Expression of *Ifgga2* and *Ifgga4* was lower in livers of $Ltg/NZO^{N/N}$ compared to $Ltg/NZO^{N/B}$ mice by 5.6-fold and 16.4-fold, respectively. An active enhancer element which harbors a FOXO1-binding motif is located upstream of both genes. $Ltg/NZO^{N/N}$ mice carry a one base pair deletion next to the FOXO1-binding motif. EMSA analysis indicates that this deletion is responsible for the reduced expression of both GTPases. Moreover, the human orthologue *IRGM* was significantly lower expressed in the liver of NAFLD patients compared to that of lean subjects.
Conclusion: A sufficient expression of *IRGM* and its orthologous, *Ifgga2* and *Ifgga4*, prevent from the hepatic accumulation of triglycerides in humans and mice.
Clinical Trial Registration Number: NCT01477957
Supported by: 82DZD00302
Disclosure: **K. Schwerbel:** None.

533

Impact of different mtDNA mutations on glucose homeostasis and fat accumulation in liver in aging mice

C. Johne, S. Schröder, M. Tiedge, S. Baltrusch;
Institute of Medical Biochemistry and Molecular Biology, Rostock University, Rostock, Germany.

Background and aims: Mutations of the mitochondrial encoded subunits of the respiratory chain lead to mitochondrial dysfunction and an impaired metabolism, two risk factors that have been discussed to promote the pathogenesis of type 2 diabetes mellitus. In a comparison of 3 different conplastic mouse strains we investigated the effects of various mtDNA point mutations on the blood glucose level and fat distribution during aging.
Materials and methods: At the age of 3, 6, 9, and 12 month mice from the following strains were examined: C57BL/6NTac (control) and the conplastic mouse strains C57BL/6NTac-mt$^{BPL/1J}$ (NADH dehydrogenase mutation und cytochrome c oxidase mutation, mtBPL), C57BL/6NTac-mt$^{NOD/LtJ}$(cytochrome c oxidase and t-RNA-Arg mutation, mtNOD) and C57BL/6NTac-mt$^{A/J}$ (t-RNA-Arg mutation). Blood glucose, serum insulin and triglycerides were measured from blood samples and fat distribution was subsequently analyzed after removal of the organs.
Results: Fasten blood glucose level at 3 month old control mice were elevated (5 mmol/l) compared to mice from all three conplastic strains (3.6–4 mmol/l). Low blood glucose level in 9 month old mtNOD mice correlated with increased serum insulin at that age. Such correlation was also found with mtBPL mice. These animals showed an increase in blood glucose level up to the age of 9 month (up to 5 mmol/l) but a decline at 12 month (4.4 mmol/l). Both control and mtNOD mice showed higher triglyceride level in serum at the age of 3 month. At later time points no differences between all 4 strains were observed. In contrast, the level of liver triglycerides increased with age in all mice, but was significantly higher in 12 month old mtBPL mice.
Conclusion: Aged 12 month old mice from the two conplastic strains mtNOD and mtBPL, which only differ in two point mutations in the mitochondrial genome, display clear differences in insulin controlled blood glucose homeostasis, fat redistribution and lipid accumulation in liver. The rate of mtDNA mutations increases with age due to missing repair mechanisms in mitochondria. Thus, mtDNA mutations could accelerate the aging process in a type of vicious circle and thereby facilitate the pathogenesis of type 2 diabetes mellitus.
Supported by: BMBF
Disclosure: **C. Johne:** None.

534

The experiment study of effects of multi-electrode renal denervation on insulin sensitivity and glucose metabolism

T. Pan[1], Y. Qian[2], G.-J. Teng[3];
[1]Southeast University, Nanjing, [2]Department of Anesthesiology, Nanjing Drum Tower Hospital, Nanjing, [3]Zhongda Hospital, Southeast University, Nanjing, China.

Background and aims: Renal denervation (RDN) may have some impact on insulin resistance and type 2 diabetes mellitus (T2DM), but the efficacy is still in controversy, and the effects and pathophysiology of multi-electrode RDN to treat T2DM are largely unknown. We sought to observe the effects of multi-electrode catheter-based RDN on insulin sensitivity and glucose metabolism in a canine model of T2DM.
Materials and methods: Thirty-three canines were divided equally into three groups: bilateral renal denervation (BRDN) group, left renal denervation (LRDN) group and sham operation (SHAM) group. Body weight and blood biochemistry were determined at baseline, 20-week and 32-week, and renal arterial angiography and computerized tomographic angiography (CTA) were determined before, 1-month, 2-month and 3-month after surgery. In addition, western blot was performed to identify the activities of gluconeogenic enzymes and insulin signaling proteins.
Results: High-fat diet feeding and streptozotocin injection succeeded leading to canine models of T2DM at 20-week. Compared with SHAM group, fasting plasma glucose, fasting insulin, homeostasis model assessment-insulin resistance, noradrenaline and angiotensin II in RDN groups had significantly decreased at 3-month follow-up. CTA and histopathological analyses did not show any dissection, aneurysm, thrombus or rupture of all the renal arteries. Western blot analyses showed that RDN modulated insulin action via the activation of insulin receptors-AKT signaling cascade in the liver, giving rise to the suppression of the gluconeogenetic genes.
Conclusion: These findings identified that multi-electrode catheter-based RDN could effectively decrease gluconeogenesis and glycogenolysis, resulting in improvements in insulin sensitivity and glucose metabolism in canines with T2DM.
Supported by: National Natural Science Foundation of China General Project (81671795)
Disclosure: **T. Pan:** None.

535

Next generation of spontaneous diabetic model of ZDSD rats with intact leptin signalling develop cardiac dysfunction and compromised cardiac dysfunction

G. Sun, G. Zhang, V. Jackson, J. Wang;
Crown Bioscience, San Diego, USA.

Background and aims: Cardiomyopathy is the leading cause of morbidity and mortality among all complications of type 2 diabetic (T2D) and obese patients. Diabetic cardiomyopathy (DC) is characterized by an initial cardiac hypertrophy followed by thinning of the cardiac walls with declines in both systolic and diastolic functions, which ultimately leads to heart failure. No rodent models fully captured phenotypes of DC. The ZDSD rat, a new generation of T2D rat model with intact leptin signaling features with slow onset of diabetes, obesity and dyslipidemia, which closely mimics the development of the disease in patients. Here we sought to evaluate the cardiac function and reserve during the development of metabolic syndromes in ZDSD rats.
Materials and methods: 12 male ZDSD rats and age-matched SD controls were monitored for blood pressure, glucose, and cardiac function using echocardiography. Animals were also challenged with 1 mg/kg dobutamine for the assessment of cardiac reserve.
Results: ZDSD rats developed hypertension from age of 18 weeks with both systolic and diastolic blood pressure significantly higher than controls. Their left ventricular (LV) functions were compromised along with changes in cardiac morphology. At resting state, ZDSD rats showed LV hypertrophy from age of 18 to 22 weeks after which cardiac walls became thinner with larger LV volume. Concomitantly, both ejection fraction (EF) and transmitral E/A ratio of LV declined at 34 weeks old. Upon treatment with dobutamine for 5 minutes, SD rats reached almost 98% EF and 80% fractional shortening (FS), while the values of ZDSD were 91% and 60% respectively after 30 weeks old, suggesting the loss of contractility and cardiac reserve of the animals.
Conclusion: ZDSD rats which carry multiple dysmetabolic phenotypes are spontaneously hypertensive with reduction in LV function and cardiac reserve which resembles ultrasonic symptoms of diabetic cardiomyopathy patients. Therefore, ZDSD rats may serve as a suitable preclinical model to study potential therapeutic approaches to treat cardiomyopathy with presence of metabolic syndromes.
Disclosure: **G. Sun:** None.

536

Aged New Zealand Obese (NZO) mice are protected against diet-induced loss of beta cells

R. Kehm[1,2], O. Kluth[1,2], A. Schürmann[1,2], T. Grune[1,2], A. Höhn[1,2];
[1]German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke, [2]German Center for Diabetes Research (DZD), München-Neuherberg, Germany.

Background and aims: Insulin-producing, pancreatic beta-cells are key regulators of blood glucose levels responsible for the maintenance of glucose homeostasis. In aging, their secretory capacity decreases

accompanied by impaired insulin action in peripheral tissues. Additionally, these effects are deteriorated by diet-induced obesity and low physical activity. Combined with limited regeneration capacities of beta cells, this contributes to a decline in functional mass and the onset of type 2 diabetes. In the adipose and diabetes-prone New Zealand Obese (NZO) mouse strain, peripheral insulin resistance and beta cell defects occur under a specific diet regimen. NZO mice were used to investigate the effects of aging on pancreatic beta-cell integrity and functionality under glucolipotoxic conditions.

Materials and methods: Starting at the age of 7 weeks, NZO mice were fed initially without carbohydrates for 11 (young) or 32 (aged) weeks. This was followed by a carbohydrate intervention for up to 21 days to induce hyperglycemia.

Results: As previously shown, a rapid and continuous increase in plasma blood glucose levels and a visible loss of beta-cells occurred in carbohydrate-fed, young NZO mice accompanied by a decrease in plasma insulin and proinsulin levels after 4 days. GLUT-2 immunostaining indicated a carbohydrate-induced loss of beta cell glucose-transporters. In contrast, aged NZO mice on carbohydrates revealed lower plasma blood glucose levels as well as increasing plasma insulin and proinsulin levels together with higher amounts of GLUT-2. Interestingly, compared to the young mice, an extended beta-cell mass and area was observed in aged NZO mice at the end of the intervention. Immunostaining of Ki-67 as a cellular proliferation marker, revealed no difference between young and aged NZO mice, but a reduced number of PDX-1^{+}-beta cells was found in young NZOs. Furthermore, preliminary analysis of microarray-based transcriptomics of isolated islets indicated that transcription factors essential for beta-cell development and insulin gene expression are downregulated in young, carbohydrate-fed NZO mice, whereas cell cycle regulators were upregulated in aged animals.

Conclusion: These findings suggest a less harmful effect of carbohydrates in aged NZO mice, presumably mediated via improved beta-cell maturation.

Supported by: DZD

Disclosure: **R. Kehm:** None.

PS 035 Inflammation in type 2 diabetes: human studies

537

Association of serum Sestrin2 level with metabolic risk factors in newly diagnosed drug-naive type 2 diabetes

Y. Lee[1], J. Kim[1], E. Roh[1], S. Hong[1], N. Kim[2], J. Seo[2], S. Kim[2], N. Kim[2], K. Choi[1], S. Baik[1], H. Yoo[1];
[1]Korea university guro hospital, Seoul, [2]Korea university hospital, Seoul, Republic of Korea.

Background and aims: The prevalence of diabetes is continuously increasing, accelerating the morbidity and mortality of cardiovascular disease (CVD) worldwide. A novel therapeutic strategy targeting the disease-specific underlying mechanism is essential to block the vicious cycle between diabetes and CVD. Sestrin2 is a newly discovered antioxidative molecule. Sesn2 functions as a stress-inducible metabolic regulator by inhibiting oxidative stress and pro-inflammatory signaling, mainly via mechanisms dependent on AMP-dependent protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1). Previous in-vitro and in-vivo experimental studies have shown that Sestrin2 attenuates oxidative stress and the pro-inflammatory pathway, resulting in improving metabolic homeostasis. However, the relationship between circulating Sestrin2 concentration and cardiometabolic risks in humans has not been explored.

Materials and methods: 240 subjects (46 without diabetes and 194 with diabetes) were included from the Korea Guro Diabetes Program. Sestrin2 concentration was measured using a commercially available enzyme-linked mmunosorbent assay (ELISA). We used carotid intima media thickness, brachial ankle pulse wave velocity and whole body dual-energy X-ray absorptiometry to evaluate the various cardiometabolic risk factors including body composition, insulin resistance, and atherosclerosis.

Results: Sestrin2 concentration showed a trend of increasing in subjects with metabolic syndrome. After adjustment for age and gender, Sestrin2 level had a positive relationship with serum triglyceride, alanine aminotransferase (ALT), and creatinine levels, but no association with carotid atherosclerosis. Especially, in subjects with type 2 diabetes Sestrin2 concentration exhibited a significant positive correlation with body mass index ($P = 0.015$), waist circumference ($P = 0.020$), high-sensitivity C reactive protein ($P = 0.008$), Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) ($P = 0.041$), percentage body fat ($P = 0.001$), and truncal fat mass ($P = 0.005$) after adjusting age and gender. Multiple stepwise regression analysis identified age, serum ALT and creatinine levels, and percentage body fat as independent determining factors for Sestrin2 concentration in patients with type 2 diabetes ($R^2 = 0.173$).

Conclusion: This study is the first to demonstrate a trend for increased Sestrin2 level in subjects with metabolic syndrome. In particular, in subjects with type 2 diabetes, Sestrin2 was significantly related to insulin resistance and percentage body fat, suggesting its potential as a novel modulatory factor for metabolic disorders in humans.

Disclosure: **Y. Lee:** None.

538

Antagonistic functions of WNT5A and SFRP5 in hepatic glucose metabolism and inflammation

M. Carstensen-Kirberg[1,2], C. Niersmann[1,2], K. Röhrig[1,2], M. Roden[1,3], C. Herder[1,2];
[1]Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, [2]German Center for Diabetes Research (DZD), München-Neuherberg, [3]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany.

Background and aims: In mice, the pro-inflammatory wingless-related MMTV integration site (WNT)5A reduces insulin sensitivity and is

antagonised by the secreted frizzled-related protein 5 (SFRP5). In humans, the interplay between WNT5A and SFRP5 in the pathogenesis of type 2 diabetes is less well studied. This study aimed to characterise the interaction of WNT5A and SFRP5 in glucose metabolism and inflammation in human hepatocytes.

Materials and methods: The human HepaRG cell line was treated without or with (i) 5 ng/ml WNT5A, (ii) 5000 ng/ml SFRP5 as well as (iii) 5 ng/ml WNT5A and 5000 ng/ml SFRP5 for 24 h. mRNA levels of key enzymes of the gluconeogenesis were assessed using real-time PCR. Inflammatory proteins in the supernatant were analysed using a primer extension technology-based assay for 92 biomarkers. Total and phosphorylated protein content of components of inflammatory pathways was measured using Western blotting.

Results: WNT5A increased mRNA levels of phosphoenolpyruvate carboxykinase (PCK)2 and glucose-6-phosphatase catalytic subunit (G6PC) by 50% and 196% compared to control ($p < 0.05$). The co-treatment with SFRP5 in addition to WNT5A decreased PCK2 and G6PC mRNA by 36% and 48% compared to control ($p < 0.05$). Furthermore, WNT5A reduced the secretion of protein levels of 23 pro-inflammatory proteins in the supernatant on average by 56% compared to control ($p < 0.05$). Most of these proteins were chemokines (C-C motif chemokine (CCL) 2, 8, 20 and C-X-C motif chemokine (CXCL) 1, 5, 6, 8, 10, 11) and pro-inflammatory cytokines (interleukin (IL)-6, IL-18, leukemia inhibitory factor). WNT5A also decreased the phosphorylation level of NF-κB by 36% ($p < 0.01$). The co-treatment with SFRP5 led to a secretion profile similar to control.

Conclusion: In contrast to mouse models, WNT5A has anti-inflammatory effects on human hepatocytes which might be mediated by the partial inactivation of the NF-κB signalling pathway. However, WNT5A unfavourably affects the expression of gluconeogenic enzymes. Its antagonist SFRP5 counterregulates both beneficial and harmful hepatic effects of WNT5A.

Supported by: DFG (CA1497/1-1)

Disclosure: **M. Carstensen-Kirberg:** None.

539

Relationship of complement parameters in type 2 diabetic patients with non-alcoholic fatty liver disease

E. Sipter, L.J. Barkai, D. Csuka, Z. Prohaszka, N. Hosszufalusi;
3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

Background and aims: Inflammation has been identified as a component in pathomechanism of type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease. The fatty liver index (FLI) is a noninvasive method for the estimation of fatty liver, which increases the cardiovascular risk and mortality. The complement cascade is a complex system, which plays an important role in inflammatory response. The aim of our study was to compare the activity of complement pathway members in T2DM patients with and without non-alcoholic fatty liver disease.

Materials and methods: In our prospective study 100 subjects with type 2 diabetes mellitus were investigated. The fatty liver index was calculated using BMI, waist circumference, γ-glutamyltransferase and triglycerides levels. FLI higher than 60 rules in fatty liver disease. Medical history, clinical data and blood samples were collected from patients. Functional activity of the lectin complement pathway member ficolin-3, activity of mannose-binding lectin (MBL), and C3 and C4 concentrations were measured from blood samples using in-house sandwich ELISA methods. For statistical analysis we used GraphPad Prism 5.

Results: 63 patients have increased FLI and 37 patients have FLI less than 60. Regarding the routine clinical laboratory parameters no significant difference was found in HbA1c levels of patients (6.4% vs 6.85%). Significantly higher ($p < 0.05$) C3 concentration and ficolin-3 activity were found in the T2DM with fatty liver disease compared to patients with low FLI. (1.95 [1.75–2.21] μg/l vs 1.76 [1.52–1.96] μg/l and 90.82 [46.18–134.3] % vs 54.18 [23.28–99.07] %). Significantly lower ($p < 0.05$) MBL activity was found in the fatty liver disease group. (76.05 [5.65–102.0] % vs 100.7 [33.24–115.5] %). There was also significant difference between the BMI of the patients (22.19 vs 31.84 kg/m^2). In the case of C4 complement component no significant difference was found.

Conclusion: Lower activity of MBL, and higher concentration of C3 and ficolin-3 activity were found in T2DM patients with high fatty liver index. Our study suggested that complement alternative and lectin pathway can be upregulated in non-alcoholic fatty liver disease among patients with type 2 diabetes mellitus. The difference between the phenotype of the patients can direct the treatment and give the possibility to decrease the inflammation and the cardiovascular risk.

Supported by: EFSD New Horizons

Disclosure: **E. Sipter:** None.

540

Intra-acinar fat in the pancreas of non-diabetic and type 2 diabetic subjects

M. Suleiman, C. De Luca, M. Bugliani, S. Mossuto, F. Grano, P. Marchetti, L. Marselli;
University of Pisa, Pisa, Italy.

Background and aims: Little information is available on intra-acinar fat (IF) features in humans. In the present study we performed morphometric analysis of IF from overweight/obese non-diabetic (ND) and matched type 2 diabetic (T2D) organ donors.

Materials and methods: We studied 13 ND (age: 69 ± 4 years; 5M/8F; BMI: 31.3 ± 0.4 Kg/m^2, data expressed as mean ± SEM) and 15 T2D (age: 71 ± 2 years; 9M/6F; BMI: 29.8 ± 0.7 Kg/m^2) organ donors. Morphometric assessments of adipocytes, insulin (Abcam Ab) and glucagon (Sigma Ab) were performed with pancreatic tissue sections using a DM5500 Leica microscope and the MetaMorph v 1.8.0 software. Macrophages were identified by immunohistochemistry using anti-CD68 (Dako) and anti-CD163 (Thermo Scientific) antibodies. Insulin secretion was assessed from isolated islets in response to acute glucose stimulation.

Results: Adipocyte number tended to be higher in T2D (8.1 ± 1.3/mm^2) than ND (7.0 ± 1.7/mm^2) and their size was greater in T2D (8,890 ± 934 μm^2) than ND (5,439 ± 557 μm^2, $p < 0.01$). Overall, the proportion of IF area in relation to acinar tissue area trended higher (+46%, $p = 0.06$) in T2D (6.8 ± 1.2%) than ND (3.7 ± 1.0%). Insulin positive area (T2D: 0.47 ± 0.05%; ND: 0.55 ± 0.06%) as well as glucagon positive area (T2D: 0.24 ± 0.03%; ND: 0.32 ± 0.06%) did not differ significantly in the two groups. The number of adipocytes with adjacent ≥2 CD68$^+$ cells counted in 4 mm^2 of pancreatic tissue was higher in T2D than ND (6.8 ± 1.8 vs 2.7 ± 0.7%, $p = 0.05$), whereas no significant difference was seen in CD163$^+$ cells. Glucose-stimulated insulin secretion index was lower in T2D (2.1 ± 0.2) than ND (3.7 ± 0.4, $p < 0.01$). A positive correlation (Pearson analysis) was found between adipocyte size and insulin area in ND ($r = 0.68$, $p = 0.01$). No significant correlation was seen between IF features and ex-vivo insulin release.

Conclusion: At our experimental conditions, adipocyte size was higher in T2D pancreatic samples, that also showed a higher infiltration by CD68$^+$ cells. If and how these differences can affect beta cell amount and function remain to be assessed.

Supported by: RHAPSODY, Grant Agreement No. 115881

Disclosure: **M. Suleiman:** None.

541

Extracellular nicotinamide phosphoribosyltransferase (eNAMPT) induces beta cell dysfunction via p38 and STAT3 signalling

P.W. Caton[1], R. Beavil[2], D.J. Hodson[3], G.G. Lavery[4], S. Sayers[1];
[1]Diabetes Research Group, King's College London, London, [2]King's College London, London, [3]Institute of Metabolism and Systems

Research, University of Birmingham, Birmingham, [4]Institute of Metabolism and Systems Research, University of Birmingham, London, UK.

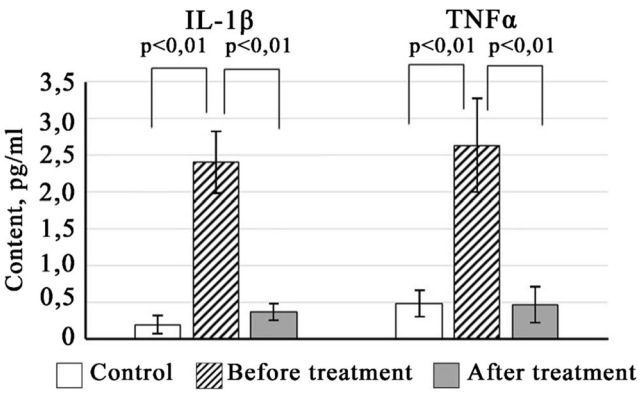
Background and aims: Elevated serum extracellular nicotinamide phosphoribosyltransferase (eNAMPT; visfatin/PBEF) levels are associated with beta-cell failure in Type 2 diabetes (T2D). Despite this, the effects of eNAMPT on beta-cell function are poorly elucidated. Understanding the effects of eNAMPT on beta-cells requires consideration of concentration and structure-function relationships. In non-diabetic serum, eNAMPT circulates at <1 ng/ml, rising to >5 ng/ml in T2D. Moreover, eNAMPT predominantly exists as a dimer, which is essential for the NAD biosynthetic capacity of eNAMPT. However, we have also reported selective elevation of a monomeric eNAMPT in mouse models of T2D. Here we examined the effects of eNAMPT-dimer and -monomer on pancreatic islets at physiological (<1 ng/ml) and pathophysiological (>5 ng/ml) concentrations.
Materials and methods: Isolated mouse and human islets were treated with monomeric or dimeric eNAMPT (24–48 h; 1 or 5 ng/ml). We assessed islet glucose-stimulated insulin secretion (GSIS; static and dynamic); calcium flux ($[Ca^{2+}]_{cyt}$; Fura-2 confocal imaging); apoptosis (Caspase-Glo 3/7 activity); islet gene expression (qPCR) and islet NAD (colourimetric assay).
Results: We identified distinct concentration- and structure-dependent effects of eNAMPT on islets. Exposure of islets to physiological levels of eNAMPT-dimer (1 ng/ml) significantly increased static and dynamic GSIS ($n = 10$, 5 size matched islets/well) and intracellular $[Ca^{2+}]_{cyt}$ (16 islets, $n = 3$ mice/treatment) ($P < 0.05$, $P < 0.01$, respectively). Moreover, gene expression markers of beta-cell identity (*PDX1*, *NKX2.2*, *INS*) were increased. These effects were associated with increases in islet NAD levels. In contrast, treatment of islets with pathophysiological concentrations of eNAMPT-dimer (5 ng/ml) or with eNAMPT-monomer (1 and 5 ng/ml) led to reduced GSIS ($P < 0.05$), calcium flux, and *PDX1*, *NKX2.2* and *INS* mRNA. Correspondingly, apoptosis ($n = 8$, 6 size matched islets/well, $P < 0.05$) and inflammatory gene expression (*CCL2*: $P < 0.01$, *Il1B*: $P < 0.05$) were enhanced following exposure to eNAMPT-dimer (5 ng/ml) or eNAMPT-monomer (1 and 5 ng/ml). These effects of eNAMPT appear to be NAD-independent. The deleterious effects of eNAMPT-dimer and -monomer were blocked when islets were co-treated with inhibitors of p38 (SB203580; 1υM) or STAT3 (NCS74859; 25 υM).
Conclusion: We report distinct dose- and structure-dependent effects of eNAMPT on pancreatic islets. 1 ng/ml eNAMPT-dimer - similar to the serum structure-concentration combination observed in non-diabetic individuals - enhanced beta-cell function and increased markers of beta-cell identity. In contrast, exposure to eNAMPT at levels/structures seen in T2D led to impaired beta-cell function, through inflammatory mechanisms involving p38 and STAT3 signalling. Together, these data suggest that at physiological levels, eNAMPT plays a key role in maintaining beta-cell function and identity, likely via NAD-dependent effects. In contrast, pathophysiological levels of eNAMPT contribute to development of T2D by through impaired beta-cell function and identity, in part via NAD-independent effects. Therefore, eNAMPT represents a novel therapeutic approach for treating T2D.
Supported by: Diabetes UK 15/0005154
Disclosure: **P.W. Caton:** None.

542

Anti-inflammatory and immune-mediated effects of metformin therapy in patients with type 2 diabetes

M.D. Tron'ko, O.V. Furmanova, V.V. Popova, Y.A. Sayenko, V.L. Orlenko, K.Y. Ivaskiva, K.M. Tron'ko, K.P. Zak;
V.P. Komisarenko Institute of Endocrinology and Metabolism of Natl. Acad. Med. Sci. Ukraine, Kyiv, Ukraine.

Background and aims: Metformin is one of the most prescribed drugs for treatment of type 2 diabetes (T2D). It is recently shown, that metformin has not only hypoglycemic properties but also beneficial effect on other important functions of the body. However, the mechanism of the pluripotent effect of metformin is not clear. Especially, the role of inflammation and the immune system is insufficiently studied, although T2D and its complications are referred to inflammatory diseases. Aim is to study some indices of immunity and inflammation in newly diagnosed patients with T2D, after their treatment with metformin.
Materials and methods: There were examined 35 patients with newly diagnosed T2D. Of these, 20 clinically homogeneous patients were selected with following administration of metformin therapy (2000 mg/day for 3 months). The control group consisted of 32 healthy individuals. The total number of leukocytes and leukocyte composition was determined by the hematological analyzer and simultaneously visually in blood smears. The index of inflammation (NLR) is calculated from the ratio of neutrophils to lymphocytes. Immunophenotype of lymphocytes (CD3+ T, CD4+ T, CD8+ T, CD20+, CD56+cells) was determined by FACS analyses and the level of cytokines (IL-1β, IL-2, IL-4, IL-6, IL-10, TNFα) and chemokynes (IL-8, IL-16) - with immunosorbent ELISA assay.
Results: In 90 days after metformin administration there was a decrease of leukocytosis (7.17 ± 0.29 vs 5.93 ± 0.22 10^9/l, $p < 0{,}01$), NLR index (3.19 ± 0.26 vs 2.06 ± 0.19, $p < 0.01$), monocyte number ($0.77 \pm 0{,}06$ vs 0.33 ± 0.06 10^9/l, $p < 0.01$) and CD4+T-cells (0.66 ± 0.07 vs 0.45 ± 0.07 10^9/l, $p < 0{,}05$) in most patients with T2D. At the same time there is a marked decrease of proinflammatory cytokine level and, in particular, IL-1β (2.4 ± 0.42 vs 0.36 ± 0.11, pg/ml, $p < 0.01$), and TNFα (2.63 ± 0.64 vs 0.46 ± 0.24 pg/ml, $p < 0.01$), and chemokine IL-16 (89.6 ± 9.3 vs 130.5 ± 11.2 pg/ml, $p < 0{,}05$), which correlated well with a decrease in % HbA1c and an improvement in the clinical status of patients.
Conclusion: An immuno-mediated anti-inflammatory effect also plays an important role in the mechanism of the curative effect of metformin in T2D.



Disclosure: **M.D. Tron'ko:** None.

543

Vitamin-D improves glycaemic outcome in prediabetes through reduced fetuin-A and systemic inflammation

M. Kumar[1], D. Dutta[2], S.A. Mondal[3], S. Mukhopadhyay[4];
[1]Department of Endocrinology, Centre for Endocrinology, Diabetes And Research (CEDAR), Patiala, [2]Department of Endocrinology, Venkateshwar Hospitals, Delhi, [3]Department of Biochemistry, IPGMER and SSKM Hospital, Kolkata, [4]Department of Endocrinology, IPGMER and SSKM Hospital, Kolkata, India.

Background and aims: The study aimed to evaluate the impact of VitD supplementation on FetA and systemic inflammation [interleukin (IL) 6,

IL1β, tumor necrosis factor (TNF) α, soluble TNF receptor (sTNFR)1, sTNFR2] in IPD, and their relation with long-term glycemic outcomes
Materials and methods: From an initially screened 2245 individuals, 207 IPD with persistent IFG and/or IGT over 2 successive OGTTs with vitD <30 ng/ml were randomized into intervention group (Group-I) (cholecalciferol 60,000 U once weekly for 8 weeks and then monthly with 1250 mg of calcium carbonate/day for study duration), and control groups [Group-C1 (Calcium Control Group: 1250 mg of calcium carbonate/day); and Group-C2 (Placebo Control Group: placebo tablet similar to calcium tablet). All received therapeutic lifestyle modification. Glucose tolerance, insulin, 25OHD, lipids, IL6, TNF-α and hsCRP were done baseline and annually. Data from IPD with at least 1-year follow up were analyzed.
Results: Data from 192 IPD (males: females = 122:70) with at least 1-year follow-up were analyzed (mean follow-up: 27.68 ± 9.72 months. At end of study, IPD in intervention group had greatest reduction in FBG, 2hPGBG, HbA1c, fetuin-A, total cholesterol, IL6, sTNFR1, sTNFR2, which was statistically significant. Group-I IPD also had greatest increase in 25OHD (Δ25OHD: 22.96 [11.54–40.30] ng/ml; $P < 0.001$). Quantum of decrease in serum triglycerides, LDL-C and HDL-C was greatest in Group-I. Placebo control group (Group-C2) had greatest increase in hip circumference and IL1β. Analysis based on glycemic outcomes revealed IPD who reverted to normoglycemia had highest baseline 25OHD, sTNFR2, along with greatest reduction (Δ change) in FetA, sTNFR1 and sTNFR2. 42.7% (41/96) IPD in intervention group reversed back to normoglycemia, in contrast to 22.92% (22/96) in control groups ($P = 0.003$). With regards to progression to T2DM, it was 10.41% (10/96) in intervention group and 17.70% (17/96) in control groups ($P = 0.144$). Cox regression revealed that FBG, 25OHD, fetuin-A, HOMA-IR, sTNFR1 and Group-I independently predicted prediabetes reversal to normoglycemia. Only FetA was an independent predictor of prediabetes progression to T2DM. Every 1 mg/dl increase in blood glucose was associated with 3.7% decreased reversal to normoglycemia. Every 1 ng/ml increase in serum 25OHD was associated with 3% increased reversal to normoglycemia. Every 1 mcg/ml increase in FetA was associated with 0.4% decreased reversal to normoglycemia and 0.6% increased progression to T2DM. Kaplan Meier analysis also showed significantly higher rates of prediabetes reversal to normoglycemia in intervention group ($P = 0.033$; log rank test [Mantel-Cox]). 42.7% IPD in intervention group reversed back to normoglycemia in contrast to 22.92% in the control groups.This evaluation achieved power of 83.7%, keeping α (Type I error) at 0.05.
Conclusion: VitD supplementation in prediabetes was associated with improved glycemic outcomes, significant reduction in serum FetA, decreased IR and systemic inflammation.
Clinical Trial Registration Number: CTRI/2011/091/000192
Supported by: We received grant from department of science and technology
Disclosure: **M. Kumar:** Grants; Department of Science and Technology (DST), Government of West Bengal, India, Grant Number 853(Sanc)/ST/P/S&T/9G-2/2011.

544

Role of O-GlcNAc glycosylation in the inflammatory effect of LPS in macrophage

H. Al-Mukh, L. Baudoin, M. Khair, L. Francese, F. Niedergang, T. Issad; Institut Cochin, INSERM U1016, CNRS UMR8104, University Paris Descartes, Paris, France.

Background and aims: O-GlcNAc glycosylation (O-GlcNAcylation) is a reversible post translational modification catalysed by O-GlcNAc transferase (OGT). O-GlcNAcylation regulates the activity of cytosolic and nuclear proteins according to glucose availability. This modification, Which corresponds to the addition of N-Acetylglucosamine (GlcNAc) on serine and threonine residues of proteins, participates in several hyperglycemia-associated complications. An important feature of metabolic diseases such as diabetes and obesity is the presence of a low-grade chronic inflammation that causes numerous complications. Chronic hyperglycemia is known to play a role in this inflammation. However, the relationships between O-GlcNAcylation and inflammatory processes remain poorly explored. The aim of this work was to evaluate potential involvement of O-GlcNAcylation in proinflammatory signalling in macrophages.
Materials and methods: The study was performed using the RAW264.7 murine macrophage cell line, peritoneal or bone marrow derived macrophages from wt or OGT-KO mice as well as macrophages differentiated from human monocytes. O-GlcNAcylation of proteins was evaluated using a BRET-based biosensor targeted to different cell compartments, or by western blot using anti-O-GlcNAc antibody.
Results: Using Thiamet G, a specific inhibitor of OGA (the enzyme that removes O-GlcNAc from proteins), we observed that increasing O-GlcNAcylation in murine macrophages potentiates the effect of LPS (lipopolysaccharide) on the expression of pro-inflammatory cytokines. Moreover, using a BRET-based biosensor, we observed that activation of Toll-like receptor 4 (TLR4) by LPS increased BRET signal at the plasma membrane, in the cytosol and in the nucleus of RAW264.7 macrophages. Similar results were observed in these cells when O-GlcNAcylation was evaluated by western-blotting with an anti-O-GlcNAc antibody. These effects were also confirmed in primary bone marrow-derived and peritoneal mouse macrophages, as well as in human monocyte derived macrophage. Precipitation of O-GlcNAcylated proteins using Wheat-germ lectin sepharose beads revealed that LPS induced O-GlcNAcylation of NFκB in macrophages. We then evaluated the consequences of OGT deletion on the expression of pro-inflammatory genes. Peritoneal-derived macrophages were prepared from OGT Lox/lox-LysM-Cre mice. We observed that whereas OGT deletion had no effect on LPS-induced NOS2 and TNFα mRNA expression, it resulted in an inhibition of LPS-induced IL1β mRNA expression by 94 ± 4% ($p < 0.001$) and that of IL6 by 78% ± 11% ($p < 0.001$). This suggest that some but not all of the pro-inflammatory effects of LPS involve the O-GlcNAcylation signaling pathway.
Conclusion: Our results indicate that activation of the O-GlcNAcylation pathway may be an integral part of the TLR4- induced signal, and suggest that this pathway is crucial to some of the pro-inflammatory effect of LPS in macrophages.
Supported by: CORDDIM
Disclosure: **H. Al-Mukh:** None.

PS 036 Inflammation in obesity and type 2 diabetes: mouse studies

545

Protective role of SIRT1 against the deleterious effect of inflammation in insulin sensitivity and thermogenesis in brown adipocytes

C. Escalona[1,2], P. Vázquez[1,2], E. García-Casarrubios[1], M. Obregón[1], Á.M. Valverde[1,2];
[1]Instituto de Investigaciones Biomédicas "Alberto Sols" (IIB, CSIC-UAM), Madrid, [2]CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM), ISCIII., Madrid, Spain.

Background and aims: Activation of brown adipose tissue (BAT) by modulation of SIRT1 activity is a promising approach to combat obesity or type 2 diabetes mellitus (T2DM). These conditions are associated with a chronic low-grade systemic inflammation, which is considered a critical underlying factor in the development of insulin resistance. Our aim was to identify the mechanisms implicated in the potential therapeutic benefit of targeting SIRT1 in BAT to ameliorate inflammation-mediated insulin resistance and defective thermogenesis.
Materials and methods: The impact of SIRT1 in BAT inflammation was studied by acute treatment of wild-type or SIRT1-Tg mice with bacterial lipopolysaccharide (LPS). We also studied the effect of resveratrol, a SIRT1 activator, in BAT from db/db mice treated for 8 weeks. Proinflammatory-mediated signaling cascades, insulin signaling and thermogenic mediators were analyzed by western blot and RT-PCR. As an in vitro model we used immortalized brown adipocytes from wild-type differentiated in the absence or presence of resveratrol or from SIRT1-Tg mice. These cells were stimulated with conditioned medium (CM) from Raw 267.4 macrophages treated or not with LPS and proinflammatory signaling, insulin signaling and noradrenaline-mediated UCP1 was assayed.
Results: Proinflammatory signalling cascades mediated by JNK, p38 MAPK, JAK-STAT3 and NFκB were enhanced in BAT from db/db mice compared to db+ lean controls. Conversely, insulin-mediated signaling was decreased. This insulin resistance was accompanied by a change in the pattern of IR isoforms showing a robust presence of IRA only in BAT from db/db mice. Treatment of db/db mice with resveratrol increased Ucp1 ($p = 0.057$), Dio2 ($p = 0.0571$) and Fgf21 ($^*p < 0.05$) mRNA levels in BAT. In a model of acute administration of LPS in lean mice a similar proinflammatory signalling in BAT was found, assessing BAT sensitivity to the proinflammatory environment. Interestingly, moderate SIRT1 overexpression decreased Il6 mRNA levels and STAT3 phosphorylation in LPS-injected mice. Differentiated brown adipocytes stimulated with CM from LPS-stimulated Raw 264.7 macrophages showed a rapid activation of inflammatory signaling and subsequently insulin resistance manifested by reduced Akt phosphorylation and glucose uptake as well as decreased response to noradrenaline in the induction of UCP1. These responses were attenuated by differentiating brown adipocytes in the presence of resveratrol or alternatively by moderate overexpression of SIRT1.
Conclusion: Our results suggest that activation of SIRT1 in brown adipocytes might play a major and beneficial role against insulin resistance and defective thermogenesis associated to inflammation.
Disclosure: **C. Escalona:** None.

546

Leukotriene B4 induces mitochondrial dysfunction in skeletal muscle by mediating oxidative stress and inflammation

M. Abu Bakar;
Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia.

Background and aims: Mitochondrial dysfunction and oxidative stress in skeletal muscle are among the detrimental events in development of type 2 diabetes (T2D). Leukotriene B4 (LTB4) is a potent leukocyte chemoattractant regulated by 5-lipoxygenase (5-LO) and leukotriene A4 hydrolase activities in the event of chronic inflammatory conditions, including T2D. However, little is known about the metabolic role of LTB4 on mitochondrial oxidative stress. Therefore in the current study, we focused on the metabolic effects of LTB4 on mitochondrial functions in skeletal muscle.
Materials and methods: The molecular mechanism underlying the roles of LTB4 on skeletal muscle was investigated *in vitro* and *in vivo* using immunoblotting, real-time RT-PCR and flow cytometry analyses.
Results: LTB4 treatment in a dose-dependent manner augmented the degree of mitochondrial superoxide generation in myotubes. Such impairments in mitochondrial integrity and dynamics by increased dynamin-related protein-1 (Drp1) and fission-1 (Fis1) protein expression but, reduced mitofusin (Mfn)-1 protein expression were noticed. Furthermore, LTB4 considerably augmented the expression of Cytochrome C and Bax, but diminished the expression of Bcl-2, thus promoting myotubes apoptosis and inflammation. Additionally, LTB4 treatment resulted in the suppression of phosphatidyl inositol 3- kinase (PI3K)/Akt signaling. Finally, impaired insulin sensitivity with severe oxidative damage and mitochondrial impairments in skeletal muscle of transgenic mice expressing human LTB4 (LTB4-Tg) mice were observed compared to wild-type C57BL/6J mice.
Conclusion: Overall, the current investigation uncovers a novel mechanism through which LTB4 leads to oxidative damage and mitochondrial dysfunction in the progression of T2D.
Supported by: USM Short Term Grant (Ref. No: 304/PTEKIND/6313329)
Disclosure: **M. Abu Bakar:** None.

547

Role of resistin/TLR4 signalling pathway in HFD induced hypothalamic inflammation and gliosis

S. Al-Rifai, D. Crepin, L. Riffault, C. Alexandre, G. Poizat, M. Taouis, Y. Benomar;
Molecular Neuroendocrinology of food intake, Paris-Saclay Institute of Neuroscience UMR9197, University Paris Sud, Orsay, France.

Background and aims: Obesity is linked to several metabolic disorders including low grade inflammation and insulin resistance, which constitutes a principal risk factor for type 2 diabetes (T2D). Increasing evidence indicates that changes in adipose-secreted factors in obesity, dramatically affect insulin sensitivity. Among these adipokines, resistin is described as a causal factor for obesity-mediated peripheral inflammation and insulin resistance. Several studies reported that resistin is also expressed in the hypothalamus, however, little is known about the molecular mechanisms mediating its effects at the neuronal level. Recently, we have shown that central resistin acts by way of hypothalamic TLR4 receptors promoting whole-body insulin resistance. Here we aim to investigate the involvement of resistin/TLR4 pathways on the onset of hypothalamic inflammation and gliosis. This study also aims to characterize the hypothalamic nuclei and neural cells expressing resistin and to establish the temporal regulation of hypothalamic resistin expression by High Fat Diet (HFD) feeding.
Materials and methods: To reach our objective, male C57BL/6 mice were fed with standard chow diet or HFD for 3 days, 8 days and 8 weeks, in addition C57BL6 TLR4$^{-/-}$ mice and their control littermates were treated for 3 days with or without resistin through ICV route. At the end of the experimental period we analyzed the impact of HFD and ICV resistin treatment on hypothalamic resistin expression, inflammation and reactive gliosis. This was assessed by combined in situ hybridization, immunohistochemistry and RTqPCR analyses.
Results: As expected, we show that HFD feeding induces hypothalamic inflammation, and reactive gliosis in the medio-basal hypothalamus (MBH). Interestingly, we report for the first time, that both long-term

and short-term HFD-induced hypothalamic inflammation is associated with a significant increase of resistin expression in tanycytes and neurons throughout the MBH, suggesting the potential involvement of resistin in the early-onset of hypothalamic inflammation. Next, we investigated whether central resistin/TLR4 pathways could directly contribute to hypothalamic inflammation; we show that central resistin infusion for 3 days markedly increases inflammatory markers in the hypothalamic arcuate nucleus and adjacent median eminence in association with reactive gliosis involving recruitment of both microglia and astrocytes. Interestingly, we report that the knockdown of TLR4 almost completely abolished resistin-dependent both hypothalamic inflammation and reactive gliosis.

Conclusion: Altogether, our findings demonstrate that Resistin/TLR4 signaling pathway constitute a crucial/key pathway promoting the onset of hypothalamic inflammation. Targeting this signaling pathway may constitute a significant breakthrough to overcome obesity-induced hypothalamic inflammation and related metabolic dysfunctions.

Disclosure: **S. Al-Rifai:** None.

548

Impaired PPAR gamma signalling but not endoplasmic reticulum stress promotes inflammation in white adipose tissue of hormone-sensitive lipase deficient mice

P. Kotzbeck[1,2], C. Haudum[1], I. Foessl[1], B. Boulgaropoulos[1,3], K. Bounab[1], T.O. Eichmann[2,4], B. Obermayer-Pietsch[1], G. Haemmerle[2], S. Cinti[5], T.R. Pieber[1,3], R. Zechner[2,6];

[1]Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria, [2]Institute of Molecular Biosciences, University of Graz, Graz, Austria, [3]Institute for Biomedicine and Health Sciences, Joanneum Research Forschungsgesellschaft mbH HEALTH, Graz, Austria, [4]Center for Explorative Lipidomics, BioTechMed-Graz, Graz, Austria, [5]Department of Experimental and Clinical Medicine, Center of Obesity, University of Ancona (Politecnica delle Marche), Ancona, Italy, [6]BioTechMed-Graz, Graz, Austria.

Background and aims: Hormone-sensitive lipase (HSL) plays a crucial role in intracellular lipolysis. Mutation or loss of HSL leads to diacylglycerol accumulation, reduced fatty acid mobilization, and impaired peroxisome proliferator-activated receptor (PPAR) gamma signaling. Diacylglycerol accumulation contributes to lipotoxicity and may induce endoplasmic reticulum (ER) stress which is able to initiate inflammatory processes. Impaired PPAR gamma signaling is also associated with inflammatory responses in adipose tissue. Previous studies have shown that HSL knock out mice exhibit macrophage infiltration and adipose tissue inflammation, but the mechanisms leading to such adipose tissue inflammation have not been studied so far. Therefore, we aimed to investigate whether impaired PPAR gamma signaling or ER stress in white adipose tissue of HSL knock out mice is causal for adipose tissue inflammation.

Materials and methods: Markers of adipose tissue inflammation (F4/80 expression, Mac-2 staining and crown-like structures quantification), adipose tissue function (adiponectin, perilipin expression) and ER stress (Chop expression, peIF2 alpha/eIF2 alpha ratio) were analyzed in different adipose tissue depots of adult, lean HSL knock out and control mice under basal conditions and after chronic treatment with the PPAR gamma agonist rosiglitazone. Additionally, diacylglycerol and ceramide concentrations were measured in white adipose tissue of HSL knock out and control mice under basal conditions and after chronic rosiglitazone treatment.

Results: HSL knock out mice exhibited 23% decreased epididymal adipose tissue mass, moderate adipocyte hypertrophy and marked macrophage infiltration represented by increased CLS count and 2.8-fold increased F4/80 mRNA expression ($p < 0.01$). Phosphorylation of eIF2 alpha was 1.9-fold ($p < 0.001$) and Chop mRNA expression 7.7-fold increased ($p < 0.01$) in white adipose tissue of HSL knock out mice. Ultrastructural analysis showed markedly dilated ER in white adipocytes of HSL knock out mice. Rosiglitazone treatment abrogated macrophage infiltration and thereby ameliorated adipose tissue inflammation. ER stress markers in contrast remained unchanged and were still elevated by 16-fold ($p < 0.01$) in white adipose tissue of rosiglitazone treated HSL knock out mice compared to the respective control mice. Lipidomic profiling of white adipose tissue showed that diacylglycerol accumulation in HSL knock out mice remained unaffected by rosiglitazone treatment and might be causal for ER stress but not inflammation.

Conclusion: Our data clearly show that impaired PPAR gamma signaling but not ER stress contributes to white adipose tissue inflammation of HSL knock out mice.

Supported by: Targeting Obesity-driven Inflammation (TOBI) contract no. 201608

Disclosure: **P. Kotzbeck:** None.

549

The role of TLR4 interactor with leucine-rich repeats (TRIL) in hypothalamic inflammation

A. Moura-Assis[1], J. de Lima Junior[1], J. Gaspar[2], R. Gaspar[1], E. R. Ropelle[3], L.A. Velloso[1];

[1]Internal Medicine, University of Campinas - Faculty of Medical Sciences, Campinas, [2]Department of Biochemistry, Federal University of Santa Catarina, Santa Catarina, [3]Internal Medicine, University of Campinas - Faculty of Applied Sciences, Limeira, Brazil.

Background and aims: The neural control of whole body energy homeostasis is controlled by key brain nuclei. In particular, the hypothalamus integrates short-term signals from the gut and long-term signals from adipose tissue to ensure correct meal initiation/termination and glucose/lipid homeostasis. In this sense, AgRP-expressing neurons and POMC-expressing neurons become more or less active in a calorically deficient state, respectively.In face of dietary excess, the obesity-associated activation of inflammatory pathways in the hypothalamus promotes caloric overconsumption and weight gain in mice. This process occurs partially via activation of TLR4 by saturated fatty acids. In other contexts, TLR4 signaling in the brain is mediated by the TLR4 interactor with leucine-rich repeats (TRIL). Our main purpose was to determine the putative involvement of TRIL in diet-induced hypothalamic inflammation. We performed bioinformatics analysis from RNA-seq database of hypothalamic neurons, using inflammatory markers, to identify genes with large negative or positive correlation with TRIL. We also knocked down the expression of TRIL in the arcuate nucleus to evaluate changes in food intake and body weight.

Materials and methods: We employed male, 6 week-old Swiss and C57BL/6J mice, fed on chow or high-fat diet for 1, 2, 4 or 8 weeks. The transcript levels in the hypothalamus were measured by RT-PCR, western blot analyses was used for determination of protein content of inflammatory markers and immunofluorescence microscopy for evaluate the distribution of TRIL in neuronal and non-neuronal cells in arcuate nucleus of hypothalamus. The bioinformatics analysis from RNA-seq database focused on the magnitude of the correlation between TRIL and inflammatory genes. Finally, we used lentiviral particles to knock-down TRIL expression in arcuate nucleus through intracerebroventricular infusion.

Results: TRIL colocalized with ACTH (POMC neurons) and a marker of activated microglia (Iba1); in addition we also found colocalization with astrocyte markers (GFAP). The expression of TRIL mRNA increased significantly after 1 and 2 weeks on high fat consumption and was back to baseline after 4 weeks, a similar expression pattern was observed for TLR4. The increased mRNA expression of TRIL and TLR4 was accompanied of increased IKK phosphorylation. The bioinformatics analysis indicated positive correlation between TRIL and inflammatory genes such as *il18*(indicative of inflammasome activation), *p2ry1* (indicative of microglial activation), *tnfrsf19* (member of the TNF-receptor

superfamily), among others. Finally, the knocked down of TRIL in mice fed on chow did not change the body and epididymal weight and food intake.
Conclusion: Collectively our results suggest that TRIL is involved in the TLR4-mediated early response to dietary fats. The expression of TRIL in neuronal and non-neuronal hypothalamic cells provides evidence that the immunologic responses to dietary excess might be mediated by TRIL. The role of TRIL inhibition in high fat-fed mice and its effect on food intake, energy expenditure and hypothalamic inflammation will be addressed in the next experiments.
Supported by: São Paulo Research Foundation (FAPESP 16/01245-5)
Disclosure: **A. Moura-Assis:** None.

550

Cystatin C alleviates obesity-associated tissue inflammation and insulin resistance

M.A. Dedual[1,2], S. Wueest[1,2], T.R.J. Aeppli[1,2], T.D. Challa[1,2], D. Konrad[1,2];
[1]Division of Pediatric Endocrinology and Diabetology, University Children's Hospital, Zurich, [2]Children's Research Center, University Children's Hospital, Zurich, Switzerland.

Background and aims: We recently demonstrated that removal of one kidney (uninephrectomy) in mice reduced high fat-diet (HFD)-induced adipose tissue inflammation and improved hepatic insulin sensitivity. Moreover, uninephrectomized mice revealed increased plasma levels of cystatin C, a circulating factor with suggested anti-inflammatory properties. We, thus, hypothesized that cystatin C alleviate obesity-associated adipose tissue inflammation.
Materials and methods: 6-week-old C57BL/6J wild type (WT) and cystatin-C-deficient mice (CysC KO) were fed a regular chow or HFD (~60% kcal fat) for 20 weeks. Glucose metabolism was assessed by intraperitoneal glucose tolerance tests (ipGTT) and by hyperinsulinaemic-euglycaemic clamp studies. After scarification, liver and fat depots were analyzed applying Western blotting, rtPCR and histological staining.
Results: HFD-induced aggravation in glucose tolerance was significantly elevated in CysC KO compared to WT mice (ΔAUC ipGTT 348 ± 85 mmol/l*min in WT vs. 803 ± 106 mmol/l*min in CysC KO, $p < 0.01$). Moreover, hyperinsulinaemic-euglycaemic clamps in HFD-fed mice revealed a significantly lower insulin-mediated inhibition of endogenous glucose production (EGP) in CysC KO compared to WT mice (24.2 ± 3.3 mg/kg*min in WT vs. 44.6 ± 3.7 mg/kg*min in CysC KO, $p < 0.05$), indicating exacerbated hepatic insulin resistance in CysC KO mice. In addition, glucose uptake into inguinal adipose tissue during clamps was ~50% reduced in CysC KO mice, paralleled by significantly reduced serine^{473} phosphorylation of Akt (1.0 ± 0.2 in WT vs. 0.3 ± 0.0 in CysC KO, $p < 0.05$). mRNA levels of the pro-inflammatory factor IL-6 (1.0 ± 0.2 in WT vs. 2.8 ± 0.6 in CysC KO, $p < 0.05$) and the pro-fibrotic factor Tgfβ1 (1.0 ± 0.2 in WT vs. 2.2 ± 0.5 in CysC KO, $p < 0.05$) were increased in epididymal fat of HFD-fed CysC KO mice. Similarly, mRNA levels of pro-inflammatory cytokines IL-6 (1.0 ± 0.1 in WT vs. 3.5 ± 0.4 in CysC KO, $p < 0.05$) and IL-1β (1.0 ± 0.1 in WT vs. 1.8 ± 0.2 in CysC KO, $p < 0.05$) were increased in livers of HFD-fed CysC KO mice, paralleled by signs of hepatocellular injury in histological sections of such mice. Moreover, *in vitro* treatment of fat explants harvested from HFD-fed WT mice with recombinant cystatin C reduced expression of pro-inflammatory cytokines.
Conclusion: Our data indicate a beneficial role of cystatin C in obesity-associated (adipose tissue) inflammation and (hepatic) insulin resistance.
Supported by: Wolfermann-Nägeli Stiftung, FZK/CRC Grant
Disclosure: **M.A. Dedual:** Grants; Research Grant by the Wolfermann-Nägeli-Stiftung, Research grant by the Forschungszentrum für das Kind of the University Children's Hospital.

551

Mouse strain-specific immunometabolic response in white adipose tissue during cold exposure

K. Adamcova[1], K. Bardova[1], P. Janovska[1], O. Horakova[1], P. Flachs[1], M. Svobodova[1], M. Rossmeisl[1], L. Madsen[2,3], K. Kristiansen[3], J. Kopecky[1];
[1]Department of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [2]National Institute of Nutrition and Seafood Research, Bergen, Norway, [3]Department of Biology, Laboratory of Genomics and Molecular Biomedicine, University of Copenhagen, Copenhagen, Denmark.

Background and aims: Cold exposure (CE) was shown to activate lipid metabolism in epididymal white adipose tissue (eWAT), with a stronger effect in obesity-resistant A/J as compared with obesity-prone C57BL/6J (B6) mice. The ability of WAT to serve as a whole-body buffer for lipids depends in part on presence of adipocytes with high lipolytic/re-esterification capacity, and possibly on the extent of WAT remodelling under the conditions of changing energy demands. Since WAT metabolism is interconnected with tissue immune responses, we investigated whether the effect of CE on eWAT metabolism was mirrored by the content and polarization of WAT macrophages.
Materials and methods: Two-month-old male A/J and B6 mice fed chow diet were maintained at thermoneutral temperature (30°C) or exposed to cold (6°C for 48 hours). eWAT was analyzed immunohistochemically and/or using quantitative PCR (qPCR); macrophages were characterized using flow cytometry. Statistical analysis was performed by SigmaStat using TwoWay ANOVA.
Results: CE decreased weight of eWAT with a more pronounced effect in A/J mice. The occurrence of UCP1-negative and ATGL- and DGAT1-positive paucilocular adipocytes was induced by CE in both strains, with a stronger induction in A/J mice. Our results document both strain-specific difference and influence of CE on eWAT abundance of macrophages. In response to CE, tissue content of M1 ($CD11c^+$, $CD206^-$) macrophages decreased in A/J, but not in B6 mice (cells/mg tissue: B6 vs. A/J; at thermoneutrality: 31 ± 8 vs. 16 ± 5; in cold: 49 ± 13 vs. 7 ± 2;). Tissue content of M2 ($CD11c^-$, $CD206^+$) macrophages was not influenced by CE in either strain of mice (cells/mg tissue: B6 vs. A/J; at thermoneutrality: 228 ± 56 vs. 89 ± 18; in cold: 211 ± 25 vs. 77 ± 22). Overall the M2/M1 ratio was increased in AJ mice by cold exposure.
Conclusion: These results suggest a causal link between the reduced content of M1 macrophages and relatively strong activation of lipid metabolism in response to CE in obesity resistant A/J mice in comparison with obesity prone B6 mice.
Supported by: 16-05151S CSF
Disclosure: **K. Adamcova:** None.

552

Microbiome inhibition of IRAK-4 by trimethylamine mediates metabolic and immune benefits in high fat diet-induced insulin resistance

M.-E. Dumas[1], J. Chilloux[1], A. Myridakis[1], L. Hoyles[1,2], A. Everard[2], H. Plovier[2], P. Cani[2,3], F. Brial[3], D. Gauguier[3,4], D. Smyth[4], L. Zhang[4], P. Liu[4];
[1]Dept of Surgery and Cancer, Div. Integrative Systems Medicine, Imperial College London, London, UK, [2]Universite catholique de Louvain, Brussels, Belgium, [3]Universite Pierre and Marie Curie, Paris, France, [4]Ottawa Heart Centre, Ottawa, Canada.

Background and aims: The interaction between high-fat diet (HFD) feeding and the gut microbiome has a strong impact on the onset of insulin resistance (IR). In particular, bacterial lipopolysaccharides (LPS) and dietary fats trigger low-grade inflammation through activation of *Toll*-like receptor 4 (TLR4), a process called metabolic endotoxemia. However, little is known about how the microbiome can mitigate this process. Here, we investigate longitudinal physiological and metabotypical responses of C57BL/6 mice to HFD feeding.

Materials and methods: We performed a series of *in vivo* experiments in High Fat Diet contexts with i) modulating choline content, ii) blocking trimethylamine (TMA) production, iii) administering TMAiv) deleting Irak4 by KO, v) inhibiting Irak4 by a chemical inhibitor.Samples were analysed by transcriptomics (liver), metabolomics (1H NMR and UPLC-MS/MS for serum and urine).We also performed in vitro kinome screens, followed by Kd and IC50 curves. We treated immortalised murine Kupffer cells (KUP5) with palmitate and TMA to assess cytokine production. These experiments were complemented by molecular dynamics simulations to model interactions between TMA and the IRAK-4 protein.
Results: We demonstrate that this microbiome-associated metabolite decouples inflammation and IR from obesity in HFD. Through *in vitro* kinome screens and *in silico* molecular dynamics studies, we reveal TMA specifically inhibits Interleukin-1 Receptor-associated Kinase 4 (IRAK-4), a central kinase integrating signals from various TLRs and cytokine receptors. Consistent with this, genetic ablation and chemical inhibition of IRAK-4 result in similar metabolic and immune improvements in HFD.
Conclusion: In conclusion, TMA is a key microbial effector inhibiting IRAK-4 and mediating metabolic and immune effects with benefits upon HFD. Thereby we highlight the critical contribution of the microbial signalling metabolome in homeostatic regulation of host disease and the emerging role of the kinome in microbial-mammalian chemical crosstalk.
Supported by: EC, MRC, FRS-FNRS, CIHR, Funds InBev-Baillet Latour
Disclosure: **M. Dumas:** Grants; European Commission (METACARDIS HEALTH-F4-2012-305312).

PS 037 Lipid metabolism in animal models

553

AGEs induce alterations of sphingolipids metabolism in the liver of genetically- and diet-induced diabetic mice

A.S. Cento[1], K. Gaens[2,3], D. Collotta[4], F. Chiazza[4], F. Barutta[5], J. Scheijen[2], M. Aragno[1], M. Collino[4], K. Wouters[2], C.G. Schalkwijk[2,3], R. Mastrocola[1,2];
[1]Dept. of Clinical and Biological Sciences, University of Turin, Turin, Italy, [2]Dept. of Internal Medicine, University of Maastricht, Maastricht, Netherlands, [3]CARIM School for Cardiovascular Diseases, University of Maastricht, Maastricht, Netherlands, [4]Dept. of Drug Science and Technology, University of Turin, Turin, Italy, [5]Dept. of Medical Sciences, University of Turin, Turin, Italy.

Background and aims: High plasma levels of the sphingolipid metabolism intermediates ceramide (Cer) and sphingosine-1-phosphate (S1P) have been found in obese and type 2 diabetic patients. Cer and S1P are reported to induce the inflammatory response leading to impaired insulin signaling in peripheral tissues. Emerging evidence suggests that accumulating Advanced Glycation End-products (AGEs) can alter the sphingolipids metabolism equilibrium, possibly contributing to the onset of insulin-resistance. We, thus, investigated whether AGEs accumulation can affect the sphingolipids metabolism in animal models of insulin resistance.
Materials and methods: To study the direct contribution of AGEs to sphingolipids metabolism and the putative role of the AGE-receptor RAGE therein, HepG2 cells were incubated with 0.5 μM control-albumin and modified N^{ε}-(carboxymethyl)lysine (CML)-albumin, pre-incubated or not with RAGE antibody. To study the role of AGEs on *in vivo* sphingolipid metabolism, C57Bl/6J (WT), and LeptrDb-/- (DbDb) mice were fed a standard diet (SD), while a group of C57Bl/6J was fed a 60% high trans-fat diet (HFD). In addition, two subgroups of SD and HFD fed mice were supplemented with the anti-AGEs compound pyridoxamine. AGEs levels were evaluated in the liver by LC-MSMS and western blotting. Cer and S1P were measured by GC-MS. The expressions of RAGE and of the enzymes involved in sphingolipid metabolism were assessed by RT-PCR and western blotting.
Results: High levels of AGEs and RAGE were detected in the liver of both DbDb and HFD mice in comparison to WT controls. Moreover, the expression of sphingolipid metabolism enzymes was altered in these mice, accompanied by increased levels of Cer and S1P. Specifically, the levels of ceramide synthase 2 and 5 and sphingosine kinase 1 were increased, while those of neutral ceramidase and S1P phosphatase were reduced. In addition, pyridoxamine supplementation to HFD mice diminished hepatic AGEs accumulation and prevented sphingolipids metabolism alterations and insulin resistance development. In line, CML administration to HepG2 cells evoked alterations similar to those observed *in vivo*, and blocking antibodies against RAGE reverted some of the altered enzyme expressions.
Conclusion: The sphingolipid metabolism is affected in different models of diabetes. The modulation of the enzymes responsible for maintenance of the sphingolipid metabolism equilibrium in CML-incubated HepG2 cells indicates the direct involvement of AGEs and their receptor RAGE in these alterations. The role of AGEs was confirmed by the *in vivo* action of pyridoxamine. These results suggest that the modulation of sphingolipids metabolism through the prevention of AGEs accumulation may reduce insulin resistance development.
Supported by: EMBO short-term fellowship
Disclosure: **A.S. Cento:** None.

554

Metabolic and hormonal alterations after orchiectomy in the rat: effect of testosterone substitution

N.L. Katsilambros[1], I.P. Doulamis[1], P. Konstantopoulos[1], A. Tzani[1], A. Daskalopoulou[1], T. Spinos[2], E. Bletsa[2], D. Mitsopoulou[2], M. Spinou[2], M. Brinia[2], K. Palaiopanos[2], L. Korou[1], D.N. Perrea[1];

[1]Laboratory for Experimental Surgery and Surgical Research, National and Kapodistrian University of Athens, Greece, Athens, [2]Student of the National and Kapodistrian University of Athens, Greece, Athens, Greece.

Background and aims: Hypogonadism is considered a risk factor for diabetes and metabolic syndrome. The association between low testosterone (T) and its underlying causes remain unclear and controversial. The present experiments aimed to possibly clarify some aspects of this subject.
Materials and methods: Twenty-nine male Wistar rats aged 11–12 weeks, were randomly divided in 3 groups: Controls (C, $n = 10$): Sham operation; Orchiectomy (ORCH, $n = 9$) and Orchiectomy+T substitution (ORCH+T, $n = 10$). Blood samples were obtained for glucose, total-, HDL-, LDL- cholesterol (T-chol, HDL-chol, LDL-chol) and triglycerides (TG) measurements at day 1 (operation), after 10 days (im injection 100 μg/100 g b.w. of testosterone enanthate), 25d (second T injection) and 40d (sacrifice). In addition, in a subgroup of the animals ($n = 6$ in each main category) insulin, irisin and resistin were measured by rat specific ELISA tests at d1, d10 and d40 time intervals. Values for biochemical and hormonal measurements are expressed as mean ± SD and median ± IQR, respectively. Parametric and non-parametric tests were applied as indicated.
Results: No significant changes in glucose, TG, HDL-chol and body weight were observed. As shown in the table T-chol and LDL-chol were higher in the ORCH group as compared to C ($p < 0.05$). In opposite, both T-chol and LDL-chol were lower in comparison to C in the ORCH+T rats. As it concerns hormonal data, orchiectomy was associated with increased levels of insulin at d10 (median ± IQR: 3.67 ± 1.40 ng/mL vs 0.85 ± 1.42 ng/mL, $p = 0.032$, for d10 and d1, respectively). Hormonal replacement significantly attenuated the negative effect of orchiectomy in insulin resistance as indicated by the observed fall both in insulin levels (median ± IQR: 4.10 ± 2.47 ng/mL vs 1.78 ± 0.68 ng/mL, $p = 0.015$, for d10 and d40, respectively) and HOMA-IR index (median ± IQR: 3.68 ± 1.87 vs 1.74 ± 0.69, $p = 0.026$, for d10 and d40, respectively). Irisin's levels followed the same pattern as the aforementioned markers in the ORCH+T group, peaking 10 days after orchiectomy and returning to its initial levels at d40 (median ± IQR: 0.27 ± 0.11 ng/mL vs 0.85 ± 0.54 ng/mL vs 0.02 ± 0.07 ng/mL, $p = 0.002$ in both cases, for d1, d10 and d40, respectively).
Conclusion: Experimental hypogonadism results in an unfavorable (atherogenic) lipid profile, which is not observed when the ORCH animals are substituted for T. In addition, the observed hormonal changes, if further confirmed, suggest the existence of post-orchiectomy insulin resistance which is improved by T administration.

Parameter	Groups	d1	d10	d25	d40
Total Cholesterol (mg/dL)	**Control**	90.00±16.25	95.00±11.00	90.00±25.00	97.50±18.75
	ORCH	90.00±22.50	102.50±13.00	110.00±18.75	117.50±17.50
	ORCH+T	82.50±21.25	95.00±25.00	102.00±10.00	92.50±30.00
LDL Cholesterol (mg/dL)	**Control**	21.50±9.25	25.50±9.00	29.00±12.00	29.00±13.25
	ORCH	24.00±12.00	33.50±13.25	42.50±20.00	42.50±14.25
	ORCH+T	18.50±18.50	30.50±17.75	31.00±11.50	27.50±12.75

Table. Mean±SD serum Total- and LDL- cholesterol values.

Supported by: SARG
Disclosure: **N.L. Katsilambros:** Grants; Funding from the Special Account for Research Grants of the National and Kapodistrian University of Athens.

555

A short bout of HFD desynchronises feeding behaviour in mice thereby affecting glucose and lipid metabolism

S. Wueest[1], M.A. Dedual[1,2], D. Konrad[1,2];
[1]Children's Hospital and Children's Research Center, University Children's Hospital, Zurich, [2]Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland.

Background and aims: A short bout of HFD impairs glucose tolerance and induces hepatic steatosis in mice. While prolonged HFD-induced metabolic complications are partly mediated by increased food intake during the light (inactive) phase, such link has not yet been established in short-term HFD-fed mice. Herein, we hypothesised that a short bout of HFD desynchronizes feeding behaviour thereby contributing to glucose intolerance and hepatic steatosis.
Materials and methods: 12-week-old C57BL/6J mice were fed a regular chow or high fat diet (HFD, ~60% kcal fat) for 4 days. Food intake was determined in metabolic cages and glucose metabolism was assessed by performing intraperitoneal glucose tolerance tests. Upon scarification, liver and fat depot weights were measured and tissue was collected for analysis. Western blots, rtPCR and a colorimetric assay for the assessment of liver triglyceride levels were performed.
Results: Changing diet from regular chow to HFD led to an immediate increase in food intake already during the 1st light phase (0.5 ± 0.2 g in chow-fed vs. 1.0 ± 0.1 g in HFD-fed, $p < 0.05$), suggesting that HFD desynchronizes feeding behaviour instantly. As expected, 4 days of ad libitum HFD-feeding significantly impaired glucose tolerance (AUC 1639 ± 76 mmol/l*min before HFD vs. 1827 ± 96 mmol/l*min after HFD, $p < 0.05$). Such effect was prevented in mice receiving intermittent HFD-feeding for 4 days (i.e. mice that had no access to food during the light phase) (AUC 1546 ± 41 mmol/l*min before HFD vs. 1690 ± 92 mmol/l*min after HFD, $p = 0.25$), indicating that desynchronized feeding behaviour contributes to short-term HFD-induced glucose intolerance. Of note, food intake was similar between the groups (2.8 ± 0.1 g/mouse*day in ad libitum HFD-fed vs. 2.5 ± 0.1 g/mouse*day in intermittent HFD-fed, $p = 0.2$), as was body weight (31.4 ± 1.0 g in ad libitum HFD-fed vs. 31.5 ± 0.5 g in intermittent HFD-fed, $p = 0.9$). However, intermittent HFD-fed mice revealed higher inguinal fat depot weights (275 ± 30 mg in ad libitum HFD-fed vs. 347 ± 22 mg in intermittent HFD-fed, $p = 0.08$), whereas liver weight was significantly lower in intermittent fed mice (1462 ± 58 mg in ad libitum HFD-fed vs. 1272 ± 59 mg in intermittent HFD-fed, $p < 0.05$). Phosphorylation of hormone sensitivity lipase (HSL) was significantly elevated in inguinal fat depots of intermittent HFD-fed mice (1.0 ± 0.1 in ad libitum HFD-fed vs. 1.5 ± 0.1 in intermittent HFD-fed, $p < 0.01$), indicating increased lipolysis. In support of increased FFA flux to the liver, hepatic PPARα mRNA expression was significantly elevated in intermittent HFD-fed mice (1.0 ± 0.2 in ad libitum HFD-fed vs. 1.8 ± 0.2 in intermittent HFD-fed, $p < 0.05$). In agreement, liver triglyceride levels were significantly increased in intermitted HFD-fed mice (20.5 ± 2.5 μmol/g liver in ad libitum HFD-fed vs. 39.1 ± 5.6 μmol/g liver in intermittent HFD-fed, $p < 0.05$).
Conclusion: Desynchronized feeding behaviour induced by a short bout of HFD impairs glucose tolerance and impacts on liver lipid metabolism.
Disclosure: **S. Wueest:** None.

556

Unsaturated fatty acids rescue GLP-1 secreting cells from a ceramide induced increase in ROS following long term exposure to saturated fatty acids

S. Ntika[1,2], K. Thombare[1], M. Aryapoor[1], C. Krizhanovskii[1,2];
[1]Cardiometabolic research, Södertälje Hospital, Södertälje, [2]Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden.

Background and aims: Studies of the effects of fatty acids (FAs) on the function of GLP-1-secreting cells show that FAs, and especially monounsaturated FAs stimulate GLP-1 release, while long term exposure to elevated levels of the saturated FA palmitate is indicated to induce lipotoxicity *in vivo* and *in vitro*. *In vitro* studies further support that cosupplementation with unsaturated FAs confers lipoprotection. Indeed,

numerous studies in various cell types demonstrate that the extent and nature of the effects induced by FAs are dependent on the molecular species of FA and the length of exposure. The present study was designed to further elucidate the mechanisms underlying the effects of saturated/unsaturated FAs on GLP-1-producing cells in terms of lipotoxicity/lipoprotection and GLP-1 secretion.
Materials and methods: GLP-1-secreting GLUTag cells were cultured in the presence/absence of saturated (16:0) and unsaturated (18:1) fatty acids (0.125 mmol/L) in different time points, followed by analyses of viability, apoptosis, GLP-1 secretion, as well as involvement of fatty acid oxidation, ROS production and ceramide kinase assay. In addition, effects on the expression of superoxide dismutase (SOD), catalase or glutathione peroxidase (GPx) were determined.
Results: Our results demonstrate that generation of intracellular ceramide and mitochondrial FA oxidation contributes to increased ROS production following long term exposure to saturated FAs in GLP-1-secreting cells. Cosupplementation with unsaturated reduces ceramide synthesis, increase the expression of ceramide kinase and attenuate ROS production, caspase-3 activity and DNA fragmentation.
Conclusion: Unsaturated FAs (18:1) reduce ceramide content - thereby attenuating the main source of the lipotoxic ROS production in response to long term exposure to elevated levels of saturated FA (16:0). Findings may be of value for nutritional interventions, as well as for identification of novel targets, to help preserve L-cell mass and potentiate GLP-1 secretion in diabesity.
Supported by: Mats Kleberg, Folksam, Tore Nilsons, Fredrik & Ingrid Thurings
Disclosure: **S. Ntika:** None.

557

Intracellular lipid mobilisation in INS-1E beta cells is required for sustained glucose-stimulated insulin secretion

L. Oberhauser, T. Brun, P. Maechler;
Department of Cell Physiology and Metabolism, University of Geneva Medical Center, Geneva, Switzerland.

Background and aims: Chronic exposure to elevated glucose levels impairs beta-cell function and eventually leads to cell death. This effect, referred to as glucotoxicity, has been described to be worsened by chronic free fatty acids (glucolipotoxicity). However, the associated turnover of the stored lipids and the consequences on glucose-stimulated insulin secretion remains a matter of debate. The aim of this study was to determine the contribution of the turnover of *de novo* lipid synthesis versus exogenous saturated and unsaturated fatty acids on the function of INS-1E beta-cells.
Materials and methods: INS-1E beta-cells were cultured for 3 days at standard 11.1 mM (control) and high 25 mM glucose in the presence or absence of BSA-complexed 0.4 mM palmitate or oleate, or a mix of both (2 × 0.2 mM) fatty acids. Lipid accumulation and mobilization were assessed by quantifying Bodipy probe fluorescent signal after the 3 days of treatment along a 12h time course at low 5.5 mM glucose. Maximal lipid storage capacity was determined by co-incubating cells with the lipase inhibitor Orlistat during the 3 days of treatment. INS-1E beta-cell function was assessed by 96-well online kinetic measurements of insulin secretion (using luciferase-based C-peptide substitution) upon glucose stimulation with or without Orlistat or insulin pre-treatment.
Results: Chronic treatment with high glucose increased cellular lipids 1.5-fold compared to control (*de novo* lipid synthesis). This effect was markedly potentiated by the addition of oleate in the medium as it increased lipid accumulation by 25-fold compared to control ($p < 0.0001$). Palmitate did so to a much lesser extent (7-fold), while the mix of both fatty acids showed an intermediary lipid accumulation profile (15-fold, $p < 0.01$). The inhibition of cell lipases by Orlistat indicated a maximal lipid accumulation capacity of 40-fold the control ($p < 0.0001$), revealing a substantial lipid turnover in INS-1E beta-cells. However, the blockade of beta-oxidation by Etomoxir during the 3 days of treatment did not further increase lipid accumulation. Once incubated at low glucose, intracellular lipids were differently mobilized according to the previous culture conditions. Over the 12h at low glucose, lipid pool from *de novo* synthesis remained constant, whereas more than 80% of the lipids stored by the cells previously treated with high glucose plus palmitate were mobilized within 3h. Concomitantly, about 75% of the lipid pool in cells treated with high glucose plus the mix of palmitate and oleate was mobilized versus only 30% for the cells treated with high glucose plus oleate. Preventing the cells from mobilizing their stored lipids using Orlistat blunted glucose-stimulated insulin secretion in all conditions. Interestingly, insulin treatment also reduced both lipid mobilization and the secretory response.
Conclusion: INS-1E beta-cells chronically exposed to free fatty acids massively store neutral lipids that can be rapidly mobilized. This turnover depends on the chemical identity of the fatty acids, the saturated palmitate being mobilized much faster than the unsaturated oleate. The mobilized lipids are preferentially required for insulin secretion rather than being used as an energy source. Moreover, insulin seems to act as a negative feedback on glucose-stimulated insulin secretion by lowering fatty-acid mobilization in INS-1E beta-cells.
Supported by: CTI/Innosuisse
Disclosure: **L. Oberhauser:** Grants; CTI/Innosuisse.

558

The protective effect of SFC on high fat diet induced NAFLD in C57BL/6J mice

S. Hong[1,2], Y. Kang[1,2];
[1]The Graduate School Ajou University, Suwon, Gyunggi-do, [2]Department of Physiology, Ajou University School of Medicine, Suwon, Gyunggi-do, Republic of Korea.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a fatty liver disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). High fat diet (HFD) generally leads to NAFLD accompanying with obesity. Augmented mobilization of large amount of fatty acids from adipose tissues to the liver is thought to be a cause for development of HFD-induced NAFLD. Thus, inhibition of fatty acid uptake into hepatocyte would be a maneuver for protection of HFD-induced NAFLD. This study was initiated to whether sodium fluorcitrate (SFC) known as a fatty acid uptake inhibitor prevents HFD-induced NAFLD in C57BL/6J mice.
Materials and methods: In hepG2 cells, we treated SFC 0.2mM for 1 hours, followed by palmitate (100uM) conjugated BODIPY-C16 fluroscence uptake for another 3 hours and detected fluorescence.
Results: Initially, we investigated the protective effect of SFC on saturated fatty acid- nduced lipotoxicity in HepG2 hepatocytes. SFC reduced BODIPY-C16 uptake into HepG2 cells and prevented fatty acid- induced lipid accumulation. In addition, SFC treatment was protective against palmitate-induced stress/inflammatory and insulin resistance signals. SFC reduced levels of palmitate-induced p-JNK, p-NFkB, CHOP and restored levels of palmitate-induced p-AKT and p-GSK. C57BL/6J mice with HFD feeding for 16 weeks showed most characteristics occurring in NAFLD. HFD increased TG content in liver and activated stress/inflammatory signals such as p-JNK and p-NFkB. HFD enhanced expression of inflammatory genes such as IL-1b, TNF-a, and MCP. Hepatic injury was also observed by increased level of plasm levels of AST and ALT in HFD-fed mice. Simultaneous treatment with SFC (10 mg/kg, 16 weeks) in HFD-fed mice reduced all markers of HFD-induced NAFLD. SFC reduced hepatic TG level and stress/inflammatory signals in HFD-fed mice. SFC restored HFD-induced insulin resistance signal and hepatic injury. SFC also prevented HFD-induced hyperglycemia and insulin resistance.
Conclusion: In conclusion, inhibition of fatty acid uptake into hepatocytes through SFC treatment can be a strategy to prevent HFD-induced NAFLD.
Disclosure: **S. Hong:** None.

559

Linking of bioenergetic function of mitochondria to tissue-specific molecular fingerprints in mice and humans

L. Kappler[1], M. Hoene[1], J. Li[2], C. Hu[2], C. von Törne[3], L. Kollipara[4], C. Hoffmann[1], A. Böhm[5,6], S. Hauck[3], G. Xu[2], H.-U. Häring[1,6], A. Peter[1,6], A. Sickmann[4,7], C. Weigert[1,6], R. Lehmann[1,6];
[1]Department of Diagnostic Laboratory Medicine, Clinical Chemistry Central Laboratory, Tuebingen, Germany, [2]Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China, [3]Research Unit Protein Science, Helmholtz Zentrum, Muenchen, Germany, [4]Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Dortmund, Germany, [5]Internal Medicine 4, Tuebingen, Germany, [6]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Zentrum Muenchen at the University of Tuebingen, Tuebingen, Germany, [7]Department of Chemistry, College of Physical Sciences, Aberdeen, UK.

Background and aims: Mitochondria are the major sites for energy production in most tissues, thus are molecularly tailored to meet tissue-specific demands. Unravelling the link of mitochondrial protein and lipid molecular fingerprints between tissues and their impact on mitochondrial function provides an important tool for the disentangling of tissue-specificities upon mitochondrial alterations facing external stimuli such as excess substrate flux by diet causing insulin resistance (IR) and diabetes.
Materials and methods: Mitochondrial function was investigated by respirometry (Oxygraph 2k) in mice and human tissue samples. LC-MS/MS-based proteomic/lipidomic analyses of ultracentrifugation-isolated mitochondria from skeletal muscle and liver of a pre-diabetic mouse model (6 weeks of control (10 kJ% fat/20 kJ% protein/70 kJ% carbohydrates) vs high-energy diet (HED; 45 kJ% fat/20 kJ% protein/35 kJ% carbohydrates) were performed ($n = 8$ each). Mitochondrial proteome was covered by non-targeted data-independent acquisition (DIA).
Results: Lipidomics revealed distinctly different lipid profiles (~280 species/50 µg mitochondrial protein) between tissues. This was specifically reflected in the acyl chain composition of phospholipids including the mitochondrial signature lipid cardiolipin (28 species) and phosphatidylethanolamine (57 species), both key regulators of oxidative phosphorylation. Despite this tissue-specificity of mitochondria, their response to HED was comparable on lipid level. However, functional analysis of isolated mitochondria from mice and human revealed that muscle oxidised more of the complex 1 substrate pyruvate (when already respiring on a fatty acid) whereas liver increased respiration more after addition of the complex 2-associated substrate succinate, indicating a routing of pyruvate towards carboxylation. Both tissues showed a higher fat oxidation capacity in the IR state. DIA identified ~2000 proteins in liver and ~900 in muscle mitochondria showing diet associated clustering by principle component analysis.
Conclusion: We linked the mitochondrial molecular composition with tissue-specific mitochondrial (dys)function generating a novel tool for elucidating mitochondrial adaptions to e.g. diet-induced IR.
Supported by: DZD
Disclosure: **L. Kappler:** None.

560

Chronic treatment with acylated analogues of apelin-13 improves glycaemic control and lipid profiles in diet induced obese diabetic mice

F.P.M. O'Harte, V. Parthsarathy, C. Hogg, P.R. Flatt;
School of Biomedical Sciences, University of Ulster, Coleraine, UK.

Background and aims: Apelin-13 is an adipokine which has promising metabolic effects but is rapidly degraded in plasma. Previous studies have shown that modified apelin analogues exhibited enzyme resistance in plasma and improved circulating half-life compared to apelin-13. This study investigated the antidiabetic effects of chronic administration of stable long acting fatty acid modified apelin analogues, namely, (Lys^{8}GluPAL)apelin-13 amide and pGlu(Lys^{8}GluPAL)apelin-13 amide, in diet induced obese-diabetic mice.
Materials and methods: Male NIH Swiss mice (groups $n = 8$) were maintained either on a high-fat diet (45% fat) from 8 to 28 weeks old, or control mice were fed a normal diet (10% fat). When diet induced obesity-diabetes was established after high-fat feeding, mice were injected i.p. once daily with apelin analogues, liraglutide (25 nmol/kg) or saline (controls).
Results: Administration of (Lys^{8}GluPAL)apelin-13 amide and pGlu(Lys^{8}GluPAL)apelin-13 amide for 28 days significantly reduced food intake and decreased body weight. Non-fasting glucose was reduced ($p < 0.01$ to $p < 0.001$) and circulating insulin concentrations elevated ($p < 0.01$ to $p < 0.001$). This was accompanied by enhanced insulin responses ($p < 0.01$ to $p < 0.001$) and significant reductions in glucose excursion after both oral ($p < 0.01$) or i.p. ($p < 0.01$) glucose challenges and feeding. Apelin analogues also significantly improved HbA_{1c} ($p < 0.01$), enhanced insulin sensitivity ($p < 0.01$), reduced triglycerides ($p < 0.001$), increased HDL-cholesterol ($p < 0.01$) and decreased LDL-cholesterol ($p < 0.01$), compared to saline treated diet induced obese mice. Cholesterol levels were decreased ($p < 0.01$) by pGlu(Lys^{8}GluPAL)apelin-13 amide and both apelin treated groups showed improved bone mineral content, reduced fat deposits and increased plasma GLP-1 concentrations. Daily treatment with liraglutide mirrored many of these changes (not on bone or adipose tissue), but unlike apelin analogues increased plasma amylase. Consumption of O_2, production of CO_2, respiratory exchange ratio and energy expenditure were improved by apelin analogues. These results indicate that long-term treatment with acylated analogues (Lys^{8}GluPAL)apelin-13 amide and particularly pGlu(Lys^{8}GluPAL)apelin-13 amide resulted in similar or enhanced therapeutic responses compared to liraglutide in high-fat fed mice.
Conclusion: Stable fatty acid modified apelin analogues represent a new and exciting development in the treatment of obesity-diabetes.
Supported by: Invest NI PoC518 award, DEL PhD Studentship for CH
Disclosure: **F.P.M. O'Harte:** Grants; Invest NI PoC518 grant funding.

PS 038 Weight regulation and obesity in humans

561

Weight loss reduces postprandial dicarbonyl stress but not AGEs in abdominally obese men

M.D.G. Van den Eynde[1,2], Y.H.A. Kusters[1,2], J.L.J. Scheijen[1], A.J.H. Houben[1], P.J. Joris[2,3], J. Plat[3], R.P. Mensink[2,3], C.D.A. Stehouwer[1], C.G. Schalkwijk[1,2];
[1]Department of Internal Medicine, School for Cardiovascular Diseases (CARIM), Maastricht University Medical Center, Maastricht, [2]Top Institute of Food and Nutrition (TIFN), Wageningen, [3]Department of Human Biology, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University Medical Center, Maastricht, Netherlands.

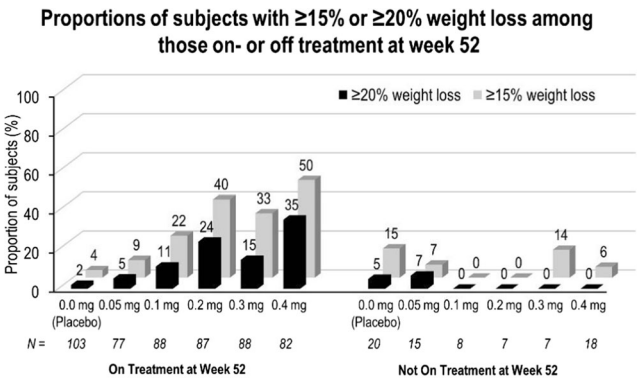
Background and aims: Dicarbonyl compounds are highly reactive byproducts of glycolysis and are a major contributor to the formation of advanced glycation endproducts (AGEs) and the development of insulin resistance and vascular complications. We recently demonstrated that increased α-dicarbonyls in the postprandial phase in patients with impaired glucose metabolism and type 2 diabetes were reduced by weight loss. However, data of α-dicarbonyls and AGEs in abdominally obese individuals are lacking. Therefore, we aim to examine fasting and postprandial levels of α-dicarbonyls and AGEs in lean and abdominally obese men and we evaluate whether a weight loss intervention reduces α-dicarbonyl stress and AGEs.
Materials and methods: Plasma samples were collected from lean ($n = 25$) and abdominally obese men ($n = 52$) in the fasting state, and in the postprandial phase during a mixed meal test. Abdominally obese men were randomized to either an 8-week dietary weight loss intervention program or their habitual diet; after 8 weeks a second mixed meal test was performed. The α-dicarbonyls, methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG) and AGEs were measured by UPLC-MS/MS. In the fasting state, skin autofluorescence (SAF) was measured using the AGE-reader. An independent samples t-test was used for the cross-sectional data, one-factor analysis of covariance with the baseline value as a covariate was used to detect differences between groups over time.
Results: Fasting MGO, GO, and 3-DG did not differ significantly between lean and abdominally obese men (MGO: 293 ± 52 nM vs 297 ± 54 nM, GO: 755 ± 195 nM vs 686 ± 139 nM, 3-DG: 958 ± 74 nM vs 993 ± 117 nM). In accordance, also AGEs and SAF did not show any difference between lean and obese individuals. However, postprandial α-dicarbonyl concentrations were significantly higher in obese men as compared to lean men (iAUC difference between lean and obese: MGO $p < 0.01$, GO $p = 0.06$, 3-DG $p < 0.01$). After an 8-week dietary weight loss intervention (−10% body weight on average), fasting α-dicarbonyls tended to decrease (MGO: from 291 ± 52 nM to 275 ± 52 nM; GO: from 674 ± 152 nM to 607 ± 136 nM; 3-DG: from 950 ± 105 nM to 938 ± 86 nM), while the weight stable group did not show such differences. AGEs and SAF measurements did not change significantly. Postprandial α-dicarbonyl levels in the weight reduction group were reduced (iAUC: MGO −75%, GO −50%, 3-DG −68%) as compared to the weight stable group (MGO $p < 0.05$, GO $p < 0.05$, 3-DG $p < 0.01$).
Conclusion: This study shows that abdominal obesity is characterized by increased postprandial α-dicarbonyl stress. While AGE and SAF levels were largely unaltered, postprandial α-dicarbonyl stress can be significantly reduced by weight reduction in abdominally obese individuals.
Clinical Trial Registration Number: NCT01675401
Supported by: Top Institute of Food and Nutrition
Disclosure: **M.D.G. Van den Eynde:** None.

562

High number of responders with pharmacotherapy-induced weight loss ≥15% in a placebo-controlled dose-ranging study of semaglutide in subjects with obesity

A.L. Birkenfeld[1], J. Wilding[2], B. McGowan[3], O. Mosenzon[4], S.D. Pedersen[5], S. Wharton[6], L. Endahl[7], C.E. Heerdegen-Jepsen[7], P.M. O'Neil[8];
[1]University Hospital and Faculty of Medicine, TU Dresden, Germany, [2]University of Liverpool, Liverpool, UK, [3]Guy's and St Thomas NHS Foundation Trust, London, UK, [4]Hadassah Hebrew University Hospital, Jerusalem, Israel, [5]C-ENDO Diabetes & Endocrinology Clinic, Calgary, Canada, [6]York University, Toronto, ON, Canada, [7]Novo Nordisk, Søborg, Denmark, [8]Medical University of South Carolina, Charleston, USA.

Background and aims: Semaglutide (SEMA) is a glucagon-like peptide 1 receptor agonist recently approved to treat type 2 diabetes and currently under investigation at higher doses for weight management. A recent phase 2 trial showed significant, dose-dependent weight loss vs placebo for once-daily SEMA at doses of 0.05–0.4 mg. In this *post-hoc* analysis, the proportion of subjects achieving ≥15% or ≥20% weight loss in this trial was assessed by assigned treatment and by whether treatment was completed.
Materials and methods: This was a randomised, double-blinded, placebo-controlled phase 2 trial of SEMA with dietary and exercise counselling in adults with obesity without diabetes. SEMA (0.05, 0.1, 0.2, 0.3 or 0.4 mg) or matching placebo was given by once-daily s.c. self-injection; starting at 0.05 mg, and escalated to the next dose level every 4 weeks until the target dose was reached. Total treatment duration (including an escalation phase ≤16 weeks) was 52 weeks. Subjects who discontinued treatment for any reason ceased subsequent visits but were encouraged to return at week 52 for off-treatment assessment. The proportions with weight loss ≥15% or ≥20% of baseline were assessed at week 52 for those with data on or off treatment with SEMA or placebo.
Results: 649 subjects (35% male) were randomised on the above schedule. Mean age was 44–48 years across dosing groups (overall range 18–77); mean weight was 111–114 kg (73–244), and mean BMI 39–40 kg/m^2 (30–80). Week 52 data were available for 600 subjects (92%); 525 on treatment, 75 off treatment. Response was dose-related and the proportion of responders in both weight-loss categories was high at doses of 0.2–0.4 mg/day; overall 32–42% of subjects lost ≥15% of baseline weight and 14–29% lost ≥20%, vs 6% and 2%, respectively, on placebo. Almost all responders in both categories at these SEMA doses were still on treatment at week 52 (97–100% across dosing arms). Among subjects who completed the full 52-week treatment period, 33–50% receiving 0.2–0.4 mg/day SEMA had a weight loss ≥15%, and 15–35% had a loss ≥20% (Figure).
Conclusion: A high proportion of subjects in this phase 2 study who completed SEMA treatment at doses of 0.2–0.4 mg/day over 52 weeks lost at least 15% of baseline body weight. For those who received 0.4 mg/day, half lost ≥15% and 35% lost ≥20% of their baseline weight.



Clinical Trial Registration Number: NCT02453711
Supported by: Novo Nordisk

Disclosure: **A.L. Birkenfeld:** Honorarium; Novo Nordisk. Other; Novo Nordisk.

563

The effects of acarbose, sitagliptin, verapamil, liraglutide and pasireotide on hypoglycaemia and glycaemic variability in Roux-en-Y gastric bypass operated subjects

C.C. Øhrstrøm[1], D.L. Hansen[2], U.L. Kielgast[1], J.J. Holst[3], D. Worm[4]; [1]Zealand University Hospital, Køge, [2]Steno Diabetes Center, Gentofte, [3]University of Copenhagen, Copenhagen, [4]Amager Hospital, Copenhagen, Denmark.

Background and aims: Hypoglycemia is a severe complication after Roux-en-Y gastric bypass (RYGB), with no effective treatment options. We investigated the glucose stabilizing effects of five therapeutic agents in RYGB operated subjects with hypoglycemia.

Materials and methods: In a randomized crossover study, 11 RYGB operated subjects with documented hypoglycemia (blood glucose <3.9 mmol/L) underwent six separate meal tolerance tests (MTT) preceded by either: no treatment, acarbose 50 mg × 6 daily for 1 week, sitagliptin 100 mg for 1 week, verapamil 120 mg for 1 week, liraglutide 1.2 mg for 3 weeks or pasireotide 300 μg as a single dose. Blood samples were drawn at fixed time intervals from −20 to 180 min. Hormonal responses were calculated as the incremental area under the curve (iAUC), and the glucose response was further specified as the area above (+iAUC) and below (−iAUC) baseline values. Furthermore, the treatment effect was evaluated in everyday life by 6 days of continuous glucose monitoring (CGM) during treatment with acarbose, sitagliptin, verapamil, liraglutide, and compared to a baseline recording with no treatment. Data were analyzed by use of linear mixed models.

Results: (See table 1) During the MTT, treatment with acarbose and pasireotide significantly increased nadir glucose, whereas treatment with sitagliptin decreased nadir glucose. Acarbose, liraglutide and pasireotide significantly reduced $-iAUC_{glucose}$, while only acarbose reduced $+iAUC_{glucose}$ and pasireotide increased this area. Time spent in hypoglycemia (<3.9 mmol/L) decreased with both acarbose and pasireotide, whereas time in hyperglycemia (>7.9 mmol/L) decreased with acarbose but increased with pasireotide. Treatment with acarbose and pasireotide significantly reduced $iAUC_{insulin}$. During the CGM recordings, treatment with liraglutide increased minimum interstitial fluid glucose (IG) and both liraglutide and acarbose reduced the standard deviation of the IG measurements. Treatment with acarbose, sitagliptin and liraglutide reduced percentage of time spent in hyperglycemia, whereas none of the treatments had any impact on time spent in hypoglycemia.

Conclusion: In response to a standardized meal, treatment with acarbose reduced both hypoglycemia and hyperglycemia, whereas pasireotide completely resolved hypoglycemia but at the cost of increased hyperglycemia. In everyday life, both acarbose and liraglutide decreased glycemic variability and reduced hyperglycemia, but had little effect on hypoglycemia. Acarbose, pasireotide and perhaps liraglutide can be considered in the treatment of hypoglycemia following RYGB.

Variable	No treatment	Acarbose	Sitaglitpin	Verapamil	Liraglutide	Pasireotide
MTT						
Nadir glucose *(mmol/L)*	3.4 ± 0.2	3.9 ± 0.2*	3.0 ± 0.2*	3.3 ± 0.2	3.3 ± 0.2	7.9 ± 0.4*
$-iAUC_{glucose}$ *(mmol*L-1*min)*	106 ± 14	49 ± 14*	112 ± 24	113 ± 15	70 ± 12*	0 ± 0*
$+iAUC_{glucose}$ *(mmol*L-1*min)*	182 ± 18	78 ± 18*	174 ± 32	177 ± 25	214 ± 26	1144 ± 62*
Time in hypoglycemia *(min)*	48 ± 12	5 ± 3*	67 ± 10	46 ± 11	55 ± 9	0 ± 0*
Time in hyperglycemia *(min)*	29 ± 4	5 ± 5*	22 ± 6	29 ± 6	29 ± 5	159 ± 6*
$iAUC_{insulin}$ *(nmol*L-1*min)*	53 ± 8	22 ± 3*	62 ± 13	50 ± 9	64 ± 9	14 ± 2*
CGM						
Minimum IG *(mmol/L)*	2.7 ± 0.2	3.0 ± 0.2	2.7 ± 0.2	2.9 ± 0.1	3.0 ± 0.2*	-
Standard deviation *(mmol/L)*	1.5 ± 0.1	1.1 ± 0.1*	1.2 ± 0.09	1.6 ± 0.1	1.2 ± 0.1*	-
Time in hypoglycemia *(%)*	6.2 ± 1.3	4.9 ± 1.1	9.4 ± 2.5	7.5 ± 3.1	9.4 ± 2.6	-
Time in hyperglycemia *(%)*	8.8 ± 1.3	4.2 ± 0.9*	4.8 ± 1.3*	10.4 ± 1.6	4.8 ± 1.0*	-

Table 1. Results from MTT and CGM (mean ± SEM).
Hypoglycemia ≤ 3.9 mmol/L; hyperglycemia ≥ 7.9 mmol/L; IG = interstitial fluid glucose; * = p ≤ 0.05.

Clinical Trial Registration Number: NCT02527993
Supported by: Novartis Healthcare,
Disclosure: **C.C. Øhrstrøm:** Grants; Novartis Healthcare A/S.

564

A randomised, phase 2, placebo- and active-controlled dose-ranging study of semaglutide for treatment of obesity in subjects without diabetes

J. Wilding[1], A.L. Birkenfeld[2], B. McGowan[3], O. Mosenzon[4], S.D. Pedersen[5], S. Wharton[6], M. Kabisch[7], C.G. Carson[7], P.M. O'Neil[8]; [1]University of Liverpool, Liverpool, UK, [2]University Hospital and Faculty of Medicine, TU Dresden, Germany, [3]Guy's and St Thomas' NHS Foundation Trust, London, UK, [4]Hadassah Hebrew University Hospital, Jerusalem, Israel, [5]C-ENDO Diabetes & Endocrinology Clinic, Calgary, Canada, [6]York University, Toronto, Canada, [7]Novo Nordisk, Søborg, Denmark, [8]Medical University of South Carolina, Charleston, USA.

Background and aims: The global rise in the prevalence of obesity and its comorbidities is a major public health challenge. The incretin glucagon-like peptide-1 (GLP-1) regulates both insulin secretion and appetite, and significant weight loss has been observed among subjects treated with the GLP-1 receptor agonists liraglutide (LIRA) and semaglutide (SEMA). The use of SEMA for treatment of obesity without diabetes was evaluated in a Phase 2 clinical trial.

Materials and methods: This was a multinational, randomised, double-blinded, dose-ranging study of SEMA versus placebo (PBO) and an active LIRA control (3 mg), each with dietary and physical activity counselling. Eligible subjects were adults with obesity (BMI ≥30 kg/m^2) without diabetes and with at least one previous non-surgical attempt at weight loss. Participants were randomised to receive PBO or active treatment with either once-daily subcutaneous SEMA at doses of 0.05, 0.1, 0.2, 0.3, or 0.4 mg (starting at 0.05 mg and escalating every 4 weeks to target dose) or with once-daily subcutaneous LIRA 3 mg (weekly escalation from 0.6 mg), in a 6:1 active:PBO ratio. Each active group had a PBO counterpart of matching injection volume and escalation schedule; all PBO groups were pooled for analysis. Two additional faster-escalation SEMA groups are not presented here. The primary endpoint was change in body weight (%) from baseline (BL) to week 52 (analysis of covariance model; treatment, region, sex, and BL body weight as covariates).

Results: A total of 957 subjects (35% male) were randomised and treated (102–103 per active arm; 136 pooled PBO). Mean (range) BL characteristics were: age 47 (18–86) years, weight 111 (70–244) kg, and BMI 39 (30–80) kg/m^2. Overall, 93% (892/957) had body weight data at Week 52 (81% on drug, 12% discontinued). Estimated mean weight losses from BL to Week 52 were −2.3% (PBO) and −7.8% (LIRA 3 mg), vs −6.0% (0.05 mg; $P = 0.001$ vs PBO), −8.6% (0.1 mg), −11.6% (0.2 mg), −11.2% (0.3 mg) and −13.8% (0.4 mg; $P < 0.0001$ vs PBO for 0.1–0.4 mg). All comparisons remained significant after adjustment for multiple testing. Mean weight loss for 0.2–0.4 mg were all $P < 0.01$ (unadjusted) vs LIRA 3 mg. Weight loss ≥5% occurred in an estimated 23% (PBO) and 66% (LIRA 3 mg) vs 54% (0.05 mg), 67% (0.1 mg), 75% (0.2 mg), 81% (0.3 mg), and 83% (0.4 mg) of subjects (all $P < 0.0001$ vs PBO). Weight loss ≥10% occurred in an estimated 10% (PBO) and 34% (LIRA 3 mg) vs 19% (SEMA 0.05 mg; P=NS vs PBO), 37% (0.1 mg), 56% (0.2 mg), 58% (0.3 mg), and 65% (0.4 mg) of subjects ($P < 0.0001$ vs PBO for 0.1–0.4 mg). All SEMA doses were generally tolerated; there were no new safety concerns observed. The most common adverse events on SEMA were dose-related gastrointestinal events as seen previously with GLP-1 receptor agonists.

Conclusion: In combination with dietary and physical activity counselling, all SEMA doses from 0.05 to 0.4 mg daily were tolerated and resulted in dose-related reductions in body weight that were superior to PBO among people with obesity without diabetes.

Clinical Trial Registration Number: NCT02453711

Supported by: Novo Nordisk
Disclosure: **J. Wilding:** Employment/Consultancy; AstraZeneca, Janssen, Lilly, Novo Nordisk, Sanofi. Grants; AstraZeneca, Novo Nordisk, Takeda. Honorarium; Astellas, AstraZeneca, Boehringer Ingelheim, Janssen, Lilly, Novo Nordisk, Sanofi. Lecture/other fees; AstraZeneca, Boehringer Ingelheim, Janssen, Novo Nordisk, Sanofi, Takeda, Lilly. Other; Astellas, AstraZeneca, Janssen, Novo Nordisk, Sanofi.

565

Serum acid uric levels as an indicator for metabolically unhealthy obesity in youth: results from a population-based cohort in Germany

E.A.A. Rocha[1], M. Vogel[2], J. Stanik[2], A. Körner[3], W. Kiess[1];
[1]University of Leipzig, Hospital for Children and Adolescents, University of Leipzig, Leipzig, [2]University of Leipzig, Centre of Paediatric Research (CPL), University of Leipzig, Leipzig, [3]Hospital for Children and Adolescents, Department of Women and Child Health, University Hospitals, University of Leipzig, Leipzig, Germany.

Background and aims: Obesity during the childhood and adolescence increases the risk of several other chronic diseases and, because of its epidemic proportions, has become a major public health problem worldwide. Increasing evidence shows, paradoxically, that some obese individuals, reported as having "metabolically healthy obesity (MHO)", seem to be protected from developing type 2 diabetes mellitus and a cardiovascular disease, in contrast to those with metabolically unhealthy obesity (MUO). The aim of this study was to improve the understanding of potential clinical and metabolic indicators that may help to distinguish between MHO from MUO phenotype. The specific aim of this study was to explore the relationship between serum uric acid levels and metabolic health in overweight and obese children and adolescents.
Materials and methods: The study involved 246 overweight/obese (ov/ob) and 212 normal weight individuals enrolled in the LIFE-Child study, aged between 6 and 18 years. LIFE-Child is a longitudinal study to evaluate how environmental, metabolic and genetic factors affect development and health from fetal life to adulthood.
Results: Among the 246 ov/ob individuals, 173 (70%) can be regarded as MHO and 73 (30%) can be regarded as MUO. Overweight/obese individuals who were completely free of metabolic cardiovascular risk, i.e., fasting serum lipids, blood pressure and glucose were classified as MHO. Individuals meeting one or more criteria of cardiovascular risk factors were classified as metabolically unhealthy obesity (MUO). The MHO individuals were younger, more likely to be male, had lower BMI-SDS and had lower levels of uric acid and C-peptide and liver enzymes. In addition, glucose metabolism was altered in MUO as indicated by increased insulin levels and reduced WBISI with normal glycemia compared to the MHO group. In logistic regression models, UA SDS, waist circumference, and C-peptide, were significant indicators of MUO after adjusting for age, sex, pubertal status, and BMI-SDS.
Conclusion: Our results show that nearly one-sixth of the individuals are already identified as MUO. Findings suggest that higher levels of uric acid are an indicator of MUO in German overweight and obese children and adolescent.
Clinical Trial Registration Number: 264-10-19042010
Supported by: CAPES-Brazil and LIFE-Child, Leipzig, Germany
Disclosure: **E.A.A. Rocha:** None.

566

Beta cells in diabetes remission and hypoglycaemia after Roux-en-Y gastric bypass surgery, visualised by 68Ga-exendin-4 PET/CT

M. Boss[1,2], L.N. Deden[2,3], H. de Boer[3], E.O. Aarts[2], E. Hazebroek[2], M. Brom[1], F.J. Berends[2], M. Gotthardt[1];
[1]Nuclear medicine, Radboudumc, Nijmegen, [2]Surgery, Rijnstate hospital, Arnhem, [3]Internal Medicine, Rijnstate hospital, Arnhem, Netherlands.

Background and aims: Roux-en-Y gastric bypass surgery (RYGB) is performed in patients with morbid obesity to achieve weight loss. In addition, remission of type 2 diabetes (T2D) occurs in >60% of patients. In rare cases, hyperinsulinaemic hypoglycaemia develops. Mechanisms underlying these responses are incompletely understood, but a role for beta cell activity (BCA) and beta cell mass (BCM) is hypothesized. Studying BCM *in vivo* is possible using radiolabeled Exendin-4, a stable analogue of glucagon-like peptide-1, that specifically accumulates in beta cells. In this study, the role of beta cell mass in differences in metabolic responses to RYGB was examined by ^{68}Ga-exendin-4 PET/CT.
Materials and methods: BCA and BCM were compared between patients with differences in glycaemic control after RYGB. Five patients with complete T2D remission (responders), five without complete T2D remission (non-responders) and five with hypoglycaemia were included. BCA was measured by arginine stimulation. BCM was studied by ^{68}Ga-exendin-4 PET/CT. Total pancreatic uptake of ^{68}Ga-exendin-4 was measured as a marker for BCM.
Results: Patient characteristics and weight loss were comparable between the groups. BCA was lower in non-responders compared to responders, (acute C-peptide responses: 0.4 ± 0.2 and 0.9 ± 0.3 nmol/l, $p = 0.02$). Pancreatic 68Ga-exendin-4 uptake was 26% lower in non-responders (83 ± 58 kBq) compared to responders (111 ± 55 kBq), although not statistically significant ($p = 0.088$). BCA and BCM did not correlate. In hypoglycaemia, mean BCM was higher than in responders (191 ± 63 kBq, $p = 0.032$).
Conclusion: This data suggests that BCM is higher in T2D responders compared to non- responders and higher in hypoglycaemia compared responders. In conclusion, BCM may play an important role patient response to RYGB. ^{68}Ga-exendin-4 PET/CT is a feasible technique to measure BCM *in vivo* and study its role in pathophysiological mechanisms underlying diabetes.
Clinical Trial Registration Number: 2014-004317-90 and 2014-005554-20
Disclosure: **M. Boss:** None.

567

Morbid obesity does not attenuate ethnic differences in regionalisation of body fat

M.B. Whyte[1,2], P. Chitongo[2], L.N. Roberts[2], R. Arya[2], S.J.B. Aylwin[2];
[1]Clinical & Experimental Medicine, University of Surrey, Guildford, [2]Medicine, King's College Hospital NHS Foundation Trust, London, UK.

Background and aims: Body mass index (BMI) is a risk factor for mortality but this relationship is weaker for black African origin than Caucasians and may relate to racial differences in body fat distribution. However, studies have primarily been made in those with a BMI of <40 kg/m^2. We examined whether racial differences in central-to-peripheral body fat distribution are present in those with morbid obesity. Due to gender differences in body composition, females only were analysed. As women increase central fat deposition during menopausal transition, we compared women of 'premenopausal' (<52 years) to 'postmenopausal' (≥52 years) age.
Materials and methods: Patients were recruited from a bariatric obesity clinic. Inclusion criteria: females, BMI ≥40 kg/m^2, age ≥18 years. Exclusion criteria: known diabetes, active cancer, history of myocardial infarction, cerebrovascular disease, bariatric surgery. Visceral adipose tissue (VAT) evaluated by single-slice computed tomography (CT) at midpoint of L4 vertebral body. Semi-automated assessment of VAT and subcutaneous adipose tissue (SAT) compartments were performed using Analyze direct 10 software (AnalyzeDirect, Kansas, USA). Adipose tissue area was determined by a Hounsfield unit range between −190 and −30. Fasting samples were collected for glucose, insulin and lipid profile. Insulin resistance was calculated using Homeostasis Model Assessment 2 (HOMA2-IR). BMI and VAT quintile, ethnicity, age category were used in a General Linear Model (GLM) for HOMA2-IR. Comparisons

between (self-reported) ethnicity groups using Kruskal-Wallis (non-parametric) and ANOVA (parametric) data. Data are mean(SD) except triglycerides: median (interquartile range).

Results: A total of $n = 71$ (Caucasian $n = 40$, black African origin (BAO) $n = 14$, Asian $n = 17$) were studied ('premenopausal' $n = 51$). There was no difference in BMI (Table 1) or age: Caucasian 45.1(9.5), BAO 49.3(11.7), Asian 42.1(11.0) years; $P = 0.158$ between ethnicities. BAO and Asians had lower triglycerides and VAT than Caucasians. There were no differences in SAT area between ethnicity, however the VAT/SAT ratio was higher in Caucasians than BAO or Asians (Table 1). HOMA2-IR was lower in BAO than Asians and Caucasians. There was no difference in VAT or SAT between age categories (Table 1). In the GLM, BAO predicted lower HOMA2-IR than Caucasians (β −0.902, 95% CI −1.671 to −0.133; $P = 0.022$). Compared to largest VAT quintile (VAT5), lowest VAT (VAT1) predicted lower HOMA2-IR (β −1.205; $P = 0.043$), as did VAT2 (β −1.485; $P = 0.009$), VAT3 (β −1.171; $P = 0.031$) and VAT4 (β −1.293; $P = 0.013$). No relationship of age or BMI category to HOMA2-IR.

Conclusion: Even in morbid obesity, differences remain in the regionalisation of body fat between ethnicities. There is greater visceral adiposity in morbidly obese Caucasian females. This might partially explain the closer relationship of BMI to mortality risk in Caucasian females than in other ethnicities.

Table 1.

	Ethnicity			Age	
	Caucasian	Black African Origin	Asian	<52 years	≥52 years
BMI (kg/m²)	49.4 (6.2)	47.6 (4.8)	52.6 (8.3)	49.8 (6.5)	49.8 (7.3)
HOMA2-IR	2.61 (0.93) [a]	1.56 (1.17)	2.45 (1.52) [b]	2.55 (1.21) [c]	1.90 (1.03)
Insulin (μU/mL)	19.5 (6.7) [a]	11.7 (9.5)	18.9 (12.0) [b]	19.4 (9.3) [c]	13.9 (7.8)
Glucose (mmol/L)	6.2 (1.6)	6.6 (2.6)	5.7 (1.2)	5.8 (1.4) [c]	7.1 (2.4)
Triglyceride (mmol/L)	1.60 (1.40-2.00) [a]	0.95 (0.70-1.20)	1.00 (0.90-1.54) [d]	1.30 (0.90-1.80)	1.35 (0.93-1.93)
Systolic blood pressure (mmHg)	126.5 (11.1)	132.9 (13.1)	131.2 (13.8)	126.8 (12.6) [c]	133.7 (10.3)
Total cholesterol (mmol/L)	5.0 (1.0)	4.4 (0.8)	4.9 (1.2)	4.8 (1.0)	4.8 (1.1)
HDL (mmol/L)	1.2 (0.4)	1.3 (0.3)	1.2 (0.3)	1.2 (0.2)	1.3 (0.5)
VAT (units)	25820 (8770)	19600 (6200) [d]	19200 (8400) [d]	21900 (8500)	26100 (8700)
SAT (units)	68800 (8800)	73400 (15000)	70900 (14300)	70900 (10300)	68800 (14200)
VAT/SAT ratio	0.39 (0.13)	0.28 (0.11) [d]	0.29 (0.20) [d]	0.32 (0.16)	0.39 (0.14)

a P<0.01 vs African; b P<0.05 vs African; c P<0.05 vs ≥52 years age; d P<0.05 vs Caucasian

Disclosure: **M.B. Whyte:** None.

568

Aberrant functional connectivity of the anterior insula with reward and inhibitory control circuits and its association with plasma levels of leptin in obese individuals

A. Ferrulli[1], F. Devoto[2], L. Zapparoli[3], R. Bonandrini[2], L.M. Sconfienza[3], G. Banfi[3], L. Luzi[4], E. Paulesu[2];
[1]IRCCS Policlinico San Donato, San Donato Milanese (MI), [2]University of Milano-Bicocca, Milan, [3]IRCCS Galeazzi, Milan, Italy, [4]University of Milan, Milan, Italy.

Background and aims: There is growing evidence for the role of the insular cortex in the integration of information coming from either sensory and somatosensory cortices and from higher-order areas. However, its role in the regulation of food intake and its interaction with hormones implied in meal termination is still poorly understood. Here, we used resting-state functional brain connectivity (FBC) to explore system-level dysfunctions in the brain of obese (OB) individuals and their correlations with the plasma levels of leptin.

Materials and methods: We started with a quantitative meta-analysis of previous fMRI data on obesity and identified the left anterior insula and the overlying frontal operculum (AI/fO) as a region with convergent hyper-activation in OB individuals exposed to food-related stimuli. This cluster was then used as a seed for a seed-based FBC analysis in 10 OB and 11 normal weight controls matched for sex, age and education. The analysis implied the calculation of the functional correlations of each and every brain voxel with the seed along the resting state fMRI time series.

Results: OB individuals, compared with the control group, showed hyper-connectivity between the left AI/fO and key regions of the reward system, such as the left medial orbitofrontal cortex (OFC), in addition to the bilateral parahippocampal gyri and the posterior cingulate gyrus; conversely, they exhibited hypo-connectivity between the seed and the left dorsolateral prefrontal cortex (DLPFC), which is a key region involved in inhibitory control (all Ps <0.05 corrected for multiple comparisons). Finally, we found a substantial trend for a negative correlation between AI/fO-OFC hyper-connectivity and plasma levels of leptin ($\rho = -0.612$, $p = 0.06$).

Conclusion: Our results provide evidence for an imbalance between reward and inhibitory control systems in OB individuals, which might be worsened by an altered response to food intake regulatory hormones (e.g., leptin), thus driving the overeating behaviour. In sum, our results suggest that the AI/fO is a privileged anatomical location capable to integrate information from both reward and inhibitory control brain regions, and might be a suitable candidate for neuro-modulatory treatments aimed at recalibrating its connectivity profile.

Supported by: IMH

Disclosure: **A. Ferrulli:** None.

PS 039 Obesity and lipid metabolism: studies in human-derived cells

569

Integral lipidomic analysis of human skeletal muscle and visceral adipose tissue biopsy samples from lean, obese and type 2 diabetic individuals

U. Loizides-Mangold[1], S. Chanon[2], M. Robert[3], E. Lefai[2], C. Dibner[1];
[1]Department of Internal Medicine Specialties, University of Geneva, Geneva, Switzerland, [2]CarMeN Laboratory, INSERM U1060, INRA 1397, University Lyon 1, Oullins, France, [3]Department of Digestive and Bariatric Surgery, Edouard Herriot Hospital, Lyon, France.

Background and aims: Type 2 diabetes is characterized by impaired insulin secretion and insulin resistance. Upon obesity, adipocyte storage capacity is eventually exceeded and lipids are released into the plasma. Chronically increased plasma lipids further promote insulin resistance as excessive accumulation of lipids and their intermediate products causes lipotoxicity in peripheral organs including pancreatic islets, liver, and muscle. In particular, lipid-induced ER stress leads to excessive UPR activation in the ER, with substantial impact on calcium homeostasis and insulin secretion. We therefore aimed to investigate the skeletal muscle and visceral adipose tissue lipidomic signatures of obesity and T2D in humans.
Materials and methods: Human tissue biopsy samples were obtained from lean, obese and obese/type 2 diabetic (T2D) human skeletal muscle ($N = 67$) and visceral adipose tissue donors ($N = 37$). Adipose biopsy samples were taken from the same individuals that were also subjected to muscle biopsy, and compared across subjects in each group, and among the groups. Lipid extracts were prepared using a modified MTBE extraction protocol on tissue biopsies with the addition of internal lipid standard. MRM based tandem mass spectrometry was performed for the identification and quantification of phospho- and sphingolipid lipid species by direct infusion on a TSQ Vantage mass spectrometer equipped with a robotic nanoflow ion source (Nanomate).
Results: Our results show that the ratio of phosphatidylcholine to phosphatidylethanolamine (PC/PE) is increased upon obesity and T2D in human skeletal muscle biopsy samples (lean vs obese, $p = 0.03$; lean vs T2D $p = 0.008$), potentially affecting ER homeostasis. Furthermore, levels of the mitochondria-specific lipid cardiolipin were decreased, reflecting the more sedentary lifestyle of obese/T2D donors (lean vs T2D $p = 0.022$). Sphingolipids were also affected in human skeletal muscle and visceral adipose tissue upon obesity and T2D. Moreover, obesity and T2D had a profound effect on the fatty acid composition of membrane lipids. Almost all major phospholipids were composed of significantly higher levels of saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFAs) with potential impact on membrane fluidity and ER homeostasis.
Conclusion: This comparative analysis between lipid profiles from muscle and adipose tissue biopsies allows for novel and important information on tissue specific lipidomic profiles upon obesity and T2D. Data from our ongoing lipidomic analyses on human skeletal muscle tissue and visceral adipose tissue derived from lean, obese and T2D donors support the hypothesis that lipid metabolic changes occur upon these pathologies.
Supported by: Sinergia Swiss National Science Foundation
Disclosure: **U. Loizides-Mangold:** None.

570

Long-chain ceramides are lipotoxicity-induced cell non-autonomous endoplasmic reticulum stress-activating secretory signals released from skeletal myocytes

L.D. Roberts[1], B.D. McNally[2], S.A. Murfitt[2], F. Sanders[2], J. Garnham[1], M. Vacca[3], K. Witte[1], A.J. Murray[4], J.L. Griffin[2];
[1]Leeds Institute of Cardiovascular & Metabolic Medicine, University of Leeds, Leeds, [2]Department of Biochemistry, University of Cambridge, Cambridge, [3]Institute of Metabolic Science, University of Cambridge, Cambridge, [4]Physiology, Development & Neuroscience, University of Cambridge, Cambridge, UK.

Background and aims: Mammalian tissues have developed responsive pathways to counter cellular stress. In type 2 diabetes (T2D), obesity and dyslipidaemia, tissues are exposed to multiple stressors including free fatty acids (FFA), which lead to lipotoxicity. The Endoplasmic Reticulum (ER) has a key role in the folding and packaging of secretory proteins, and in lipid biosynthesis. Upon the detection of stress stimuli in the ER, the unfolded protein response (UPR) is activated through three distinct branches; regulated by the proteins PERK, IRE1 and ATF6. Chronic ER stress has deleterious effects and has emerged as an underlying mechanism of FFA-induced insulin resistance in skeletal muscle. Tissue-localized ER UPRs were recently observed to activate systemic UPR activation, suggesting an, as yet unknown, ER stress-propagating cell non-autonomous intra- and inter-organ signal. Determining the mechanisms of this stress communication across tissues is crucial to understanding the role of ER stress in T2D aetiology. This work aims to identify lipotoxicity-induced signals released from myocytes and skeletal muscle which function to propagate the ER stress response.
Materials and methods: Human and mouse C2C12 myoblasts were differentiated into myotubes and treated with palmitate (100 and 200 μM) throughout. RT-qPCR analysis of ER stress markers in the myoblasts was performed (EDEM1, ATF4, ATF3, HSPA5). Exosomes were isolated from conditioned myocyte media. *Pectoralis major* muscle biopsies and plasma were obtained from 80 patient volunteers with Body Mass Indices (BMI) ranging from 17.8–40.8. Gastrocnemius muscle and plasma was harvested from C57BL6 mice fed either a western or 60% High fat diet for 12 weeks. Lipidomic analysis of cell media and exosomes, mouse and human skeletal muscle and plasma was performed using Liquid Chromatography-Mass Spectrometry (LC-MS).
Results: Lipotoxicity and ER stress were induced in murine C2C12 and human primary skeletal myotubes using the FFA, palmitate. Conditioned media from these myotubes induced the expression of the UPR genes in naïve myotubes (EDEM1 2-fold t-test $P < 0.01$, ATF4 3.5-fold t-test $P < 0.001$, HSPA5 1.3-fold, $P < 0.05$). The ER stress-inducing ability of the conditioned media was retained in boiled media (protein denaturation), in the isolated media lipid fraction, and in exosomes isolated from the media, suggesting a lipid signal secreted within exosomes. Using lipidomics, long-chain ceramides (Cer 34:1, Cer 40:1, Cer 40:2 and Cer 42:1) were found to be enriched in exosomes from the media of FFA-treated myotubes, via a PERK-dependent ER stress pathway. Ceramide 40:1 and 42:1 induced ER stress in human and murine myocytes (Cer 40:1 ATF4 2-fold t-test $P < 0.05$, HSPA5 1.5-fold, $P < 0.05$, Cer 42:1 ATF4 3-fold $P < 0.01$, HSPA5 1.5-fold $P < 0.05$) and were enriched in the skeletal muscle of both diet induced mouse models of diabetes, and in obese humans.
Conclusion: These data suggest long-chain ceramides may be a novel skeletal myocyte non-autonomous intra-organ signal propagating ER stress resultant from FFA-induced lipotoxicity.
Clinical Trial Registration Number: 11/YH/0291
Supported by: BDM - MRC Studentship, LDR - Diabetes UK RD Lawrence Fellowship
Disclosure: **L.D. Roberts:** None.

571

Structure-toxicity relationships of different saturated and unsaturated free fatty acids with respect to mechanisms of toxic action in human EndoC-βH1 beta cells

T. Plötz[1], B. Krümmel[1], I. Mehmeti[2], S. Lenzen[1];
[1]Institute of Experimental Diabetes Research, Hannover Medical School, Hannover, [2]Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany.

Background and aims: Type 2 diabetes is associated with chronically increased plasma concentrations of free fatty acids (FFAs), which trigger β-cell dysfunction and apoptosis, also referred to as lipotoxicity. This lipotoxicity has been linked to an excessive beta-oxidation of FFAs within the mitochondria and peroxisomes, thereby leading to oxidative stress due to increased production of toxic reactive oxygen species. Moreover, ER-stress and formation of lipid droplets are considered as further mechanisms involved in lipotoxicity. However, the underlying mechanism of FFA-mediated β-cell apoptosis has not yet been fully elucidated. Therefore, the aim of this study was to gain a deeper insight into the mechanism of lipotoxicity with structure-toxicity relationships using various long-chain (LC) and very long-chain (VLC) saturated, mono- and poly-unsaturated FFAs in the human EndoC-βH1 β-cell line.
Materials and methods: EndoC-βH1 tissue culture β-cells were incubated with various LC and VLC saturated and unsaturated FFAs. Toxicity was determined by using caspase-3 assay. The formation of β-cell-toxic hydrogen peroxide (H_2O_2) by FFAs was analyzed by expression of the H_2O_2-sensitive fluorescence protein HyPer in mitochondria and peroxisomes. Lipid droplets within EndoC-βH1 β-cells were determined by fluorescence microscopy after fixation and staining with *Oil Red O*. The respective ER-stress genes were quantified after FFA incubation by RT-qPCR using specific primers.
Results: Only LC and VLC saturated and mono-unsaturated FFAs (≥16 carbon atoms) were toxic to human insulin-producing EndoC-βH1 β-cells as documented by caspase-3 activation. The toxicity increased with increasing chain length. However, we observed in EndoC-βH1 β-cells a decrease in toxicity as the number of double bonds in FFAs increased and, in addition, as the double bond approached the carboxyl group. Hence, the position and the number of double bonds affected the toxicity. Generally, the toxicity correlated with the metabolism of LC and VLC FFAs in the peroxisomal beta-oxidation, in which the toxic H_2O_2 is generated. Interestingly, there was a difference between effects of saturated and mono-unsaturated FFAs upon the ER, where only saturated VLC FFAs were able to induce ER-stress genes in human EndoC-βH1 β-cells. Neither unsaturated VLC FFAs nor LC FFAs had an effect on the gene expression of any of the analyzed ER-stress genes. In contrast to the induction of ER-stress, saturated VLC FFAs did not form lipid droplets, whereas all other analyzed LC and VLC FFAs generated lipid droplets in human EndoC-βH1 β-cells.
Conclusion: Only LC (C16-C18) and even more so VLC (C20-C22) saturated and mono-unsaturated FFAs were toxic to human EndoC-βH1 β-cells. This chain length dependent toxicity correlated with the metabolic preference for their metabolism in the peroxisomal beta-oxidation, where toxic H_2O_2 is generated, which could be proved by experiments with the H_2O_2-sensitive protein HyPer. In contrast poly-unsaturated FFAs showed a decrease in toxicity in human EndoC-βH1 β-cells. When comparing lipid droplet formation and ER stress gene induction an inverse correlation could be documented, even though there was no difference in the toxicity of FFAs irrespective of their chemical structure.
Disclosure: **T. Plötz:** None.

572

The importance of adipocyte H2S-synthesising enzymes in human adipose tissue adipogenesis and systemic insulin action

J. Moreno-Navarrete[1], F. Comas[1], J. Latorre[1], M. Arnoriaga-Rodríguez[1], F. Ortega[1], M. Kern[2], O. Cusso[3], M. Sabater[1], W. Ricart[1], X. Ribas[3], M. Costas[3], M. Blüher[2], J. Fernández-Real[1];
[1]Institut d'Investigació Biomèdica de Girona (IdIBGi), Girona, [2]University of Leipzig, Leipzig, Germany, [3]Institut de Química Computational i Catàlisi (IQCC), Girona, Spain.

Background and aims: Recent studies in 3T3-L1 cells and mice suggest a possible role of H_2S biosynthesis in adipogenesis. Here, we aimed to investigate the possible role of H_2S-synthesising enzymes [cystathionine γ-lyase (CTH), cystathionine β-synthase (CBS), mercaptopyruvate sulfurtransferase (MPST)] in human adipose tissue (AT) adipogenesis and systemic insulin action.
Materials and methods: Both visceral (VAT) and subcutaneous (SAT) *CTH*, *CBS* and *MPST* mRNA and protein levels were measured in five independent (2 cross-sectional and 3 longitudinal) cohorts. Hydrogen sulfide levels were measured by fluorescent probe. *Ex vivo* cysteine and piridoxal phosphate coadministration (H_2S-synthesising enzyme activators) was performed in AT explants ($n = 16$) according to systemic insulin sensitivity (hyperinsulinemic-euglycemic clamp). *In vitro* experiments using lentiviral-induced specific gene knockdown and chemical inhibitors during human adipocyte differentiation were also performed.
Results: Both VAT and SAT *CTH*, *CBS* and *MPST* mRNA and protein levels were significantly decreased in participants with obesity and type 2 diabetes, highly expressed in adipocyte fraction, and positively correlated with expression of adipogenic- and insulin signaling-related genes, while in inverse proportion to genes linked to adipose tissue dysfunction (inflammation and senescence) in two independent cohorts. Concordantly, *CTH* in subcutaneous adipose tissue was positively correlated with insulin sensitivity, while the improvement in insulin action induced by bariatric surgery, physical activity and diet -induced weight loss, all resulted in increased expression of H_2S-synthesising enzymes in parallel to *ADIPOQ*. *Ex vivo* experiments demonstrated that the activation of H_2S biosynthesis resulted in increased adipogenesis, which was enhanced in those participants with increased insulin sensitivity. *In vitro* experiments revealed that *CTH*, *CBS* and *MPST* gene knockdown or the CTH inhibitor DL-propargylglycine inhibited adipocyte differentiation, insulin action and H_2S biosynthesis, whereas GYY4137 (an H_2S donor) exerted opposite effects.
Conclusion: These data demonstrated that transsulfuration pathway is required for human adipogenesis and exerts an important role in the relationship between adipose tissue physiology and systemic insulin action.
Supported by: FIS (PI16/01173) from ISCIII; SED
Disclosure: **J. Moreno-Navarrete:** None.

573

Effects of glycated insulin on adipogenic differentiation

A. Piccoli[1], T. Bisogno[1,2], M. Maccarrone[1,3], P. Pozzilli[1,4], N. Napoli[1,5], R. Strollo[1];
[1]Universita Campus Bio-Medico di Roma, Rome, Italy, [2]National Research Council, Pozzuoli, Italy, [3]European Center for Brain Research (CERC)/Santa Lucia Foundation IRCCS, Rome, Italy, [4]Queen Mary, University of London, London, UK, [5]Washington University in St Louis, St Louis, USA.

Background and aims: Hyperglycaemia and chronic low-grade inflammation associated with obesity and diabetes are a main source of reactive oxidants (ROS). High levels of ROS may generate oxidative post-translational modification (oxPTMs) that may impair proteins biological functions. We have shown that insulin could also be a target of these modifications that include glycation, oxidation and chlorination. Adipose tissue is a primary target of this hormone and a key player in insulin resistance (IR): evidence from animal studies show that the glycation of insulin or other proteins result in a significant impairment of biological function of adipose tissue, affecting adipokines expression and insulin sensitivity. However, how oxPTMs such as glycation interfere with insulin function and their involvement in IR and type 2 diabetes (T2D) is still unknown. We hypothesize that insulin glycation may be involved in IR and in the pathogenesis of T2D. Therefore, the aim of this study was to assess the possible effects of glycated vs. native insulin on adipocyte differentiation.
Materials and methods: Human recombinant insulin was glycated *in vitro* following our established protocol. Insulin glycation was monitored through PAGE. Human preadipocytes (HPAd), a primary cell line from

subcutaneous adipose tissue, and adipose-derived stem cells (ASCs), isolated from tissues of lean and obese subjects (BMI <25 and BMI >30), were differentiated with standard adipogenic medium in presence of native or glycated insulin. Cellular differentiation was assessed by gene and protein expression analyses of adipogenic markers (adiponectin, peroxisome proliferator-activated receptor gamma -*Pparγ*-, fatty acid binding protein 4 -*Fabp4*-, glucose transporter 4 -*Glut4*-) and of receptors for adiponectin (*AdipoR1*) and for insulin (*InsR*), through real time RT-PCR and immunoblot respectively.

Results: Our preliminary results showed that preadipocytes (both HPAd and ASCs) exposed to glycated insulin had impaired differentiation capacities: Oil Red O-staining showed that glycated insulin reduced adipocyte differentiation. Considering gene expression, we observed an overall downregulation of adipogenic markers, with a significant decrease of adiponectin both in leans- and in obese-derived cells ($p = 0.007$ and $p = 0.017$ respectively), lower levels of *Glut4* and a significant lower expression of *Fabp4* ($p = 0.002$) in cells from leans differentiated with glycated insulin. Moreover, we also observed a strong increased of *Pparγ* in normal weight subjects, with a significance of $p = 0.013$, and raised levels of *AdipoR1* ($p = 0.005$) in obese-derived cells, treated with modified insulin. Unlike gene expression analysis, protein levels of INSR presented a significant down regulation in cells treated with glycated insulin, with a p value of 0.0266 in leans and of 0.011 in obese subjects.

Conclusion: In conclusion, our data highlight for the first time that glycated insulin affect human adipocytes differentiation and impair insulin receptor expression, suggesting to deeper investigate of its role as a possible mediator in the pathogenesis of T2D.

Supported by: Campus Bio-Medico University Research Grant

Disclosure: **A. Piccoli:** None.

574

MicroRNAs regulate expression of uncoupling protein 2 gene (UCP-2) in visceral adipose tissues of obese individuals

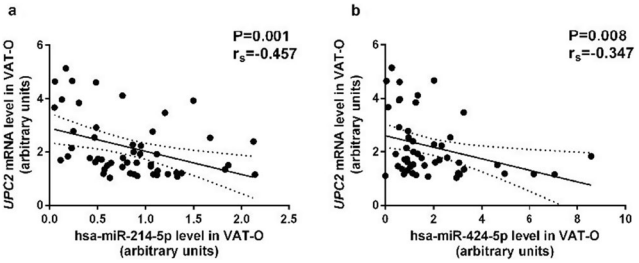
A. Kurylowicz[1], M. Owczarz[1], J. Polosak[1], M. Jonas[1], M. Jonas[2], W. Lisik[2], M. Puzianowska-Kuznicka[1];
[1]Department of Human Epigenetics, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [2]Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland.

Background and aims: Impaired thermogenesis may contribute to the positive energy balance and therefore promote obesity. Indeed, adipose tissues of obese individuals are characterized by the lower expression of the key genes involved in thermogenesis, e.g. a gene encoding uncoupling protein 2 (*UCP-2*). However, mechanisms underlying this phenomenon are unknown. In recent years there has been great progress in the understanding of the role of microRNA (miRNA) in the regulation of expression of various genes, including those related to adipocyte differentiation and function. The aim of the study was to examine if *UCP-2* mRNA levels in adipose tissues of obese individuals correlate with the expression of the relevant miRNAs, potentially interfering with *UCP-2* mRNA 5′UTR sequence.

Materials and methods: We used *in silico* analysis with the TargetScanHuman, miRanda-mirSVR and the Pictar programs to identify miRNA potentially interfering with *UCP-2* mRNA 5′UTR sequence. Next generation sequencing (NGS) was applied as a screening analysis to measure expression of the selected miRNA in 37 samples of visceral (VAT) and subcutaneous (SAT) adipose tissues from normal-weight (N) and obese (O) individuals. Subsequently, miRNAs expression was validated by the *real-time* PCR method in 164 samples of VAT and SAT (from 55 obese and 27 normal weight patients) and correlated with the expression of the *UCP-2* on the mRNA level.

Results: *UCP-2* mRNA levels were significantly lower in VAT and SAT of obese subjects compared to the normal-weight individuals ($P = 0.002$ and $P = 0.009$, respectively). Based on the *in silico* analysis 6 miRNAs potentially interfering with *UCP-2* mRNA were selected (namely: hsa-miR-15a-5p, hsa-miR-15b-5p, hsa-miR-16-5p, hsa-miR-185-5p, hsa-miR-214-5p and hsa-miR-424-5p). Screening analysis by NGS only in case of hsa-miR-214-5p and hsa-miR-424-5p confirmed significant differences in their expression between the investigated tissues. These results were verified by the *real-time* PCR and correlated with *UCP-2* mRNA levels. Significant negative correlations between hsa-miR-214-5p as well as hsa-miR-424-5p expression levels and *UCP-2* mRNA were found in VAT of obese individuals ($P = 0.001$, $r_s = -0.457$ and $P = 0.008$, $r_s = -0.347$, respectively; Figure 1).

Conclusion: Decreased expression of the *UCP-2* mRNA in VAT of obese individuals may result from the interference with hsa-miR-214-5p and hsa-miR-424-5p. The mechanisms responsible for the lower expression of *UCP-2* in SAT of obese subjects remain unknown.



Supported by: National Science Centre Poland grant 2012/05/B/NZ5/01536

Disclosure: **A. Kurylowicz:** None.

575

Comparative and functional analysis of plasma membrane-derived extracellular vesicles from obese vs nonobese women

J. Latorre[1,2], F. Santamaria-Martos[3], I. Benítez[3], J. Moreno-Navarrete[1,2], M. Sabater[1,2], W. Ricart[1,2], M. Sanchez de la Torre[3,4], S. Mora[5], J. Fernández-Real[1,2], F. Ortega[1,2];
[1]Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta, Salt, Spain, [2]Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [3]Group of Translational Research in Respiratory Medicine, Hospital Universitari Arnau de Vilanova i Santa Maria, IRB Lleida, Lleida, Spain, [4]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [5]Department of Molecular and Cellular Physiology, Institute of Translational Medicine (ITM), University of Liverpool, Liverpool, UK.

Background and aims: Since the discovery of plasma membrane-derived extracellular vesicles (EVs) as vehicles for exchange of regulatory microRNAs (miRNAs), RNA-based cell-to-cell communication through circulating EVs has attracted many studies endorsing the concept that EVs and their cargo are of most relevance in physiology and physiopathology. Today we know that EVs are released to the circulation by cells found in adipose tissue, namely mature adipocytes, transferring miRNAs that may mediate the adaptive response of recipient cells. This study investigated plasma EVs from obese vs. nonobese women and their functional impact in adipocytes.

Materials and methods: Plasma EVs were isolated by ultracentrifugation. Concentration and size were examined by nanoparticle tracking analysis (NanoSight). Functional analyses were performed in human adipocytes using isolated plasma EVs from obese and nonobese women. RNA was purified from plasma and plasma EVs of 45 women (47 ± 12 years, 58% of obesity), and profiles of mature miRNAs were assessed.

Results: Smaller plasma EVs were found in obese when compared to nonobese women. Positive associations were identified between circulating EVs number and parameters of impaired glucose tolerance. Treatments with EVs from obese subjects led to a significant reduction of genes involved in lipid biosynthesis, while increasing the expression of IRS1 (12.3%, $p = 0.002$) in human adipocytes. Almost 40% of plasma cell-free miRNAs were also recovered from isolated plasma EVs (defined as Ct values<37 in ≥75% of samples). BMI together with parameters of insulin resistance were major contributors to EVs-incorporated miRNA patterns.
Conclusion: Size, concentration, and the miRNA cargo of plasma EVs are independently and significantly associated with obesity and parameters of insulin resistance, and may mediate intercellular communication relevant to metabolism in fully-differentiated adipocytes.
Supported by: CIBEROBN, PERIS 2016, AGAUR, FEDER
Disclosure: **J. Latorre:** None.

576

CEBP-beta regulates RAP1 expression in visceral adipose tissue of obese patients

C. Formichi[1,2], G. Sebastiani[1,2], N. Brusco[2], S. Cantara[1], G.E. Grieco[2], C. Maccora[1], A. Tirone[3], G. Vuolo[3], C. Ciuoli[1], F. Dotta[1,2];
[1]University of Siena, Siena, [2]Umberto Di Mario Foundation, Toscana Life Science, Siena, [3]Le Scotte Hospital, Siena, Italy.

Background and aims: The prevalence of obesity and its associated comorbidities has dramatically increased in the last decades. However, factors involved in the development of metabolic complications of obesity are still to be fully elucidated. Previous studies demonstrated that RAP1 protein - a component of shelterin complex, which binds and protects mammalian *telomeres* - has a role in regulation of metabolism and that RAP1 whole-body knockout mouse show adult-onset obesity and metabolic impairment. Our aim was to evaluate RAP1 expression and regulation in different human adipose depots.
Materials and methods: We performed real time PCR to measure RAP1 expression in visceral (VAT) and subcutaneous (ScAT) adipose tissue of 49 obese patients and 14 metabolically healthy normal-weight subjects. To elucidate the cause of differential RAP1 expression in VAT, we searched for predicted transcription factors, using predictive algorithms, and selected the adipogenic transcription factor CEBPβ. CEBPβ mRNA can be translated into different protein isoforms: the activating isoform LAP (*liver-enriched transcriptional activating protein*) and the inhibiting isoform LIP (*liver-enriched transcriptional inhibitory protein*). Indeed, previous studies demonstrated that the ratio between these two isoforms is critical for CEBPβ-mediated gene expression. We performed Western Blot analysis to evaluate CEBPβ expression in VAT of 10 obese patients and 4 controls. In addition, we performed ChIP analysis on chromatin samples from cultured visceral preadipocytes.
Results: RAP1 mRNA expression was significantly reduced in VAT of obese patients with metabolic syndrome ($n = 37$) compared to metabolically healthy obese ($n = 12$) and controls (respectively, $p = 0.042$ e $p < 0.0001$); no difference was found in RAP1 expression in ScAT between obese and controls ($p = 0.246$). Next, we analyzed CEBPβ expression in VAT: obese patients showed an inhibitory expression pattern of CEBPβ, with higher LIP/LAP ratio compared to controls ($p = 0.024$). Of note, obese patients with higher LIP/LAP ratio showed a higher body weight, total cholesterol and triglycerides levels and lower HDL levels ($p = 0.0095$, $p = 0.042$, $p = 0.038$ and $p = 0.014$, respectively) compared to obese patients with lower LIP/LAP ratio. ChIP analysis confirmed that CEBPβ binds RAP1 promoter.
Conclusion: In conclusion, our data highlight the role of an altered expression pattern of transcription factor CEBPβ in RAP1 reduced expression in VAT; this mechanism could be involved in the development of obesity-related metabolic complications. Interestingly, we showed that the differential expression of CEBPβ isoforms can identify subsets of obese patients with a different metabolic profile.
Disclosure: **C. Formichi:** None.

PS 040 Adipose tissue biology: animal studies

577

Glucagon-like peptide 2 (GLP-2): an underestimated signal in metabolic control

J.J. Vendrell[1], M. Ejarque[1], D. Beiroa[2], E. Calvo[1], N. Keiran[1], C. Nuñez Roa[1], R. Nogueiras[3], S. Fernandez-Veledo[1];
[1]Endocrinology, Institut d'Investigacio Sanitaria Pere Virgili (IISPV), Tarragona, [2]Department of Physiology, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela, [3]Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela, Spain.

Background and aims: Glucagon-like peptide-2 (GLP-2) is a gastrointestinal hormone released in response to dietary nutrients as GLP-1, which acts through a specific receptor, the GLP-2 receptor (GLP-2R). To date, GLP-2R expression has been mainly located in gut, where it exerts potent trophic effects, and in brain, where it acts as an anorexigenic hormone. Nonetheless, some in vivo observations suggest a wider expression profile than expected. This work aims to gain insight into the molecular function of GLP-2/GLP-2R axis on energetic metabolism, focusing on its potential modulatory function on adipose tissue (AT).
Materials and methods: SGBS cell line was used as an *in vitro* model of human subcutaneous pre-adipocytes. Gene and protein expression studies were performed. *Animal studies.* Chow and high-fat diet (HFD) mice were treated once a day with GLP-2 analogue (Teduglutide) for 4 weeks, and different metabolic studies were performed
Results: In agreement with previous data, short-term GLP-2 agonist centrally-treated mice showed a decreased food intake and weight gain. Surprisingly, these effects were not maintained in a chronic treatment of 14 days. Chronic modulation of central GLP-2R of diet-induced obesity (DIO) mice did not affect metabolism either. Conversely, chronic activation of peripheral GLP-2R provided body weight-independent glucose tolerance. Interestingly, Teduglutide shown an anti-inflammatory effect on visceral AT meanwhile it had lipogenic effects on subcutaneous AT, suggesting that GLP-2 agonism may have a direct effect on AT. Remarkably, our results demonstrate that GLP-2R is also expressed in AT, mainly in adipocyte fraction. Different from what is seen in intestine, i*n vitro* studies revealed no effects of GLP2 on adipocytes proliferation. However, and consistent with in vivo data, GLP-2 treatment produced an increase on lipids accumulation with an up-regulation of lipogenic genes in subcutaneous adipocytes.
Conclusion: Overall, our data identify AT as a new target for GLP-2 activity. Understanding the role of GLP-2 in the metabolic events that take place in AT may help to define new GLP-2 receptor analogs as potential indications of clinical usefulness in obesity.
Supported by: PI14/00228; PI17/01503, SAF2015-65019-R; ERDF. EFSD/Lilly Fellowship
Disclosure: **J.J. Vendrell:** None.

578

Evaluation of active brown adipose tissue by the use of hyperpolarised [1-13C] pyruvate MRI in mice

M. Riis-Vestergaard[1], P. Breining[1], S. Pedersen[1], C. Laustsen[2], H. Stødkilde-Jørgensen[2], P. Borghammer[3], N. Jessen[4], B. Richelsen[1];
[1]Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus C, [2]MR Research Center, Aarhus University, Skejby, [3]Nucelar Medicine and PET Centre, Aarhus University Hospital, Aarhus C, [4]Clinical Pharmacology, Aarhus University Hospital, Aarhus C, Denmark.

Background and aims: Since evidence of persisting brown adipose tissue (BAT) in adults humans emerged in the late 2000's, BAT has been of great interest as a putative pharmacological target for treatment of

metabolic disorders such as obesity, insulin resistance and metabolic syndrome. Activated BAT has the capacity to dissipate energy as heat through uncoupling of the inner mitochondrial membrane and thereby increase energy expenditure. Currently, assessment of the potential of BAT activation in humans is mainly provided by FDG PET scans but in order to expand and supplement these investigations introduction of radiation-free methods are warranted. Thus, the aim is to determine if hyperpolarized [1-^{13}C] pyruvate MRI (HP-MRI) which is a radiation-free method is able to determine BAT activity in mice following chronic cold exposure.

Materials and methods: Long-term cold (6 C) and thermo-neutral (30 C) acclimated mice were scanned with HP-MRI for assessment of the interscapular BAT (iBAT) activity. Likewise, another group of acclimated mice were scanned with the conventional method FDG PET/MRI. Finally, the mice were sacrificed, iBAT was removed and evaluated for gene expression and protein levels of the specific thermogenic marker UCP1.

Results: Cold exposure as compared with thermo-neutrality was found to increase the activity of iBAT by 4–13 fold assessed by the HP-MRI method dependent of the pyruvate metabolite examined ([1-^{13}C]bicarbonate, [1-^{13}C]lactate or [1-^{13}C]alanine). This was in agreement with the findings of a 5-fold increment in FDG uptake ($p < 0.05$) after cold exposure using FDG PET/MRI. Finally, by direct investigation of iBAT, cold exposure resulted in both increased UCP1 mRNA expression and UCP1 protein levels by 3–4 fold ($p < 0.05$).

Conclusion: We found that iBAT activity in mice could be detected using the hyperpolarized [1-^{13}C] pyruvate MRI method revealing changes in metabolites downstream from pyruvate. Besides detection of iBAT this new imaging modality also introduces the potential of detection of changes in intracellular metabolism and may therefore add useful information to the conventional FDG PET studies. HP-MRI may also be a promising radiation-free tool for repetitive BAT studies in humans.

Disclosure: **M. Riis-Vestergaard:** None.

579

Absence of Interleukin-1 Receptor (IL-1RI) alters adipogenesis and adipokine secretion in high-fat diet-induced obesity

K.A.J. Mitchelson[1], J.C. Ralston[1], C.R. Strain[2], Y.M. Lenighan[1], E.B. Kennedy[1], G.M. Lynch[1], F.C. McGillicuddy[1], P.W. O'Toole[3], H.M. Roche[1];
[1]University College Dublin, Dublin, [2]Teagasc Food Research Centre, Fermoy, [3]University College Cork, Cork, Ireland.

Background and aims: IL1 is a cytokine family involved in immune regulatory and inflammatory response. Lack of IL-1 Receptor (IL-1RI-/-) protects against high-fat diet (HFD)-induced insulin resistance after 3 months (mo) HFD, but this protection was lost after 6 mo HFD. After 6 mo HFD IL-1RI-/- mice were more glucose intolerant and insulin resistant, compared to wildtype (WT) mice. IL-1RI-/- mice displayed loss of adipose functionality, increased adipocyte hypertrophy and reduced TNFα and IL-6 secretion from stromal vascular fraction (SVF). However it was unknown if/how lack of IL-1RI impeded pre-adipocyte differentiation. It has been suggested that microbiome transfer can affect weight gain and phenotype in mice with inflammation-induced disease. The primary aim of this study was to determine how the lack of IL-1RI affected adipogenetic potential and inflammation in adipose tissue

Materials and methods: IL-1R-/- mice on C57BL/6 background breeding pairs were bred to obtain male WT and IL-1R-/- offspring. Mice were fed a HFD for 6 mo (45% kcal) and weight and feed intake was monitored weekly. WT and IL-1R-/- mice were cohoused in a subset of cages to determine whether microbiome transfer can affect phenotype. Glucose tolerance (1.5 g/kg) and insulin tolerance (0.5 U/kg) were examined. We investigated the gene expression of PPAR-γ and FASN and lipogenesis in preadipocytes and differentiated adipocytes. Also IL-6 secretion in adipose tissue organ culture (ATOC) in WT versus IL-1RI-/-. Pre-adipocytes were isolated, differentiated, and expression of adipogenic markers was measured by real time PCR. Triacylglycerol (TAG) accumulation was quantified by Oil Red O absorbance. IL-6 secretion from ATOC following IL-1β/TNFα stimulation was measured by ELISA. Statistical analysis was completed by two-way repeated measures ANOVA for GTT/ ITT, one-way ANOVA for PCR and unpaired t-test for TAG accumulation.

Results: IL-1RI-/- mice gained more body weight than WT mice ($P \leq 0.05$) and increased in adipose tissue (AT) weight. IL-1RI-/- and WT mice displayed similar glucose tolerance. Insulin resistance was greater in IL-1RI-/-, though not statistically significant. When IL-1RI-/- and WT mice were cohoused together, both groups showed higher glucose tolerance. Adipogenesis analysis showed *Ppar-γ* expression was 37- fold higher in differentiated adipocytes from WT mice compared to IL-1RI-/- mice. *Fasn* expression was 11- fold higher in differentiated adipocytes from IL-1RI-/- mice compared to WT mice. TAG accumulation was 50% higher in differentiated adipocytes from WT mice compared to IL-1RI-/- mice. IL-6 secretion from WT ATOC was higher than IL-1RI-/- after stimulation with TNFα and/or IL-1β. The impact of the microbiome was determined and will be presented.

Conclusion: While IL-1RI-/- mice show an increase in AT weight, our results show 6 mo HFD impairs adipocyte differentiation and reduces IL-6 secretion. This indicates that although AT is increased in IL-1R-/-, the amount of mature adipocytes and related adipose inflammation present may be lower, which may provide protection from HFD induced inflammation. The microbiome transfer in co-housed mice was reflected in total weight gain which suggests a mechanism for reduction in total adipose tissue while maintaining the beneficial decrease in inflammation

Supported by: Irish DAFM "ImmunoMet" (Grant number: 14/F/828)

Disclosure: **K.A.J. Mitchelson:** None.

580

Characterisation of the exosomal proteins and their potential as regulators of systemic metabolism

R. Garcia Martin, E. Altindis, B.B. Brandao, C. Kahn;
Joslin Diabetes Center and Harvard Medical School, Boston, USA.

Background and aims: Intercellular communication is essential for metabolic processes. Our lab recently showed that tissues can communicate *in vivo* through secretion of exosomal miRNAs which induce changes in gene expression and metabolic adaptation in other tissues. In addition to miRNAs, exosomes are loaded with proteins. However, little is known about how these vary depending on tissue source or their role in the physiological regulation of metabolism. In this study, we aimed to identify both common and unique proteins in exosomes secreted by white/brown adipocytes, hepatocytes, muscle and endothelial cells, and identify the pathways that might be regulated by these proteins

Materials and methods: Murine brown and white adipocytes, AML12 hepatocytes, C2C12 muscle cells and vascular endothelial cells were grown in culture, and exosomes released into the media isolated by ultracentrifugation and filtration. Protein cargo was identified by using tandem mass tag (TMT)-labeling and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results were confirmed by immunoblotting and compared to cellular content to identify enrichment in exosome versus cell pellet.

Results: By comparing the exosomal proteome released by different cell types, we identified general and cell type-specific exosomal proteins. Thus, adiponectin and LPL were only present in white adipocyte exosomes, whereas SPARC and IGFBP5 were only in myotube exosomes. Similarly, EGF receptor, myosin-9 and thrombospondin-5 were uniquely found in exosomes of hepatocytes, endothelial cells and brown adipocytes, respectively. Several exosomal proteins secreted by hepatocytes were also secreted by muscle cells, including members of the Serpin family, some complement factors and proteins involved in iron/copper metabolism. In contrast, white and brown adipocyte- and

endothelial cell-secreted proteins that were similar included proteins of carbohydrate metabolism (PGK1 and UGP2) and proteosomal proteins. When compared to the relative abundance of these proteins in cells, it was clear that loading of proteins into exosomes was selective, and that some proteins were enriched in different cell types and not in others despite similar abundance in the cell pellets. Several exosomal proteins identified here have been linked to the development of diseases such as obesity and diabetes including SPARC and LGALS3BP, which raises the possibility that these factors present in the exosomal compartments might contribute to the pathology of these metabolic diseases.

Conclusion: Exosomes contain novel and cell type-specific proteins that could be involved in tissue communication in healthy and disease

Supported by: RGM is supported by a DFG fellowship

Disclosure: **R. Garcia Martin:** None.

581

Increased FGF21 expression in brown adipose tissue of TH heterozygous mice: implications for cold-adaptative mechanisms

P. Vázquez[1,2], C. Hernández[3,2], L. Pereira[4,2], J. Balsinde[4,2], F. de Pablo[3,2], Á.M. Valverde[1,2];

[1]Instituto de Investigaciones Biomédicas Alberto Sols (CSIC/UAM), Madrid, [2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas (CIBERdem), ISCIII, Madrid, [3]Centro de Investigaciones Biológicas (CSIC), Madrid, [4]Instituto de Biología y Genética Molecular (CSIC), Universidad de Valladolid, Valladolid, Spain.

Background and aims: Obesity is an important global health problem that results from an imbalance between energy intake and expenditure. Whereas white adipocytes accumulate energy in the form of triglyceride depots, brown adipocytes are responsible for non-shivering thermogenesis, and control energy expenditure. Additionally, BAT is a secretory tissue producing *batokines*, one of which is fibroblast growth factor 21 (FGF21). Classical activation of thermogenesis occurs via noradrenaline (NA) released from the sympathetic nervous system being Tyrosine Hydroxylase (TH) the first enzyme catalyzing catecholamines synthesis. We have examined how *Th*-haploinsufficiency in mice affects the response to the stress induced by cold exposure in BAT, and whether FGF21 plays a role in this process.

Materials and methods: Wild-type and *Th* heterozygous ($Th^{+/-}$) mice were maintained at thermoneutrality (29°C) during one week after that one group from each genotype was exposure to cold (4°C) for 6 h. Catecholamines content in BAT was measured after cold exposure by ELISA. The expression of genes involved in BAT and iWAT activation was analyzed by RT-qPCR and different signaling mediators were studied by Western blot. Lipidomic analysis was performed to identify different lipid species in BAT.

Results: The reduced catecholaminergic response (dopamine and noradrenaline content) found in $Th^{+/-}$ mice did not impair cold adaptation since these mice maintained the induction of uncoupling protein-1 (UCP1) as the wild-type littermates. However, we found elevations in *Prdm16* ($^{**}p < 0.01$) and *Fgf21* ($^{*}p < 0.05$) expression, key genes in BAT activation, in $Th^{+/-}$ mice at thermoneutrality. Both were already increased in e18.5 embryos. Moreover, plasma FGF21 ($^{*}p < 0.05$) and liver expression of *Fgf21* ($^{*}p < 0.05$) were also elevated in $Th^{+/-}$ mice. Since endoplasmic reticulum (ER) stress triggers FGF21 production, we analyzed ER stress markers and found increased CHOP levels as well as IRE1 and JNK phosphorylation in BAT of $Th^{+/-}$ mice at thermoneutrality. Also, increased lipolysis in BAT of $Th^{+/-}$ mice during cold challenge was manifested by elevations in hormone-sensitive lipase (HSL) phosphorylation and diacylglycerol (DAG) and free fatty acids (FFA) content.

Conclusion: Our results suggest the existence of a *Th-Prm16-Fgf21-* axis in BAT under basal conditions that might operate under stress conditions to maintain BAT functionality/homeostasis. Thus, in a situation of *Th* haploinsuficiency, the elevation in *Fgf21*, probably as a result of the subsequent ER stress, together with the elevation in *Prdm16* already detectable before birth might ensure a normal response to cold exposure. This axis might also operate in iWAT. This compensation may be facilitated by the higher content of DAG and increased activity of HSL in BAT from $Th^{+/-}$ mice, yielding more availability of FFAs that ultimately are the fuels for UCP-1 activation and thermogenesis.

Disclosure: **P. Vázquez:** None.

582

Unravelling the molecular mechanisms involved in adaptation of adipose tissue to cold

J. Rodó[1], M. Garcia[2], E. Casana[2], V. Sacristan[1], C. Jambrina[2], S. Muñoz[2], C. Mallol[2], X. Leon[2], S. Darriba[1], I. Grass[1], S. Franckhauser[2], V. Jimenez[2], F. Bosch[2];

[1]Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona, Bellaterrra, [2]Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona and Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Bellaterrra, Spain.

Background and aims: Type 2 diabetes (T2D) and obesity are strongly associated and consitute a major health problem. Novel and safe approaches are needed to prevent and combat the current T2D-obesity epidemic. New therapeutic strategies aiming at increasing metabolic rate thorough enhancement of non-shivering thermogenesis may hold great potential for the future treatment of obesity and T2D. To unravel the molecular mechanisms and factors capable of inducing browning of white adipose tissue (WAT), activation of brown adipose tissue (BAT) and/or improving glucose metabolism we performed an exhaustive, comparative transcriptomic analysis of mRNA and lncRNA isolated from brown and white adipose depots of adult mice exposed to cold.

Materials and methods: Mice were exposed either to cold (4°C) or room temperature for 4 days and RNAs from these tissues were extracted from interscapular BAT (iBAT), inguinal WAT (iWAT), epididymal WAT (eWAT) using specific kits. The expression of mRNAs and lncRNA was analyzed using the Affymetrix GeneChip Mouse Gene 2.1 ST microarray technology in 4 biological replicates. Microarray data was analysed by well-established statistic and bioinformatic tools.

Results: Principal component analysis (PCA) suggested that the gene expression profile of BAT exposed to RT or 4°C did not differ significantly. Similarly, no major differences were also observed in eWAT after cold exposure. However, the gene expression pattern of iWAT after cold exposure resembled that of BAT. Differentially expressed genes and lncRNA were then evaluated in each individual fat pad. Specifically, 243, 247 and 50 genes were upregulated in iBAT, iWAT and eWAT, respectively, whereas 87, 218 and 672 were downregulated in the same tissues. Similar tendency was observed for lncRNA within each tissue. The hierarchical clustering analysis of iBAT, iWAT and eWAT revealed that differentially expressed genes grouped in five different clusters. Functional analysis of this data further demonstrated that iWAT exposed to cold resembled BAT. iWAT also showed the highest number of enriched pathways and gene ontologies with significant statistically differences. Moreover, both pathway enrichment and gene ontology results indicated that major differences were due to metabolism- and mitochondrial-related genes. A second bioinformatic pipeline using several complementary approaches such as logical set relation, pattern matching, gene functional, co-expression and interaction networks analyses, was carried out to unravel novel cold-induced factors potentially capable of improving energy and glucose metabolism. The role of the most robust factors was then tested in mice fed a high fat diet (HFD) after gene transfer using adeno-associated viral (AAV) vectors. These factors were able to decrease body and, white adipose tissue and liver weight and to increase the expression levels of thermogenic genes in adipose tissue.

Conclusion: This study contributes to better understand the molecular mechanisms underlying adaptation of adipose tissue to cold and identifies novel factors able to enhance non-shivering thermogenesis.
Supported by: EFSD/MSD, SAF2014-54866R, EFSD/LILLY, ICREA Academia Award to F.B.
Disclosure: **J. Rodó:** Grants; SAF2014-54866R, 2014-SGR-1669, ICREA Academia Award to F.B., EFSD/MSD.

583

Adenosine metabolism is deregulated in brown adipose tissue in diet-induced type 2 diabetes

I.B. Martins, B.F. Melo, C.S. Prego, J.F. Sacramento, S.V. Conde;
CEDOC, NOVA Medical School, Lisbon, Portugal.

Background and aims: Obesity is the major cause of type 2 diabetes (T2D). Brown adipose tissue (BAT) has been described as a potential target to control and treat these metabolic disorders, since is involved in body thermogenesis and energy expenditure. Moreover, BAT activity is inversed correlated with obesity and insulin resistance (IR). Adenosine (Ado) is a purine nucleoside that has been shown to be involved in glucose homeostasis. Ado has an insulin-sensitizer effect and the administration of Ado antagonists, as caffeine, increases IR. Ado is known to be involved in the regulation of lipolysis and inflammation, being expressed in adipose tissue and recently it was shown that Ado activates BAT via A_{2A} receptors. However, the role of Ado in BAT metabolism and dysfunction in metabolic disorders is unclear. Herein, we hypothesize that Ado metabolism is deregulated in BAT and that its amelioration will improve the metabolic profile. Therefore, in the present work we investigated if alterations in Ado metabolism and function in BAT contribute to metabolic dysfunction in T2D.
Materials and methods: Eight weeks male *Wistar* rats were divided in 2 groups: the control group that fed a standard chow, and the high-fat high-sucrose group (HFHSu), a model of T2D, that fed a combined diet (35% sucrose in drinking water and 60% fat chow) for 25 weeks. Animals were anesthetized with pentobarbital (60 mg/kg, i.p) and BAT was collected and weighted. Glucose uptake was evaluated by evaluating the uptake of 2-deoxyglucose (2-DG) into BAT in the presence and in the absence of Ado receptors agonists CPA 30nM (A_1 agonist), CGS-21680 30 nM (A_{2A} agonist) and Bay-606583 1 μM (A_{2B} agonist). For evaluation of Ado content and release, BAT was incubated in vitro, in the presence and/or absence of EHNA (Ado deaminase inhibitor, 25 μM) and NBTI (equilibrative nucleoside transport inhibitor, 5 μM) and Ado in the medium and within the tissue was quantified by HPLC. The expression of A_1, A_{2A} and A_{2B} receptors in BAT was investigated by western-blot as well as the expression of HIF-1α, ENT-1 and CD73. Statistical analysis was performed in GraphPad Prism 6.0 software and data compared using a t-test, One-Way or Two-Way ANOVA with Bonferroni post-tests.
Results: 2-DG uptake in BAT in control conditions (without Ado receptors agonists) was 4.55 ± 0.33 nmol/mg tissue. CPA increased glucose uptake by 33% (6.04 ± 0.30 nmol/mg, $p \leq 0{,}001$), while CGS and Bay decreased glucose uptake by 20% (3.64 ± 0.12 nmol/mg, $p \leq 0.05$) and 32% (3.09 ± 0.20 nmol/mg, $p \leq 0{,}001$), respectively. In HFHsu animals, BAT exhibited an increase by 30% (CTL = 1.79 ± 0.17 g/kg; HFHSu = 2.55 ± 0.14 g/kg). Ado content in BAT in control animals was 1.43 ± 0.21 nmol/g tissue, 1.42 ± 0.27 nmol/g and 1.09 ± 0.27 nmol/g after 10, 30 and 60 min of incubation. HFHSu diet significantly decreased Ado content by 61, 83 and 65% when tissues were incubated during 10, 30 and 60 min, respectively, without altering Ado release. HFHSu diet increased respectively CD73, ENT-1 and A_{2A} receptor expression by 135% ($p \leq 0.001$), 36% ($p \leq 0.05$), and 34% ($p \leq 0.01$), decreased A_{2B} expression by 54% ($p \leq 0.001$) and did not change A_1 expression. HFHsu diet increased BAT HIF-1α expression by 40%.
Conclusion: We conclude that glucose uptake in BAT in control conditions is modulated mainly via A1 receptors. Moreover, we demonstrate that BAT of T2D animals exhibit alterations in Ado metabolism and function suggesting that the reversion of these alterations will contribute to improve metabolic function in T2D.
Supported by: BF Melo by PD/BD/128336/2017; JF Sacramento by PD/BD/105890/2014
Disclosure: **I.B. Martins:** None.

584

Characterising the function of the Arl15 gene and its role in the development of metabolic traits

Y. Bai[1,2], Y. Wu[1], L. Bentley[1], R.D. Cox[1];
[1]MRC Harwell Institute, Oxford, [2]Department of Biochemistry, University of Oxford, Oxford, UK.

Background and aims: The *ARL15* human Genome Wide Association (GWA) locus is associated with increased risk of type 2 diabetes, lower plasma adiponectin, higher fasting plasma insulin, sexual dimorphic altered body composition and lower HDL cholesterol. Knocking down *ARL15* in a human β cell line and confluent 3T3-L1 cell line is reported to result in impaired insulin secretion and impaired differentiation, respectively. *ARL15* belongs to the small GTPase ARF family and is predicted to be involved in vesicle trafficking. The aim is to test the *Arl15* gene as a functional candidate underlying the GWAS associated metabolic traits.
Materials and methods: We have used the 3T3-L1 cell line to study the effect of knocking down *Arl15* in pre-adipocytes and during differentiation using siRNAs. Further, post-translational modification of ARL15 and its sub-cellular localisation were studied. Global, adipose tissue and islet specific *Arl15* knockout mouse models were generated to investigate the *in vivo* physiological function of ARL15.
Results: Knocking down *Arl15* in sub-confluent pre-adipocytes increased differentiation as measured by q-PCR of differentiation markers and increased triglyceride content ($P = 0.0098$). *Arl15* RNA levels were significantly reduced compared to control cells for 5 days after silencing (D0 $P < 0.001$, D3 $P < 0.001$, D5 $P < 0.001$). The master regulator of adipogenesis *Pparγ* was up-regulated from Day 5 ($P < 0.001$). *Fasn*, *Fabp4*, *Cebpα*, *Plin1*, *Glut4*, *Lipe* and *AdipoQ* were up-regulated from Day 3 ($P < 0.001$). By over-expressing C-Myc, N-Myc and N-EGFP tagged ARL15 constructs in 3T3-L1 and C2C12, ARL15 was co-localised with the Golgi marker RCAS1. Sub-cellular fractionation also confirmed the localisation of ARL15 in Golgi. Using a resin assisted capture assay, we found that ARL15 was palmitoylated in the Golgi. To study adiponectin secretion, cellular and secreted total adiponectin on Day 7 of differentiation was measured and found to be increased in silenced 3T3-L1 cells. However, Western blotting of non-denatured culture media showed an increase in the higher molecular weight form of adiponectin in silenced cells, indicating a potential role for ARL15 in adiponectin assembly and secretion pathways. *Arl15* homozygous global KO mice exhibit cleft palate and die postnatally. Heterozygous female mice on high fat diet showed lower fasting adiponectin levels at 20 weeks of age ($P = 0.0373$). Decreased body weight ($P = 0.0318$), gWAT ($P = 0.0459$) and BAT ($P = 0.0135$) weight was found post mortem at 32 weeks of age. Isolated islets from heterozygous male mice at 17 weeks of age in a static assay did not show a difference in glucose stimulated insulin secretion. Cohorts of adipose tissue specific KO and pancreatic β cell specific KO mice are being generated to investigate adiposity and insulin secretion in *Arl15* homozygotes.
Conclusion: Silencing *Arl15* in pre-adipocytes leads to increased adipogenesis *in vitro* and may alter adiponectin assembly and secretion. ARL15 is palmitoylated and localised to Golgi and we plan to investigate its role in protein trafficking. In the mouse, ARL15 is essential for normal palate development and for postnatal viability. Further, reduction of *Arl15* in heterozygous female mice altered plasma adiponectin levels and body weight and composition. Future experiments will focus on the physiological effects of tissue specific homozygous deletion of *Arl15.*
Supported by: MRC funding MC_U142661184
Disclosure: **Y. Bai:** None.

PS 041 Lipid metabolism in humans and in cell models

585

Suppression of hepatic VLDL production after weight loss is associated with the recovery of beta cell function in type 2 diabetes

A. Al-Mrabeh[1], S.V. Zhyzhneuskaya[1], C. Peters[1], A.C. Barnes[1], B.S. Aribisala[1], K.G. Hollingsworth[1], N. Sattar[2], M.E.J. Lean[2], R. Taylor[1];
[1]Newcastle University, Newcastle upon Tyne, [2]University of Glasgow, Glasgow, UK.

Background and aims: Overproduction of hepatic very low density lipoprotein (VLDL) is a characteristic of type 2 diabetes. This is driven mainly by the VLDL1 subclass with its higher triglyceride content. The Diabetes Remission Clinical Trial (DiRECT) has demonstrated reversal of diabetes in 46% of participants at 12 months. Previous work has indicated that this may be a consequence of decreased systemic delivery of triglyceride from the liver. DiRECT allows examination of the relationship between change in hepatic VLDL1-TG production and insulin secretion.
Materials and methods: The Tyneside sub-group of DiRECT underwent detailed metabolic tests. 64 participants (type 2 diabetes <6 years duration) were randomized to receive low calorie diet (825–853 kcal/d). We report here data from the Intervention group on 56 participants who have paired VLDL1-TG data between baseline and after weight loss. A non-isotopic competitive method was employed to measure VLDL1-TG production. Beta cell function was assessed using a Stepped Insulin Secretion Test with Arginine stimulation (SISTA). First phase insulin response was taken as the 6 minute increment in C-peptide concentration.
Results: Caloric restriction induced major weight loss (−15.2 ± 1.0 kg). Within those who achieved remission ($n = 38$), the group who had >10% decrease in VLDL-1 TG production ($n = 25$) achieved substantial increase in first phase insulin response compared with the group that had <10% change in VLDL-1 TG production (0.22 [−010–0.55] nmol/l, vs. 0.12 [−0.27–0.30] nmol/l, $p = 0.02$). In the whole intervention group there was a negative correlation between the change in VLDL-TG production and the first phase insulin response ($r = −0.33$, $p = 0.01$). Total plasma triglyceride and VLDL1-TG decreased only when VLDL1-TG decrease was >10% after weight loss (VLDL-TG: 0.63 ± 0.07 to 0.31 ± 0.05 mmol/l, $p < 0.0001$ vs. 0.82 ± 0.16 to 0.69 ± 0.10 mmol/l, $p = 0.38$; Total triglyceride: 1.8 ± 0.17 to 1.1 ± 0.10 mmol/l, $p < 0.0001$ vs. 2.0 ± 0.23 to 1.84 ± 0.30, $p = 0.52$). Similarly, VLDL1-TG pool decreased in the group with Δ VLDL1-TG >10% (2406.5 ± 301.2 to 944.6 ± 173.4 mg, $p < 0.0001$ vs. 2521.6 ± 541.8 to 1862.0 ± 304.8 mg, $p = 0.19$). The group who achieved >10% change in VLDL-TG production had significantly higher VLDL1-TG production at baseline (609.9 ± 37. vs. 466.2 ± 46.5 mg/kg/day, $p = 0.02$). This group was also characterized by higher fasting plasma glucose and insulin levels (8.9 ± 0.5 vs. 7.0 ± 0.4 mmol/l, $p = 0.007$; and 126.9 ± 13.1 vs. 69.8 ± 9.9 pmol/l, $p = 0.001$, respectively). This was accompanied with a lower baseline C-peptide level (0.01 [−0.30–0.67] nmol/l vs. 0.05 [−0.09–0.57] nmol/l, $p = 0.05$).
Conclusion: Overproduction of VLDL1-TG in early type 2 diabetes is associated with elevated levels of fasting plasma glucose and insulin and a weak acute insulin secretion response. The return of beta cell function after weight loss was associated with a greater suppression in VLDL1-TG production.
Supported by: Diabetes UK
Disclosure: **A. Al-Mrabeh:** Grants; Diabetes UK.

586

Correlates of pentraxin 3 serum concentration in men and women with type 2 diabetes

M. Walus-Miarka[1], M. Kapusta[2], A. Trojak[3], A. Hebda-Szydlo[1], P. Miarka[4], E. Kawalec[3], B. Idzior-Walus[1];
[1]Metabolic Diseases, Jagiellonian University, Krakow, [2]Biochemistry, Jagiellonian University, Krakow, [3]Jagiellonian University, Krakow, [4]Nephrology, Jagiellonian University, Krakow, Poland.

Background and aims: Pentraxin 3 (PTX3) is an essential component of innate immunity and a member of the long pentraxin superfamily, which are soluble proteins induced by various inflammatory stimuli. Several clinical investigations have demonstrated that elevated plasma PTX3 levels are associated with the risk of developing cardiovascular and chronic kidney diseases and retinopathy. PTX3, a marker of inflammation, is produced locally in relevant cells such as endothelial cells, macrophages and granulocytes. There are some data, that PTX3 also possess anti-microbial, anti-inflammatory and cardioprotective properties. **Aim**: In this study we aimed to determine factors associated with PTX3 serum concentrations in men and women with type 2 diabetes (DM2).
Materials and methods: Material included consecutive patients with DM2 from outpatient diabetic clinic. In each patient standardized questionnaire, anthropometric measurements, and laboratory tests, including fasting serum lipids, glucose, glycated hemoglobin HbA1c, PTX3 and apolipoproteins (apo) A1, B 100, B 48 and C3 were performed. Serum fasting lipids concentrations were determined by enzymatic methods using Roche reagents, HbA1c by HPLC, PTX3 by ELISA method using Cloud-Clone corp. reagents. Apolipoproteins A1 and B 100 were determined by immunoturbidimetry, apo C3 and apo B 48 by ELISA. Descriptive statistics and Spearman's/Pearson's correlation analyses were performed.
Results: We examined 116 patients with DM2, 67 men and 49 women. In the whole studied group of patients mean (sd) age was 59.1 (11.07) years, diabetes duration was 9,3 (7,5) years, HbA1c 8.6 (2.3)%, BMI 32.74 (5.79) kg/m^2. Men were characterized by significantly lower age and higher uric acid, creatinine and bilirubin concentrations, and as expected, higher WHR than women. In women LDL-C levels were higher than in men. In men median (IR) values of PTX3 were 4.02 (1.99) and in women 4.53 (3.31) ng/ml (ns).
In men PTX3 concentrations correlated significantly positively with total cholesterol ($r = 0.40$, $p < 0.0012$), triglycerides ($r = 0.51$, $p = 0.001$), apo C3 ($r = 0.48$, $p < 0.001$), apo B 48 ($r = 0.34$, $p = 0.0048$), glucose ($r = 0.35$, p 0.0128), and creatinine ($r = 0.32$, $p = 0.0092$) levels. In women PTX3 correlated significantly with total cholesterol and LDL-C ($r = 0.53$, $p = 0.0002$) and $r = 0.50$, $p = 0.0008$) respectively, and with apo B 100 ($r = 0.41$, $p = 0.0033$). We did not find any correlations between PTX3 and glycemic control, transaminases, GGTP or obesity parameters.
Conclusion: The results of our study indicate that in patients with type 2 diabetes in both sexes there is significant association between PTX3 and total cholesterol, while in men also with triglycerides, apoC3, apo B 48, glucose and creatinine. In women PTX3 correlated with LDL-C and apo B 100, strong cardiovascular risk factors. The results of our study suggest that there are different correlates between inflammation marker PTX3 and examined lipids and apolipoproteins in men and women, what potentially could be of importance in prevention of vascular complications in these patients.
Supported by: K/ZDS/5595
Disclosure: **M. Walus-Miarka:** None.

587

Increased glycerophosphocholine concentration is associated with insulin resistance and hyperglycaemia in skeletal muscle

G.D. Clarke[1], J.A. Vasquez[1], C. Solis[2], M. Abdul-Ghani[2], D. Tripathy[2], R.A. DeFronzo[2];
[1]Radiology, University of Texas Health Science Center at San Antonio, San Antonio, [2]Medicine (Diabetes), University of Texas Health Science Center at San Antonio, San Antonio, USA.

Background and aims: We previously reported, using quantitative phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS), that phosphocreatine, [PCr], and [ATP] concentrations are lower in skeletal muscle of type 2 diabetic (T2D) versus normal glucose tolerant (NGT) subjects. Reduced PCr also was significantly and inversely correlated with measures of glycemia, i.e. HbA1c and fasting plasma glucose (FPG), but not with indexes of insulin resistance (IR). Increased phosphodiester (PDE)

signals relative to ATP (PDE/ATP) have been associated with reduced mitochondrial activity and insulin resistance (Szendroedi et al, 2011). The PDE signal mainly is due to glycerophosphocholine (GPC), a lipid intermediate involved in membrane homeostasis, skeletal muscle contraction and mitochondrial NAD+/NADH metabolism. In the present study we related *in vivo* [PDE] in NGT and T2D subjects to HbA1c, Matsuda index (MI) of insulin sensitivity, FPG, insulin-mediated rate of glucose disposal (Rd) (insulin clamp) and intramyocellular lipid concentration [IMCL].

Materials and methods: Fourteen NGT (age = 55 ± 11 y, A1c = 5.5 ± 0.3%, BMI = 28.6 ± 3.9 kg/m^2) and 11 T2D (age = 48 ± 12 y, A1c = 8.1 ± 1.3%, BMI = 30.8 ± 5 kg/m^2) subjects received an OGTT to quantitate glucose tolerance status and Matsuda index. On a separate day, subjects were scanned in a 3T MRI system (Siemens Trio) with a dual-tuned ^{1}H-^{31}P TX-RX coil to measure [PCr], [ATP], and [PDE] in resting vastus lateralis (VL) muscle. Roughly one week after the MRS study, subjects returned for a 4-hour euglycemic insulin clamp study. ^{31}P-MRS concentrations were calibrated to an external phosphate standard, as previously described. VL [IMCL] was measured with hydrogen-1 MRS. The AMARES fitting algorithm in jMRUI 5.0 was used for spectral analysis. Student's two-tailed t-test and Pearson's correlation were performed using R with significance set at $p < 0.05$.

Results: [PDE] was increased in T2D vs NGT subjects (3.11 ± 0.82 mM versus 4.04 ± 0.98 mM, $p = 0.02$). PDE/ATP also was significantly increased in T2D (5.35 ± 1.6 versus 3.11 ± 0.82 for NGT, $p = 0.005$) due to the combination of increased [PDE] and reduced [ATP] in skeletal muscle. [PDE] also was correlated negatively with the Matsuda Index of insulin sensitivity ($r = -0.43$, $p = 0.03$) and insulin-mediated Rd ($r = -0.44$, $p = 0.04$) and was positively correlated with HbA1c ($r = 0.57$, $p = 0.003$) and FPG ($r = 0.602$, $p = 0.001$) across all subjects. Increased [PDE] was positively correlated with [IMCL] ($r = 0.64$, $p < 0.005$) across all subjects. This novel observation has not reported previously.

Conclusion: Absolute [PDE] is significantly increased in skeletal muscle of T2D individuals and is closely associated with muscle insulin resistance and poor glycemic control. In mammalian cells, hydrolysis of GPC to glycerol-3-phosphate and choline is mediated by the glycerophosphocholine phosphodiesterase isoform, GDE5. We infer that the buildup of GPC in the skeletal muscle in insulin resistant T2D subjects is linked to a bottleneck in GPC catabolism due to reduced expression of GDE5, consistent with reduced mRNA expression in gastrocnemius muscles of diabetic KK-Ay mice (Okazaki et al, 2010). These results suggest that glycerophosphocholine is a key lipotoxic molecule that contributes to skeletal muscle insulin resistance in T2D individuals.

Supported by: This work was supported by NIH grants DK24092-34 and K25DK089012.

Disclosure: **G.D. Clarke:** None.

588

Acute hyperketonaemia does not reduce glucose or palmitate uptake in abdominal organs or skeletal muscle

E. Søndergaard[1], K.M. Lauritsen[1], N. Møller[1], L.C. Gormsen[2];
[1]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus C, [2]Department of Nuclear Medicine and PET Center, Aarhus University Hospital, Aarhus C, Denmark.

Background and aims: SGLT-2 inhibitors have been shown to prevent cardiovascular disease through mechanisms not fully understood. It has been hypothesized that the cardioprotective effects are mediated through an increase in circulating ketone bodies, which are energy-efficient oxidative fuels due to a lower oxygen consumption compared to glucose and fatty acid oxidation. We have previously shown that infusion of ketone bodies reduces myocardial glucose uptake by 50%, but it is still unknown whether an increase in ketone bodies affect substrate metabolism in other organs. In this study, we performed PET analysis of glucose and lipid metabolism in abdominal organs and skeletal muscle in healthy subjects during a ketone body infusion.

Materials and methods: The study was performed on eight healthy subjects [3 women; age: 60(50–68) years; BMI: 25.5 (21.5–34.6) kg/m^2] as a single-blinded, randomized cross-over study with a ketone study day (0.18 g/kg/hour Na-3-β-hydroxy-butyrate) and a placebo study day (0.9% saline). On both study days, subjects underwent a hyperinsulinemic-euglycaemic clamp (0.3 mIE/kg/min). PET/CT scans were performed dynamically (50 min each) with radiotracers ^{11}C-palmitate (≈280 MBq) and ^{18}F-FDG (≈200 MBq). Volumes of interest (VOI's) were drawn in erector spinae and biceps muscle, kidney, spleen, bone marrow, liver, visceral and subcutaneous fat. Relative substrate uptake rates were then calculated using linear fitting of input vs. tissue activity curves and Patlak or Logan plots as appropriate. Absolute uptake rates were calculated as k-values times substrate concentrations for the Patlak plots. Statistical comparison of organ metabolism during ketone body infusion vs. saline infusion was performed by a paired t-test.

Results: Ketone body infusion did not alter glucose uptake compared to saline infusion in erector spinae muscle (0.03 ± 0.01 vs. 0.03 ± 0.01 mmol/g/min, $p = 0.42$) or biceps muscle (0.05 ± 0.03 vs. 0.09 ± 0.15 mmol/g/min, $p = 0.49$), nor did it affect the volume of distribution (Vd (ml plasma/ml tissue)) in the kidneys (1.22 ± 0.17 vs. 1.40 ± 0.23, $p = 0.11$), spleen (0.80 ± 0.11 vs. 0.92 ± 0.40, $p = 0.30$), liver (1.19 ± 0.13 vs. 1.15 ± 0.14, $p = 0.60$), visceral fat (0.31 ± 0.23 vs. 0.27 ± 0.21, $p = 0.21$) or subcutaneous fat (0.20 ± 0.12 vs. 0.21 ± 0.15, $p = 0.92$). Ketone body infusion significantly increased glucose Vd in bone marrow (0.64 ± 0.08 vs. 0.51 ± 0.12, $p = 0.04$). Absolute palmitate uptake was low due to suppression of lipolysis by the hyperinsulinemic-euglycaemic clamp and was unaffected by ketone infusion in all organs. However, ketone body infusion led to an increased palmitate uptake capacity (Patlak k-values (ml plasma/ml tissue/min)) in erector spinae muscle (0.03 ± 0.01 vs. 0.02 ± 0.00, $p = 0.02$) and bone marrow (0.05 ± 0.01 vs. 0.03 ± 0.01, $p = 0.01$).

Conclusion: Even pronounced acute hyperketonaemia does not reduce glucose or palmitate uptake in abdominal organs or skeletal muscle. This underscores the priority of ketone body utilisation in the heart and brain in preference to other substrates during a state of energy deficiency. In addition, ketone bodies increase bone marrow glucose uptake. We speculate this is due to an increased haematopoiesis, which could contribute to the increased haematocrit values observed during SGLT-2 inhibition.

Clinical Trial Registration Number: NCT02814474
Supported by: The Danish Council for Strategic Research

Disclosure: **E. Søndergaard:** None.

589

GlycA and lipoprotein by 1HNMR in subjects with type 2 diabetes, prediabetes and control and the association with clinical and inflammatory factors during a programme of exercise

L. Brugnara[1,2], S. Murillo[1,2], N. Amigò[3], R. Fuentes[3,4], X. Correig[4,2], A. Novials[1,2];
[1]IDIBAPS/Hospital Clínic, Barcelona, [2]CIBERDEM, Barcelona, [3]Biosfer Teslab SL, Reus, [4]Universitat Rovira i Virgili (URV-IISPV), Reus, Spain.

Background and aims: GlycA, a pro-inflammatory glycoprotein biomarker, is elevated in several chronic inflammatory diseases and recently associated with incident type 2 diabetes (T2D), cardiovascular (CV) events and mortality. The implementation of a program of exercise could improve the levels of GlycA, lipoprotein subfractions and clinical parameters in subjects with T2D.

Materials and methods: A total of 34 subjects (11 control [CT], 10 prediabetes [PD] and 13 T2D), between 40–65 years old, sedentary (physical activity <2 times/week), BMI <35, without CV or microvascular complications, were included in the study. The exercise program included 16 weeks of aerobic and strength exercise twice a week. Body fat composition (anthropometric and DXA measurements), physical fitness (1RM [repetition maximum] for strength capacity; VO_2max and Wmax for aerobic capacity) and biochemical analysis were evaluated before and

after the exercise program. Glycoprotein A (GlycA) and lipoprotein subfractions were analyzed by nuclear magnetic resonance (1HNMR) by Biosfer Teslab.

Results: Subjects were 52.2 ± 8.8, 58 ± 10.1 and 58.8 ± 5.8 years old in CT, PD and T2D, respectively. After the exercise program, there were no changes in body weight. However, there was a reduction in body fat composition by DXA in CT (1.6% in total body fat, $p = 0.004$; 5.1% in abdominal fat, $p = 0.007$; 10.4% in visceral fat, $p = 0.012$), and in T2D (2.1% in total body fat, $p = 0.026$; 2.1% in abdominal fat, $p = 0.033$), but not in PD. Also, fitness parameters improved in the three groups: in CT (23.1% in 1RM, $p = 0.005$; 18.8% in VO_2max, $p = 0.019$; 16.2% in Wmax, $p < 0.001$); in PD (13.9% in 1RM, $p = 0.005$; 12.2% in Wmax, $p = 0.007$); and in T2D (19% in 1RM, $p = 0.001$; 25.1% in Wmax, $p = 0.001$). There was a reduction in fasting glycemia in the CT group (from 85.1 ± 10.3 to 79.1 ± 8 mg/dl, $p = 0.043$). No changes in HbA1c, HOMA-IR, inflammatory parameters, lipoprotein subfractions or GlycA were identified in the three groups after exercise. In pre-exercise, the groups CT and T2D presented different results, respectively: GlycA (4.4 ± 0.6 vs 5.4 ± 1.2 A.U., $p = 0.024$), small-LDL-particle (376 ± 53 vs. 446 ± 46 nmol/L, $p = 0.004$), medium-HDL-particle (11.5 ± 2.7 vs. 8.7 ± 1.6 μmol/L, $p = 0.006$), VLDL-size (42.2 ± 0.2 vs. 42 ± 0.2 nm, $p = 0.04$), LDL-size (21.2 ± 0.2 vs. 20.8 nm, $p = 0.002$) and HDL-size (8.3 ± 0.05 vs. 8.2 ± 0.06 nm, $p = 0.011$). GlycA presented a direct correlation ($p < 0.05$, $R > 0.34$) with insulinemia, HOMA-IR, triglycerides, TNFalpha, VLDL-C, VLDL-TG, total-VLDL-particle, medium-VLDL-particle, small-VLDL-particle, small-LDL-particle, and inverse correlation with adiponectin, total-HDL-particle and medium-HDL-particle, but not with clinical variables.

Conclusion: GlycA was associated with several parameters of CV risk. GlycA and a more atherogenic lipoprotein profile were higher in subjects with T2D when compared to controls. The present program of exercise, despite improving fitness and body composition, was not able to improve GlyA, lipoprotein profile or metabolic parameters, indicating that the status of carbohydrate tolerance is more important than the effects of the program of exercise.

Supported by: Sardà Farriol Research Program/Generalitat de Catalunya

Disclosure: **L. Brugnara:** None.

590

Energy metabolism and lipid turnover are altered in superficial subcutaneous adipose tissue of male type 2 diabetes patients

K. Bódis[1,2], J. Lundbom[1,3], T. Jelenik[1,3], D. Markgraf[1,3], A. Strom[1,3], O.-P. Zaharia[1,3], Y. Karusheva[1,3], V. Burkart[1,3], K. Müssig[1,2], M. Ouni[3,4], J.-H. Hwang[1,3], D. Ziegler[1,3], A. Schürmann[3,4], M. Roden[1,2], J. Szendroedi[1,2];

[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Duesseldorf, [2]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Duesseldorf, [3]German Center for Diabetes Research (DZD), München-Neuherberg, [4]German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany.

Background and aims: Insulin resistant humans are characterized by lower mitochondrial gene expression and lipogenesis in subcutaneous adipose tissue (SAT). Furthermore, human carriers of a hormone sensitive lipase (*HSL)* gene mutation exhibited hepatic steatosis and increased risk for type 2 diabetes (T2D). SAT is composed of deep (DSAT) and superficial layers (SSAT). SSAT was assumed to have protective function in T2D, but sex specific differences have not been studied yet. This study aimed to characterize sex specificity and differences in SSAT between T2D patients and glucose-tolerant humans (CON) by assessment of mitochondrial efficiency, markers of lipogenesis and lipolysis in SSAT.

Materials and methods: We included 20 T2D and compared them to 20 CON of similar sex distribution, age and body mass index (BMI) (6/14 female/male per group; 52 ± 2 vs 54 ± 2 years, 32 ± 1 vs 31 ± 1 kg/m^2). All participants underwent euglycemic-hyperinsulinemic clamp tests to assess insulin sensitivity (M-value) as well as magnetic resonance spectroscopy to assess liver fat content (HCL) and visceral adipose tissue volume (VAT). Ultrasound guided biopsies were performed to obtain targeted samples of SSAT. In SSAT, mitochondrial efficiency from respiratory control ratio (RCR = state 3/state 4) was measured by respirometry. Two markers of lipogenesis were assessed in SSAT: stearic-to-palmitic acid ratio (18:0/16:0) by gas chromatography-mass spectrometry and stearoyl-CoA desaturase (*SCD*) mRNA by PCR. Additionally, lipolysis markers adipocyte triglyceride lipase (*ATGL*) and *HSL* mRNA were assessed by PCR.

Results: In insulin resistant T2D, HCL was 63% ($p < 0.01$) and VAT 22% higher ($p < 0.05$) compared to CON. T2D patients had lower RCR (−19%) and 18:0/16:0 ratios (−17%) as well as lower *SCD* (−50%), *ATGL* (−70%) and *HSL* (−49%) mRNA levels in SSAT (all $p < 0.05$). While also in males these differences were present, in female T2D, only *ATGL* was lower compared to female CON. In female T2D, VAT was 22% lower as well as RCR 37% and *HSL* mRNA 70% higher compared to male T2D patients (all $p < 0.01$).

Conclusion: Lower mitochondrial efficiency, lipogenesis and lipolysis gene expression in SSAT of male, but not female T2D patients suggest inadequate energy metabolism and lipid turnover in SSAT of male patients with T2D.

Clinical Trial Registration Number: NCT01055093
Supported by: BMBF, DZD

Disclosure: **K. Bódis:** None.

591

Dissociation of insulin sensitivity and bioactive lipid content in endurance-trained athletes

D. Pesta[1], E. Anadol-Schmitz[1], S. Gancheva[1], D. Markgraf[1], O.-P. Zaharia[1], H. Katsuyama[1], Y. Kupriyanova[1], J.-H. Hwang[1], D. Zhang[2], G. Shulman[2], M. Roden[1];

[1]German Diabetes Center, Düsseldorf, Germany, [2]Yale University School of Medicine, New Haven, USA.

Background and aims: Increased concentrations of bioactive lipids such as diacylglycerol (DAG) in the cell membrane of skeletal muscle have been associated with insulin resistance due to activation of protein kinase C (PKC) isoforms and subsequent disruption of insulin signaling. This study aimed at testing the hypothesis that cellular localization and molecular species of DAG differently affect insulin action in sedentary and endurance-trained individuals.

Materials and methods: Endurance-trained athletes (ATH; $n = 9$) and sedentary individuals (SED; $n = 12$) with comparable total IMCL, as measured by ^{1}H-magnetic resonance spectroscopy, who underwent spiroergometry and hyperinsulinemic-euglycemic clamps to assess maximal oxidative capacity and insulin sensitivity, respectively were included in this study. In skeletal muscle biopsies, translocation of protein kinase C (PKC) θ and ε were determined by Western blotting and concentrations of DAG were measured using targeted LC-tandem mass spectrometry upon separating fractions of cellular membranes, lipid droplets and cytosol by ultracentrifugation.

Results: Maximal oxidative capacity and insulin sensitivity were 46% and 47% higher in ATH than in SED (both $p < 0.01$), respectively. The membrane:cytosol ratio of PKCθ, which reflects PKCθ activity, was 62% lower in ATH consistent with their increased insulin sensitivity ($p < 0.01$), while PKCε was not different between both groups. In SED, PKCθ activation associated negatively with membrane and lipid-droplet DAG, while PKCε was negatively associated with total sn-1,2 DAG and membrane DAG. In ATH, PKCθ associated negatively with cytosolic and lipid droplet DAG, while PKCε associated negatively with lipid droplet and positively with cytosolic and membrane DAG. Concentrations of DAG in total and membrane (40% and 48%, both $p < 0.01$), but not in lipid droplet

and cytosolic fractions, were higher in ATH. In SED, insulin sensitivity correlated inversely with all stereoselective subspecies of lipid-droplet DAG. On the other hand, cytosolic sn-1,2 (C16:0–C18:2) and sn-1,3 (C18:1–C18:0) DAG correlated positively with insulin sensitivity in ATH.

Conclusion: Differences in the stereo-selectivity and/or sub-compartmentalization of cellular DAG between athletes and insulin resistant sedentary individuals may explain the lower muscle PKCθ activation and in turn higher insulin sensitivity in endurance trained athletes.

Clinical Trial Registration Number: NCT03314714

Supported by: MIWF NRW, BMG, BMBF, DZD e.V., Schmutzler Stiftung

Disclosure: **D. Pesta:** None.

592

Both fructose and glucose contribute substantially to intestinal triglyceride synthesis in vitro

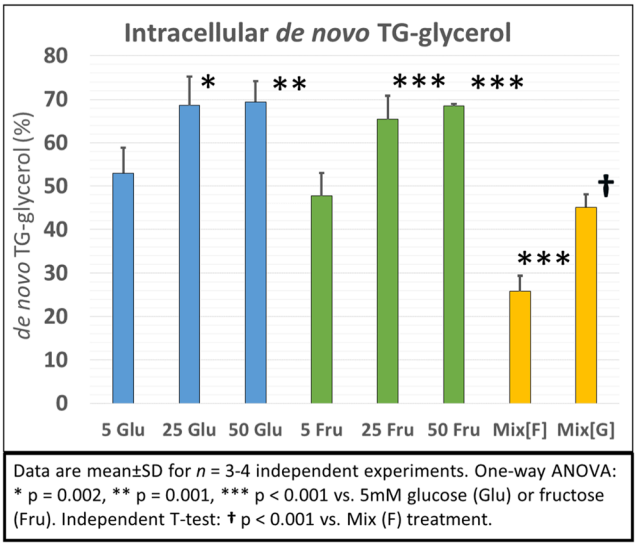
S. Steenson[1], K.G. Jackson[2], A.M. Umpleby[1], F. Shojaee-Moradie[1], N. Jackson[1], J.A. Lovegrove[2], B.A. Fielding[1];
[1]Department of Nutritional Sciences, University of Surrey, Guildford, [2]Department of Food & Nutritional Sciences, University of Reading, Reading, UK.

Background and aims: Fructose consumption has been associated with postprandial hypertriglyceridaemia. The cellular metabolism of fructose is an unregulated process, potentially providing excess three-carbon precursors for several metabolic pathways, including the formation of glycerol (glyceroneogenesis), which may be utilised for triglyceride (TG) synthesis. While the effects of fructose on hepatic TG metabolism have been widely reported, recent evidence suggests that the small intestine may in fact be the major site of fructose metabolism. The present study used Caco-2 cells, a well-characterised intestinal cell line, to determine the concentration dependent effects of fructose, and glucose, on TG-glycerol synthesis.

Materials and methods: Caco-2 cells (passage 49–53) were grown for 21 days on Corning Transwell® supports, ensuring maximal differentiation and TG synthesis capacity. Cells were treated for 96 h with carbohydrate-free media containing either 5, 25 or 50 mmol/l fructose or glucose (5, 25 or 50 Fru/Glu), or an equimolar mixture of 12.5 mmol/l fructose and 12.5 mmol/l glucose ("Mix"). In addition, a 0.5 mmol/l mixture of fatty acids was added, with a composition representing the UK diet. Stable isotope tracers were also added, accounting for 20% of the total glucose/fructose concentration in each treatment, to measure the proportion of TG-glycerol carbons derived from either fructose ($^{13}C_6$-fructose) or glucose ($^{13}C_6$-glucose). Following treatment, TG was extracted from the cells (intracellular) and media (secreted), hydrolysed to obtain the glycerol moiety, and the ^{13}C-enrichment measured via gas chromatography-mass spectrometry.

Results: A substantial proportion of TG-glycerol was derived from both fructose (range 25–69%) and glucose (33–70%) carbons, across all treatment types. Intracellular TG (see figure) contained significantly higher *de novo* TG-glycerol (%) after treatment with 25Fru, 50Fru, 25Glu and 50Glu versus either 5Fru or 5Glu, respectively (all $p \leq 0.002$). The same pattern was observed for secreted TG-glycerol (all $p \leq 0.003$). For the "Mix" treatments containing either fructose (Mix [F]) or glucose (Mix [G]) isotope tracer, Mix [G] yielded a higher percent intracellular ^{13}C-glycerol than for Mix [F] ($p < 0.001$).

Conclusion: The present study further highlights the potential importance of the small intestine in fructose metabolism. Glucose appears to be the preferred substrate for TG-glycerol synthesis within a sugar mixture. However, fructose is also highly utilised by intestinal enterocytes, both for TG directed toward lipoprotein formation and secretion, as well as for storage within intracellular lipid droplets. Whether a similar level of TG-glycerol may be derived from fructose *in vivo*, and its possible implications for post-prandial hypertriglyceridaemia, warrant further study in human subjects.



Data are mean±SD for n = 3-4 independent experiments. One-way ANOVA: * p = 0.002, ** p = 0.001, *** p < 0.001 vs. 5mM glucose (Glu) or fructose (Fru). Independent T-test: † p < 0.001 vs. Mix (F) treatment.

Supported by: BBSRC Food Security DTP

Disclosure: **S. Steenson:** None.

PS 042 Adipose tissue biology in humans

593

Cytoskeletal transgelin 2 (TAGLN2) is associated with sex-dependent adipose tissue expandability

F. Ortega[1,2], J. Moreno-Navarrete[1,2], M. Gómez-Serrano[3], E. García-Santos[3], J. Latorre[1], M. Sabater[1,2], E. Caballano-Infantes[1,2], R. Guzmán[2,4], A. Vidal-Puig[5], M. Malagón[2,4], B. Peral[4], A. Zorzano[6,7], J. Fernández-Real[1,2];
[1]Diabetes, Endocrinology and Nutrition (UDEN), Institut d'Investigació Biomèdica de Girona (IdIBGi), Girona, Spain, [2]CIBEROBN (ISCIII), Madrid, Spain, [3]Endocrinology, Physiopathology and Nervous System, Instituto de Investigaciones Biomédicas 'Alberto Sols' (IIBM), Consejo Superior de Investigaciones Científicas (CSIC) and Universidad Autónoma de Madrid (UAM), Madrid, Spain, [4]Cell Biology, Physiology and Immunology, Instituto Maimonides de Investigaciones Biomedicas de Cordoba (IMIBIC)/University of Cordoba/Reina Sofia University Hospital, Córdoba, Spain, [5]Metabolic Research Laboratories, Welcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Addenbrooke's Hospita, Cambridge, UK, [6]Bioquímica i Biología Molecular, Facultat de Biologia, Universitat de Barcelona, Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain, [7]CIBERDEM (ISCIII), Madrid, Spain.

Background and aims: Adipose tissue (AT) expands to accommodate increased energy uptake by initiating proliferation and differentiation of preadipocytes, leading to its hyperplastic cytoarchitecture. Preadipocytes' shape changes dramatically during adipogenesis, in parallel with lipid accumulation and cytoskeletal reorganization. Failure to do so may impact the flexibility to shift between lipid storage and mobilization, leading to impaired metabolism and insulin resistance. Cytoskeletal Transgelin 2 (TAGLN2) was identified in obese AT, being closely associated with AT expandability and inflammation.
Materials and methods: We evaluated the impact of surgery stress *in vivo* and macrophages *in vitro*. Weight loss was chosen as an anti-inflammatory model, so TAGLN2 was analyzed in samples collected before and after bariatric surgery. Associations with inflammatory and metabolic parameters were analyzed in nonobese and obese subjects, in *ex vivo* isolated adipocytes/stromal-vascular cells (SVC), and *in vitro* cultured adipocytes. Causal characterization was assessed by silencing *Tagln2* in preadipocytes, over-expression in AT (aP2-Tagln2 mice), and the study of genetic variants modulating *TAGLN2* gene expression.
Results: *TAGLN2* was increased in obese AT in humans and mice, up-regulated with inflammation, and appropriately decreased after weight loss. *Tagln2* knockdown in preadipocytes prevented adipogenesis, mitochondrial biogenesis and mitosis, and was associated with down-regulation of genes related to growth, biosynthetic and oxidation-reduction processes in differentiated adipocytes. Female transgenic mice over-expressing *Tagln2* in fat exhibited enlarged AT and adipocyte hypertrophy not associated with insulin resistance. Conversely, male transgenic mice failed to expand their AT and showed impaired distribution of small/large adipocytes, in association with decreased glucose tolerance. The relevance of this phenotype was outlined by the existence of common variants within *TAGLN2* gene associated with increased expression and impaired AT expansion in men, as evidence by two independent cohorts and protection from ischemic heart disease in public datasets.
Conclusion: Current findings highlight the contribution of cytoskeletal *TAGLN2* regulation to AT expansion and protection from cardiometabolic disease in a sex-dependent manner.
Supported by: FIS, CIBEROBN, CIBERDEM, PERIS 2016, AGAUR, FPI-UAM, FEDER
Disclosure: **F. Ortega:** None.

594

Betatrophin predicts cardiovascular events independently from the presence of type 2 diabetes and coronary artery disease

A. Leiherer[1,2], A. Muendlein[1,2], K. Geiger[1], C.H. Saely[3,1], E.-M. Brandtner[1], J. Ebner[1], B. Larcher[3,1], A. Mader[3,1], P. Fraunberger[4,2], H. Drexel[1,5];
[1]Vorarlberg Institute for Vascular Investigation and Treatment, Feldkirch, Austria, [2]Private University in the Principality of Liechtenstein, Triesen, Liechtenstein, [3]Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [4]Medical Central Laboratories Feldkirch, Feldkirch, Austria, [5]Division of Angiology, Swiss Cardiovascular Center, University Hospital Berne, Berne, Switzerland.

Background and aims: Betatrophin, also known as ANGPTL8 or lipasin is a nutritionally-regulated protein secreted by the liver and adipose tissue. It is associated with type 2 diabetes mellitus (T2DM) and lipid metabolism. Whether betatrophin is associated with the risk for cardiovascular events is unknown and is addressed in the present study.
Materials and methods: We measured betatrophin in 553 patients undergoing coronary angiography for the evaluation of established or suspected stable coronary artery disease (CAD) and prospectively recorded cardiovascular events in these patients during a follow-up period of up to 8 years.
Results: During follow-up, 301 cardiovascular events occurred. The incidence of cardiovascular events was significantly higher in patients with T2DM ($n = 161$) than in those who did not have diabetes (47.2% vs. 34.4%; $p = 0.005$). Betatrophin was significantly and inversely associated with cardiovascular events both univariately (HR 0.64 [95%CI 0.47–0.87], $p = 0.004$) and after full adjustment including T2DM and baseline CAD (HR 0.55 [95%CI 0.40–0.76], $p < 0.001$). The inclusion of betatrophin to a basic prediction model for the cardiovascular event risk significantly increased model performance (NRI = 0.188, $p < 0.01$).
Conclusion: In conclusion, this study for the first time shows that betatrophin predicts cardiovascular events independently from conventional risk factors including the presence of T2DM.
Disclosure: **A. Leiherer:** None.

595

Dysregulated urocortin 3 expression and its modulation with physical exercise in adult humans with obesity and diabetes

S.A. Kavalakatt, A. Khadir, A. Tiss;
Research Division, Dasman Diabetes Institute, Dasman, Kuwait.

Background and aims: Obesity and Type 2 diabetes (T2D) are characterized by inflammation, disturbed insulin secretion and insulin resistance due to reduced insulin action in target tissues such as muscle, liver and adipose tissue. Urocortin3 (Ucn3) is a molecular marker for mature pancreatic β-cells that regulates glucose-stimulated insulin secretion. However, their release and expression by human adipose tissue and role in obesity and diabetes is still not well investigated. The aim of this study was to assess the effects of obesity and diabetes on circulating Ucn3 and its expression in adipose tissue. Also, to further assess if these levels are affected by physical exercise.
Materials and methods: Adult male and female human subjects consisting of 41 non-diabetic normal-weight ($20 \leq BMI < 25$ kg/m^2) and 205 overweight ($25 \leq BMI < 40$ kg/m^2, 107 non-diabetic and 98 diabetic) were enrolled in the study followed by a 3-month moderate exercise program. Subcutaneous adipose tissue (SAT) biopsies and venous peripheral blood were collected before and after exercise along with anthropometric measurements and blood biochemistry analysis. Plasma levels of inflammatory and metabolic markers were measured using Bioplex-200 system. The expression and circulating levels of Ucn3 were assessed using ELISA, RT-PCR, western blot and confocal microscopy.
Results: Ucn3 release was significantly decreased in non-diabetic overweight when compared to normal weight subjects and increased in

diabetic compared to non-diabetic overweight. On the contrary, in SAT, Ucn3 expression was increased in non-diabetic and decreased with diabetic overweight. Our 3-month supervised physical exercise protocol, increased the levels of circulating Ucn3 in non-diabetic overweight concomitantly with reduced insulin levels. Furthermore, significant decrease of Ucn3 levels was observed in the SAT of both diabetic and non-diabetic overweight subjects. In non-diabetic subjects Ucn3 levels negatively correlated with insulin, C-peptide and HOMA1. Interestingly, Ucn3 circulating levels strongly correlated with Visfatin in both diabetic and non-diabetic subjects.

Conclusion: Expression of Ucn3, representing the feedback loop linking insulin secretion and glucose, are disrupted not only in established diabetic subjects but also in overweight non-diabetic where glucose tolerance is only minimally impaired. Also, following physical exercise insulin levels were inversely related with circulating Ucn3 in both diabetic and non-diabetic subjects. These data suggest that Ucn3 might be a key player in the pathophysiology of diabetes and hence further studies are warranted to investigate its role in the onset and progression of diabetes.

Supported by: KFAS

Disclosure: **S.A. Kavalakatt:** None.

596

Skin autofluorescence measurement and carotid intima media thickness in morbid obese patients

D. Gogas Yavuz[1], M. Uygur[2], D. Yazici[3];
[1]Endocrinology and Metabolism, Marmara University School of Medicine, Istanbul, Kadikoy, [2]Endocrinology and Metabolism, Marmara University School of Medicine, Istanbul, [3]Endocrinology and Metabolism, Koc University School of Medicine, Istanbul, Turkey.

Background and aims: Obesity associated with accelerated atherosclerosis independent of hyperglycemia. Advanced glycation end-products (AGEs) are known to play an important role in the pathogenesis of atherogenesis.The present study aimed to evaluate the skin autofluorescence (AF) as a marker of skin AGE accumulation and carotid intima media thickness(CIMT) as an early marker of atherosclerosis in diabetic and nondiabetic morbid obese patient

Materials and methods: 322 morbid obese patients (36 ± 10 year, F/M: 200/122) who were evaluated for bariatric surgery were included in the study. Skin AGE were measured using skin autofluorescence (SAF) in the forearm with an AGE Reader™ (DiagnOptics Technologies,Netherlands). Autofluorescence measurements were presented as arbitrary units (AU). CIMT was measured by ultrasonography. Fasting blood glucose (FPG) and HbA1c levels were measured, Body mass index(BMI) was calculated.Study parameters were compared between diabetic and nondiabetic obese patients. Students t test and spearman correlation tests were used.

Results: BMI were similar between diabetic and non diabetic patients (48.1 ± 7 kg/m^2 and 47.7 ± 7 p:0.1). FBG and HbA1c levesl were higher in the diabetic patients than nondiabetic obese patients ($p < 0.0001$). Skin AF was higher in diabetic obese patients (1.975 ± 0.44 AU)compared to non diabetic obese patients (1.773 ± 0.39 AU, $p < 0.0001$). CIMT measurements were 0.6019 ± 0.13 mm and 0.5691 ± 0.12 mm for diabetic and non diabetic obese patients respectively (p:0.003). CIMT and skin AF measurements have shown a positive correlation in the whole group. ($r = 0.19$, $p < 0.0006$).

Conclusion: Advanced glycation end product accumulation in skin measures as skin AF was higher in diabetic obese patients. Skin AF was found to be positively associated with CIMT in the whole group. Advanced glycation pathway may be accelerated independently in the presence of hyperglycemia in morbid obese patients. Further prospective studies are needed to clarify the association between AGE and atherosclerosis in nondiabetic obese patients.

Disclosure: **D. Gogas Yavuz:** None.

597

Role of adipose tissue in development of insulin resistance in healthy non-obese male offspring of type 2 diabetes patient

M. Koc[1], E. Krauzová[1,2], M. Šiklová[1], L. Beranová[1], M. Štěpán[1,2], J. Gojda[2], V. Šrámková[1], L. Rossmeislová[1], V. Štich[1,2];
[1]Department for Study of Obesity and Diabetes, Charles University, Prague, [2]Second Department of Internal Medicine, University Hospital Kralovske Vinohrady, Prague, Czech Republic.

Background and aims: The risk of developing type 2 diabetes (T2DM) is higher in the offspring of diabetic patients. Although the development of T2DM is often associated with obesity, people with genetic predisposition to T2DM may exhibit signs of impaired insulin sensitivity already before the onset of excessive adipose tissue accumulation. Thus, the aim of our study was to investigate whether the early disturbances of glucose and lipid metabolism in non-obese first degree relatives of T2DM patients (FDR) could be partially based on the alteration of adipose tissue (AT) metabolism and expandability.

Materials and methods: 45 non-obese men (FDR $n = 24$; controls without family history of T2DM $n = 21$) were investigated. The groups were matched for age (35.7 ± 3.5 vs. 36.3 ± 4 years) and BMI (25.9 ± 1.1 kg/m^2 vs. 24.9 ± 0.5 kg/m^2). The subjects were healthy, non-smokers, without hypertension or any systemic disease. All subjects underwent oral glucose tolerance test (OGTT), blood examination, CT of abdominal L3 region to evaluate subcutaneous and visceral fat mass, and euglycemic hyperinsulinemic clamp (HEC). Abdominal subcutaneous AT was obtained by needle biopsy and used for the analysis of mRNA expression (qPCR on the microfluidic platform Fluidigm Biomark) and establishment of primary cultures of AT derived stem cells. These cells were in vitro differentiated into adipocytes. Insulin sensitivity of cells was assessed by the Western blot (detection of phosphoSer473-Akt levels) and by the ability of insulin to suppress Br-cAMP induced lipolysis.

Results: Compared to control subjects, FDR were characterized with higher fasting glycemia (5.6 mmol/l ± 0.1 vs. 5.3 mmol/l ± 0.1; $p < 0.05$) and insulinemia (8.1 mU/l ± 0.1 vs. 5.0 mU/l ± 0.1; $p < 0.05$). No significant difference was observed in glucose disposal evaluated by HEC (M value/FFM [mg/kg FFM/min] 5.8 ± 0.5 vs. 6.7 ± 0.4; $p = 0{,}29$). Despite similar BMI, FDR had higher visceral AT mass (312 ml ± 38 vs. 189 ± 24; $p < 0.05$). Insulin sensitivity in AT measured as suppression of lipolysis (FFA blood levels) at the 2 hour point of OGTT was lower in FDR compared to control group (89% ± 0.9 vs. 93% ± 1.0; $p < 0.01$). The extent of this suppression negatively correlated with visceral but not subcutaneous fat mass. In vitro, the levels of Akt phosphorylation in response to insulin and insulin dependent suppression of lipolysis were similar in differentiated subcutaneous adipocytes from both groups. Subcutaneous AT gene expression of pro- and anti-adipogenic regulators and markers of lipid metabolism, as well as in vitro proliferative and adipogenic capacity of subcutaneous AT derived stem cells, was not different between the groups.

Conclusion: The early disturbances of glucose and lipid metabolism detected in non-obese FDR were not associated with worsened characteristics of subcutaneous abdominal AT and adipocytes but appeared to be linked with the expansion of visceral AT depot. Thus, alteration of visceral rather than subcutaneous AT qualities could contribute to the onset of insulin resistance in non-obese men with genetic predisposition to T2DM.

Supported by: GACR project 16-14048S, PROGRES Q36 of Charles University

Disclosure: **M. Koc:** None.

598

Relationship of pigment epithelium derived factor to metabolic syndrome and vascular damage in patients with type 2 diabetes

D. Karasek[1], J. Spurna[1], O. Krystynik[1], V. Kubickova[2];
[1]Third Department of Internal Medicine – Nephrology, Rheumatology and Endocrinology, Faculty of Medicine and Dentistry, University

Hospital and Palacky University Olomouc, Olomouc, [2]Department of Clinical Biochemistry, University Hospital Olomouc, Olomouc, Czech Republic.

Background and aims: Pigment epithelium derived factor (PEDF) may participate in insulin resistance and vascular damage of patients with dysfunctional adipose tissue. Aim of the pilot study was to compare its circulating levels in type 2 diabetes patients with and without metabolic syndrome (MS) to healthy controls. Relationship to risk metabolic parameters and markers of vascular damage were also investigated.
Materials and methods: Fifty individuals with type 2 diabetes (23 men, 27 women) and forty healthy controls (15 men, 25 women) were included to the study. PEDF, lipids, anthropometric parameters, markers of insulin resistance and diabetes compensation were investigated in all subjects. Diabetics were divided into two groups: with ($n = 30$; 11 men, 19 women) and without ($n = 20$; 12 men, 8 women) MS. Von Willebrand factor (vWF) and plasminogen activator inhibitor-1 served as markers of endothelial dysfunction. Augmentation index (AI) and pulse wave velocity (PWV) were measured as markers of arterial stiffness.
Results: Compared to healthy controls only diabetics with MS had higher levels of PEDF [14160 (10240–16000) ng/ml versus 11120 (8560–14400) ng/ml; $p < 0.05$]. In all subjects PEDF significantly ($p < 0.05$) correlated: positively with BMI, waist, hs-CRP, triglycerides, non-HDL cholesterol, apolipoprotein B, fasting glucose, glycated hemoglobin, C-peptide and insulin; negatively with HDL-cholesterol and apolipoprotein A1. Additionally, in diabetics with MS a negative correlation of PEDF with vWF ($\rho = -0.46$ $p < 0.05$) were found. There were no significant correlations with AI, or with PWV.
Conclusion: Patients with type 2 diabetes and MS had significantly higher levels of PEDF. They were associated with obesity, dyslipidemia and insulin resistance. A negative correlation of PEDF with vWF may point out its vascular protective role, despite its association with adverse metabolic parameters.
Supported by: MH CZ DRO (FNOl, 00098892) and IGA_LF_2018_010
Disclosure: **D. Karasek:** None.

599

Estrogen and glucocorticoid effects on lipocalin 2 expression in human adipose tissue: A role of ERβ pathway in insulin resistance?

P.G. Kamble, M.J. Pereira, G.J. Boersma, K.E. Almby, J.W. Eriksson; Medical Sciences, Uppsala University, Uppsala, Sweden.

Background and aims: Recently, we showed that the adipokine lipocalin 2 (LCN2) can produce insulin resistance in human adipose tissue (AT). Its expression was increased by a synthetic glucocorticoid, dexamethasone (Dex), in AT from pre-menopausal women but not in post-menopausal women or men, implying a role of sex steroids in regulating LCN2 expression in AT. Here, we aimed to study the direct effects of estrogen on LCN2 expression in AT. In this context, we also studied if any cross-talk occurs between estrogen and glucocorticoids in the regulation of AT LCN2 expression.
Materials and methods: Subcutaneous AT from non-diabetic post-menopausal women ($n = 42$, 68 ± 6 Y, BMI 28 ± 4 kg/m^2) was incubated with or without 17 β-hydroxy estradiol (E2, 0.01–100 nM), Dex (0.3 μM) or the combination for 24 h and LCN2 mRNA levels were measured. AT was co-treated with E2 (1 nM) or (E2+dex) and 100 nM of either estrogen receptor (ER) α or β antagonist, MPP or PHTPP, respectively. Moreover, AT was treated with ERβ specific agonist (DPN, 100 nM) for 24 h. In addition, LCN2 gene expression was measured in freshly snap frozen subcutaneous AT from pre- ($n = 23$, 32 ± 9 y, 31.5 ± 13.1) and post-menopausal ($n = 51$, 48 ± 18 Y, BMI 26.1 ± 3.2 kg/m^2) women and men ($n = 32$, 51.5 ± 17 Y, BMI 29.2 ± 9.5 kg/m^2).
Results: LCN2 gene expression in men was higher by 2 fold than pre-menopausal women ($p < 0.05$). Post-menopausal women showed a trend of a higher LCN2 expression than pre-menopausal women ($p = 0.079$). E2 (0.001 to 100 nM) dose-dependently increased LCN2 gene expression in AT up to 3.5 fold ($p < 0.01$, $n = 5$ to 18). Furthermore, E2 at 1 nM also increased LCN2 protein expression by 2.7 fold ($p < 0.05$, $n = 6$). E2 in the presence of ERα antagonist could significantly increase LCN2 gene expression by 4.8 fold ($p < 0.05$, $n = 11$). In contrast, the effect was blocked ($p < 0.05$, $n = 10$) by addition of ERβ antagonist. In addition, treatment with ERβ specific agonist alone increased LCN2 gene expression by 5.2 ($p < 0.01$, $n = 18$) fold compared to control. AT treated with E2 (1 nM) did not show any effect on ERα gene ($n = 10$) or protein ($n = 3$) expression compared to control. Interestingly, E2 incubation increased ERβ gene expression by 1.8 fold ($p < 0.05$, $n = 11$). Dex alone or E2 + Dex did not show any significant effect on LCN2 gene expression. However, E2 + Dex could significantly increase LCN2 gene expression by about 20 fold ($p < 0.5$, $n = 6$) in presence of ERα antagonist, but not in presence of ERβ antagonist ($n = 6$). Long-term Dex (0.3 μM) exposure significantly decreased ERα gene by 20% ($n = 24$, $p < 0.01$), and protein expression by 35% ($p < 0.01$, $n = 5$,), but increased ERβ gene expression by 120% ($p < 0.01$, $n = 24$). In bivariate analysis, Dex-induced reduction in expression of ERα was significantly correlated with BMI ($r = 0.569$, $p < 0.01$), waist circumference ($r = 0.565$, $p < 0.01$) fasting insulin ($r = 0.531$, $p < 0.05$) and C-peptide ($r = 0.560$, $p < 0.01$). In a stepwise multivariate analysis, BMI remained as an independent factor (standard β coefficient = 0.626, $p < 0.01$; model: $r^2 = 0.392$).
Conclusion: Our data suggest that E2 can increase LCN2 expression in subcutaneous AT from post-menopausal women and this action seems to be mediated via ERβ. The up-regulation of ERβ together with down-regulation of ERα seen following dexamethasone can be of importance for glucocorticoid-induced insulin resistance in human AT. Taken together, our data suggest that the ERβ and ERα pathways interact with glucocorticoid action and have reciprocal effects on LCN2 expression.
Supported by: SRC, EXODIAB, EF, SDF, ALF
Disclosure: **P.G. Kamble:** None.

600

Differences in extracellular matrix expression in pancreatic fat cells of non-diabetic, pre-diabetic and diabetic individuals

D. Siegel-Axel[1,2], F. Gerst[1,2], S. Ullrich[1,2], N. Stefan[1,2], B. Sipos[3], S. Haen[4], F. Fend[4], A. Koenigsrainer[3], E. Schleicher[1,2], H.-U. Haering[1,2]; [1]Department of Internal Medicine, Medical Clinic IV, Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, Tuebingen, [2]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tuebingen and German Center for Diabetes Research (DZD), Muenchen-Neuherberg, [3]Department of General Visceral and Transplantation Surgery, University of Tuebingen, Tuebingen, [4]Institute of Pathology and Neuropathology, University of Tuebingen, Tuebingen, Germany.

Background and aims: Extracellular matrix (ECM) affects metabolic function in many tissues. Pancreatic β-cell's function maybe influenced by a specific microenvironment composed of ECM surrounding the islet. Laminin and collagen isoforms were found to enhance insulin gene transcription and secretion, β-cell survival rates and proliferation. Recently we found that pancreatic (pre)adipocytes (PA) and islets influence each other and that the crosstalk of the hepatokine fetuin-A/palmitate with PA and islets induces inflammatory responses in this specific pancreatic microenvironment. In the present study we determined the basal expression of a variety of ECM components in PA, as well as under the influence of fetuin-A/palmitate. In addition, ECM distribution and amount was examined histologically in human pancreatic resections.
Materials and methods: PA were isolated from each 6 non-diabetic, pre-diabetic and diabetic subjects. PA were treated with 600 μg/ml fetuin-A in combination with 60 μmol/L palmitate for 24 h. mRNA levels of collagen I, III, IV, fibronectin, decorin, laminin, elastin, tenascin and the growth factors transforming growth factor beta (TGF-β) and connective tissue

growth factor (CTGF) were quantified by real-time PCR analysis. ECM was analysed in pancreatic resections of the same individuals by (immuno)stainings against collagens, elastin and basement membrane (BM) proteins.
Results: Predominantly collagens but also fibronectin, decorin, laminin and elastin were expressed strongly while TGF-β and CTGF mRNA were less intensely expressed. Collagens, Elastin and CTGF were expressed significantly higher in PA of pre-diabetic patients compared to the other groups. Fetuin-A/palmitate decreased the mRNA expression of the ECM proteins collagen I, III and IV, fibronectin, elastin and CTGF significantly in PA of pre-diabetic subjects. In PA from diabetic patients the mRNA expression of the BM components collagen IV and laminin, as well as TGF-β was downregulated by fetuin-A/palmitate. In contrast no effect was observed in PA from healthy people. Individuals containing PA highly expressing ECM proteins showed also larger amounts of ECM (collagens, elastin) around fat cells and islets in their pancreatic resections.
Conclusion: Our study shows that the amount of ECM and BM components expressed by PA varies between non-, pre-, and diabetic individuals and that the crosstalk with the fatty liver by fetuin-A/palmitate influences the mRNA expression of structural and BM proteins in PA from pre-diabetic and diabetic subjects. Since we could show interactions between pancreatic fat cells and islets, the ECM and BM protein production by PA might influence islet function. This supports other studies showing that incompletely isolated islets that retain some ECM have reduced apoptosis rates and better function.
Supported by: BMBF/DZD 01GI0925
Disclosure: **D. Siegel-Axel:** None.

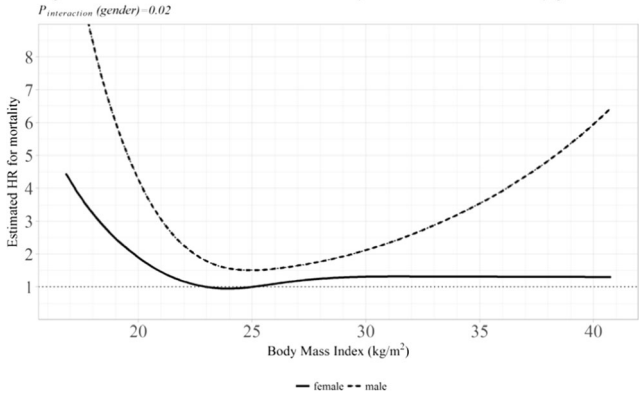
PS 043 Weight regulation and obesity in humans and rodent models

601

The association between BMI and mortality in type 1 diabetes is modified by gender, age at diabetes onset and diabetic kidney disease
E.H. Dahlström[1,2], N. Sandholm[1,2], C. Forsblom[1,2], L. Thorn[1,2], V. Harjutsalo[1,3], F.J. Jansson[1,2], P.-H. Groop[1,2], The FinnDiane Study group; [1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, [2]Abdominal Center, Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, [3]Chronic Disease Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland.

Background and aims: The association between BMI and mortality is U-shaped, with an increased risk of premature mortality both for low and high BMI values. BMI is, however, influenced by many factors such as gender and age, but in patients with type 1 diabetes further by age at onset of diabetes, glycemic control, diabetes duration and the presence of diabetic complications. Thus, the aim of this study was to evaluate if the association between BMI and mortality in type 1 diabetes is modified by these factors.
Materials and methods: Totally, 5,836 adult individuals with type 1 diabetes from the Finnish Diabetic Nephropathy Study (FinnDiane) with data on BMI available, were followed until death or end of 2015. Mortality data were obtained from the Finnish National Death Register. Associations between mortality and BMI were evaluated by a Cox proportional hazard model with a restricted cubic spline transformation of the BMI variable using 4 knots. The reference value for HRs in the restricted cubic spline model was set to a BMI value of 23 kg/m^2. Effect modification was evaluated by introducing interaction terms one at a time, between the BMI spline variable and the variables that could possibly affect the relationship between BMI and mortality. Continuous variables were dichotomized by their median value into two similarly sized groups before included as interaction terms.
Results: During a median of 13.7 follow-up years (Inter-quartile range: 6.4–16.2), 876 individuals (15%) died. BMI was significantly associated with the risk of premature mortality; with both high and low BMI levels conferring increased risk. The nadir BMI (BMI value with lowest mortality risk) was in the normal weight region (24.3–24.8 kg/m^2) but when analyses were restricted to individuals with diabetic kidney disease, the nadir BMI located in the overweight region (25.9–26.1 kg/m^2). Diabetic kidney disease, age at diabetes onset and gender significantly modified the relationship between BMI and mortality ($P_{interaction} < 0.05$). In interaction analyses with gender (Figure1), the risk of mortality observed with higher BMI values appeared to be driven by individuals of male gender. Same was observed for age at diabetes onset, were mortality risk with higher BMI values was more marked among those with a later onset of diabetes.
Conclusion: BMI is nonlinearly associated with premature mortality and the association is modified by gender, age at diabetes onset and diabetic kidney disease. This study highlights the possibility that reducing obesity could potentially translate into reduced premature death in type 1 diabetes, particularly for individuals of male gender and later diabetes onset.

Figure 1. Association between BMI and mortality with effect modification by gender



Supported by: Folkhälsan Research Foundation, Nylands Nation, W. & E. Stockmann Foundation
Disclosure: **E.H. Dahlström:** None.

602

The role of weight in modulating metabolic parameters and vascular complications in patients with type 1 diabetes

R.J. Helliwell[1], H.L. Warnes[1], R.J. King[2], R.A. Ajjan[1];
[1]University of Leeds, Leeds, [2]Taunton and Somerset NHS Foundation Trust, Taunton, UK.

Background and aims: Type 1 diabetes (T1DM) is characterised by insulin deficiency, but some of these individuals can become overweight thus developing a type 2 diabetes phenotype, a group described as having double diabetes. Our aim was to analyse the prevalence of double diabetes in patients originally classified as having T1DM and investigate whether this population is at a higher risk of developing vascular complications of diabetes.
Materials and methods: Patients with T1DM from a single centre (n = 2365) were included in this cross-sectional analysis. They were classified per body mass index (BMI) to normal weight (BMI 18–25 kg/m^2), overweight (25–30 kg/m^2) and obese (>30 kg/m^2). The following metabolic and vascular parameters were investigated: HbA1c, alanine transaminase (ALT), total cholesterol, triglycerides, retinopathy, creatinine, urine albumin creatinine ratio (UACR) and composite cardiovascular outcomes (CV) of stroke and cardiac events.
Results: BMI data was available for 2313 patients (98%) distributed as follows: normal BMI 38.7%, overweight 38.0% and obese 23.3%. Lower HbA1c was observed in overweight patients compared to those with a normal BMI (67.6 mmol/mol vs. 69.8 mmol/mol, p = 0.032), while obese individuals had a similar HbA1c to normal BMI patients (70.2 mmol/mol, p = 0.201). When analysed by gender, normal weight and obese males had similar HbA1c (70.3 and 68.1 mmol/mol, respectively, p = 0.662), which was higher than overweight males (66.9 mmol/mol, p = 0.023). In contrast, female obese patients had higher HbA1c compared with normal weight patients (72.3 mmol/mol vs. 68.7 mmol/mol, p = 0.011). ALT was lower in normal weight patients at 20.7 iu/L than overweight and obese patients (22.6 iu/L and 26.1 iu/L; p = 0.026 and <0.001, respectively). Prevalence of retinopathy, defined as any abnormality other than background changes, was more prominent in obese patients compared with those who had normal weight [OR = 1.77 (1.33–2.34), p < 0.001] with only a trend detected in those who were overweight [OR = 1.29 (0.99–1.67), p = 0.056]. A gender difference was detected with overweight and obese females showing increased prevalence of retinopathy compared with normal weight patients, whereas males failed to show such a pattern. Creatinine and UACR were not different between the three BMI groups and were not affected by gender. Although triglycerides levels were raised in overweight and obese patients compared with normal BMI individuals and total cholesterol was raised in obese female patients, we failed to detect a difference in CV events in various BMI groups.
Conclusion: Almost two thirds of patients with T1DM have raised BMI, whilst higher BMI was not associated with worse glycaemic control, it appears to increase the risk of microvascular complications. Higher BMI was also associated with an abnormal lipid profile but no relationship was found with macrovascular disease, likely due to small sample size and limited number of events. Future longitudinal studies in overweight and obese T1DM patients are warranted to establish whether weight reduction modulates microvascular risk and improves the dyslipidaemic profile.
Disclosure: **R.J. Helliwell:** None.

603

Effects of irisin on leptin and ghrelin secretion and expression of the major appetite regulators in mouse brain

N. Marrano, A. Natalicchio, R. Spagnuolo, L. Dipaola, G. Biondi, A. Cignarelli, S. Perrini, L. Laviola, F. Giorgino;
Department of Emergency and Organ Transplantation, University of Bari Aldo Moro, Bari, Italy.

Background and aims: Irisin is a newly discovered muscle-derived hormone, produced by cleavage of the membrane protein fibronectin type III domain-containing protein 5 (FNDC5) and proposed to bridge exercise with metabolic homeostasis. In mammals, irisin acts in different organs and tissues, improving energy homeostasis. However, the role of irisin on body weight and food intake is still unclear. The aim of this study was to assess the effects of intraperitoneal injections of irisin on leptin and ghrelin secretion, mRNA expression of the major appetite regulators (anorexigenic genes: cocaine- and amphetamine-regulated transcript [CART] and pro-opiomelanocortin [POMC]; orexigenic genes: agouti related neuropeptide [AgRP], neuropeptide Y [NPY], orexin [HCRT] and pro-melanin-concentrating hormone [PMCH]; UCP2 and brain-derived neurotrophic factor [BDNF]), and feeding behaviour in healthy mice.
Materials and methods: Twelve male 6-weeks old C57BL/6 mice were randomized into two groups, and intraperitoneally injected daily with irisin (0.5 microg/g body weight) or vehicle (PBS) for 14 days. On the last day, animals were sacrificed and brains and sera were collected. Leptin and ghrelin levels were measured by ELISA assays. mRNA expression levels were analyzed by quantitative RT-PCR.
Results: Irisin administration did not change leptin and ghrelin serum concentration. Furthermore, irisin injection increased CART, POMC, NPY and BDNF mRNA levels, without affecting the mRNA expression of AgRP, orexin, PMCH and UCP2. Finally, over the short time frame of this observation, body weight and feeding behaviour were unaltered.
Conclusion: In conclusion, intraperitoneal injection of irisin is able to increase the expression of anorexigenic genes in mice. However, this does not translate into significant differences in feeding behaviour and body weight over a 14-day treatment period with this muscle-derived hormone.
Disclosure: **N. Marrano:** None.

604

Effect of ARHGAP21 reduction upon energy homeostasis of diet-induced obese mice

G.M. Soares[1], L. Zangerolamo[1], S.T.O. Saad[2], A.C. Boschero[1], H.C.L. Barbosa-Sampaio[1];
[1]Department of Structural and Functional Biology, Biology Institute, University of Campinas, Campinas, [2]Haematology Centre, School of Medicine, University of Campinas, Campinas, Brazil.

Background and aims: GTPase activating proteins (GAP's) seems to impact glycemic homeostasis. The reduction of ARHGAP21, a GAP, alters glucose-induced insulin secretion as well as body composition of diet-induced obese mice, suggesting changes in the energetic metabolism. However, the possible role of ARHGAP21 in energy homeostasis

remains unknown. Here we used ARHGAP21 haplodeficient mice, aiming to explore the involvement of ARHGAP21 in energy homeostasis.

Materials and methods: 30 days old C57BL/6 mice, ARHGAP21 haplodeficient or not, were fed on chow (Ctl and Het) or a high-fat diet (Ctl-HFD and Het-HFD) for 10 weeks. Body composition was analyzed by body weight gain during all experimental period and also by perigonadal fat content. We also evaluated the food intake and energy homeostasis through voluntary physical activity and energy expenditure, by indirect calorimetry. Data were analyzed by Student's t-test. Data are mean ± SEM, and the difference between the groups were considered statistically significant if $P \leq 0.05$.

Results: Het mice displayed reduced body weight compared with Ctl mice (28.44 g ± 0.33 Ctl x 26.26 g ± 0.30 Het). Besides that, Het animals present a reduction in food intake (13.35 Kcal ±0.34 Ctl x 10.80 Kcal ±0.15 Het) and increase of O_2 consumption (light period: 2048 ml/Kg/hr ± 46.76 x Ctl x 3016 ml/Kg/hr ± 22.67 Het; dark period: 3695 ml/Kg/hr ± 71.02 x Ctl x 4002 ml/Kg/hr ± 80.32 Het), in heat rate (light period: 0.42 Kcal/h ± 0.003 x Ctl x 0.45 Kcal/h ± 0.02 Het; dark period: 0.56 Kcal/h ± 0.01 x Ctl x 0.60 Kcal/h ± 0.003 Het) and in the ambulatory activity (3739 Row means ±47.52 Ctl x 5436 Row means ±182.1 Het). As expected, Ctl-HFD mice developed the deleterious effects inherent in the consumption of the high fat diet. Interestingly, Het-HFD animals did not become obese (35.51 g ± 0.66 Ctl-HFD x 27.66 g ± 0.68 Het-HFD) and had less accumulation of fat deposits (3.22% body weight ± 0.24 Ctl-HFD x 1.72% body weight ± 0.06 Het-HFD), which may be due to a decreased food consumption (15.40 Kcal ±0.21 Ctl-HFD x 13.05 Kcal ± 0.06 Het-HFD) and an increased energy expenditure. Het-HFD mice displayed increased energy expenditure, through the increase of O_2 consumption (light period: 3450 ml/Kg/hr ± 55.90 x Ctl-HFD x 3819 ml/Kg/hr ± 113.0 Het-HFD; dark period: 4155 ml/Kg/hr ± 74.25 x Ctl-HFD x 4407 ml/Kg/hr ± 48.79 Het-HFD), respiratory exchange ratio (RER) (dark period: 0.76 RER ± 0.01 x Ctl-HFD x 0.95 ml/Kg/hr ± 0.01 Het-HFD) and heat rate (light period: 0.53 Kcal/h ± 0.01 x Ctl-HFD x 0.64 Kcal/h ± 0.02 Het-HFD; dark period: 0.64 Kcal/h ± 0.01 x Ctl-HFD x 0.74 Kcal/h ± 0.02 Het-HFD). Moreover, during some points of the dark period, the ambulatory activity was significantly higher in the Het-HFD group when compared to Ctl-HFD group (2279 Row means ±180.3 Ctl-HFD x 3934 Row means ±142.5 Het-HFD).

Conclusion: Taken together, our study indicates a possible role of ARHGAP21 in whole body metabolism, impacting on body weight gain, by reducing food consumption and increasing energy expenditure, regardless of diet composition. The data suggest that GAP protein member as a potential candidate to prevent and treat obesity and related diseases.

Clinical Trial Registration Number: 3783-1

Supported by: CNPq, Capes and Fapesp.

Disclosure: **G.M. Soares:** None.

605

Medi0382, a GLP-1/glucagon receptor dual agonist, reduces weight and improves metabolism via central and peripheral actions

D. Baker[1], S. Oldham[2], S. Will[2], C. Church[1], G. Davies[1], L. Burke[1], L. Brown[1], M. Sulikowski[1], J.-M. Lapointe[1], A. Lewis[1], H. Jouihan[2], P. Barkholt[3], R. Veggerby Grønlund[3], C. Rhodes[2], J. Trevaskis[2];
[1]MedImmune, Cambridge, UK, [2]MedImmune, Gaithersburg, USA, [3]Gubra, Hørsholm, Denmark.

Background and aims: MEDI0382, a balanced GLP-1/glucagon dual receptor agonist, is under development for the treatment of Type 2 Diabetes and nonalcoholic steatohepatitis. Here we characterize the effect of MEDI0382 on metabolic endpoints in leptin-deficient *ob/ob* mice. We further characterize the central actions of MEDI0382 via identification of key neuronal regions activated by subcutaneous delivery and explore the sub-chronic effect of MEDI0382 on improving leptin sensitivity in diet induced obese (DIO) mice.

Materials and methods: MEDI0382 (10, 20 or 30 nmol/kg; s.c., q.d.), liraglutide (Lira; 30 nmol/kg; a GLP-1 analog), or G1437 (30 nmol/kg; a lipidated glucagon receptor agonist) was administered to *ob/ob* mice for 21 days. Body weight, GTT, liver fat and gene expression in adipose tissue was examined. To determine the central sites activated by MEDI0382, 4 h fasted lean C57BL6J mice ($n = 4$/group) were injected s.c with MEDI0382, Lira or G1437 (3 nmol/kg) or vehicle. After 2 h, mice were sacrificed and brains removed and immersion-fixed in formalin (24–48 h). The brains were cut into 3 mm serial slices and processed to paraffin blocks and immunohistochemically stained for c-fos using a rabbit anti-fos polyclonal, with haematoxylin counterstain. To determine effects on leptin sensitivity, DIO mice were administered vehicle, MEDI0382 (10 nmol/kg; s.c., q.d.) or vehicle and pair-fed to MEDI0382-treated mice ($n = 15$–16/group). On day 10, half of the mice from each group were administered vehicle or murine leptin (10 mg/kg) and the mice sacrificed 45 min later for assessment of phosphorylated STAT3 (pSTAT3) in various brain regions.

Results: In *ob/ob* mice, body weight was reduced by MEDI0382 compared to vehicle, with 15% weight loss observed at the highest 30 nmol/kg dose, whereas Lira reduced body weight by 8% and G1437 by 29% (both $p < 0.0001$ vs. vehicle and MEDI0382). GTT was improved by MEDI0382, similar to Lira, but worsened by G1437. Liver fat was reduced in a dose-dependent manner (11%, 14% and 22%, respectively) by MEDI0382 compared to vehicle. Both Lira (12%) and G1437 (10%) also reduced liver fat content (both $p < 0.05$ vs. vehicle and MEDI0382 30 nmol/kg). Expression of *Ucp1*, *Ppargc1a* and *Adrb3* tended to be increased in BAT and WAT by MEDI0382 administration. In lean mice, MEDI0382 increased c-fos in the area postrema and nucleus tract of the solitaries vs. vehicle, whereas Lira and G1437 showed no difference from vehicle in these regions. There was no c-fos activation in the arcuate nucleus in any treatment group. DIO mice treated with MEDI0382 exhibited 11% weight loss ($p < 0.01$ vs. vehicle), whereas pair-fed mice lost a similar weight as MEDI0382 group during the first 5 days, but rebounded such that final weight loss was 3% from baseline by day 10 ($p < 0.05$ vs. vehicle and MEDI0382). Acute administration of exogenous leptin resulted in increased levels of pSTAT3 in the arcuate nucleus of the hypothalamus in MEDI0382-treated but not vehicle or pair-fed mice.

Conclusion: MEDI0382 induced significant metabolic improvement in *ob/ob* mice, superior to Lira without the glycemic liabilities of glucagon. These effects may be mediated via improved peripheral energy utilization in peripheral tissues as well as activation of key central sites that regulate metabolism, including enhanced leptin action.

Disclosure: **D. Baker:** None.

606

ICA6150349, a highly selective glucagon agonist, in combination with exenatide significantly reduces weight and glucose in obese and diabetic rats

M. Paulik[1], T. Tlusty[1], M. Grizzle[1], M. Copeland[1], S. Weng[1], W. Blackwell[1], V. Srivastava[1], J. Way[1], S. Roller[1], D. Zane[2], R. Hodge[1], A. Young[1], P. Feldman[1];
[1]Intarcia Therapeutics Inc, Research Triangle Park, [2]Intarcia Therapeutics Inc, Hayward, USA.

Background and aims: ICA6150349, a 38-amino acid analog of glucagon, is peptidase resistant and highly selective for the human glucagon receptor. Our objectives were to determine the magnitude and timing of effects of 27 days continuous subcutaneous (s.c.) infusion of ICA6150349 alone and in combination with exenatide (GLP-1 agonist) on body weight, body composition, food intake, HbA1c, glucose and serum chemistries in rodent models of obesity and T2D.

Materials and methods: Male Long Evans (LE) Diet Induced Obese (DIO) rats at 18 weeks of age (14 weeks on high fat diet) were s.c. implanted with two Alzet osmotic mini-pumps delivering 7.5, 24 or 50 mcg/kg/d of ICA6150349 with or without exenatide (10 mcg/kg/d;

n = 8/group). Male Zucker Diabetic Fatty (ZDF) rats at 8 weeks of age were similarly implanted with mini-pumps delivering ICA6150349 (15 or 50 mcg/kg/d) with or without exenatide (10 mcg/kg/day; n = 10/group).
Results: ICA6150349 continuously s.c. infused at 50 mcg/kg/d in DIO LE rats reduced weight (21%), fat mass (37%), and food intake (17%) and normalized triglycerides and cholesterol to lean control levels. ICA6150349 (50 mcg/kg/d) in combination with exenatide (10 mcg/kg/d) further significantly reduced weight (38%), fat mass (70%), and food intake (52%) and also normalized glucose and lipids to lean control levels. ICA6150349 continuously infused at 50 mcg/kg/d in ZDF rats significantly increased HbA1c (1.3%), reduced weight (29%), fat mass (42%), and food intake (13%) and normalized triglycerides (66%) and cholesterol (38%) to lean control levels. ICA6150349 (50 mcg/kg/d) in combination with exenatide (10 mcg/kg/d) significantly decreased HbA1c (1.5%), off-setting the increase seen with ICA6150349 monotherapy. The ICA6150349 and exenatide combination significantly reduced weight (19%), fat mass (25%), food intake (29%) cholesterol (27%) and triglycerides (41%).
Conclusion: In rodent models of obesity and T2D, the selective glucagon agonist ICA6150349, in combination with exenatide can significantly reduce weight, fat mass, glucose and lipids, sometimes normalizing these parameters to lean control levels.

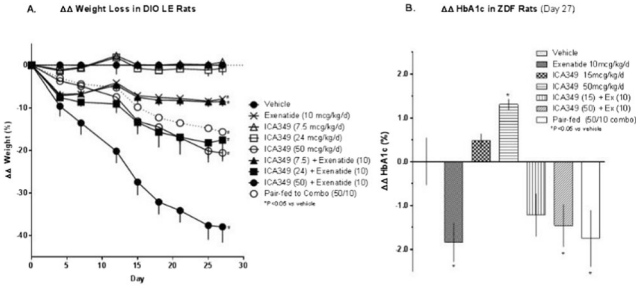


Figure 1. Chronic Effects of ICA6150349 (ICA349) and Exenatide Alone and in Combination on (A) ΔΔ Weight loss in DIO LE Rats and (B) ΔΔ HbA1c % in ZDF Rats

Disclosure: **M. Paulik:** Employment/Consultancy; Intarcia Therapeutics Inc. Stock/Shareholding; Intarcia Therapeutics Inc.

607

ATG7-expression and chemerin secretion are co-regulated in adipocytes

S. Heinitz[1], C. Gebhardt[2], P. Piaggi[3], J. Krüger[2], H. Heyne[4], J. Weiner[5], J. Heiker[5], M. Stumvoll[1], M. Blüher[1], L. Baier[6], A. Rudich[7], P. Kovacs[1], A. Tönjes[1];
[1]Department of Medicine, Division of Endocrinology and Nephrology, University of Leipzig, Leipzig, Germany, [2]IFB Adiposity Diseases, Leipzig University Medical Center, Leipzig, Germany, [3]Obesity and Diabetes Clinical Research Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, USA, [4]Broad Institute, Cambridge, USA, [5]Institute of Biochemistry, University of Leipzig, Leipzig, Germany, [6]Diabetes Molecular Genetics Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, USA, [7]The National Institute for Biotechnology in the Negev Ltd., Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Background and aims: In obese individuals, adipocyte endocrine function is affected by altered autophagy. In a recent genome-wide association study (GWAS) genetic variants in autophagy-related gene 7 (*ATG7*) correlated with serum chemerin (*RARRES2*) concentrations. To investigate a possible interplay between chemerin and ATG7, how it may relate to autophagy-mediated adipocyte dysfunction in obesity, and the functional relevance of genetic variants in *ATG7*.
Materials and methods: Adipose *ATG7*-mRNA expression and adiposity measures were available in 100 Caucasians and 83 Native Americans. The effect of a 12-week high-calorie diet on adipose *RARRES2* and *ATG7*-expression was investigated in mice. In 3T3L1-adipocytes, the effect of *ATG7*-knockdown on chemerin expression and secretion was studied. The influence of single nucleotide polymorphisms (SNPs) in linkage disequilibrium with the tag-SNP from recent GWAS on *ATG7*-transcription and chemerin physiology were investigated using a luciferase assay.
Results: *ATG7*-mRNA expression in human subcutaneous adipose tissue positively correlated with BMI, fat mass, body weight ($r > 0.27$, $P < 0.01$), and measures of adipocyte cell size ($r > 0.42$, $P < 0.02$). In mice fed a high-calorie diet, adipose *ATG7*-expression did not parallel an increase in*RARRES2*-expression. *ATG7*-knockdown in 3T3L1-adipocytes decreased chemerin secretion by 25% ($P < 0.01$; CI 0.6, 0.9). Rs2606729 in *ATG7* was predicted to alter *ATG7*-transcription and induced higher luciferase activity *in vitro* ($P < 0.0001$; CI 2.6, 24.8).
Conclusion: *ATG7*-mRNA expression in human adipose tissue relates to measures of adiposity. *ATG7* regulates chemerin secretion from adipocytes *in vitro* supportive of a functional interplay between *ATG7* and chemerin in autophagy-mediated adipocyte dysfunction.
Clinical Trial Registration Number: NCT00340132
Supported by: FKZ: 01EO1501 (AD2-060E, AD2-6E99), CRC 1052; C01, B01, B02, B03; DHFD, NIH
Disclosure: **S. Heinitz:** None.

608

Plasma lipidomics are associated with cardiometabolic risk factors in overweight or obese non-diabetic adults

B. de Courten[1], A. Mousa[1], N. Naderpoor[1], J. Johnson[1], N. Mellett[2], P. Meikle[2];
[1]Monash University, Melbourne, [2]Baker Heart & Diabetes Institute, Melbourne, Australia.

Background and aims: Dyslipidaemia is a key risk factor for type 2 diabetes and cardiovascular disease. Novel lipidomics methods are providing new insights into the pathophysiology of these diseases. However, human studies are limited and no previous human studies have examined relationships between the plasma lipidome and insulin secretion using gold-standard methods. The aim of this study was to examine whether dysregulation of certain lipid species or classes was associated with reduced insulin sensitivity and/or impaired insulin secretion measured by gold-standard methods, as well as other cardiometabolic risk factors in overweight or obese, non-diabetic adults.
Materials and methods: In 65 adults, we performed lipid profiling of 459 lipid species across 26 lipid classes (liquid chromatography mass spectrometry). Gold-standard methods were used to assess body composition (% body fat, dual X-ray absorptiometry), insulin sensitivity (hyperinsulinaemic-euglycaemic clamps) and insulin secretion (intravenous glucose tolerance tests). Additional measures included anthropometry (BMI, waist-to-hip ratio [WHR]), oral glucose tolerance tests (OGTT) for fasting and 2-hour post-OGTT glucose concentrations, and measurement of serum inflammatory markers including high-sensitivity C-reactive protein (hsCRP by immunoturbidimetry), tumor necrosis factor (TNF), and several interleukins (ILs) and adipokines (multiplex assay; flow cytometry). Multivariable regression was performed with adjustment for age, sex, and % body fat, and all analyses were adjusted for multiple testing using Benjamini-Hochberg correction.
Results: The sample comprised 35 males and 19 females, with a mean age of 31.3 ± 8.5 years and BMI of 31.5 ± 5.2 kg/m^2 (mean ± SD). On univariable analyses, BMI, WHR, fasting glucose, 2-hour post-OGTT glucose, fasting insulin, and insulin sensitivity were not associated with lipid species or classes (all $p > 0.05$). Total % body fat was associated with nine lipid classes; however, after adjustment for age and sex, only an inverse relationship between % body fat and the alkylphosphatidylethanolamine

(PE-plasmalogen) lipid class was observed ($p = 0.01$). Total and second-phase insulin secretion were positively associated with the lysophosphatidylinositol (LPI) lipid class ($\beta = 781.9$ mU/L, $p = 0.01$ and $\beta = 521.3$ mU/L, $p = 0.01$, respectively). After adjustment for age, sex, and % body fat, LPI remained associated with total ($p = 0.02$) and second-phase insulin secretion ($p = 0.01$), and insulin sensitivity became negatively associated with the dihydroceramide (dhCer) lipid class ($p = 0.01$) and several diacylglycerol and triacylglycerol lipid species (all $p < 0.05$). IL-1β was positively associated with the cholesterol ester (CE) lipid class ($\beta = 11.9$ pg/ml, $p = 0.04$) and adiponectin was inversely associated with several diacylglycerol (DG) and triacylglycerol (TG) lipid species (all $p < 0.05$); however these associations were attenuated after adjustment for age, sex, and body fat.

Conclusion: Using gold-standard methods in a well-characterised cohort of overweight or obese non-diabetic adults, we found that decreased PE-plasmalogens and increased dhCer and LPI were associated with greater adiposity, reduced insulin sensitivity, and impaired insulin secretion, respectively. Our findings suggest that these lipid classes may be involved in the pathophysiology of type 2 diabetes.

Disclosure: **B. de Courten:** None.

PS 044 Are SGLT2 inhibitors effective and safe in type 1 diabetes?

609

The inTandem1 study: 52-week efficacy and safety of sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, as adjunct therapy to insulin in adults with type 1 diabetes

S.K. Garg[1], J. Buse[2], J. Rosenstock[3], T. Bailey[4], P. Banks[5], B.W. Bode[6], T. Danne[7], J.A. Kushner[8], W.S. Lane[9], P. Lapuerta[5], D.K. McGuire[10], A. Peters[11], J. Reed[12], S. Sawhney[5], P. Strumph[5];
[1]Department of Medicine and Pediatrics, University of Colorado Denver, Aurora, USA, [2]Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, USA, [3]Dallas Diabetes Research Center at Medical City, Dallas, USA, [4]AMCR Institute, Escondido, USA, [5]Lexicon Pharmaceuticals, Inc., The Woodlands, USA, [6]Emory University School of Medicine, Atlanta, USA, [7]Department of Diabetes, Endocrinology, and Clinical Research, Hannover Medical School, Hannover, Germany, [8]Baylor College of Medicine and Texas Children's Hospital, Houston, USA, [9]Mountain Diabetes and Endocrine Center, Asheville, USA, [10]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, USA, [11]Keck School of Medicine of the University of Southern California, Los Angeles, USA, [12]Endocine Research Solutions, Inc., Roswell, USA.

Background and aims: Sotagliflozin (SOTA), a dual SGLT1 and SGLT2 inhibitor, is currently in development as an oral adjunct to insulin in type 1 diabetes (T1D).

Materials and methods: In this double-blind, 52-week North American trial, 793 adults with T1D treated with multiple daily insulin injections (40%) or pump (60%) were randomized 1:1:1 to placebo ($n = 268$), SOTA 200 mg ($n = 263$) or SOTA 400 mg ($n = 262$) once daily after 6 weeks of insulin optimization. Primary endpoint was change from baseline in A1C at Week 24. Other endpoints included A1C, body weight, bolus insulin dose and FPG changes at Week 52, patient (pt) reported outcomes and net clinical benefit (NCB), assessing the proportion of pts with A1C <7.0% without severe hypoglycemia (SH) or diabetic ketoacidosis (DKA).

Results: Baseline characteristics were similar between groups. Compared with placebo, SOTA 200 and 400 mg improved A1C and pt satisfaction at Week 24 and reduced A1C, weight, bolus insulin, FPG and pt distress at Week 52. More pts achieved NCB with SOTA vs placebo (each $P < 0.05$; Table). The highest incidence of SH was in the placebo arm; more genital mycotic infections, diarrhea and DKA events (> patients using a pump than MDI) were in the SOTA arms (Table).

Conclusion: SOTA 200 and 400 mg provided statistically significant A1C reductions that were sustained ($P < 0.05$) at Week 52. SOTA was associated with an increased (but low) rate of DKA, and a lower incidence of SH, when compared with placebo.

	Placebo n=268	SOTA 200 mg n=263	SOTA 400 mg n=262
Mean A1C at Baseline, after 6-week insulin optimization, %	7.54	7.61	7.56
Outcomes at Week 24			
A1C LSM diff from placebo, % ± SE (P-value)	-	−0.36±0.05(<0.001)	−0.41±0.05 (<0.001)
Outcomes at Week 52			
A1C LSM diff from placebo, % ± SE (P-value)	-	−0.25±0.06 (P<0.001)	−0.31±0.06 (P<0.001)
FPG LSM diff from placebo, mmol/L ± SE	-	−0.68±0.31 (P=0.028)	−1.08±0.31 (P<0.001)
Body weight LSM diff from placebo, kg ± SE (P-value)	-	−3.14±0.34 (P<0.001)	−4.32±0.35 (P<0.001)
Mean daily bolus insulin dose at Baseline, IU	31.7	30.3	30.8
Bolus insulin dose mean change from Baseline, % ± SE	7.01±3.40	−1.48±3.40	−3.63±3.42
Bolus insulin dose LSM diff from placebo, % ± SE (P-value)	-	−5.53±4.59 (P=0.23)	−15.83±4.60 (P<0.001)
Net clinical benefit at Week 52			
A1C <7.0% without SH and without DKA, n (%)	51 (19.0)	69 (26.2)	85 (32.4)
Safety outcomes over 52 weeks			
Any TEAE, n (%)	216 (80.6)	215 (81.7)	209 (79.8)
TEAEs leading to study discontinuation, n (%)	11 (4.1)	13 (4.9)	17 (6.5)
Treatment-emergent serious adverse events, n (%)	20 (7.5)	27 (10.3)	29 (11.1)
Death, n (%)	1 (0.4)	0	0
DKA, n (%)[1]	1 (0.4)	9 (3.4)	11 (4.2)
Severe hypoglycemia, n (%)[1]	26 (9.7)	17 (6.5)	17 (6.5)
Diarrhea, n (%)	18 (6.7)	22 (8.4)	27 (10.3)
Genital mycotic infection, n (%)	9 (3.4)	24 (9.1)	34 (13.0)
Patient-reported outcomes			
DTSQ score LSM diff from placebo at Week 24 ± SE (P-value)	-	2.5±0.40 (P<0.001)	2.5±0.40 (P<0.001)
DDS2 score LSM diff from placebo at Week 52 ± SE (P-value)	-	−0.4±0.15 (P=0.003)	−0.5±0.15 (P<0.001)

DDS2, two-item Diabetes Distress Screening Scale; DKA, diabetic ketoacidosis; DTSQ, diabetes treatment satisfaction questionnaire; LSM diff, least squares mean; mITT, modified intent-to-treat; SD, standard deviation; SE, standard error; SH, severe hypoglycemia; SOTA, sotagliflozin; TEAE, treatment emergent adverse events. [1]Positively-adjudicated events.

Clinical Trial Registration Number: NCT02384941
Supported by: Lexicon Pharmaceuticals, Inc/Sanofi

Disclosure: **S.K. Garg:** Grants; Grant support paid to the Barabara Davis Center for Diabetes, U Colorado Denver from Eli Lilly, Dexcom, Sanofi, Dario, NCI, T1D Exchange, NIDDK, JDRF, Animas, Lexicon, Novo Nordisk, Medtronic.

610

inTandem1 and inTandem2: increased time in range with sotagliflozin as adjunct therapy to insulin in adults with type 1 diabetes by 24-week continuous glucose monitoring

T. Danne[1], B. Cariou[2], J.B. Buse[3], S.K. Garg[4], J. Rosenstock[5], P. Banks[6], J.A. Kushner[7], D.K. McGuire[8], A.L. Peters[9], S. Sawhney[6], P. Strumph[6];
[1]Department of Diabetes, Endocrinology, and Clinical Research, Hannover Medical School, Hannover, Germany, [2]Department of Endocrinology, CHU Nantes, Nantes, France, [3]Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, USA, [4]Department of Medicine and Pediatrics, University of Colorado Denver, Aurora, USA, [5]Dallas Diabetes Research Center at Medical City, Dallas, USA, [6]Lexicon Pharmaceuticals, Inc., The Woodlands, USA, [7]Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, USA, [8]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, USA, [9]Keck School of Medicine of the University of Southern California, Los Angeles, USA.

Background and aims: Sotagliflozin (SOTA), a dual SGLT1 and SGLT2 inhibitor, is currently in development as an oral adjunct to insulin in type 1 diabetes (T1D).
Materials and methods: This was a pooled analysis of the inTandem1 and inTandem2 trials in adults with T1D treated with multiple daily insulin injections or pump therapy who were randomized 1:1:1 to placebo, SOTA 200 mg or SOTA 400 mg once daily after 6 weeks of insulin optimization. Of these, 278 patients participated in a blinded continuous glucose monitoring (CGM) sub-study. The primary endpoint of this sub-study was the percentage of time in the target glucose range (70 mg/dL–180 mg/dL). Mean daily glucose and amplitude of all glycemic excursions (MAGE) of <70 and >180 mg/dL were also assessed.
Results: Baseline characteristics were similar among groups. Compared with placebo, treatment with SOTA 200 and 400 mg significantly increased the time in range (+1.3 and +2.8 hours/day, respectively) and reduced MAGE at Week 24, while SOTA 400 mg also significantly reduced mean daily glucose (Table).
Conclusion: SOTA used as adjunct therapy in T1D, provided glycemic control beyond decreasing A1C as seen by improvements in the time in target glucose range compared with placebo.

	Optimized insulin + placebo n=93	Optimized insulin + SOTA 200 mg n=89	Optimized insulin + SOTA 400 mg n=96
Percentage of readings in the range of 70–180 mg/dL (3.9–10.0 mmol/L)			
Baseline mean ± SD	52 ± 14	52 ± 15	51 ± 15
Week 24 mean ± SD	52 ± 15	58 ± 16	64 ± 14
LSM change from baseline ± SE	−1.3 ± 1.8	+4.1 ± 1.8	+10.5 ± 1.7
Mean change from baseline hr/day*	−0.3 hr/day	+1.0 hr/day	+2.5 hr/day
LSM diff from placebo (95% CI)	NA	+5.4 (+0.6, +10.1)	+11.7 (+7.1, +16.3)
P-value	NA	0.026	<0.001
Mean difference from placebo hr/day*	NA	+1.3 hr/day	+2.8 hr/day
Mean daily glucose (mg/dL)			
Baseline mean ± SD	175 ± 31	176 ± 33	178 ± 32
Week 24 mean ± SD	176 ± 32	167 ± 32	156 ± 23
LSM change from baseline ± SE	+2.0 ± 3.6	−5.9 ± 3.6	−16.9 ± 3.4
LSM diff from placebo (95% CI)	NA	−7.9 (−17.2, +1.3)	−18.9 (−27.9, −9.9)
P-value	NA	0.09	<0.001
MAGE mg/dL			
Baseline mean ± SD	166 ± 35	163 ± 34	158 ± 36
Week 24 mean ± SD	159 ± 32	146 ± 38	131 ± 33
LSM change from baseline ± SE	−3.0 ± 4.2	−15.7 ± 4.2	−25.1 ± 3.9
LSM diff from placebo (95% CI)	NA	−12.7 (−23.6, −1.8)	−22.1 (−32.7, −11.5)
P-value	NA	0.022	<0.001

CGM, continuous glucose monitoring; CI, confidence interval; LSM diff, least squares mean difference, MAGE; mean amplitude of glucose excursion, NA; not applicable; SD, standard deviation; SE, standard error. Statistical comparisons of each SOTA arm with placebo were pre-planned and performed using a generalized linear model with repeated measures statistics.
*Assuming 100% daily CGM data available for analysis, 1.0% of daily CGM time = 0.24 hours.

Clinical Trial Registration Number: NCT02384941/NCT02421510
Supported by: Lexicon Pharmaceuticals, Inc/Sanofi

Disclosure: **T. Danne:** Employment/Consultancy; Consultant, advisory board member, steering committee member, or speaker for Abbott, Medtronic, Roche, Lexicon, Menarini, Boehringer Ingelheim, AstraZeneca, Novo Nordisk, Sanofi, Dexcom, and Eli Lilly. Grants; Received research grants from Abbott, AstraZeneca, Novo Nordisk, Medtronic, and Sanofi.

611

Sotagliflozin in combination with optimised insulin therapy reduced HbA_{1c} levels with a decreased daily insulin requirement after 52 weeks in adults with type 1 diabetes

J. Pettus[1], S. Weinzimer[2], R. McCrimmon[3], F. Ampudia-Blasco[4], J. Stewart[5], P. Strumph[6], J. Oliveira[7], P. Lapuerta[8], R. Castro[7];
[1]University of California, San Diego, San Diego, USA, [2]Yale School of Medicine, New Haven, USA, [3]University of Dundee, Dundee, UK, [4]Hospital Clínico Universitario de Valencia, Valencia, Spain, [5]Sanofi, Westmount, Canada, [6]Lexicon Pharmaceuticals, Woodlands, USA, [7]Sanofi, Bridgewater, USA, [8]Lexicon Pharmaceuticals, Princeton, USA.

Background and aims: In most patients with T1D, adequate glycemic control is not achieved with insulin therapy alone, and intensifying insulin therapy can increase the risk of hypoglycemia. Sotagliflozin (SOTA) is a dual SGLT1 & SGLT2 inhibitor, which blunts and delays postprandial hyperglycemia and reduces renal glucose reabsorption. The goal of this analysis was to determine the effect of SOTA on HbA1c levels and insulin requirements in patients with T1D.
Materials and methods: In two 52 weeks phase 3 studies adults with T1D were randomized 1:1:1 to placebo + insulin (PBO), SOTA 200 mg + insulin or SOTA 400 mg + insulin, once daily, after 6 weeks of insulin therapy optimization. HbA1c change over 52 weeks, along with change of daily insulin dose and safety, were assessed using a pooled analysis. Statistical comparisons of each SOTA arm to placebo were preplanned and performed using a generalized linear model with repeated measures statistics. Proportion of insulin reduction attributable to bolus or basal insulin was calculated by LS Mean of absolute bolus or basal insulin dose change (IU) from baseline over bolus + basal absolute dose change from baseline (IU). Number of patients on which the raw means and LS means are not the same for each type of insulin.
Results: Significant HbA1c reductions were observed with SOTA 200 or 400 vs. PBO at week 24 and sustained to 52 weeks, with a concomitant decrease in total daily insulin dose, mainly due to reduction in bolus insulin (BI), as 70–80% of the total insulin reduction was from BI for both SOTA doses. At 52 weeks, patients in SOTA groups had a lower incidence of severe hypo (SH) but more genital mycotic infections, DKA and diarrhea than PBO.
Conclusion: SOTA demonstrated additional and sustained reduction of HbA1c on top of optimized insulin while reducing total daily insulin dose (mainly BI doses), with less incidence of SH. This may be an additional therapy for patients with T1D without good glycemic control despite optimized insulin therapy.

	Placebo n = 526		SOTA 200 mg n = 524			SOTA 400 mg n = 525		
HbA1c at baseline (%) Mean (SD)	7.89 (0.01)		7.88 (0.77)			7.84 (0.78)		
Outcomes at Week 24 HbA1c LSM difference from PBO, % (p-value)	N/A		0.36 (p=0.001)			0.56 (p<0.001)		
Outcomes at Week 52 HbA1c LSM difference from PBO, % (p-value)	N/A		0.23 (p=0.001)			0.32 (p<0.001)		
	Change from baseline		Change from baseline		vs. PBO LSM (SE)	Change from baseline		vs. PBO
Total insulin	%	IU	%	IU	%	%	IU	LSM (SE) %
LSM (SE), p-value	2.12 (0.999), p: 0.028	0.10 (0.05?), p: 0.70	-4.99 (0.994), p=0.001	-3.01 (0.994), p<0.001	-7.10 (1.301), p<0.001	-8.21 (0.998), p<0.001	-5.86 (0.994), p<0.001	-10.33 (1.303), p<0.001
Bolus insulin								
LSM (SE), p-value	5.11 (2.330), p: 0.028	1.45 (0.511), p: 0.004	-1.48 (2.332), p=0.52	-3.06 (0.508), p<0.001	-6.63 (3.182), p=0.037	0.59 (2.330), p=0.001	-4.26 (0.510), p<0.001	-13.73 (3.187), p<0.001
Basal insulin								
LSM (SE), p-value	-4.79 (1.137), p<0.001	1.56 (0.337), p<0.001	-2.36 (1.139), p: 0.038	0.94 (0.338), p=0.066	-7.11 (1.554), p<0.001	-4.50 (1.136), p<0.001	-1.72 (0.338), p<0.001	-9.25 (1.554), p<0.001
Proportion of insulin reduction attributable to bolus insulin%	N/A		82.8			71.3		
Safety outcomes over 52 wk								
Any TEAE, n (%)	374 (71.1)		390 (75.0)			390 (74.3)		
DKA,** n (%)	1 (0.2)		15 (2.9)			20 (3.8)		
Severe hypoglycemia,* n (%)	36 (7.4)		30 (5.7)			23 (4.4)		
Diarrhea,^ n (%)	27 (5.1)		34 (6.5)			46 (8.8)		
Genital mycotic infection, n (%)	15 (2.9)		48 (9.2)			63 (12.0)		

DKA, diabetic ketoacidosis; IU, international units; LSM (sd) (upon) mean; SD, standard deviation; SE, standard error; TEAE, treatment emergent adverse event. *Adjudicated event of special interest. **DKA cases were adjudicated as 'yes with certainty', 'yes, probably', 'no with certainty', 'unclear/labile' and 'insufficient data'.^Positively adjudicated cases were classified as either 'with certainty' or 'probably'. ^Discontinuation of drug due to diarrhea was: 0.0% placebo; 0.4% SOTA 200 mg; and 0.8% SOTA 400 mg.

Clinical Trial Registration Number: NCT02384941; NCT02421510
Disclosure: **J. Pettus:** Employment/Consultancy; Sanofi, Novo Nordisk Inc, Valeritas, Inc, Insulet Corporation, MannKind Corporation, Senseonics, Dexcom, Inc. Lecture/other fees; Sanofi, Valeritas, Inc.

612

Pooled data analysis of composite endpoints from the DEPICT-1 and DEPICT-2 studies using dapagliflozin compared to placebo added to adjustable insulin in type 1 diabetes

P. Dandona[1], F. Thorén[2], A.M. Langkilde[2], L. Hansen[3], J. Xu[4], C. Mathieu[5];
[1]State University of New York at Buffalo, Buffalo, USA, [2]AstraZeneca, Mölndal, Sweden, [3]MedImmune, Gaithersburg, USA, [4]AstraZeneca, Gaithersburg, USA, [5]University of Leuven, Leuven, Belgium.

Background and aims: Treatment with insulin (INS) is the mainstay of therapy for patients with type 1 diabetes (T1D). Besides INS, adjunct therapy with INS-independent antihyperglycaemic agents has been proposed for adequate glycaemic control in T1D patients. In this sub-analysis of pooled data from the DEPICT 1 and DEPICT 2 studies, we evaluated the effect of dapagliflozin (DAPA), a selective sodium glucose cotransporter-2 inhibitor, in T1D patients on adjustable INS treatment on two composite endpoints: (a) proportion of patients with a reduction in HbA1c of ≥0.5% from baseline to week 24 with no weight gain and; (b) proportion of patients with a reduction in HbA1c of ≥0.5% from baseline to week 24 with no severe hypoglycaemia and no diabetic ketoacidosis (DKA).
Materials and methods: T1D patients (n = 1591) with inadequate glycaemic control (HbA1c ≥7.7% and ≤11.0%) on INS for ≥12 months with a total INS dose of ≥0.3 U/kg/day for ≥3 months prior to screening, a BMI of ≥18.5 kg/m^2 and C-peptide <0.7 ng/mL, were randomised in a 1:1:1 ratio to receive either DAPA 5 mg/day plus INS (n = 530) or DAPA 10 mg/day plus INS (n = 529) or placebo (PBO) plus INS (n = 532). Logistic regression, with adjustment for study, baseline HbA1c and randomisation stratification factor, was performed to evaluate the two composite endpoints for patients on 5 mg DAPA/INS, 10 mg DAPA/INS, and PBO/INS.
Results: At baseline, patients included in this sub-analysis (n = 1591) had a mean (SD) age of 42.6 (13.6) years, BMI of 28.0 (5.4) kg/m^2, duration of T1D of 19.8 (11.8) years and HbA1c of 8.5% (0.7%). Overall, demographics and baseline characteristics of patients were comparable across study groups. More patients in the 5 mg DAPA/INS (38.5%) and 10 mg DAPA/INS (42.4%) groups achieved a reduction in HbA1c of ≥0.5%, without weight gain, than in the PBO/INS group (10.8%). A larger proportion of patients on 5 mg DAPA/INS and10 mg DAPA/INS versus those on PBO/INS showed a reduction in HbA1c of ≥0.5%, without hypoglycaemia or DKA (43.9% and 45.3% vs. 22.1%, respectively). The ORs of patients achieving both composite endpoints were greater in the DAPA/INS groups versus the PBO/INS group (Table).
Conclusion: DAPA as an adjunct therapy to INS may facilitate improvement in glycaemic control with less risk of weight gain and with more patients achieving improved glycaemic control without severe hypoglycaemia or DKA, in patients with inadequately controlled T1D treated with adjustable INS.

Table: Composite endpoint analysis

	5 mg DAPA/INS (n=530)	10 mg DAPA/INS (n=529)	PBO/INS (n=532)
HbA1c reduction ≥0.5% + no body weight gain (baseline to week 24)			
Number of patients	522	521	526
Patients achieving composite endpoint, n (%)	201 (38.5)	221 (42.4)	57 (10.8)
Odds ratio vs. PBO/INS (95% CI)	5.4 (3.9, 7.6)	6.5 (4.7, 9.1)	-
HbA1c reduction ≥0.5% + no severe hypoglycaemia or DKA (baseline to week 24)			
Number of patients	522	521	526
Patients achieving composite endpoint, n (%)	229 (43.9)	236 (45.3)	116 (22.1)
Odds ratio vs. PBO/INS (95% CI)	2.9 (2.2, 3.9)	3.2 (2.4, 4.2)	-

n (%) is the number of patients in the full analysis dataset with non-missing baseline and week 24 (LOCF) values.
Weight (kg) gain is defined as that weight at week 24 (LOCF), which is larger than baseline weight.
DAPA/INS, dapagliflozin plus insulin; DKA, diabetic ketoacidosis; PBO/INS, placebo plus insulin.

Clinical Trial Registration Number: NCT02460978 and NCT02268214
Supported by: Funding for this analysis was supported by AstraZeneca Pharmaceuticals LP.
Disclosure: **P. Dandona:** Employment/Consultancy; AbbVie, AstraZeneca, Boehringer Ingelheim, Merck, Intarcia, Novo Nordisk, Sanofi. Grants; AbbVie, AstraZeneca, Boehringer Ingelheim, Merck, Novo Nordisk, Sanofi.

613

Pooled analysis of the duration of type 1 diabetes in dapagliflozin vs placebo on adjustable insulin therapy from DEPICT 1 and 2: effects on glycaemia, weight and insulin dosage

J. Lüdemann[1], T. Schaum[2], C. Mathieu[3], J. Xu[4], F. Thorén[5];
[1]Diabetes-Falkensee, Diabetes Centre and Centre for Clinical Studies, Falkensee, Germany, [2]Sana Kliniken Ostholstein GmbH, Oldenburg In Holstein, Germany, [3]University of Leuven, Leuven, Belgium, [4]AstraZeneca, Gaithersburg, USA, [5]AstraZeneca, Mölndal, Sweden.

Background and aims: In Type 1 diabetes (T1D), add-on therapy with insulin (INS)-independent agents is likely to enhance glycaemic control compared to INS monotherapy. In DEPICT 1 and 2 (double-blind, Phase 3) studies, improvement in glycaemic control, weight loss and reduction in INS dose were reported for dapagliflozin (DAPA) vs. placebo (PBO), without increases in hypoglycaemia. In this subgroup analysis, pooled data from DEPICT 1 and 2, were analysed on T1D duration on the effect of DAPA.
Materials and methods: T1D pts (n = 1591; aged 18–75y; HbA_{1c} ≥7.7% and ≤11.0%; BMI ≥18.5 kg/m^2; C-peptide <0.7 ng/mL; prescribed INS for ≥12 months [m] before enrolment with a total INS dose of ≥0.3 IU/kg/day for ≥3 m prior to screening) were randomised (1:1:1) to add DAPA 5 mg (n = 530) or DAPA 10 mg (n = 529) or PBO (n = 532) to INS. Endpoints were adjusted mean change in HbA_{1c}, percent changes in total daily INS dose and total body weight; 24-hour mean glucose readings and amplitude of glycaemic excursion (MAGE), the proportions of pts maintaining glucose within 70–180 mg/dL using continuous glucose monitoring, and HbA_{1c} reduction ≥0.5% without severe hypoglycaemia from baseline to Week 24. Endpoints were analysed by T1D duration tertiles (<12.9, ≥12.9–≤23.5 and ≥23.5y) for each treatment arm. No formal statistical testing was done.
Results: Pts with the longest T1D duration tended to have slightly lower HbA_{1c} reductions and greater weight loss (**Table**). No noticeable changes in HbA_{1c} reduction or weight loss were observed in pts on PBO in any of the three age groups (**Table**). When plotted as continuous variables, the correlation coefficients between change in HbA_{1c} vs. T1D duration for DAPA 5 mg, DAPA 10 mg and PBO were 0.05635, 0.11214 and −0.09277, respectively. No trends were evident for INS dose, or the other analysed parameters with respect to duration of T1D.
Conclusion: Pts with T1D on DAPA added on to INS with longest T1D duration demonstrated relatively lower reductions in HbA_{1c}; however, were able to achieve greatest weight loss.

Table: Changes in major endpoints from baseline to week 24

	<12.9 years Baseline mean age 37.6 years (SD 13.99)			≥12.9-≤23.5 years Baseline mean age 40.1 years (SD 12.88)			>23.5 years Baseline mean age 50.0 years (SD 10.40)		
	Baseline, mean (SD)	24 weeks, mean (SD)	Adj. mean change from baseline at week 24 (95% CI)	Baseline, mean (SD)	24 weeks, mean (SD)	Adj. mean change from baseline at week 24 (95% CI)	Baseline, mean (SD)	24 weeks, mean (SD)	Adj. mean change from baseline at week 24 (95% CI)
HbA_{1c} (%)									
PBO*	8.54 (0.65)	8.63 (0.94)	0.13 (0.01, 0.24)	8.50 (0.66)	8.39 (0.86)	-0.05 (-0.16, 0.07)	8.38 (0.66)	8.29 (0.85)	-0.08 (-0.19, 0.02)
DAPA 5 mg**	8.62 (0.73)	8.09 (0.94)	-0.46 (-0.57, -0.35)	8.48 (0.71)	8.13 (0.84)	-0.34 (-0.46, -0.23)	8.36 (0.64)	7.96 (0.78)	-0.40 (-0.51, -0.29)
DAPA 10 mg***	8.54 (0.72)	8.01 (0.86)	-0.51 (-0.62, -0.40)	8.51 (0.63)	8.02 (0.78)	-0.43 (-0.54, -0.32)	8.35 (0.61)	8.00 (0.75)	-0.36 (-0.47, -0.25)
Total body weight (kg)									
PBO*	76.09 (16.05)	76.40 (16.59)	0.19 (-0.47, 0.85)	82.84 (20.45)	82.56 (21.27)	-0.39 (-1.02, 0.25)	85.31 (18.39)	85.80 (18.38)	0.10 (-0.51, 0.71)
DAPA 5 mg**	77.39 (16.97)	75.80 (17.01)	-2.92 (-3.53, -2.31)	80.25 (17.26)	78.46 (16.89)	-3.00 (-3.60, -2.39)	82.29 (19.28)	79.87 (18.89)	-3.35 (-3.96, -2.73)
DAPA 10 mg***	77.84 (18.10)	75.42 (16.95)	-2.98 (-3.58, -2.37)	82.00 (17.93)	78.70 (17.55)	-3.87 (-4.49, -3.25)	83.29 (17.35)	79.93 (17.28)	-4.30 (-4.89, -3.71)

*n=521; **n=521; ***n=524. DAPA, dapagliflozin; PBO, placebo; pts, patients.

Clinical Trial Registration Number: NCT02268214 (DEPICT 1) and NCT02460978 (DEPICT 2)
Supported by: The study was funded by AstraZeneca Pharmaceuticals LP.
Disclosure: **J. Lüdemann:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Sanofi. Grants; Bristol-Myers Squibb, Gan and Lee Pharmaceuticals, Eli Lilly, Novo Nordisk, Sanofi.

614

Sotagliflozin further improves percentage of patients achieving HbA_{1c} goal without weight gain in adults with type 1 diabetes after insulin therapy optimisation

R. Henry[1], H. Rodbard[2], A. Giaccari[3], R. Lajara[4], J. Stewart[5], P. Strumph[6], J. Oliveira[7], P. Lapuerta[8], R. Castro[7];
[1]UC San Diego, San Diego, USA, [2]Endocrine & Metabolic Consultants, Rockville, USA, [3]Fondazione Policlinico A. Gemelli, Rome, Italy, [4]DCoA - DFW, Plano, USA, [5]Sanofi, Westmount, USA, [6]Lexicon, Woodlands, USA, [7]Sanofi, Bridgewater, USA, [8]Lexicon, Princeton, USA.

Background and aims: Data indicate that more than 50% of adults with T1D are overweight or obese. Although intensive insulin therapy improves glycemic control and reduces the incidence and progression of diabetes-related complications, it is also associated with potential undesirable effects, including increased risk of hypoglycemia and notably weight gain. Sotagliflozin (SOTA) is a dual SGLT1 and SGLT2 inhibitor, which blunts and delays postprandial hyperglycemia while also reducing renal glucose reabsorption. The goal of this analysis was to determine if SOTA, combined with insulin, could significantly increase the percentage of adults with T1D who achieve HbA1c goals without weight gain.
Materials and methods: In two 52-week phase 3 studies, adults with T1D were randomized 1:1:1 to placebo + insulin (PBO), SOTA 200 mg + insulin, or SOTA 400 mg + insulin, once daily after 6 weeks of insulin therapy optimization. Statistical comparisons of each SOTA arm to PBO were preplanned and performed using a generalized linear model with repeated measures statistics. A pooled data analysis was conducted using an integrated dataset of 1,575 patients (mean BMI: 28.72 kg/m^2 [s.d. 5.28]) to evaluate the percentage of patients achieving HbA1c target of <7% and that had reductions of HbA1c from baseline of ≥0.5%, change of body weight, and safety.
Results: Significant HbA1c reductions were observed with SOTA 200 mg or 400 mg vs. PBO at week 24 and still statistically significant at 52 weeks. Analyses of change from baseline showed significant reduction in weight (kg) at 24 weeks with SOTA vs. PBO (200 mg: −2.17 kg; 400 mg: −3.02 kg) and sustained at 52 weeks (200 mg: −2.67 kg; 400 mg: −3.64 kg), $p < 0.001$, in patients receiving SOTA + insulin vs. PBO. A significantly greater percentage of patients achieved HbA1c reduction of <7% with no weight gain ($p < 0.001$) at both SOTA doses vs. PBO at weeks 24 and 52. Similar results were obtained for patients who achieved HbA1c reductions of ≥0.5% at 24 and 52 weeks ($p < 0.001$). At 52 weeks, patients in both SOTA groups had a lower incidence of severe hypoglycemia (SH) but higher incidence of genital mycotic infections, DKA and diarrhea vs. PBO.
Conclusion: SOTA added to optimized insulin demonstrated significant and sustained reduction of HbA1c and body weight, with a greater percentage of patients reaching their HbA1c goals, without weight gain and with fewer events of SH. More patients on SOTA experienced DKA, genital mycotic infections and diarrhea vs. PBO. In conclusion, for T1D with suboptimal control, adding SOTA to optimized insulin therapy offers a treatment option that improves glycemic control with no increase in body weight and less severe hypoglycemia.

	PBO n = 526	SOTA 200 mg n = 524	SOTA 400 mg n = 525
HbA1c (%) Mean (SD)	7.66 (0.81)	7.68 (0.77)	7.64 (0.78)
Weight (kg) Mean (SD)	84.25 (17.56)	84.46 (18.13)	84.23 (18.11)
HbA1c change from Baseline			
Week 24 Treatment Comparison vs. PBO			
LS Mean (SE), %, *p*	N/A	−0.36 (0.037), *p*<0.001	−0.38 (0.037), *p*<0.001
Week 52 Treatment Comparison vs. PBO			
LS Mean (SE), %, *p*	N/A	−0.23 (0.046), *p*<0.001	−0.32 (0.046), *p*<0.001
Weight (kg), % change at Week 24			
Vs Baseline LS Mean (SE), *p*	0.54 (0.161), *p*<0.001	−2.11 (0.161), *p*<0.001	−3.02 (0.161), *p*<0.001
Treatment Comparison vs. PBO LS Mean (SE), *p*	N/A	−2.64 (0.222), p<0.001	−3.56 (0.222), *p*<0.001
Weight (kg), % change at Week 52			
Vs Baseline LS Mean (SE), *p*	0.91 (0.209), *p*<0.001	−2.31 (0.208), *p*<0.001	−3.33 (0.209), *p*<0.001
Treatment Comparison vs. PBO LS Mean (SE), *p*	N/A	−3.21 (0.291), *p*<0.001	−4.24 (0.291), *p*<0.001
No Weight Gain at Week 52 (SOTA vs. PBO)			
Percentage HbA1c <7.0%	9.1	21.8	26.1
Absolute difference in %, *p* [a]	N/A	12.6, *p*<0.001	17.0, *p*<0.001
Percentage with absolute reduction in HbA1c ≥0.5%	8.8	23.7	28.8
Absolute difference in %, *p* [a]	N/A	14.9, *p*<0.001	20.0, *p*<0.001
Safety outcomes over 52 weeks			
Any TEAE, n (%)	374 (71.1)	393 (75.0)	390 (74.3)
DKA, [b, c] n (%)	1 (0.2)	15 (2.9)	20 (3.8)
Severe hypoglycemia, [b] n (%)	39 (7.4)	30 (5.7)	23 (4.4)
Diarrhea, [d] n (%)	27 (5.1)	34 (6.5)	46 (8.8)
Genital mycotic infection, n (%)	15 (2.9)	48 (9.2)	63 (12.0)

DKA, diabetic ketoacidosis; LSM, least square mean; N/A, Not applicable; SD, standard deviation; SE, standard error; SH, severe hypoglycemia; TEAE, Treatment-emergent adverse event. [a] *p*-value based on Wald test. [b] Number of patients with ≥1 positively adjudicated event. [c] DKA cases were as "yes with certainty," "yes, probably," "no, unlikely," "no with certainty," "unclassifiable," and "insufficient data."
Positively adjudicated cases were classified as either "with certainty" or "probably." [d] Discontinuation of drug due to diarrhea was: 0.4% PBO, 0.4% SOTA 200 mg, and 0.8% SOTA 400 mg.

Clinical Trial Registration Number: NCT02384941; NCT02421510
Disclosure: **R. Henry:** Employment/Consultancy; Abbott, AstraZeneca, Boehringer Ingelheim, Elcelyx, Intarcia, Ionis, Ligand, Merck, Sanofi-Aventis. Lecture/other fees; Servier. Other; AstraReal, Eli Lilly, Hitachi, Viacyte, Lexicon Pharmaceuticals.

615

Patients with type 1 diabetes value increased glucose stability and associate it with improved well-being: exit interviews from Sotagliflozin Phase 3 study

P. Lapuerta[1], V.N. Joish[1], C. Ervin[2], E. Evans[2], M. Reaney[3], R. Preblick[3], B. De Fanti[3], R. Castro[3], D. DiBenedetti[2];
[1]Lexicon Pharmaceuticals, Inc., The Woodlands, [2]RTI Health Solutions, Research Triangle Park, [3]Sanofi, Bridgewater, USA.

Background and aims: Sotagliflozin (SOTA) is a dual SGLT1 and SGLT2 inhibitor in Phase 3 development for type 2 and as adjunct to insulin in type 1 diabetes (T1D). In a Phase 3 study (inTandem 1) after 24 weeks, SOTA 200 and 400 mg adjunctive to insulin resulted in higher glycemic control versus insulin alone (plus placebo). A qualitative study was conducted with inTandem 1 participants to better understand experiences with T1D before and during the study.
Materials and methods: All participants who completed or discontinued early from US and Canadian clinical sites, within the past 12 months, were invited to participate in an individual phone interview. Trial sites referred participants sequentially, starting with those who exited the study most recently. All parties were blinded to treatment assignment. Open-ended questions were posed to ascertain a full understanding of the participants' experiences both before and during the trial, including their experience related to trial-related ketone monitoring. Follow-up probes addressed treatment satisfaction and the importance and impact of reported treatment benefits. Importance of symptom improvement was rated on a 5-point scale ranging from "Not at all important" to "Extremely important". Thematic methods were used to analyze the interview transcripts and descriptive statistics were computed for quantitative data. All study procedures were approved by central Institutional Review Boards.
Results: Results were pooled across treatment arms. Thirty-two participants with characteristics representative of the overall study population, completed a phone interview. All participants reported challenges in achieving glycemic control prior to the trial, characterized by frequent "high" ($n = 31$) and "low" ($n = 24$) blood sugar events, included high HbA_{1c} levels ($n = 29$), lack of glucose stability ($n = 27$), and increased insulin use ($n = 18$). Participants reported feelings of stress/worry about T1D ($n = 21$), low energy levels that reduced productivity/physical activity ($n = 20$), and negative impacts on mood including feeling depressed and/or irritable ($n = 21$). Participants who reported greater glucose instability generally reported greater negative impacts across various areas of their lives. Twenty-six participants (81%) reported at least 1 improvement

during the trial, including reduction in the frequency of hyperglycemic ($n = 26$) and hypoglycemic ($n = 14$) events, greater glucose stability and reduced insulin use (both $n = 23$), and lower HbA_{1c} levels ($n = 21$). Improvements most frequently reported as "very important" or "extremely important" included reductions inHbA_{1c} levels (95%) and greater glucose stability (91%). Improvements in T1D symptoms were associated with feeling happier, less stressed/worried, and more in control of T1D. Participants also noted increased energy and improved quality of life related to improved glucose stability. The ketone monitoring requirement was accepted by participants and was not noted as burdensome. No new safety signals were identified.

Conclusion: In this Phase 3 study, interview participants consistently reported that improvement in glycemic stability/control was meaningful and that this improvement had a significant positive impact both clinical and emotional on their lives.

Clinical Trial Registration Number: NCT02384941

Supported by: This study was funded by Sanofi.

Disclosure: **P. Lapuerta:** Employment/Consultancy; Lexicon Pharmaceuticals, Inc. Stock/Shareholding; Lexicon Pharmaceuticals, Inc.

616

Off-label use of SGLT-2 inhibitors in type 1 diabetes: a German/Austrian DPV multicentre analysis of 119,819 patients

B. Bohn[1,2], J. Seufert[3], T. Danne[4], H. Kalscheuer[5], F. Kopp[6], S. Kress[7], P. Fasching[8], R.W. Holl[1,2], on behalf of the DPV-initiative;
[1]Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany, [2]German Center for Diabetes Research (DZD), Munich-Neuherberg, Germany, [3]Division of Endocrinology & Diabetology, Dep of Medicine II, Medical Faculty, University of Freiburg, University Hospital of Freiburg, Freiburg, Germany, [4]Diabetes Center for Children and Adolescents, Kinder- und Jugendkrankenhaus AUF DER BULT, Hannover, Germany, [5]First Department of Medicine, University of Lübeck, Lübeck, Germany, [6]Diabetes Center, Augsburg Clinical Center, Augsburg, Germany, [7]Medical Clinic I, General Internal Medicine, Gastroenterology, Hepatology, Endocrinology, Diabetes, Vinzentius Hospital Landau, Landau, Germany, [8]5th Medical Department, Wilhelminenspital, Vienna, Austria.

Background and aims: Sodium-glucose cotransporter type-2 inhibitors (SGLT-2i) are not approved for the treatment of type 1 diabetes (T1D). Randomized controlled trials (RCTs) revealed that adding SGLT-2i to insulin therapy in T1D could be advantageous for metabolic control and bodyweight (BW). But an increase in the risk of diabetic ketoacidosis (DKA) was reported as well. Extent and effects of off-label use of SGLT-2i therapy in T1D in real-life diabetes care are currently not known. The aim of this analysis was, therefore, to identify and characterize patients with T1D and SGLT-2i from the German/Austrian diabetes patients follow-up (DPV) registry.

Materials and methods: 119,819 patients with T1D were selected from the DPV registry. Off-label use of SGLT-2i was detected in 184 patients. For each patient, the last year of treatment (before adding SGLT-2i) was analyzed. Sociodemographic and clinical differences were compared between patients initiating add-on therapy with SGLT-2i and those without SGLT-2i. Linear regression models were implemented for hemoglobin A1c (HbA1c), body mass index (BMI) and daily insulin dose/kgBW. Logistic regression was conducted for the proportion of patients with at least one severe hypoglycemia, hypoglycemic coma, or DKA. Models were adjusted for age, sex, and diabetes duration. *P*-level was set at <0.05.

Results: Off-label use of SGLT-2i in T1D ($n = 184$) was predominantly observed in adult patients (≥18 years of age: 90.1%). Patients initiating therapy with SGLT-2i were older (median age: 52.0 years (Q1: 37.4; Q3: 62.9) compared to those without SGLT-2i (17.9 years (14.8; 37.6); $p < 0.0001$) and had a longer diabetes duration (14.8 years (4.1; 25.6) vs. 7.1 years (2.9; 13.7); $p < 0.0001$). Subjects starting with SGLT-2i displayed a higher HbA1c (8.6% vs. 8.3%; $p = 0.0315$) and a higher BMI (28.2 kg/m^2 vs. 23.3 kg/m^2; $p < 0.0001$) compared to those without SGLT-2i. Daily insulin dose/kgBW was comparable (0.81 IU vs. 0.79 IU; $p = 0.4907$). The proportion of patients with at least one severe hypoglycemia, hypoglycemic coma, or DKA did not differ between patient groups (severe: 7.1% vs. 8.2%; $p = 0.5116$/coma: 3.6% vs. 3.1%; $p = 0.6266$/DKA: 0.8% vs. 1.4%; $p = 0.5829$).

Conclusion: SGLT-2i off-label use in T1D was observed in patients with generally worse metabolic control and a higher bodyweight. Potential benefits of SGLT-2i in T1D reported by RCTs could have contributed to the prescription behavior. Further research is needed to investigate efficacy and safety (especially DKA) of adding SGLT-2i therapy in selected patients with T1D in real-life diabetes care.

Supported by: DZD, DDG

Disclosure: **B. Bohn:** Grants; EFSD.

PS 045 Microvascular effects of SGLT2 inhibitors: focus on kidneys and eyes

617

Dapaglifozin preserves renal function in patients with type 2 diabetes: a longitudinal meta-analysis of eGFR across clinical trials

S. Johansson[1], B. Hamrén[1], M. Åstrand[1], R. Penland[2], D. Boulton[3];
[1]Quantitative Clinical Pharmacology, Early Clinical Development, IMED Biotech Unit, AstraZeneca R&D, Gothenburg, Sweden, [2]Quantitative Clinical Pharmacology, Early Clinical Development, IMED Biotech Unit, AstraZeneca R&D, Boston, USA, [3]Quantitative Clinical Pharmacology, Early Clinical Development, IMED Biotech Unit, AstraZeneca R&D, Gaithersburg, USA.

Background and aims: Results from the recently-completed cardiovascular outcomes trials (CVOTs) testing the sodium-glucose transport protein 2 (SGLT2) inhibitors empaglifozin and canaglifozin (EMPA-REG and CANVAS/R, respectively) indicated renal benefit and lower rates of clinically relevant renal events for SGLT2 inhibitor-treated subjects' vs placebo. Dapaglifozin, an SGLT2-inhibitor approved for the treatment of T2DM is being studied in the ongoing CVOT, DECLARE and in renal outcome. We have done a first, cross-trial model-based analysis with the aim to assess whether dapaglifozin could have renal benefit properties.
Materials and methods: Integrating data cross clinical trials can allow better quantitative insights than assessment of individual trials. We did a model-based meta-analysis, that includes individual serial estimated glomerular filtration rate (eGFR) measurements in T2DM subjects from 8 randomized, placebo-controlled double-blind Phase 2 and 3 dapaglifozin trials. A linear mixed-effect model was used to estimate the effect of placebo and dapaglifozin over time. A baseline model was developed, accounting for the differences in baseline eGFR. Next, a placebo model was added using serial data from placebo subjects, accounting for differences in placebo response observed across trials. Lastly and including all data, dapaglifozin dose-response model was developed characterizing the dapaglifozin effect on eGFR. The effect of population characteristics on the rate of eGFR change, eg duration of T2DM, body mass index, age, gender and concomitant/prior treatment was explored and included if significant ($p < 0.05$) using a chi-square test.
Results: 4.894 subjects with T2DM having a total of 58626 eGFR measurements from 8 dapaglifozin clinical studies were included in this analysis. Subjects were treated with placebo, 1, 2.5, 5 or 10 mg dapaglifozin once daily for up to 2 years. The pooled mean baseline characteristics were an age of 60 years, an HbA1c of 8.2% and an eGFR of 78.5 ml/min/1.73 m^2. On average, the eGFR in placebo treated subjects declined steadily over time (0.3 ml/min/1.73 m^2/year). Subjects treated with dapaglifozin showed a small and immediate decline (~2 ml/min/1.73 m^2, placebo-corrected) as seen with other SGLT2-inhibitors. After the initial, small eGFR drop, dapaglifozin then slowed any further loss of eGFR leading to a dose-dependent net preservation and increase of eGFR over the two-year treatment period. In the average patient, the placebo-corrected treatment effect, post the initial drop was an increase in the rate of eGFR change of +1.2 and +1.4 ml/min/1.73 m^2/year for 5 and 10 mg dapaglifozin respectively. Females had a somewhat larger treatment effect compared to males, whereas no other covariates were identified which indicates similar benefit across the subgroups studied.
Conclusion: This is the first integrated and model-based analysis which shows that dapaglifozin prevents the progressive loss of renal function in T2DM over the two years treatment studied. These results build confidence in the ongoing dapaglifozin renal outcome study.
Disclosure: **S. Johansson:** Employment/Consultancy; AstraZeneca R&D.

618

SGLT2 inhibitor dapagliflozin is renoprotective via mitigation of protein O-GlcNAcylation in streptozotocin-induced diabetes

D.B. Balogh[1,2], J. Hodrea[1], L. Lenart[1], A. Hosszu[1,3], C.K. Mezei[1,2], A. Vannay[4], L.J. Wagner[3], A.J. Szabo[2,4], A. Fekete[1,2];
[1]MTA-SE „Lendület" Diabetes Research Group, Hungarian Academy of Sciences, Budapest, [2]1st Department of Pediatrics, Semmelweis University, Budapest, [3]Department of Transplantation and Surgery, Semmelweis University, Budapest, [4]MTA-SE Pediatrics and Nephrology Research Group, Hungarian Academy of Sciences, Budapest, Hungary.

Background and aims: Normalizing hyperglycaemia is vital in slowing the progression of diabetes and preventing devastating secondary consequences such as diabetic kidney disease (DKD). Sodium-glucose cotransporter 2 inhibitors (SGLT2i) have recently been approved as new anti-hyperglycaemic drugs. To-date SGLT2i have only been approved in type 2 diabetes mellitus, while only a few studies are currently underway in type 1 diabetes mellitus (T1DM) using SGLT2i as an add-on to insulin therapy. Growing body of clinical data support that SGLT2i have renoprotective effects; but the mechanism of action is still not fully clarified. We previously showed that enhanced *O*-GlcNAcylation (increased addition of single *O*-GlcNAc moieties by *O*-GlcNAc tranferase (OGT) or decreased removal by *O*-GlcNAcase (OGA)) in DKD induces cellular processes leading to kidney fibrosis. Since there is still an unmet need for oral therapies in T1DM here we investigated the antidiabetic effect and the underlying mechanisms of renoprotection of the highly selective SGLT2 inhibitor dapagliflozin (DAPA) in a streptozotocin-induced model of T1DM.
Materials and methods: Diabetes (D) was induced by streptozotocin (65 mg/bwkg, *ip.*) in adult, male Wistar rats. Following the onset of D, animals were treated for six weeks with dapagliflozin (D+DAPA, 1 mg/bwkg/day, *po.*). Metabolic, renal parameters and kidney fibrosis were evaluated. *O*-GlcNAc, OGT and OGA protein levels were measured. Specific markers of tubular damage (NGAL, KIM-1), profibrotic factors (TGF-β, CTGF, PDGF, PAI-1) and proinflammatory cytokines (IL-6, IL-1β, TNF-α) were determined.
Results: Development of DKD was confirmed by functional impairment, massive proteinuria and structural damage of kidneys. DAPA reduced weight loss (D: 256 ± 10.9 *vs.* D+DAPA: 333 ± 13.9 g; $p < 0.01$), decreased blood glucose levels (D: 37 ± 2.7 *vs.* D+DAPA: 18 ± 5.6 mmol/L; $p < 0.05$) and improved renal function (creatinine clearance: D: 3.8 ± 0.4 *vs.* D+DAPA: 8.91.0 mL/min; $p < 0.01$). DAPA treatment minimized hyperglycaemia-induced protein *O*-GlcNAcylation and OGT levels. Subsequently profibrotic growth factor (CTGF, PDGF, TGF-β, PAI-1) expressions and renal fibrotic tissue accumulation were reduced. Renal proinflammatory cytokine (TNF-α, IL-1β, IL-6) expressions were also decreased by DAPA.
Conclusion: Our results support that DAPA is an effective and safe treatment in experimental DKD. Here we identified a novel mechanism: inhibition of OGT by DAPA results in decreased *O*-GlcNAcylation which leads to alleviated kidney fibrosis and improved renal function.
Supported by: LP008/2017, OTKA-K112629-FK124491-NN-114607, VKE-2017-00006, EEMOFAKT
Disclosure: **D.B. Balogh:** None.

619

Effect of dapagliflozin on renal and cardiac function in patients with type 2 diabetes and albuminuria: a randomised study

M.K. Eickhoff[1], F.J. Olsen[2], M. Frimodt-Møller[1], L.J. Diaz[1], M.T. Jensen[2], P. Rossing[1,3], F. Persson[1];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]Herlev & Gentofte Hospital, Gentofte, [3]University of Copenhagen, Copenhagen, Denmark.

Background and aims: To evaluate the effect of dapagliflozin treatment on renal and myocardial funtion assessed with advanced echocardiography,

and cardiac biomarkers when added to standard care in patients with type 2 diabetes (T2D).

Materials and methods: This is a sub-study of a double-masked randomized placebo-controlled crossover trial of 12 weeks treatment with dapagliflozin 10 mg once daily or matching placebo. All patients were on RAS blocking treatment. Included patients ($n = 40$) had T2D and albuminuria at baseline. At the end of the treatment period echocardiography (TTE) was performed, ^{51}Cr-EDTA, albuminuria and 24h blood pressure were measured, as well as cardiac markers in plasma; troponin I, MRproANP, MRproADM and copeptin.Global longitudinal strain (GLS) was the primary systolic echocardiographic endpoint. To assess diastolic function a combined diastolic endpoint was used including mean early diastolic myocardial velocity (e'), ratio between early transmitral inflow velocity (E) and e' (E/e'), atrial volume, and pulmonary pressure (TRmaxPG). Values at the end of treatment periods (placebo vs. dapagliflozin) were compared using mixed model analysis. Change in the four variables estimating change in diastolic function were weighted with an individual z-score and combined with a computed weighted average with weights equal to the inverse estimated variance.

Results: Baseline geometric mean urinary albumin creatinine ratio (UACR) was 147 (IQR 75–289) mg/g, mean eGFR 85 (SD ±19.7) ml/min/1.72 m^2. Baseline HbA_{1c} was 73 (SD ±15) mmol/mol, 24 h blood pressure 148/82 (SD ±12.5/7.7) mmHg, diabetes duration 16.5 (SD ±4.8) years, age 65 (SD ±8) years and 90% were male. After 12 weeks treatment with dapagliflozin vs. placebo, GFR decreased by 10.9 (5–16) ml/min ($p < 0.01$), UACR was reduced by 36% (95% CI 16–56%), HbA_{1c} was reduced by 7.4 (5–10) mmol/mol ($p < 0.01$) and 24 h blood pressure decreased 4.8/2.7 mmHg ($p = 0.023/0.031$). Left ventricular ejection fraction after placebo was 55.5 (SD ±6.7) % and 53.7 (SD ±6.7) % after active treatment - a non-significant change. GLS did not change whereas diastolic function improved significantly with 19.8% (3.3–36.3%, $p = 0.021$). Plasma concentration of NTproBNP , troponin, MRproANP, and MRproADM did not change. Plasma concentration of copeptin increased with 32.3% ($p < 0.0001$).

Conclusion: Treatment of dapagliflozin 10 mg vs. placebo in patients with albuminuria was associated with a clinically significant reduction in albuminuria, 24 h blood pressure, and GFR, paralleled by improvement in diastolic function but not GLS, demonstrating simultaneous beneficial renal and cardiac effects of SGLT2 inhibition.

Clinical Trial Registration Number: NCT02914691

Supported by: AstraZeneca

Disclosure: **M.K. Eickhoff:** Grants; Steno Diabetes Center Copenhagen received support from AstraZeneca for this study.

620

Composite retinopathy outcome in patients treated with empagliflozin versus placebo in the EMPA-REG OUTCOME trial

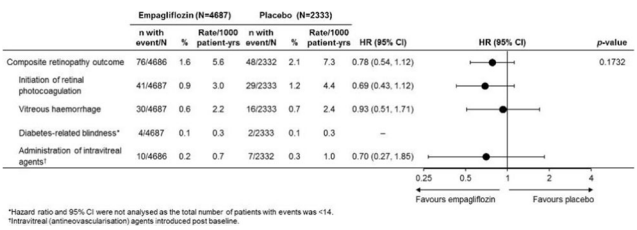
S.E. Inzucchi[1], C. Wanner[2], U. Hehnke[3], J. Lee[3], S. Kaspers[3], D. Clark[3], J.T. George[3], B. Zinman[4];
[1]Section of Endocrinology, Yale University School of Medicine, New Haven, USA, [2]Department of Medicine, Würzburg University Clinic, Würzburg, Germany, [3]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [4]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Canada.

Background and aims: Recently, questions have been raised about the effect of glucose-lowering drugs on retinopathy. In the EMPA-REG OUTCOME trial in patients with type 2 diabetes and established cardiovascular (CV) disease, empagliflozin (EMPA) given in addition to standard of care reduced the relative risk of 3-point major adverse CV events by 14%, driven by a 38% reduction in CV death. In addition, the relative risk of a pre-specified composite microvascular outcome of time to first initiation of retinal photocoagulation, vitreous haemorrhage, diabetes-related blindness, or incident or worsening nephropathy was reduced by 38% vs placebo (PBO), driven by the renal component. We analysed the effect of EMPA on retinopathy in the EMPA-REG OUTCOME trial.

Materials and methods: Patients were randomised to receive EMPA 10 mg, EMPA 25 mg, or PBO. Background glucose-lowering therapy was to remain unchanged for 12 weeks then be adjusted to achieve glycaemic control according to local guidelines. In post hoc analyses, we assessed the risk of a composite outcome of time to first initiation of retinal photocoagulation, vitreous haemorrhage, diabetes-related blindness, or administration of intravitreal agents. Differences in risk between the pooled EMPA and PBO groups were assessed using a Cox proportional hazards model in patients treated with ≥1 dose of study drug. To assess the potential impact of glucose lowering on retinopathy, we assessed the risk of the composite outcome after week 12 in subgroups by reduction in HbA1c at week 12 (<1% and ≥1%).

Results: There was no difference between EMPA and PBO in the risk of the composite retinopathy outcome (HR 0.78 [95% CI 0.54, 1.12]; $p = 0.1732$), nor any of its components (Figure). In subgroup analyses, the risk of the composite retinopathy outcome with EMPA vs PBO after week 12 was similar irrespective of whether patients had a reduction in HbA1c at week 12 of <1% (HR 0.87 [95% CI 0.58, 1.31]) or ≥1% (HR 0.40 [95% CI 0.14, 1.14]) ($p = 0.1755$ for treatment by subgroup interaction).

Conclusion: In the EMPA-REG OUTCOME trial in patients with T2DM and established CV disease, there was no difference in risk of retinopathy between patients treated with EMPA and PBO.



Clinical Trial Registration Number: NCT01131676

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **S.E. Inzucchi:** Honorarium; AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo, Eisai, Intarcia Therapeutics, Inc., Janssen, Lexicon Pharmaceuticals, Merck & Co, Novo Nordisk, Sanofi, vTv Therapeutics.

621

Assessment of adverse renal effects in patients with type 2 diabetes receiving ertugliflozin

S.G. Terra[1], S. Patel[2], A. Hickman[3], R. Frederich[4], B. Lauring[2], S. Johnson[4], S. Huyck[2], J.P. Mancuso[3];
[1]Pfizer Inc., Andover, [2]Merck & Co., Inc., Kenilworth, [3]Pfizer Inc., Groton, [4]Pfizer Inc., Collegeville, USA.

Background and aims: Ertugliflozin (ERTU) is a selective inhibitor of sodium-glucose cotransporter 2 (SGLT2) for the treatment of adults with type 2 diabetes mellitus (T2DM). Based on the mechanism of action of SGLT2 inhibitors, the risk of adverse renal effects was assessed in patients with T2DM receiving ERTU.

Materials and methods: Adverse renal effects were assessed in adults with T2DM receiving ERTU relative to placebo (PBO) or active comparators in pooled analyses of 7 randomised, double-blind, Phase 3 trials. Patients ($N = 4859$) received ERTU 5 mg ($n = 1716$), ERTU 15 mg ($n = 1693$) or non-ERTU (PBO, glimepiride or sitagliptin; $n = 1450$) for up to 2 years.

Results: Mean age was 57.8 y and 25.8% were ≥65 y; 51.8% were males. Mean eGFR was 85.3 ml min^{-1} 1.73 m^{-2} and ~12% had moderate renal impairment (RI). The mean duration of T2DM was 7.9 y and mean baseline HbA_{1C} was 66 mmol/mol (8.2%). The incidence of renal-

related adverse effects (AEs) was low across all groups (ERTU 5 mg, 0.6%; ERTU 15 mg, 0.8%; non-ERTU, 0.4%); few were serious (0.1% in all groups) or led to treatment discontinuation (0.1%, 0.2% and 0.1%, respectively). Subgroup analyses demonstrated that in patients with moderate RI, renal-related AEs were more frequent in the ERTU groups relative to the non-ERTU group (ERTU 5 mg, 3.6%; ERTU 15 mg, 2.1%; non-ERTU, 1.1%). Incidence of renal-related AEs was similar across groups for patients ≥65 y old (excluding those with moderate RI at baseline) (ERTU 5 mg, 0.3%; ERTU 15 mg, 1.0%; non-ERTU, 0.8%) or those using diuretics (ERTU 5 mg, 1.2%; ERTU 15 mg, 0.8%; non-ERTU, 0.8%) or ACE inhibitors/angiotensin-receptor blockers (ERTU 5 mg, 0.9%; ERTU 15 mg, 0.9%; non-ERTU, 0.5%). Independent blinded adjudication for causality was performed for prespecified renal-related AEs and renal laboratory changes from baseline. Four patients had renal events that were adjudicated as causally related to study medication ("possible" or "very likely"; 1 in the ERTU 5 mg group, 2 in the ERTU 15 mg group and 1 in the non-ERTU group).

Conclusion: There was no evidence of increased risk of adverse renal effects in patients with T2DM receiving ERTU relative to PBO or active comparators, except in those with baseline moderate RI.

Clinical Trial Registration Number: NCT01958671, NCT02033889, NCT02036515, NCT02226003, NCT01999218, NCT02099110, NCT01986855

Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer Inc.

Disclosure: **S.G. Terra:** Employment/Consultancy; Pfizer Inc. Stock/Shareholding; Pfizer Inc.

622

Effect of dapagliflozin (DAPA) on cardiovascular and renal risk factors in patients with type 2 diabetes treated with or without renin-angiotensin system inhibitors (RASi)

D.H. van Raalte[1], R. Correa-Rotter[2], R. Toto[3], H.J.L. Heerspink[4], V. Cain[5], B.V. Stefansson[6], C.D. Sjöström[6], P. Sartipy[6];
[1]Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands, [2]Nephrology and Mineral Metabolism, National Medical Science and Nutrition Institute Salvador Zubirán, Mexico City, Mexico, [3]University of Texas Southwestern Medical Center, Dallas, USA, [4]Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, Netherlands, [5]Bogier Clinical and IT Solutions Inc, Raleigh, USA, [6]Cardiovascular, Renal and Metabolic Disease, AstraZeneca, Gothenburg, Sweden.

Background and aims: Reduction of urinary protein excretion and optimal blood pressure (BP) control are the main focus of current therapeutic strategies to prevent and slow progression of diabetic proteinuric kidney disease in type 2 diabetes (T2D). RASi are the most effective treatment options but significant residual proteinuria remains. Novel therapeutic strategies, such as sodium-glucose cotransporter 2 inhibitors (SGLT2i) may complement RASi by offering additional proteinuria reduction and renal protection. This study investigated the effects of the SGLT2i DAPA in patients with T2D with micro- and macro-albuminuria treated with or without RASi at baseline.

Materials and methods: In this post hoc analysis, we evaluated the effect of DAPA 10 mg/day over 24 weeks (w) across 13 placebo-controlled studies in patients with T2D with urinary albumin: creatinine ratio (UACR) ≥30 mg/g at baseline. The patient population was divided into 2 subgroups based on treatment (Yes/No) with RASi at baseline.

Results: A total of 957 and 302 patients were included in the groups with or without RASi treatment at baseline, respectively. Demographic and baseline characteristics were similar between groups, but patients without RASi treatment had shorter duration of diabetes (7 vs 12 years), higher estimated glomerular filtration rate (eGFR) (90 vs 79 mL/min/1.73 m^2), and slightly lower UACR, serum uric acid (sUA), body weight, and systolic blood pressure (SBP) at baseline. Placebo-adjusted treatment effects of DAPA 10 mg on UACR, eGFR, HbA_{1c}, and hematocrit over 24 w were similar across groups (Table). Mean reductions in body weight and sUA were more distinct in patients treated without RASi at baseline. The placebo-adjusted treatment effect of DAPA 10 mg on UACR in the overall population was independent of other covariates (age, race, body weight, SBP, eGFR, and RASi use). Adverse event (AE) profiles were similar between placebo and DAPA 10 mg in each group, consistent with known DAPA safety profile. There were more serious AEs and hypoglycaemia in patients with RASi.

Conclusion: Treatment with DAPA over 24 w provides similar clinically relevant improvements in metabolic and haemodynamic parameters and similar reductions in UACR in patients with T2D with albuminuria treated with or without RASi at baseline. Further investigation of cardiovascular and renal outcomes in patients with T2D treated with DAPA on top of RASi is warranted.

Table I. Adjusted change from baseline at week 24

	Adjusted change from baseline at week 24, difference vs PBO*		
	With RASi at baseline	Without RASi at baseline	*P* value for overall treatment by subgroup interaction
UACR (%)**	-19.0 (-28.5, -8.2)	-17.3 (-35.7, 6.4)	0.7499
SBP (mm Hg)	-3.4 (-5.0, -1.7)	-6.3 (-9.1, -3.6)	0.1449
DBP (mm Hg)	-1.9 (-2.9, -0.9)	-2.8 (-4.6, -0.9)	0.7249
eGFR (mL/min/1.73 m^2)	-1.4 (-3.1, 0.2)	-2.0 (-5.1, 1.1)	0.3808
HbA_{1c} (%)	-0.4 (-0.5, -0.3)	-0.5 (-0.7, -0.2)	0.4116
Body weight (kg)	-1.7 (-2.1, -1.3)	-2.5 (-3.3, -1.7)	0.0198
sUA (mg/dL)	-0.4 (-0.5, -0.2)	-0.6 (-0.8, -0.4)	0.0433
Haematocrit (%)	2.9 (2.5, 3.2)	2.5 (1.8, 3.1)	0.5944

*Numbers are mean and 95% CI; ** UACR log transformed percent change from baseline, exponentiated back for presentation.
Abbreviations: DBP: diastolic BP; eGFR: estimated glomerular filtration rate; PBO: placebo; RASi: renin-angiotensin system inhibitors; SBP: systolic BP; sUA: serum uric acid; UACR: urine albumin : creatinine ratio

Supported by: AstraZeneca Pharmaceuticals LP.

Disclosure: **D.H. van Raalte:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Sanofi. Grants; AstraZeneca, Boehringer Ingelheim, Novo Nordisk, Sanofi.

623

Acute renal outcomes with sodium glucose co-transporter 2 inhibitors: real world data analysis

C. Melzer Cohen[1], A. Cahn[2,3], R. Pollack[2,3], V. Shalev[1,4], G. Chodick[1,4];
[1]Medical division, Maccabi Healthcare Services, Tel Aviv, [2]Diabetes Unit and Endocrinology and Metabolism Unit, Hadassah Hebrew University Hospital, Jerusalem, [3]Diabetes Clinic, Maccabi Healthcare Services, Jerusalem, [4]School of Public Health, Tel Aviv University, Tel Aviv, Israel.

Background and aims: Sodium glucose co-transporter 2 inhibitors (SGLT2-i) ameliorate hyperglycemia by blocking renal glucose reabsorption and are indicated for the treatment of adults with type 2 diabetes mellitus (T2DM). We aimed to evaluate short-term renal outcomes in a real-world cohort of new users of SGLT2-i compared to new users of Dipeptidyl Peptidase-4 inhibitors (DPP-4i).

Materials and methods: Included in this retrospective cohort study were patients with T2DM who initiated SGLT2-i or DPP-4i during 1/4/2015–30/6/2017, had a baseline serum creatinine (SCr) measurement within 180 days prior to treatment initiation and had a second SCr measurement, hospitalization with acute kidney injury (AKI) or death within 24 weeks of index date. Primary endpoints were (1) ≥30% reduction in estimated glomerular filtration rate (eGFR) from baseline; and (2) hospitalization with AKI, initiation of dialysis, or sustained eGFR of <15 mL/min/1.73 m^2. Additional endpoints included deterioration in chronic kidney disease (CKD) category, doubling of SCr, hospitalization for AKI, all-cause mortality and hospitalization for any cause.

Results: Overall, 6418 and 5604 patients initiated SGLT2-i and DPP-4i, respectively. Baseline mean (SD) eGFR was higher among SGLT2-i users (88.3 [17.4] mL/min/1.73 m^2) vs. DPP-4i users (82.8 [23.7] mL/

min/1.73 m^2), yet eGFR levels were similar between groups when stratified by CKD stages. Fewer patients with eGFR ≤60 mL/min/1.73 m^2 were in the SGLT2-i (n = 503, 7.8%) vs. DPP4-i group (n = 1059, 18.9%). The adjusted OR (95% CI) for ≥30% reduction in eGFR with SGLT2-i vs. DPP4-i was 0.70 (0.49–1.00) and adjusted ORs ranged from 1.97 (0.62–6.26) to 0.45 (0.21–0.99) in patients with baseline eGFR 30–45 and ≥90 mL/min/1.73 m², respectively. The adjusted OR for CKD stage deterioration was 1.01 (0.90–1.14). Although not statistically significant, a similar trend was observed with adjusted ORs ranging from 1.95 (0.96–3.97) to 0.85 (0.68–1.08) among patients with baseline eGFR 30–45 and 60–90 mL/min/1.73 m², respectively. There was a lower risk of hospitalization for AKI with SGLT2-i vs. DPP-4i (adjusted OR (95% CI) 0.47 (0.27–0.80)) with grossly similar ORs across eGFR categories. Two patients initiated dialysis, both in the DPP-4i group. All-cause mortality, and hospitalizations were reduced with SGLT2-i vs. DPP-4i as well (adjusted OR (95% CI) 0.43 (0.20–0.95) and 0.66 (0.56–0.78) respectively).

Conclusion: This real-world data analysis supports evidence from previous randomized clinical trials demonstrating a reduction in hospitalizations for AKI, any hospitalizations and mortality among SGLT2-i users across all CKD categories. Nevertheless, due to the more prominent decrease in eGFR in patients with moderate CKD, cautious use of these agents in patients with low eGFR is advisable.

Disclosure: **C. Melzer Cohen:** None.

624

Effect of sotagliflozin on renal threshold for glucose reabsorption and on gastrointestinal glucose absorption in subjects with type 2 diabetes: a model based quantification

H.-C. Schneider, A. Strougo, B. Göbel, T. Klabunde, R. Dahmen, M. Riz;
Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany.

Background and aims: The dual SGLT1 and 2 inhibitor sotagliflozin reduces plasma glucose by blocking renal SGLT2 and by inhibiting intestinal SGLT1, thus increasing urinary glucose excretion and protracting gastrointestinal glucose absorption. The reduced rate of oral glucose appearance has previously been shown in healthy volunteers using glucose tracer techniques. Here, we investigated the effect of sotagliflozin on renal threshold for glucose excretion (RTg) and gastrointestinal glucose absorption in type-2-diabetic (T2D) subjects using the oral glucose minimal model and clinical data of a Phase IIa study with sotagliflozin in T2D subjects.

Materials and methods: In the Phase IIa study, after a 14-day metformin washout period, subjects were randomized to receive placebo or 150 mg or 300 mg QD sotagliflozin in an oral liquid formulation for 4 weeks. Oral glucose tolerance tests (OGTT) were performed in each arm before the treatment period (Day -2) and at the end of treatment (Day 27). First, the effect on RTg was quantified using total amount of glucose excreted via the urine and 8-point self-measured blood glucose. This first step allowed including urinary glucose excretion induced by SGLT2 inhibition into the oral glucose minimal model. The validity of the resulting model to estimate the protraction in gastrointestinal glucose absorption was shown using data of a glucose tracer study in healthy volunteers. This validated, modified version of the oral glucose minimal model was then applied to the OGTT data to evaluate the rate of oral glucose appearance in T2D subjects.

Results: The first step of the analysis showed that both sotagliflozin doses significantly lowered RTg from 212.4 ± 44.4 to 108.8 ± 26.3 mg/dL (mean ± SD) under 150 mg QD and from 226.5 ± 27.6 to 96.2 ± 24.0 mg/dL under 300 mg QD. Moreover, both sotagliflozin doses reduced the amount of glucose absorbed during the first hour after glucose intake. The area under the curve (AUC) for the rate of oral glucose appearance in the first hour was reduced from Day -2 to Day 27 by 33.3% (from 347.6 ± 68.3 to 232.0 ± 38.0 mg/kg) under 150 mg QD sotagliflozin and by 42.3% (from 385.4 ± 70.1 to 222.5 ± 53.4 mg/kg) under 300 mg QD sotagliflozin, while there was no significant effect in the placebo group. No difference was found in AUC between 0 and 3 hours from Day -2 to Day 27 indicating that sotagliflozin mediated inhibition of intestinal SGLT1 protracts but does not generally block glucose absorption.

Conclusion: The oral glucose minimal model could be extended to analyze the rate of oral glucose appearance under sotagliflozin treatment from OGTT data in T2D subjects without the need for complex and expensive glucose tracer studies. The analysis showed that after 4 weeks of treatment sotagliflozin significantly reduced the renal threshold for glucose excretion. In addition, the model based analysis demonstrated that sotagliflozin in T2D subjects protracts the time course of oral glucose absorption, but the total amount of glucose absorbed during an OGTT is not reduced. This additional effect is attributed to the inhibition of gastrointestinal SGLT1 and is expected to add beneficial effects of this dual SGLT1 and 2 inhibitor in the post-prandial phase.

Clinical Trial Registration Number: NCT00962065

Disclosure: **H. Schneider:** Employment/Consultancy; Employee of Sanofi-Aventis Deutschland GmbH.

PS 046 Efficacy and safety of the SGLT2 inhibitor ertugliflozin

625

A pooled analysis of the efficacy and safety of ertugliflozin as add-on therapy to metformin

R.A. Calle[1], J. Liu[2], S. Huyck[2], L. Wu[2], A. Pong[2], J.P. Mancuso[3], S.G. Terra[4], B. Lauring[2];
[1]Pfizer Inc., Cambridge, [2]Merck & Co., Inc., Kenilworth, [3]Pfizer Inc., Groton, [4]Pfizer Inc., Andover, USA.

Background and aims: Ertugliflozin, an oral sodium-glucose cotransporter 2 inhibitor, improves glycaemic control in adult patients with type 2 diabetes mellitus (T2DM). This pooled analysis characterised the efficacy and safety of ertugliflozin when used as add-on therapy to metformin.
Materials and methods: Pooled data from two randomised, double-blind, placebo-controlled Phase 3 studies with similar design and patient population (VERTIS MET and VERTIS SITA2) were analysed. Adult patients with T2DM inadequately controlled on metformin (± sitagliptin) with HbA1c 53–91 mmol/mol (7.0–10.5%) were randomised to placebo, ertugliflozin 5 mg or 15 mg for 26 weeks.
Results: Mean baseline characteristics of included patients ($N = 1083$) were similar across pooled treatment groups (age 57.7 years; T2DM duration 8.6 years; HbA1c 65 mmol/mol (8.1%); body weight 85.7 kg; systolic blood pressure [SBP] 130.6 mmHg; estimated GFR 89.4 mL min^{-1} 1.73 m^{-2}). Changes in HbA1c, body weight and SBP after 26 weeks are shown in the Table. Relative to placebo, more patients receiving ertugliflozin had HbA1c <53 mmol/mol (7.0%), body weight reduction of ≥5%, or SBP <130 mmHg (among patients with baseline SBP ≥130 mmHg) at Week 26. Ertugliflozin had an overall similar safety profile to placebo, except for a higher incidence of adverse events (AEs) of genital mycotic infections and of AEs related to osmotic diuresis.
Conclusion: Addition of ertugliflozin to metformin (± sitagliptin) provides reductions in HbA1c, body weight and SBP, resulting in more patients achieving metabolic treatment goals.

Table. Treatment effects on HbA1c, body weight and SBP at Week 26

		Placebo (n=362)	Ertugliflozin 5 mg (n=363)	Ertugliflozin 15 mg (n=358)
HbA1c†	LS mean change from baseline at Week 26 (95% CI), mmol/mol‡	−0.6 (−1.7, 0.4)	−8.2 (−9.2, −7.2)	−9.7 (−10.7, −8.7)
	Placebo-adjusted difference‡	–	*−7.6 (−8.9, −6.2)*	*−9.1 (−10.4, −7.7)*
	LS mean change from baseline at Week 26 (95% CI), %‡	−0.1 (−0.2, 0.0)	−0.8 (−0.8, −0.7)	−0.9 (−1.0, −0.8)
	Placebo-adjusted difference‡	–	*−0.7 (−0.8, −0.6)*	*−0.8 (−1.0, −0.7)*
	Patients with HbA1c <53 mmol/mol (7.0%) at Week 26, n (%)§	59 (16.3)	123 (33.9)	143 (39.9)
Body weight†	LS mean change from baseline at Week 26 (95% CI), kg‡	−1.3 (−1.6, −1.0)	−3.2 (−3.4, −2.9)	−3.0 (−3.3, −2.7)
	Placebo-adjusted difference‡	–	*−1.8 (−2.3, −1.4)*	*−1.7 (−2.1, −1.2)*
	Patients with body weight reduction ≥5% at Week 26, n (%)§	39 (10.8)	115 (31.7)	103 (28.8)
SBP†	Least squares mean change from baseline at Week 26 (95% CI), mmHg‡	−0.8 (−2.0, 0.5)	−4.1 (−5.3, −2.9)	−4.9 (−6.1, −3.7)
	Placebo-adjusted difference‡	–	*−3.4 (−5.1, −1.7)*	*−4.1 (−5.8, −2.4)*
	Patients with baseline SBP ≥130 mmHg (N) and with SBP <130 mmHg (n) at Week 26, n/N (%)§	32/171 (18.7)	70/192 (36.5)	71/189 (40.7)

†Analyses conducted on pooled data from randomised, treated patients, who had at least one measurement of the relevant parameter at or after baseline; data obtained after initiation of glycaemic rescue were excluded from the analyses.
‡Longitudinal data analysis model with fixed effects for treatment, time, study, baseline estimated GFR, and the interaction of time by treatment.
§Analysis performed using the Miettinen and Nurminen method, with missing data at Week 26 considered as 'non-responders'.
LS, least squares.

Clinical Trial Registration Number: NCT02033889, NCT02036515
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer, Inc
Disclosure: **R.A. Calle:** Employment/Consultancy; Pfizer Inc. Stock/Shareholding; Pfizer Inc.

626

Long-term efficacy and safety of ertugliflozin in patients with type 2 diabetes inadequately controlled with metformin monotherapy: 104-week VERTIS MET Trial

S. Gallo[1], B. Charbonnel[2], A. Goldman[3], H. Shi[4], S. Huyck[5], A. Darekar[6], B. Lauring[5], S.G. Terra[7];
[1]Pfizer Deutschland GmbH, Berlin, Germany, [2]University of Nantes, Nantes, France, [3]Pfizer Inc., Collegeville, USA, [4]Pfizer Inc., New York, USA, [5]Merck & Co., Inc., Kenilworth, USA, [6]Pfizer Ltd., Tadworth, UK, [7]Pfizer Inc., Andover, USA.

Background and aims: Ertugliflozin (ERTU) is a selective inhibitor of sodium-glucose cotransporter 2 for treatment of adults with type 2 diabetes mellitus (T2DM). The present study evaluated the long-term safety and efficacy of ERTU in patients with T2DM inadequately controlled with metformin (MET) monotherapy.
Materials and methods: This Phase 3 randomised, double-blind study in adults with T2DM (HbA_{1C} 53–91 mmol/mol [7.0–10.5%]) on MET (≥1500 mg/d ≥8 wk) included a 26-wk placebo (PBO)-controlled period followed by a 78-wk extension where non-rescued PBO patients with fasting finger-stick glucose ≥6.1 mmol/l received blinded glimepiride

(GLIM). Efficacy, safety and effect on bone mineral density (BMD) of ERTU 5 mg and 15 mg once daily at Wk 104 are reported.

Results: Patients ($N = 621$) had baseline mean ± SD: age 56.6 ± 8.8 y; T2DM duration 8.0 ± 6.0 y; BMI 31.1 ± 4.7 kg/m^2; HbA_{1C} 65.2 ± 9.9 mmol/mol (8.1 ± 0.9%); 41% were post-menopausal women. At Wk 104, ERTU 5 mg and 15 mg reduced HbA_{1C}, fasting plasma glucose, body weight (BW) and BP compared with baseline, and increased the proportion of patients with HbA_{1C} <53 mmol/mol (7%) (Table). Incidence of female genital mycotic infections was higher with ERTU 5 mg (7.3%; $P = 0.017$) and 15 mg (9.8%; $P = 0.003$) vs PBO/GLIM (0.9%). The incidence of symptomatic hypoglycaemia was lower with ERTU 5 mg (5.8%; $P = 0.009$) and 15 mg (5.9%; $P = 0.009$) vs PBO/GLIM (13.4%). ERTU had no impact on BMD vs PBO/GLIM, except total hip where BMD reduction was greater for ERTU 15 mg (Table). Fractures occurred in 3 (1.4%), 2 (1.0%) and 7 (3.3%) patients in the ERTU 5 mg, 15 mg and PBO/GLIM groups, respectively.

Conclusion: ERTU added to MET improved glycaemic control, BW and BP over 104 wk in patients with inadequately controlled T2DM. ERTU was well tolerated, with no clinically meaningful impact on BMD.

Table: Change in efficacy (vs baseline) and bone mineral density (vs PBO/GLIM) endpoints

		ERTU 5 mg	ERTU 15 mg
Efficacy endpoints[a] (change vs baseline)			
Change from baseline at Wk 104, LS mean (95% CI)[b]	HbA_{1C}, mmol/mol	–5.4 (–6.9, –4.0)	–8.4 (–9.8, –6.9)
	%	–0.5 (–0.6, –0.4)	–0.8 (–0.9, –0.6)
	FPG, mmol/l	–0.9 (–1.3, –0.6)	–1.5 (–1.8, –1.2)
	Body weight, kg	–3.6 (–4.2, –3.0)	–3.5 (–4.1, –2.9)
	Systolic BP, mm Hg	–3.9 (–5.8, –2.0)	–2.8 (–4.8, –0.9)
	Diastolic BP, mm Hg	–2.3 (–3.6, –1.1)	–1.2 (–2.5, 0.0)
Proportion of subjects with HbA_{1C} <53 mmol/mol (7%)[c]		27.1%	36.6%
BMD endpoints (change vs PBO/GLIM)			
% Change from baseline at Wk 104: Difference in LS mean (95% CI) vs PBO/GLIM[b]	Lumbar spine	–0.3 (–1.1, 0.5)	–0.2 (–1.0, 0.6)
	Femoral neck	0.1 (–0.7, 0.9)	0.3 (–0.6, 1.1)
	Total hip	–0.5 (–1.1, 0.1)	–0.8 (–1.4, –0.2)
	Distal forearm	0.2 (–0.5, 0.9)	–0.1 (–0.8, 0.7)

[a] All efficacy analyses were based on the full analysis set and excluding data after initiation of glycaemic rescue therapy; 23 (11.1%), 22 (10.7%) and 51 (24.4%) patients in the ERTU 5 mg, ERTU 15 mg and PBO/GLIM groups, respectively, received glycaemic rescue through Wk 104 (out of all patients randomised and treated).
[b] Based on cLDA model with fixed effects for treatment, time, prior anti-hyperglycaemic medication, baseline eGFR, menopausal status and the interaction of time by treatment.
[c] Missing data imputed using the cLDA model used for efficacy analyses.
BMD, bone mineral density; cLDA, constrained longitudinal data analysis; FPG, fasting plasma glucose; GLIM, glimepiride; LS, least squares.

Clinical Trial Registration Number: NCT02033889
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer Inc.
Disclosure: **S. Gallo:** Employment/Consultancy; Pfizer Inc. Stock/Shareholding; Pfizer Inc.

627

Safety and efficacy of ertugliflozin compared with glimepiride after 104 weeks in patients with type 2 diabetes inadequately controlled on metformin: VERTIS SU extension

P. Hollander[1], J. Liu[2], J. Hill[2], J. Johnson[2], Z.W. Jiang[3], D. Wang[3], G. Golm[2], S. Huyck[2], S. Terra[4], J.P. Mancuso[5], S.S. Engel[2], B. Lauring[2]; [1]Baylor Endocrine Center, Dallas, USA, [2]Merck & Co., Inc., Kenilworth, USA, [3]MSD R&D (China) Co., Ltd, Beijing, China, [4]Pfizer Inc., Andover, USA, [5]Pfizer Inc., Groton, USA.

Background and aims: Ertugliflozin, an oral sodium-glucose cotransporter 2 (SGLT2) inhibitor, improves glycaemic control in adults with type 2 diabetes mellitus (T2DM). This 52-week extension to the 52-week base study compared the long-term safety and efficacy of ertugliflozin with that of glimepiride over 104 weeks.

Materials and methods: In this double-blind, Phase 3 study (VERTIS SU), adults with HbA1c 53–75 mmol/mol (7.0–9.0%) on metformin ≥1500 mg/day were randomised 1:1:1 to ertugliflozin 5 mg, 15 mg, or glimepiride (initiated at 1 mg/day, up-titrated to 6 or 8 mg/day dependent on local labelling or maximum tolerated). The primary time point for efficacy was Week 52; treatment was continued until Week 104 in a blinded extension.

Results: Baseline characteristics of randomised, treated patients eligible for analysis ($n = 1315$) were similar across groups (mean age 58.2 years, HbA1c 62 mmol/mol [7.8%], duration of T2DM 7.5 years, estimated GFR [eGFR] 87.2 mL min^{-1} 1.73 m^{-2}). Approximately 60% of patients in each group completed Week 104 on study medication. Mean dose of glimepiride at Weeks 52 and 104 was 3.0 mg/day and 3.5 mg/day, respectively. As previously reported, ertugliflozin 15 mg was non-inferior to glimepiride in reducing HbA1c at Week 52. After 104 weeks, reductions in HbA1c from baseline were observed in all treatment groups; greater reductions in body weight and systolic BP were seen with ertugliflozin relative to glimepiride (Table). A similar incidence of adverse events (AEs) (Table) and serious AEs was reported across groups at Week 104. A total of 7 (1.6%), 2 (0.5%), and 1 (0.2%) patients receiving ertugliflozin 5 mg, 15 mg, or glimepiride, respectively, died; none of the AEs leading to death were considered drug-related. The incidence of symptomatic hypoglycaemia was significantly lower with ertugliflozin 5 mg and 15 mg compared with glimepiride ($p < 0.001$ for each comparison). Incidence of severe hypoglycaemia was lower with ertugliflozin (1 [0.2%] in each group) compared with glimepiride (15 [3.4%]). Incidence of genital mycotic infection (GMI) was significantly higher with ertugliflozin 5 mg and 15 mg than glimepiride ($p < 0.05$ for each comparison). Incidences of urinary tract infection and hypovolaemia were similar across groups. Mean change from baseline in eGFR at Week 104 was 0.7, 0.7 and −1.9 mL min^{-1} 1.73 m^{-2} in the ertugliflozin 5 mg, 15 mg, and glimepiride groups, respectively.

Conclusion: In patients with T2DM inadequately controlled on metformin, ertugliflozin led to clinically meaningful reductions in HbA1c over 104 weeks, similar to those observed with glimepiride. Relative to glimepiride, the incidence of symptomatic and severe hypoglycaemia was lower, and incidence of GMI was higher, for both ertugliflozin 5 mg and 15 mg doses.

Table. Summary of efficacy and safety analyses

		ERTU 5 mg	ERTU 15 mg	GLIM
Efficacy analysis LS mean change from baseline (95% CI)[†]	**Week 52**[‡]	n=448	n=440	n=437
	HbA1c, mmol/mol	–6.1 (–7.1, –5.1)	–7.0 (–7.9, –6.0)[§]	–8.1 (–9.0, –7.1)
	HbA1c, %	*–0.6 (–0.6, –0.5)*	*–0.6 (–0.7, –0.5)[§]*	*–0.7 (–0.8, –0.7)*
	Week 104[#]	n=445	n=435	n=435
	HbA1c, mmol/mol	–3.4 (–4.5, –2.2)	–4.0 (–5.1, –2.8)	–4.6 (–5.7, –3.4)
	HbA1c, %	*–0.3 (–0.4, –0.2)*	*–0.4 (–0.5, –0.3)*	*–0.4 (–0.5, –0.3)*
	Body weight, kg	–2.9 (–3.3, –2.5)	–3.4 (–3.9, –2.9)	1.0 (0.5, 1.4)
	Sitting SBP, mmHg	–2.0 (–3.3, –0.7)	–1.2 (–2.5, 0.0)	2.1 (0.9, 3.4)
Safety analysis at Week 104 Number of patients, n (%)	Adverse events	312 (70.1)	310 (71.3)	303 (69.7)
	Symptomatic hypoglycaemia[¶]	17 (3.8)**	28 (6.4)**	96 (22.1)
	GMI (men)[¶]	12/227 (5.3)**	5/191 (2.6)*	0/224 (0)
	GMI (women)[¶]	20/218 (9.2)**	30/244 (12.3)**	3/211 (1.4)
	UTI[¶]	40 (9.0)	39 (9.0)	36 (8.3)
	Hypovolaemia[¶]	8 (1.8)	8 (1.8)	3 (0.7)

[†]Longitudinal analysis model with fixed effects for treatment, time, prior antihyperglycaemic medication (monotherapy or dual therapy), baseline estimated GFR (continuous) and the interaction of time by treatment; [‡]These results have previously been reported; [§]Non-inferiority was declared as the upper bound of the two sided 95% CI for the mean difference was <3.3 mmol/mol (0.3%); [#]As the primary time point was Week 52, between-group comparisons were not performed for efficacy endpoints at Week 104; in total, 1315 randomised, treated patients were eligible for analysis at Week 104; [¶]Between group testing performed using the Miettinen and Nurminen method, with no control for multiplicity.

**p<0.001 vs glimepiride; *p=0.015 vs glimepiride.

ERTU, ertugliflozin; GLIM, glimepiride; GMI, genital mycotic infection; LS, least squares; SBP, systolic BP; UTI, urinary tract infection.

Clinical Trial Registration Number: NCT01999218
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer, Inc
Disclosure: **P. Hollander:** Grants; Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Honorarium; Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, Novo Nordisk.

628

Effects of ertugliflozin monotherapy or combination therapy on glycaemic control, body weight, and blood pressure in patients with type 2 diabetes: a pooled analysis

B. Lauring[1], J. Liu[1], L. Tarasenko[2], S.G. Terra[3], S. Huyck[1], L. Wu[1], A. Pong[1], R.A. Calle[4], S. Gallo[5], A. Darekar[6], J.P. Mancuso[7];
[1]Merck & Co., Inc., Kenilworth, USA, [2]Pfizer Inc., New York, USA, [3]Pfizer Inc., Andover, USA, [4]Pfizer Inc., Cambridge, USA, [5]Pfizer Pharma GmbH, Berlin, Germany, [6]Pfizer Ltd, Walton Oaks, UK, [7]Pfizer Inc., Groton, USA.

Background and aims: Ertugliflozin, an oral sodium-glucose cotransporter 2 inhibitor, improves glycaemic control in adult patients with type 2 diabetes mellitus (T2DM). This pooled analysis assessed changes from baseline in HbA1c, body weight (BW) and systolic blood pressure (SBP), with ertugliflozin 5 mg and 15 mg relative to placebo across three placebo-controlled Phase 3 studies.
Materials and methods: The analyses were conducted on pooled data from three randomised, double-blind, placebo-controlled Phase 3 studies with similar design and patient population (VERTIS MONO, VERTIS MET and VERTIS SITA2). Adult patients with T2DM inadequately controlled on diet and exercise only, or on metformin alone, or on metformin and sitagliptin, were randomised to receive placebo, ertugliflozin 5 mg, or ertugliflozin 15 mg for 26 weeks. Change from baseline to Week 26 in HbA1c and BW (predefined analyses), and in SBP (post-hoc analysis), for each ertugliflozin group was compared with placebo. Analyses were also conducted by baseline subgroup categories (including age, sex, race, ethnicity, region, body mass index, HbA1c, estimated GFR [eGFR], and duration of T2DM).
Results: Mean baseline characteristics of included patients ($n = 1544$) were similar across treatment groups (overall mean age 57.3 years; T2DM duration 7.5 years; eGFR 88.9 mL min^{-1} 1.73 m^{-2}). Overall, 89% of patients were overweight or obese, and 68% had a diagnosis of hypertension at baseline. Mean baseline HbA1c, BW and SBP were similar across groups (HbA1c: 65.1, 64.9 and 65.7 mmol/mol [8.1, 8.1 and 8.2%]; BW: 88.0, 88.5 and 87.3 kg; SBP: 129.7, 131.0 and 130.5 mmHg; in the placebo, ertugliflozin 5 mg, and ertugliflozin 15 mg groups, respectively). At Week 26, greater reductions from baseline in HbA1c, BW and SBP were observed with ertugliflozin compared with placebo (Table). More patients receiving ertugliflozin had HbA1c <53 mmol/mol (7.0%), a BW reduction of ≥5%, or SBP <130 mmHg (among patients with baseline SBP ≥130 mmHg) at Week 26 relative to placebo (Table). Clinically meaningful reductions in HbA1c, BW and SBP with ertugliflozin compared with placebo were generally consistent across subgroup categories analysed. The safety profile of ertugliflozin was similar to that of placebo, except for a higher incidence of drug-related adverse events that was primarily driven by genital mycotic infections and adverse events related to osmotic diuresis.
Conclusion: After 26 weeks of treatment in patients with T2DM, ertugliflozin led to greater reductions from baseline in HbA1c, BW and SBP compared with placebo, resulting in more patients achieving metabolic treatment goals.

Table. Treatment effects on HbA1c, BW and SBP at Week 26

		Placebo (n=515)	Ertugliflozin 5 mg (n=519)	Ertugliflozin 15 mg (n=510)†
HbA1c‡	LS mean change from baseline at Week 26 (95% CI), mmol/mol§	0 (–0.9, 0.9)	–8.3 (–9.2, –7.4)	–9.9 (–10.8, –9.0)
	Placebo-adjusted difference§	–	*–8.3 (–9.5, –7.1)*	*–9.9 (–11.1, –8.7)*
	LS mean change from baseline at Week 26 (95% CI), %§	0 (–0.1, 0.1)	–0.8 (–0.8, –0.7)	–0.9 (–1.0, –0.8)
	Placebo-adjusted difference§	–	*–0.8 (–0.9, –0.7)*	*–0.9 (–1.0, –0.8)*
	Patients with HbA1c <53 mmol/mol (7.0%) at Week 26, n (%)#	79 (15.3)	167 (32.2)	197 (38.7)
BW‡	LS mean change from baseline at Week 26 (95% CI), kg§	–1.4 (–1.6, –1.1)	–3.2 (–3.4, –2.9)	–3.2 (–3.4, –2.9)
	Placebo-adjusted difference§	–	*–1.8 (–2.2, –1.4)*	*–1.8 (–2.2, –1.4)*
	Patients with a BW reduction ≥5% at Week 26, n (%)#	54 (10.5)	155 (29.9)	147 (28.8)
SBP‡	LS mean change from baseline (95% CI) at Week 26, mmHg§	–1.2 (–2.3, –0.1)	–4.6 (–5.6, –3.6)	–4.7 (–5.7, –3.7)
	Placebo-adjusted difference§	–	*–3.4 (–4.8, –2.0)*	*–3.5 (–4.9, –2.0)*
	Patients with baseline SBP ≥130 mmHg (N) and with SBP <130 mmHg (n) at Week 26, n/N (%)#	47/242 (19.4)	100/275 (36.4)	98/262 (37.4)

†n=509 for the HbA1c analysis.
‡Analyses conducted on pooled data from randomised, treated patients, who had at least one measurement of the relevant parameter at or after baseline; data obtained after initiation of glycaemic rescue were excluded from the analyses.
§Longitudinal data analysis model with fixed effects for treatment, time, study, baseline estimated GFR, and the interaction of time by treatment.
#Analyses performed using the Miettinen and Nurminen method, with missing data at Week 26 imputed with the LDA model (A1C goal analysis), or considered as 'non-responders' (BW and SBP goal analyses).
LDA, longitudinal data analysis; LS, least squares.

Clinical Trial Registration Number: NCT01958671, NCT02033889, NCT02036515
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer, Inc
Disclosure: **B. Lauring:** Employment/Consultancy; Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Stock/Shareholding; Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

629

Design and baseline characteristics of the eValuation of ERTugliflozin effIcacy and Safety CardioVascular outcomes trial (VERTIS-CV)

D.K. McGuire[1], C.P. Cannon[2], R. Pratley[3], S. Dagogo-Jack[4], J. Mancuso[5], S. Huyck[6], B. Charbonnel[7], W.J. Shih[8], S. Gallo[9], U. Masiukiewicz[5], G. Golm[6], F. Cosentino[10], B. Lauring[6], S.G. Terra[11];
[1]University of Texas Southwestern Medical Center, Dallas, USA, [2]Harvard Medical School, Baim Institute for Clinical Research, Boston, USA, [3]Florida Hospital Translational Research Institute for Metabolism and Diabetes, Orlando, USA, [4]University of Tennessee Health Science Center, Memphis, USA, [5]Pfizer Inc., Groton, USA, [6]Merck & Co., Inc., Kenilworth, USA, [7]University of Nantes, Nantes, France, [8]Rutgers Cancer Institute of New Jersey, New Brunswick, USA, [9]Pfizer Inc., Berlin, Germany, [10]Unit of Cardiology, Karolinska University Hospital Solna, Stockholm, Sweden, [11]Pfizer Inc., Andover, USA.

Background and aims: Ertugliflozin (ERTU) is a selective inhibitor of sodium-glucose cotransporter 2 (SGLT2). The VERTIS cardiovascular (CV) outcomes trial has a primary objective to demonstrate noninferiority of ERTU vs placebo (PBO) on time to the first major cardiac event outcome (CV death, nonfatal myocardial infarction or nonfatal stroke). Secondary objectives are to demonstrate superiority of ERTU vs PBO on time to first event of CV death or hospitalisation for heart failure; CV death; and first event of renal death, dialysis/transplant or doubling of serum creatinine from baseline.
Materials and methods: Patients ≥40 years old with type 2 diabetes mellitus (T2DM) (HbA_{1C} 53–91 mmol/mol [7.0–10.5%]) and established atherosclerotic vascular disease of the coronary, cerebral and/or peripheral arterial systems were randomised 1:1:1 in a double-blind fashion to PBO or ERTU 5 mg or 15 mg added to existing therapy.
Results: 8246 patients were randomised and 8238 patients received ≥1 dose of study drug. Coronary artery disease, cerebrovascular disease and peripheral arterial disease were present in 76.3%, 23.1% and 18.8% of patients, respectively. In all, 21.6% of patients had Stage III kidney disease (eGFR 30 to <60 ml min^{-1} 1.73 m^{-2}); 30.2% had micro- and 9.2% had macroalbuminuria; 11.0% were ≥75 years old. Baseline characteristics are in the table compared with other SGLT2 inhibitor CV outcome trials. VERTIS-CV is ongoing and data are preliminary.
Conclusion: VERTIS-CV enrolled patients with T2DM with a history of established atherosclerotic vascular disease, and enrolment of a substantial proportion of patients with renal impairment, heart failure and older patients. Notably, the proportion of participants with established CV disease has varied across the SGLT2 inhibitor CV outcome trials.

	VERTIS-CV (N=8238) Ertugliflozin	EMPA-REG OUTCOME (N=7034) Empagliflozin	CANVAS (N=10,142) Canagliflozin	DECLARE (N=17,160) Dapagliflozin
Age, years	64.4 ± 8.1	63.1 ± 8.6	63.3 ± 8.3	63.8 ± 6.8
Males, n (%)	5764 (70)	5026 (72)	6509 (64.2)	10,738 (62.6)
HbA_{1c}, mmol/mol	67.08 ± 9.88	NA	NA	NA
%	8.3 ± 0.9[a]	8.1 ± 0.8	8.2 ± 0.9	8.3 ± 1.2
eGFR, ml min^{-1} 1.73 m^{-2}	76.0 ± 20.9	74 ± 21	76.5 ± 20.5	86.1 ± 21.8
≥90 ml min^{-1} 1.73 m^{-2}	2044 (24.8)	1534 (22)	2474 (24.4)	6855 (39.9)
60 to <90 ml min^{-1} 1.73 m^{-2}	4387 (53.3)	3671 (52)	5620 (55.5)	8739 (50.9)
30 to <60 ml min^{-1} 1.73 m^{-2}	1776 (21.6)	1796 (26)	2010 (19.8)	1565 (9.1)[c]
Established CV disease, %	99.9	>99	65.6	40.6
Myocardial infarction	3942 (47.9)	3275 (47)	2956 (29.2)	3580 (20.9)
Coronary revascularisation				
CABG	1808 (21.9)	1738 (25)	1427 (14.1)	1678 (9.8)
PCI	3408 (41.4)	NA	2558 (25.3)	3655 (21.3)
Stroke	1728 (21.0)	1631 (23)	1291 (12.8)	1107 (6.5)[d]
Peripheral arterial disease	1547 (18.8)	1449 (21)	2113 (20.8)	1025 (6.0)
History of heart failure	1844 (22.4)	706 (10.1)[b]	1461 (14.4)	1698 (9.9)

Data are n (%) or mean ± standard deviation, unless otherwise shown;
eGFR by Modification of Diet in Renal Disease Study equation.
[a]HbA_{1c} data from screening visit; [b]Percentage based on 7020 patients; [c]<60 ml min^{-1} 1.73 m^{-2}; [d]Ischaemic stroke.
CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention.

Clinical Trial Registration Number: NCT01986881
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer Inc.
Disclosure: **D.K. McGuire:** None.

630

Incidence of urinary tract infections in patients with type 2 diabetes receiving ertugliflozin, placebo or active comparator

M.A. Hickman[1], S. Patel[2], B. Lauring[2], S.G. Terra[3], S. Johnson[4], S. Huyck[2], J.P. Mancuso[1];
[1]Pfizer Inc., Groton, [2]Merck & Co., Inc., Kenilworth, [3]Pfizer Inc., Andover, [4]Pfizer Inc., Collegeville, USA.

Background and aims: Ertugliflozin, an oral sodium-glucose cotransporter 2 inhibitor (SGLT2), improves glycaemic control in adult patients with type 2 diabetes mellitus (T2DM). Although an increased risk of genital mycotic infections has been consistently observed with SGLT2 inhibitors, data on urinary tract infections (UTIs) are not consistent among this class of agents. This study aimed to assess the risk of UTIs, including complicated UTIs, in adult patients with T2DM receiving ertugliflozin relative to placebo or active comparators.
Materials and methods: Pooled analyses were performed on seven randomised, double-blind, Phase 3 studies (VERTIS studies MONO, MET, SITA2, FACTORIAL, SU, SITA, RENAL). Adult patients with T2DM received ertugliflozin 5 mg, 15 mg, placebo or active comparators (glimepiride or sitagliptin) for ≥26 weeks. Analyses were conducted on a broad pool (all studies) and a placebo pool (subset of studies). UTIs were identified using a pre-specified custom MedDRA query (CMQ). Complicated UTIs were defined as serious adverse events (SAEs) that were UTIs or UTIs considered to be potentially medically significant; complicated UTIs were analysed in the broad pool. Subgroup analyses by baseline demographics, including sex, age and renal function were also conducted in the broad pool.
Results: The broad pool comprised 4859 patients (n = 1450 non-ertugliflozin [placebo/active comparator]; n = 1716 ertugliflozin 5 mg; n = 1693 ertugliflozin 15 mg) with a mean treatment duration of ~355 days. The placebo pool comprised 1544 patients (n = 515 placebo; n = 519 ertugliflozin 5 mg; n = 510 ertugliflozin 15 mg) with a mean treatment duration of ~172 days. At baseline in the broad pool, mean age was 57.8 years, 25.8% were ≥65 years and 51.8% were males. Mean estimated GFR was 85.3 mL min^{-1} 1.73 m^{-2}. The mean duration of T2DM was 7.9 years and mean HbA1c was 66 mmol/mol (8.2%). Similar baseline characteristics were observed in the placebo pool. The incidence of UTIs in the broad pool was similar across groups (7.9%, 6.9% and 7.0% for non-ertugliflozin, ertugliflozin 5 mg and 15 mg, respectively), as was the incidence in the placebo pool (3.9%, 4.0% and 4.1% for placebo, ertugliflozin 5 mg and 15 mg, respectively). In the broad pool, ≥90% of UTIs in all groups were assessed by the investigator as mild or moderate in intensity. Few UTIs (<0.5%) in all groups were SAEs or led to drug discontinuation. The incidence of complicated UTIs, including pyelonephritis and urosepsis, was low across groups (0.3%, 0.2% and 0.6% for non-ertugliflozin, ertugliflozin 5 mg and 15 mg, respectively). In subgroup analyses by age, sex, and renal function, the incidence of UTIs was similar across treatment groups.
Conclusion: The risk of UTIs, including complicated UTIs, was not increased in patients with T2DM receiving ertugliflozin relative to placebo or active comparators.
Clinical Trial Registration Number: NCT01958671; NCT02033889; NCT02036515; NCT02099110; NCT01999218; NCT02226003; NCT01986855
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer, Inc
Disclosure: **M.A. Hickman:** Employment/Consultancy; Pfizer Inc. Stock/Shareholding; Pfizer Inc.

631

Evaluation of fractures, bone mineral density and bone biomarkers in patients with type 2 diabetes receiving ertugliflozin

U. Masiukiewicz[1], A. Hickman[1], R. Frederich[2], S. Patel[3], S. Gallo[4], B. Lauring[3], S.G. Terra[5], S. Johnson[2], S. Huyck[3], J.P. Mancuso[1];
[1]Pfizer Inc., Groton, USA, [2]Pfizer Inc., Collegeville, USA, [3]Merck & Co., Inc., Kenilworth, USA, [4]Pfizer Deutschland GmbH, Berlin, Germany, [5]Pfizer Inc., Andover, USA.

Background and aims: Ertugliflozin (ERTU) is a sodium-glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes mellitus (T2DM) in adults. As other members of the SGLT2 inhibitor class have been associated with an increased risk of bone fracture, the risk of adverse bone effects was assessed in patients with T2DM receiving ERTU.
Materials and methods: The risk of fracture was assessed in adults with T2DM receiving ERTU relative to placebo (PBO) or active comparator in pooled analyses of 7 randomised, double-blind, Phase 3 trials. Patients (N = 4859) received ERTU 5 mg (n = 1716), ERTU 15 mg (n = 1693) or non-ERTU (PBO, glimepiride or sitagliptin; n = 1450) for up to 2 y. In an add-on to metformin study (VERTIS MET; N = 621 [41.1%

postmenopausal (PM) women]; ERTU 5 mg, $n = 207$; ERTU 15 mg, $n = 205$; PBO, $n = 209$), bone mineral density (BMD) was measured at the lumbar spine, femoral neck, total hip and distal forearm through 2 y. Biomarkers of bone metabolism were also measured.

Results: In pooled analyses, mean age was 57.8 y and 25.8% were ≥65 y; 51.8% were male. Mean eGFR was 85.3 ml min^{-1} 1.73 m^{-2}. Mean duration of T2DM was 7.9 y and mean baseline HbA_{1C} was 66 mmol/mol (8.2%). The incidence of adjudication-confirmed fractures was similar across groups (ERTU 5 mg, 0.5%; ERTU 15 mg, 0.5%; non-ERTU, 0.6%). At Wk 26, serum calcium did not change from baseline when compared across groups, but there were small mean increases in serum magnesium (0.06 mmol/l vs −0.01 mmol/l) and phosphate (0.08 mmol/l vs 0.01 mmol/l) for all ERTU vs PBO. In VERTIS MET at Wk 104, overall and in the PM subgroup ($n = 255$), ERTU had no impact on BMD, except at the total hip where small reductions in BMD were observed (−0.84% overall; −1.17% PM) for ERTU 15 mg vs PBO. At Wk 104, there were no meaningful differences from baseline in procollagen type 1 N-terminal propeptide (P1NP; ERTU 5 mg, 10.1%; ERTU 15 mg, 24.2%; PBO, 19.4%) or parathyroid hormone (ERTU 5 mg, 8.2%; ERTU 15 mg, 5.5%; PBO, 10.1%) across groups. A small non-sustained increase in serum C-terminal telopeptide of type 1 collagen (CTX) was noted at Wk 26 and Wk 52 with ERTU vs PBO, which attenuated at Wk 104 (ERTU 5 mg, 26.9%; ERTU 15 mg, 32.5%; PBO, 19.3%).

Conclusion: There was no increased risk of fracture and no clinically meaningful change in BMD or bone biomarkers in patients with T2DM receiving ERTU.

Clinical Trial Registration Number: NCT01958671, NCT02033889, NCT02036515, NCT02226003, NCT01999218, NCT02099110, NCT01986855

Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer Inc.

Disclosure: **U. Masiukiewicz:** Employment/Consultancy; Pfizer Inc. Stock/Shareholding; Pfizer Inc.

PS 047 SGLT2 inhibitors around the world: evidence from clinical trials and registries

632

Safety and tolerability of empagliflozin in East Asian patients with type 2 diabetes: pooled analysis of phase I-III clinical trials

D. Yabe[1,2], A. Yasui[3], L. Ji[4], M.-K. Lee[5], R. Ma[6], T.-J. Chang[7], T. Okamura[3], C. Zeller[8], S. Kaspers[9], J. Lee[9], S. Kohler[9], Y. Seino[1,10];
[1]Kansai Electric Power Medical Research Institute, Kobe, Japan, [2]Kyoto University, Kyoto, Japan, [3]Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan, [4]Peking University People's Hospital, Beijing, China, [5]Sungkyunkwan University School of Medicine, Seoul, Republic of Korea, [6]The Chinese University of Hong Kong, Hong Kong SAR, China, [7]National Taiwan University Hospital, Taipei City, Taiwan, [8]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, [9]Boehringer Ingelheim International GmbH & Co. KG, Ingelheim, Germany, [10]Kansai Electric Power Hospital, Osaka, Japan.

Background and aims: We investigated the safety and tolerability of empagliflozin in East Asian patients with type 2 diabetes (T2DM).

Materials and methods: Data were pooled from patients with T2DM randomized 1:1:1 to placebo, empagliflozin 10 mg, or empagliflozin 25 mg in 15 Phase I-III trials. Adverse events (AEs) were analyzed in the subgroup of patients from East Asian countries.

Results: In total, 709, 724 and 708 East Asian patients received placebo, empagliflozin 10 mg and empagliflozin 25 mg, respectively; total exposure was 953, 1072, and 1033 patient-years in these groups, respectively. The incidence of any AEs, severe AEs, serious AEs, and AEs leading to discontinuation was not higher in patients treated with empagliflozin than placebo. The incidence of hypoglycemia differed according to glucose-lowering medication used at baseline. The incidence of events consistent with urinary tract infection was numerically lower with empagliflozin (5.3–5.8/100 patient-years) than placebo (7.2/100 patient-years). Events consistent with genital infection occurred more frequently with empagliflozin (1.5–1.7/100 patient-years) than placebo (0.2/100 patient-years). The incidence of AEs consistent with volume depletion was similar across treatment groups (0.8–1.4/100 patient-years) but higher with empagliflozin 25 mg vs placebo in patients aged ≥65 years (3.5 vs 2.0/100 patient-years). Rates of bone fractures, renal AEs, venous thromboembolic events, hepatic injury, lower limb amputation and diabetic ketoacidosis were similar or not higher with empagliflozin than placebo.

Conclusion: In this pooled analysis, empagliflozin was well tolerated in East Asian patients with T2DM based on >3,000 patient-years' exposure, consistent with results from the overall analysis population.

Supported by: Boehringer Ingelheim

Disclosure: **D. Yabe:** None.

633

Safety and efficacy of ertugliflozin in Asian patients with type 2 diabetes inadequately controlled with metformin monotherapy: VERTIS-Asia

M. Yang[1], L. Ji[2], Y. Liu[3], H. Miao[4], Y. Xie[5], W. Wang[1], Y. Mu[1], P. Yan[1], S. Pan[6], B. Lauring[7], S. Liu[8], S. Huyck[7], D. Wang[8], S.G. Terra[9];
[1]Pfizer (China) R&D Co., Shanghai, China, [2]Peking University People's Hospital, Beijing, China, [3]Yancheng First People's Hospital, Yancheng, China, [4]The Second Affiliated Hospital of Nanjing Medical University, Nanjing, China, [5]Pingxiang People's Hospital, Pingxiang, China, [6]Pfizer Inc., New York, USA, [7]Merck & Co., Inc., Kenilworth, USA, [8]Merck & Co., Beijing, China, [9]Pfizer Inc., Andover, USA.

Background and aims: Ertugliflozin (ERTU), a selective inhibitor of sodium-glucose cotransporter 2 (SGLT2), was recently approved in the United States for treatment of adult patients with type 2 diabetes mellitus (T2DM). This Phase 3, randomised, double-blind, 26-week (wk) multicentre study evaluated the efficacy and safety of ERTU vs placebo

(PBO) in Asian adults with T2DM inadequately controlled on metformin (MET) monotherapy.

Materials and methods: 506 Adult Asian patients from mainland China, Hong Kong, Republic of Korea, Philippines and Taiwan with T2DM and inadequate glycaemic control (HbA_{1C} 53–91 mmol/mol [7.0–10.5%]) on MET monotherapy (≥1500 mg/d for ≥8 wk) were randomised 1:1:1 to receive PBO ($n = 167$) or ERTU 5 mg ($n = 170$) or 15 mg ($n = 169$) once daily.

Results: Overall, 480 (94.9%) patients completed the study and 465 (91.9%) completed study medication. Baseline characteristics (mean ± SD) were similar among treatment groups: age 56.5 ± 9.1 y; T2DM duration 6.95 ± 5.08 y; body weight 70.3 ± 11.5 kg; BMI 26.0 ± 3.2 kg/m^2; HbA_{1C} 8.1 ± 0.9%; eGFR 99.3 ± 19.7 mL min^{-1} 1.73 m^{-2}. At Wk 26, patients randomised to ERTU 5 mg or 15 mg had significantly greater reductions (all $P < 0.001$) in HbA_{1C}, fasting plasma glucose, body weight and systolic BP and were significantly more likely to have an HbA_{1C} <53 mmol/mol (7.0%) vs PBO (Table). At Wk 26, 16.2%, 38.2% and 40.8% had an HbA_{1C} <53 mmol/mol (7.0%) with PBO, ERTU 5 mg and ERTU 15 mg, respectively. Mean reduction in diastolic BP with ERTU 5 mg and 15 mg was numerically greater vs PBO. Incidence of adverse events (AEs) was 59.3%, 56.5% and 53.3% with PBO, ERTU 5 mg and ERTU 15 mg, respectively. Serious AEs were more frequent with ERTU 5 mg (5.3%) and 15 mg (5.9%) vs PBO (1.2%). Overall, 1.8%, 1.2% and 0.6% of patients receiving PBO, ERTU 5 mg and 15 mg, respectively, discontinued study medication due to an AE. The incidence of genital mycotic infection, urinary tract infection and hypovolemia was similar with PBO, ERTU 5 mg and ERTU 15 mg. The incidence of symptomatic hypoglycaemia was higher with ERTU 15 mg (4.7%) compared with PBO (0.6%; $P = 0.019$), and the incidence was 2.4% with ERTU 5 mg.

Conclusion: ERTU significantly improved glycaemic control and reduced body weight and systolic BP in Asian patients with T2DM inadequately controlled on MET monotherapy. Significantly more patients treated with ERTU had an HbA_{1C} <53 mmol/mol (7.0%) at Wk 26 compared with PBO. Ertugliflozin was well tolerated.

	PBO (n=167)	ERTU 5 mg (n=170)	ERTU 15 mg (n=169)
Baseline HbA_{1C}, mean ± SD, mmol/mol	65.3 ± 10.5	65.4 ± 9.7	64.9 ± 10.0
%	8.1 ± 1.0	8.1 ± 0.9	8.1 ± 0.9
Wk 26 HbA_{1C}, mean ± SD, mmol/mol	61.1 ± 10.4	54.4 ± 8.5	55.2 ± 8.9
%	7.7 ± 1.0	7.1 ± 0.8	7.2 ± 0.8
Efficacy endpoints	**ERTU 5 mg vs PBO**		**ERTU 15 mg vs PBO**
Change from baseline at Wk 26: difference in LS mean[a] (95% CI)			
HbA_{1C}, mmol/mol	−8.7 (−10.6, −6.9)		−7.5 (−9.3, −5.7)
%	−0.8 (−1.0, −0.6)		−0.7 (−0.9, −0.5)
	P<0.001		*P*<0.001
Fasting plasma glucose, mmol/l	−1.7 (−2.0, −1.4)		−1.5 (−1.9, −1.2)
	P<0.001		*P*<0.001
Body weight, kg	−1.8 (−2.3, −1.3)		−2.0 (−2.5, −1.5)
	P<0.001		*P*<0.001
Systolic BP, mm Hg	−5.3 (−7.7, −2.9)		−4.1 (−6.5, −1.7)
	P<0.001		*P*<0.001
Diastolic BP, mm Hg	−1.4 (−3.0, 0.2)		−1.4 (−3.0, 0.2)
	P=0.081		*P*=0.086
HbA_{1C} <53 mmol/mol (7.0%) at Wk 26: odds ratio (95% CI)[b]			
	4.6 (2.5, 8.4)		4.6 (2.5, 8.4)
	P<0.001		*P*<0.001

[a] The LS mean for change from baseline to Wk 26 was estimated from a constrained longitudinal data analysis model.
[b] Adjusted odds ratio (95% CI) derived from a logistic regression model.
Analyses included all randomised patients receiving ≥1 dose of study treatment and having ≥1 measurement of the analysis endpoint during 26 weeks (including baseline). The mean and SD for baseline and Wk 26 are based on the number of patients with non-missing values at the specific time point. LS, least squares.

Clinical Trial Registration Number: NCT02630706
Supported by: Merck Sharp & Dohme Corp. (Merck & Co., Inc., USA, subsidiary); Pfizer Inc.
Disclosure: **M. Yang:** Employment/Consultancy; Pfizer. Stock/Shareholding; Pfizer.

634

Stroke safety with Sodium-Glucose Cotransporter-2 (SGLT-2) inhibitor use: a systematic review and meta-analysis

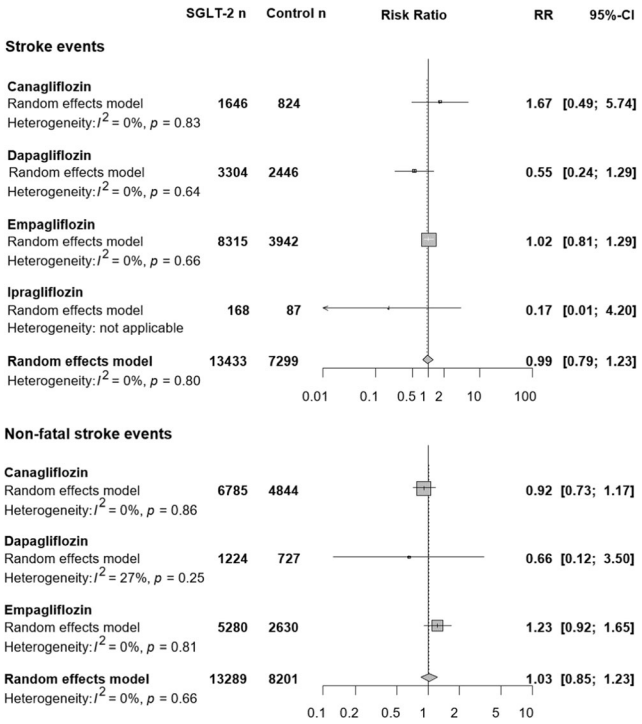
A.J. Roddick[1], S.L. Zheng[2];
[1]King's College London, London, [2]Imperial College Healthcare NHS Foundation Trust, London, UK.

Background and aims: Sodium-Glucose Cotransporter-2 (SGLT-2) inhibitors are among the most recent treatments to be approved for the treatment of type 2 diabetes. SGLT-2 inhibitors have been shown to be efficacious not only for glycaemic control but also in reducing all-cause and cardiovascular mortality. However, previous meta-analysis has indicated an increased risk of harm from non-fatal stroke with SGLT-2 inhibitor use. The aim of the present study is to investigate stroke safety in this treatment class.

Materials and methods: MEDLINE, Embase and CENTRAL databases and published meta-analyses were searched from inception through October 11th, 2017 for phase 2–4 randomised controlled trials of greater than 12 weeks' duration, enrolling patients with type 2 diabetes, and comparing SGLT-2 inhibitors with placebo. Study outcomes were any and non-fatal stroke events. Random-effects pairwise meta-analysis was undertaken using the Mantel-Haenzel method.

Results: For any stroke outcome, 25 trials of four different SGLT-2 inhibitor types were included for meta-analysis, comprising 358 events in 20,732 patients. Stroke safety was not negatively impacted by SGLT-2 inhibitors compared to placebo (RR 0.99; 95% CI 0.79 to 1.23; $P = 0.92$). For non-fatal stroke safety, 12 trials from three different SGLT-2 inhibitor types were included, accounting for 501 events in 21,490 patients. Non-fatal stroke was not associated with SGLT-2 inhibitor use compared to placebo (RR 1.03; 95% CI 0.86 to 1.23; $P = 0.78$). For both outcomes, no individual type of SGLT-2 inhibitor was associated with increased risk of any or non-fatal stroke events, and between-study heterogeneity was low ($I^2 = 0\%$ for both outcomes).

Conclusion: In this comprehensive meta-analysis of stroke safety in SGLT-2 inhibitors, we find no evidence of increased risk of any or non-fatal stroke with SGLT-2 inhibitors compared to placebo treatment. The inclusion of data from the CANVAS program contributes considerable strength to this study and permits expansion upon previous meta-analyses. Our data suggest that SGLT-2 inhibition does not negatively impact on stroke safety in patients with type 2 diabetes.



Disclosure: **A.J. Roddick:** None.

635

Lower cardiovascular risk with SGLT-2 inhibitors vs other glucose-lowering drugs: real world data from Asia Pacific, North America, Europe and Middle East: the CVD-REAL study

M. Kosiborod[1], C.S.P. Lam[2], S. Kohsaka[3], D. Kim[4], A. Karasik[5], S.-Y. Goh[6], J. Shaw[7], N. Tangri[8], A. Norhammar[9], J. Franch-Nadal[10], M. Thuresson[11], F. Surmont[12], N. Hammar[13,14], P. Fenici[15], CVD-REAL Investigators and Study Group;
[1]Saint-Luke's Mid. America Health Institute, Kansas City, USA, [2]Singapore and SingHealth Duke-NUS, National Heart Centre, Singapore, Singapore, [3]Keio University School of Medicine, Tokyo, Japan, [4]Ajou University School of Medicine, Suwon, Republic of Korea, [5]Tel Aviv University, Tel Aviv, Israel, [6]Singapore General Hospital, Singapore, Singapore, [7]Baker IDI Heart and Diabetes Institute, Melbourne, Australia, [8]Department of medicine, University of Manitoba, Winnipeg, MB, Canada, [9]S:t Göran Hospital, Stockholm, Sweden, [10]Institut Universitari d'investigació en Atenció Primaria (IDIAP Jordi Gol), Barcelona, Spain, [11]Statisticon AB, Uppsala, Sweden, [12]AstraZeneca, Luton, UK, [13]Institute of Environmental Medicine, Stockholm, Sweden, [14]AstraZeneca, Gothenburg, Sweden, [15]AstraZeneca, Cambridge, UK.

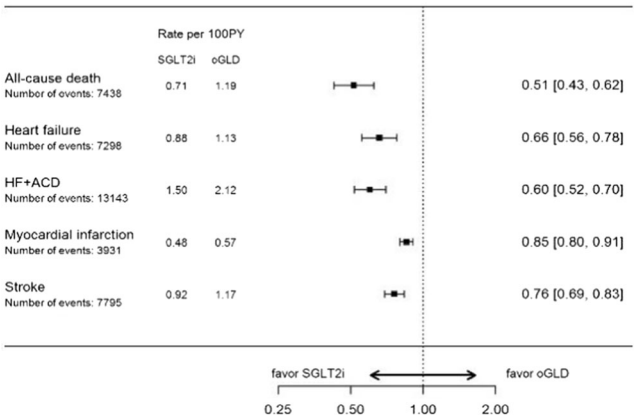
Background and aims: Results from CVD-REAL have been previously reported, showing consistently lower risk of cardiovascular events, death and heart failure hospitalization (HHF) with sodium glucose cotransporter-2 inhibitors (SGLT-2i) vs other glucose-lowering drugs (oGLD) in patients with type 2 diabetes (T2D). The aim of this analysis was to extend the CVD-REAL analyses across larger number of countries and patients, and longer duration of follow up.

Materials and methods: New users of SGLT2i or oGLD were included from established data sources in South Korea, Japan, Singapore, Israel, Spain, Sweden, Canada, the US and Australia. Propensity scores for SGLT-2i initiation were developed in each country by a standardized protocol, with 1:1 matching. Hazard ratios for death, hospitalization for heart failure (HHF), death or HHF, MI and stroke were derived using proportional hazards regression (intent-to-treat approach), and pooled in a weighted meta-analysis.

Results: After propensity-match, there were 360,356 episodes of treatment initiation in each group; ~29% had established CVD. Baseline characteristics were well balanced between treatment groups. As a proportion of total exposure in the SGLT-2i group, 53.8% of treatment episodes were with dapagliflozin, 20.1% canagliflozin, 18.3% empagliflozin and 7.8% other SGLT-2i. Mean follow up was 410 and 421 days for SGLT-2i and oGLD, respectively. Initiation of SGLT-2i vs oGLD was associated with lower risk of HHF and death; and modestly lower risks of myocardial infarction and stroke (Figure).The results were directionally consistent across countries in various geographic regions.

Conclusion: In a large cohort of patients with T2D from clinical practice in Asia-Pacific, Europe, the Middle East and North America, initiation of SGLT-2i was associated with lower risks of HHF, death and major cardiovascular events.

Figure: Hazard ratios for death and CV events after initiation of SGLT-2i vs oGLD



Clinical Trial Registration Number: NCT02993614
Supported by: AstraZeneca
Disclosure: **M. Kosiborod:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Sanofi, GSK, Janssen, Intarcia, Merck (Diabetes), Novo Nordisk, Glytec, ZS Pharma. Grants; AstraZeneca, Boehringer Ingelheim. Honorarium; AstraZeneca, Boehringer Ingelheim, Sanofi, Glytec, Novo Nordisk, ZS Pharma, Janssen, Merck (Diabetes), Novartis.

636

CANadian CAnagliflozin REgistry (CanCARE): a prospective, observational, assessment of canagliflozin (CANA) treatment in type 2 diabetes; 12 month results

V. Woo[1], A. Bell[2], M. Clement[3], F. Camacho[4], N. Georgijev[5], J.B. Rose[5], W. Rapattoni[5], H.S. Bajaj[6];
[1]University of Manitoba, Winnipeg, MB, [2]University of Toronto, Toronto, [3]University of British Columbia, Vancouver, [4]University of Waterloo, Waterloo, [5]Janssen Inc., Toronto, [6]LMC Diabetes & Endocrinology and Mt. Sinai Hospital, Brampton and Toronto, Canada.

Background and aims: CanCARE is a Canadian multicenter, prospective cohort study that enrolled SGLT2 inhibitor-naïve adult patients with T2DM, with HbA1c ≥7% on a stable anti-hyperglycemic agent (AHA) regimen at baseline and eGFR ≥60 mL/min/1.73 m^2, who were initiated on CANA as part of their usual treatment.

Materials and methods: This real-world (RW) study assessed the effectiveness and safety outcomes of the enrolled cohort of 527 subjects (mean age 60.7 yrs, mean baseline A1c 8.3%) over 12 months.

Results: Mean A1c reduction was −1.06 (1.12), with an observed dose response: −0.96 for CANA 100 mg, −1.20 for CANA 300 mg. 84.9%, 57.9% and 33% of subjects experienced >0%, ≥3%, ≥5% weight loss, respectively. Overall, 38.8% of subjects achieved A1c <7.0%, while 41% achieved the composite endpoint of A1c reduction ≥0.5%, body weight loss ≥3%. 17.9% subjects discontinued CANA. Safety data showed 37.4% subjects had ≥1 Adverse Event (AE), 3.5% had serious AEs, 14.5% had AEs special Interest: GMI (9.5%), polyuria (3.7%), UTI (1.5%), severe hypoglycemia (0.9%) and volume-related AE (0.7%). No reports to date of diabetic ketoacidosis or amputations.

Conclusion: CANA shows sustained, clinical meaningful improvement in cardiometabolic (CM) parameters in the RW, confirming findings from Phase 3 trials.

Table 1: Baseline characteristics and response of CM parameters at 6 and 12 months after initiation with CANA

Vital of Lab Value	Baseline	6 Months	12 Months	Change 6 Months from Baseline mean (std)	Change 12 Months from Baseline mean (std)
Hemoglobin A1C (%)	8.4	7.4	7.3	-0.90 (1.16)	-1.06 (1.12)
Hemoglobin A1C (mmol/mol)	68.8	57.7	56.2	-33.34 (12.68)	-35.06 (12.23)
Weight (kg)	90.1	88.5	89.4	-2.87 (4.62)	-3.24 (4.81)
Body Mass Index (kg/m2)	32.1	31.6	31.8	-1.02 (1.61)	-1.15 (1.68)
Waist Circumference (cm)	107.4	104.7	105.9	-3.11 (9.39)	-2.91 (11.12)
Diastolic Blood Pressure (mmHg)	78.3	75.3	75.3	-2.90 (8.78)	-3.50 (8.68)
Systolic Blood Pressure (mmHg)	130.8	126.1	126.3	-4.52 (13.64)	-4.65 (13.04)
Glomerular Filtration Rate Corrected (mL/min/1.73m^2)	85.8	81.3	81.4	-3.96 (10.72)	-5.55 (11.50)
Potassium (mmol/L)	4.4	4.5	4.5	0.04 (0.37)	0.05 (0.40)
HDLCholesterol (mmol/L)	1.2	1.2	1.2	0.02 (0.43)	0.06 (0.31)
LDLCholesterol (mmol/L)	1.9	1.9	2.0	0.01 (0.57)	0.01 (0.65)
Cholesterol (mmol/L)	3.9	4.0	3.9	0.09 (0.76)	0.06 (0.84)
Triglycerides (mmol/L)	1.9	1.8	1.7	-0.08 (0.96)	-0.24 (1.17)
Triglycerides (mg/dL)	172.5	162.0	151.8	-7.53 (85.28)	-21.27 (103.27)
Albumin/Creatinine (mg/mmol)	4.7	3.5	4.1	-0.28 (5.66)	-0.70 (7.96)
Albumin/Creatinine (mg/g)	41.6	31.3	36.3	-2.47 (50.11)	-6.16 (70.48)

Clinical Trial Registration Number: NCT02688075

Disclosure: **V. Woo:** Grants; Janssen Inc., Novo Nordisk A/S, Eli Lilly and Company, Merck Sharp & Dohme Corp, Boehringer Ingelheim Pharmaceuticals, Inc., Bristol-Myers Squibb Company, Sanofi, AstraZeneca, Johnson & Johnson Diabetes Institute, LLC., Roche Pharma, Abbott.

637

2 year metabolic outcomes in the ABCD nationwide canagliflozin audit

A. Puttanna[1], M. Yadagiri[1], P. Sen Gupta[1], I. Gallen[2], A. Bickerton[3], S. Phillips[4], A. Evans[4], D. Sennik[5], R. Ryder[1];
[1]Diabetes and Endocrinology, Sandwell and West Birmingham NHS Trust, Birmingham, [2]Diabetes and Endocrinology, Royal Berkshire Hospitals NHS Trust, Berkshire, [3]Diabetes and Endocrinology, Yeovil District Hospital NHS Trust, Yeovil, [4]Diabetes and Endocrinology, Cheltenham General Hospital, Cheltenham, [5]Diabetes and Endocrinology, Princess Alexandra Hospital, Harlow, UK.

Background and aims: The ABCD nationwide canagliflozin audit was launched in January 2016 to evaluate the efficacy of canagliflozin in a real world setting of clinical use in the United Kingdom (UK).
Materials and methods: Two year follow up data from 21 centres across the UK on 690 patients treated with canagliflozin. Male 60.2%, mean age (±SD) 58.9 ± 10.9 years, weight 101.3 ± 22.2 kg, BMI 34.0 ± 6.9, Hba1c 76.3 ± 16.3 mmol/mol. Patients with baseline, first return and second return follow up data were included in the analysis.
Results: Mean Hba1c fell by 9.0 ± 13.4 mmol/mol at first return and 11.1 ± 14.7 mmol/mol at second return ($n = 297$, $p < 0.001$) with 2.1 mmol/mol fall between first and second return ($p = 0.001$). Mean weight fell by 2.8 ± 4 kg at first return and 4.0 ± 5.4 kg at second return ($n = 242$, $p < 0.001$) with 1.3 kg fall between first and second return ($p < 0.001$).Mean alanine aminotransferase (ALT) fell by 3.8 ± 23.2 U/L at first return ($p < 0.031$) and 5.6 ± 18 U/L at second return ($n = 177$, $p < 0.001$) with 1.8 U/L fall between first and second return ($p = 0.25$). Mean systolic blood pressure (SBP) fell by 1.9 ± 15.4 mmHg at first return ($p = 0.035$) and 3.7 ± 16.2 mmHg at second return ($n = 285$, $p < 0.001$) with 1.8 mmHg fall between first and second return ($p = 0.05$). Mean diastolic blood pressure (DBP) fell by 1.0 ± 10.1 mmHg at first return ($p = 0.086$) and 2.6 ± 11.1 mmHg at second return ($n = 284$, $p < 0.001$) with 1.5 mmHg fall between first and second return ($p = 0.006$). Median range of weeks for follow up for first and second returns (IQR) were 21 (15–30) and 44.9 (34.3–58.9) for Hba1c, 26.8 (15.5–41.6) and 54.6 (38.6–75) for weight, 30 (19–48.3) and 57.6 (42.7–77.5) for ALT, 27.3 (17.4–42.7) and 53.1 (40.4–70.3) for SBP, 27.2 (17.3–42.8) and 50 (40.3–71) for DBP.
Conclusion: Canagliflozin showed statistically significant and sustained reduction in Hba1c, weight, ALT, systolic and diastolic blood pressure across a wide range of real-world UK patients with type 2 diabetes. Further benefit was seen between first and second returns with statistically significant reductions in Hba1c, weight, systolic and diastolic blood pressures.

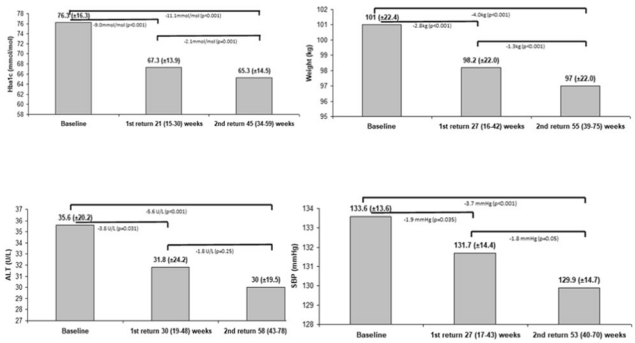


Figure: Mean (±SD) HbA1c (n=297) , weight (n=242), ALT (n=177) and systolic blood pressure (n=285), baseline vs first and second return (after median (interquartile range) weeks) to clinic following commencement of canagliflozin.

Disclosure: **A. Puttanna:** None.

638

Temporal trends in the use of sodium-glucose cotransporter-2 inhibitors: the global DISCOVER study

L. Ji[1], H. Chen[2], J. Cid-Ruzafa[3], P. Fenici[4], M.B. Gomes[5], N. Hammar[6], K. Khunti[7], S. Pocock[8], M.V. Shestakova[9], I. Shimomura[10], F. Surmont[11], F. Tang[12], M. Kosiborod[12];
[1]Peking University People's Hospital, Beijing, China, [2]AstraZeneca, Gaithersburg, USA, [3]Evidera, Barcelona, Spain, [4]AstraZeneca, Cambridge, UK, [5]Rio de Janeiro State University, Rio de Janeiro, Brazil, [6]AstraZeneca Gothenburg, Mölndal, Sweden, [7]University of Leicester, Leicester, UK, [8]London School of Hygiene and Tropical Medicine, London, UK, [9]Endocrinology Research Center, Diabetes Institute, Moscow, Russian Federation, [10]Osaka University, Osaka, Japan, [11]AstraZeneca, Luton, UK, [12]Saint Luke's Mid America Heart Institute, Kansas City, USA.

Background and aims: Sodium-glucose cotransporter-2 inhibitors (SGLT-2is) reduce the risk of cardiovascular (CV) events in patients with type 2 diabetes (T2D), first demonstrated in the EMPA-REG OUTCOME trial (EMPA-REG). We examined trends in the use of SGLT-2is during the first year of DISCOVER, a 3-year observational study of patients with T2D initiating second-line glucose-lowering therapy in 37 countries.
Materials and methods: Patients who weren't receiving an SGLT2i at first-line and who had data on glucose-lowering medications at baseline, 6 and 12 months were included ($N = 11\,706$). We assessed the numbers and characteristics of patients who were initiated on an SGLT-2i (initiation of second-line therapy at baseline, or later-line therapy during the first year of follow-up) overall and according to whether the date of initiation was before or after EMPA-REG.
Results: Overall, 1138 patients were prescribed an SGLT-2i at baseline or during follow-up (9.7%; across-country range: 0.0–68.9%). There were no substantial differences in patient characteristics among those who were prescribed an SGLT-2i before or after EMPA-REG: 10.4% (before) and 14.4% (after) of patients had a history of macrovascular complications, compared with 12.9% of patients prescribed a different class of medication ($p = 0.24$). At baseline (initiation of second-line therapy), the proportion of patients who received an SGLT-2i was higher when the initiation occurred after vs before EMPA-REG (8.4% vs 5.9%, $p < 0.001$).
Conclusion: The overall proportion of patients prescribed an SGLT-2i was low, but varied greatly across countries. SGLT-2i use increased modestly after EMPA-REG. Although the CV benefits of SGLT-2i use have been best demonstrated in people with established macrovascular disease,

the clinical decision to prescribe these agents does not appear to be primarily driven by the presence of macrovascular complications.
Clinical Trial Registration Number: NCT02322762
Supported by: AstraZeneca
Disclosure: **L. Ji:** Grants; Roche, Sanofi, Merck Sharp & Dohme, AstraZeneca, Novartis, Eli Lilly, Bristol-Myers Squibb. Honorarium; Eli Lilly, Bristol-Myers Squibb, Novartis, Novo Nordisk, Merck Sharp & Dohme, Takeda, Sanofi, Roche, AstraZeneca, Bayer, Boehringer Ingelhiem.

639

Economic evaluation of dapagliflozin as add-on to metformin in type 2 diabetes in the Israeli healthcare setting

S. Moshel, M. Hirsch Vexberg, O. Shavit, Y. Toledano;
Meuhedet health fund, Tel Aviv, Israel.

Background and aims: Dapagliflozin is a potential for combination therapy with metformin in T2DM. However, its cost-effectiveness relative to other alternatives in the Israeli healthcare setup remains unknown.The purpose of this work was to evaluate the cost-effectiveness of dapagliflozin 10 mg as add-on to metformin, compared to common alternatives (sitagliptin 100 mg, glimepiride 2 mg, liraglutide 1.2 mg) based on Meuhedet health services database.
Materials and methods: A cost-effectiveness evaluation was performed using the Cardiff diabetes model. A cohort of 1000 T2DM patients (ages 21 and older) who had received additional medication to metformin was randomly chosen from Meuhedet's database. Baseline values for demographic and clinical variables prior to the add-on therapy, along with data from clinical trials served as inputs to the model. Simulation was performed for each drug, calculating its total costs and benefits (QALYs). The model's time horizon was set to 40 years, annual discount rate for both costs and benefits was 3.5% and incremental cost effectiveness ratio (iCER) threshold was £20,000/QALY. Finally, single-variable and multivariable sensitivity analyses were performed.
Results: In the base-case scenario, dapagliflozin was found cost-effective compared to sitagliptin, liraglutide and glimepiride (iCER values of £1,232, £-16,517 and £13,476, respectively). For all comparisons, iCER was driven by differences in costs, while differences in QALYs were minimal. Dapagliflozin remained cost-effective even after performing sensitivity analyses. However, when performing the simulation under comparators' generic competition scenario, dapagliflozin was no longer cost-effective compared to liraglutide (iCER = £24,900/QALY).
Conclusion: Dapagliflozin as add-on treatment to metformin was cost-effective compared to several alternatives in T2DM patients in Israel's healthcare system. Additional research is needed in order to evaluate the effects of evolving new clinical data.
Supported by: AstraZeneca
Disclosure: **S. Moshel:** Grants; AstraZeneca.

PS 048 Glycaemic and metabolic effects of SGLT2 inhibitors

640

DAPADream: improvement of time in range after SGLT2-add-on-medication in youth and young adults with type 1 diabetes during unannounced meals under full closed loop CSII

T. Biester[1], A.K. Nieswandt[1], S. Biester[1], K. Remus[1], I. Muller[2], E. Atlas[2], M. Philip[3], R. Nimri[3], T. Battelino[4], N. Bratina[4], K. Dovc[4], M.F. Scheerer[5], O. Kordonouri[1], T. Danne[1];
[1]Diabetes-Centre for Children and Adolescents, AUF DER BULT, Hannover, Germany, [2]DreaMed Diabetes Ltd., Petah Tikvah, Israel, [3]Schneider Children's Medical Center, Petah Tikvah, Israel, [4]University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia, [5]Astra Zeneca GmbH, Wedel, Germany.

Background and aims: Therapeutic aims such as HbA_{1c} are often hard to reach - particularly in youth and young adults with Type 1 Diabetes (T1D). The SGLT2-inhibitor dapagliflozin (DAPA) has been submitted for regulatory approval in the EU as adjunct to insulin therapy in adult T1D patients to improve glycemic control. The CE-marked DreaMed Substance Administration System© with a fuzzy logic closed loop algorithm was proven to be safe and effective in hybrid closed loop settings. In full closed loop (FCL) setting, postprandial time has so far been associated with a prolonged phase of hyperglycemic excursions. The aim of this trial is to investigate the effect of DAPA on glucose levels overnight and after two unannounced meals under FCL conditions.
Materials and methods: For this monocentric, double-blind, randomized, placebo-controlled cross-over trial, eligible patients (T1D, CSII, non-severe obese) were admitted to the research center on two occasions for 24 hours. 10 mg DAPA or placebo were administered twice: First in the evening and second on the following morning. After the latter application, two consecutive mixed meal tests (MTT) were performed 6 hours apart. Glucose control was achieved automatically by DreaMed FCL. Primary outcome was "Time in Range 70–180 mg/dl" (TIR). For safety, ß-Hydroxybutyrate (BHB), Glucagon, Insulin, Proinsulin and GIP were measured during the first MTT.
Results: 15 adolescents [age 15.3 ± 1.5 years, diabetes duration (DD) 9.8 ± 3.5 years, HbA1c 8.3 ± 0.9%] and 15 young adults [19.0 ± 0.8; DD 11.9 ± 4.3; 8.4 ± 1.0%] completed the trial. In both age groups, TIR increased significantly in the DAPA-arm compared to placebo (adolescents 68.7 ± 6.6 vs. 50.7 ± 11.9% $p < 0.001$; adults 66.5 ± 6.9 vs. 49.8 ± 14.0%, $p = 0.001$). In the combined analysis, nocturnal glucose was significantly lower with DAPA versus placebo (112 ± 15 vs. 132 ± 30 mg/dl; $p = 0.003$) without an increase in hypoglycemic episodes (3.3 ± 6.0 vs 3.1 ± 5.2% <70 mg/dl, $p = 0.75$).Urinary glucose excretion was increased threefold using DAPA (135.4 ± 42.3 vs. 44.4 ± 18.8 g/24 h). However, no abnormal elevated BHB-values were observed using the FCL Setting.
Conclusion: The SGLT2 inhibitor was able to improve glycemic control by increasing TIR on average by 259 minutes per day and reducing glycemic variability significantly without any signals for hypoglycemia or ketoacidosis. For achieving in-range post prandial glucose control after MTT challenges during FCL condition, pre-meal boluses should be considered even if using DAPA.

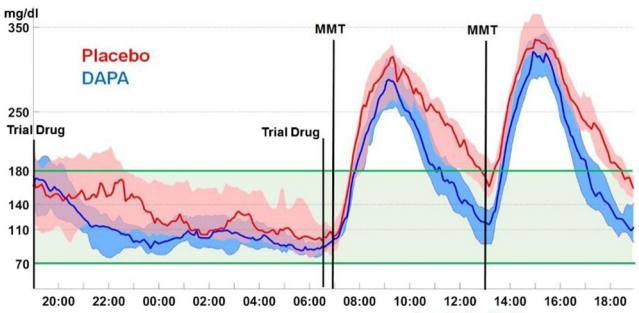


Fig.1: Median (25., 75. Quart.) Glucose Sensor curves under Full closed loop control with Dapagliflozin or Placebo

Clinical Trial Registration Number: 2016-002212-41
Disclosure: **T. Biester:** Honorarium; Medtronic, DexCom, Ypsomed.

641

Effect of combination therapy with liraglutide plus canagliflozin on HGP, plasma hormones and HbA_{1c} versus each therapy alone in type 2 diabetes

M. Abdul-Ghani, A. Ali, R. Martinez, J. Adams, E. Cersosimo, C. Triplitt, R.A. DeFronzo;
UTHSCSA, San Antonio, USA.

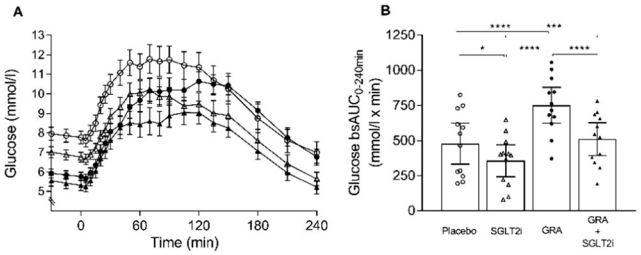
Background and aims: We previously have shown that SGLT2 inhibitors cause an increase in HGP accompanied with an increase in plasma glucagon concentration. We hypothesized that the increase in plasma glucagon concentration is, at least in part, responsible for the increase in HGP. The aim of the present study was to examine whether inhibition of glucagon secretion by liraglutide can prevent the increase in HGP.
Materials and methods: 51 T2DM patients (age = 51 ± 1 yr; 40% female; BMI = 34.6 ± 0.7; diabetes duration = 6.8 ± 0.8 yr; FPG = 175 ± 7; HbA1c = 8.3 ± 0.1%) were randomized to receive for 16 weeks: (i) canagliflozin 300 mg; (ii) liraglutide 1.8 mg; or (iii) canagliflozin 300 mg plus liraglutide 1.8 mg. HGP (measured with 3-^{3}H-glucose infusion) and plasma glucagon concentration were measured before and after 16 weeks of treatment.
Results: Canagliflozin monotherapy caused a significant reduction in HbA1c (−1.1 ± 0.2%, $p < 0.01$) accompanied with an increase in plasma glucagon concentration (by 28%, $p < 0.05$) and HGP (by 15%, $p < 0.05$) which lasted for 16 weeks. Conversely, liraglutide monotherapy caused a 1.6 ± 0.5% ($p < 0.01$) reduction in HbA1c accompanied by a small (6%) reduction in HGP (P=NS) without significant change in fasting plasma glucagon concentration. The combination of canagliflozin plus liraglutide caused a greater reduction in HbA1c (1.9 ± 0.5%, $p < 0.05$ vs canagliflozin and p = NS vs liraglutide) and attenuated the increase in fasting plasma glucagon and basal HGP at 16 weeks.
Conclusion: These results: (1) support a possible role for increased plasma glucagon levels in the long term maintenance of increase in HGP caused by SGLT2i, and (2) suggest that factors other than/in addition to glucagon contribute to the initiation of the increase in HGP.
Clinical Trial Registration Number: NCT02324842
Supported by: Janssen Pharmaceutical
Disclosure: **M. Abdul-Ghani:** None.

642

Individual and combined glucose-lowering effects of glucagon receptor antagonism and sodium-glucose cotransporter 2 inhibition

S. Haedersdal[1], A. Lund[1], H. Maagensen[1], E. Nielsen-Hannerup[1], J. Holst[2], F. Knop[1,3], T. Vilsbøll[4,3];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Hellerup, [2]Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, [3]Department of Clinical Medicine, Health and Medical Sciences, Copenhagen University, Copenhagen, [4]Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: Studies suggest that sodium-glucose cotransporter 2 inhibitors (SGLT-2i) induce hyperglucagonaemia, which might counteract its glucose-lowering ability in type 2 diabetes.
Materials and methods: In a randomised, placebo-controlled, double-dummy, double-blinded, cross-over study, 12 patients with type 2 diabetes (Age [mean (SD)]: 59.5 (5.8) years, BMI 30.3 (5.6) kg/m^2; HbA1c: 47.3 (6.2) mmol/mol) underwent four 4-hour liquid mixed meal tests preceded by single-dose administration: 1) Placebo, 2) SGLT-2i (25 mg empagliflozin) 2 hours before the meal, 3) glucagon receptor antagonist (GRA) (300 mg LY2409021) 10 hours before the meal, and 4) GRA + SGLT-2i. Indirect calorimetry, plasma glucose, C-peptide, glucagon and paracetamol (paracetamol absorption test) was measured. Stable isotopes were infused for glucose and glycerol turnover and endogenous glucose production.
Results: The SGLT-2i and GRA individually lowered fasting plasma glucose (FPG) compared to placebo and the combination further decreased FPG. SGLT-2i reduced postprandial glucose excursions as assessed by baseline-subtracted area under curve (bsAUC) whereas GRA increased bsAUC compared to placebo (Figure). The paradoxical GRA-induced increment in bsAUC was annulled by a potent SGLT-2i-induced reduction of bsAUC during the combination of SGLT-2i and GRA (Figure).
Conclusion: The SGLT-2i and GRA combined reduce FPG beyond their individual capacity in patients with type 2 diabetes, and in the context of GRA, SGLT-2i's beneficial effect on postprandial glucose excursions seems to be potentiated.



Plasma glucose excursions (A) following a mixed meal tolerance test preceded by single doses of 1) placebo (open circles), 2) SGLT2i (open triangles), 3) Glucagon receptor antagonist (GRA) (filled circles) and 4) GRA+SGLT2i (filled triangles). Baseline-subtracted AUCs (bsAUC) (B) of the postprandial glucose excursion. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

Clinical Trial Registration Number: NCT02792400
Supported by: Danish Diabetes Association supported by the Novo Nordisk Foundation
Disclosure: **S. Haedersdal:** None.

643

Continuous glucose monitoring glycaemic profiles are more favourable for dapagliflozin plus saxagliptin compared to glimepiride when added to metformin in type 2 diabetes

D.C. Simonson[1], M.A. Testa[2], J. Maaske[3], R. Garcia-Sanchez[3], E. Johnsson[4], M. Su[2], J.P. Frias[5];
[1]Endocrinology, Brigham and Women's Hospital, Boston, [2]Biostatistics, Harvard T. H. Chan School of Public Health, Boston, [3]AstraZeneca, Gaithersburg, [4]AstraZeneca, Mölndal, Sweden, [5]National Research Institute, Los Angeles, USA.

Background and aims: Within-day glycaemic excursions and variability characterized by short-term oscillations in glucose are gaining increasing clinical interest independent of A1C and hypoglycaemia in treatment of patients with type 2 diabetes (T2D). This study compares glycaemic profiles of dapagliflozin (DAPA) plus saxagliptin (SAXA) versus glimepiride (GLIM) when added to metformin (MET).
Materials and methods: Masked continuous glucose monitoring (CGM) data were analyzed from patients with T2D inadequately controlled on MET in a substudy of n = 118 of 443 patients participating in an international randomized, 52-week, double-blind trial of DAPA co-administered with SAXA (n = 61) compared to GLIM (n = 57) when added to MET. Six-day CGM (288 glucoses/day) and A1C were measured at baseline and Week 52.
Results: At baseline, patients had mean ± SD age 55.1 ± 10.7 years, A1C 8.7 ± 0.8%, diabetes duration 10.3 ± 6.7 years, and were 45.8% male. Using linear mixed models, the primary CGM substudy endpoint, Week 52 change from baseline in the mean amplitude of glycaemic excursion (MAGE) for Week 52 per protocol (PP) completers not requiring rescue medication (addition of insulin or other glucose-lowering agent), favored

DAPA+SAXA+MET (mean ± SE −0.89 ± 0.22 mmol/L) vs GLIM+MET (0.40 ± 0.26 mmol/L), nominal $p = 0.0003$. Secondary CGM measures also favored DAPA+SAXA+MET by demonstrating lower mean glucose and variability (see Table). One subject (1.6%) in DAPA+SAXA+MET and 8 (14.0%) in the GLIM+MET required rescue therapy prior to 52 weeks, $p = 0.033$. For the intent to treat (ITT) sample including all subjects with evaluable Week 52 data, the differences were more pronounced (see Table). The percent time spent in hypoglycaemia was low in both groups and did not show a difference. In the substudy, 52-week A1C changes were −1.43 ± 0.22% for DAPA+SAXA+MET and −1.10 ± 0.21% for GLIM+MET. In the full study population corresponding changes were −1.35 ± 0.07% for DAPA+SAXA+MET ($n = 193$) vs −0.98 ± 0.07% for GLIM+MET ($n = 171$), $p < 0.001$.

Conclusion: In this substudy, CGM glycaemic profiles and variability measures demonstrated relatively strong differences between the two therapeutic regimens that were not apparent using A1C alone. Subjects on DAPA+SAXA+MET had lower average 24-hour glucose levels, less glycaemic variability, and spent more time in the normal glucose range and less time with hyperglycaemia as compared to subjects on GLIM+MET. The CGM summary measures and indices that are derived from diagnostic CGM can provide important clinical insight for optimizing diabetes treatment regimens and should be used to supplement the standard measures of A1C, fasting plasma glucose and patient reports of hypoglycaemic episodes.

Change from Baseline in CGM Measures at Week 52 for Per Protocol (PP) Sample [LS Mean (SE)]

CGM Endpoint	DAPA+SAXA+MET (N = 46)	GLIM+MET (N = 32)	p-value (PP)	p-value (ITT)[3]
MAGE[1] (mmol/L)	-0.89 (0.22)	+0.40 (0.26)	0.0003	< 0.0001
Mean 24-hr glucose (mmol/L)	-2.32 (0.25)	-1.41 (0.30)	0.0244	0.0005
Within-Subject/Day SD (mmol/L)	-0.35 (0.08)	0.10 (0.09)	0.0004	< 0.0001
Nocturnal Within-Subject/Day SD (mmol/L)	-0.23 (0.05)	0.04 (0.07)	0.0018	0.0002
Nocturnal Within-Subject, Between Day SD	-8.29 (1.62)	-1.62 (1.73)	0.0639	0.0065
% time glucose 3.9 mmol/L to 10.0 mmol/L	30.28 (3.05)	17.02 (3.62)	0.0067	< 0.0001
% time glucose < 3.9 mmol/L	0.41 (0.28)	0.79 (0.33)	0.3887	0.5923
GRADE[2]	-5.43 (0.58)	-3.15 (0.69)	0.0144	0.0002
High Blood Glucose Index (HBGI)	-6.43 (0.70)	-3.46 (0.83)	0.0081	< 0.0001

Footnotes: [1]Mean Amplitude Glycaemic Excursion, [2]Glycaemic Risk Assessment Diabetes Equation, [3] Intent to Treat sample p-value with N = 47 in DAPA+SAXA+MET and N = 42 in GLIM+MET

Clinical Trial Registration Number: NCT02419612
Supported by: AstraZeneca
Disclosure: **D.C. Simonson:** None.

644

Head to head comparison of efficacy between dapagliflozin and canagliflozin in long standing type 2 diabetes

C. Mahesh Babu;
Diabetology, Meenakshi mission hospital and research centre, Madurai, India.

Background and aims: the study is done in patients with long standing diabetes for more than 10 years. we compared the efficacy of dapagliflozin and canagliflozin in reducing the fasting plasma glucose (FPG), postprandial plasma glucose (PPG), glycosylated hemoglobin (HbA1c), body weight (BW) and systolic BP (SBP).

Materials and methods: 200 type 2 diabetes patients were randomized into two groups each group having 100 patients. One gropu was given dapagliflozin 10 mg and other was given canagliflozin 100 mg per day in addition to the existing oral drugs. All patients were already on sulphonylurea and metformin combination without good control. All patients had HbA1c more than 10%. Both the groups were matched on gender and age distribution. Both groups were followed for one year and results were analysed.

Results: FPG dropped from a baseline of (180 ± 58 mg/dL) to (97 ± 9 mg/dL) in dapagliflozin group and (158 ± 45 mg/dL) to (94 ± 9 mg/dL) in canagliflozin group. PPG dropped from a baseline of (289 ± 86 mg/dL) to (163 ± 22 mg/dL) in dapagliflozin group and (267 ± 76 mg/dL) to (159 ± 22 mg/dL) in canagliflozin group. HbA1c dropped from a baseline of (9.9 ± 1.9%) to (7.4 ± 0.6%) in dapagliflozin group and (9.5 ± 1.7%) to (7.4 ± 0.7%) in canagliflozin group. 4 mmHg systolic BP reduction was seen in both the groups.Body weight dropped from a baseline of (72 ± 12 kg) to (69 ± 11 kg) in dapagliflozin group and (74 ± 12 kg) to (70 ± 10 kg) in canagliflozin group. Incidence of genital mycotic infection(GMI) was 2% in dapagliflozin group and 3% in canagliflozin group. Incidence of urinary tract infection (UTI) was 3% in both the groups. The UTI and GMI were occuring only during the first 3 months of therapy

Conclusion: Both dapagliflozin and canagliflozin are equally effective in long standing diabetes with uncontrolled hyperglycemia when added to existing oral drugs. They produced a meaningful reduction in FPG, PPG, HbA1c, BW and SBP. The incidence of UTI and GMI are negligible after first 3 months of therapy.

Disclosure: **C. Mahesh Babu:** None.

645

Dose-ranging effects of SGLT2 inhibitors in patients with type 2 diabetes: a systematic review and meta-analysis

L.C. Pinto, D.R. Rados, L.R. Remonti, M.V. Viana, C.B. Leitão, J.L. Gross;
Endocrine Division, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Background and aims: The lowest dosage of empagliflozin (10 mg) produced similar benefits on glycated hemoglobin (HbA1c), body weight, blood pressure, total and cardiovascular mortality in comparison with the highest available dose (25 mg) in EMPAREG trial. It is uncertain if canagliflozin and dapagliflozin behave similarly. Aims: To compare the effect of different doses of SGLT2 inhibitors in HbA1c and body weight of patients with type 2 diabetes.

Materials and methods: MEDLINE, Cochrane and Embase databases were searched for randomized controlled trials of SGLT2 inhibitors in type 2 diabetes patients, lasting at least 12 weeks. HbA_{1c} and body weight variations were described as standard mean difference. We performed direct, indirect meta-analysis, as well as a metarregression with medications' doses as covariates.

Results: Eighteen studies were included (16,095 patients). In direct meta-analysis, canagliflozin, dapagliflozin and empagliflozin lead to similar effects on HbA_{1c} (−0.62%; 95% CI −0.66 to −0.59) and body weight (−0.60 kg; 95% CI −0.64 to −0.55). Indirect meta-analysis showed that canagliflozin 300 mg had the greatest effect in both HbA1c and body weight reduction (−0.79%; 95% CI −0.84 to −0.75; −2.35 kg; 95% CI −2.73 to −1.97), however the differences from the other medications or dosages was small and probably not clinically relevant (−0.15 to −0.44% in HbA1c and −0.28 to −1.04 kg in body weight). All SGLT2 inhibitors in different doses were associated with similar increased risk for genital infections.

Conclusion: Different doses of SGLT2 inhibitors results in similar reductions of HbA1c and body weight. Whether these glycemic and weight effects reflect on mortality and cardiovascular events is still uncertain and may be a topic of further studies.

Clinical Trial Registration Number: CRD42015006975
Supported by: CNPq
Disclosure: **L.C. Pinto:** None.

646

Comparison of ipragliflozin and metformin for visceral fat reduction in elderly patients with type 2 diabetes: a prospective, blinded-endpoint, randomised controlled study

M. Koshizaka, K. Ishikawa, R. Ishibashi, H. Yoko, K. Sakamoto, T. Ishikawa, Y. Maezawa, M. Takemoto, K. Yokote, PRIME-V Study Group;
Chiba University Hospital, Chiba, Japan.

Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors have low risk of side effects, such as hypoglycemia, and are often used as the first drug of choice in elderly patients with type 2 diabetes (T2DM) in Japan. However, T2DM in some patients is not controlled sufficiently with DPP-4 inhibitor and requires multiple drug treatment. To determine the effects of either sodium glucose transporter 2 (SGLT2) inhibitor or metformin on reducing visceral fat and other effects in Asian elderly patients with T2DM inadequately controlled with DPP-4 inhibitor, we performed a sub-analysis of a prospective, multicenter, blinded-endpoint, randomized controlled study on elderly patients aged 65 and older.
Materials and methods: The study was conducted to evaluate the efficacy of treatment with SGLT2 inhibitor (ipragliflozin) or metformin added to sitagliptin for reducing visceral fat and glucose control in 103 patients with T2DM. Patients with T2DM, taking sitagliptin for more than 12 weeks and having BMI of ≥22 kg/m^2 and HbA1c of ≥7% and <10% were included. They were randomized (1:1) to receive ipragliflozin (50 mg/day) or metformin (1000–1500 mg/day). The primary outcome was the rate of change in visceral fat area, measured using computed tomography (CT) after 24 weeks of therapy. Two radiologists, blinded to the information, analyzed the images. The secondary outcome included total and subcutaneous fat area, muscle volume, bone mineral density measured by CT, fasting blood glucose, insulin level, HbA1c, and grip strength. Among the patients, we selected those aged ≥65 years for sub-analysis.
Results: The reduction rate in visceral fat area was significantly greater in the ipragliflozin group than that of the metformin group (−12.1% vs. −3.7%, $P < 0.05$). Sub-analysis was performed with a full analysis set of 14 patients in the ipragliflozin group and 14 patients in the metformin group, and no difference was found between the two groups. Patients mean age of 69.2 were included. The mean HbA1c was 8.0 ± 0.8 percent. The rate of reduction in visceral fat area was significantly greater in the ipragliflozin group than the metformin group (−16.5% vs. 0.6%, $P = 0.02$). There was no significant difference in changes on muscle volume and bone mineral density between the groups. However, grip strength in metformin group improved more compared to the ipragliflozin group (−1.8% vs. 11.4%, $P = 0.01$).
Conclusion: As the second agent to be used in combination with DPP-4 inhibitors, ipragliflozin significantly reduced the visceral fat area compared with metformin. For elderly patients with the risk of sarcopenia, metformin might increase grip strength; and ipragliflozin could reduce visceral fat for elderly patients with abdominal obesity. Our results can help with drug selection for the Asian elderly patients with T2DM inadequately controlled on DPP-4 inhibitor.

	Ipragliflozin		Metformin		
	Mean	SD	Mean	SD	*P*-value
Age (year)	68.6	2.5	69.2	2.8	0.57
BMI (kg/m^2)	26.5	3.7	27.2	3.9	0.67
Female sex (%)	57.1		50.0		0.70
Visceral fat area change rate (%)	-16.5	17.5	0.6	18.6	0.02
Subcutaneous fat area change rate (%)	-2.4	14.1	5.7	24.5	0.30
Total fat area change rate (%)	-9.5	13.6	3.7	18.5	0.04
Waist circumference change rate (%)	-1.8	3.6	0.3	5.9	0.25
Bone mineral density change rate (%)	0.0	10.3	-4.5	10.4	0.27
Muscle volume change rate (%)	-3.3	6.4	-1.5	3.1	0.35
HbA1c change rate (%)	5.8	9.2	10.7	9.8	0.19
Fasting blood glucose change rate (%)	9.9	24.1	16.3	13.3	0.39
Insulin level change rate (%)	-61.2	240.9	17.8	33.6	0.29
Hand grip strength change rate (%)	-1.8	8.6	11.4	15.1	0.01

Clinical Trial Registration Number: UMIN000015170
Supported by: Astellas Pharma Inc.
Disclosure: **M. Koshizaka:** Grants; Astellas Pharma Inc.

647

Dapagliflozin plus saxagliptin add-on to metformin reduces liver fat and adipose tissue volume in patients with type 2 diabetes

L. Johansson[1], P. Hockings[1], E.K. Johnsson[2], J. Maaske[3], R. Garcia-Sanchez[3], J.P. Wilding[4];
[1]Antaros Medical AB, Bioventure Hub, Mölndal, Sweden, [2]Global Medicines Development, AstraZeneca, Gothenburg, Sweden, [3]Global Medicines Development, AstraZeneca, Gaithersburg, USA, [4]Obesity & Endocrinology Research Group, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK.

Background and aims: Oral administration of a fixed-dose combination of the sodium glucose co-transporter (SGLT)-2 inhibitor dapagliflozin (DAPA) and the dipeptidyl peptidase 4 (DPP-4) inhibitor saxagliptin (SAXA) is approved for improving glycaemic control in adult patients with type 2 diabetes (T2D).
Materials and methods: A 52-week, multicentre, randomised, double-blind, parallel-group trial evaluated the efficacy and safety of DAPA 10 mg/day + SAXA 5 mg/day vs titrated glimepiride (GLIM) 1–6 mg/day in 443 patients with T2D (A1C, 7.5%–10.5%) on metformin (MET) ≥1500 mg/day background. In a sub-study, we used magnetic resonance imaging (MRI) to assess effects on liver fat (proton density fat fraction [PDFF]) and visceral and subcutaneous adipose tissue volumes over 52 weeks of treatment. An MRI was performed on 59 patients; liver fat and adipose tissue volumes were analysed for 59 and 57 patients, respectively.
Results: There was a significant >30% reduction from baseline in liver fat ($P = 0.007$) and a >10% reduction in visceral and subcutaneous adipose tissue volumes ($P < 0.01$) with DAPA + SAXA + MET at week 52 vs GLIM + MET (Table). In the full study population, DAPA + SAXA + MET decreased body weight and serum levels of alanine aminotransferase and aspartate aminotransferase over 52 weeks.
Conclusion: DAPA + SAXA significantly decreased liver fat and adipose tissue volume vs GLIM, and reduced serum liver enzyme levels, indicating a favourable metabolic profile of DAPA + SAXA in patients with T2D on MET therapy.

Table

	DAPA + SAXA + MET	GLIM + MET	*P* value*
Sub-study	**n=35**	**n=24**	
Mean ± SD liver fat (PDFF%)			
Baseline	14.3 ± 6.4	13.7 ± 8.3	
Week 52	9.9 ± 7.1	12.9 ± 8.6	0.007
Mean ± SD visceral adipose tissue (L)			
Baseline	3.6 ± 1.1	2.9 ± 1.1	
Week 52	3.2 ± 1.1	3.0 ± 1.1	<0.001
Mean ± SD subcutaneous adipose tissue (L)			
Baseline	4.7 ± 2.2	4.1 ± 1.8	
Week 52	4.2 ± 2.0	4.1 ± 1.7	0.006
Full study	**N=227**	**N=216**	
Mean ± SD body weight (kg)			
Baseline	90.8 ± 19.7	88.4 ± 17.0	
Week 52	88.4 ± 18.1	90.6 ± 17.4	<0.001
Mean ± SD serum ALT concentration (U/L)			
Baseline	28.2 ± 15.4	28.7 ± 15.7	
Change at week 52 (95% CI)	−5.3 (−6.9, −3.6)	2.1 (−0.2, 4.4)	
Mean ± SD serum AST concentration (U/L)			
Baseline	22.5 ± 11.2	23.5 ± 12.2	
Change at week 52 (95% CI)	−2.4 (−3.7, −1.2)	1.4 (−0.9, 3.7)	

*Nominal *P* value for least squares mean difference in change from baseline to week 52 between groups
ALT: alanine aminotransferase; AST: aspartate aminotransferase; DAPA: dapagliflozin; GLIM: glimepiride; MET: metformin; PDFF: proton density fat fraction; SAXA: saxagliptin

Clinical Trial Registration Number: NCT02419612
Supported by: AstraZeneca Pharmaceuticals
Disclosure: **L. Johansson:** Employment/Consultancy; Antaros Medical.

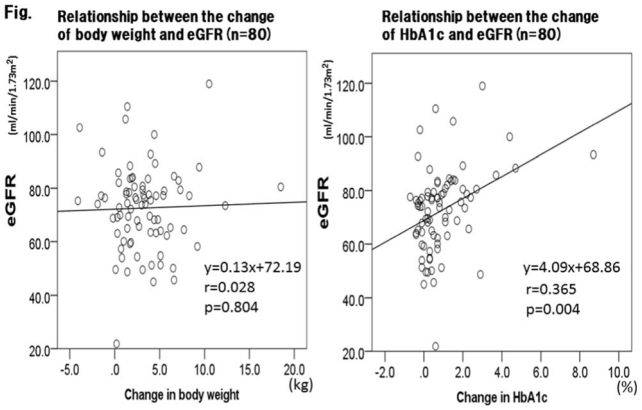
648

An inhibitor of sodium-glucose cotransporter 2 shows a rational effect for reducing body weight but not for lowering plasma glucose in type 2 diabetes with mild renal failure

S. Taneda, K. Tsuchida, H. Bando, S. Hagiwara, Y. IIjima, K. Misawa, M. Kikuchi, K. Ohta, H. Nakayama, N. Manda;
Internal Medicine, Manda Memorial Hospital, Sapporo, Japan.

Background and aims: Sodium-glucose cotransporter 2 (SGLT2) inhibitor reduces rates of hyperglycemia and body weight in patient with type 2 diabetes (T2DM) by decreasing renal glucose reabsorption, thereby increasing urinary glucose excretion. However, the effects supposed to be impaired in the state of renal failure , because It does not reach the target, i.e. SGLT2 existed on the membrane of proximal tubular epithelium. So, treatment with SGLT2 inhibitor for the T2DM patient with renal failure is not recommended in Japan. In addition, the effects of reducing plasma glucose and body weight may not be paralleled each other. In this presentation, we try to clarify the difference between the two effects of SGLT2 inhibitor In T2DM with mild renal failure.
Materials and methods: 80 T2DM patients (male 54, female 26) were newly administrated standard dose of SGLT2 inhibitors (Dapagliflozin 32, Empagliflozin 23, Tofogliflozin 22,Ipragliflozin 3). Their estimated glomerular filtration rate (eGFR) was distributed from 21.9 to 110.5 ml/min/1.73 m², and we devided into 2 groups i.e. 64 cases of normal renal function group (eGFR >60) and 16 cases of mild renal failure group (eGFR <60). The changes of plasma glucose and body weight were tested every month by 6 months in both groups, and the relationship between the changes of these parameters and their renal function before intake of SGLT2 inhibitors was analyzed.
Results: HbA1c lowering effects is more markedly in normal renal function group than in mild renal failure group (−1.03% ± 1.48 vs −0.43% ± 0.72), contrary body weight was reduced equally in both groups (−3.3 kg ± 2.7 vs −3.2 kg ± 3.6). Renal function was unchanged during these 6 months except one month after administration in both groups. By Analyzing in all cases, we found that body weight was reduced regardless of eGFR, however, the decrease of HbA1c was correlated with eGFR (Fig).
Conclusion: SGLT2 inhibitor is effective even in T2DM patients with mild renal failure, especially body weight lowering effects is shown regardless of renal function. Body weight lowering effects of SGLT2 inhibitor seems to be due to an accumulation of energy loss, contrary plasma glucose lowering effect reflects the balance of the amount of urinary glucose extraction and plasma glucose. SGLT2 inhibitor has various clinical effects and each effect does not appear in a parallel. It is very important to consider which effects will be expected for the T2DM patients mostly when we use SGLT2 inhibitors, especially for the patients with an impairment of renal function.

Fig.



Disclosure: **S. Taneda:** None.

PS 049 Novel aspects of SGLT2 inhibitors

649

Effects of sodium glucose cotransporter-2 inhibitors on circulating stem and progenitor cells in patients with type 2 diabetes

B. Bonora, R. Cappellari, A. Avogaro, G. Fadini;
University of Padova, Padova, Italy.

Background and aims: Reduction in the levels of circulating stem cells (CSCs) and endothelial progenitor cells (EPCs) predicts development or progression of micro- and macroangiopathy in patients with type 2 diabetes (T2D). Since sodium glucose cotransporter-2 (SGLT2) inhibitors improve cardiovascular and renal outcomes, we tested whether treatment with SGLT2 inhibitors affects the levels of CSCs and EPCs in patients with T2D.
Materials and methods: Thirty-one patients with T2D were randomized to receive dapagliflozin 10 mg ($n = 16$) or placebo ($n = 15$) for 3 months. CSCs ($CD34^+$) and EPCs ($CD34^+KDR^+$) were measured by flow cytometry at baseline, at 3 months and after an open-label extension period. CSCs and EPCs were also quantified at baseline and after 3 months of open-label treatment with empagliflozin 10 mg ($n = 15$).
Results: After 3 months, CSCs non-significantly declined in the dapagliflozin group (from 321 ± 25 to 270 ± 29/10^6; $p = 0.13$) and remained stable in the placebo group (from 328 ± 29 to 332 ± 28/10^6; $p = 0.88$), but the change from baseline was not significantly different between the two groups ($p = 0.21$). EPCs non-significantly declined in the dapagliflozin group (from 12.9 ± 2.1 to 8.7 ± 1.9/10^6; $p = 0.11$) and non-significantly increased in the placebo group (from 10.5 ± 1.9 to 15.9 ± 3.1/10^6; $p = 0.19$), and the change from baseline was significantly different between the two groups ($p = 0.042$). After an open-label extension period of 12 months, CSCs remained stable over time (reaching 294 ± 35/10^6 in the dapagliflozin and 357 ± 29/10^6 in the placebo group), while EPCs significantly increased in patients who received dapagliflozin during the randomized and/or open-label periods (26.6 ± 6.6/10^6; $p = 0.047$ versus baseline; $p = 0.033$ versus 3 months). In all patients, irrespectively of treatment, EPCs increased significantly from baseline to end of observation, concomitantly with improvement in HbA1c, which declined from 8.2 ± 0.1 to 7.7 ± 0.3% ($p < 0.001$). In a cohort of 15 patients who received open-label empagliflozin for 3 months, CSCs non-significantly declined (from 281 ± 35 to 222 ± 31/10^6; $p = 0.22$), whereas EPCs remained stable (from 11.0 ± 1.8 to 11.5 ± 1.6/10^6; $p = 0.82$).
Conclusion: During 3 months of treatment, SGLT2 inhibitors do not significantly increase CSCs or EPCs. Improvement in glucose control over >1 year, is associated with a significant increase in EPC levels
Clinical Trial Registration Number: NCT02327039
Disclosure: **B. Bonora:** None.

650

Dapagliflozin preserves renal vasodilating capacity in hypertensive patients with type 2 diabetes

R. Bruno, L. Giannini, A. Dardano, E. Biancalana, M. Seghieri, S. Taddei, L. Ghiadoni, A. Solini;
University of Pisa, Pisa, Italy.

Background and aims: Mechanisms through which SGLT-2 inhibitors achieve cardiovascular and renal protection are still unknown. We investigated whether dapagliflozin (Dapa) modulates Na and water balance and systemic and renal vascular parameters like endothelial function, arterial stiffness and renal vasodilating capacity, exploring the epigenetic regulation behind it.
Materials and methods: Two groups of hypertensive patients with type 2 diabetes were studied at baseline (V0) and after four weeks (V1) of Dapa 10 mg ($N = 20$) or hydroclorothiazide 12.5 mg (HCT, $N = 20$), collecting blood and urinary samples for routine analyses, determination of plasma renin activity, aldosterone, norepinephrine, adrenaline and 24

hour-urinary electrolytes. Flow-mediated dilation of the brachial artery (FMD), baseline (RI) and dynamic renal resistive index (DRIN), carotid-femoral pulse-wave velocity (PWV) and Augmentation Index (AIx) were also measured. Circulating miRNA related to chronic heart failure (miR27a-3p, miR30e-5p, miR199a-3p) and renal function (miR130b-3p, miR21-5p) were assessed.

Results: The two groups were comparable for age, sex, BMI and HbA1c. Both Dapa and HCT marginally lowered systolic and diastolic BP values, with no effect on heart rate. Fasting glucose did not significantly vary. Estimated GFR (by CKD-EPI equation) was unmodified in both groups after treatment. Serum magnesium concentration significantly rose in Dapa group (from 1.88 ± 0.27 to 2.01 ± 0.22 mg/dl, $p = 0.02$ for time*treatment interaction), while urinary magnesium was unchanged. 24h diuresis and glycosuria and osmolar clearance increased in Dapa ($p < 0.001$ for time*treatment interaction), with no changes in sodium excretion and creatinine clearance. Dapa induced also a rise in aldosterone ($p = 0.02$ for time*treatment interaction), with no influence on cathecolamines. Nor DAPA neither HCT modified FMD and PWV. Interestingly, in Dapa group DRIN remained unmodified, while tended to increase in HCT group ($p = 0.05$ for time*treatment interaction). Preliminary data show differences in circulating miRNAs between the two groups.

Conclusion: 4-week treatment with Dapa did not significantly influence BP, glucose values, eGFR and systemic indices of vascular function. However, in comparison to HCT, renal vasodilating capacity was preserved in Dapa group, indicating a selective effect on renal vascular function, which may act as a mechanism of nephroprotection. Furthermore, the increase in serum magnesium might contribute to cardiovascular protection.

Supported by: Unresticted grant from Astra Zeneca

Disclosure: **R. Bruno:** Grants; Astra Zeneca.

651

Small increases in serum magnesium levels by dapagliflozin and normalisation of hypomagnesaemia in patients with type 2 diabetes

R. Toto[1], R. Goldenberg[2], G.M. Chertow[3], V. Cain[4], B.V. Stefansson[5], C.D. Sjöström[5], P. Sartipy[5];
[1]University of Texas Southwestern Medical Center, Dallas, USA, [2]LMC Diabetes & Endocrinology, Thornhill, Canada, [3]Stanford School of Medicine, Stanford, USA, [4]Bogier Clinical and IT Solutions, Raleigh, USA, [5]Cardiovascular, Renal and Metabolic Disease, AstraZeneca, Gothenburg, Sweden.

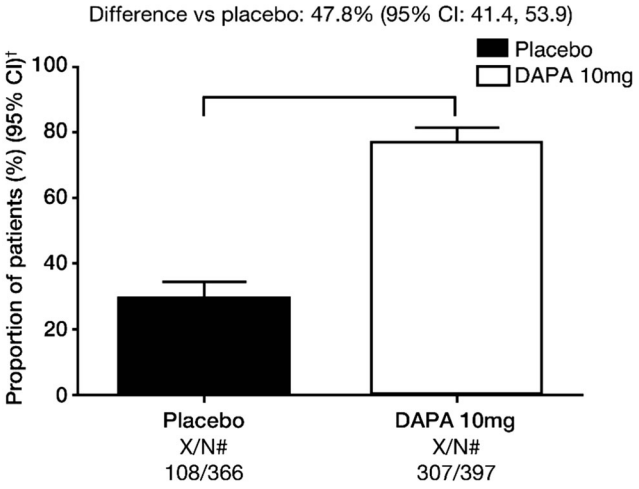
Background and aims: Hypomagnesaemia (serum magnesium [Mg] concentration <0.74 mmol/L) is common in patients with type 2 diabetes mellitus (T2DM). It is associated with increased risk of cardiometabolic complications, insulin resistance, ventricular arrhythmia, and chronic kidney disease. Small increases in serum Mg have been reported in patients treated with sodium-glucose co-transporter 2 inhibitors (SGLT2i). This study investigated the effect of the SGLT2i dapagliflozin (DAPA) on serum Mg in patients with T2DM.

Materials and methods: This post hoc analysis evaluated the effect of DAPA 10 mg on serum Mg level over 24 weeks (w) across 10 placebo-controlled studies in patients with T2DM. The patient population was subdivided based on serum Mg levels at baseline (<0.74 [$n = 773$] or ≥0.74 mmol/L [$n = 3625$]).

Results: Demographic and baseline characteristics were similar between subgroups. Patients with hypomagnesaemia (<0.74 mmol/L) had slightly longer average mean duration of T2DM (11 vs 9 years) and numerically higher frequency of reported cardiovascular-related disease history (67% vs 47%). Treatment with daily DAPA 10 mg increased placebo-adjusted serum Mg concentrations in patients with hypomagnesaemia and serum Mg concentrations ≥0.74 mmol/L (0.08 and 0.05 mmol/L, respectively). Similar changes occurred in patients with different estimated GFRs at baseline. The proportion of patients with baseline serum Mg <0.74 mmol/L achieving serum Mg ≥0.74 mmol/L at 24 w was >48% with DAPA 10 mg vs placebo (Figure). The proportions of patients with hypermagnesaemia (defined as >1.05 mmol/L) at baseline in the DAPA 10 mg and placebo groups were 2.2% (39/1811) and 1.3% (24/1781), respectively. These proportions remained similar post 24 w treatment with DAPA 10 mg and placebo (1.7% [31/1811] and 0.7% [13/1781], respectively). While treatment with DAPA 10 mg over 24 w reduced systolic blood pressure (SBP) vs placebo (~3 mm Hg), no clinically meaningful differences were observed in placebo-adjusted changes over 24 w in SBP and heart rate between patients in both subgroups at baseline. The frequency of reported adverse events under the MedDRA system organ class 'Cardiac Disorders' was numerically lower post 24 w treatment with DAPA 10 mg vs placebo in patients with serum Mg <0.74 mmol/L at baseline (3.5% vs 5.7%, respectively) and similar post 24 w treatment in the DAPA 10 mg and placebo groups (3.5% in both) in patients with serum Mg ≥0.74 mmol/L at baseline.

Conclusion: In patients with T2DM, 24 w treatment with DAPA 10 mg normalised serum Mg levels more than placebo in hypomagnesaemic patients. There was no increase from baseline to 24 w in the proportion of patients displaying hypermagnesaemia post treatment with DAPA 10 mg.

Figure: Proportion of patients with baseline serum Mg<0.74 mmol/L achieving serum Mg ≥0.74 mmol/L at 24 w



†Proportion of patients with low baseline serum Mg (<0.74 mmol/L) whose serum Mg level increased into normal range (≥0.74 mmol/L) at 24 weeks after treatment with either placebo or DAPA 10 mg.
CI: confidence interval; DAPA: dapagliflozin; Mg: magnesium; PBO: placebo; vs: versus; w: week; x: number of patients in subgroup; N: total number of patients in subgroup

Supported by: AstraZeneca Pharmaceuticals LP

Disclosure: **R. Toto:** Employment/Consultancy; Amgen, AstraZeneca, Bayer, Boehringer-Ingelheim, Novo Nordisk, Reata, Relypsa, ZS Pharma, Quintiles.

652

SGLT-2 inhibitors efficacy in type 2 diabetic patients with bariatric surgery

J.P. Valderas[1], Y. Preiss[2], M. Sepulveda[3], F. Crovari[4], C. Boza[5];
[1]Medical Sciences, Universidad de Antofagasta, Antofagasta, [2]Center of obesity treatment, Hospital Dipreca, Santiago, [3]Surgery, Universidad Diego Portales, Santiago, [4]Surgery, Pontificia Universidad Catolica de Chile, Santiago, [5]Surgery, Clinica Las Condes, Santiago, Chile.

Background and aims: The Bariatric Surgery (BS) is part of the treatment of Type 2 Diabetes (T2D) in patients with obesity at worldwide, but

a great percentage of these patients undergo hyperglycemia after the procedure and require antidiabetic drugs. The same algorithm employed with non-operated diabetic subjects is used with these patients. However, the anatomical and physiological changes produced by BS could affect the efficacy of the drugs as well increase the adverse effects. The SGLT-2 inhibitors (iSGLT-2) are a new antidiabetic drugs with positive effects in body weight, blood pressure, cardiovascular events and nephropathy. Aim: To evaluate the efficacy of iSGLT-2 in T2D patients with a BS

Materials and methods: A retrospective study on 17 consecutive T2D patients with BS from 3 chilean centers. We report the changes in Hba1c and weight 6 months after the use of iSGLT-2, as well the main adverse effects.

Results: The patients had 54 ± 7.6 years old, a BMI of 27.6 ± 4.4 kg/m^2, and a Hba1c of 7.9 ± 0.9%. 11 subjects had a gastric bypass and 6 had a sleeve gastrectomy. The procedure was performed between 6 to 72 months ago (median of 26). At baseline 14 patients were on metformin, eight used insulin and two were off drugs. All patients had an Hba1c greater then 7% and 14 had overweigth. 13 patients started empagliflozin and four started dapagliflozin on the recommended doses. After 6 months, Hba1c was reduced in 0.9% [95% CI 0.6 to 1.1] $p < 0.0001$. Body weight decreased 0.7 kg [95% CI 0.7 to 2.9] $p = 0.003$. Two patient suspended insulin and nobody augmeted the hypoglycemic therapy. One patient had a mycotic balanitis that responded to topical treatment without withdraw the treatment. One additional patient developted a vulvovaginitis that did not relieve with topical and oral treatment and stopped the iSGLT-2 at 2 weeks, therefore she was not included in this report. No other adverse effects were observed.

Conclusion: In our cohort of T2D patients with bariatric surgery the SGLT-2 inhibitors preserved the hypoglycemic efficacy and this was similar than published in non-operated subjects. A small but significant weight loss was also observed. The genital infection was the only adverse effect observed but it can induce the withdrawal of the treatment. iSGLT-2 could be a good alternative in the management of hyperglycemia in bariatric patients.More studies are needed to develop an special pharmacological algorithm for the bariatric patients

Supported by: FONDECYT Project N° 1120877

Disclosure: **J.P. Valderas:** None.

653

Effects of dapagliflozin on urine and plasma metabolome in patients with type 2 diabetes: preliminary results

V. Tsimihodimos[1], E. Ferrannini[2], S. Filippas-Dekouan[1], E. Bletsa[1], A. Dimou[3], E. Bairaktari[3], M. Elisaf[1];
[1]University of Ioannina, Ioannina, Greece, [2]Institute of Clinical Physiology, CNR (Consiglio Nazionale delle Ricerche), Pisa, Italy, [3]Laboratory of Clinical Chemistry, University of Ioannina, Ioannina, Greece.

Background and aims: SGSGLT2 inhibitors alter the pattern of substrate utilisation in patients with type 2 diabetes. Metabolomics has been used to predict, diagnose and monitor metabolic disorders, including diabetes. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is a rapid and reproducible technique, requires minimum sample pretreatment and quantitates multiple metabolites. We employed a targeted NMR-based metabolomics approach to evaluate the effects of dapagliflozin on serum and urine metabolic profile

Materials and methods: Twenty-five patients with type 2 diabetes [14 males, age 60.3 ± 9.3 years, BMI 32.8 ± 5.4, HbA1c 8.3 ± 1.5%], inadequately controlled with metformin monotherapy, were included. All patients received 10 mg dapagliflozin daily for 3 months. Blood and urine samples were collected after a 12-hour fast at baseline and at the end of the active treatment period. For targeted metabolomics, the ^{1}H NMR spectra of the deproteinized serum samples and urine were recorded on a 700 MHz Bruker Avance DRX spectrometer

Results: Dapagliflozin administration resulted in several significant alterations of the metabolic profile in both serum and urine. As expected, serum levels of ketone bodies (acetone, 3-hydroxybutyrate and acetoacetate) showed a significant increase ($p < 0.01$), which was accompanied by a significant increase in their urine excretion ($p < 0.05$). Also, a significant decrease in serum lactate ($p < 0.05$) was observed while the urine levels of this metabolite were slightly but non-significantly increased. Dapagliflozin significantly increased the serum concentrations of branched-chain amino acids (leucine, isoleucine and valine: $p < 0.001$, $p < 0.05$ and $p < 0.001$, respectively). Of note, a smaller increase in the renal excretion of these amino acids was observed, suggesting that their reabsorption in proximal tubular cells was preserved and not affected by dapagliflozin. Depletion of urinary citrate has been attributed to either an impairment of the tricarboxylic acid cycle or to renal tubular acidosis, which typically appears as part of a generalized proximal tubule dysfunction. We found an increase in urine levels of citrate ($p < 0.05$) after dapagliflozin administration, which may indicate an improvement in the function of proximal tubular cells. Finally, an increase in the urine concentrations of various endogenous osmolytes (such as betaine, myoinositol and trimethylamine-N-oxide, $p < 0.001$ for all) was observed after dapagliflozin administration, which may result from an enhanced medullary production of these molecules. These changes may represent an adaptive response to the large amount of glucose that reaches the renal medulla and the resulting extracellular hypertonicity.

Conclusion: Dapagliflozin increases the availability of ketones and branched-chain amino acids as fuel to tissues; effects on the kidney include increased excretion of citrate and osmolytes

Clinical Trial Registration Number: NCT02798757
Supported by: Astra Zeneca research grant

Disclosure: **V. Tsimihodimos:** None.

654

Effects of SGLT2 inhibitors in cells (human myeloid angiogenic cells and platelets) pivotally involved in atherosclerotic plaque (in)stability/thrombosis

V. Spigoni, F. Fantuzzi, C. Carubbi, G. Pozzi, E. Masselli, D. Galli, G. Gobbi, P. Mirandola, M. Vitale, R.C. Bonadonna, A. Dei Cas;
University of Parma, Parma, Italy.

Background and aims: The rapid emergence of cardiovascular (CV) benefits in SGLT2 inhibitor (SGLT2i) trials might suggest a direct effect on atherosclerotic plaque vulnerability and/or thrombosis. Circulating myeloid angiogenic cells (MAC; CD14$^+$ CD31$^+$ KDR$^+$ cells) and platelets (PLT) are pivotally implicated in these processes. We hypothesized that SGLT2i may influence MAC and/or PLT function. The aim of this study was to assess the effects of SGLT2i in reducing a) inflammation, oxidant stress and apoptosis in a published model of stearic acid (SA)-induced lipotoxicity in MAC; b) PLT activation. The possible involvement of the Na^+/H^+ exchanger (NHE) was also explored.

Materials and methods: MAC and PLT were isolated from peripheral blood of healthy subjects and incubated with/without SGLT2i [empagliflozin (EMPA) and dapagliflozin (DAPA) 1–100 μM] to assess the effects on: a) SA (100 μM)-induced cytokine expression, oxidant stress (qPCR) and apoptosis (caspase activation) in MAC and b) flow cytometric expression of PLT activation markers (CD62p and PAC-1) after ADP-stimulation. Digital/qPCR and western blot were used to test the expression of NHE isoforms and SGLT2 in MAC and purified PLT; NHE involvement was assessed with amiloride (aspecific NHE inhibitor) or cariporide (NHE1 specific inhibitor).

Results: NHE isoforms (1.6–9), but not SGLT2 expression, were detected in MAC and PLT. EMPA and DAPA (100 μM) reduced SA-induced inflammation (IL1β, IL8, TNFα, MCP1; $p < 0.005$), oxidant stress (SOD2, TXN, HO1; $p < 0.05$), but not apoptosis in MAC. EMPA and DAPA (both 1 μM) reduced PLT activation (CD62p and PAC1

expression; $p < 0.05$). SGLT2i effects were mimicked by amiloride, but not cariporide, in MAC, and by both inhibitors in PLT.
Conclusion: The effects of EMPA and DAPA - reduced lipotoxicity in MAC and PLT activation, possibly via NHE-inhibition - point to plaque stabilization and/or thrombosis inhibition as potential mechanism(s) involved in SGLT2i-mediated CV protection.
Supported by: Fondazione Diabete e Ricerca ONLUS in collaboration with Eli Lilly Italia
Disclosure: **V. Spigoni:** Grants; "Fondazione Diabete e Ricerca" in collaboration with Eli Lilly Italia.

655

Determinants of the improved arterial stiffness observed after empagliflozin treatment

R.E. Schmieder, A. Bosch, S. Jung, K. Striepe, M.V. Karg, D. Kannenkeril, T. Dienemann, C. Ott;
Universitätsklinikum, Erlangen, Germany.

Background and aims: The selective sodium-glucose cotransporter 2 inhibitor empagliflozin has been shown to reduce cardiovascular morbidity and mortality, but the underlying pathogenetic mechanisms are poorly understood. Most recently, a reduction in central systolic blood pressure (BP) and pulse pressure, both parameters reflecting arterial stiffness, has been observed. Factors triggering the improvement of arterial stiffness are now in detail analyzed.
Materials and methods: Fifty eight male and female patients with diagnosed type 2 diabetes mellitus participated in an investigator-initiated prospective, double-blind, randomized, placebo-controlled, interventional clinical trial. Patients received either 6-weeks treatment with 25 mg empagliflozin orally once daily or placebo, followed by the second 6-week treatment with the other compound (crossover). Radial artery waveforms were recorded by the SphygmoCor System (AtCor Medical) and corresponding central (aortic) waveforms were then automatically generated through a validated transfer function. Now, we investigated the influence of parameters of glucose metabolism, volume status, sympathetic activation and inflammation on vascular parameters of arterial stiffness using multivariate regression analysis.
Results: Therapy with empagliflozin improved arterial stiffness as indicated by reduced central systolic BP (113.6 ± 12.1 vs 118.6 ± 12.9 mmHg, $p < 0.001$), central pulse pressure (39.1 ± 10.2 vs 41.9 ± 10.7 mmHg, $p = 0.027$) forward (27.1 ± 5.69 vs 28.7 ± 6.23 mmHg, $p = 0.031$) as well as reflected wave amplitude (18.9 ± 5.98 vs 20.3 ± 5.97 mmHg, $p = 0.045$) compared to placebo. The results of the multivariate regression analysis revealed that besides 24-hour ambulatory systolic BP ($p = 0.016$), age ($p = 0.022$) and sex ($p = 0.092$), change in high sensitive CRP (hsCRP) ($p = 0.029$) emerged as a significant determinant of empagliflozin induced reduction in central pulse pressure, whereas copeptin, hematocrit, heart rate, HbA1c and LDL-cholesterol did not emerge as significant determinants. When replacing HbA1c with fasting plasma glucose, again the change in hsCRP ($p = 0.020$) was an independent determinant of central pulse pressure. Repeating the analysis with "improvement of reflected wave amplitude", change in hsCRP ($p = 0.037$) predicted significantly the change in the reflected wave amplitude ($p = 0.037$).
Conclusion: Besides age, sex and change in systolic 24 hour ambulatory BP, change in high sensitive CRP was an independent determinant of empagliflozin induced improvement of arterial stiffness, whereas parameters of change in glucose metabolism and volume status had no significant influence. Our analysis revealed a signal that empagliflozin exerts, at least to some extent, beneficial vascular effects via anti-inflammatory mechanisms.
Clinical Trial Registration Number: NCT02471963
Supported by: Unrestricted grant by Boehringer Ingelheim Pharma GmbH & Co.KG
Disclosure: **R.E. Schmieder:** None.

656

SGLT2 inhibitors effect on fatty liver disease in patients with Berardinelli-Seip lipodystrophy

E.B. Parente[1], V.R.F. Simoes[1], M.A. Medeiros[1], I.E. Bacha[2], E.R. Parisi[2], J.E.N. Salles[1];
[1]Internal Medicine- Endocrinology, Faculty of Medical Science of Santa Casa of Sao Paulo, São Paulo, [2]Medicine- Endocrinology, Federal University of Sao Paulo, São Paulo, Brazil.

Background and aims: Berardinelli-Seip congenital lipodystrophy (BSCL) is a rare autosomal recessive disease with almost complete absence of adipose tissue and severe insulin resistance. Its prevalence worldwide is 1 in 12 million people. BSCL patients usually develop severe diabetes mellitus (DM), hypertriglyceridemia and fatty liver disease. The glucose control in BSCL is very difficult due the severity of insulin resistance, so they need high doses of insulin. Because of few options of treatment available for these patients, we tried to associate iSGLT2 to insulin, aiming to improve glucose control, liver disease and reduce insulin doses. There is no data in the literature regarding the use of iSGLT2 in patients with BSCL.
Materials and methods: This is a retrospective, descriptive study of four patients with BSCL using more than 1 ui/kg of insulin and fatty liver disease that received iSGLT2 for 12 months. Liver disease was evaluated by elastography (FibroScan 502) considering a value of CAP >200 dB/m as steatosis and a kPa >6.0 for liver fibrosis. Patients were submitted to elastography before, 6 and 12 months after iSGLT2. A1c, fasting triglycerides (TG), liver enzymes, fasting glucose, body weight were measured at the same time.
Results: Patient 1: 28 year-old woman, 50.4 kg, DM since 14 years old. At diagnosis: TG 896 mg/ml and A1c 14.2%. Leptin 0.6 ng/mL. Before empagliflozin 10 mg: A1c 9.4%, using 1.2 ui/kg of insulin, CAP 341, kPa 4.8. After 6 months: A1c 10%, insulin 1.3 uikg, CAP 351, kPa 6.6. After one year: A1c 10%, insulin 1.2 uikg, CAP 311, kPa 5.9 Patient 2: 33 year old woman, 64.4 kg, DM since puberty. At diagnosis: TG 678 mg/ml A1c 12.6%. Leptin 0.8 ng/mL. Before empagliflozin 10 mg: A1c 6.0% using 2.4 ui/kg of insulin, CAP 161, kPa 3.7. After 6 months: A1c 5.9%, insulin 2.4 uikg, CAP 234, kPa 4.9. After one year: A1c 6.1%, insulin 2.5 uikg, CAP 245, kPa 5.2.Patient 3: 59-year-old woman, 58.1 kg, DM since 40 years old. At diagnosis: TG 792 mg/ml and A1c 8.6%. Leptin 1.9 ng/mL. Before dapagliflozin 10 mg: A1c 8.5% using 2.0 ui/kg of insulin, CAP 304, kPa 8.1. After 6 months: A1c 7.5%, insulin 1.8 uikg, CAP 390, kPa 7.3. After one year: A1c 8.5%, insulin 1.8 uikg, CAP 320, kPa 7.1.Patient 4: 61-year-old woman, 54.4kg, DM since 47 years old. At diagnosis: TG 937 mg/ml and A1c 8.1%. Leptin 0.5 ng/mL. Before empagliflozin 10 mg: A1c 9.1% using 1.1 ui/kg of insulin, CAP 364, kPa 6.1. After 6 months: A1c 8.0%, insulin 1.1 uikg, CAP 287, kPa 5.8. After one year: A1c 8.5%, insulin 1.2 uikg, CAP 309, kPa 5.3.The use of iSGLT2 with insulin in patients with BSCL did not reduce A1c, insulin doses, weight nor triglycerides, but improved liver fibrosis in the two patients that presented this condition. The period of exposure to metabolic disturbances seems to be an important factor for liver fibrosis, since it was present in the two oldest patients. After one year of iSGLT2, without glycemic, body weight or steatosis improvements, the two patients with liver fibrosis reduced it in 12.3% and 13.1%. Maybe it was a direct effect of iSGLT2 in liver fibrosis, but as this is a rare disease we will a greater sample size to conclude this effect.
Conclusion: The association of iSGLT2 to insulin for one year in four patients with BSCL did not improve steatosis, glycemic control neither insulin doses, but reduced liver fibrosis when it was present.
Disclosure: **E.B. Parente:** None.

657

Dapagliflozin improves liver dysfunction in parallel with a decrease in serum soluble DPP-4/CD26 level in type 2 diabetic patients with non-alcoholic fatty liver

H. Kishi, K. Suzuki, K. Kato, M. Shimizu, T. Jojima, T. Iijima, I. Usui, Y. Aso;
Dokkyo Medical University, Mibu, Japan.

Background and aims: Serum levels of soluble DPP-4/CD26 are elevated in patients with type 2 diabetes and non-alcoholic fatty disease (NAFLD). Hepatocyte-secreted sDPP-4 induce directly inflammation and insulin resistance in the liver. Similar to a carbohydrate-restricted diet, SGLT2 inhibitors reduce hepatic steatosis by inhibiting hepatic de novo lipogenesis. We investigated effects of dapagliflozin, an SGLT2 inhibitor, on liver dysfunction, hepatic steatosis, and serum levels of sDPP-4 in patients with type 2 diabetes and NAFLD.
Materials and methods: Fifty-seven patients with type 2 diabetes and NAFLD were randomized into a dapagliflozin (5 mg/day) treatment group ($n = 33$) or an active placebo group ($n = 24$) for 24 weeks. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volumes were measured using a dual bioelectrical impedance analysis (Dual Scan). Hepatic steatosis was evaluated by controlled attenuated parameter (CAP) using a transient ultrasound elastography. Serum levels of sDPP-4 were measured with a commercially available ELISA kit.
Results: At baseline, serum sDPP-4 showed positive correlations with aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), and HOMA-IR, an index of hepatic insulin resistance, in a total of 57 patients. Both VAT and SAT significantly decreased in the dapagliflozin group, but not in the placebo group. Serum level of AST, ALT, and GGT showed a significant decrease at 24 weeks in the dapagliflozin group, while they were unchanged in the placebo group. CAP also significantly decreased in the dapagliflozin group. Although both groups showed a significant reduction in serum sDPP-4 at 24 weeks after treatment, the magnitude of sDPP-4 reduction was greater in the dapagliflozin group. Changes in liver enzymes (AST, ALT, and GGT) after treatment with dapagliflozin correlated positively with those in serum sDPP-4 levels, but not those in VAT (Fat volume) or HbA1c.
Conclusion: A improvement of liver dysfunction was associated with a decrease in serum sDPP-4, but not that in VAT or HbA1c, after treatment with dapagliflozin in patients with type 2 diabetes and NAFLD, suggesting that reducing serum sDPP-4 by SGLT2 inhibitors may be a therapeutic strategy for treatment of NAFLD/non-alcoholic steatohepatitis in people with type 2 diabetes.
Clinical Trial Registration Number: UMIN000022155
Disclosure: **H. Kishi:** None.

658

Canagliflozin prevents development of hepatocellular carcinoma (HCC) from non-alcoholic steatohepatitis (NASH) in a novel mouse model of NASH-HCC under diabetic state

T. Jojima, H. Kishi, T. Iijima, I. Usui, Y. Aso;
Dokkyo Medical Unversity, Mibu, Japan.

Background and aims: Non-alcoholic steatohepatitis (NASH), which is characterized by hepatocellular lipid accumulation (steatosis) along with lobular inflammation, hepatocellular ballooning, and fibrosis, can result in liver cirrhosis and hepatocellular carcinoma (HCC). Several studies have shown that some SGLT2 inhibitors alleviate hepatic steatosis or steatohepatitis in type 2 diabetic mice or rats.We investigated the effects of canagliflozin (CANA), an SGLT2 inhibitor, on steatohepatitis and fibrosis in a novel diabetes-non-alcoholic steatohepatitis (NASH) -HCC progression model mice. Moreover, we investigated inhibitory effects of CANA on development of HCC in this model mice
Materials and methods: Mice aged 5 weeks were divided into two groups (vehicle and CANA 30 mg/kg) and fed for 3 more weeks. Next, mice aged 5 weeks were divided into three groups of 9 animals: vehicle, CANA initial administration 30 mg/kg (5 to 9 W), CANA continuous administration 30 mg/kg (5 to 16W).
Results: The histological non-alcoholic fatty liver disease activity score (NAS) was lower in the CANA group than in the vehicle group at age of 8 weeks. The expression of type 1 and 3 collagen mRNA was reduced in the CANA group.CANA continuous administration 30 mg/kg (5 to 16W). CANA continuous treated mice had greater survival at 16 weeks of age compared with vehicle mice (89% vs 56%, $P < 0.01$). At the age of 16 weeks, the NAS was significantly lower in the CANA continuous administration group than in the vehicle or the CANA initial administration groups. The number of hepatic tumors was significantly smaller in the CANA continuous administration group than in the vehicle group. Immunohistochemistry showed that expression of glutamine synthetase, a marker of HCC, was reduced in the CANA continuous administration group compared with the vehicle group. The CANA continuous administration significantly decreased expression of mRNAs for α-fetoprotein, a tumor maker of HCC, compared with the vehicle administration
Conclusion: Canagliflozin attenuates development of NASH showing anti-steatotic and anti-inflammatory effects, and prevents progression of HCC from NASH, leading to prolongation of survival in STAM mice.
Supported by: Tanabe Mitsubishi
Disclosure: **T. Jojima:** None.

PS 050 SGLT2 inhibitors: What do the CVOTs tell us?

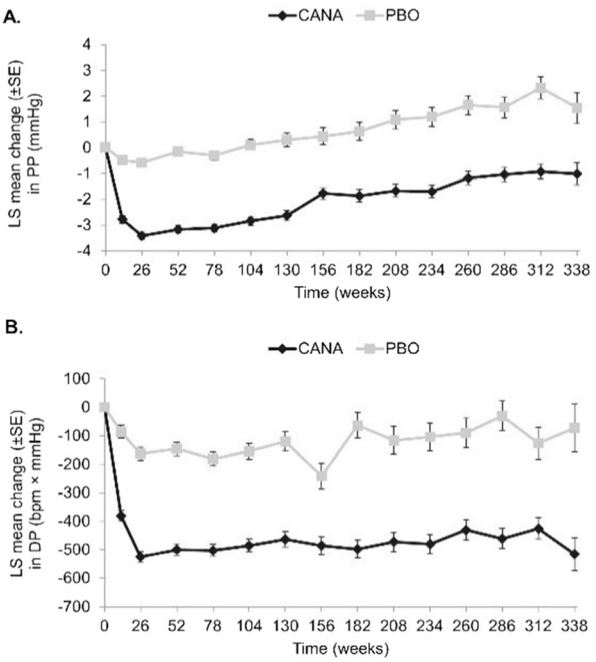
659

Improvements in blood pressure and markers of arterial stiffness with canagliflozin in the CANVAS Program

S. Genovese[1], R.R. Townsend[2], M. Dharmalingham[3], A. Steele[4], J. Arenas León[5], A. Slee[6], E. Fabbrini[7], K.W. Mahaffey[8];
[1]Diabetes Endocrine and Metabolic Disease Unit, IRCCS Centro Cardiologico Monzino, Milan, Italy, [2]Hospital of the University of Pennsylvania, Philadelphia, USA, [3]Ramaiah Medical College, Bengaluru, Karnataka, India, [4]Lakeridge Health, Oshawa, Canada, [5]Hospital Angeles Centro Médico del Potosí, San Luis, Mexico, [6]Axio Research, Seattle, USA, [7]Janssen Research & Development, LLC, Raritan, USA, [8]Stanford Center for Clinical Research, Department of Medicine, Stanford University School of Medicine, Stanford, USA.

Background and aims: The CANagliflozin cardioVascular Assessment Study (CANVAS) Program demonstrated a reduced risk of cardiovascular (CV) and renal outcomes with the SGLT2 inhibitor canagliflozin (CANA) versus placebo (PBO) in adults with type 2 diabetes mellitus (T2DM) and established CV disease or ≥2 CV risk factors ($N = 10,142$; mean age, 63.3 y; systolic blood pressure [SBP], 136.6 mmHg; diastolic blood pressure [DBP], 77.7 mmHg). This analysis assessed the effects of CANA on blood pressure (BP), pulse, and markers of arterial stiffness, including pulse pressure (PP) and double product (DP), in the CANVAS Program.
Materials and methods: SBP, DBP, and pulse were measured in the CANVAS Program and were used for post hoc analyses of least squares (LS) mean change in PP (PP = SBP − DBP) and DP (DP = pulse × SBP) over time.
Results: CANA lowered SBP and DBP compared with PBO over the CANVAS Program (mean differences of −3.93 mmHg [95% confidence interval [CI]: −4.30, −3.56]) and −1.39 mmHg [95% CI: −1.61, −1.17]; both $P < 0.001$). There were no meaningful differences in pulse over 104 weeks; a ~0.5 bpm reduction in pulse was seen with CANA at Week 26, which remained stable over time, while pulse with PBO was similar to baseline over 104 weeks. CANA provided reductions in PP and DP compared with PBO (**Figure**). There was an initial reduction in PP that increased over time with CANA, but remained lower compared with PBO. CANA also provided an initial reduction in DP that was sustained over time.
Conclusion: Favourable effects of CANA on BP, pulse, and markers of arterial stiffness were observed in patients with T2DM with CV risk factors or established CV disease, which may contribute to the beneficial effects of CANA on CV outcomes.

Figure. Change in (A) PP and (B) DP with CANA versus PBO over time.



Clinical Trial Registration Number: NCT01032629, NCT01989754
Supported by: Janssen Global Services, LLC
Disclosure: **S. Genovese:** Employment/Consultancy; Abbott Diabetes Care, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Bruno Farmaceutici, Eli Lilly, Hikma Pharmaceuticals, Janssen, Johnson & Johnson, Merck Sharp & Dohme, Novartis, Novo Nordisk, Sanofi, Takeda. Lecture/other fees; Abbott Diabetes Care, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Bruno Farmaceutici, Eli Lilly, Hikma Pharmaceuticals, Janssen, Johnson & Johnson, Merck Sharp & Dohme, Novartis, Novo Nordisk, Sanofi, Takeda.

660

Effects of canagliflozin on HbA_{1c} and changes in antihyperglycaemic agents in the CANVAS Programme

G. Fulcher[1], M. Davies[2], M. Tsoukas[3], M. Desai[4], C. Wysham[5];
[1]The Royal North Shore Hospital and University of Sydney, Sydney, Australia, [2]University of Leicester, Leicester Diabetes Centre, Leicester, UK, [3]McGill University Health Centre, Montreal, Canada, [4]Janssen Research & Development, LLC, Raritan, USA, [5]University of Washington, Spokane, USA.

Background and aims: Canagliflozin (CANA) improved glycaemic control by reducing HbA1c in a broad range of patients with type 2 diabetes (T2D) across its clinical trial program. This analysis examines the effects of CANA on HbA1c and changes in use of antihyperglycaemic agents (AHAs) in patients with high cardiovascular (CV) risk in the CANagliflozin cardioVascular Assessment Study (CANVAS) Program.
Materials and methods: The CANVAS Program randomly assigned 10,142 participants with T2D and established CV disease or ≥2 CV risk factors to treatment with CANA or placebo (PBO). Least squares (LS) mean changes from baseline HbA1c and initiation of new AHAs were analysed in the on-treatment population (patients receiving CANA/PBO or within 30 days after discontinuation; $n = 10,134$).
Results: From a baseline mean HbA1c of 8.2%, the maximum difference between CANA and PBO (PBO-subtracted difference [95% CI]: −0.64% [−0.68, −0.61]; mean 8.1% vs 7.5%) was observed at Week 26. After Week 26, the curves began to converge, reaching a minimum difference between CANA and PBO of −0.23% (95% CI: −0.33, −0.14; 8.2% vs

8.0%) at Week 286. At the end of the study (Week 338), the mean difference HbA1c with CANA versus PBO was −0.24% (95% CI: −0.37, −0.10; 8.1% vs 7.9%), with a mean reduction of −0.58% (95% CI: −0.61, −0.56) with CANA versus PBO over the entire follow-up period. Discontinuation from study drug was about the same in both treatment groups during the first year of the study; after Week 52, more patients discontinued PBO versus CANA. At baseline, the treatment groups were well-balanced with respect to AHA use. Almost all patients (98.6%) were being treated with ≥1 AHA at baseline (18.7% on 1 AHA, 43.6% on 2 AHAs, and 36.3% on ≥3 AHAs). The most common AHAs at baseline were biguanides (77.2%), insulin (50.3%), and sulfonylureas (43.0%). Over the course of the study, approximately 22% of patients initiated new AHAs during treatment with study drug, with DPP-4 inhibitors and insulin being the most common newly initiated AHAs (**Table**). A higher proportion of PBO-treated patients initiated AHA therapy across all classes, including insulin. Through the first and second years of the study, patients in the PBO group initiated new AHAs approximately twice as often as CANA-treated patients (first year: 6.3% and 14.7% with CANA and PBO; first 2 years: 11.3% and 22.9% with CANA and PBO).
Conclusion: In the CANVAS Program, patients treated with CANA had greater reductions in HbA1c compared with patients treated with PBO. After 52 weeks of treatment, the difference in HbA1c between the CANA and PBO groups began to decrease likely due to the joint effects of discontinuation of randomized therapy and a higher rate of initiating new AHA therapies in the PBO group.

Table. Newly Initiated Concomitant AHA Medications

Concomitant medication	PBO (n = 4344)	CANA (n = 5790)	Total (n = 10,134)
Patients with concomitant therapy, n (%)	1173 (27.0)	1029 (17.8)	2202 (21.7)
Alpha glucosidase inhibitors	59 (1.4)	59 (1.0)	118 (1.2)
Biguanides	209 (4.8)	188 (3.2)	397 (3.9)
DPP-4 inhibitors	385 (8.9)	340 (5.9)	725 (7.2)
GLP-1 receptor agonists	148 (3.4)	138 (2.4)	286 (2.8)
Insulin	380 (8.7)	271 (4.7)	651 (6.4)
SGLT2 inhibitors	21 (0.5)	29 (0.5)	50 (0.5)
Sulfonylureas	251 (5.8)	218 (3.8)	469 (4.6)
Thiazolidinediones	81 (1.9)	53 (0.9)	134 (1.3)

DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; SGLT2, sodium glucose co-transporter 2.

Clinical Trial Registration Number: NCT01032629, NCT01989754
Supported by: Janssen Global Services, LLC
Disclosure: **G. Fulcher:** Employment/Consultancy; Janssen, Novo Nordisk, Boehringer Ingelheim, Merck Sharp and Dohme. Grants; Novo Nordisk.

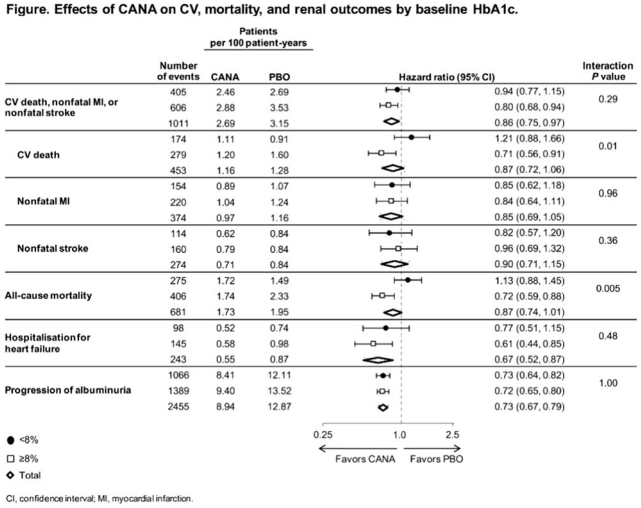
661

Relatively consistent effects of canagliflozin on outcomes regardless of baseline HbA_{1c} in the CANVAS Program

D.R. Matthews[1], J. Rosenstock[2], G. Bantwal[3], J. Wilding[4], R. Dumas[5], G. González-Gálvez[6], A. Slee[7], F. Vercruysse[8], K.W. Mahaffey[9], D. de Zeeuw[10], G. Fulcher[11];
[1]Oxford Centre for Diabetes, Endocrinology and Metabolism and Harris Manchester College, University of Oxford, Oxford, UK, [2]Dallas Diabetes Research Center at Medical City, Dallas, USA, [3]St. John's Medical College and Hospital, Bangalore, India, [4]University of Liverpool, Liverpool, UK, [5]Laval Clinic Research Center, Montreal, Canada, [6]Jalisco Institute of Diabetes and Obesity Research, Guadalajara, Mexico, [7]Axio Research, Seattle, USA, [8]Janssen Research & Development, Beerse, Belgium, [9]Stanford Center for Clinical Research, Department of Medicine, Stanford University School of Medicine, Stanford, USA, [10]University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [11]The Royal North Shore Hospital and University of Sydney, Sydney, Australia.

Background and aims: The CANagliflozin cardioVascular Assessment Study (CANVAS) Program evaluated cardiovascular (CV) safety of canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, compared with placebo (PBO) in participants with type 2 diabetes mellitus (T2DM) and established CV disease (CVD) or with ≥2 CV risk factors. The primary composite outcome of CV death, nonfatal myocardial infarction, or nonfatal stroke was significantly reduced with CANA vs. PBO by 14% (hazard ratio [HR] 0.86; 95% confidence interval [CI], 0.75, 0.97; $P = 0.02$). In this prespecified analysis, the effects of CANA on the risk of CV, mortality, and renal outcomes were assessed in patients by baseline HbA1c.
Materials and methods: Outcomes were assessed in CANVAS Program participants by baseline HbA1c (<8% and ≥8%). The number of events, incidence rates, HRs, and 95% CIs were calculated for each outcome.
Results: Among CANVAS Program participants, 4,411 (43%) had baseline HbA1c <8% (mean HbA1c 7.4%; prior CVD 67%; age 64 y; body mass index [BMI] 32 kg/m^2; and blood pressure [BP] 136/77 mmHg) and 5,731 (57%) had baseline HbA1c ≥8% (mean HbA1c 8.9%; prior CVD 65%; age 63 y; BMI 32 kg/m^2; and BP 137/78 mmHg). The effects of CANA on CV and renal outcomes were similar overall in participants with baseline HbA1c <8% or ≥8%, but there was evidence of statistical heterogeneity between subgroups for CV death and all-cause mortality, where effects on these adverse outcomes were better in participants with HbA1c ≥8% (**Figure**).
Conclusion: CV and renal outcomes appeared better in CANA-treated participants regardless of HbA1c in this hypothesis-generating subanalysis with a suggestion of greater effects on lowering mortality risk in those with higher HbA1c levels at baseline.

Figure. Effects of CANA on CV, mortality, and renal outcomes by baseline HbA1c.



CI, confidence interval; MI, myocardial infarction.

Clinical Trial Registration Number: NCT01032629, NCT01989754
Supported by: Janssen Global Services, LLC
Disclosure: **D.R. Matthews:** Employment/Consultancy; Novo Nordisk, Novartis, Eli Lilly, Sanofi-Aventis, Janssen, Servier. Grants; Janssen. Lecture/other fees; Novo Nordisk, Servier, Sanofi-Aventis, Eli Lilly, Novartis, Janssen, Mitsubishi Tanabe, Aché Laboratories.

662

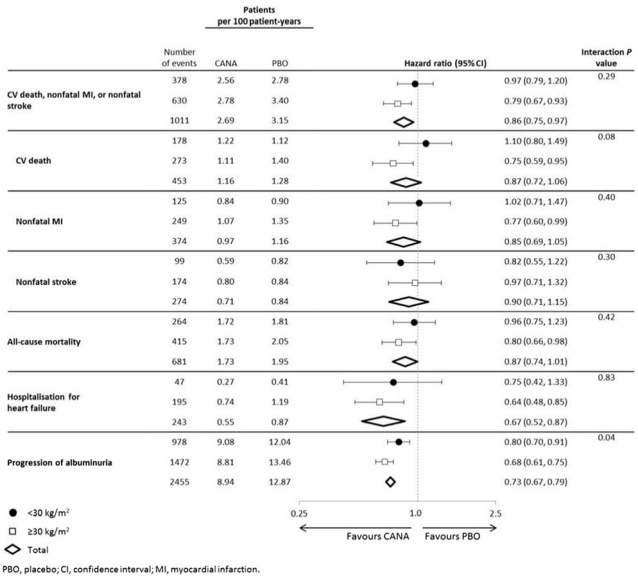
Improved cardiovascular and renal outcomes in the CANVAS Program irrespective of baseline body mass index

L. Van Gaal[1], H. Bays[2], S.R. Aravind[3], N. Aggarwal[4], R. Violante[5], A. Slee[6], W. Shaw[7], K.W. Mahaffey[8], D. de Zeeuw[9], D. Matthews[10];
[1]Antwerp University Hospital, Antwerp, Belgium, [2]Louisville Metabolic and Atherosclerosis Research Center, Louisville, USA, [3]Diacon Hospital, Bangalore, India, [4]Aggarwal and Associates Clinical Research, Brampton, Canada, [5]Center for Metabolic and Cardiovascular Research Studies, Tampico, Mexico, [6]Axio Research, Seattle, USA, [7]Janssen Research & Development, LLC, Raritan, USA, [8]Stanford Center for

Clinical Research, Department of Medicine, Stanford University School of Medicine, Stanford, USA, [9]University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [10]Oxford Centre for Diabetes, Endocrinology and Metabolism and Harris Manchester College, University of Oxford, Oxford, UK.

Background and aims: The CANagliflozin cardioVascular Assessment Study (CANVAS) Program evaluated the cardiovascular (CV) safety of canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, compared with placebo (PBO) in patients with type 2 diabetes and established CV disease or with ≥2 CV risk factors. The results showed that CANA reduced the risk of CV and renal outcomes. Overweight and obesity are known risk factors for heart and kidney disease. In this prespecified analysis, the effects of CANA on the risk of CV, mortality, and renal outcomes were assessed in patients by baseline body mass index (BMI).
Materials and methods: Outcomes were assessed in the CANVAS Program participants (N = 10,142) by baseline BMI (<30 or ≥30 kg/m^2). The number of events, incidence rates, hazard ratios, and 95% confidence intervals were calculated for each outcome.
Results: At baseline compared to patients with BMI <30 kg/m^2, more patients with BMI ≥30 kg/m^2 had systolic blood pressure (BP) ≥140 or diastolic BP ≥90 mmHg (47.9% vs 40.3%) and were taking renin-angiotensin-aldosterone system (RAAS) inhibitors (83.8% vs 74.6%). Effects of CANA on the primary outcome (CV death, nonfatal myocardial infarction, or nonfatal stroke) and other CV outcomes were consistent between BMI subgroups (**Figure**). CANA decreased progression of albuminuria in both BMI subgroups with the effect appearing greater in patients with BMI ≥30 kg/m^2. Further statistical testing did not show a qualitative interaction (Gail-Simons P = 0.875). The comparative benefits of CANA on albuminuria in patients with higher BMI may be due to greater reductions in body weight (PBO-subtracted difference at 1 y: −2.1 and −2.8 kg for BMI <30 and ≥30 kg/m^2) and BP (systolic BP: −4.2 and −4.6 mmHg; diastolic BP: −1.0 and −1.7 mmHg for BMI <30 and ≥30 kg/m^2); HbA1c reductions were similar with BMI <30 and ≥30 kg/m^2 (−0.60% and −0.63%).
Conclusion: While small statistical heterogeneity may exist, BMI <30 versus ≥30 kg/m^2 does not appear to be a major determinant of the observed improvements in CV outcomes with CANA.

Figure. CV and renal outcomes by BMI subgroups.



PBO, placebo; CI, confidence interval; MI, myocardial infarction.

Clinical Trial Registration Number: NCT01032629, NCT01989754
Supported by: Janssen Global Services, LLC

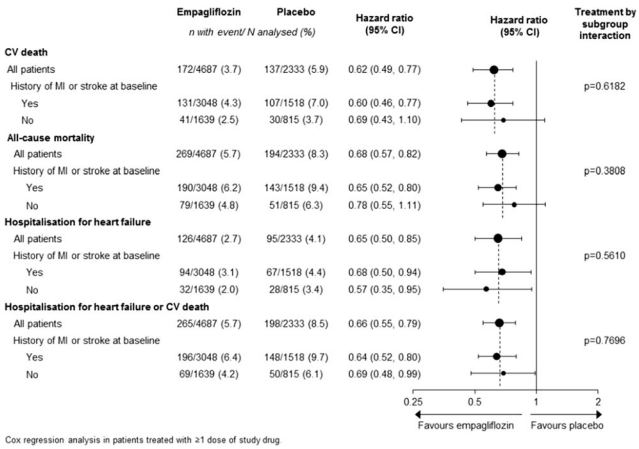
Disclosure: **L. Van Gaal:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck MSD, Novo Nordisk, Sanofi, Servier. Grants; European Union (HEPADIP and Resolve consortium), National Research Funds, Belgium.

663

Empagliflozin reduces mortality and hospitalisation for heart failure in patients with or without a history of myocardial infarction or stroke at baseline

S. Sambevski[1], D. Fitchett[2], S.E. Inzucchi[3], S. Kaspers[1], E. Pfarr[1], J.T. George[1], B. Zinman[4];
[1]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [2]St Michael's Hospital, Division of Cardiology, University of Toronto, Toronto, Canada, [3]Section of Endocrinology, Yale University School of Medicine, New Haven, USA, [4]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Canada.

Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin added to standard of care reduced cardiovascular (CV) death by 38% (HR 0.62 [95% CI 0.49, 0.77]), all-cause mortality by 32% (HR 0.68 [95% CI 0.57, 0.82]) and hospitalisation for heart failure (HHF) by 35% (HR 0.65 [95% CI 0.50, 0.85]) vs placebo in patients with type 2 diabetes and established CV disease. We investigated whether a history of myocardial infarction (MI) or stroke at baseline influenced the effect of empagliflozin on these outcomes.
Materials and methods: Patients were randomised to empagliflozin 10 mg, empagliflozin 25 mg, or placebo. Median observation time was 3.1 years. CV death, all-cause mortality, HHF and the composite of HHF or CV death were assessed for empagliflozin pooled vs placebo in subgroups by history MI or stroke at baseline using Cox regression analyses. P values for treatment by subgroup interaction were obtained from tests of homogeneity of treatment group differences among subgroups with no adjustment for multiple testing.
Results: Of 7020 patients treated, 65% in both treatment groups had a history of MI or stroke at baseline. Effects of empagliflozin on CV death, all-cause mortality, HHF and HHF or CV death were consistent in patients with and without MI or stroke.
Conclusion: Reductions in mortality and HHF with empagliflozin in patients with type 2 diabetes and established CV disease in the EMPA-REG OUTCOME trial were consistent in patients with or without a history of MI or stroke at baseline.



Cox regression analysis in patients treated with ≥1 dose of study drug.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

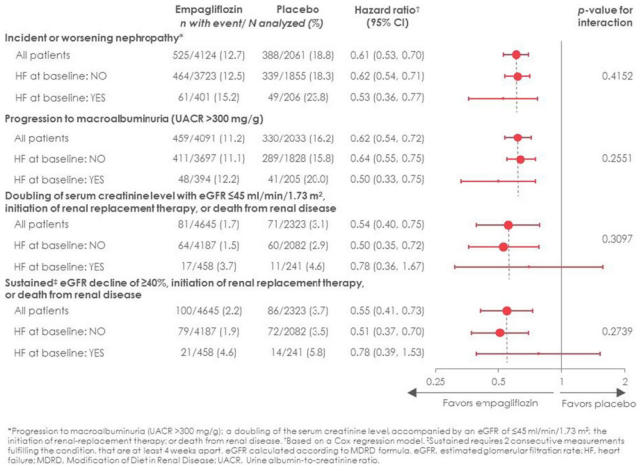
Disclosure: **S. Sambevski:** Employment/Consultancy; Employee of Boehringer Ingelheim.

664

Empagliflozin and kidney outcomes in patients with or without heart failure at baseline: insights from the EMPA-REG OUTCOME trial

J. Butler[1], F. Zannad[2], D. Fitchett[3], B. Zinman[4], A. Koitka-Weber[5,6], M. von Eynatten[7], U. Hehnke[7], J. George[7], M. Brueckmann[7], A.K. Cheung[8], C. Wanner[9];
[1]Dept of Medicine, University of Mississippi, Jackson, USA, [2]Institut Lorrain du Coeur et des Vaisseaux, Nancy, France, [3]St Michael's Hospital, University of Toronto, Toronto, Canada, [4]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital and Division of Endocrinology, University of Toronto, Toronto, Canada, [5]Boehringer Ingelheim International GmbH, Biberach, Germany, [6]Dept of Diabetes, Central Clinical School, Monash University, Melbourne, Australia, [7]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [8]University of Utah, Salt Lake City, USA, [9]Dept of Medicine, Division of Nephrology, Würzburg University Clinic, Würzburg, Germany.

Background and aims: Chronic kidney disease (CKD) is common and portends worse prognosis in patients with heart failure (HF), especially in the presence of type 2 diabetes (T2D). In the EMPA-REG OUTCOME trial, the sodium-glucose co-transporter 2 inhibitor empagliflozin (EMPA) significantly reduced the risk of cardiovascular (CV) death by 38% vs placebo (PBO) in patients with T2D and established CV disease (CVD). EMPA also reduced the risk of incident or worsening nephropathy by 39%, and slowed progression of CKD. Here, we report *post-hoc* kidney outcomes in patients with or without HF at baseline from EMPA-REG OUTCOME.
Materials and methods: Patients were randomised (1:1:1) to EMPA 10 mg, 25 mg or PBO. The composite kidney outcome of incident or worsening nephropathy (defined as progression to macroalbuminuria, doubling of serum creatinine, initiation of renal replacement therapy, or death from renal disease) and its components were analysed in subgroups of patients with or without HF at baseline. The incidence of first sustained decline in eGFR from baseline of ≥40% combined with initiation of renal replacement therapy, or renal death was also evaluated. Cox proportional hazards models were used to investigate the consistency of treatment effect across subgroups.
Results: Of 7020 treated patients, 706 (10.1%) had HF at baseline. For each of the four kidney outcomes, EMPA was associated with a consistent and lower relative risk versus PBO across the baseline HF subgroups (the *p* values for treatment by subgroup interaction across all kidney outcomes were >0.05) (Figure).
Conclusion: In EMPA-REG OUTCOME, EMPA reduced the risk of clinically relevant kidney events in patients with T2D and HF at baseline. The renoprotective effects were observed on a background of standard of care and were consistent with those reported for the overall study population. The potential of EMPA to slow CKD progression in patients with HF (with or without diabetes) is being further investigated in ongoing HF-trials (EMPEROR-trials).



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **J. Butler:** Non-financial support; Boehringer Ingelheim.

665

Design of the EMPERIAL trials of empagliflozin in patients with chronic heart failure with reduced or preserved ejection fraction

W.T. Abraham[1], S.D. Anker[2], A. Salsali[3], B. Peil[4], C. Zeller[5], M. Brun[6], H. Macesic[7], M. Brueckmann[4], J. Lindenfeld[8], P. Ponikowski[9];
[1]The Ohio State University, Columbus, USA, [2]Charité University, Berlin, Germany, [3]Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, USA, [4]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [5]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, [6]Boehringer Ingelheim France, Reims cedex, France, [7]Boehringer Ingelheim (Canada) Ltd, Burlington, ON, Canada, [8]Vanderbilt University Medical Center, Nashville, USA, [9]Wrocław Medical University, Wroclaw, Poland.

Background and aims: Besides increasing the risks of cardiovascular death and hospitalisation, chronic heart failure (HF) with either reduced (HFrEF) or preserved (HFpEF) ejection fraction is associated with a high symptom burden, and impaired physical functioning. Empagliflozin is a sodium-glucose cotransporter-2 inhibitor that was shown in the EMPA-REG OUTCOME trial to reduce the risk of cardiovascular events, hospitalisations and mortality in patients with type 2 diabetes and established cardiovascular disease. Two Phase III trials, EMPERIAL-reduced and EMPERIAL-preserved, have been initiated to investigate the effects of empagliflozin on functional outcomes in patients with HFrEF and HFpEF, respectively.
Materials and methods: The EMPERIAL trials (Effect of EMPaglifozin on ExeRcise ability and HF-symptoms, In patients with chronic heArt faiLure) are two randomised, double-blind, placebo-controlled trials designed to evaluate the effect of empagliflozin on exercise capacity and symptoms in patients with either HFrEF (left ventricular ejection fraction ≤40%) or HFpEF (left ventricular ejection fraction >40%). Inclusion criteria include 6-minute walk test (6MWT) distance of 100 m to ≤350 m and elevated N-terminal pro-brain natriuretic peptide (NT-proBNP) >600 pg/mL in HFrEF, or >300 pg/mL (>600 pg/mL with atrial fibrillation) in HFpEF, respectively. In addition, patients with HFpEF must present with structural heart disease (left atrial enlargement and/or left ventricular hypertrophy) documented by echocardiogram and/or hospitalisation for HF within the previous 12 months. Patients must be clinically stable and on appropriate medical therapy for HF consistent with current guidelines, with doses stable for ≥4 weeks (or ≥2 weeks

for diuretics). Approximately 300 patients in each trial will be randomised 1:1 to receive empagliflozin 10 mg or placebo once daily for 12 weeks. The primary endpoint is the change from baseline in 6MWT distance at week 12. Key secondary endpoints are changes from baseline in Kansas City Cardiomyopathy Questionnaire, total symptom score and in Chronic Heart Failure Questionnaire Self-Administered Standardized format dyspnoea score at week 12. Patient Global Impression of change questionnaires and change from baseline in NT-proBNP at week 12 are other secondary endpoints.

Results: Recruitment for this trial has started in 2018.

Conclusion: The findings of the EMPERIAL-reduced and EMPERIAL-preserved trials will determine the effects of empagliflozin on symptoms, exercise capacity and patient reported outcome in patients with heart failure. The effects of empagliflozin on cardiovascular death and hospitalisation for heart failure in patients with chronic heart failure are being investigated in the EMPEROR-reduced and EMPEROR-preserved trials.

Clinical Trial Registration Number: NCT03448419 and NCT03448406
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **W.T. Abraham:** Employment/Consultancy; Consulting fees for my role as an Executive Committee member for the EMPERIAL trials.

666

Design and rationale of the EMPEROR trials of empagliflozin in patients with chronic heart failure with reduced or preserved ejection fraction

S.D. Anker[1], F. Zannad[2], J. Butler[3], G. Filippatos[4], A. Salsali[5], K. Kimura[6], J. Schnee[5], C. Zeller[7], S. Pocock[8], J.T. George[9], M. Brueckmann[9], M. Packer[10];
[1]Charité - Universitätsmedizin, Berlin, Germany, [2]Institut Lorrain du Coeur et des Vaisseaux, Nancy, France, [3]Department of Medicine, University of Mississippi, Jackson, USA, [4]University of Athens, Athens, Greece, [5]Boehringer Ingelheim Pharmaceuticals, Ridgefield, USA, [6]Boehringer Ingelheim Canada Ltd., Burlington, ON, Canada, [7]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, [8]London School of Hygiene and Tropical Medicine, London, UK, [9]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [10]Baylor Heart and Vascular Institute, Dallas, USA.

Background and aims: Despite available therapies for heart failure (HF) with reduced ejection fraction (HFrEF), rates of hospitalization and mortality remain high. HF with preserved ejection fraction (HFpEF) increasingly contributes to HF hospitalizations with outcomes as poor as in patients with HFrEF, and there is no evidence-based therapy for HFpEF patients. In the EMPA-REG OUTCOME trial in patients with type 2 diabetes mellitus (T2DM) and established cardiovascular (CV) disease, the sodium-glucose co-transporter-2 (SGLT-2) empagliflozin reduced the risk CV mortality by 38% and the risk of HF hospitalizations (HHF) by 35%. Several mechanisms have been put forward to explain these benefits, which may go beyond the blood glucose lowering effect of empagliflozin. This raises the possibility of using empagliflozin as treatment for patients with established HF regardless of the presence of diabetes.

Materials and methods: Two phase III randomized, double-blind trials, EMPEROR-Reduced and EMPEROR-Preserved, will explore the efficacy and safety of once daily empagliflozin 10 mg compared with placebo, in patients with chronic HFrEF or HFpEF. The EMPEROR-Reduced trial includes patients with left ventricular ejection fraction (EF) ≤40% and elevated NT-proBNP levels (cut-offs for patients without/with atrial fibrillation are: NT-proBNP ≥2500/5000 pg/ml with EF 36–40%, ≥1000/2000 pg/ml with EF 31–35%, ≥600/1200 pg/ml with EF ≤30%). Alternatively, patients with EF ≤40% qualify, if they had a HHF within 12 months and present with NT-proBNP levels of ≥600 pg/ml (without AF) or ≥1200 pg/ml (with AF). In EMPEROR-Preserved, patients with chronic HFpEF with left ventricular EF >40% will be included. Patients are required to have elevated NT-proBNP levels (>300 pg/ml for patients without AF, or >900 pg/ml for patients with AF), and structural heart disease or documented HHF within 12 months. The composite primary endpoint in both trials is the time to first adjudicated CV death or HHF. Approximately 2850 and 4130 patients are planned to be randomized in EMPEROR-Reduced and EMPEROR-Preserved, respectively. The number of patients in these event-driven trials may be increased based on a blinded assessment of the primary event rate. The incidence of adjudicated HHF and the renal outcome of eGFR slope of change from baseline are key secondary endpoints.

Results: Recruitment for both trials started in 2017.

Conclusion: The EMPEROR-Reduced study evaluates empagliflozin in patients with chronic HF with reduced ejection fraction. As yet, EMPEROR-Preserved is the only phase III outcome study to evaluate an SGLT-2 inhibitor in patients with chronic HFpEF. Together, these trials are expected to deliver conclusive insights regarding the value of empagliflozin treatment for patients with heart failure.

Clinical Trial Registration Number: NCT03057977 and NCT03057951
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **S.D. Anker:** Employment/Consultancy; Boehringer Ingelheim, Astra Zeneca.

667

Effect of empagliflozin on cardiovascular events including recurrent events in the EMPA-REG OUTCOME trial

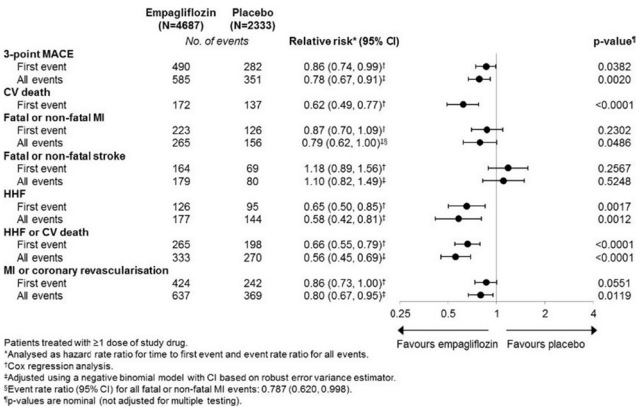
S.S. Lund[1], D.K. McGuire[2], B. Zinman[3], S.E. Inzucchi[4], S.D. Anker[5], C. Wanner[6], S. Kaspers[1], J.T. George[1], U. Elsasser[1], H.J. Woerle[1], D. Fitchett[7];
[1]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [2]University of Texas Southwestern Medical Center, Dallas, USA, [3]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Canada, [4]Section of Endocrinology, Yale University School of Medicine, New Haven, USA, [5]Division of Cardiology and Metabolism – Heart Failure, Cachexia & Sarcopenia; Department of Cardiology (Campus CVK) & Berlin-Brandenburg Center for Regenerative Therapies (BCRT); Charité - Universitätsmedizin Berlin, Berlin, Germany, [6]Würzburg University Clinic, Würzburg, Germany, [7]St Michael's Hospital, Division of Cardiology, University of Toronto, Toronto, Canada.

Background and aims: In the EMPA-REG OUTCOME trial in patients with type 2 diabetes and established CV disease, empagliflozin reduced the risk of 3-point MACE (composite of CV death, MI, or stroke) by 14%, CV death by 38% and hospitalisation for heart failure (HHF) by 35% vs placebo in analyses of time to first event. We assessed the effect of empagliflozin on all (first and recurrent) CV events.

Materials and methods: Patients were randomised to receive empagliflozin 10 mg, empagliflozin 25 mg, or placebo in addition to standard of care. We assessed the effects of empagliflozin pooled vs placebo based on all adjudicated CV events using a negative binomial model with confidence intervals based on robust error variance estimators to account for within-subject correlation.

Results: A total of 7020 patients were treated (mean [SD] age 63 [9] years, 71% male, 47% with history of MI, 23% with history of stroke, 10% with HF). In analyses including all events, the event rate ratio (95% CI) with empagliflozin vs placebo was 0.78 (0.67, 0.91; $p = 0.0020$) for 3-point MACE, 0.79 (0.620, 0.998; $p = 0.0486$) for MI, 1.10 (0.82, 1.49; $p = 0.5248$) for stroke, 0.58 (0.42, 0.81; $p = 0.0012$) for HHF, 0.56 (0.45, 0.69; $p < 0.0001$) for the composite of CV death or HHF, and 0.80 (0.67, 0.95; $p = 0.0119$) for the composite of MI or coronary revascularisation. Results were consistent with analyses of first events (Figure).

Conclusion: Analyses of all (first and recurrent) CV events in the EMPA-REG OUTCOME trial complement previous analyses and confirm the consistency of the results.



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **S.S. Lund:** Employment/Consultancy; Employee of Boehringer Ingelheim.

PS 051 Tackling glucose and fat with novel agents

668

Liver directed FGF21 gene therapy reverses obesity and insulin resistance

C. Jambrina, V. Jimenez, E. Casana, V. Sacristan, S. Muñoz, S. Darriba, J. Rodó, M. Garcia, S. Marco, A. Ribera, I. Elias, A. Casellas, I. Grass, J. Ruberte, F. Bosch;
Universitat Autònoma de Barcelona, Bellaterra, Spain.

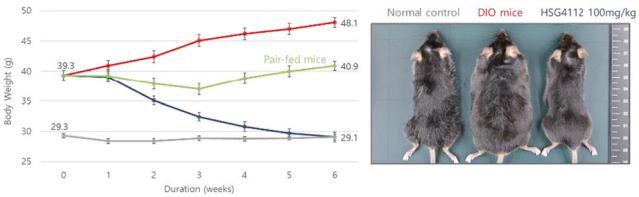
Background and aims: The prevalence of type 2 diabetes (T2D) and obesity is increasing worldwide. Currently available therapies are not suited for all patients in the heterogeneous obese/T2D population, and there is a need for novel treatments. Fibroblast growth factor 21 (FGF21) is considered a promising therapeutic agent for T2D/obesity. Native FGF21 has, however, poor pharmacokinetic properties, making gene therapy an attractive strategy to achieve sustained circulating levels of this protein. Thus, the aim of this study was to study the efficacy and safety of a gene therapy approach to overexpress native FGF21 in the liver, the main tissue from where circulating endogenous FGF21 is derived.
Materials and methods: We used adeno-associated viral vectors (AAV) and a liver specific promoter to genetically engineer the liver to achieve long-term FGF21 overexpression in two murine models of obesity and insulin resistance. Two-month-old ("Young adults") or seven-month-old ("Adults") C57Bl6 mice were fed either a chow or a HFD for 10 weeks and then administered intravenously with different doses of AAV vectors encoding a murine optimized FGF21 coding sequence or non-coding null vectors as controls. Following AAV delivery, mice were maintained on chow or HFD feeding for about 1 year, and body weight and metabolic parameters were monitored regularly. To further test the anti-obesogenic and anti-diabetic effects of FGF21 gene transfer to the liver, we performed a similar study in 8-week-old ob/ob mice.
Results: Treatment of animals fed a high-fat diet for a long time with AAV-FGF21 vectors resulted in marked reductions in body weight, adipose tissue hypertrophy and inflammation, hepatic steatosis, inflammation and fibrosis and insulin resistance for >1 year. This therapeutic effect was achieved in the absence of side effects on bones despite continuously elevated serum FGF21. Additionally, AAV treatment with AAV8-FGF21 vectors reduced the incidence of liver neoplasms associated to long-term-HFD-feeding. The therapeutic efficacy of FGF21 gene transfer to the liver was further confirmed in the genetically obese ob/ob mouse model.
Conclusion: Altogether, our results demonstrate that a single administration of AAV-FGF21 vectors to obese animals enabled a long-lasting increase in FGF21 levels in circulation, which resulted in sustained counteraction of obesity, insulin-resistance and NASH in the absence of adverse events. To the best of our knowledge, this is the longest follow up ever reported for an FGF21-based treatment in HFD-fed mice, and demonstrates both the efficacy and the safety of our therapeutic approach.
Supported by: SAF2014-54866R, 2014-SGR-1669, ICREA Academia Award to F.B., EFSD/MSD
Disclosure: **C. Jambrina:** Grants; Ministerio de Economía y Competitividad (MINECO) and FEDER, Plan Nacional I+D+I (SAF2014-54866R), Generalitat de Catalunya (2014 SGR 1669 and ICREA Academia award to F.B.), European Commission (MYOCURE, PHC-14-2015-667751), European Foundation for the Study of Diabetes (EFSD/MSD European Research Programme on Novel Therapies for Type 2 Diabetes, 2013). Honorarium; V.J. was recipient of a post-doctoral reasearch fellowship from EFSD/Lilly, E.C., V.S. and C.M. received a predoctoral fellowship from Ministerio de Educación, Cultura y Deporte, J.R. received a predoctoral fellowship from Ministerio de Economía y Competitividad. Other; Veronica Jimenez, Claudia Jambrina and Fatima Bosch are coinventors on a patent application for the use of AAV vectors for the treatment of metabolic disorders.

669

Efficacy and safety of HSG4112, a novel anti-obesity oral agent in diet-induced obesity (DIO) mice

K.-W. Lim, K. Kim, S.-K. Yoo, I. Jo;
Glaceum Inc., Suwon, Republic of Korea.

Background and aims: Obesity is defined by excess adipose mass and adipose tissue expansion, which occurs through adipocyte hypertrophy and hyperlasia. Hypertrophic adipocytes (sick fat) secrete pro-inflammatory cytokines, such as, TNF-α, IL-6, resistin and MCP1 etc., which lead to chronic inflammation that causes problems with triglyceride metabolism. The currently existing drugs for obesity are altering either appetite or absorption of calories. Yet, there are no approved drugs to solve the fundamental problem of triglyceride metabolism. The purpose of this study was to evaluate HSG4112 as a new drug candidate to resolve the fundamental energy metabolism in obesity.
Materials and methods: HSG4112 is a novel small molecule anti-obesity oral agent discovered and developed by Glaceum Inc. and its efficacy was tested in DIO mice. HSG4112 was given orally once daily for 7 days for 6 consecutive weeks (qd × 7 × 6). The test consisted of a normal control group (normal diet), a vehicle control group (high fat diet), a test group (HSG4112 100 mg/kg) and a pair-fed group.
Results: After 6 weeks, the total mean weight loss was 10.2 g (−26.0%) in the test group. The contribution made by reduced food intake was 38.0% and the energy expenditure effect was 62.0%. Increase of O_2 consuming and CO_2 generating rates and decrease of 5' Adenosine Monophosphate-activated Protein Kinase (AMPK) activity in hypothalamus after oral administration of HSG4112 to DIO mice were observed. Furthermore, enhanced browning effect with the characteristics of high mitochondria content was observed in scapular brown adipose tissue. As a result, lean and fit body shape was achieved in the test group. Single oral dose toxicity study of HSG4112 was performed with rat and dog. The Maximum Tolerated Dose (MTD) 2,000 mg/kg was observed from both rat and dog.
Conclusion: In nonclinical studies, HSG4112 demonstrated its weight reduction efficacy mainly by increased energy consumption with excellent safety profiles. These results suggest HSG4112 as a promising new drug candidate to resolve the fundamental energy metabolism in the treatment of obesity.



Disclosure: **K. Lim:** None.

670

Results of an interim analysis of a phase 2, randomised, double-blind, placebo-controlled clinical trial of ZGN-1061 in patients with obesity and type 2 diabetes

T. Kim, D. Zhuang, T.E. Hughes, D.D. Kim, K. Taylor;
Zafgen, Inc., Boston, USA.

Background and aims: ZGN-1061 (1061) is a methionine aminopeptidase 2 (MetAP2) inhibitor being developed to improve glycemic control in type 2 diabetes. This clinical trial investigated the effect of 12 weeks of 1061 (0.05, 0.3, 0.9 mg or placebo) administered s.c. every 3 days on A1C, safety, and tolerability. Stable noninsulin diabetes therapy was permitted.
Materials and methods: The study included 129 patients; an interim analysis was conducted prior to study completion on a subset of patients with Week 8 measurements ($N = 57$). Of these, 41 patients also had a Week 12 A1C measurement.
Results: Baseline characteristics of the interim intent-to-treat population ($N = 57$) were: 54% male, 77% white, (mean ± SD) age 54 ± 8 years, A1C 8.7 ± 1.0%, BMI 37.2 ± 6.4 kg/m^2. At Week 8, the least squares (LS) mean ± SE change in A1C for placebo was 0.2 ± 0.1% ($N = 13$) vs −0.4 ± 0.2% with 0.9 mg 1061 ($N = 13$, LS mean ± SE difference −0.6 ± 0.2%, $p < 0.02$). In patients with Week 12 measurements, the change in A1C was 0.4 ± 0.2 ($N = 12$) vs −0.5 ± 0.2 ($N = 8$, LS mean ± SE difference −0.9 ± 0.3, $p < 0.01$). There were trends for weight loss and improved fasting plasma glucose with 0.9 mg 1061 vs placebo as well as improvements in biomarkers, including hsCRP (Week 8 −2.1 ± 2.2 mg/L vs 1.0 ± 2.1, ns), adiponectin (1.0 ± 0.3 ug/mL vs −0.1 ± 0.3, $p < 0.05$), leptin (−6.9 ± 1.9 ng/mL vs 2.1 ± 1.7, $p < 0.05$), FGF21 (0.026 ± 0.014 ng/mL vs 0.000 ± 0.013; ns), and postprandial glucose AUC ($p < 0.01$). Improvements in efficacy measures with the lower doses of 1061 were variable. There were no serious or severe adverse events (AEs) and no subjects withdrew due to an AE. The most common AE (1061 > placebo) was upper respiratory tract infection. The 1061 pharmacokinetic profile indicated that exposure was within target levels for efficacy and safety and there were no changes in thrombosis markers (eg, D-dimer).
Conclusion: In this interim analysis, 1061 produced improvements in glycemic control and metabolic biomarkers that are consistent with MetAP2 inhibition. 1061 was also well tolerated with no safety signals in all doses tested. Week 12 results from the full analysis dataset will be presented.
Clinical Trial Registration Number: NCT03254368
Supported by: Zafgen, Inc.
Disclosure: **T. Kim:** Employment/Consultancy; Zafgen. Stock/Shareholding; Zafgen.

671

ZGN-1061 improves metabolic parameters and hepatic pathology in an obese mouse model of diet-induced and biopsy-confirmed nonalcoholic steatohepatitis

J.E. Vath[1], M. Illemann[2], S.S. Veidal[2], M. Feigh[2], B.F. Burkey[1], T.E. Hughes[1];
[1]Zafgen, Inc., Boston, USA, [2]Gubra, Hørsholm, Denmark.

Background and aims: ZGN-1061 (1061) is a methionine aminopeptidase 2 (MetAP2) inhibitor being developed to improve glycemic control in type 2 diabetes. In diet-induced obese (DIO) mice, 1061 reduces fat mass and improves glycemic control, lipid metabolism, and other metabolic parameters.
Materials and methods: This study investigated 8 weeks of s.c. treatment with 1061 (0.3 mg/kg, $N = 11$) or vehicle ($N = 10$) on metabolic parameters, hepatic pathology, and nonalcoholic fatty liver disease (NAFLD) activity score (NAS; a composite measure of steatosis, inflammation, and ballooning degeneration; range 0–8) in male mice with diet-induced and biopsy-confirmed nonalcoholic steatohepatitis (DIO-NASH).
Results: At Week 8, there was a vehicle-corrected weight loss of 13.1% with 1061 ($p < 0.001$). 1061-treated mice had reduced liver weight (mean ± SE: 2.6 ± 0.4 g) vs vehicle (3.8 ± 0.8 g, $p < 0.001$) and a 21% reduction in liver triglyceride content ($p < 0.05$). There was no change in food intake. NAS was unchanged in vehicle-treated mice (baseline: 6.2 ± 0.1, Week 8: 6.4 ± 0.2, ns). 1061-treated mice had reduced NAS (baseline: 6.2 ± 0.2, Week 8: 5.0 ± 0.3, $p = 0.002$). Two of the NAS component measures improved: steatosis in vehicle-treated mice was unchanged (baseline: 3.0 ± 0.0, Week 8: 3.0 ± 0.0, ns), whereas 1061 reduced steatosis (baseline: 2.9 ± 0.1, Week 8: 2.5 ± 0.2, $p = 0.02$), and hepatocellular ballooning was unaffected in vehicle-treated mice (baseline: 0.7 ± 0.2, Week 8: 0.6 ± 0.2, ns), whereas 1061 significantly reduced ballooning severity (baseline: 0.6 ± 0.2, Week 8: 0.0 ± 0.0, $p = 0.006$). There was no treatment effect on fibrosis stage or liver collagen 1A. However, liver

galectin-3 was reduced with 1061 vs vehicle. 1061 produced a reduction in terminal plasma alanine aminotransferase and aspartate aminotransferase (AST) vs vehicle.

Conclusion: In DIO-NASH mice, 1061 markedly reduced body weight in conjunction with liver weight and triglyceride content. Importantly, 1061 improved liver function, steatohepatitis, and NAS composite score. These findings introduce 1061 as a promising therapy for obesity-related NASH.

Supported by: Zafgen, Inc.

Disclosure: **J.E. Vath:** Employment/Consultancy; Zafgen. Stock/Shareholding; Zafgen.

672

ZGN-1061, a novel MetAP2 inhibitor, and liraglutide combine to improve glycaemic control and reduce body weight in a rat model of diet-induced obesity

B.F. Burkey[1], P.G.J. Pedersen[2], T.X. Pedersen[2], M. Feigh[2], J.E. Vath[1], T.E. Hughes[1];
[1]Zafgen, Inc., Boston, USA, [2]Gubra, Hørsholm, Denmark.

Background and aims: ZGN-1061 (1061) is a methionine aminopeptidase 2 (MetAP2) inhibitor being developed to improve glycemic control in type 2 diabetes. In diet-induced obese (DIO) mice, 1061 reduces fat mass and improves glycemic control, lipid metabolism, and other metabolic parameters.

Materials and methods: This study investigated 5 weeks of once-daily s.c. treatment with a submaximal 0.3 mg/kg dose of 1061, a maximal 0.4 mg/kg dose of liraglutide (lira), 0.3 mg/kg 1061 + 0.4 mg/kg lira (1061+lira), or vehicle ($N = 10$/group) on body weight, food intake/preference, and glycemic control in the Gubra DIO rat model (ad libitum access to standard chow and highly palatable high-fat, high-sugar [HFHS] diet). Fasting (4h) blood glucose was collected weekly and a semifasted (access to 50% of daily energy requirement in the prior 16 hours) OGTT was performed at Week 4.

Results: At Week 5 (Day 33), body weight changed by +1.7% with vehicle vs −6.0% with 1061, −9.1% with lira, and −16.9% with 1061+lira (all $p < 0.001$). Weight loss occurred earlier with lira (Day 1) than 1061 (Day 5). Starting at Day 6, intake of the HFHS diet was modestly reduced (15–30%) with 1061 vs vehicle. In contrast, lira and 1061+lira induced rapid and sustained 40–50% and 50–80% reductions in intake of the HFHS diet, respectively, over the first 8–9 days. Cumulative (Day 0–35) intake of the HFHS diet was reduced vs vehicle with 1061 (13.4%, $p = 0.07$), lira (17.8%, $p < 0.01$), and 1061+lira (38.2%, $p < 0.001$). Cumulative intake of standard chow was increased with 1061 and 1061+lira but not lira alone. There was a modest reduction in fasting glucose (mean Week 1–5) with 1061 vs vehicle ($p = 0.08$), a significant reduction with lira, and 1061+lira produced the largest reduction (both $p < 0.001$) that was significantly lower than either agent alone (both $p < 0.01$). In the OGTT, 1061 reduced the glucose AUC vs vehicle by 37% ($p = 0.07$), lira by 60% ($p < 0.001$) and 1061+lira by 80% ($p < 0.001$), achieving an AUC that was no different from lean rats.

Conclusion: In this DIO rat model, 1061 and lira had complementary effects on reducing body weight and normalizing glycemic control. Combination treatment with 1061 and liraglutide may yield greater weight loss and glycemic control than either agent alone in patients with type 2 diabetes.

Supported by: Zafgen, Inc.

Disclosure: **B.F. Burkey:** Employment/Consultancy; Zafgen. Stock/Shareholding; Zafgen.

673

Novel GPR40 agonist CPL207-280CA independently improves glycaemia and mitigates neuropathic pain in diabetic rodents

P. Buda, K. Bazydlo, M. Mach, F. Stelmach, R. Dzida, K. Dubiel, J. Pieczykolan, M. Wieczorek;
CelonPharma, Kielpin, Poland.

Background and aims: The new generation drug in type 2 diabetes (T2D) is expected to effectively control glycaemia, be safe, ensure infrequent dosage and, importantly, manage diabetic complications. GPR40 activation in pancreatic β-cells improves glycaemic control in T2D through enhancement of glucose-stimulated insulin secretion (GSIS). This process is mediated by Ca^{2+} release from intracellular stores. Similar mechanism was proposed in endogenous pain inhibitory system regulating pain sensation in the brain. These properties of GPR40 make its agonists promising agents acting on the frontier of both diabetes and its complication - neuropathy. However, the most clinically advanced GPR40 agonist fasiglifam was withdrawn from phase 3 due to high drug retention in the liver and bile acid transporters inhibition. In this study we aimed at designing more effective GPR40 agonist devoid of fasiglifam drawbacks. We also investigated its capacity to alleviate allodynia (exaggerated pain sensation) in diabetes-induced neuropathy.

Materials and methods: EC_{50} of CPL207-280CA was assessed in Ca^{2+} influx assay in CHO cells expressing human GPR40. Insulin secretion *in vitro* was measured in MIN6 cells treated with 20mM glucose. *In vivo* compound efficacy was assessed by intraperitoneal glucose tolerance test (IPGTT) in Wistar rats. In pharmacokinetic studies test compounds were administered p.o. and i.v. to C57BL6/cmdb mice and drug concentration in blood was measured at different time points up to 24h (assessed by mass spectrometry). Neuropathy was studied in STZ-treated (200 mg/kg) Albino Swiss mice, which developed diabetes (glucose >300 mg/dl) after 20 post-treatment days accompanied by allodynia manifestation (pain feeling threshold drop from 3.30 ± 0.20 to 1.75 ± 0.07 g). Mechanical allodynia in mice was assessed by paw withdrawal threshold in von Frey test.

Results: CPL207-280CA showed substantially higher efficacy as compared to fasiglifam in Ca^{2+} influx assay ($EC_{50} = 70$ vs 250 nM, respectively). Moreover, it also showed 3.9-times greater enhancement of GSIS in MIN6 cells (at 10 μM). Similarly, in glucose-challenged Wistar rats CPL207-280CA, compared to fasiglifam, improved GSIS 2.5-times and reduced glucose level 2-times without causing hypoglycaemia. Strikingly, the glucose lowering effect was present also during second glucose challenge performed 6 h later, indicating long-lasting activity of the compound allowing infrequent dosing. Pharmacokinetic studies in C57BL6/cmdb mice revealed different organ distribution of the compounds. In contrast to fasiglifam mostly penetrating to the liver, CPL207-280CA was present mainly in serum. Interestingly, only CPL207-280CA managed to pass blood-brain barrier reaching a concentration of 1.6 μM in the brain, well above its EC_{50}. When administered p.o. to neuropathic pain model STZ-mice, CPL207-280CA proved as effective as pregabalin (well acknowledged agent for neuropathic pain treatment) already after 1h treatment, presenting superior features to other glucose-regulating agents. Improved pain threshold (at the level of 2.30 ± 0.10 g) was observed at 10 mg/kg CPL207-280CA while full nominal pain sensation recovery was reached at 30 mg/kg.

Conclusion: We conclude that CPL207-280CA is a candidate for a potent new generation drug in T2D, which can safely and durably improve glucose control, and in parallel manage neuropathic pain, placing it well ahead among competitor drugs.

Supported by: NCBiR, Poland

Disclosure: **P. Buda:** Grants; NCBiR Poland.

674

6-amino-6-deoxy paramylon improved obesity and glucose metabolism in a diet-induced obesity mouse model

S. Suzuki[1], T. Hashimoto[2], K. Shibata[3], M. Nakano[2], N. Onaka[4], G. Tsubouchi[4], M. Takahashi[4], A. Akashi[4], N. Hirawa[1], Y. Toya1[1], K. Ohashi[5], Y. Goshima[2], K. Tamura[1];
[1]Department of Cardiorenal Medicine, Yokohama City University School of Medicine, Kanagawa, [2]Department of Pharmacology, Yokohama City University School of Medicine, Kanagawa, [3]Artisan-ab Co.,Ltd, Kanagawa, [4]KOBELCO ECO-SOLUTIONS Co., Ltd., Hyogo, [5]Department of Pathology, Yokohama City University School of Medicine, Kanagawa, Japan.

Background and aims: Obesity and type 2 diabetes are preventable causes of death. Bile acid binding resins are beneficial for obesity and diabetes, but they are more likely to accompany abdominal distention and constipation. These side effects can be prevented by using dietary fiber. β-glucan is one of the dietary fiber and is reported to improve obesity and glucose metabolism. Barley or oat β-glucan increases fecal output of fat and bile acids synthesis from cholesterol in the liver. Paramylon is one of the component of green algae, Euglena, and consists of β-glucan. Besides barley or oat β-glucan, paramylon is reported to have no effect on hyperglycemia in diabetic rats. Here we generate cationized 6-amino-6-deoxy paramylon, which is just like bile acid binding resins. The effect of 6-amino-6-deoxy paramylon on obesity and glucose metabolism were examined in a diet-induced obesity mouse model.
Materials and methods: As an in vitro experiment, bile acids were incubated with 6-amino-6-deoxy paramylon in a fed state simulated human intestinal fluid. After that, the adsorption rate of bile acid was measured. Furthermore, in an experiment using animals, male C57BL/6J mice were fed high-fat (HF) diets supplemented with 6-amino-6-deoxy paramylon for 5 weeks. Body weight, blood sugar, serum LDL cholesterol, fecal bile acid composition and fecal lipid content were assessed. Hepatic small heterodimer partner (SHP) and cholesterol 7 alpha-hydroxylase (CYP7A1) gene expression levels were also analyzed to investigate the effect of 6-amino-6-deoxy paramylon on bile acids synthesis.
Results: Although paramylon barely absorbed bile acids, 6-amino-6-deoxy paramylon absorbed effectively in a simulated intestinal fluid. In mice, supplementation of 1% or 2% 6-amino-6-deoxy paramylon in a HF diet led to significant weight gain reduction (HF, 1%, 2%; +7.72 + 3.1, +4.16 + 1.4, +0.15 + 0.86 g). Significant decreases in concentrations of blood sugar (HF, 1%, 2%; 161 + 42, 138 + 23, 95 + 41 mg/dl) and serum LDL cholesterol (HF, 1%, 2%; 16.0 + 1.2, 9.4 + 2.1, 8.8 + 2.5 mg/dl) were shown. Strongly positive staining for Sudan IV in fecal smears indicated altered fat absorption when 6-amino-6-deoxy paramylon was supplemented in the HF diet. The composition of fecal bile acids were completely different, secondary bile acids were almost absent and primary bile acids like tauro-βmuricholic acids were dominated in feces of mice supplemented with 6-amino-6-deoxy paramylon. Moreover, decreased levels of SHP mRNA and increased levels of CYP7A1 mRNA were shown in the liver of mice supplemented with 6-amino-6-deoxy paramylon. 6-amino-6-deoxy paramylon had no effect on the food consumption and histology of digestive systems.
Conclusion: 6-amino-6-deoxy paramylon increased fecal output of fat and increases bile acids synthesis from cholesterol in the liver, thus improved obesity and glucose metabolism in a diet-induced obesity mouse model.
Supported by: KOBELCO ECO-SOLUTIONS Co
Disclosure: **S. Suzuki:** None.

675

Improvement of type 2 diabetes in hypogonadal men with long-term testosterone therapy (TTh) is sustained for up to 10 years compared to untreated controls

U. Wissinger[1], A. Haider[2], K.S. Haider[2], G. Doros[3], A. Traish[4], F. Saad[5]; [1]Medical Affairs Established Products, Bayer AG, Berlin, Germany, [2]Private Urology Practice, Bremerhaven, Germany, [3]Boston University School of Public Health, Boston, USA, [4]Boston University School of Medicine, Boston, USA, [5]Medical Affairs Andrology, Bayer AG, Berlin, Germany.

Background and aims: Numerous experimental and clinical studies have shown beneficial effects of TTh in hypogonadal men with T2DM. In an ongoing registry study in a urological setting, men with T2DM were analysed as a subgroup.
Materials and methods: 805 men with hypogonadism (total testosterone ≤12.1 nmol/L) participate in an ongoing observational registry. Of 311 (38.6%) with T2DM which was diagnosed elsewhere, 141 men opted for TTh and received testosterone undecanoate (TU) injections 1000 mg every 12 weeks (T-group), 170 against TTh and served as controls (CTRL). Measurements were performed 1–4 times a year. Mean change over time between groups were compared by mixed effects model for repeated measures with random effect for intercept and fixed effects for time, group and their interaction and adjusted for age, weight, waist circumference, fasting glucose, blood pressure and lipids to account for baseline differences between groups.
Results: Mean follow-up: 7.5, median: 8 years. Mean age: 61.8 ± 5.3 (T-group), 63.5 ± 4.9 (CTRL). Fasting glucose decreased from 7.7 ± 1.2 to 5.3 ± 0.1 mmol/L at 10 years in the T-group with statistical significance vs. previous year for the first two years and increased from 6.3 ± 0.7 to 8.2 ± 2.7 mmol/L in CTRL. Estimated adjusted difference between groups: −3.6 mmol/L ($p < 0.0001$ for all). Mean HbA_{1c} decreased from 9.0 ± 1.2 to 5.9 ± 0.3% at 10 years in the T-group with statistical significance vs. previous year for the first 7 years and increased from 7.8 ± 0.7 to 10.6 ± 1.7% in CTRL. Estimated adjusted difference between groups: −6.2% ($p < 0.0001$ for all). At baseline, 61 patients in the T-group were on insulin at a mean dose of 34 ± 11.1 units/d, and 63 in CTRL at a mean dose of 30.7 ± 6 units/d. The mean dose requirement in the T-group declined to 19.9 ± 10.5 with statistical significance vs. previous year for the first 8 years. In CTRL, insulin dose was increased to 42.2 ± 8.5 units/d with statistical significance vs. previous year for the entire observation time. Estimated adjusted difference between groups: −33.1 units/d ($p < 0.0001$ for all). In the T-group, 113 patients (80.1%) achieved HbA_{1c} <6.5% and 128 (90.8%) <7.0% at last measurement. Men not reaching targets were those with the shortest treatment duration. In CTRL, no patient achieved either of the two targets. All men had an increase in HbA_{1c}. In the T-group, weight decreased from 113.4 ± 13.9 to 90.7 ± 8.6 kg at 10 years ($p < 0.0001$) with statistical significance vs. previous year for the first 9 years. Waist circumference decreased from 112.6 ± 10.7 to 99.6 ± 5.2 cm ($p < 0.0001$) with statistical significance vs. previous year for the first 9 years. In CTRL, weight and waist circumference remained stable. Since all injections were administered in the doctor's office and documented, there was a 100% adherence to TTh.
Conclusion: Long-term TTh with TU in hypogonadal men with T2DM improved glycaemic control which deteriorated in untreated controls. In patients in the T-group on insulin, the dose could be substantially reduced. Reductions of weight and waist circumference in the T-group may have contributed to the observed effects. Correcting hypogonadism in men with T2DM supports standard diabetes treatment.
Supported by: Partial funding from Bayer AG for statistical analyses and data entry
Disclosure: **U. Wissinger:** Employment/Consultancy; Bayer AG. Stock/Shareholding; Bayer AG.

676

A network meta-analysis for the best procedures of bariatric surgery for cure of diabetes

S. Kodama, K. Fujihara, C. Horikawa, D. Ishii, D. Ishiguro, M. Harada, Y. Matsubayashi, T. Yamada, S. Tanaka, H. Sone;
Niigata University Faculty of Medicine, Niigata, Japan.

Background and aims: There is strong evidence that bariatric surgery leads to a higher remission rate for type 2 diabetes mellitus (T2DM) than non-surgical treatment (NST). However, it remains unsolved which surgical procedure is most efficacious.This network meta-analysis aimed to compare and rank surgical procedures for the treatment of T2DM.
Materials and methods: Electronic literature searches were conducted for randomized controlled trials (RCTs) in which at least one surgical treatment was included among multiple arms and the diabetes remission rate was included in study outcomes. A random effects network meta-analysis was performed within a frequentist framework. The hierarchy of

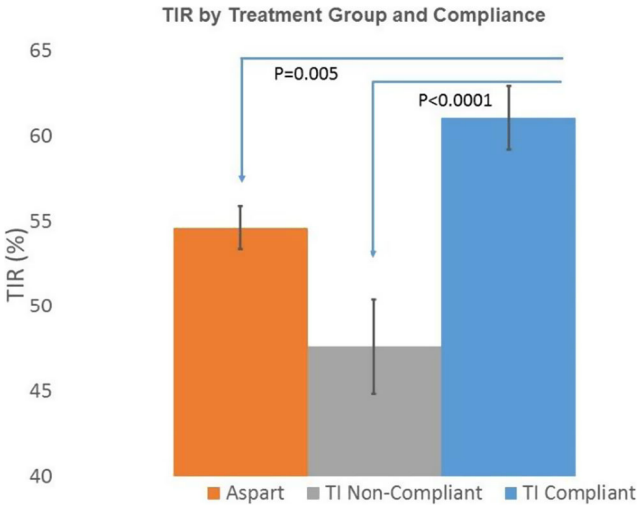
treatments was expressed as an under the cumulative ranking curve (SUCRA) value.
Results: There were 25 eligible RCTs covering NST and 8 kinds of surgical treatments: biliopancreatic diversion with duodenal switch (BPD/DS), biliopancreatic diversion without duodenal switch (BPD), Roux-en Y gastric bypass (RYGBP), mini gastric bypass (mini-GBP), laparoscopic adjustable gastric banding (LAGB), laparoscopic sleeve gastrectomy (LSG), greater curvature plication (GCP), and duodenal-jejunal bypass (DJB). The surgical treatments except for DJB were significantly more efficacious than NST. BPD, BPD/DS, mini-GBP, RYGBP, and LSG were significantly more efficacious than LAGB and GCP. Mini-GBP was more efficacious than LSG (relative risk (RR) (95% confidence interval (CI)), 1.85 (1.15–2.97)) and was borderline more significantly efficacious than RYGBP (RR (95% CI), 1.64 (0.99–2.71)). Overall and sensitivity analyses indicated that BPD and mini-GBP had the highest SUCRA values of these 9 treatments.
Conclusion: BPD or mini-GBP is the best option among surgical treatments in terms of diabetes remission.
Supported by: the Japan Society for the Promotion of Science
Disclosure: **S. Kodama:** None.

677

Improved time-in-range on continuous glucose monitor with Technosphere insulin compared to insulin Aspart in adults with type 1 diabetes: Stat study per protocol analysis

J. Snell-Bergeon[1], H.K. Akturk[1], E.A. Beck[1], L.J. Klaff[2], B.W. Bode[3], A. Peters[4], T.S. Bailey[5], S.K. Garg[1];
[1]Barbara Davis Center for Diabetes, University of Colorado AMC, Aurora, [2]Rainier Clinical Research Center, Renton, [3]Atlanta Diabetes Associates, Atlanta, [4]USC Westside Center for Diabetes, Beverly Hills, [5]AMCR Clinic, Escondido, USA.

Background and aims: Effective control of post-prandial hyperglycemia remains a challenge. In order to meet this challenge, a number of faster acting insulins have been developed for clinical use. However, inhaled Technosphere insulin (TI) has consistently shown a more rapid insulin absorption and clearance. This investigator-led, collaborative open-label pilot clinical trial evaluated the efficacy of TI in improving post-prandial blood glucose (PPBG), post-prandial glucose excursions (PPGE) and glucose Time-In-Range (TIR) (70–180 mg/dL) as measured by continuous glucose monitor (CGM) over a 4-week treatment period.
Materials and methods: Sixty patients with T1D on multiple daily injections were randomized in a multi-center study, stratified by A1c values (≤8% or >8%) to the control arm using aspart ($n = 34$) vs TI group ($n = 26$). Two patients in the TI arm discontinued from the study; and 2 had inadequate CGM data for analysis. Patients in the TI arm were instructed per protocol to take insulin doses pre-meal, and at 1 and 2 hours after meals based on observed PPBG values. Patients with at least 80% compliance with TI use were included in the PPT analysis ($n = 15$) and were compared to aspart treated individuals using usual mealtime administration guidelines and non-adherent TI users ($n = 7$). Baseline characteristics of the study group were compared to the randomization group using a student's t-test, and CGM data were analyzed using ANOVA models. Co-primary outcomes were TIR (70–180 mg/dl) and PPGE 1–4 hours after meals.
Results: Study participants did not differ on any baseline characteristic by randomization group, including: age, HbA1c, basal and bolus insulin doses, and forced expiration volume. Per protocol analysis, the TI-compliant group had significantly higher TIR (Figure) than both the aspart group and the TI-non-compliant group. The TI-compliant group also had lower PPGE 1–4 hours after meals than the aspart group (Least square mean ± SE = 113.5 ± 6.4 mg/dL vs. 133.8 ± 4.4 mg/dL, $p = 0.01$) but similar PPGE 1–4 hours after meals than the TI-non-compliant group (Least square mean ± SE = 115.9 ± 9.6 mg/dL, $p = 0.83$). Further, secondary endpoints including mean sensor glucose, SD for glucose, and time spent in hypoglycemic range (<70 mg/dL) were significantly lower in the TI-compliant when compared to the aspart group (all $p < 0.05$, data not shown).
Conclusion: These data uniquely demonstrate that the use of inhaled insulin (TI) with proper supplemental dosing significantly improves CGM measured TIR and lowers early PPGE values when compared to insulin aspart. In addition, the data suggests that TI can reduce time in hypoglycemia in adult patients with type 1 diabetes when compared to aspart.



Clinical Trial Registration Number: NCT03143816
Supported by: Support was provided by Mannkind, Inc for this investigator-developed study
Disclosure: **J. Snell-Bergeon:** Grants; Mannkind, Inc.

PS 052 Quality of nutrients and meals: How important are they?

678

Time of fat and carbohydrate intake affects substrate oxidation and adipokine secretion in subjects with impaired glucose metabolism

O. Pivovarova[1,2], K. Kessler[1,2], S. Hornemann[1,2], M. Markova[1,2], K.J. Petzke[3], M. Kemper[1,2], N. Rudovich[1,4], A. Kramer[5], A.F.H. Pfeiffer[1,2];
[1]Dept. of Clinical Nutrition, German Institute of Human Nutrition, Nuthetal, Germany, [2]German Center for Diabetes Research (DZD), München-Neuherberg, Germany, [3]Research Group Physiology of Energy Metabolism, German Institute of Human Nutrition, Nuthetal, Germany, [4]Spital Bülach, Bülach, Switzerland, [5]Laboratory of Chronobiology, Charité University of Medicine, Berlin, Germany.

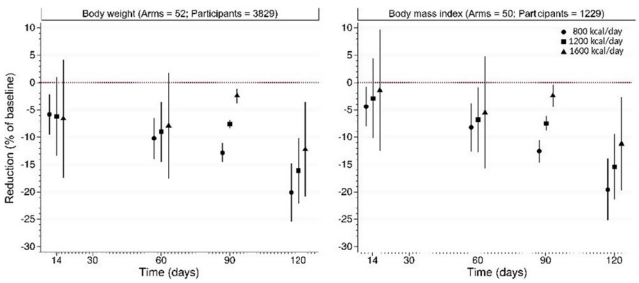
Background and aims: We recently showed that a diet where fat is mainly eaten in the morning and carbohydrates mainly in the evening (compared to the reverse order) worsens glycemic control in people with prediabetes. Here, we investigated effects of the same dietary patterns on energy metabolism and daily profiles of circulating lipids, adipokines and inflammatory markers.
Materials and methods: In a randomized controlled cross-over trial, 29 non-obese men (with normal glucose tolerance (NGT) $n = 18$; or impaired fasting glucose/glucose tolerance (IFG/IGT) $n = 11$) underwent two isocaloric 4-week diets: (1) carbohydrate-rich meals until 13.30 and fat-rich meals between 16.30 and 22.00 (HC/HF) *versus* (2) inverse sequence of meals (HF/HC). After each intervention period, two meal tolerance tests were performed, at 09.00 and 15.40, respectively, according to the previous intervention. Substrate oxidation and levels of circulating lipids, adipokines and cytokines were assessed pre- and postprandially. Postprandial inflammatory response in leukocytes was analyzed ex vivo.
Results: Fasting levels of blood markers did not differ between diets. However, diurnal distribution of carbohydrates and fat modulated daily profiles of carbohydrate and lipid oxidation, as well as patterns of beta-hydroxybutyrate, triglycerides, LDL cholesterol, leptin, visfatin and of the LPS-induced inflammatory response in blood leukocytes. On the HF/HC diet, daily respiratory quotient was lower ($p = 0.026$) and lipid oxidation rate higher ($p = 0.008$) in IFG/IGT subjects compared to NGT subjects. The HF/HC diet decreased average daily leptin levels in the whole cohort ($p = 0.017$) and adiponectin in IFG/IGT subjects ($p = 0.037$).
Conclusion: The HF/HC diet induces a deterioration of metabolic flexibility in IFG/IGT subjects and affects adipokine secretion confirming the unfavorable effects of the HF/HC diet in subjects with impaired glucose metabolism.
Clinical Trial Registration Number: NCT00390637
Supported by: DFG grant KFO218 PF164/16-1 OP, AK, AFHP; DDG 2015, OP; DZD82DZD0019G, OP
Disclosure: **O. Pivovarova:** None.

679

Efficacy of low- and very-low-calorie diets in overweight and obese patients with type 2 diabetes: a meta-analysis of intervention studies

D.E. Kloecker, F. Zaccardi, M.J. Davies, M. Dario, K. Khunti, D.R. Webb;
University of Leicester, Leicester, UK.

Background and aims: In overweight and obese patients with type 2 diabetes mellitus recommendations based on calorie restriction with Very-Low-Calorie Diets (VLCD) are based mainly on evidence from observational studies. We undertook a comprehensive systematic review and meta-analysis of relevant intervention studies to clarify existing evidence about the effects of calorie restriction on body weight in type 2 diabetes.
Materials and methods: We electronically searched articles on MEDLINE, EMBASE, and CINAHL from inception to March 2018 to identify intervention studies (single or multiple arms) reporting the effect of Low-Calorie Diets (LCD; ≤1600 kcal/day) and VLCDs (≤800 kcal/day) on the outcomes body weight and body mass index (BMI) in people with type 2 diabetes. Given the non-linear effects of diets over time, we modelled outcomes using restricted cubic splines and accounting for study-specific estimate precision (inverse of study variance). Egger's test was used to assess publication bias.
Results: Of 803 records identified, 47 met the inclusion criteria comprising 55 study arms and 3883 participants (45% male); mean age was 51.9 (SD 7.0) and duration of diabetes 7.0 (3.8) years; mean weight and BMI before the interventions were 102.8 (11.7) kg and 36.6 (4.6) kg/m^2, respectively. The median duration of intervention was 56 (IQR 28–84; range 6–112) days and the mean daily target was 729 (SD 381, range 23.2–1600) kcal. Studies were published between 1978 and 2017 and were generally of low-moderate quality. There was a significant publication bias for body weight (Egger's $p < 0.001$) but not for BMI ($p = 0.057$). For 800 kcal/day, estimated reduction in body weight was already evident at 14 days (−5.8%; 95%CI: −9.5 to −2.2) and progressively increased at 60, 90, and 120 days while for 1200 kcal/day the effect was present only from 60 days onwards (Figure). Conversely, for 1600 kcal/day there was no effect at 14 and 60 days, a small reduction at 90 days, and an uncertain effect at 120 days. BMI followed the same pattern of body weight change.
Conclusion: Short-term efficacy of VLCDs and some LCDs is supported by evidence from reported intervention studies. Uncertainty remains over the true effect of diets on body weight and BMI due to a paucity of high-quality studies, the possibility of publication bias, and the relatively short duration of published interventions. This highlights a need for RCTs with larger sample size and longer duration to confidently quantify the efficacy of VLCDs and LCDs in overweight and obese people with type 2 diabetes.



Disclosure: **D.E. Kloecker:** None.

680

Dietary patterns and non-alcoholic fatty liver disease in a Greek case-control study

I.P. Kalafati[1], D. Borsa[1], M. Dimitriou[1], K. Revenas[2], A. Kokkinos[3], G. Dedoussis[1];
[1]Dietetics and Nutritional Sciences, Harokopio University of Athens, Athens, [2]Radiology Department, Laiko General Hospital, Athens, [3]First Department of Propaedeutic Medicine, Laiko General Hospital, Athens, Greece.

Background and aims: Dietary patterns of non-alcoholic fatty liver disease (NAFLD) patients have not been defined yet. Up to now, there are only a handful of studies on the field and none in a European population.
Aims: We examined the relationship of a posteriori derived dietary patterns with NAFLD risk and NAFLD-related biomarkers in a Greek case-control study.
Materials and methods: A total of 351 individuals were recruited (134 NAFLD patients, 217 controls). Disease was diagnosed with abdominal ultrasound (U/S). Dietary intake data were collected with a 172 semi-quantitative Food Frequency Questionnaire (FFQ) and dietary patterns were derived by factor analysis. Consumption of dietary patterns was

divided into quartiles. Multivariate logistic and linear regression models were applied to investigate associations of dietary patterns with NAFLD risk and NAFLD-associated biomarkers.
Results: Four dietary patterns were identified. Adherence to the "Fast-food type" pattern was independently associated with higher odds for NAFLD. However, results were statistically significant only for the highest vs the lowest consumption (OR = 3.9, $p = 0.003$). On the contrary, individuals in the 2nd quartile of the "Unsaturated fatty acids" pattern had 55.7% reduced risk of developing NAFLD compared to those in the 1st quartile, after adjusting for the main confounders. The "Fast-food type" pattern was further associated with higher levels of CRP and uric acid and the "Unsaturated fatty acids" pattern with reduced levels of insulin and HOMAIR ($p < 0.05$). The "Prudent" dietary pattern was associated with triglycerides and uric acid levels (beta = −5.960, $p = 0.037$ and beta = −0.153, $p = 0.035$, respectively).
Conclusion: This is the first study to indicate associations of dietary patterns with NAFLD in a European population. More studies are needed in order to shed light on the exact role of diet in NAFLD development and treatment.
Supported by: Cooperation 890/2009
Disclosure: **I.P. Kalafati:** None.

681

The effect of two energy- and macronutrient-matched meals on glucose metabolism and gastrointestinal hormones: a randomised cross-over study
H. Kahleova[1], M. Klementova[1], L. Belinova[1], M. Haluzik[1], R. Pavlovicova[1], M. Hill[2], T. Pelikanova[1];
[1]IKEM, Prague, [2]Institute of Endocrinology, Prague, Czech Republic.

Background and aims: Gastrointestinal hormones play a key role in glucose metabolism, energy homeostasis, and regulation of body weight.
Materials and methods: A randomized cross-over study was used to test the effects of two energy- (514 kcal) and macronutrient-matched plant-based and processed-meat meals (45% carbohydrates, 16% protein, and 39% lipids) on glucose metabolism, plasma concentrations of gastrointestinal hormones, and satiety in subjects with T2D ($n = 20$), obese subjects ($n = 20$) and healthy controls ($n = 20$). Plasma concentrations of glucose, immunoreactive insulin, C-peptide, GLP-1, GIP, amylin, and PYY, along with satiety, were determined at 0, 30, 60, 120 and 180 min. Repeated-measures ANOVA was used for statistical analysis.
Results: An increase in stimulated secretion of immunoreactive insulin was observed in T2D and obese subjects ($p = 0.005$, and $p = 0.045$, respectively) after the plant-based meal. We observed an increase in stimulated secretion of C-peptide in all groups after the plant-based meal ($p < 0.001$ for T2D, $p = 0.014$ for obese subjects, and $p = 0.001$ for healthy controls). An increase in post-prandial plasma concentrations of amylin was observed in T2D and healthy controls after the plant-based meal ($p < 0.001$ for both groups). An increase in stimulated secretion of GLP-1 was observed in T2D obese subjects and healthy controls ($p < 0.001$, and $p = 0.01$, respectively) after the plant-based meal. A decrease in peak concentrations of GIP (at 60 min.), and an increase in peak concentrations of PYY were observed in all groups after the plant-based meal. The participants in all groups reported greater satiety after the plant-based meal ($p = 0.004$ for T2D, $p < 0.001$ for obese subjects, and $p < 0.001$ for healthy controls).
Conclusion: Our study suggests that plant-based meals may be effective in increasing stimulated secretion of GLP-1, insulin, amylin, and PYY, as well as in promoting satiety.
Clinical Trial Registration Number: NCT02474147
Supported by: AZV15-27338A, MZCR 00023001
Disclosure: **H. Kahleova:** None.

682

Dietary intake and glycaemia in individuals with type 1 diabetes
A.J. Ahola[1,2], C. Forsblom[1,2], M. Saraheimo[1,2], P.-H. Groop[1,2];
[1]Folkhälsan Research Center, Helsinki, [2]Abdominal Center Nephrology, Helsinki University Central Hospital, Helsinki, Finland.

Background and aims: Dietary intake plays an important role in the glycaemic control of patients with type 1 diabetes. Current evidence in this field is, however, somewhat mixed. Importantly, in isoenergetic conditions an increase in the intake of one macronutrient is accompanied by a decrease in another macronutrient(s). Most of the previous studies have not taken the macronutrient substitution into consideration. Moreover, a number of studies have not adjusted their analyses for fibre intake, which may be important in glycaemic control. We investigated the association between dietary intake and glycaemia in a large population of individuals with type 1 diabetes taking part in the Finnish Diabetic Nephropathy Study.
Materials and methods: Food records from a minimum of 3 days were available from 1000 individuals (42% men, mean ± standard deviation age 48 ± 14 years). Along with reporting food and beverage consumption, in these records, participants also reported physical activity, insulin dosing, and self-monitored blood glucose (SMBG) values (median, interquartile range number of measurements, 3.8, 2.7–5.0). Daily average energy and nutrient intakes, and the mean and coefficient of variation (CV) of the reported SMBG measurements were calculated for each participant. The SMBG means and CVs were used as continuous variables in the generalised linear regression, where macronutrient substitution method was applied. Moreover, based on the median of the mean SMBG values and the median of the CVs, participants were divided into two respective groups. In these groups, the role of nutrient intake was investigated using logistic regression analysis.
Results: In the logistic regression analysis, adjusted for age, sex, BMI, triglyceride concentration, insulin dosing, physical activity, and other dietary variables, fibre intake (g/MJ) was associated with having mean SMBG (Exp(B), 95% CI, *P*, 1.403, 1.094–1.799, 0.008) below the median. In the multivariable macronutrient substitution analyses, mean SMBG values increased when energy intake from fats was increased at the expense of proteins. Similarly, increased consumption of saturated fatty acids, in place of either monounsaturated or polyunsaturated fatty acids, was associated with higher mean SMBG. After further adjustment with fibre intake, however, these observations were no longer significant. In the fully adjusted models, favouring the intake of proteins at the expense of either carbohydrates (−0.026, −0.013–0.040, <0.001), fats (−0.018, −0.004–0.033, 0.014), or alcohol (−0.026, −0.045–0.006, 0.010), or fats at the expense of carbohydrates (−0.009, −0.001–0.017, 0.030) were all associated with lower variability in the measured BG values.
Conclusion: Our observations highlight the important role of dietary fibre in the management of glycaemia. Moreover, even when adjusted for fibre intake, proteins, when consumed in place of excess carbohydrates, fats, or alcohol, or fats in place of carbohydrates, may reduce glycaemic excursions and thus impact glycaemia.
Supported by: Multiple grants, including: Academy of Finland and Novo Nordisk Foundation
Disclosure: **A.J. Ahola:** Grants; Diabetes Wellness Finland.

683

The immediate clinical effects of a carbohydrate-reduced high-protein diet on glycaemic variability in well-controlled type 2 diabetes: a randomised controlled study
M.N. Thomsen[1], M.J. Skytte[1], A. Astrup[2], C.F. Deacon[3], J.J. Holst[3,4], S. Madsbad[5], T. Krarup[1], S.B. Haugaard[1], A. Samkani[1];
[1]Dept. of Endocrinology, Copenhagen University Hospital, Bispebjerg, Copenhagen, [2]Dept. of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, [3]Endocrinology Research Section, Dept. of Biomedical Sciences, University of Copenhagen, Copenhagen, [4]Section

for Translational Physiology, Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, [5]Dept. of Endocrinology, Copenhagen University Hospital, Amager Hvidovre, Copenhagen, Denmark.

Background and aims: High glycaemic variability (GV) is associated with late complications in Type 2 Diabetes (T2D). We hypothesized that a carbohydrate-reduced high-protein (CRHP) diet compared with a conventional diabetes (CD) diet would reduce GV acutely in patients with T2D.
Materials and methods: In this randomized, controlled, crossover study, 16 patients with metformin-treated T2D (median (IQR) age: 64.0 (58.8–68.0) yrs; HbA1c: 47 (43–57) mmol/mol; duration of T2D: 5.5 (2.8–10.3) yrs; mean ± SD BMI: 30.1 ± 4.4 kg/m^2) were randomly assigned to a CRHP diet (31E% carbohydrate, 29E% protein, 40E% fat) or an energy-matched CD diet (54E% carbohydrate, 16E% protein and 30E% fat) for two separate 48-hour intervention periods. Interstitial continuous glucose monitoring (CGM) was performed to assess measures of GV, i.e. standard deviation (SD); coefficient of variation (CV); mean amplitude of glucose excursions (MAGE); continuous overlapping net glycaemic action ($CONGA_n$) of observations n hours apart; and mean absolute glucose (MAG) change. Differences between diet interventions were compared using paired sample *t* tests or Wilcoxon signed-rank tests if assumptions of normality were not met.
Results: All indices of glycaemic variability (mean ± SD) were significantly reduced during CRHP diet compared with CD diet; including SD (1.6 ± 0.5 (CD) vs 1.0 ± 0.3 mmol/l (CRHP)), CV (19.3 ± 5.5 vs 12.3 ± 3.8%), MAGE (4.2 ± 1.3 vs 2.3 ± 0.9 mmol/l), $CONGA_1$ (1.5 ± 0.4 vs 0.8 ± 0.3 mmol/l), $CONGA_2$ (2.1 ± 0.7 vs 1.2 ± 0.4 mmol/l), $CONGA_4$ (2.5 ± 0.8 vs 1.4 ± 0.5 mmol/l), and MAG change (1.4 ± 0.4 vs 0.9 ± 0.3 mmol/l/h) ($p < 0.001$ for all). Compared with the CD diet, the CRHP diet improved the diurnal glucose profile by reducing 24-hour mean sensor glucose (MSG) (8.6 ± 2.0 vs 7.7 ± 1.6 mmol/l); postprandial glucose (PPG) levels after breakfast and lunch (10.1 ± 2.4 vs 8.5 ± 1.9 mmol/l and 9.4 ± 2.3 vs 8.0 ± 1.5 mmol/l, respectively); and total 24-hour peak levels (12.6 ± 2.5 vs 10.1 ± 2.0 mmol/l), ($p < 0.001$ for all), respectively. Patients spent significantly more time with blood glucose below 10 mmol/l (median (IQR)) (80.4% (62.2–96.7) vs 97.2% (94.5–100)) and correspondingly less time above 10 mmol/l (18.0% (3.1–37.8) vs 0.78% (0.0–5.5)) ($p = 0.003$ for both).
Conclusion: In T2D patients, two days of iso-energetic replacement of dietary carbohydrates by protein and fat reduced glycaemic variability when compared with a conventional diabetes diet. These data support reduction of carbohydrates as dietary advice for T2D patients.
Clinical Trial Registration Number: NCT02472951
Supported by: DDRF
Disclosure: **M.N. Thomsen:** None.

684

The effect of three different types of diet on glycaemic control assessed by continuous monitoring in patients with type 1 diabetes on multiple daily insulin treatment

C. Dimosthenopoulos[1], S. Liatis[2], A. Tentolouris[2], E. Athanasopoulou[2], S. Driva[2], K. Makrilakis[2], N. Tentolouris[2], A. Kokkinos[2];
[1]Clinical Nutrition, Laiko General Hospital, Athens, [2]First Department of Propaedeutic Medicine, Medical School, National and Kapodistrian University, Laiko General Hospital, Athens, Greece.

Background and aims: Medical Nutritional Therapy (MNT) in diabetes is an essential part of the overall treatment. In recent decades, dietary recommendations have been greatly modified and various types of dietary patterns are currently recommended by large organisms. The aim of the present study was to compare the effects of three diets differing in the ratio of macronutrients, on glycemic variability and insulin needs in patients with type 1 diabetes (T1DM) undergoing multiple daily insulin (MDI) treatment.
Materials and methods: Patients with T1DM on MDI therapy were recruited from the outpatient diabetes clinic of Laiko General Hospital. They were adults, with an HbA1c <8%, and no hypoglycemia unawareness or clinically significant diabetic complications. Each participant followed three different isocaloric diets for three separate weeks, in a random order, and with a 7-day washout period between them: a reference (REF) diet corresponding to previous ADA recommendations 50% carbohydrate, 20% protein, 30% fat), a high protein-low carb (HPD) diet (20% carbohydrate, 40% protein, 40% fat), and a Mediterranean style/low GI (<60) (LGI) diet (40% carbohydrate 25% protein, 35% fat). The diets were tailored to the needs and preferences of each patient, but strictly defined and absolutely equivalent among patients regarding the proportion of macronutrients and the glycemic index. Glucose values were monitored with the iPro2 continuous glucose monitoring (CGM) device.
Results: Fifteen patients (5 males, mean age 26.1 ± 10 years, mean diabetes duration 12.4 ± 9.8 years, mean BMI 22.8 ± 3.8 kg/m^2) participated in the study. The mean percentage of time spent within the euglycemic range (70–140 mg/dl) differed significantly among the three diets (REF: 48.3 ± 13.7%, HPD: 55.7 ± 14.0% and LGI: 49.7 ± 12.6%, $p = 0.041$), the difference in *post hoc* analysis being statistically significant between the HPD and the REF diet ($p = 0.027$). Furthermore, glycemic variability, as expressed by the standard deviation of the mean interstitial tissue glucose value, was lower during the HPD as compared to the REF diet (46.1 ± 12.8 mg/dl vs. 52.7 ± 10.8 mg/dl, $p = 0.005$) but not to the LGI diet (51.4 ± 14.3 mg/dl, $p = 0.077$). In addition, the area under the curve (AUC) of time spent in the hypoglycemic range (<70 mg/dl) was lower during the HPD than during the REF diet (1.1 ± 1.0 vs. 2.4 ± 2.3, $p = 0.044$) but not the LGI diet (1.6 ± 1.9, $p = 0.76$). No significant differences were observed between the three diets regarding the mean interstitial tissue glucose value, the time spent or the AUC of time spent in the hyperglycemic range (>140 mg/dl) and the time spent in the hypoglycemic range. During the HPD, patients received less bolus insulin by 3.3 and 2.3 units per day compared to the REF and the LGI diet, respectively.
Conclusion: An HPD dietary pattern resulted in less hypoglycemic episodes, overall lower glucose, better glycemic variability, and less total insulin use vs a reference and a LGI dietary pattern. Modifying traditional dietary counseling by increasing protein intake improves glycemic indices and may be beneficial for people with T1DM. The use of CGM is useful in uncovering such differences which otherwise may had gone unnoticed.
Disclosure: **C. Dimosthenopoulos:** None.

685

A carbohydrate-reduced high-protein diet significantly reduces HbA_{1c}, diurnal and prandial plasma glucose in weight stable subjects with type 2 diabetes

M.J. Skytte[1], A.A. Samkani[1], A.D. Petersen[1], M.N. Thomsen[1], A. Astrup[2], E. Chabanova[3], J. Frystyk[4], J.J. Holst[5], H. Thomsen[3], S. Madsbad[6], T.M. Larsen[2], S.B. Haugaard[1], T. Krarup[1];
[1]Dept. of Endocrinology, Copenhagen University Hospital Bispebjerg, Copenhagen, [2]Dept. of Nutrition, Exercise and Sports, Copenhagen University, Copenhagen, [3]Dept. of Radiology, Copenhagen University Hospital Herlev, Copenhagen, [4]Dept. of Endocrinology and Internal Medicine, Aarhus University, Aarhus, [5]Center for Basic Metabolic Research, Copenhagen University, Copenhagen, [6]Dept. of Endocrinology, Copenhagen University Hospital Hvidovre, Copenhagen, Denmark.

Background and aims: The carbohydrate content of the diet has been proved to be of crucial importance for the increase in postprandial plasma glucose in subjects with type 2 diabetes mellitus (T2D). The long term metabolic effect of lowering the carbohydrate content in the diet has yet to be determined. The aim of the study was to investigate whether 6 weeks of fully controlled carbohydrate reduced high protein (CRHP) diet compared with 6 weeks of conventional diabetes (CD) diet improved metabolic control in well controlled, weight stable subjects with T2D.

Materials and methods: Twenty-eight patients with T2D, 20 males, age 64 (±7.7) years (mean ± SD), duration of T2D 7 (±5.4) years, BMI 30.1 (±5.2) kg/m², HbA1c 59.6 (±8.4) mmol/mol on oral antidiabetic agents were randomized to 6 weeks of CRHP or CD diet (carbohydrate 30/50 E%, protein 30/17 E%, fat 40/33 E%, respectively) followed by 6 weeks of the opposite diet. All meals were provided in accordance to participant estimated daily total energy expenditure based on a dual-energy X-ray absorptiometry scan and continuously adjusted to reinforce weight stability. At baseline, week 6 and week 12 metabolic measurements were performed. A linear mixed effects model was used to assess differences between diets adjusted for body weight loss.
Results: Compared with the CD diet, a significant reduction in HbA_{1c} was found on the CRHP diet (0.8 (±5.3) vs. 6.2 (±4.2) mmol/mol, $p < 0.01$, respectively) (Fig.). Furthermore, the CRHP vs. CD diet reduced 24 h mean glucose concentrations (8.02 (±1.2) vs. 9.24 (±1.9) mmol/L, $p < 0.01$), 4 hour meal tolerance test net area under curve (AUC) of glucose (1.25 (±1.1) vs. 3.1 (±1.3) mmol/L × 240 min, $p < 0.01$) and insulin (194.7 (±107) vs. 239.2 (±191) pmol/L × 240 min, $p = 0.04$), respectively. No difference in body weight loss was found between diets.
Conclusion: A CRHP diet significantly improved glycaemic control during a 6 week intervention with full diet provision in body weight stable patients with T2D compared with a CD diet. The improved glucose metabolism following a CRHP diet was not mediated by an increased insulin secretion.

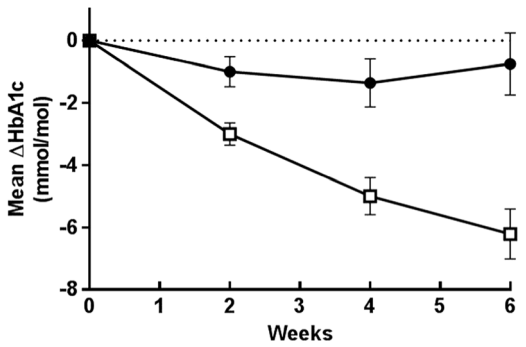


Figure. Effect of 6 weeks of conventional diabetes diet (●) and carbohydrate-reduced high-protein diet (□) on ΔHbA1c in 28 subjects with T2D. Values are means with standard error.

Clinical Trial Registration Number: NCT02764021
Supported by: AFH, CBMR, BFH, DC, RG, JI A/S, NEXS, KU, ARLA
Disclosure: **M.J. Skytte:** None.

PS 053 Dietary supplements: Which is best?

686

The common food additive carrageenan increases intestinal permeability without affecting whole-body insulin sensitivity in humans

R. Wagner[1], J. Buettner[2], M. Heni[1], L. Fritsche[1], S. Kullmann[3], M. Wagmüller[1], A. Peter[1], J. Machann[4], U.-F. Pape[5], G. Van Hall[6], P.S. Plomgaard[7], R. Klein[8], H.-U. Häring[1], A. Fritsche[1], N. Stefan[1];
[1]University Hospital Tübingen, Tuebingen, Germany, [2]Department of Hepatology and Gastroenterology, Charité Universitätsmedizin, Berlin, Germany, [3]Institute of Medical Psychology and Behavioral Neurobiology/fMEG Center, Eberhard Karls University Tübingen, Tuebingen, Germany, [4]Section on Experimental Radiology, Department of Diagnostic and Interventional Radiology, University Hospital Tübingen, Tuebingen, Germany, [5]Department of Hepatology and Gastroenterology, Charité Universitätsmedizin, Berlin, Berlin, Germany, [6]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark, [7]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Copenhagen, Denmark, [8]Division of Haematology, Oncology, Immunology, Rheumatology and Pulmonology, Department of Internal Medicine, University Hospital, Eberhard-Karls-University Tübingen, Tuebingen, Germany.

Background and aims: The increasing global diabetes epidemic is strongly associated with the western lifestyle. While increased intake of high-energy nutrients is a major dietary component of this lifestyle, it is not known whether food additives, such as the widely used carrageenan, also play a role in the pathogenesis of diabetes. Animal data suggest that increased carrageenan consumption causes insulin resistance and diabetes, mainly by interfering with hepatic insulin signaling. This is the first trial in humans that tested whether carrageenan added to the diet affects insulin sensitivity, inflammatory markers, body-fat distribution and intestinal permeability.
Materials and methods: We conducted a randomized, double-blind, placebo-controlled, cross-over trial. Healthy males ($N = 20$) were randomly allocated to 14 days of carrageenan (250 mg twice daily) or matching placebo. After a mean (±SD) washout-period of 30 ± 7 days they received the other compound. At the end of each treatment phase, subjects underwent an oral glucose tolerance test (OGTT), a hyperinsulinemic-euglycemic clamp with labeled glucose (6·6-$^2H_2$ glucose), whole-body MR tomography and ^{1}H-MR-spectroscopy measurements of body fat mass and distribution, liver fat content, blood immune cell phenotyping and a lactulose-mannitol test for investigating intestinal permeability.
Results: The subjects had a mean (±SD) age of 27.6 ± 4.8 years and a BMI of 24.4 ± 2.6 kg/m^2. Intestinal permeability significantly increased after carrageenan vs placebo exposure (lactulose-mannitol-ratio 0.0196 vs 0.015, $p = 0.03$). Whole-body insulin sensitivity did not differ between placebo and carrageenan exposure ($p = 0.5$ for both clamp-derived whole-body insulin sensitivity and OGTT-derived insulin sensitivity). No changes of hepatic fat content, body fat mass and distribution, liver transaminases, C-reactive protein and interleukin-6 levels were observed (all $p > 0.4$).
Conclusion: In young healthy males, carrageenan intake over a relatively short period of time resulted in an increased gut permeability, which was not accompanied by changes of whole-body insulin sensitivity, markers of subclinical inflammation, body fat mass and fat distribution and liver fat content.
Clinical Trial Registration Number: NCT02629705
Supported by: BMBF, DZD
Disclosure: **R. Wagner:** None.

687

Why resveratrol is effective in some but not all individuals: a combined data-analyses

M. de Ligt[1], E. Phielix[1], B. Havekes[2], V.B. Schrauwen-Hinderling[3], P. Schrauwen[1];

[1]Nutrition and Movement Sciences, Maastricht University, Maastricht, [2]Internal Medicine, Division of Endocrinology, Maastricht University Medical Center, Maastricht, [3]Radiology, Maastricht University, Maastricht, Netherlands.

Background and aims: Results from pre-clinical studies have suggested that resveratrol may have metabolic health effects and can prevent insulin resistance. However, the translation from animal to human studies turned out to be challenging. Even with similar treatment duration and dose of resveratrol, the efficacy differs between participant populations. Here we investigated which subject characteristics predict efficaciousness of resveratrol on a number of metabolic health parameters in humans.
Materials and methods: Data from three placebo-controlled, cross-over human clinical trials was combined. In all three clinical trials the same dose (150 mg/d) and treatment duration (30 days) was applied, but the populations varied from healthy obese men, to men at risk of type 2 diabetes (T2D) and to patients with T2D. Paired sample t-test was applied to evaluate effects of resveratrol treatment on a whole group basis. Pearson correlation coefficient analysis followed by stepwise linear regression was used to detect which baseline participant characteristics are determinants of resveratrol-induced changes in metabolic health outcome parameters.
Results: Overall, resveratrol had beneficial effects on systolic blood pressure (137 ± 1.8 upon placebo vs. 134 ± 1.8 mmHg upon resveratrol $p = 0.038$) and *ex vivo* mitochondrial function ($p < 0.001$, for all mitochondrial states) and decreased sleeping metabolic rate (7.71 ± 0.12 upon placebo vs. 7.51 ± 0.10 MJ/day upon resveratrol; $p = 0.029$), but not on plasma glucose or HOMA index. Remarkably, despite similar dosing, circulating plasma resveratrol levels were markedly different between the three groups ($p = 0.008$), but plasma resveratrol levels did not predict outcome. Baseline serum bilirubin was found to significantly predict resveratrol-induced change in diastolic blood pressure ($p = 0.044$), ALT ($p = 0.030$), intrahepatic lipid content ($p = 0.020$), and plasma glucose ($p = 0.004$). The latter was especially profound when also baseline serum albumin levels were taken into account ($p = 0.002$).
Conclusion: Baseline serum bilirubin was identified as the most relevant predictor of resveratrol-induced change in metabolic health outcome parameters. Thus, higher baseline serum bilirubin levels were associated with improvements in metabolic health upon resveratrol treatment. It has been suggested that bilirubin is a surrogate of UGT1A1 activity, which is an enzyme involved in glucuronidation of *trans*-resveratrol. Although speculative, our data may suggest that bilirubin levels reflect activity of glucuronidation enzymes, implying glucuronidation is important for efficacy of resveratrol. Further studies are needed to investigate the importance of glucuronidation of resveratrol.
Supported by: The Dutch Diabetes Foundation and the Dutch Heart Foundation
Disclosure: **M. de Ligt:** Grants; Dutch Diabetes Foundation: 2012.00.1525, Dutch Heart Foundation: CVON2014-02 ENERGISE.

688

Spirulina liquid extract prevents glucose intolerance and NAFLD in mouse

M. Coué[1,2], J. Falewee[1,3], A. Tesse[4], L. Fizanne[5], M. Krempf[1,2], J.-M. Pommet[6], O. Lépine[6], K. Ouguerram[1,3];
[1]INRA, UMR 1280, Physiopathologie des Adaptations Nutritionnelles, Nantes, [2]Centre de Recherche en Nutrition Humaine, Nantes, [3]Université de Nantes, Nantes, [4]INSERM, UMR 1087, Institut du Thorax, Nantes, [5]Université d'Angers, EA 3859, Laboratoire Intéraction Fibrose et Invasivité Tumorale Hépatique, Angers, [6]AlgoSource Technologies, Saint-Nazaire, France.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) concerns 85% of obese people and can progress to non-alcoholic steatohepatitis (NASH) and cirrhosis. Insulin resistance and oxidative stress are thought to play a pivotal role in the initiation and worsening of the disease spectrum. The aim of this work is to study the potential beneficial effect of supplementation with a liquid extract of Spirulina, a cyanobacteria rich in an antioxidant (AO) pigment, the phycocyanin (PC), on NAFLD establishment and its progression in mouse.
Materials and methods: C57Bl/6 male mice were submitted to western diet (WD) containing 23% of lipids and 2% of cholesterol and to drinking water rich in fructose (42 g/l). Supplemented group mice received the same diet but supplemented with a liquid extract of spirulina (WD-Spi) added in drinking water (10 mg PC/mouse/day). This protocol was conducted for 25 weeks. Mice were weighted once per week and different plasmatic and liver physiological parameters as well as expression of some key genes in glucose and lipid metabolisms were analyzed. Statistical tests were realized on 10 mice/group and data are presented as mean ± SEM.
Results: At 25 weeks of diet, body weight and subcutaneous adipose tissue were decreased in WD-Spi group by 20% ($p < 0.001$) and 52% ($p < 0.001$) respectively compared to WD group. The supplemented group ate significantly more food related to their body weight than WD mice (+19%) (WD: 0.076 ± 0.0008 and WD-Spi: 0.090 ± 0.0011; $p < 0.001$). Furthermore, spirulina supplementation improved fasting blood glucose (WD: 169 ± 3.7 mg/dl; WD-Spi: 153 ± 4.6 mg/dl; $p = 0.01$) and glycemic control reflected by area under the curve from glucose tolerance test ($p = 0.01$) compared to WD mice. This is accompanied by a reduced liver weight to body weight ratio (−25%, $p = 0.003$) and plasma aspartate amino transferase (−33%, $p = 0.004$) in WD-Spi group. These mice showed a lessening hepatic expression of sterol regulatory element-binding protein (SREBP-1), stearoyl-CoA desaturase-1 and of microsomal TG transfer protein. Hepatic expression of AO genes (catalase, glutathione peroxidase, superoxide dismutase) were diminished in WD-Spi mice.
Conclusion: Our data indicate that spirulina liquid extract protects against some deleterious effects of the WD known to affect liver function. We are now analyzing more specifically liver to unravel underlying mechanisms associated to spirulina effects.
Supported by: AlgoSource Technologies
Disclosure: **M. Coué:** Employment/Consultancy; AlgoSource Technologies.

689

Impact of isoflavones on several primary outcomes associated with diabetes: a meta-analysis of randomised controlled human trials

R. Menezes[1,2], A.F. Raimundo[1,2], F. Félix[1,2], A. Raimundo[3], A. Rodriguez-Mateos[4], A. González-Sarrías[5], B. Wang[4], C.N. Santos[1,2], I. Cruz[3], J.V.G. Lopes[6], M. Schär[7], M.-T. García-Conesa[5], R. Andrade[3], R.T. Ribeiro[3,8], P. Pinto[1,9];
[1]iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, [2]ITQB-NOVA, Oeiras, Portugal, [3]APDP, Lisbon, Portugal, [4]King's College London, London, UK, [5]CEBAS-CSIC, Murcia, Spain, [6]CEDOC-FCM, Lisbon, Portugal, [7]University of Reading, Reading, UK, [8]iBiMed - UA, Aveiro, Portugal, [9]Instituto Politécnico de Santarém, Santarém, Portugal.

Background and aims: Isoflavones are naturally occurring isoflavanoids found in legumes, particularly in soybeans. As a phytoestrogen resembling human estrogen, isoflavones might provide many health benefits including antidiabetic properties. The reduction of blood glucose and insulin levels are some of the effects attributed to isoflavones, however, the association between isoflavones intake and diabetes remains inconclusive. Thus, this meta-analysis aimed to evaluate the effect of isoflavone-rich products intake on primary diabetes outcomes.
Materials and methods: A systematic search was conducted in several databases and articles were selected for meta-analysis based on the following criteria: human randomized controlled trials with diabetic or pre-diabetic patients; primary outcomes of diabetes such as glucose, insulin,

insulin resistance (HOMA-IR) and glycated hemoglobin (HbA1c), and intervention with a (poly)phenol. Selected articles were used for data extraction regarding the measured outcomes, individual factors of the participants and characteristics of the study, including quality. Results of meta-analysis are presented as standardized mean difference (SDM).

Results: This meta-analysis studied the effect of interventions with isoflavone-rich products, in diabetic and pre-diabetic patients. From eighty-nine interventions with different (poly)phenols, only eleven studies used isoflavones as the bioactive compound. Four studies were discarded: three due to inexistence of a proper control, and one did not measure any of the previously defined primary outcomes. Seven studies were selected for the meta-analysis, with 1208 participants (668 treated with isoflavones and 604 with placebo). Four outcomes were measured: glucose (6 studies), insulin (6 studies), Hb1Ac (4 studies) and HOMA-IR (5 studies). Results from the meta-analyses show a significant reduction in insulin (SDM = −0.26; 95% CI [−0.45, −0.07]; $p = 0.011$) and HOMA-IR (SDM = −0.25; 95% CI [−0.45, −0.06]; $p = 0.011$), but not on glucose (SDM = −0.16; 95% CI [−0.35, 0.03]; $p = 0.097$) and Hb1Ac (SDM = −0.33; 95% CI [−0.74, 0.09]; $p = 0.123$).

Conclusion: Based on the results of this meta-analysis, prolonged intake of isoflavone-rich products can contribute to reduce insulin resistance both in diabetic and pre-diabetic patients.

Disclosure: **R. Menezes:** None.

690

Vitamin D supplementation and body weight status in overweight or obesity: a systematic review and meta-analysis

J. Sun[1], C. Wu[2], L. Li[2];
[1]Shaoxing University Medical School, Shaoxing, [2]Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China.

Background and aims: Our studies have found that vitamin D supplementation was associated with improved beta cell function and insulin sensitivity among persons who were at high risk for diabetes. Obesity has an increase in the risk for type 2 diabetes. Overweight and obese subjects typically have low circulating 25-hydroxyvitamin D [25(OH)D] levels. Although observational studies have suggested an increased risk of vitamin D deficiency in obese subjects, randomized controlled trials (RCTs) that evaluated the effects of vitamin D supplementation have produced inconsistent results. We evaluated whether vitamin D supplementation affects the body weight and composition. We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) involving vitamin D supplementation with or without calcium or caloric restriction on overweight or obesity.

Materials and methods: We used PubMed, Web of Science and the Cochrane Library to search for pertinent studies published through January 2017.

Results: Seven trials provided the required data, including body mass index (BMI), weight, and waist circumference. Vitamin D supplementation had no effect on BMI [standardized mean difference (SMD) −0.02 (−0.15 to 0.12), $P = 0.80$), weight (SMD −0.01 (−0.15 to 0.12), $P = 0.85$], and waist circumference [SMD 0.07 (−0.16 to 0.18), $P = 0.92$] of overweight or obese patients. According to the results of a meta-regression analysis, none of the potential moderating factors [age, gender, BMI, baseline 25(OH)D] was a significant predictor of changes in obesity measures.

Conclusion: Although Vitamin D supplementation could be effctive at improving glycemic control, it did not decrease measures of adiposity in overweight and obese subjects.

Supported by: National Natural Science Foundation of China (No 81570739)

Disclosure: **J. Sun:** None.

691

Effects of glutamine on gastric emptying of low- and high-nutrient drinks in healthy young subjects: impact on glycaemia

Y.T. Du[1,2], D. Piscitelli[1,3], S. Ahmad[1,3], L.G. Trahair[1], J.R. Greenfield[4], D. Samocha-Bonet[4], C.K. Rayner[1,5], M. Horowitz[1,5], K.L. Jones[1,5];
[1]The University of Adelaide, Adelaide, [2]Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, [3]University of South Australia, Adelaide, [4]Garvan Institute of Medical Research, Sydney, [5]NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, Adelaide, Australia.

Background and aims: Glutamine is a potent stimulus for the release of glucagon-like peptide-1 (GLP-1), which increases postprandial insulin secretion and slows gastric emptying. It has, accordingly, been suggested that glutamine may be useful in the management of type 2 diabetes. The rate of gastric emptying, which exhibits a substantial inter-individual variation, is a major determinant of postprandial glycaemic excursions in both health and diabetes. The aims of this study were to determine the effects of glutamine on the rate of gastric emptying, and glycaemic responses to, low- and high-nutrient drinks in healthy individuals.

Materials and methods: Eight healthy males (mean age 21.6 ± 0.7 years and BMI 22.9 ± 0.7 kg/m^2) were studied on 4 separate occasions on which they consumed both low- (beef soup; 18 kcal) and high-nutrient (75 g dextrose; 255 kcal) drinks, each with or without 30 g of glutamine (120 kcal), in a randomised, crossover design. Gastric emptying was measured using 2D ultrasound (to calculate the 50% emptying time; T50), and venous blood sampled for measurements of blood glucose and plasma insulin concentrations before and after each drink. Data are presented as mean ± SEM.

Results: Glutamine slowed gastric emptying of both low- (T50: 45 ± 3 min vs 26 ± 2 min, $P < 0.001$), and high-nutrient, (T50: 100 ± 5 min vs 77 ± 5 min, $P = 0.03$) drinks. However, there was no difference in gastric emptying of the high nutrient drinks when gastric emptying was expressed as kcal/min (3.39 ± 0.21 kcal/min vs 3.81 ± 0.20 kcal/min, $P = 0.25$) i.e. the prolongation of the T50 by glutamine reflected its caloric content. There was no change in blood glucose after the low-nutrient drinks with or without glutamine, despite a modest increase in plasma insulin with glutamine ($P = 0.007$). The rise in blood glucose following the high-nutrient drink ($P = 0.0001$) was attenuated during the first 60 min by glutamine (AUC^{0-60} 443 ± 17 mmol/L.min vs 473 ± 22 mmol/L.min, $P = 0.007$) without any difference in the plasma insulin response (AUC^{0-60} 1717 ± 233 mU/L.min vs 2250 ± 549 mU/L.min, $P = 0.19$).

Conclusion: In healthy subjects, glutamine in a dose of 30 g slows gastric emptying of both low- and high-nutrient drinks comparably and attenuates the rise in blood glucose after oral glucose, possibly via effects on gastric emptying.

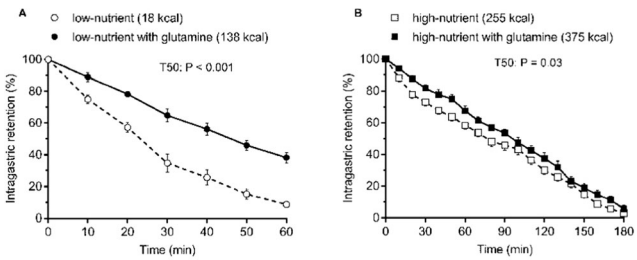


Figure: Gastric emptying of (A) low- and (B) high-nutrient drinks, with or without glutamine (30g)

Disclosure: **Y.T. Du:** None.

692

Short-term dietary restriction of branched-chain amino acids (BCAA) decreases insulin secretion in type 2 diabetes

Y. Karusheva[1,2], T. van Gemert[1,3], M.-C. Simon[1,2], D. Markgraf[1,2], K. Strassburger[2,4], T. Jelenik[1,2], D. Schmoll[5], V. Burkart[1,2], K. Müssig[1,3], J. Szendroedi[1,3], M. Roden[1,3];
[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, [2]German Center for Diabetes Research (DZD), München-Neuherberg, [3]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, [4]Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, [5]Sanofi-Aventis Deutschland GmbH, Industriepark Hoechst, Frankfurt, Germany.

Background and aims: Serum levels of BCAA (valine, leucine, isoleucine) are elevated in insulin resistant animals and humans with type 2 diabetes (T2D) suggesting a role of BCAA in the development of insulin resistance. BCAA proved to reduce the serum levels of fibroblast-growth factor (FGF) 21, a novel metabolic regulator which improves glucose uptake in adipose tissue at the background of insulin resistance. We examined the hypothesis that reducing dietary BCAA for 1 week improves insulin sensitivity (IS) and oxidative phosphorylation in T2D and increases the levels of FGF 21 in humans.
Materials and methods: We designed a randomized, placebo-controlled, double-blinded cross-over study including 12 patients (8 male, 4 female; age 54 ± 4 years, body mass index 30.8 ± 2.8 kg/m^2, HbA1c 6.6 ± 0.9%, 49 ± 10 mmol/mol) with known T2D duration of <5 years. Patients were on a 4-week isocaloric diet with constant protein intake of 1 g/kg body weight. The diet consisted of either the complete amino acid set (BCAA$^+$) or a 60% reduction in BCAA (BCAA$^-$) for 1 week. Beta-cell function was assessed by mixed-meal tolerance tests (MMTT). Mitochondrial efficiency was assessed from the respiratory control ratio (RCR) measured by high-resolution respirometry in muscle and adipose tissue. FGF 21 serum levels were quantified by ELISA.
Results: MMTT showed lower increases in insulin and C-peptide after BCAA$^-$ than BCCA$^+$ diet (incremental insulin area under the curve: 21 ± 11 vs 29 ± 19 mU*ml^{-1}*4 h^{-1}, $p < 0.01$). The BCAA$^-$ diet also increased FGF 21 levels by 25.4% ($p < 0.05$). RCR in adipose tissue was 1.7-fold higher (1.2 ± 0.7 vs 2.1 ± 0.8, $p < 0.05$) after BCAA$^-$ diet but unchanged in skeletal muscle which points to improved mitochondrial efficiency in adipose tissue only.
Conclusion: Short-term dietary BCAA restriction decreases meal-induced insulin secretion, increases FGF 21 and stimulates adipose tissue mitochondrial efficiency.
Clinical Trial Registration Number: NCT03261362
Supported by: Sanofi-Aventis Deutschland GmbH
Disclosure: **Y. Karusheva:** Grants; Branched-chain amino acids reduced intake under weight maintenance in overweight patients with type 2 diabetes.

693

Insulin influences mindset-induced brain response and behaviour on portion size selection for lunch

S. Kullmann[1], R. Veit[1], M. Heni[1,2], L. Horstman[1], M. Hege[1], P.J. Rogers[3], J.M. Brunstrom[3], H.-U. Häring[1,2], A. Fritsche[1,2], H. Preissl[1];
[1]Institute for Diabetes Research and Metabolic Diseases (IDM)of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany, [2]Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, Department of Internal Medicine, Eberhard Karls University Tübingen, Tübingen, Germany, [3]Nutrition and Behaviour Unit, University of Bristol, Bristol, UK.

Background and aims: Food intake increases when people are served larger portion sizes. For decisions on the consumed portion size, attentional focus plays an important role. Currently, it is not known whether attentional modulation can influence pre-meal planning on portion size selection in overweight and obese individuals and whether these processes are influenced by insulin.
Materials and methods: In the current study, we asked normal-weight, overweight and obese participants ($n = 34$) how much they intent to consume for lunch prior to eating (free choice condition) whilst undergoing functional magnetic resonance imaging (fMRI). To investigate the important role of attentional focus, participants adopted different mindsets (eat with pleasure, considering health aspects and being full until dinner) while selecting their portion size for lunch. Blood samples were taken after fMRI measurement.
Results: Compared with a free choice condition, mindsets induced behavioral changes in portion size selection associated with specific neuronal processes. Both lean and obese participants reduced their portion size when considering health, which was accompanied by increased left prefrontal cortex activation. However, obese and overweight subjects chose larger portion sizes during the pleasure mindset compared to the lean group. This was related to an increased response in parts of primary gustatory cortex in overweight and obese participants. Moreover, insulin levels determined portion size selection when asked to choose with pleasure, leading to higher portion sizes with low insulin levels. Interestingly, activation of the right lateral prefrontal cortex, recently reported as vulnerable to insulin resistance, was related to individuals' plasma insulin levels when asked to choose a portion size while adopting the health mindset.
Conclusion: Mindsets induced specific behavioral and neural changes modulating portion size selection for lunch in an insulin dependent manner. Understanding these behavioral differences during pre-meal planning can inform the development of effective strategies for healthy weight management.
Supported by: European Union Seventh Framework Programme (FP7/2007-2013)
Disclosure: **S. Kullmann:** None.

PS 054 All what you need to know for a healthy diet

694

Coffee and tea consumption in relation to impaired glucose metabolism and diabetes

A.A.M. Berendsen, G. Abma, D. Sluik, E. Feskens;
Human Nutrition, Wageningen University & Research, Wageningen, Netherlands.

Background and aims: Earlier studies suggest that higher coffee and tea consumption are associated with a decreased diabetes incidence, however these associations are still controversial. In this research, we investigated the association between coffee and tea consumption, impaired glucose metabolism (IGM) and diabetes.
Materials and methods: The study population comprised participants from five population studies (Lifelines, the New Zealand Adult Nutrition Survey (NZANS), the Cardiovascular Risk in Young Finns Study (YFS), the Nutrition Questionnaires plus study (NQplus) and the Quebec Family Study (QFS)), which are included in the PREVIEW study. In our cross-sectional analyses, 144,452 participants were included. The prospective analyses on diabetes included 86,051 participants and the prospective analyses on impaired glucose metabolism included 60,419 participants. Each population was divided into categories of coffee (<1 (=ref.), 1–<4 and ≥4 cups/day) and tea (0 (=ref.), >0–<2 and ≥2 cups/day) consumption. Study-specific prevalence ratios (PR) and incidence ratios (IR) and 95% confidence intervals (95%CI) were calculated applying Cox regression with robust variance estimation. Results were adjusted for general characteristics, medical history, anthropometric measurements, lifestyle and dietary factors. Study-specific results for the highest compared to the lowest category of intake were combined in random effect meta-analyses.
Results: Results from our meta-analyses, cross-sectionally showed a lower diabetes prevalence (PR ≥4 cups/d vs. <1 cup/d = 0.79 [95% CI 0.71, 0.87]) and prospectively a decreased diabetes incidence (IR ≥4 cups/d vs. <1 cup/d = 0.75 [95% CI 0.59, 0.95]) for consumption of ≥4 cups of coffee per day compared to consumption of <1 cup per day. No overall association between diabetes and higher tea consumption was observed. Moreover, based on our meta-analyses, no cross-sectional and prospective associations between coffee and tea consumption and IGM were observed.
Conclusion: Combining results from the five studies in a meta-analysis, we showed that consumption of ≥4 cups of coffee per day compared to <1 cup per day was associated with a 21% lower diabetes prevalence and a 25% decreased diabetes incidence. No overall association between tea and diabetes was observed. We also showed no overall associations between higher coffee and tea consumption and IGM. Between-study heterogeneity limits the possibility to draw consistent conclusions. Further research is needed to study if the association between coffee consumption and diabetes is causal, to explore the associations for different types of coffee and tea and to study the impact of the preparation method.
Clinical Trial Registration Number: ISRCTN31174892
Supported by: EUSFP, NZHRC, MESRST
Disclosure: **A.A.M. Berendsen:** None.

695

Snacks at post-dinner increases the mean amplitude of glycaemic excursion whereas snacks at mid-afternoon decreases it in young healthy women

S. Imai[1], S. Kajiyama[2,3], A. Nitta[1], T. Miyawaki[1], S. Matsumoto[1], Y. Hashimoto[3], M. Tanaka[3], S. Kajiyama[3], N. Ozasa[4], M. Fukui[3];
[1]Kyoto Women's University, Kyoto, [2]kajiyama Clinic, Kyoto, [3]Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, , [4]Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Background and aims: Postprandial hyperglycaemia and glycaemic spikes are associated with cardiovascular diseases even in people without diabetes. The best timing of eating snacks on postprandial glucose levels has not been extensively studied. The aim of this study was to evaluate the effect of consuming snacks at different time of the day on glycaemic parameters in Japanese young women without diabetes.
Materials and methods: This is a randomized controlled three-treatment crossover study. Seventeen women (21.2 ± 0.8 years, BMI 20.7 ± 2.5 kg/m^2, HbA1c 5.14 ± 0.15%: mean ± SD) wore continuous glucose monitors for 7 days. During the test period, each participant consumed identical test meals (total energy 2,060 kcal, protein 70.5 g, fat 70.5 g, carbohydrate 288 g) of breakfast at 07:00, lunch at 12:00, and dinner at 19:00 from the second to the sixth day. The energy ratio of test meals was 58, 13, and 29% from carbohydrates, proteins, and fat, respectively. The half of the participants consumed snack (baked cake 498 kcal, carbohydrate 53.6 g, protein 8.0 g, fat 28.0 g) at 12:30 (post-lunch) on the third day, at 15:30 (mid-afternoon) between lunch and dinner on the fourth day, and at 19:30 (post-dinner) on the fifth day at home. The rest of the participants consumed snacks at post-dinner on the third day, at mid-afternoon on the fourth day, and at post-lunch on the fifth day. The daily glucose parameters were compared during the study period.
Results: The standard deviation of glucose showed higher in consuming snacks at post-dinner than those at mid-afternoon (1.20 ± 0.11 vs. 0.92 ± 0.07 mmol/L, mean ± SE, $p = 0.002$), and the mean amplitude of glycaemic excursion (MAGE) was higher compared to those at post-lunch (3.54 ± 0.32 vs. 3.03 ± 0.27 mmol/L, $p = 0.049$) and at mid-afternoon (2.73 ± 0.20 mmol/L, $p = 0.013$), although mean glucose values did not differ among timings of eating snack (post-lunch, 5.81 ± 0.09; mid-afternoon 5.77 ± 0.11; post-dinner 5.80 ± 0.12 mmol/L). The incremental area under the curve for glucose (IAUC) 12:00–07:00 was higher in consuming snacks at post-dinner compared to that at post-lunch (986 ± 89 vs. 870 ± 112 mmol/L × min, $p = 0.049$) and at mid-afternoon (716 ± 88 mmol/L × min, $p = 0.013$). Additionally IAUC 07:00–10:00 (the following post-breakfast) in consuming snacks at post-dinner was not different from that at post-lunch (142 ± 17 vs. 105 ± 9 mmol/L × min, $p = 0.210$), but higher than that at mid-afternoon (104 ± 12 mmol/L × min, $p = 0.013$).
Conclusion: This study demonstrated that consuming snacks at post-dinner affects the MAGE and the postprandial glucose levels even in the following post-breakfast. On the other hand, consuming snacks at mid-afternoon could be a successful strategy for reduction of glucose excursions in healthy women.

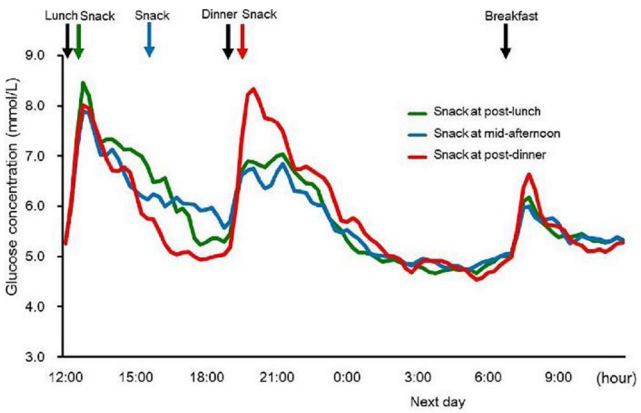


Figure The mean glucose profiles during the study period in healthy women (n = 17)

Clinical Trial Registration Number: 9465
Supported by: KAKENHI, Kyoto Women's University
Disclosure: **S. Imai:** None.

696

Association between brown rice consumption and circulating microRNAs in Japanese subjects with prediabetes

H. Suzuki[1], R. Araki[2], K. Sasaki-Fukatsu[3], K. Fujie[2], M. Sekiya[1], Y. Nakagawa[1], T. Matsuzaka[1], Y. Nakata[2], N. Yahagi[1], K. Hashimoto[2], H. Shimano[1];
[1]Department of Internal Medicine (Endocrinology and Metabolism), Faculty of Medicine, University of Tsukuba, Tsukuba-shi, [2]Course of Clinical Research and Regional Innovation Donated by the Japanese Agriculture Ibaraki Publ, Faculty of Medicine, University of Tsukuba, Tsukuba-shi, [3]Department of Food Sciences, College of Life Sciences, Ibaraki Christian University, Hitachi-shi, Japan.

Background and aims: Consumption of whole grains like brown rice has beneficial effects for obesity and diabetes. MicroRNAs (miRNAs) are one type of non-coding RNA, which suppress target gene expression post transcriptionally. MiRNAs are detected in blood and could be useful biomarkers in many pathologies. The effects of whole grain consumption on circulating miRNA levels are not clear. The current study aimed at examining how whole grain consumption affects circulating miRNAs levels.
Materials and methods: This was a post-hoc analysis of a 12-week randomized controlled trial published previously. Middle age overweight participants with prediabetes ($n = 36$) were randomly allocated to receive either brown rice (BR) or white rice (WR). Serum miRNAs levels were analyzed by quantitative RT-PCR and the ΔΔCt method using miR-423 as a reference. Lipoprotein fractions were analyzed using HPLC.
Results: After the 12-week intervention period, the pooled serum levels of 51 circulating miRNAs of the BR group were significantly different compared to the WR group. Given their expression levels and their biological significance, we focused the analysis on miR-29a-3p and miR-92a-3p, which are known to inhibit lipogenesis in mouse liver and brown adipose tissue activity, respectively. The levels of miR-92a-3p in the WR group showed a trend of increase in comparison to the BR group (Table 1). However, ΔΔCt of miR-92a-3p levels were not significantly correlated with percentage of changes in body weight. Although miR-29a-3p levels were not significantly different between the two groups, the ΔΔCt of miR-29a-3p significantly correlated with percentage of changes in insulin levels ($r = -0.351$, $P = 0.045$), HOMA-IR levels ($r = -0.424$, $P = 0.014$), LDL cholesterol levels ($r = -0.505$, $P = 0.002$), number of large VLDL particles ($r = -0.364$, $P = 0.034$), number of total LDL particles ($r = -0.414$, $P = 0.015$), large LDL cholesterol levels ($r = -0.414$, $P = 0.015$) and medium LDL cholesterol levels ($r = -0.477$, $P = 0.004$).
Conclusion: BR consumption might increase circulating miR-92a-3p levels. Therefore, it is unlikely that brown adipose tissue activation is a cause of body weight reduction by BR consumption. miR29a-3p levels are associated with insulin resistance and apolipoprotein B containing lipoprotein metabolism.

Table 1. ΔCt and ΔΔCt of circulating levels of miR-29a-3p and miR-92a-3p

		Brown Rice	White Rice	*P*
n		18	18	
Age (years)		55 ± 7	52 ± 7	0.282
Changes in body weight (kg)		-2.4 ± 2.0	-0.2 ± 1.1	0.000
Changes in waist circumference (cm)		-3.1 ± 2.9	-0.4 ± 1.4	0.002
miR-29a-3p	ΔCt at 0-week	2.7 ± 0.9	2.7 ± 1.0	0.868
	ΔCt at 12-week	2.4 ± 1.6	2.5 ± 1.1	0.785
	ΔΔCt	-0.4 ± 2.0	-0.2 ± 1.5	0.682
miR-92a-3p	ΔCt at 0-week	-3.9 ± 0.9	-3.7 ± 1.1	0.688
	ΔCt at 12-week	-4.0 ± 0.9	-3.0 ± 0.8	0.001
	ΔΔCt	-0.1 ± 1.4	0.8 ± 1.2	0.052

Data are mean ± SD.

Clinical Trial Registration Number: UMIN000016293
Supported by: KAKENHI and NARO
Disclosure: **H. Suzuki:** None.

697

Effect of meal composition on subsequent eating behaviour

G. Freckmann, D. Waldenmaier, S. Ulbrich, S. Pleus, C. Haug;
Institut für Diabetes-Technologie, Forschungs- und Entwicklungsgesellschaft mbH an der Universität Ulm, Ulm, Germany.

Background and aims: An energy intake that exceeds the energy requirement promotes the progression of obesity. The intake of food with a low glycemic index (GI) might prevent the progression of obesity. This study examined the effect of meals with different GI on eating behavior at a subsequent meal in subjects without diagnosed diabetes mellitus.
Materials and methods: On two consecutive study days, 36 subjects received two standardized test meals, containing equal amounts of carbohydrate (50 grams) but differing in GI (high GI meal: "HGI", low GI meal: "LGI"), for breakfast and lunch. HGI mainly consisted of simple carbohydrates, whereas LGI contained complex carbohydrates and a high fat and protein content. On both study days, test meals were consumed in alternating sequence. For the subsequent dinner (unrestrictedly selected from a buffet), energy and macronutrient content were calculated. Before and after each meal, subjects rated their feeling of satiety on a scale ranging from −3 = extremely hungry to 3 = extremely full.
Results: The energy content of the dinner selected after LGI for breakfast and HGI for lunch (1270 ± 531 kcal) was on average 168 kcal higher than after the opposite test meal sequence (1102 ± 331 kcal; $p = 0.004$), although relative macronutrient composition was comparable. This difference corresponds to 8% of the calculated mean daily energy demand of the subjects. After test meal sequence LGI-HGI the feeling of satiety before dinner was on average −2.4, whereas after the opposite sequence the mean feeling was −1.6.
Conclusion: The composition of the previously consumed meal influenced meal selection at a subsequent meal more than the preceding cumulative energy intake. A low GI meal promoted the feeling of satiety for a longer time and decreased the risk of an increased energy intake at a subsequent meal.
Clinical Trial Registration Number: NCT03405415
Disclosure: **G. Freckmann:** None.

698

Measurement of gastric emptying using scintigraphy and a ^{13}C-octanoic acid breath test with Wagner-Nelson analysis in type 2 diabetes

L.G. Trahair[1,2], M.A. Nauck[3], T. Wu[1,2], M.D.M. Buttfield[1,2], J.E. Stevens[4,2], S. Hatzinikolas[1,2], C.K. Rayner[1,2], J.J. Meier[3], M. Horowitz[1,2], K.L. Jones[1,2];
[1]Adelaide Medical School, The University of Adelaide, Adelaide, Australia, [2]NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, Adelaide, Australia, [3]Diabetes Center Bochum-Hattingen, Medical Department I, St. Josef-Hospital, Bochum, Germany, [4]School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia.

Background and aims: Disordered gastric emptying (GE) occurs frequently in diabetes. While scintigraphy remains the 'gold standard' measurement, GE of ^{13}C-labelled substrates has been applied widely in both clinical and research settings given the simplicity of the technique and lack of a radiation burden. A variety of mathematical approaches have been utilised to generate a GE curve from the % $^{13}CO_2$ measured in breath samples following a test meal, most simply, through the fitting of a non-linear exponential curve (conventional analysis). Wagner-Nelson (WN) analysis is another mathematical model that has been proposed; this method has hitherto not been assessed in a diabetic population. We compared WN analysis with (i) scintigraphy and (ii) conventional breath test modelling to evaluate GE in type 2 diabetes (T2DM).
Materials and methods: Thirteen patients with T2DM (age 68.1 ± 1.5 yr, BMI 31.0 ± 0.9 kg/m^2 duration of known diabetes 4.3 ± 0.9 yr, HbA1c

6.3 ± 0.2%) consumed a standardised mashed potato meal comprising 65 g powdered potato and 20 g glucose reconstituted with 250 ml water and an egg yolk labelled with 100 μL ^{13}C-octanoic acid, mixed with 20 MBq ^{99m}Tc-calcium phytate. Scintgraphic data were acquired and breath samples collected for 4 hours after meal ingestion, with the subject seated with their back against a gamma camera. Decay and attenuation-corrected GE curves were generated for the scintigraphic data. GE curves were derived from the breath test data using both WN analysis (with a range of values for the elimination constant (K_{el}) from 0.5–0.7) and conventional analysis. The 50% GE time (T50) and intragastric retention at 60 min were compared. Data are mean ± SEM.

Results: With WN analysis, a $K_{el} = 0.60$ best approximated the scintigraphic GE curve (Figure). There was a correlation between the T50 calculated with scintigraphy and the T50 calculated by $WN_{Kel=0.60}$ ($r^2 = 0.44$, $P < 0.05$) or conventional analysis ($r^2 = 0.44$, $P < 0.05$). Similarly, the intragastric retention at 60 min calculated with scintigraphy correlated with the $WN_{Kel=0.60}$ ($r^2 = 0.61$, $P < 0.01$) and conventional analysis ($r^2 = 0.51$, $P < 0.01$). The T50 calculated with scintigraphy (68.5 ± 4.8 min) and $WN_{Kel=0.60}$ (71.3 ± 4.5 min) were not different, whereas the T50 calculated by conventional analysis was much longer at 164.7 ± 6.0 min ($P < 0.001$).

Conclusion: In T2DM GE of a mashed potato meal assessed using a ^{13}C-octanoic acid breath test with $WN_{Kel=0.60}$ closely reflects measurements obtained with scintigraphy whereas, in absolute terms, the conventional breath test analysis does not.

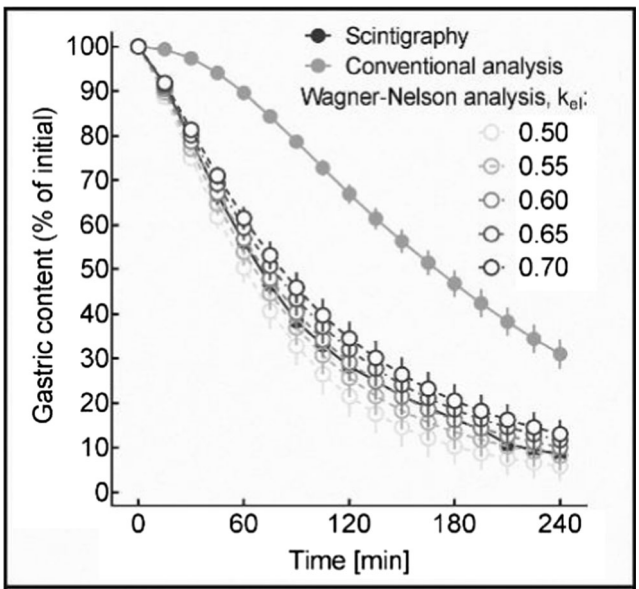


Figure: Gastric emptying of a mashed potato meal measured using scintigraphy and a breath test with both Wagner-Nelson analysis (with values for the elimination constant (Kel) ranging from 0.5-0.7) and a standard non-linear exponential model (conventional analysis) in n=13 type 2 patients.

Clinical Trial Registration Number: NCT02324010
Supported by: RAH clinical project grant
Disclosure: **L.G. Trahair:** None.

699

Influence of dietary and haematobiochemical profile in patients with type 2 diabetes belong to Umbria clinic: "TOSCA.IT" study

A. Tantucci, A. Timi, G. Perriello;
University of Perugia, Perugia, Italy.

Background and aims: TOSCA.IT is a multicentre, randomised, open label, parallel-group clinical trial designed to compare the long-term effects of pioglitazone versus sulfonylureas, given in addition to metformin, on cardiovascular events in patients with type 2 diabetes mellitus. The aim of the study is to analyze the habitual diet of 138 partecipants of TOSCA.IT study (95 men and 43 women) belong to Umbria Clinics, to explore the association of different proportions of the various macronutrients of the diet within the ranges recommended by the various authorities and also to verify any gender differences

Materials and methods: First, subjects were administered two tests to assess the degree of knowledge of diabetes and its complications and to investigate physical health, psychological state, social and environmental context, relationships and quality of life; then, they performed a food interview. Anthropometric variables considered were body mass index and waist circumference. In addition, several blood parameters to evaluate the glycometabolic and cardiovascular state of patients were taken into consideration: glycosylated hemoglobin, total cholesterol, triglycerides, LDL and HDL cholesterol fractions, C-reactive Protein.

Results: Regarding lipid intake, the glycated hemoglobin is significantly higher ($p = 0.021$) in adherents men compared to non-adherents, while HDL are lower in adherents women compared to non-adherents ($p = 0.042$); as regards carbohydrate intake, glycated hemoglobin is higher in non-adherents men ($p = 0.007$) than in adherents and the HDL are lower in non-adherents women ($p = 0.02$) compared to adherents. As for the fiber, the HDL are lower in non-adherents men than in adherents ($p = 0.049$), the same situation regarding cholesterol for triglycerides ($p = 0.03$); very interesting data regarding the daily consumption of alcohol: in non-adherents women the weight and the BMI are higher compared to adherents women ($p = 0.006$ and $p = 0.03$ respectively), the same situation for PCR ($p = 0.042$).

Conclusion: Although nutritional therapy is a central point in diabetic pathology, it is difficult to achieve optimal adherence to recommendations, especially in the long term; our study, even if performed on a small cohort of people, shows that adherence or no-adherence to the nutritional recommendations can affect cardiometabolic and inflammatory risk factors, regardless the use of drugs and cigarettes. Despite of an ideal "diet" for the diabetic patient, it is necessary to "personalize" it according to who we are facing, following the DNSG/SID recommendations on the Mediterranean diet; therefore, we need interventional and not only observational studies to compare different dietetic strategies on diabetic patients, that involve a large cohort of people, representative the general diabetic population commonly present in the real clinical practice.

Clinical Trial Registration Number: NCT00700856
Supported by: SID
Disclosure: **A. Tantucci:** None.

700

Orthodox religious fasting in practice: a comparative evaluation between Greek Orthodox general population fasters and Athonian monks

S.N. Karras[1], T. Koufakis[1], A. Petróczi[2], D. Folkerts[3,2], M. Kypraiou[1], H. Mulrooney[2], D.P. Naughton[2], P. Zebekakis[1], D. Skoutas[4], K. Kotsa[1];
[1]Division of Endocrinology and Metabolism, First Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA University Hospital, Thessaloniki, Greece, [2]School of Life Sciences, Pharmacy and Chemistry, Kingston University London, London, UK, [3]Department of Sport Psychology, Institute of Sport and Exercise Sciences, University of Muenster, Muenster, Germany, [4]Sarafianos Private Hospital, Thessaloniki, Greece.

Background and aims: Orthodox religious fasting (OF), a periodical vegetarian subset of the Mediterranean diet, has been proven to exert beneficial effects on human health. Athonian fasting is a pescetarian OF variation, where red meat is strictly restricted throughout the year. Previous studies have examined the OF nutritional synthesis and health

impact in general population fasters (GF) and Athonian monks (AM), separately. This is the first study to comparatively evaluate the characteristics and effects of this nutritional advocacy between the two populations.

Materials and methods: 43 general population male fasters (aged 20–45 years) and 57 age-matched male monks following Orthodox fasting were included in the study. Dietary intake data were collected in both groups during a restrictive (RD) and a non-restrictive (NRD) day. Nutritional, cardiometabolic and anthropometric parameters were compared between the two cohorts.

Results: AM compared to GF, presented lower daily total caloric intake for both RD (1362.42 ± 84.52 vs 1575.47 ± 285.96 kcal, $p < 0.001$) and NRD (1571.55 ± 81.07 vs 2137.80 ± 470.84 kcal, $p < 0.001$). They also demonstrated lower Body Mass Index (23.77 ± 3.91 vs 28.92 ± 4.50 kg/m^2, $p < 0.001$), Body Fat mass (14.57 ± 8.98 vs 24.61 ± 11.18 kg, $p = 0.001$), Fasting Insulin concentrations (4.61 ± 3.16 vs 11.64 ± 9.21 μg/ml, $p < 0.001$) and Homeostatic Model Assessment for Insulin Resistance values (0.98 ± 0.72 vs 2.67 ± 2.19 mmol/l, $p < 0.001$), compared to GF. GF and AM demonstrated a comparable profile for Total Cholesterol (189.1 ± 45.08 vs 183.00 ± 40.87 mg/dl respectively, $p = 0.470$) and Low-Density Lipoprotein (120.68 ± 45.92 vs 119.97 ± 36.70 mg/dl respectively, $p = 0.930$), while High-Density Lipoprotein concentrations were in the low-normal range for both (43.20 ± 11.05 vs 47.83 ± 14.11 mg/dl respectively, $p = 0.061$). Secondary hyperparathyroidism (Parathyroid Hormone concentrations: 116.08 ± 49.74 pg/ml), as a result of profound hypovitaminosis D [25(OH)D: 9.27 ± 5.81 ng/ml], were evident in the AM group.

Conclusion: The results of the present study highlight the unique characteristics of Athonian Orthodox fasting and its value as a health-promoting diet. The impact of limitation of specific vitamins and minerals during fasting warrants further investigation.

Cardiometabolic markers in general population fasters and Athonian Monks

Biochemical Marker	Group 1 General population fasters	Group 2 Athonian monks	Test Statistics
CHOL (mg/dl)	189.1 ± 45.08	183.00 ± 40.87	t(99.758)= 0.725, d = 0.142, p = .470
TRIG (mg/dl)	113.22 ± 79.09	73.82 ± 31.68	t(105)= 3.460, d = 0.711, p = .001
HDL (mg/dl)	43.20 ± 11.05	47.83 ± 14.11	t(103.733)= -1.898, d = -0.368, p = .061
LDL (mg/dl)	120.68 ± 45.92	119.97 ± 36.70	t(93.582)= 0.088, d = 0.017, p = .930
Calcium (mg/dl)	9.01 ± 1.27	9.06 ± 0.42	t(112)= -0.270, d = -0.059, p = .788
Insulin (μg/ml)	11.64 ± 9.21	4.61 ± 3.16	t(100)= 5.150, d = 1.137, p < .001
PTH (pg/ml)	37.69 ± 16.36	116.09 ± 49.75	t(110)= -11.283, d = -2.372, p < .001
25(OH)D (ng/ml)	28.26 ± 39.66	9.27 ± 5.81	t(103)= 3.448, d = 0.839, p = .001
Glucose (mmol/l)	5.12 ± 0.32	4.71 ± 0.60	t(114)= 4.645, d = 0.891, p < .001
HOMA-IR (mmol/l)	2.67 ± 2.19	0.98 ± 0.72	t(100)= 5.229, d = 1.162, p < .001

Abbreviations: CHOL: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; PTH: parathyroid hormone; TRIG: triglycerides; 25(OH)D: 25-hydroxyvitamin-D; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

Disclosure: **S.N. Karras:** None.

701

Carbohydrate tolerance at near-normoglycaemia remission in obese African American patients with hyperglycaemic crises

P. Vellanki[1], D. Stefanovski[2], V. Narwani[1], I. Anzola[1], D.D. Smiley[1], L. Peng[3], G.E. Umpierrez[1];
[1]Emory University School of Medicine, Atlanta, [2]University of Pennsylvania, Atlanta, [3]Emory University, Atlanta, USA.

Background and aims: Many obese African American (AA) patients with new-onset DKA and severe hyperglycemia achieve near-normoglycemia remission (HbA1c <7%, fasting blood glucose [BG] <130 mg/dl) with intensive insulin treatment. Glycemic status at insulin remission varies from normal glucose tolerance (NGT), prediabetes or diabetes on oral glucose tolerance test (OGTT). We hypothesized that patients with NGT at near-normoglycemia remission will have higher insulin sensitivity (S_i) and insulin secretion, and longer hyperglycemia relapse-free survival.

Materials and methods: A total of 135 obese AA new-onset of diabetes presenting with DKA and severe hyperglycemia (BG >400 mg/dl without DKA) were consented. Seventy-eight patients (58%) achieved near-normoglycemia remission and 75 of these patients underwent 2-h 75-gm OGTTs a week after insulin remission and every 6 months till hyperglycemia relapse. After initial OGTT, subjects were randomized to metformin, sitagliptin, pioglitazone or placebo for a median of 336 days (range 19–1186). Because baseline characteristics did not differ significantly between DKA and severe hyperglycemia, data was combined for the analysis. S_i was calculated using the OGTT minimal model analysis. Insulin secretion was calculated as incremental area under the curve of insulin ($IncreAUC_i$) with insulin levels from OGTT. Disposition index (DI) was calculated as S_i x $IncreAUC_i$. Hyperglycemia relapse was defined as fasting BG ≥130 mg/dl, HbA1c >7% or 2 random BG ≥180 mg/dl.

Results: There were 33 patients with DKA and 42 with severe hyperglycemia. On initial OGTT, 12% had NGT, 45% had prediabetes and 43% had diabetes. There were no differences in baseline characteristics. DI was higher in patients with NGT vs prediabetes vs diabetes (1.88 ± 1.63 vs 1.05 ± 1.14 vs 0.35 ± 0.92, $p < 0.001$). The difference in DI was explained by higher S_i than $IncreAUC_i$ in NGT compared to prediabetes and diabetes (S_i: 4.2 ± 4.4 vs 2.4 ± 3.5 vs 1.2 ± 2.5 10^{-4}.(mU/l)$^{-1}$.min^{-1}, $p < 0.001$; $IncreAUC_i$: 6571 ± 3451 vs 6585 ± 4947 vs 4450 ± 3051, $p = 0.14$ mU/ml^2). At a median follow-up of 336 days, of the patients with NGT, 43% remained NGT, 43% and 14% developed prediabetes and diabetes respectively. Of the patients with prediabetes, 42% remained with prediabetes, 13% achieved NGT and 46% developed diabetes. Of the patients with diabetes, 79% remained with diabetes, 18% and 4% achieved prediabetes and NGT respectively. At last follow-up, DI was significantly higher in patients with NGT vs prediabetes and diabetes (2.49 ± 3.1 vs 1.21 ± 1.50 vs 0.61 ± 0.96, $p = 0.02$). S_i ($p = 0.17$) and $IncreAUC_i$ ($p = 0.25$) did not differ significantly among the groups at follow-up. Multivariate Cox regression adjusting for age, sex, DKA presentation and initial OGTT status showed that treatment with metformin, sitagliptin or pioglitazone was significantly associated with long-term relapse-free survival (Hazard ratio: 0.42, 95% confidence interval 0.20, 0.92) compared to placebo.

Conclusion: In obese AA patients, attaining NGT at near-normoglycemia remission is characterized by higher insulin sensitivity and disposition index rather than higher insulin secretion. However, none of these markers were associated with long-term hyperglycemia-free survival.

Clinical Trial Registration Number: NCT01099618, NCT00426413
Supported by: NIH, Jacobs Family Foundation

Disclosure: **P. Vellanki:** Employment/Consultancy; Boehringer-Ingelheim. Grants; Astra Zeneca, Boehringer-Ingelheim.

PS 055 New clues on metformin, sulfonylureas and insulin

702

OCT1 is a target of metformin and regulates pancreatic stellate cell activity

C. Wu, S. Qiu, X. Zhu, L. Li;
Departmentof Endocrinology, affiliated ZhongDa Hospital of Southeast University, Nanjing, China.

Background and aims: Metformin treatment is reported to be associated with a lower incidence of and mortality from pancreatic cancer (PC) in type 2 diabetes patients. Activated pancreatic stellate cells (PSCs) are key stroma cells responsible for pancreatic fibrogenesis and PC progression. However, little research is about the influence of metformin on PSCs. Given the potential beneficial effects of metformin on PC, pancreatic tumour stroma is an important target for new therapeutics. We observed the effects of metformin on PSCs. We investigated the effects of metformin on human PSCs proliferation and the production of extracellular matrix (ECM) proteins.
Materials and methods: Cells were cultured with different concentrations of metformin (0–10 mmol/L). Cell proliferation was determined by immunofluorescence staining for nuclear Ki67 labelling. ECM production was studied by quantitative real-time polymerase chain reaction, immunoblotting and immunofluorescence microscopy. Adenosine monophosphate-activated protein kinase (AMPK), an important regulatory molecule responsible for metformin action, and the organic cation transporter member 1 (OCT1), which is believed to be the most important transporter for the pharmacological action of metformin, were investigated for their possible involvements in metformin-induced proliferation and ECM production.
Results: Our results showed that metformin inhibited PSCs proliferation and decreased the production of ECM proteins by activation of AMPK phosphorylation. Silencing of OCT1 expression resulted in a reduction in the effects of metformin on AMPK phosphorylation.
Conclusion: Collectively, the data indicate that OCT1 is important for metformin regulation of PSCs activity. OCT1 is a target of metformin in regulating PSCs activity.
Supported by: No 81570739
Disclosure: **C. Wu:** None.

703

Metformin-induced alterations of transcriptome profile in healthy individuals

M. Ustinova[1], I. Silamiķelis[1], I. Elbere[1], I. Kalniņa[1], L. Zaharenko[1], I. Radoviča-Spalviņa[1], V. Rovite[1], I. Konrāde[2], V. Pirags[1], J. Klovins[1];
[1]Latvian Biomedical Research and Study Centre, Riga, [2]Riga Stradins University, Riga, Latvia.

Background and aims: Metformin is the first-line antidiabetic agent used in pharmacotherapy of type 2 diabetes to improve glucose homeostasis. Nevertheless, additional therapeutic directions such as cancer prevention, treatment of polycystic ovary syndrome and neurodegenerative diseases have been highlighted lately justifying the pleiotropic effect of the drug. Despite the identification of AMP-activated protein kinase and mitochondrial respiratory-chain complex 1 as the major mediators of its effects, exact mechanisms of metformin action remain obscure. The aim of the study was to evaluate metformin-induced alterations of transcriptome profile, in order to identify novel mechanisms of action at the molecular level in non-diabetic individuals.
Materials and methods: The longitudinal study enrolled 25 healthy volunteers of European descent, receiving oral 850 mg dose of metformin twice-daily for 7 days. RNA was isolated from whole blood samples, collected at three consecutive time points: before metformin administration, 10 hours after the first dose and at the end of metformin treatment. RNA-seq was performed on Ion Proton™ System and Ion PI™ Chip. For bioinformatic analysis Trimmomatic 0.36, STAR 2.5.3a, edgeR and DAVID 6.8. tools were applied.
Results: In total 681 differentially expressed genes were identified (FDR <0.05), among them genes related to inflammatory responses, promoting enrichment of intestinal immune network for IgA production and cytokine-cytokine receptor interaction pathways. In addition, differential expression of four functional gene clusters were revealed after consideration of subject-specific effects, including upregulation of ribosomal genes, snoRNAs and genes relevant to insulin production (HNF1B, HNF1A, HNF4A, GCK, INS, NEUROD1, PAX4, PDX1, ABCC8, KCNJ11), and downregulation of genes contributing in cholesterol homeostasis (APOB, LDLR, PCSK9).
Conclusion: In healthy individuals universal metformin-induced alterations of global gene expression profiles in white blood cells are associated with immune responses, while subject-specific effects, which tendency to be more permanent are related to energy metabolism.
Clinical Trial Registration Number: 2016-001092-74
Supported by: ERDF Project Nr. 1.1.1.1/16/A/091
Disclosure: **M. Ustinova:** None.

704

Physiological effects of pioglitazone and metformin in patients with ataxia-telangiectasia

L.J. McCreight[1], L. Coppin[2], N. Jackson[2], A.M. Umpleby[2], A. Mari[3], E.R. Pearson[1];
[1]University of Dundee, Dundee, UK, [2]University of Surrey, Guildford, UK, [3]National Research Council, Padova, Italy.

Background and aims: Ataxia telangiectasia (A-T) is a rare genetic condition associated with diabetes, caused by homozygous recessive loss of function of the A-T mutated (*ATM*) gene. OGTT data show that adults with A-T are insulin resistant, with normal fasting glucose and insulin, but large, prolonged excursions after a glucose load. *ATM*-ko mice have glucose intolerance, insulin resistance and abnormal fat distribution, which improved with TZD treatment. In people with diabetes, a SNP at a locus containing *ATM* was associated with greater response to metformin. Our study aimed to characterise the insulin resistance seen in A-T and to determine the physiological effects of pioglitazone and metformin in these patients.
Materials and methods: This open label, non-randomised, crossover study compared 8 non-diabetic people with A-T and 15 healthy controls over 17 weeks. The study consisted of two treatment periods, with one week washout, and three study visits: baseline, post-metformin and post-pioglitazone. At each visit, a dual tracer mixed meal test was used to measure fluxes in glucose, insulin, and c-peptide over 6 hours following a meal. Glucose absorption, beta cell function and insulin sensitivity were modelled.
Results: The groups were well matched for age, BMI and HbA1c. Adults with A-T are insulin resistant compared to controls, with higher fasting insulin secretion rate (ISR) (89.3 ± 13.3 v 58.1 ± 7.0 pmol/min/m^2, $p = 0.04$), despite comparable fasting glucose, glucose clearance (CL) and endogenous glucose production (GP). Post meal, they have greater total insulin secretion (96.1 ± 14.6 v 53.3 ± 6.1 pmol/L, $p = 0.005$) and glucose (6.4 ± 0.3 v 5.3 ± 0.1 mmol/L, $p = 0.001$). In the A-T group, metformin treatment resulted in increased CL (2.7 ± 0.2 v 2.5 ± 0.2 mmol/min/kg, $p = 0.04$) during fasting, in the context of reduced insulin (85.3 ± 33.9 v 99.5 ± 36.1, $p = 0.04$), and unchanged GP. Post-meal, metformin treatment caused a greater suppression in GP in the first hour compared to baseline (24 vs 4%, $p = 0.04$), in the absence of altered insulin or CL. After metformin treatment the control group had increased CL at fasting (3.1 ± 0.2 v 2.6 ± 0.1, $p < 0.001$), in the context of unchanged insulin. However, their fasting GP was increased (13.5 ± 0.8 v 11.2 ± 0.5 mmol/min/kg, $p = 0.001$). Post meal there was no significant difference in insulin, CL, or GP. Pioglitazone reduced fasting (58.2 ± 17.5 v 99.5 ± 36.1,

$p = 0.007$) and mean (522 ± 170 v 1065 ± 388, $p = 0.015$) insulin in the A-T group, in the context of unchanged fasting glucose but reduced mean glucose (5.9 ± 0.02 v 6.4 ± 0.3, $p = 0.02$), with unchanged CL or GP. In the controls, pioglitazone treatment had no significant effect on insulin or glucose concentration.

Conclusion: Metformin improves peripheral insulin sensitivity in the fasting state in both groups. In adults with A-T, there is increased suppression of EGP in the hour post-meal, in keeping with an improvement in hepatic insulin sensitivity. In contrast, the controls do not demonstrate any improvement in hepatic insulin sensitivity with metformin, but in fact have an increased EGP, which may represent compensation for an increase in fasting glucose clearance. Pioglitazone treatment in the A-T group improved both peripheral and hepatic insulin sensitivity. Pioglitazone reduced glucose excursion post-meal, where metformin did not. These data suggest that pioglitazone may be appropriate as first line treatment for diabetes in A-T.

Clinical Trial Registration Number: NCT02733679

Supported by: Funded by Wellcome Trust New Investigator Award (102820/Z/13/Z) held by ERP

Disclosure: **L.J. McCreight:** None.

705

Distribution of dose and up-titration patterns for patients initiating metformin monotherapy in the UK

K. Iglay[1], B. Sawhney[2], A.Z. Fu[2], G. Fernandes[1], M. Crutchlow[1], S. Rajpathak[1], K. Khunti[3];

[1]Merck & Co., Inc., Kenilworth, USA, [2]Complete HEOR Solutions (CHEORS), North Wales, USA, [3]Diabetes Research Centre, University of Leicester, Leicester, UK.

Background and aims: International guidelines recommend treatment with metformin as initial pharmacotherapy for patients with type 2 diabetes mellitus (T2DM). Dosing guidelines for metformin recommend starting with a low dose and up-titrating the dose over a period of 1–2 months to a maximally effective (~2000 mg/day) dose, unless tolerability-limited; however, the extent to which this occurs in the real world setting is unknown. To address this question, the current study sought to determine the dose distribution among new users of metformin monotherapy at initiation, 3, 6, and 12 months, as well as the pattern of up-titration following initiation of therapy.

Materials and methods: Patients ≥21 years of age with a T2DM diagnosis between 2012 and 2017 were identified using the Clinical Practice Research Datalink (CPRD) database. Patients with an HbA1c value in the 6 months prior to metformin monotherapy initiation, and continuous enrollment in the database 1 year prior and 15 months following metformin monotherapy initiation were included. Data were censored once patients discontinued metformin or added another diabetes medication if these occurred during the follow-up period. Descriptive statistics were used to describe dose distribution and up-titration patterns for patients on metformin monotherapy. Time to highest dose was estimated using a cumulative incidence function.

Results: Of the 6174 eligible patients, 42.7% were female, median age was 61 years, and median HbA1c prior to initiation was 7.8%. At 12 months post-initiation, 58.2% of the initial cohort remained on metformin monotherapy, while 41.8% were censored (33.8% discontinued metformin without initiating another anti-hyperglycemic agent (AHA) at the time of discontinuation, 4.8% had another AHA added to metformin, and 3.2% switched from metformin to another AHA). The table below describes the distribution of metformin dose and up-titration during the follow-up period. The majority (71.6%) of patients initiated metformin at ≤1000 mg/day. At 3, 6, and 12 months, 71.6%, 68.6% and 68.1% of metformin monotherapy patients, respectively, were on doses ≤1000 mg/day. Cumulatively, 0.03%, 6.7%, and 10.8% of patients were up-titrated from their initial dose at 3, 6 and 12 months, respectively. Among those who up-titrated during follow-up ($n = 667$), median (Q1, Q3) highest dose of metformin was 2000 mg/day (1000 mg/day, 2000 mg/day) and median (Q1, Q3) time to highest dose was 175 (162, 348) days.

Conclusion: Almost two-thirds of patients remained on metformin monotherapy 12 months after initiation. The majority were not up-titrated and remained on a sub-maximally effective dose of metformin at 12 months. Further research needs to determine potential reasons for failure to intensify metformin therapy.

Distribution of Dose and Up-titration Patterns for Patients on Metformin Monotherapy

	Initiation (n=6174)	3 Months (n=6155)*	6 Months (n=4820)†	12 Months (n=3592)‡
Median (Q1, Q3) Dose of Metformin (mg/day)	1000 (750, 1500)	1000 (850, 1500)	1000 (1000, 1500)	1000 (1000, 1500)
Distribution of Daily Dose of Metformin, n (%)				
>0 mg to ≤500 mg	1511 (24.5%)	1500 (24.4%)	1086 (22.5%)	812 (22.6%)
>500 mg to ≤1000 mg	2909 (47.1%)	2906 (47.2%)	2222 (46.1%)	1633 (45.5%)
>1000 mg to ≤1500 mg	1026 (16.6%)	1024 (16.6%)	767 (15.9%)	513 (14.3%)
>1500 mg to ≤2000 mg	716 (11.6%)	793 (11.6%)	726 (15.1%)	619 (17.2%)
>2000 mg	12 (0.2%)	12 (0.2%)	19 (0.4%)	15 (0.4%)
Cumulative proportion of patients up-titrated from their initial dose of metformin, n (%)§	NA	2 (0.03%)§	414 (6.7%)§	667 (10.8%)§

*19 patients discontinued metformin without initiating another AHA and were removed from the 3 month analysis; †1335 patients were removed from the 6 month analysis (1106 discontinued metformin without initiating another AHA, 137 had another AHA added to metformin, and 92 switched from metformin to another AHA); ‡1228 were removed from the 12 month analysis (960 discontinued metformin without initiating another AHA, 162 had another AHA added to metformin, and 106 switched from metformin to another AHA); §Denominator is the initial cohort (n=6174).

Disclosure: **K. Iglay:** Employment/Consultancy; Merck & Co., Inc. Stock/Shareholding; Merck & Co., Inc.

706

Associations between metformin use and the risks of vitamin B_{12} deficiency, anaemia and neuropathy in patients with diabetes: a meta-analysis

W. Yang[1], X. Cai[1], H. Wu[2], L. Ji[1];

[1]Endocrinology and Metabolism, Peking University People's Hospital, Beijing, [2]Eisai China Inc., Shanghai, China.

Background and aims: Metformin is the first line therapy for patients with type 2 diabetes. However, numerous studies reported that metformin treatment led to decreased vitamin B12 level in patients, and clinically meaningful decrease in vitamin B12 level in patients could lead to diseases such as macrocrytic anemia and neuropathy. The aim of this meta-analysis was to find the associations between metformin use and the risks of vitamin B12 deficiency, anemia and neuropathy in patients with diabetes.

Materials and methods: The databases of PubMed, Web of Knowledge, Cochrane Library and Embase were searched to identify studies including all controlled studies on relationships between metformin use and vitamin B12, anemia or neuropathy in patients with diabetes, which were published in English prior to March 2018. The pooled risk ratio (RR) and 95% confidence interval (CI) were calculated with Cochrane Review Manager 5.3 (RevMan 5.3) to compare the risks of vitamin B12 deficiency, anemia and neuropathy in diabetic patients on metformin treatment vs those without metformin use. The mean difference (MD) and 95%CI were calculated to compare the levels and changes of serum vitamin B12 concentration (pmol/l) from baseline in diabetic patients on metformin treatment vs those without metformin use. A random-effect model was first used for the analyses, and a fixed effect model were used for those analyses with a low-to-moderate heterogeneity ($I^2 < 50\%$).

Results: A total of 30 studies were included in the analyses. Compared to patients without metformin use, patients with metformin treatment had significantly higher risk of vitamin B12 deficiency (RR = 2.28; 95% CI [1.63, 3.21]; $P < 0.001$; $I^2 = 62\%$), significantly lower levels of serum vitamin B12 concentration (MD = −64.71 pmol/l; 95% CI [−75.52, −53.91] pmol/l; $P < 0.001$; $I^2 = 87\%$), and significantly greater decrease in serum vitamin B12 concentration from baseline (MD = −14.68%; 95% CI [−17.98, −11.39]%; $P < 0.001$; $I^2 = 33\%$). Additionally, analyses of the few currently available studies did not reveal significant difference in risk

of anemia (four studies with 4070 patients, RR = 0.93; 95% CI [0.79, 1.09]; $P = 0.36$; $I^2 = 0\%$) and neuropathy (six studies with 1058 patients, (RR = 0.84; 95% CI [0.62, 1.13]; $P = 0.25$; $I^2 = 60\%$) between patients on metformin therapy vs those who were not.

Conclusion: Metformin use was significantly associated with the risk of vitamin B12 deficiency and the level of vitamin B12 was significantly lower in patients with metformin use compared with those without metformin use. As vitamin B12 deficiency could potentially have serious clinical consequences, affordable and non-toxic vitamin B12 supplement such as methylcobalamin is recommend to prevent and treat vitamin B12 deficiency in patients with diabetes on metformin therapy. In addition, more quality studies are needed to further elucidate whether metformin use was associated with anemia and neuropathy in patients with diabetes.

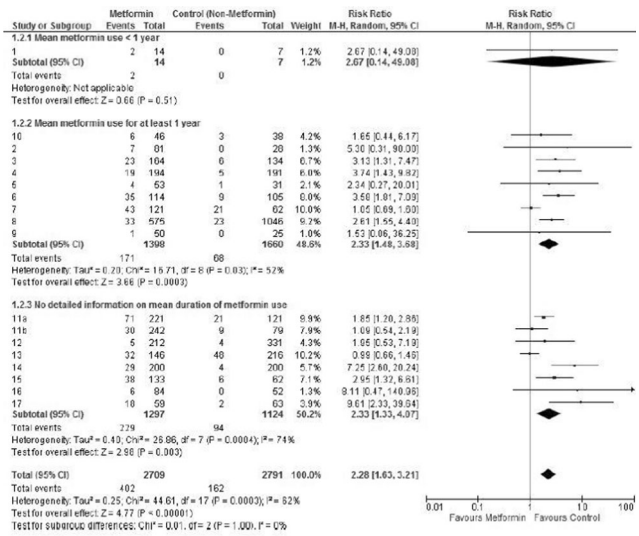


Figure 1 Risk of vitamin B12 deficiency between patients with metformin use vs controls (non-metformin use)

Supported by: Eisai China Inc. Medical Support
Disclosure: **W. Yang:** None.

707

Trends in medication utilisation, glycaemic control and rate of severe hypoglycaemia among type 2 diabetes patients at a tertiary referral centre in Singapore from 2007 to 2017

Y.Z. Tan[1], M.H.H. Cheen[1], P.S. Lim[1], G.Y. Khee[1], J. Thumboo[2], S.-Y. Goh[3], Y.M. Bee[3];
[1]Pharmacy, Singapore General Hospital, Singapore, [2]Rheumatology & Immunology, Singapore General Hospital, Singapore, [3]Endocrinology, Singapore General Hospital, Singapore, Singapore.

Background and aims: Singapore has one of the highest prevalence of type 2 diabetes (T2DM) amongst developed countries, and this is estimated to rise from 11.3% in 2010 to 15.0% in 2050. Use of glucose-lowering medications has been a cornerstone in combating T2DM. Over the past decade, novel agents with more favourable adverse effects profiles have been introduced and subsequently incorporated into treatment guidelines. We report the temporal trends in the utilization of glucose-lowering medications, glycaemic control and rate of severe hypoglycaemia in T2DM patients managed at a tertiary referral centre in Singapore.

Materials and methods: We analysed data of 36,925 T2DM patients from the SingHealth Diabetes Registry seen at the Singapore General Hospital from 2007 to 2017. Annual age-, sex- and ethnicity-standardized proportions of patients (a) who were prescribed with each class of glucose-lowering agent; (b) who were on monotherapy with either an oral agent or insulin, a combination of oral agents, or on both insulin and oral agents(s); and (c) with glycated haemoglobin A1c (HbA_{1c}) of <6%, 6 to <7%, 7 to <8%, 8 to <9% and ≥9%, were estimated using logistic regression. Annual age-, sex- and ethnicity-standardized rates of severe hypoglycaemia were estimated using Poisson regression.

Results: Use of metformin (45.9% to 59.6%, from 2007 to 2017), insulin (24.4% to 57.9%, from 2007 to 2017), DPP-4 inhibitors (1.2% to 31.2%, from 2008 to 2017) and SGLT-2 inhibitors (0.50% to 7.4%, from 2014 to 2017) increased significantly, while utilization of sulphonylureas (52.0% to 44.9%, from 2007 to 2017) and acarbose (15.3% to 5.7%, from 2007 to 2017) decreased (all $p < 0.001$). Proportion of patients with a single oral agent decreased (40.1% to 18.0%), but increased for those on treatment regimen containing both insulin and oral agent(s) (17.2% to 46.0%; all $p < 0.001$). The proportion of patients with HbA_{1c} ≥9% increased (18.1 to 20.3%) and decreased for those with HbA_{1c} ≤7% (40.7% to 38.5%; all $p < 0.001$). The rate of severe hypoglycaemia increased (5.5 to 9.4 per 100 patient-years; $p < 0.001$) during the study period.

Conclusion: Medication utilization patterns have changed significantly over the past 11 years with a shift towards newer agents. Overall glycaemic control amongst those managed at our centre did not improve, and the rate of severe hypoglycaemia increased. Further analysis is required before causal relationships between medication utilization, glycaemic control and hypoglycaemia can be inferred.

Disclosure: **Y.Z. Tan:** None.

708

Different sulfonylureas induce the apoptosis of proximal tubular epithelial cell differently via closing K_{ATP} channel

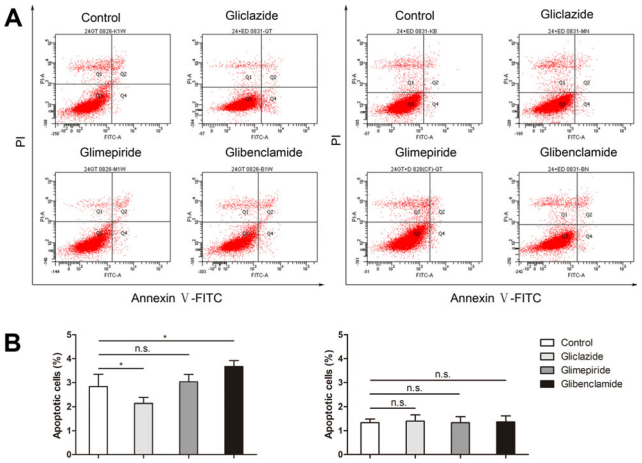
R. Zhang[1], X. Zhou[1], X. Shen[2], T. Xie[2], C. Xu[1], Z. Zou[1], J. Dong[3], L. Liao[1];
[1]Shandong provincial Qianfoshan hospital, Shandong university, Jinan, [2]Shandong university of Traditional Chinese Medicine, Jinan, China, [3]Qilu Hospital of Shandong university, Jinan, China.

Background and aims: Sulfonylureas (SUs) are widely prescribed for the treatment of type 2 diabetes (T2DM). Sulfonylurea receptors (SURs) are their main functional receptors. These receptors are also found in kidney, especially the tubular cells. However, the effects of SUs on renal proximal tubular epithelial cells (PTECs) were unclear. The aim of present study is to investigate if different SUs have different effects on the apoptosis of PTECs.

Materials and methods: HK-2 cells were exposed to SUs for 24 h prior to exposure to 30 mM glucose, the apoptosis rate was evaluated by Annexin/PI flow cytometry. Bcl-2, Bax and the ratio of LC3II to LC3I were also studied *in vitro*.

Results: Treatment with glibenclamide aggravated the apoptosis of HK-2 cells in high-glucose, as indicated by a significant decrease in the expression of Bcl-2 and increase in Bax ($P < 0.05$). Additionally, the decreased LC3II/LC3I reflects that the autophagy was inhibited by glibenclamide ($P < 0.01$). Similar but less pronounced change was found in glimepiride group, however, nearly opposite effects were found in gliclazide group ($P < 0.01$). Further, the effects of glibenclamide on apoptosis promotion and the decreased LC3II/LC3I were ameliorated obviously by treatment with 100 uM diazoxide ($P < 0.05$, $P < 0.01$). The potential protection effect of gliclazide was also inhibited after opening the K_{ATP} channel ($P < 0.05$).

Conclusion: The results suggest that, the effects of glibenclamide and glimepiride on PTECs apoptosis, especially the former, were achieved in part by closing the K_{ATP} channel. In contrast to glibenclamide and glimepiride, therapeutic concentrations of gliclazide showed an inhibitory effect on apoptosis of PTECs, which may have a benefit in the preservation of functional PTECs mass.



Supported by: NSFC 81570742; JSTDP 21601172
Disclosure: **R. Zhang:** None.

709

Comparison of medical resources, costs, and health utilities among patients with CHD and impaired glucose tolerance in the Acarbose Cardiovascular Evaluation Trial (ACE)

L. Mc Morrow[1], F. Becker[1], J. Leal[1], A.M. Gray[1], R. Coleman[2], H.C. Gerstein[3], L. Rydèn[4], R.R. Holman[5];
[1]Health Economics Research Centre, University of Oxford, Oxford, UK, [2]Diabetes Trials Unit, University of Oxford, Oxford, UK, [3]McMaster University, Hamilton, Canada, [4]Karolinska Institutet, Stockholm, Sweden, [5]DiabetesTrials Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, Diabetes Trials Unit, University of Oxford, Oxford, UK.

Background and aims: ACE assessed the effects of acarbose, an α-glucosidase inhibitor, in 6,522 patients with CHD and impaired glucose tolerance from 176 hospital outpatient clinics in China. This randomized, double-blind, placebo-controlled, phase-4 trial with a five year median follow-up showed acarbose did not reduce the risk of major adverse cardiovascular events, but reduced the incidence of diabetes by 18% ($p = 0.005$). We aimed to compare medical resource use, costs and health utilities between treatment arms.
Materials and methods: Medical resource use data were collected throughout the trial. Hospitalisations, medications and outpatient visits were valued using Chinese costs from, respectively, the China Health and Family Planning Statistical Yearbook (2016), the Beijing Medicine Sunshine Purchase Platform, and published studies. Medication use is represented as drug days, with all cardiovascular and diabetes drugs summed across the follow-up period for each patient. Health utilities were measured using the Euro-Qol-5-Dimension three level (EQ-5D-3L) questionnaire. An available-case analysis was performed using regression analyses (hierarchical generalized linear models) to compare resource use, costs, and health utilities accounting for between-site variation. Costs were discounted at 3% per annum.
Results: There were no significant differences in hospitalisations, inpatient days, outpatient visits or drug days between treatment arms. However, mean (standard error) diabetes drug days per patient (excluding study drug), as part of total drug days, were significantly lower in the acarbose group compared with the placebo group (91 ± 6.08 vs. 118 ± 6.99, $p = 0.04$). Costs over the trial period for inpatient care, outpatient care, medications and total costs (excluding study drug) did not differ significantly between groups. On average, the study drug (acarbose) cost ¥6,594 (€857, 1241 drug days) per patient during the trial follow-up period. Total costs per patient for the acarbose group were significantly higher than for the placebo group (Table). Health utilities were similar at baseline in the acarbose and placebo groups (0.94 ± 0.002 vs. 0.94 ± 0.002) indicating a trial population with few health problems. No significant between group differences in health utilities were detected during the trial ($p = 0.42$).
Conclusion: Total costs during the follow-up period were significantly higher in the acarbose arm once the study drug costs were added. Future research will explore the impact of acarbose on resource use, costs and quality adjusted survival over the lifetime horizon.

Comparing Resource Use and Costs across Treatment Groups (available case analysis)

Resource Use/Cost *(Chinese Yuan 2017)**	Acarbose *n/Mean (SE)*		Placebo *n/Mean (SE)*		Difference Mean (SE)	*P-value**
Hospitalisations (n)	3,272	0.5 (0.02)	3,250	0.4 (0.02)	0.02 (0.02)	0.40
Inpatient Days (n)	3,226	4.7 (0.24)	3,222	4.7 (0.22)	-0.01 (0.32)	0.54
Outpatient Care Visits (n)	3,250	30.2 (0.74)	3,231	29.8 (0.76)	0.36 (1.06)	0.44
Total Drug Days excluding study drug (n)	3,260	5,025 (64.58)	3,237	4,966 (65.27)	59 (91.81)	0.14
Inpatient Care Costs	3,272	4,878 (242.48)	3,250	4,897 (244.64)	-19 (330.64)	0.57
Outpatient Care Costs	3,250	10,052 (244.23)	3,231	9,920 (251.09)	132 (350.26)	0.72
Total Medication Costs excluding study drug	3,260	10,673 (210.34)	3,237	10,404 (186.77)	269 (281.42)	0.47
Acarbose Costs	3,272	6,594 (75.34)	3,250	0 (0)	6,594 (75.34)	N/A
Total Costs excluding study drug	3,249	23,011 (444.95)	3,231	22,806 (443.01)	205 (627.90)	0.82
Total Costs	3,247	28,524 (492.36)	3,231	22,806 (443.01)	5,718 (662.53)	<0.01

*P-value of treatment effect variable in GLMM model with log link function, log of follow-up as an offset variable and a negative binomial distribution for resource use, and gamma distribution for costs. ^ ¥1 = US$0.16; ¥1 = €0.13. N/A not applicable.

Clinical Trial Registration Number: ISRCTN91899513.
Supported by: Bayer AG.
Disclosure: **L. Mc Morrow:** Grants; Bayer AG.

710

HbA_{1c} is highly variable in people with type 2 diabetes on stable therapy in both trial and real-world settings: implications for clinical practice

A. McGovern[1], J. Dennis[1], B. Shields[1], E. Pearson[2], A. Hattersley[1], A. Jones[1];
[1]University of Exeter, Exeter, [2]University of Dundee, Dundee, UK.

Background and aims: HbA1c is the measure most widely used to make decisions about changing treatment in people with type 2 diabetes (T2D). However within individuals HbA1c fluctuates considerably over time. It is unknown how much this noise in HbA1c obscures true deterioration or treatment response. A detailed understanding of how HbA1c fluctuates over time in people on stable therapy is essential for understanding how to interpret HbA1c changes at an individual level. We assessed the variability in HbA1c over time in people with T2D on stable treatment in a large randomised controlled trial and a large UK based real world cohort. We subsequently analysed the impact of clinical characteristics on HbA1c variability.
Materials and methods: We used data from the ADOPT randomised clinical trial ($n = 3380$) and a large UK primary care database (CPRD; Clinical Practice Research Datalink, $n = 82,439$) to analyse variation in HbA1c over time in people with non-insulin treated T2D on stable therapy. HbA1c variability was defined as the standard deviation (SD) of 4

measurements; measured every 3 months in ADOPT and 6 months in CPRD. Stable therapy was defined as no discontinuation or addition of any glycaemic lowering medication in the preceding six months or during follow up. The outcome SD in HbA1c was stratified by baseline HbA1c. We analysed the impact of age, gender, ethnicity, and body mass index (BMI), and drug class on glycaemic variability in multivariable regression after adjusting for baseline HbA1c.

Results: In ADOPT, SD of HbA1c was non-linearly correlated with baseline HbA1c, substantially increasing in those with the highest HbA1c (SD = 2.8 mmol/mol for baseline <48 mmol/mol, 3.5 mmol/mol for 48–64 mmol/mol, and 7.0 mmol/mol for >64 mmol/mol). Small but significant increases in SD were seen with younger age (0.33; 95%CI 0.24, 0.42 mmol/mol per decade), male gender (0.19; 95%CI 0.01, 0.38 mmol/mol), and Black race relative to White (0.63; 95%CI 0.39, 0.88 mmol/mol). Asian race was associated with less variation (−0.46; 95%CI −0.62, −0.30 mmol/mol). There was no association with BMI. Relative to metformin, glyburide had higher SD (0.42; 95%CI 0.21, 0.64 mmol/mol) and rosiglitazone lower (−0.28; 95%CI −0.49, −0.07 mmol/mol). In CPRD, the SD in HbA1c had a similar association with baseline HbA1c but was larger; (SD = 4.5 mmol/mol for baseline HbA1c <48 mmol, 5.4 mmol/mol for 48–64 mmol/mol, 7.9 mmol/mol for 64–86 mmol/mol, and 11.4 mmol/mol for >86 mmol/mol). Increases in SD were seen with younger age (0.42; 95%CI 0.39, 0.47 mmol/mol per decade), male gender (0.36; 95%CI 0.28, 0.46 mmol/mol), and Black race (0.63; 95%CI 0.38, 0.88 mmol/mol). There no association with BMI or Asian race relative to White. The lowest SD by drug class was with SGLT2 inhibitors (−1.71; 95%CI −1.98, −1.42 mmol/mol) and the highest with sulphonylureas (1.11; 95%CI 1.02, 1.21 mmol/mol) relative to metformin.

Conclusion: The amount of variability in HbA1c on stable therapy is large, particularly in patients with poor glycaemic control where it is similar to the HbA1c reductions expected from oral therapies. Therefore, a single post treatment HbA1c measure is unlikely to be sufficient to determine the effectiveness of a treatment, or need for therapy intensification for an individual. The high level of HbA1c variability was not substantially modified by any clinical characteristic.

Supported by: NIHR

Disclosure: **A. McGovern:** None.

711

Impact of proximal intestinal exclusion with EndoBarrier on key metabolic parameters and cardiovascular risk (UKPDS risk engine) in the first NHS-UK EndoBarrier service

E.N. Fogden[1], P. Sen Gupta[2], M. Yadagiri[2], S.P. Irwin[2], W. Burbridge[2], T. Bashir[3], R. Allden[1], J.P. Bleasdale[4], M.R. Anderson[1], R.E.J. Ryder[2]; [1]Gastroenterology, City Hospital, Birmingham, [2]Diabetes, City Hospital, Birmingham, [3]Dietetics, City Hospital, Birmingham, [4]Anaesthetics, City Hospital, Birmingham, UK.

Background and aims: Background and aims: The leading cause of death in patients with type 2 diabetes is cardiovascular disease (CVD). The United Kingdom Prospective Diabetes Study (UKPDS) CVD risk engine version 2.0 uses recognised risk factors to calculate future CVD risk. Our aim was to investigate the impact of proximal intestinal exclusion using EndoBarrier, a device implantable for up to 1 year, on 10 year CVD risk.

Materials and methods: We report the first 46 patients who have had their devices removed after (mean ± SD) 11.5 ± 2.2 months, of 62 who have so far received devices in our NHS service (2015–2018). We measured all factors utilised by the risk engine: age, duration of diabetes, sex, atrial fibrillation, ethnicity, smoking, systolic blood pressure, HbA1c, total cholesterol and HDL cholesterol.

Results: The table shows baseline characteristics of the patients and the impact on metabolic and CVD risk factors of EndoBarrier treatment. There were highly significant falls in all parameters involved in CVD risk assessment other than HDL cholesterol which remained unchanged. The UKPDS risk engine mean ± SD 10 year coronary heart disease (CHD) risk fell by 7.3 ± 8.3% from 17.1 ± 12.6% to 9.8 ± 6.9% ($p < 0.001$). 10 year fatal CHD risk fell by 6.1 ± 7.2% from 12.5 ± 10.6% to 6.3 ± 5.4% ($p < 0.001$). 10 year stroke risk fell by 0.94 ± 1.35% from 5.92 ± 4.27% to 4.98 ± 3.45% ($p < 0.001$). 10 year fatal stroke risk fell by 0.27 ± 0.41% from 0.93 ± 0.78% to 0.66 ± 0.49% ($p < 0.001$). Additionally, weight, which is not a factor utilised in the UKPDS risk engine, fell by 15.7 ± 8.8 kg from 124.0 ± 30.1 to 108.3 ± 31.3 kg ($p < 0.001$), and BMI by 5.6 ± 3.3 kg/m^2 from 41.9 ± 8.2 to 35.3 ± 8.5 kg/m^2 ($p < 0.001$); serum alanine-aminotransferase (marker of hepatic steatosis) fell by 13.6 ± 19.7 U/L from 32.5 ± 20.1 to 18.6 ± 10.5 U/L ($p < 0.001$). Median (IQR) total daily insulin dose reduced from 104 (60–140) to 40 (0–80) units ($p < 0.001$), $n = 27$. 7/27 (26%) insulin treated patients discontinued insulin.

Conclusion: In addition to reducing the requirement for insulin and a liver fat biomarker, EndoBarrier treatment reduced 10-year CVD risk by clinically useful amounts in patients with poorly controlled diabetes and obesity. These data suggest that EndoBarrier treatment in 100 patients could prevent between 8 and 9 events of CHD or stroke and save between 6 and 7 lives over the next 10 years, if effects were maintained. The results are likely to be an underestimate of the true reductions as the UKPDS risk engine does not take into account weight or BMI which were significantly impacted by EndoBarrier treatment.

Parameter	Baseline	At Removal	Difference	P value (n=46)
Age (years)	51.2±6.9			
Sex (%male)	60.9			
Ethnicity: % White % Afro-Caribbean % Asian-Indian	50 21.7 28.3			
Diabetes Duration (Median (IQR) years)	13.7 (8.0-20.0)			
Smoking: % Never Smoked % Past Smoker % Current Smoker	54.3 28.3 17.4			
Taking insulin (%)	59			
Weight (kg)	124.0±30.1	108.3±31.3	-15.7±8.8	<0.001
BMI (kg/m^2)	41.9±8.2	35.3±8.5	-5.6±3.3	<0.001
HbA1c (mmol/mol)	84.3±23.0	58.0±13.1	-26.3±23.0	<0.001
HbA1c (%)	9.9±2.1	7.5±1.2	-2.4±2.1	<0.001
Systolic Blood Pressure (mm Hg)	138.4±15.6	125.4±15.1	-13.0±16.9	<0.001
Serum total cholesterol (mmol/L)	4.87±1.45	3.94±0.89	0.94±1.23	<0.001
Serum HDL cholesterol (mmol/L)	1.11±0.28	1.11±0.32	0.00±0.21	0.90
Serum alanine aminotransferase (U/L)	32.5±20.1	18.6±10.5	-13.6±19.7	<0.001
Total Daily Insulin Dose* [median(IQR), n=27]	104(60-140)	40(0-80)	-64	<0.001

Table: Baseline characteristics of the 46 patients who completed treatment with Endobarrier along with outcomes at the time of removal as mean±SD or median(interquartile range [IQR]). P-values reflect change from baseline. Removal at 1 year in 43/46 (94%) patients with early removal due to complications in 3/46 patients (2 x GI bleed, 1x hepatic abscess). *7/27 (26%) patients discontinued insulin.

Disclosure: **E.N. Fogden:** None.

PS 056 Metabolic effects of novel, dual and triple incretin agonists

712

Preclinical effects of efpeglenatide, a long-acting glucagon-like peptide-1 receptor agonist, compared with liraglutide and dulaglutide

M. Trautmann[1], I. Choi[2], J. Kim[2], C. Sorli[3];
[1]ProSciento, Inc., Chula Vista, USA, [2]Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea, [3]Sanofi, Bridgewater, USA.

Background and aims: Efpeglenatide is a long-acting glucagon-like peptide-1 receptor agonist (GLP-1 RA) in development for the treatment of type 2 diabetes.
Materials and methods: The effects of efpeglenatide vs those of liraglutide and dulaglutide on glucodynamics and weight/lipid profiles were studied over 4 weeks in mouse models of diabetes (db/db) and obesity (diet-induced obesity [DIO]), respectively; doses tested were efpeglenatide 1.45, 2.89, or 4.35 nmol/kg once every 2 days (Q2D); twice-daily liraglutide 30 nmol/kg (db/db study) or 50 nmol/kg (DIO study); and dulaglutide 0.98 or 1.96 nmol/kg Q2D (human equivalent doses/key results in Table).
Results: At the highest doses tested, efpeglenatide was significantly more effective at lowering blood glucose (vs liraglutide and dulaglutide) and reducing the increase in HbA_{1c} from Day 0 (vs liraglutide) in db/db mice. Efpeglenatide also improved insulin sensitivity (vs liraglutide and dulaglutide) and postprandial glucose control (vs dulaglutide). In DIO mice, efpeglenatide significantly reduced body weight and mesenteric fat mass (vs dulaglutide), and cholesterol (vs liraglutide and dulaglutide; at highest doses).
Conclusion: Overall, efpeglenatide showed greater reductions in blood glucose, HbA_{1c} increase, weight, and cholesterol vs other GLP-1 RAs in mice. These effects may be due to the distinct receptor-binding properties of efpeglenatide, which enhance intracellular signalling and insulin release in β-cells. Further studies are needed to assess the clinical relevance of these findings.

Table. Summary of the effects of GLP-1 RAs on glycaemic, weight, and lipid endpoints in db/db and DIO mice

	Vehicle	Efpeg 4.35 nmol/kg, Q2D (HED: 6.0 mg/wk)	Lira 30 nmol/kg, BID (HED: 1.8 mg/day)	Dula 1.96 nmol/kg, Q2D (HED: 3.0 mg/wk)	Comparison between efpeg vs GLP-1 RA
Glycaemic effects in db/db mice over 28 days					
Non-fasting blood glucose AUC (mg/dL * days)[a]	12254.2	3678.8***	7370.5***	5799.0***	p<0.001 vs lira; p<0.05 vs dula
Increment in HbA_{1c} (%) from baseline[b]	4.03	0.12***	1.47***	1.12***	p<0.01 vs lira
ipITT AUC (% mg/dL vs 0 hr * hrs)[c]	144.3	84.9***	121.7	115.4**	p<0.001 vs lira; p<0.01vs dula
ipGTT AUC (Δ mg/dL vs 0 hr * hrs)[d]	897.1	307.2***	468.1***	521.4***	p<0.05 vs dula
	Vehicle	**Efpeg 4.35 nmol/kg, Q2D (HED: 6.0 mg/wk)**	**Lira 50 nmol/kg, BID (HED: 3.0 mg/day)**	**Dula 1.96 nmol/kg, Q2D (HED: 3.0 mg/wk)**	**Comparison between efpeg vs GLP-1 RA**
Weight and lipid effects in DIO mice over 28 days					
Body weight change (% vs baseline)	3.42	−22.18***	−18.55***	−7.15**	p<0.001 vs dula
Mesenteric fat mass at Day 28 (g)	0.88	0.44***	0.50***	0.67*	p<0.05 vs dula
Total cholesterol (mg/dL)	310	167***	206***	243***	p<0.05 vs lira; p<0.001 vs dula

P-value vs vehicle: *p<0.05, **p<0.01, and ***p<0.001
[a]Non-fasting blood glucose (mg/dL) was monitored every day for the first week and then twice weekly; AUC of blood glucose was calculated (mg/dL * days)
[b]HbA_{1c} was measured at Day 0 and again at Day 28; the increment of HbA_{1c} (Δ %) was obtained by subtracting the value at Day 0 (baseline) from the value at Day 28
[c]For ipITT, the % change of blood glucose from 0 hr (immediately prior to the ipITT) was calculated at several time points (0.5, 1, 2, and 4 hrs) and used to calculate the AUC of % change of blood glucose from 0 hr
[d]For ipGTT, change in blood glucose from 0 hr (just before glucose loading) was calculated at several time points (0.25, 0.5, 1, and 2 hrs) and used to calculate the AUC of change in blood glucose from 0 hr. Both ipITT and ipGTT were conducted at study end
AUC=area under the curve; BID=twice daily; dula=dulaglutide; efpeg=efpeglenatide; HED=human equivalent dose; hr=hour; ipGTT=intraperitoneal glucose tolerance test; ipITT=intraperitoneal insulin tolerance test; lira=liraglutide, wk=week

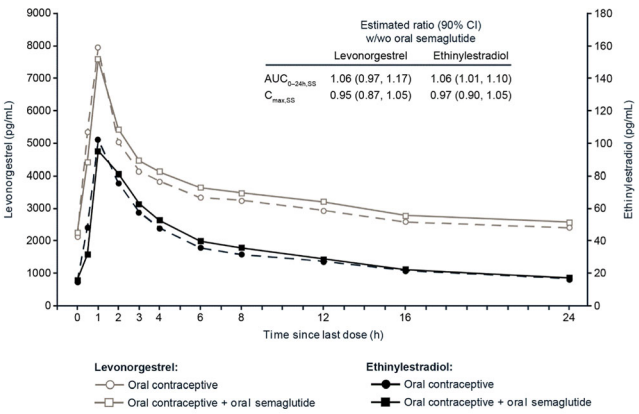
Supported by: Hanmi
Disclosure: **M. Trautmann:** Employment/Consultancy; Prosciento Inc, Hanmi, Cequr Inc, Kinexum, Intarcia, Servier. Stock/Shareholding; Eli Lilly.

713

Oral semaglutide does not affect the bioavailability of the combined oral contraceptive, ethinylestradiol/levonorgestrel

A.B. Jordy[1], A. Breitschaft[2], E. Christiansen[1], C. Granhall[1], C.W. Hansen[1], A. Houshmand-Øregaard[1], T.A. Bækdal[1];
[1]Novo Nordisk A/S, Søborg, Denmark, [2]Parexel International GmbH, Berlin, Germany.

Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue co-formulated with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), to allow oral administration. The effect of oral semaglutide on the pharmacokinetics of the combined oral contraceptive ethinylestradiol (0.03 mg)/levonorgestrel (0.15 mg) was assessed in an open-label, one sequence crossover trial.
Materials and methods: Healthy post-menopausal females ($n = 25$) received 8 days of oral contraceptive alone and 8 days of oral contraceptive with oral semaglutide (dose escalated to steady state at week 6: 1 week at 3 mg dose, 1 week at 7 mg dose, 4 weeks at 14 mg dose). Primary endpoints were total exposure to ethinylestradiol and levonorgestrel as measured by the areas under the plasma concentration-time curve during a dosing interval (0–24 h) at steady state ($AUC_{0-24h,SS}$). Secondary endpoints included other pharmacokinetic parameters, safety and tolerability.
Results: $AUC_{0-24h,SS}$ and maximum plasma exposure ($C_{max,SS}$) to ethinylestradiol and levonorgestrel appeared similar for oral contraceptive alone vs. oral contraceptive with oral semaglutide, and $AUC_{0-24h,SS}$ and $C_{max,SS}$ ratios were within the predefined no effect interval (0.8–1.25) (Figure). Adverse events with oral semaglutide at applied doses were consistent with previous trials and expected GLP-1 receptor agonist class effects.
Conclusion: These data indicate that oral semaglutide does not affect the bioavailability of the combined oral contraceptive ethinylestradiol and levonorgestrel.



For both $AUC_{0-24h,SS}$ and $C_{max,SS}$: no effect criterion is met if 90% CI is within the pre-defined interval 0.80–1.25.

Clinical Trial Registration Number: NCT02845219
Supported by: Novo Nordisk A/S
Disclosure: **A.B. Jordy:** Employment/Consultancy; Novo Nordisk A/S.

714

A trial to investigate the effect of oral semaglutide on the pharmacokinetics of furosemide and rosuvastatin in healthy subjects

T.A. Bækdal[1], M. Albayaty[2], E. Manigandan[3], T.W. Anderson[1], S. Skibsted[1];
[1]Novo Nordisk A/S, Søborg, Denmark, [2]Parexel, London, UK, [3]Novo Nordisk, Bangalore, India.

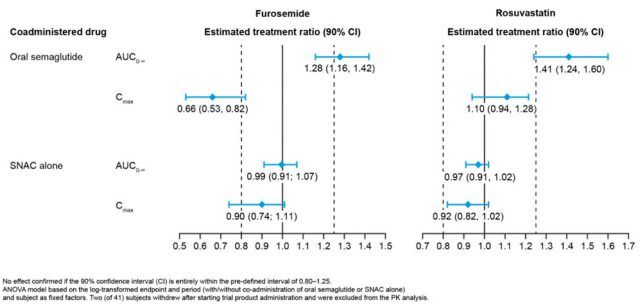
Background and aims: Oral semaglutide is a human glucagon-like peptide-1 (GLP-1) analogue co-formulated with an absorption enhancer,

sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), for oral administration. *In vitro* assessments have indicated that SNAC may inhibit drug transporters (OAT1/3, BCRP, OATP1B1), potentially leading to increased plasma levels of transporter substrates such as furosemide (OAT1/3 substrate) or rosuvastatin (BCRP/OATP1B1 substrate). This open-label, one-sequence crossover trial investigated the effect of oral semaglutide and SNAC alone on the pharmacokinetics of furosemide and rosuvastatin.

Materials and methods: The trial consisted of three treatment periods during which 41 healthy subjects (39 of whom completed the trial) received single doses of furosemide 40 mg and rosuvastatin 20 mg alone, co-administered with SNAC 300 mg, and co-administered with oral semaglutide (dose escalated to steady state at week 6: 1 week at 3 mg dose, 1 week at 7 mg dose, 4 weeks at 14 mg dose). Primary endpoints were area under the furosemide or rosuvastatin plasma concentration-time curves from zero to infinity ($AUC_{0-\infty}$) after single doses. It was prespecified to conclude no effect of oral semaglutide or SNAC alone on exposure of furosemide or rosuvastatin if the 90% confidence interval (CI) for the treatment ratio (with/without oral semaglutide or SNAC alone) was entirely within the pre-defined interval (0.80–1.25). Secondary endpoints were other pharmacokinetic parameters, safety and tolerability.

Results: Co-administration of steady-state oral semaglutide with single-dose furosemide resulted in a 28% increase in total furosemide exposure ($AUC_{0-\infty}$) and a 34% decrease in maximum furosemide concentration (C_{max}). The no effect criterion was not met for either AUC or C_{max} (Figure). When co-administered with SNAC alone, there was no effect on the $AUC_{0-\infty}$ of single-dose furosemide while C_{max} decreased by 10%. Co-administration of steady-state oral semaglutide with single-dose rosuvastatin resulted in increases in both $AUC_{0-\infty}$ (41%) and C_{max} (10%) and the no effect criterion was not met for either endpoint. $AUC_{0-\infty}$ and C_{max} of rosuvastatin were not affected by co-administration of SNAC alone. The safety profile was as expected for the GLP-1 receptor agonist class and consistent with previous trials of oral semaglutide.

Conclusion: These data indicate that the SNAC component of oral semaglutide does not inhibit the transporters OAT1/3, BCRP or OATP1B1. Changes in exposure of furosemide and rosuvastatin when co-administered with oral semaglutide may be related to the known gastric emptying delaying effect of the GLP-1 component, which may influence both the rate and the extent of absorption of co-administered drugs. The observed changes in furosemide and rosuvastatin exposure are not expected to be clinically relevant.



No effect confirmed if the 90% confidence interval (CI) is entirely within the pre-defined interval of 0.80–1.25.
ANOVA model based on the log-transformed endpoint and period (with/without co-administration of oral semaglutide or SNAC alone) and subject as fixed factors. Two (of 41) subjects withdrew after starting trial product administration and were excluded from the PK analysis.

Clinical Trial Registration Number: NCT03010475
Supported by: Novo Nordisk A/S
Disclosure: **T.A. Bækdal:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

715
WITHDRAWN

716
Investigations into tissue distribution and inhibition of food consumption with efpeglenatide

Y. Park[1], I. Choi[1], M. Trautmann[2], M. Hompesch[2], C. Sorli[3];
[1]Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea, [2]ProSciento, Inc., Chula Vista, USA, [3]Sanofi, Bridgewater, USA.

Background and aims: Efpeglenatide, a long-acting glucagon-like peptide-1 receptor agonist in development for type 2 diabetes, induces weight loss through satiety and reduced food intake. Studies in Sprague Dawley rats investigated how peripheral and central mechanisms contribute to the anorectic effects of efpeglenatide.

Materials and methods: Tissue distribution of efpeglenatide was assessed by whole-body autoradioluminography with ^{14}C-efpeglenatide. The contribution of vagal-nerve signalling to food-intake control was assessed in vagotomized rats treated with exenatide (0.15 or 0.45 nmol/kg twice daily) or efpeglenatide (0.71 or 2.1 nmol/kg every 2 days).

Results: Pharmacokinetic parameters of ^{14}C-efpeglenatide in selected organs/tissues are shown in the Table. Peak concentration (C_{max}) of ^{14}C-efpeglenatide was highest in the kidney cortex; C_{max} at all other sites was similar to/lower than in blood, relatively high in the intestine and pituitary, with below-quantifiable levels in the cerebellum/cerebrum. ^{14}C-efpeglenatide levels were highest in the kidney cortex at all time points (3, 24, 48, 144, 312, 480, 648, 888, and 1128 h). In blood, intestinal content, liver, lung, myocardium, pancreas, pituitary, and spleen, ^{14}C-efpeglenatide levels were below the limit of quantification or were not detected by 480 h. At all time points assessed, radioactivity levels in the cerebellum and cerebrum were below the limit of detection. Inhibition of food intake was not attenuated in vagotomized vs sham-operated rats at higher doses of efpeglenatide (3-day cumulative intake: 47.4 vs 49.4 g) or exenatide (54.9 vs 60.7 g), suggesting a non-vagal mechanism for these anorectic effects.

Conclusion: These findings suggest that central mechanisms are involved in the anorectic effects of efpeglenatide in this animal model; potential cell-signalling effects of efpeglenatide in the intestine and pituitary need to be investigated further.

Table. Pharmacokinetic parameters in select organs and tissues following single subcutaneous administration of radiolabelled efpeglenatide at a target dose level of 24 nmol/kg in male Sprague Dawley rats

Tissue	T_{max} (h)	C_{max} (μmol/L)	AUC_{last} (h·μmol/L)
Kidney cortex	144	1.24683	391.77027
Blood	24	0.0586	5.83519
Liver	48	0.01456	1.3384
Intestinal content	48	0.05296	NA
Lung	48	0.06625	13.54497
Myocardium	48	0.01911	1.81044
Pancreas	48	0.01174	1.22135
Pituitary	48	0.02291	2.26935
Spleen	48	0.01805	3.67717
Cerebellum	NA	NA	NA
Cerebrum	NA	NA	NA

AUC_{last}=area under serum concentration-time curve from time zero to the last detectable concentration; C_{max}=maximal observed serum concentration; NA=not applicable; T_{max}=time of the maximal observed concentration

Supported by: Hanmi
Disclosure: **Y. Park:** Employment/Consultancy; Hanmi Pharm Co. Stock/Shareholding; Hanmi Pharm Co.

717

Mono- and co-activation of the GIP and GLP-1 receptors inhibits bone resorption

N.C. Bergmann[1,2], A. Lund[1,3], S.M. Heimbürger[1], L.S. Gasbjerg[1,4], N.R. Jørgensen[5,6], L. Jessen[2], B. Hartmann[4], J.J. Holst[4], M.B. Christensen[1,7], T. Vilsbøll[1,8], F.K. Knop[1,8];
[1]Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, University of Copenhagen, Hellerup, [2]Zealand Pharma A/S, Glostrup, [3]Department of Medicine, Gentofte Hospital, Hellerup, [4]Department of Biomedical Sciences and Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, [5]Department of Clinical Chemistry, Rigshospitalet, University of Copenhagen, Glostrup, [6]OPEN, Odense Patient data Explorative Network, Odense University Hospital, Odense, [7]Department of Clinical Pharmacology, Bispebjerg Hospital, Copenhagen, [8]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Background and aims: This study investigated the effect of mono- and co-administration of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) on bone resorption and formation.
Materials and methods: Seventeen overweight/obese men without diabetes were included in a randomised, double-blinded, placebo-controlled, cross-over study and subjected to a 50 g OGTT and four isoglycaemic intravenous glucose infusions (IIGI), mimicking the glucose excursions from the OGTT, with concomitant infusions of GIP (4 pmol/kg/min), GLP-1 (1 pmol/kg/min), GIP+GLP-1 (4 and 1 pmol/kg/min, respectively) or placebo. Bone resorption and formation were assessed by measurements of carboxy-terminal type I collagen crosslinks (CTX), and procollagen type 1 N-terminal propeptide (P1NP), respectively.
Results: Suppression of CTX is shown in Fig. 1. During OGTT the baseline-subtracted AUC (bsAUC) was significantly lower than during IIGI+saline infusion (−9,117 ± 566 (mean ± SEM) vs −4333 ± 583% × min, $p < 0.0001$). Infusion of GLP-1 and GIP significantly potentiated CTX suppression to −10,751 ± 799% × min and −13,714 ± 440% × min, respectively. Co-administration of GIP+GLP-1 suppressed CTX to −15,466 ± 268% × min. P1NP levels were unaffected.
Conclusion: Our data suggest that GLP-1, like GIP, may be involved in bone homeostasis and that co-administration of GIP+GLP-1 inhibits bone resorption more than each hormone alone.

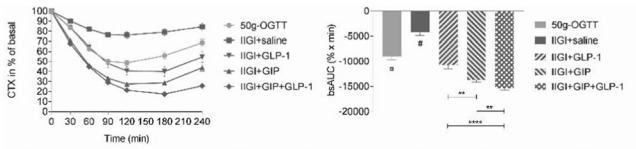


Figure 1. A) Carboxy-terminal type I collagen crosslinks (CTX) values in % of basal during 50g OGTT and four isoglycaemic intravenous glucose infusions (IIGI) with co-infusion of saline, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and GIP+GLP-1, respectively. B) baseline-subtracted AUC values. Data are means±SEM, n=17. ¤The 50g OGTT differed from the other study days, $p<0.05$. # The IIGI−saline days differed from the other study days, $p<0.0001$. **$p<0.005$, ****$p<0.0001$

Clinical Trial Registration Number: NCT02598791
Supported by: Innovation Fund Denmark, Zealand Pharma A/S and the Vissing Foundation
Disclosure: **N.C. Bergmann:** Employment/Consultancy; Zealand Pharma A/S. Grants; The Innovation Fund Denmark, The Vissing Foundation.

718

MEDI0382, a glucagon-like peptide/glucagon receptor dual agonist, in patients with type 2 diabetes: a multiple-ascending-dose study

D. Robertson[1], M. Stumvoll[2], M. Posch[3], T. Heise[4], L. Plum-Moerschel[5], G. Klein[6], L.-F. Tsai[7], M. Petrone[1], B. Hirshberg[7], C. Rondinone[7], V. Parker[1], L. Jermutus[1], P. Ambery[1];
[1]MedImmune, Cambridge, UK, [2]University Hospital Leipzig, Leipzig, Germany, [3]Charité Research Organisation GmbH, Berlin, Germany, [4]Profil, Neuss, Germany, [5]Profil, Mainz, Germany, [6]APEX GmbH, Munich, Germany, [7]MedImmune, Gaithersburg, USA.

Background and aims: MEDI0382 is a glucagon-like peptide 1 (GLP-1)/glucagon receptor dual agonist under development for the treatment of type 2 diabetes mellitus. and nonalcoholic steatohepatitis. This double-blind study evaluated MEDI0382 in patients with type 2 diabetes and a body mass index of 27–40 kg/m^2.
Materials and methods: Subjects ($n = 61$) were randomized to once-daily subcutaneous MEDI0382 or placebo at different dosing levels and uptitration schedules (100 μg [cohort 1], 150 μg [cohort 2], 200 μg [cohort 3], and 300 μg [2 uptitration schedules, cohorts 4 and 5]).
Results: Marked reduction in fasting and postprandial glucose was achieved with $a \approx 40\%$ decrease in glucose $AUC_{0\text{-}4h}$ after mixed-meal testing across all cohorts ($P \leq 0.0102$) (Table). Weight loss was observed at all dose levels and reached statistical significance ($P = 0.024$) at 300 μg (cohort 4) (Table). Ambulatory systolic blood pressure decreased nonsignificantly from baseline in the 300-μg cohort (cohort 4, −4.9 vs −6.5 mmHg; cohort 5, −9.1 vs −5.0 mmHg). Similar to other marketed GLP-1 analogs, MEDI0382 increased pulse rate vs placebo (cohort 4, 8.4 vs −2.4 bpm, $P = 0.0039$; cohort 5, 5.8 vs 0.2 bpm, $P = 0.0796$). Treatment-related adverse events occurred more often with MEDI0382 than with placebo (86% [36/42] vs 47% [9/19]); most events were mild or moderate in severity. Nausea and vomiting occurred more frequently with MEDI0382 vs placebo; however, their occurrence was not dose related.
Conclusion: In conclusion, MEDI0382 administered for up to 22 days showed a marked reduction in both fasting and postprandial glucose with weight reduction and an acceptable tolerability profile.

Table. Change from baseline in efficacy endpoints in subjects treated with MEDI0382 vs placebo

Cohort (days on treatment)	Glucose $AUC_{0\text{-}4h}$ post-MMTT (%)[a]	Body weight (kg)[b]	Fasting glucose (mg/dL)[a]
1 (8)	−41.9 vs −14.3 ($P = 0.0102$)	−2.1 vs −1.7 ($P = 0.6549$)	−73.6 vs −34.2 ($P = 0.0019$)
2 (12)	−37.1 vs −14.3 ($P = 0.0026$)	−1.5 vs −1.0 ($P = 0.4286$)	−60.5 vs −37.1 ($P = 0.0316$)
3 (16)	−37.0 vs −5.6 ($P = 0.0003$)	−4.5 vs −3.3 ($P = 0.3251$)	−54.2 vs −23.1 ($P = 0.0044$)
4 (22)	−41.7 vs −14.5 ($P = 0.0001$)	−3.4 vs −0.7 ($P = 0.0240$)	−53.6 vs −33.8 ($P = 0.0694$)
5 (17)	−36.7 vs −8.0 ($P < 0.0001$)	−2.1 vs −0.9 ($P = 0.3596$)	−52.0 vs −23.5 ($P = 0.0216$)

[a]LS mean (cohorts 1–3: MEDI0382 [$n = 6$], placebo [$n = 3$]; cohort 4: MEDI0382 [$n = 10$], placebo [$n = 5$]; cohort 5: MEDI0382 [$n = 11$], placebo [$n = 5$]).
[b]LS mean (cohorts 1 and 2: MEDI0382 [$n = 6$], placebo [$n = 3$]; cohort 3: MEDI0382 [$n = 7$], placebo [$n = 3$]; cohorts 4 and 5: MEDI0382 [$n = 11$], placebo [$n = 5$]).
AUC = area under the curve; LS = least squares; MMTT = mixed-meal tolerance test.

Clinical Trial Registration Number: NCT02548585
Disclosure: **D. Robertson:** Employment/Consultancy; MedImmune. Stock/Shareholding; AstraZeneca.

719

A novel combination of a long-acting GLP-1/GIP/Glucagon triple agonist and once weekly basal insulin offers improved glucose lowering and weight loss in diabetic animal model

J. Lee, J. Kim, J. Choi, S. Jung, S. Lee, I. Choi, S. Kim;
Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea.

Background and aims: Obesity is an established risk factor for the aggravation of pre-existing diabetes by negatively affecting lipid metabolism, insulin response, and eventually β-cell function. In this respect,

effective body weight loss (BWL) not only prevents or delays the onset of T2DM, but also enhances the response to conventional diabetes drugs including basal insulin in T2DM patients. Recently, we developed a novel long-acting GLP-1/GIP/Glucagon triple agonist, HM15211, which showed potent BWL in various obese animal models. Thus, we hypothesized that when combined with basal insulin, HM15211 could maximize the exogenous insulin response by providing potent BWL and following insulin sensitivity improvement. Here, we investigated the therapeutic potential of HM15211 and long-acting basal insulin combination for T2DM treatment by evaluating 1) drug-to-drug interaction (DDI) *in vitro* and *in vivo*, and 2) glycemic and BW control efficacy in diabetic animal models.

Materials and methods: *In vitro* human insulin receptor (hIR) phosphorylation potency by long-acting basal insulin (HM12460A) or its analog (HM12480) was evaluated in CHO cell stably expressing hIR in the presence or absence of HM15211. Similarly, cAMP accumulation potency by HM15211 was evaluated in CHO cells stably expressing respective receptors (human GLP-1R, GCGR, or GIPR) in the presence or absence of insulin counter partners. For PK assessment, HM15211 and/or HM12460A (or HM12480) were subcutaneously administered in SD rats. Blood samples were collected at indicated time points, and their blood concentration were determined using in-house established ELISA. To evaluate the *in vivo* efficacy, *db/db* mice and DIO/STZ rats were chronically administered with HM15211 and/or HM12460A (or HM12480), and blood glucose and BW were monitored. At the end of the treatment, HbA1c levels were measured to determine overall glycemic control efficacy.

Results: Firstly, hIR phosphorylation potency of either HM12460A or HM12480 was not affected by concomitant treatment of HM15211 (EC_{50} = 249.4 *vs.* 203.2 nM for HM12460A mono *vs.* COMBO, EC_{50} = 433.8 *vs.* 491.7 nM for HM12480 mono *vs.* COMBO). Similar results were also observed when cAMP accumulation potency of HM15211 was determined (EC_{50} = 0.027 *vs.* 0.030, and 0.030 nM for HM15211 mono *vs.* HM12460A COMBO, and HM12480 COMBO, for hGCGR/CHO cell activation). Secondly, both HM15211, HM12460A, and HM12480 showed favorable PK profiles as indicated by prolonged half-lives, and did not affect counter partners' PK properties in SD rats, demonstrating no DDI *in vivo*. Of note, compared to HM12460A, HM12480 showed more harmonized PK profiles with HM15211. Lastly, HM15211 combination either with HM12460A or HM12480 showed enhanced blood glucose lowering and more HbA1c reduction, compared to insulin mono treatment both in *db/db* mice and DIO/STZ rats. Most interestingly, HM15211 combination efficiently reduced BW, not just BW gain neutralization observed in insulin/GLP-1 combination, demonstrating their therapeutic benefits in BW control as well as glycemic control.

Conclusion: Based on these observations, we propose that HM15211 may be a novel combination partner of our long-acting basal insulin such as HM12460A and HM12480 by providing more favorable BW control as well as improved glycemic control in obese and T2DM patients.

Disclosure: **J. Lee:** None.

PS 057 Lipids and fatty liver: What GLP1 receptor agonists can do

720

The effect of liraglutide treatment on postprandial remnant particles, apoCIII, liver fat and de novo lipogenesis in adequately controlled type 2 diabetes

N. Matikainen[1,2], S. Söderlund[2], K.H. Pietiläinen[2], E. Björnson[3], A. Hakkarainen[4], N. Lundbom[4], J. Borén[3], M.-R. Taskinen[2];
[1]Endocrinology, Helsinki University Hospital, Helsinki, Finland, [2]Research Programs Unit, Diabetes and Obesity, Helsinki, Finland, [3]Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden, [4]HUS Medical Imaging Center, Radiology, Helsinki, Finland.

Background and aims: Subjects with type 2 diabetes remain in high cardiovascular disease (CVD) risk despite of multifactorial interventions targeted against hyperglycemia, hyperlipidemia and hypertension. Incretin-based therapy with liraglutide reduces CVD risk by partly unexplained mechanisms. Previous data suggest that reversal of the incretin effect alleviates postprandial lipemia and may reduce liver fat. Here, we tested the combined effect of liraglutide on ectopic fat depots and postprandial lipid metabolism. To elucidate whether these metabolic effects are driven by weight reduction we aimed at similar weight loss in the liraglutide and placebo groups.

Materials and methods: We examined the effect of liraglutide on background of simvastatin and metformin therapy on 22 subjects with adequately controlled type 2 diabetes with mean HBA1c of 7% and 6.3% at baseline. Subjects were randomly allocated in 2:1 ratio to single-blinded liraglutide 1.8 mg o.d. or placebo treatments for 16 weeks. Subjects in placebo group received dietary counselling in order to lose 3 kg of body weight. Hepatic, intra-abdominal and subcutaneous fat depots, de novo lipogenesis (DNL), fat oxidation, postprandial lipids, apoproteins, glucose and insulin during a high-fat mixed meal were measured before and at the end of the treatment period.

Results: Weight loss at week 16 was similar between the two groups; −2.4 kg (−2.5%) in the liraglutide group and −2.1 kg (−2.2%) in placebo group. HBA1c improved by 0.6% in the liraglutide group (p = 0.006). Liver fat was reduced by 31% in liraglutide group and by 18% in the placebo group. Although liver fat decreased in both groups, no changes occurred in DNL or β-hydroxybutyrate level (a surrogate marker of fat oxidation). Despite similar weight loss, we found significant postprandial decreases of plasma, chylomicron and VLDL triglycerides and postprandial remnant particle cholesterol concentrations at week 16 in the liraglutide group. Both fasting and postprandial apoCIII concentrations were decreased at week 16 in the liraglutide group and these changes were closely related to improvement in glycemia. In relative importance analysis, less than 10% of changes in postprandial lipids were explained by improvements in weight, glycaemic control, insulin AUC or liver fat. Instead, reductions in apoCIII concentrations explained about 50% of the variation in these variables.

Conclusion: We report that treatment with liraglutide for 16 weeks produces multiple improvements in cardiometabolic risk factors not seen in the placebo group with similar weight loss. Importantly, liraglutide treatment resulted in a marked reduction of postprandial atherogenic remnant particles. The underlying mechanism is consistent with the hypothesis that improvement of glycaemic control leads to reduced expression of apoCIII, a key regulator of triglyceride metabolism in type 2 diabetes subjects.

Clinical Trial Registration Number: NCT02765399
Supported by: Novo Nordisk, Juselius Foundation, EU Seventh Framework Program

Disclosure: **N. Matikainen:** None.

721

Effect of the exenatide plus dapagliflozin combination on fatty liver index and insulin resistance in type 2 diabetes patients: the DURATION-8 trial

C. Guja[1], E. Repetto[2], J. Han[3], E. Hardy[2], S.A. Jabbour[4];
[1]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [2]AstraZeneca, Gaithersburg, USA, [3]Pharmapace Inc., San Diego, USA, [4]Thomas Jefferson University, Philadelphia, USA.

Background and aims: Type 2 diabetes mellitus (T2DM) is often associated with obesity and the risk of developing hepatic steatosis. Exenatide once weekly (ExQW) and dapagliflozin (DAPA) reduce glycaemia and body weight in T2DM patients. This analysis explored the effects of ExQW+DAPA vs ExQW+placebo (PBO) or DAPA+PBO on weight, fatty liver index (FLI) and insulin resistance in T2DM patients inadequately controlled by metformin.
Materials and methods: DURATION-8 was a multicentre, double-blind, randomized, phase 3 trial. Adults with T2DM and inadequate glycaemic control (baseline HbA_{1c} 8%–12%) despite stable metformin monotherapy were randomly assigned to receive ExQW 2 mg plus once daily oral DAPA 10 mg, ExQW with DAPA matched oral PBO or DAPA with ExQW matched PBO injections for 28 weeks. Patients entered a 24-week extension period where they continued to receive randomised active treatment. Changes in body weight, FLI (as determined by an algorithm based on body mass index, waist circumference, triglycerides and gamma-glutamyltransferase) and insulin resistance (homeostasis model assessment-insulin resistance [HOMA-IR]) from baseline to weeks 28 and 52 were studied and within-treatment and between-group comparisons were performed using analysis of covariance (ANCOVA). Correlations between FLI and HOMA-IR were evaluated.
Results: In total, 231, 230 and 233 patients received ExQW+DAPA, ExQW+PBO and DAPA+PBO, respectively. Baseline characteristics were similar across treatment groups (Table). ExQW+DAPA was significantly superior to either drug alone in achieving weight loss at 28 and 52 weeks ($P < 0.001$). Significant improvement in FLI was seen with ExQW+DAPA at week 28 (least square [LS {SEM}]: −6.81 [0.87], $P < 0.0001$) and was maintained at week 52 (LS [SEM]: −6.23 [1.02], $P < 0.0001$). HOMA-IR significantly improved in the ExQW+DAPA group at week 28 (geometric LS mean ratio to baseline [95% CI]: 0.81 [0.75–0.88], $P < 0.0001$) and week 52 (LS mean ratio to baseline [95%CI]: 0.80 [0.74–0.87], $P < 0.0001$). Changes in FLI (from baseline) correlated with HOMA-IR (ratio of week 28 or week 52 values to the baseline value) in all treatment groups (week 28: $r = 0.37$, 0.09 and 0.23; week 52: $r = 0.12$, 0.30 and 0.27 for ExQW+DAPA, ExQW+PBO and DAPA+PBO, respectively).
Conclusion: Combination of ExQW and DAPA has demonstrated efficacy in reducing body weight and other metabolic parameters in T2DM patients inadequately controlled on metformin. ExQW+DAPA also showed an improvement in FLI and insulin resistance that was sustainable up to 52 weeks.

Table. Study parameters at baseline, week 28 and week 52

Parameters		ExQW+DAPA (N=228)	ExQW+PBO (N=227)	DAPA+PBO (N=230)
Weight (kg)	Baseline	91.79 (22.24)	89.77 (20.22)	91.06 (19.71)
	Week 28	88.35 (20.57)[a]	87.62 (18.05)[b]	88.64 (18.90)[c]
	Week 52	89.44 (20.99)[d]	89.69 (18.00)[e]	89.49 (16.85)[f]
GGT (U/L)	Baseline	39.90 (36.24)[g]	41.30 (37.81)[h]	37.80 (27.06)[i]
	Week 28	34.00 (40.76)[j]	35.30 (27.60)[k]	33.10 (22.81)[l]
	Week 52	31.20 (25.23)[m]	35.90 (28.77)[n]	37.80 (43.73)[o]
FLI (overall)	Baseline	78.10 (22.77)[p]	76.22 (24.27)[m]	78.53 (22.15)[o]
	Week 28	71.50 (24.64)[p]	72.72 (23.33)[m]	74.69 (22.78)[o]
	Week 52	71.95 (25.41)[p]	73.82 (23.29)[p]	73.92 (23.24)[q]
FLI (<60 subgroup)	Baseline	39.85 (16.22)[r]	33.93 (17.08)[s]	38.81 (15.68)[t]
	Week 28	35.24 (16.94)[r]	37.45 (18.23)[s]	38.76 (17.12)[t]
	Week 52	34.92 (20.36)[r]	39.12 (19.52)[s]	40.87 (19.21)[t]
FLI (≥60 subgroup)	Baseline	87.35 (11.84)[u]	86.42 (11.12)[v]	87.19 (11.16)[w]
	Week 28	80.27 (17.02)[u]	81.23 (14.93)[v]	82.52 (15.02)[w]
	Week 52	80.91 (17.03)[u]	81.85 (15.03)[v]	81.22 (16.83)[w]
*HOMA-IR (geometric mean [SEM])	Baseline	1.618 (0.0757)[x]	1.417 (0.0598)[z]	1.587 (0.0642)[†]
	Week 28	1.348 (0.0634)[x]	1.517 (0.0683)[z]	1.231 (0.0542)[†]
	Week 52	1.331 (0.0598)[y]	1.537 (0.0698)[#]	1.266 (0.0562)[‡]

DAPA, dapagliflozin; ExQW, exenatide once weekly; FLI, fatty liver index; GGT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment-insulin resistance; PBO, placebo; Data presented as mean (SD) unless mentioned otherwise.
*HOMA-IR was calculated based on plasma glucose and insulin. HOMA-IR was analysed at logarithm scale and then back-transformed to the original scale.
[a]n=194, [b]n=184, [c]n=196, [d]n=133, [e]n=115, [f]n=118, [g]n=231, [h]n=230, [i]n=233, [j]n=198, [k]n=189, [l]n=200, [m]n=180, [n]n=164, [o]n=190, [p]n=182, [q]n=192, [r]n=37, [s]n=35, [t]n=34, [u]n=153, [v]n=145, [w]n=156, [x]n=172, [y]n=179, [z]n=168, [#]n=175, [†]n=178

Clinical Trial Registration Number: NCT02229396
Supported by: Funding for this analysis was supported by AstraZeneca Pharmaceuticals LP.
Disclosure: **C. Guja:** Employment/Consultancy; AstraZeneca, Bayer AG, Eli Lilly, Merck KGaA, MSD, Novartis, Novo Nordisk, Sanofi.

722

Liraglutide in combination with metformin reduces lipolysis and lipid oxidation in patients with well controlled type 2 diabetes and ischaemic heart disease

C. Anholm[1,2], P. Kumarathurai[3], L.R. Pedersen[3], A. Samkani[4], O.P. Kristiansen[3], O.W. Nielsen[3], M. Fenger[5], S. Madsbad[6], A. Sajadieh[3], R.C. Boston[7], S.B. Haugaard[4,2];
[1]Department of Internal Medicine, Copenhagen University Hospital, Glostrup, Glostrup, Denmark, [2]Department of Internal Medicine, Copenhagen University Hospital, Amager, Copenhagen, Denmark, [3]Department of Cardiology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark, [4]Department of Endocrinology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark, [5]Department of Clinical Biochemistry, Copenhagen University Hospital, Hvidovre, Hvidovre, Denmark, [6]Department of Endocrinology, Copenhagen University Hospital, Hvidovre, Hvidovre, Denmark, [7]Department of Clinical Studies–New Bolton Center, School of Veterinary Medicine,, Pennsylvania, USA.

Background and aims: Type 2 diabetes (T2DM) is associated with dyslipidemia which are a risk factor for cardiovascular disease and cardiovascular mortality. Elevated levels of non-esterified fatty acids (NEFA), i.e. lipotoxicity, plays a role in the development of insulin resistance, impaired beta-cell function and is also a major denominator of the abnormal atherogenic lipid profile characterizing obese T2DM. It is hypothesized that the GLP-1 receptor agonist liraglutide in combination with metformin may reduce lipolysis and, thereby improves insulin sensitivity and beta-cell function.
Materials and methods: A randomized, double-blind, placebo-controlled, cross-over trial in 12 + 12-week periods with ≥2-weeks wash-

out. Intervention: liraglutide/metformin vs. placebo/metformin. Based on plasma NEFA and glucose levels measured during a standard 180 minutes insulin modified frequently sampled intravenous glucose tolerance test, NEFA kinetics was estimated by the Boston NEFA minimal model. First phase insulin response (AIRg) and insulin sensitivity (Si) were estimated by MINMOD minimal model analysis.

Results: Of 41 patients randomized thirty completed all study visits, n = 28 had data sets available for minimal modelling and paired analysis. Liraglutide in combination with metformin reduced lipolysis by 30% from baseline 36.6 (10.4) μmol/L/min ($p < 0.001$). Metformin in combination with placebo increased lipid oxidation by 2.4 (0.9) %/min ($p < 0.001$) from baseline level 4.4 (0.8) %/min, however adding liraglutide to metformin halved lipid oxidation (−49%, $p < 0.001$). Insulin sensitivity Si did not differ while AIRg improved 3-fold with liraglutide treatment compared with placebo.

Conclusion: Liraglutide exerted a remarkable reduction in both lipolysis and lipid oxidation during an intravenous glucose tolerance test, which may be explained by improved insulin secretion. This mechanism of action of liraglutide may add to the explanation of the improved long-term outcome in in patients with ischaemic heart disease and T2DM treated with liraglutide.

Clinical Trial Registration Number: NCT01595789

Supported by: A grant for investigator-initiated studies from Novo Nordisk A/S

Disclosure: **C. Anholm:** Grants; Novo Nordisk, The AP Moller Foundation, The Bispebjerg Hospital Research Foundation, The Danish Heart Foundation, Department of Internal Medicine at Amager Hospital, Clinical Research Centre Hvidovre Hospital.

723

The effect of efpeglenatide on lipid profiles and overall metabolism in patients with type 2 diabetes and obese patients without diabetes

K.-H. Yoon[1], J. Kang[2], I. Choi[2], M. Trautmann[3], M. Hompesch[3], C. Sorli[4], R. Pratley[5];
[1]The Catholic University of Korea, Seoul, Republic of Korea, [2]Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea, [3]ProSciento, Inc., Chula Vista, USA, [4]Sanofi, Bridgewater, USA, [5]Translational Research Institute for Metabolism and Disease, Florida Hospital, Orlando, USA.

Background and aims: Once-weekly (QW), subcutaneous efpeglenatide is a long-acting glucagon-like peptide-1 receptor agonist (GLP-1 RA) in development for type 2 diabetes (T2D). In a 12-week Phase 2 study in patients with T2D (EXCEED 203), treatment with efpeglenatide 3 and 4 mg QW was associated with significant ($p < 0.05$) reductions in HbA_{1c} (−1.41% [baseline: 7.82%] and −1.61% [baseline: 7.99%], respectively) vs placebo (−0.40% [baseline: 7.99%]). Fasting plasma glucose was also reduced in the efpeglenatide 3- and 4-mg groups (−2.25 mmol/L [baseline: 9.09 mmol/L] and −2.50 mmol/L [baseline: 9.15 mmol/L], respectively) vs placebo (−0.55 mmol/L [baseline: 9.06]). In a 20-week Phase 2 study in patients with obesity without diabetes (BALANCE 205), treatment with efpeglenatide 4 or 6 mg QW was associated with significant ($p < 0.05$) reductions in body weight (−6.63 kg [baseline: 100.81 kg] and −7.32 kg [baseline: 101.68 kg], respectively) vs placebo (−0.13 kg [baseline: 97.54 kg]).

Materials and methods: To assess whether these effects were associated with benefits on lipids, the effects of efpeglenatide QW on total cholesterol, LDL-cholesterol (LDL-C), HDL-C, VLDL-C, and triglycerides (TG) were assessed in patients from these Phase 2 trials (VLDL-C was measured in BALANCE 205 only).

Results: In the 12-week EXCEED 203 study in patients with T2D, efpeglenatide 4 mg QW led to significant ($p < 0.05$) reductions vs placebo in LDL-C and TG (Table). In the 20-week BALANCE 205 study in non-diabetic patients with obesity, efpeglenatide QW led to significant reductions vs placebo in total cholesterol, VLDL-C, TG (4-mg and 6-mg doses), and LDL-C and HDL-C (6 mg only). The safety profile of efpeglenatide in both trials was consistent with GLP-1 RAs; the proportions of patients who experienced any treatment-emergent adverse events were 62.2%, 66.7%, and 66.7% with placebo, efpeglenatide 3 mg, and efpeglenatide 4 mg, respectively, in EXCEED 203 and 80.0%, 86.4%, and 91.5% with placebo, efpeglenatide 4 mg, and efpeglenatide 6 mg, respectively, in BALANCE 205.

Conclusion: These findings suggest that loss of body weight and glycaemic effects with efpeglenatide are accompanied by significant benefits on lipid profiles, providing an overall improvement in the abnormal metabolic state associated with T2D.

Table. Lipid profile at baseline and change from baseline to study end (EXCEED 203: Week 13; BALANCE 205: Week 21)

		EXCEED 203: 12-week, randomized, double-blind, placebo-controlled Phase 2 trial in patients with T2D			BALANCE 205: 20-week, randomized, double-blind, placebo-controlled Phase 2 trial in patients with obesity and without T2D		
		Placebo (n=37)	Efpeglenatide 3 mg QW (n=36)	Efpeglenatide 4 mg QW (n=36)	Placebo (n=60)	Efpeglenatide 4 mg QW (n=59)	Efpeglenatide 6 mg QW (n=59)
Total cholesterol (mmol/L)	Baseline[a] (SD)	NA	NA	NA	4.78 (0.85)	4.73 (0.78)	4.60 (0.83)
	Change[b] (SE)	NA	NA	NA	0.11 (0.08)	−0.23 (0.08)**	−0.32 (0.08)***
LDL-C (mmol/L)	Baseline[a] (SD)	2.87 (0.79)	2.72 (0.74)	2.73 (0.95)	3.07 (0.77)	3.01 (0.70)	2.83 (0.76)
	Change[b] (SE)	0.09 (0.09)	−0.06 (0.10)	−0.22 (0.10)*	0.26 (0.07)	0.00 (0.08)	−0.10 (0.08)***
HDL-C (mmol/L)	Baseline[a] (SD)	1.20 (0.31)	1.22 (0.38)	1.09 (0.24)	1.30 (0.30)	1.31 (0.35)	1.31 (0.34)
	Change[b] (SE)	0.02 (0.03)	−0.02 (0.03)	−0.01 (0.03)	0.01 (0.02)	−0.05 (0.02)	−0.06 (0.02)*
VLDL-C (mmol/L)	Baseline[a] (SD)	NA	NA	NA	0.61 (0.28)	0.60 (0.30)	0.62 (0.28)
	Change[b] (SE)	NA	NA	NA	0.01 (0.02)	−0.08 (0.03)*	−0.08 (0.03)*
TG (mmol/L)	Baseline[a] (SD)	2.00 (1.20)	1.88 (1.23)	2.44 (1.55)	1.33 (0.62)	1.30 (0.65)	1.35 (0.60)
	Change[b] (SE)	−0.09 (0.13)	−0.41 (0.15)	−0.59 (0.15)**	0.01 (0.05)	−0.17 (0.06)*	−0.17 (0.06)*

Difference in LS mean change from baseline with efpeglenatide vs placebo: *p<0.05, **p<0.01, and ***p<0.001
[a]Data are means (SD)
[b]Data are LS means (SE) from mixed model repeated measures; change from baseline to study end
LS=least squares; NA=not available; SE=standard error

Clinical Trial Registration Number: NCT02057172 (EXCEED 203), NCT02075281 (BALANCE 205)

Supported by: Hanmi

Disclosure: **K. Yoon:** None.

724

Liraglutide and metformin may rock and roll the most atherogenic LDL fraction in patients with diabetes and ischaemic heart disease on stable statin therapy: a randomised trial

S.B. Haugaard[1,2], C. Anholm[3,2], P. Kumarathurai[4], L.R. Pedersen[4], A. Samkani[1], R.L. Walzem[5], O.P. Kristiansen[4], O.W. Nielsen[4], M. Fenger[6], S. Madsbad[7], A. Sajadieh[4];
[1]Department of Endocrinology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark, [2]Department of Internal Medicine, Copenhagen University Hospital, Amager, Copenhagen, Denmark, [3]Department of Internal Medicine, Copenhagen University Hospital, Glostrup, Glostrup, Denmark, [4]Department of Cardiology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark, [5]Department of Poultry Science and Faculty of Nutrition, Texas A&M University, College Station, USA, [6]Department of Clinical Biochemistry, Copenhagen University Hospital, Hvidovre, Hvidovre, Denmark, [7]Department of Endocrinology, Copenhagen University Hospital, Hvidovre, Copenhagen, Denmark.

Background and aims: Atherosclerosis in type 2 diabetes (T2DM) and obesity is multifactorial and associated with low-grade inflammation and dyslipidemias, especially small low dense lipoprotein particles are atherogenic. The glucagon-like peptide-1 receptor agonist, liraglutide, reduces cardiovascular events by unclear mechanisms. We investigated the effect of liraglutide combined with metformin on lipoprotein density profiles and low-grade inflammation and in patients with ischaemic heart disease and newly diagnosed well-controlled T2DM on stable statin therapy.

Materials and methods: A randomized, double-blind, placebo-controlled, cross-over trial in 12 + 12-weeks periods with ≥2-weeks washout. Intervention: liraglutide/metformin vs. placebo/metformin. Biochemical analysis was done at the beginning and end of each 12-weeks periods. Lipoproteins were separated by continuous density gradient ultracentrifugation and divided into HDL and LDL subfractions (5 each). The amount of particles in each subfraction was determined as the AUC of the density profile curve. Plasma C-reactive protein (CRP) and TNF-α were determined by the enzyme-linked immunosorbent-assay.

Results: Of 41 patients randomized thirty completed all study visits and were included in paired analysis, except from one patient, who had statin

dosage changed during the study. At baseline 95% of the patients were on statin therapy with LDL 2.3 (0.7) mmol/L (Friedwalds formula). LDL was divided in subfractions between 226–270 Å, considering particle sizes ≤255 Å as the most atherogenic. Liraglutide in combination with metformin reduced the most atherogenic subfraction LDL_5 by 17% ($p = 0.03$) from baseline level. Subgroup analysis comparing the "liraglutide-first" to "placebo-first" groups revealed that liraglutide significantly suppressed the LDL_5 fraction by 16% ($p = 0.03$) during treatment, but the subfraction rebounded significantly ($p = 0.03$) to pre-treatment value at the end of the wash-out period. HDL subfractions were not significantly affected by liraglutide combined to metformin. Markers of low-grade inflammation were at baseline slightly elevated, but the combination of liraglutide/metformin improved only CRP but not TNF-α.

Conclusion: Liraglutide in combination with metformin significantly improved the most atherogenic LDL subfraction and CRP in patients on stable statin therapy, with ischaemic heart disease and newly diagnosed well-controlled T2DM.

Clinical Trial Registration Number: NCT01595789

Supported by: A grant for investigator-initiated studies from Novo Nordisk A/S

Disclosure: **S.B. Haugaard:** Grants; Novo Nordisk A/S, The AP Moller Foundation, The Bispebjerg Hospital Research Foundation, The Danish Heart Foundation, Department of Internal Medicine at Amager Hospital, Clinical Research Centre Hvidovre Hospital.

725

Effects of liraglutide versus placebo on gallbladder events: results from the LEADER trial

M.A. Nauck[1], H.A. Sævereid[2], M.L.M. Ghorbani[2], E. Kreiner[2], J. Buse[3], LEADER Publication Committee on behalf of the LEADER Trial Investigators;
[1]Diabetes Center Bochum-Hattingen, St Josef Hospital (Ruhr-Universität Bochum), Bochum, Germany, [2]Novo Nordisk A/S, Søborg, Denmark, [3]University of North Carolina School of Medicine, Chapel Hill, USA.

Background and aims: Patients with type 2 diabetes (T2D) have an increased risk of gallbladder disease. In the LEADER trial, cholelithiasis and cholecystitis were more frequent in patients with T2D randomised to liraglutide 1.8 mg vs placebo (PBO). The present post hoc analysis further explored the clinical features of the imbalance in gallbladder events in the liraglutide and PBO groups.

Materials and methods: In LEADER, 9340 patients with T2D and high cardiovascular (CV) risk were randomised 1:1 to liraglutide 1.8 mg or PBO, on top of standard of care, and followed for 3.5–5 years. Acute gallstone disease was a predefined area of special interest and events were systematically captured. Events were defined as gallbladder disease including biliary colic, symptomatic cholelithiasis and cholecystitis, and identified for analysis using predefined Medical Dictionary for Regulatory Activities (MedDRA) search criteria. All captured events were blind-reviewed post hoc by medically qualified persons, and grouped into 4 categories based on the clinical features described: uncomplicated gallbladder stones (gallbladder stones supported by imaging, but no cholecystitis, bile duct stones, pain or signs of cholestasis, invasive procedures or surgery), complicated gallbladder stones, cholecystitis (with/without gallbladder stones) or biliary obstruction. Cholecystectomy due to the events was also evaluated. Time to first event by treatment (liraglutide vs PBO) was analyses using Cox regression independently for all categories.

Results: The MedDRA search captured 275 events, most frequently reported as cholelithiasis (119), acute cholecystitis (59) and cholecystitis (26). Based on the post hoc review, 7 events (5 liraglutide, 2 PBO) were found to be unrelated to biliary disease and excluded from analyses.

No differences at baseline were seen in patients experiencing an event in the liraglutide group vs PBO with respect to weight, BMI, age, gender, diabetes duration, triglycerides or LDL cholesterol, and no apparent differences were observed in patients with an event vs those without. Overall, acute gallstone disease was more common with liraglutide vs PBO (141 patients [3.0%] vs 88 [1.9%]; HR 1.60, 95% CI 1.23–2.09), and more common with liraglutide vs PBO for all 4 categories: uncomplicated gallbladder stone (16 patients [0.3%] vs 5 [0.1%]; HR 3.19, 95% CI 1.17–8.70), complicated gallbladder stone (52 patients [1.1%] vs 40 [0.9%]; HR 1.30, 95% CI 0.86–1.96), cholecystitis (51 patients [1.1%] vs 33 [0.7%]; HR 1.54, 95% CI 0.99–2.39) and biliary obstruction (25 patients [0.5%] vs 16 [0.3%]; HR 1.56, 95% CI 0.83–2.91). Cholecystectomy was more common in patients treated with liraglutide vs PBO (81 patients [1.74%] vs 52 [1.11%]; HR 1.56, 95% CI 1.10–2.20).

Conclusion: These detailed post hoc analyses of gallbladder-related events in LEADER demonstrate increases in the incidence with liraglutide- vs PBO-treatment in 4 categories, representative of different clinical diagnoses. In addition, the increased incidence of cholecystectomies is correlated with the increase in gallbladder-related events. Further research should explore relevant mechanisms and measures to prevent gallbladder-related events with glucagon-like peptide-1 receptor agonists.

Clinical Trial Registration Number: NCT01179048

Supported by: Novo Nordisk

Disclosure: **M.A. Nauck:** Other; Novo Nordisk.

726

HbA_{1c} changes in subjects with obesity without diabetes receiving semaglutide for weight management

O. Mosenzon[1], J. Wilding[2], A.L. Birkenfeld[3], B. McGowan[4], S.D. Pedersen[5], S. Wharton[6], P. Auerbach[7], L. Endahl[7], C.G. Carson[7], P.M. O'Neil[8];
[1]Hadassah Hebrew University Hospital, Jerusalem, Israel, [2]University of Liverpool, Liverpool, UK, [3]University Clinic Dresden, Dresden, Germany, [4]Guy's and St Thomas NHS Foundation Trust, London, UK, [5]C-ENDO Diabetes & Endocrinology Clinic, Calgary, AB, Canada, [6]York University, Toronto, Canada, [7]Novo Nordisk, Søborg, Denmark, [8]Medical University of South Carolina, Charleston, USA.

Background and aims: Elevated HbA_{1c} below diabetic levels is common in obesity and associated with an increased risk of type 2 diabetes (T2D). Semaglutide (SEMA) is a human glucagon-like peptide 1 analogue approved for T2D and under evaluation for weight management. A randomised, placebo (PBO)-controlled phase 2 trial of once-daily SEMA (0.05, 0.1, 0.2, 0.3, or 0.4 mg s. c. following escalation every 4 weeks [q4w] or 2 weeks) in adults with obesity without diabetes (screening HbA_{1c} <6.5%) showed mean week 52 weight loss on q4w SEMA between −6.0% (0.05 mg) and −13.8% (0.4 mg), vs −2.3% on PBO. We report a *post hoc* analysis of HbA_{1c} changes in this study.

Materials and methods: Baseline (BL) to week 52 changes in HbA_{1c} and body weight (%-points) were evaluated for 0.3 and 0.4 mg/day q4w SEMA and the PBO group (overall $N = 341$), in subgroups with normal HbA_{1c} (<5.7%) or elevated HbA_{1c} (≥5.7%) at BL. All available on- or off-treatment data were used in an analysis of covariance model (treatment, region and sex as fixed factors). Missing data were imputed from the PBO group (jump-to-reference multiple imputation).

Results: At BL, 65% (222/341) had normal HbA_{1c} and 35% (119/341) elevated HbA_{1c}. Across the evaluated groups, median normal HbA_{1c} was 5.3–5.4% (range 4.3–5.6%), vs 5.8–5.9% (5.7–7.0%) for elevated HbA_{1c}. By week 52, 75–77% with elevated BL HbA_{1c} achieved normal HbA_{1c} on SEMA, vs 24% on PBO. Only 2–5% progressed from normal to elevated HbA_{1c} on SEMA, vs 9% on PBO (Table). SEMA-related changes in HbA_{1c} were larger in those with elevated BL HbA_{1c}. Estimated mean change in HbA_{1c} vs PBO for 0.3 and 0.4 mg SEMA was −0.32% (95% CI −0.47; −0.16) and −0.45% (−0.61; −0.29), respectively, in the elevated group, −0.18% (−0.27; −0.09) and −0.19% (−0.28; −0.10) in the normal group. Mean estimated body-weight change vs PBO for 0.3 and 0.4 mg SEMA was −9.2% (−12.6; −5.8) and −10.2% (−13.7; −6.7),

respectively, for elevated BL HbA_{1c}, −8.4% (−11.2; −5.5) and −12.2% (−15.0; −9.4) for normal BL HbA_{1c} (all $P \leq 0.0001$). Treatment was generally tolerated, with no severe or documented symptomatic hypoglycaemic episodes reported in these groups.

Conclusion: A third of individuals evaluated had elevated BL HbA_{1c}, and most treated with SEMA 0.3 or 0.4 mg achieved normal levels by week 52. HbA_{1c} reductions were greater in those with elevated HbA_{1c} than in those without, but this did not appear to correlate with differences in weight loss. Further studies are needed to determine whether SEMA reduces progression to T2D in people with obesity and elevated HbA_{1c}.

	Normal Baseline HbA_{1c} (<5.7%)		Elevated Baseline HbA_{1c} (≥5.7%)	
Observed data % (yes/n)	**Still normal at week 52**	**Elevated at week 52**	**Normal at week 52**	**Still elevated at week 52**
SEMA 0.3 mg	94.6 (53/56)	5.4 (3/56)	77.4 (24/31)	22.6 (7/31)
SEMA 0.4 mg	98.1 (53/54)	1.9 (1/54)	75.0 (21/28)	25.0 (7/28)
PBO	90.9 (60/66)	9.1 (6/66)	24.3 (9/37)	75.7 (28/37)

n=total observed at week 52 in indicated subgroup (normal or elevated HbA_{1c} at baseline)

Clinical Trial Registration Number: NCT02453711
Supported by: Novo Nordisk
Disclosure: **O. Mosenzon:** Grants; AstraZeneca, Bristol-Myers Squibb, Novo Nordisk. Honorarium; Novo Nordisk, Eli Lilly, Sanofi, Merck Sharp & Dohme, Boehringer Ingelheim, Jansen and Jansen, Novartis, AstraZeneca. Lecture/other fees; Novo Nordisk, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Sanofi, Novartis, Merck Sharp & Dohme, Boehringer Ingelheim. Other; Novo Nordisk, Eli Lilly, Sanofi, Merck Sharp & Dohme, Boehringer Ingelheim, Jansen and Jansen, AstraZeneca, Bayer, Lexicon, Gluco-Vista.

727

MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, significantly reduces hepatic fat content in subjects with type 2 diabetes

M. Jain[1], L.-F. Tsai[2], D. Robertson[1], B. Hirshberg[2], P. Hockings[3], L. Johansson[3], P. Ambery[1];
[1]MedImmune, Cambridge, UK, [2]MedImmune, Gaithersburg, USA, [3]Antaros Medical AB, Molndal, Sweden.

Background and aims: MEDI0382 is a balanced glucagon-like peptide 1 (GLP-1)/glucagon receptor dual agonist in development for type 2 diabetes mellitus and nonalcoholic steatohepatitis. We investigated the effects of MEDI0382 on hepatic fat content in overweight/obese subjects with type 2 diabetes and a body mass index of 27–40 kg/m^2.

Materials and methods: In a phase 2a study, 51 subjects were randomized to receive MEDI0382 200 μg or placebo daily during a 41-day treatment period. Quantification of liver proton density fat fraction, subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) was performed as an exploratory analysis in a subset of patients (MEDI0382, $n = 17$; placebo, $n = 21$) who had magnetic resonance imaging scans taken at baseline and on day 41.

Results: Mean (SD) liver fat content at baseline was 15.74% (8.97) and 18.07% (8.38) in the MEDI0382 and placebo groups, respectively. Patients treated with MEDI0382 showed a highly significant relative reduction from baseline in hepatic fat content versus placebo (Table). This was positively correlated with reductions in both body weight ($r = 0.49$, $P = 0.002$) and alanine aminotransferase ($r = 0.42$, $P = 0.009$). A significant decrease in liver volume with MEDI0382 vs placebo was also observed (Table). Treatment with MEDI0382 was also associated with reductions in both SAT and VAT versus placebo.

Conclusion: These data demonstrate the efficacy of MEDI0382 in reducing liver fat and its potential for the treatment of non-alcoholic fatty liver disease, including steatohepatitis.

	Change from Baseline, Least Squares Mean (90% CI)		
Measurement	**MEDI0382 (*n* = 17)**	**Placebo (*n* = 21)**	***P***
Hepatic fat content, absolute change (%)	−5.98 (−7.67, −4.29)	−3.17 (−4.68, −1.65)	0.017
Hepatic fat content, relative change (%)	−39.12 (−49.11, −29.14)	−19.51 (−28.49, −10.53)	0.006
Hepatic volume (L)	-0.22 (-0·3, -0·2)	0.09 (-0·2, 0·0)	0.012
SAT (L)	−0.39 (−0.48, −0.30)	−0.21 (−0.30, −0.13)	0.008
VAT (L)	−0.36 (−0.52, −0.19)	−0.14 (−0.29, 0.01)	0.057

Clinical Trial Registration Number: NCT02548585
Disclosure: **M. Jain:** Employment/Consultancy; MedImmune. Stock/Shareholding; AstraZeneca.

PS 058 More on GLP1 receptor agonists and diabetes complications

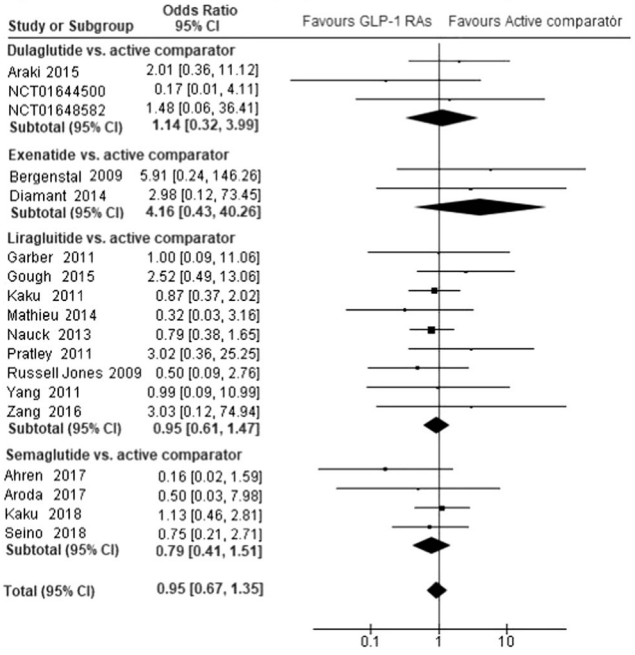
728

Effect of GLP-1 receptor agonists on microvascular endpoints in type 2 diabetes: a systematic review and meta-analysis

I. Avgerinos[1], T. Karagiannis[1], K. Malandris[1], A. Liakos[1], M. Mainou[1], P. Andreadis[1], E. Bekiari[1], D.R. Matthews[2], A. Tsapas[1,3];
[1]Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK, [3]Harris Manchester College, University of Oxford, Oxford, UK.

Background and aims: Effect of GLP-1 receptor agonists (GLP-1 RAs) on microvascular outcomes in patients with type 2 diabetes (T2DM) is controversial. Aim of this systematic review and meta-analysis was to assess the effect of GLP-1 RAs on renal and eye-related outcomes in adult patients with T2DM.
Materials and methods: We searched PubMed, Embase, the Cochrane Library and grey literature sources. We included randomized controlled trials of at least 12 weeks' duration, comparing a GLP-1 RA with placebo or another antidiabetic agent in adults with T2DM. Outcomes included change from baseline in urinary albumin-to-creatinine ratio (UACR, mg/g), and incidence of diabetic retinopathy, macular oedema and vitreous haemorrhage. Two reviewers independently screened search results and extracted data from included studies. We calculated weighted mean differences (WMDs) for UACR and odds ratios (ORs) for retinopathy related outcomes.
Results: We included 55 studies ($n = 58,789$ patients) in the meta-analysis. Compared with placebo (13 studies, $n = 17,794$ patients), GLP-1 RAs slightly reduced UACR (WMD −2.25 mg/g, 95% CI −3.88 to −0.62), while no effect was evident when they were compared to other antidiabetic agents (WMD −5.03 mg/g, 95% CI −10.68 to 0.63, 13 studies, $n = 5769$). Treatment with GLP-1 RAs was not associated with an increase in the risk for diabetic retinopathy compared with placebo (OR 1.05, 95% CI 0.91 to 1.20, 13 studies, $n = 30,981$ patients) or against any other antidiabetic agents (figure). Similarly, GLP-1 RAs were safe regarding incidence of macular oedema (OR 0.89, 95% CI 0.47 to 1.69 against placebo, and OR 1.14, 95% CI 0.34 to 3.84 against active comparator). Finally, incidence of vitreous haemorrhage was higher in patients treated with GLP-1 RAs compared with placebo (OR 2.04, 95% CI 1.16 to 3.59, 6 trials, $n = 20,723$ patients).
Conclusion: Treatment with GLP-1 RAs is safe regarding albuminuria and overall incidence of diabetic retinopathy. However, caution is needed for incidence of vitreous hemorrhage.

Figure. Odds Ratios for diabetic retinopathy. GLP-1 RAs vs active comparator.



Disclosure: **I. Avgerinos:** None.

729

The effect of DPP-4 protected GLP-1 (7-36) on coronary microcirculation in obese adults

M. Nilsson[1], K. Bové[2], E. Suhrs[2], T. Hermann[2], S. Madsbad[3], J.J. Holst[4], E. Prescott[2], M. Zander[1];
[1]Department of Endocrinology, Bispebjerg Hospital, Copenhagen, [2]Department of Cardiology, Bispebjerg Hospital, Copenhagen, [3]Department of Endocrinology, Hvidovre Hospital, Copenhagen, [4]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark.

Background and aims: Recently, glucagon-like-peptide-1 (GLP-1) receptor analogues have been shown to reduce cardiovascular events in patients with type 2 diabetes. However, the mechanism behind is still unknown. We hypothesized that GLP-1 would improve coronary microcirculation. In the absence of stenosis in major coronary arteries, coronary flow velocity reserve (CFVR) reflects the function of the coronary microcirculation. Impaired CFVR is associated with type 2 diabetes and obesity as well as increased cardiovascular mortality. The aim of the study was to investigate the effect of native GLP-1 on coronary microcirculation in overweight adults with neither diabetes nor coronary artery disease.
Materials and methods: Twelve obese adults (7 male/5 female; mean age, 54 ± 9.7 years; BMI, 30.9 ± 2.9 kg/m^2) participated in this double-blinded randomized cross-over study. They underwent a treadmill exercise test with echocardiography, to exclude coronary macrovascular disease, prior to participation. Effects of infusions of native GLP-1 (1.5 pmol/kg/min) were compared with a saline infusion on separate days. A DPP-IV inhibitor (Januvia 100 mg) was administered the night before and in the morning of the examination day to block the degradation of intact GLP-1 (7–36) to the GLP-1 metabolite (9–36). Coronary microcirculation, assessed by CFVR, was measured before and after 2 hours of infusion. Blood samples were collected every 30 minutes for measurements of GLP-1 (intact and metabolite), insulin, glucagon and glucose.
Results: Plasma GLP-1 (7-36) was significantly increased during GLP-1 infusion compared to saline (AUC 15037 ± 977 vs. 36 ± 19 pmol/L × min, $p < 0.0001$) with a mean difference of 117 pmol/L between the two infusions just before CFR measurement. Plasma glucose decreased

during infusion with GLP-1 (AUC 691 ± 22 vs. 794 ± 24 pmol/L × min, $p < 0.0001$) with a mean difference in glucose concentrations between the two infusions of 0.5 mmol/L just before CFR measurement (4.9 vs 5.4 mmol/l). However, plasma glucose stayed within normal range throughout infusions. Plasma glucagon showed no significant changes (AUC 691 ± 97 vs. 804 ± 108 pmol/L × min, with GLP-1 and saline, respectively, $p = 0.132$) whereas C-peptide increased slightly with GLP-1 (AUC 95539 ± 12049 vs. 63379 ± 7340 pmol/L × min, $p = 0.0009$). No effect of GLP-1 infusion was found on CFVR compared to saline infusion (ΔCFVR 0.376 ± 0.267 vs. ΔCFVR 0.713 ± 0.298, $p = 0.349$).

Conclusion: We found no effect of acute infusion of GLP-1, protected from DPP-4 mediated degradation, on coronary flow velocity reserve in overweight adults without diabetes. Thus the effect of GLP-1 on cardiovascular function does not seem to be mediated through improvement in coronary microcirculation.

Clinical Trial Registration Number: NCT02333591

Disclosure: **M. Nilsson:** None.

730

Neuroprotective property of liraglutide

A. Simanenkova, M. Makarova, L. Vasina, T. Vlasov, E. Shlyakhto; Pavlov First Saint-Petersburg State Medical University, Saint-Petersburg, Russian Federation.

Background and aims: Type 2 DM complications are main reasons of mortality and invalidity. Ischemic insult and chronic brain discirculation are much more frequent in type 2 DM. This requires a search for glucose-lowering drug having protective effect for the brain. Aim of the present study was to evaluate neuroprotecive effect of GLP-1 receptor agonist liraglutide (LIR).

Materials and methods: 45–75 aged type 2 diabetic patients ($n = 52$) with glycated hemoglobin (HbA1C) 7.5–9.0% on metformin (MET) therapy less than 2000 mg were included in the study. MET dose was titrated for 3 months until euglycemia or until 3000 mg. 3 months later patients who reached HbA1C less than 7.5% were included in group 1 and continued MET monotherapy for the following 6 months. Subjects with HbA1C more than 7.5% formed group 2 - they were co-administered LIR for next 6 months. At baseline, in 3, 6 and 9 months neuron-specific enolase (NSE) and S100 protein as neuroglial damage markers were evaluated.

Results: Baseline HbA1C level did not differ in groups 1 and 2 (8.4 (7.5; 9.0) % and 7.8 (7.54; 8.7) %, respectively). In 3 months 28 persons reached HbA1C less than 7.5% (6.75 (6.3; 7.2) %) and formed group 1, 24 patients were included in group 2 (HbA1C 8.2 (7.68; 8.68) %). In 6 months HbA1C remained satisfactory in group 1 (6.89 (6.45; 7.5) %) and demonstrated positive dynamics (decrease 0.5% and more for 3 months) in group 2 after LIR co-administration to metformin (7.6 (7.2; 8.3) %). In 9 months 3 patients in group 1 had worsening of glycemic profile (HbA1C 8.2 (7.6; 8.7) %), 25 still had HbA1C 6.7 (6.2; 7.0) %. Similarly, 5 patients in group 2 had increase of HbA1C (8.1 (7.75; 8.6) %), the rest 18 had further positive dynamics (7.35 (6.43; 7.75) %). Baseline level of NSE was higher in group 2, than in group 1 (26.55 (15.06; 43.95) mcg/L and 8.74 (4.52; 23.9) mcg/L, respectively, $p = 0.044$). S100 baseline level did not differ in group 1 (73.2 (21.98; 176.05) ng/L) and 2 (136.85 (79.55; 296.03 ng/L), $p = 0.246$, but prominently exceeded normal. No correlation was observed between neuroglial markers levels and/or DM duration, HbA1C. Glucose lowering in group 1 in 3 months led to NSE (6.81 (4.28; 12.0) mcg/L) and S100 (34.62 (19.45; 128.5) ng/L) normalization, they remained normal in 6 moths (NSE 6.67 (4.54; 10.28) mcg/L and S100 20.81 (13.1; 70.5) ng/L). In 9 months patients in group 1 with satisfactory glucose control had normal NSE (4.04 (3.39; 5.78) mcg/L) and S100 (19.2 (14.16; 59.52) ng/L), glycemia worsening led to NSE (6.43 (3.48; 6.43) mcg/L) and S100 (67.4 (19.33; 86.7) ng/L) increase. NSE (14.58 (7.42; 26.8) mcg/L) and S100 (38.8 (23.71; 82.6) ng/L) decreased in group 2 in 3 months even under unsatisfactory glycemic conditions, but this decrease was more prominent in 6 months after LIR administration, these parameters normalized (NSE 5.87 (4.18; 8.66) mcg/L and S100 44.28 (22.61; 69.15) ng/L). Importantly, NSE level did not differ in the part of group 2 having glycemia improvement (4.86 (3.5; 5.84) mcg/L) and worsening (4.58 (3.99; 6.53) mcg/L), $p > 0.05$. Similarly, S100 concentration remained normal and did not differ in patients having euglycemia under MET+ LIR therapy (29.54 (12.88; 45.24) ng/L) and having glycemic control worsening (23.2 918.19 (50.68) ng/l.

Conclusion: Type 2 DM leads to neuroglial damage not connected with glycemic control or disease duration, which manifests in NSE and S100 increase. Glycemic control improvement helps to decrease neuroglial damage. LIR has neuroprotective effect in type 2 DM not connected with its glucose-lowering action.

Disclosure: **A. Simanenkova:** None.

731

Liraglutide treatment fails to show neuronal repair or neuroprotective effects in patients with type 1 diabetes and diabetic symmetric polyneuropathy

C. Brock[1,2], C.S. Hansen[3], J.S. Karmitsholt[4,5], H.J. Møller[6], A.H. Juhl[7], A.D. Farmer[8], A.M. Drewes[1], S. Riahi[9], H. Lervang[4], P. Jakobsen[4,5], B. Brock[10,5];

[1]Mech-Sense, Department of Gastroenterology and Hepatology & Clinical Institute, Aalborg University Hospital, Aalborg, Denmark, [2]Department of Pharmacotherapy and Development, University of Copenhagen, Copenhagen, Denmark, [3]Complications research, Steno Diabetes Center Copenhagen, Gentofte, Denmark, [4]Department of Endocrinology, Aalborg University Hospital, Aalborg, Denmark, [5]Steno Diabetes Center North, Steno Diabetes Center North, Aalborg, Denmark, [6]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark, [7]Department of Neurophysiology, Aalborg University Hospital, Aalborg, Denmark, [8]Centre for Neuroscience and Trauma, Blizard Institute, Wingate Institute of Neurogastroenterology, Barts and the London School of Medicine & Dentistry, Queen Mary University of London, London, UK, [9]Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark, [10]Clinical research, Steno Diabetes Center Copenhangen, Gentofte, Denmark.

Background and aims: The pathogenesis of diabetic neuropathy is heterogenic and involves vascular-, metabolic-, immune-mediated - and inflammatory pathways. Studies have shown that glucagon-like-peptides-1 receptor agonists (GLP-1 RA) possess anti-inflammatory effects. The primary objective of this study was to evaluate the efficacy of treatment with the GLP-1 RA liraglutide on neuro inflammation and neuronal activity in a multi-level neurophysiological model.

Materials and methods: This randomized, double-blinded, single-centre, placebo-controlled trial carried out at Aalborg University Hospital comprised of 39 type 1 diabetes patients above 18 years, with HbA_{1c} >6.5 (48%) and confirmed neuropathy according to the Toronto criteria and assigned 1:1 to liraglutide (1.2–1.8 mg/day) or placebo. Primary outcome was in electrically evoked brain potentials at the motor threshold of the median nerve. Secondary outcomes were 24-h heart rate variability (HRV), sympatico-vagal balance; assessed by cardiac vagal tone (CVT) and cardiac sensitivity to the baroreceptor (CSB). Neurophysiological evaluation of peripheral sensory and motor nerves of upper and lower extremities was done. Pro-inflammatory cytokines; IL1β, TNF-α, IL6, IL-8 and IL-10 and macrophage markers sCD163 and sCD206 were assessed. Tertiary outcomes were alterations in HbA1C, insulin use and weight.

Results: In comparison to placebo, liraglutide elicited no differences in evoked potentials, peripheral nerve function, HRV parameters or measures of sympatico-vagal balance. Liraglutide reduced several inflammatory markers, only IL-6 was significantly reduced by −22.6% (95%CI: −38.1; −3.2; $P = 0.025$), and induced a weight loss of 3.38 kg (95%CI:

−5.29; −1.48, $P < 0.001$), but no change HbA1C: 1.18% (95%CI: −4.31; 6.98) or insulin use: −1.57% (95%CI: −18.14; 18.34) $P > 0.6$.

Conclusion: Liraglutide did not induce changes in evoked brain potentials, HRV parameters, measures of sympatico-vagal balance, nerve function of the peripheral nerves or glycaemic control. However liraglutide lowered levels of pro-inflammatory cytokines, however only significantly for IL-6, this independently of glycose metabolism. Liraglutide-induced reduction in neuro-inflammation may not have an effect on late-state neuropathy. Treatment effects on nerve function in patients with early-stage neuropathy remains to be explored.

Clinical Trial Registration Number: EUDRA CT: 2013-004375-12
Supported by: Unrestricted grants from Novo Nordisk Scandinavia AS; Empowering Industry

Disclosure: **C. Brock:** None.

732

Liraglutide with an effect on asymmetric dimethylarginine might be superior in nephroprotection compared to linagliptin and vildagliptin

A. Piljac[1], A. Jazbec[2], L. Duvnjak[1], S. Ljubic[1];
[1]Vuk Vrhovac University Clinic, Zagreb, [2]University of Zagreb, Zagreb, Croatia.

Background and aims: Incretins emerged as important in cardiovascular risk reduction in diabetes. As nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA) is responsible for increased risk of angiopathy, it also predicts the progression of nephropathy. The significance of liraglutide in comparison to linagliptin and vildagliptin for nephroprotection was studied through an impact on factors of angiopathy.

Materials and methods: ADMA, BNP, ApN, some other markers of inflammation, BP, HbA1c and other cardiovascular (CV) risk factors were determined. A total of 243 type 2 diabetics divided into three groups treated with linagliptin (Group [Gr] 1), vildagliptin (Gr 2) and liraglutide (Gr 3) were studied during a 6-month follow-up period. Patients differed in their estimated GFR calculated using the CKD-EPI formula (Gr 1: eGFR >90, Gr 2: eGFR:90–60, and Gr 3: eGFR <60 ml/min/1.73 m^2), and albumin/cretinine ratio (ACR <2.5 and ≥2.5).

Results: A significant among-group difference was found in ApN (Gr 1 vs Gr 2 vs Gr 3 = 4.84 ± 1.81 vs 5.33 ± 1.74 vs 10.08 ± 2.82; ANOVA: F(2.36) = 14.06; $p < 0.0001$), ADMA (Gr 1 vs Gr 2 vs Gr 3 = 0.47 ± 0.07 vs 0.54 ± 0.05 vs 0.66 ± 0.16; F(2.38) = 13.05; $p < 0.0001$), and homocysteine (HCY) (Gr 1 vs Gr 2 vs Gr 3 = 12.92 ± 3.43 vs 14.4 ± 4.6 vs 21.03 ± 5.25; F(2.37) = 6.84; $p = 0.003$). Tukey post hoc test showed significant differences ($p < 0.05$) in ApN and HCY between Gr 1 and Gr 3, and Gr 2 and Gr 3, in ADMA between Gr 1 and Gr 3. No differences in variables according to ACR were dtermined (Mann Whitney test). Mean values for ADMA at the beginning of the study were 0.56 ± 0.14, 0.49 ± 0.07 and 0.54 ± 0.04 in the Gr 1, 2, 3 respectively, and were significantly reduced by 0.07 (95% CI: 0.02–0.14), 0.06 (95% CI: 0.02–0.1) and 0.04 (95% CI: 0.02–0.06) on average in all three groups, with greater reduction of Gr 3 in comparison with Gr 1 and 2. Mean values for high-sensitivity C-reactive protein (hs-CRP) at the beginning of the study were 3.86 ± 3.64, 2.67 ± 2.52 and 5.31 ± 2.37 in the Gr 1, 2, 3 respectively, and were significantly reduced by 0.63 (95% CI: 0.1–1.15), 1.35 (95% CI: −0.26–2.97) and 1.71 (95% CI: 0.57–2.84) on average in all three groups, with greater reduction of Gr 3 in comparison with Gr 2 (*t* paired test). HbA1c mean values at the beginning were 8.01 ± 0.79, 7.36 ± 0.87 and 8.01 ± 0.95 in the Gr 1, 2, 3 respectively, and were significantly reduced by 0.94 (95% CI: 0.73–1.15), 0.69 (95% CI: 0.05–1.32) and 1.15 (95% CI: 0.35–1.95) on average, with no difference between groups. BMI mean values in Gr 3 at the beginning were 39.3 ± 4.5 and were significantly reduced by 2.65 (95% CI: 1.35–3.94) on average, whereas SBP reduction was significant from baseline (137.5 ± 16.9) in Gr 2 by 9.0 (95% CI: −0.05–18.55). Postprandial C-peptide and triglycerides (TG) were reduced in Gr 3 by −0.32 (95% CI: −0.65–0.01) and 0.67 (95% CI: −0.09–1.43) on average, however, not significantly. ADMA correlated significantly with alanine aminotransferase ($r = -0.38$) and eGFR ($r = -0.64$) and ApN ($r = 0.43$).

Conclusion: ADMA, ApN and HCY accompanied impaired renal function. Liraglutide was superior in ADMA, hs-CRP, BMI and TG reduction, therefore it is credible to argue its advantage in diabetic angiopathy protection. Reducing ADMA, together with glycemic regulation, CRP, BMI and Tg reduction, liraglutide could be superior in nephroprotection.

Disclosure: **A. Piljac:** None.

733

Liraglutide reduces cardiovascular events and mortality in type 2 diabetes independent of LDL cholesterol and statin use: results of the LEADER trial

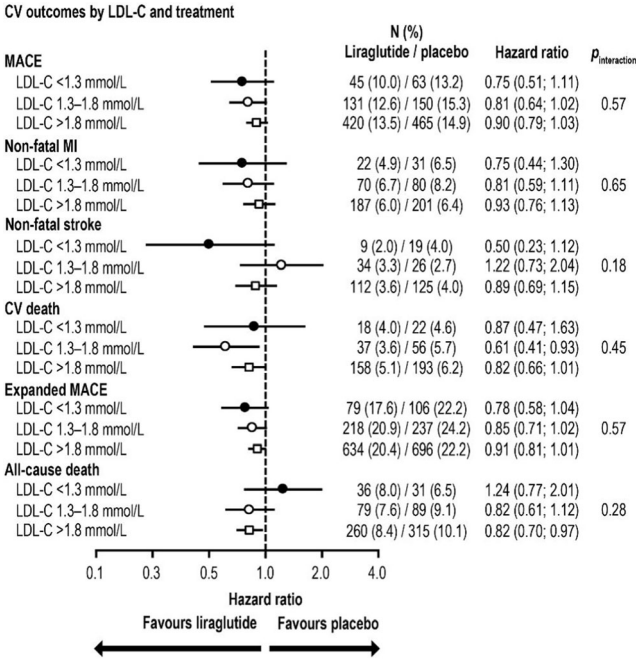
L.A. Leiter[1], S. Verma[2], C. Mazer[1], S.C. Bain[3], J. Buse[4], S. Marso[5], M.A. Nauck[6], B. Zinman[7], H. Bosch-Traberg[8], H. Frimer-Larsen[8], M.M. Michelsen[8], D.L. Bhatt[9], LEADER Publication Committee on behalf of the LEADER Trial Investigators;
[1]Li Ka Shing Knowledge Institute, St. Michael's Hospital and Univ. of Toronto, Toronto, Canada, [2]St. Michael's Hospital and Univ. of Toronto, Toronto, Canada, [3]Institute of Life Science, Swansea Univ., Swansea, UK, [4]Univ. of North Carolina School of Medicine, Chapel Hill, USA, [5]HCA Midwest Health Heart & Vascular Institute, Kansas City, USA, [6]Diabetes Center Bochum-Hattingen, St. Josef-Hospital (Ruhr-Universität Bochum), Bochum, Germany, [7]Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Univ. of Toronto, Toronto, Canada, [8]Novo Nordisk A/S, Søborg, Denmark, [9]Brigham and Women's Hospital Heart and Vascular Center and Harvard Medical School, Boston, USA.

Background and aims: The relationships among LDL cholesterol levels, statin use and cardiovascular (CV) outcomes are well established. In LEADER, the human glucagon-like peptide 1 analogue liraglutide reduced CV events in patients with type 2 diabetes (T2D) at high CV risk. This post hoc analysis evaluated liraglutide effects on CV outcomes by baseline LDL and statin use.

Materials and methods: LEADER studied liraglutide (1.8 mg or maximum tolerated dose) vs placebo, both in addition to standard care, in 9340 patients with T2D and high CV risk. Primary outcome: composite of CV death, non-fatal myocardial infarction, or non-fatal stroke (major adverse CV events, MACE). The key secondary expanded outcome (expanded MACE) also included hospitalisation for unstable angina or heart failure, or revascularisation. Cox regression evaluated liraglutide effect on CV outcomes by baseline LDL <1.3 mmol/L, 1.3–1.8 mmol/L and >1.8 mmol/L, and baseline statin use.

Results: In LEADER, 9187 patients had LDL measured: 926 (10.1%), 2021 (22.0%) and 6240 (67.9%) had baseline LDL <1.3 mmol/L, 1.3–1.8 mmol/L or >1.8 mmol/L, respectively. Baseline characteristics were relatively similar between the 3 groups, except that patients with LDL >1.8 mmol/L had lower statin use and lower proportion of CV events in their medical history. Within groups by LDL level, baseline characteristics were well-balanced across treatment groups. Liraglutide consistently reduced MACE vs placebo irrespective of baseline LDL (HR 0.75, 95% CI 0.51–1.11 vs HR 0.81, 95% CI 0.64–1.02 vs HR 0.90, 95% CI 0.79–1.03, *p* interaction = 0.57). Similarly for expanded MACE: HR 0.78, 95% CI 0.58–1.04 vs HR 0.85, 95% CI 0.71–1.02 vs HR 0.91, 95% CI 0.81–1.01, *p* interaction = 0.57) (Figure). The HR for MACE, with liraglutide vs placebo, was 0.83 (95% CI 0.73–0.94) in statin users (72%) and 0.97 (95% CI 0.79–1.20) in non-statin users (28%) (*p* interaction = 0.19). Results were similar in models adjusted for baseline characteristics.

Conclusion: In LEADER, liraglutide was associated with lower risk of major CV events in patients with T2D across LDL levels and statin use, with event reduction even in patients with lowest LDL.



N (%), number of patients with an event (as a proportion of the full analysis set). CV, cardiovascular; LDL-C, low-density lipoprotein cholesterol; MACE, major adverse cardiovascular event; MI, myocardial infarction

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **L.A. Leiter:** Other; Funding: Novo Nordisk A/S.

734

Semaglutide consistently reduces cardiovascular events in both male and female subjects with type 2 diabetes

I. Hramiak[1], C. DeSouza[2], J. Seufert[3], T. Hansen[4], J. Pettersson[4], D. Thielke[4], I. Lingvay[5];
[1]Western University, London, Canada, [2]University of Nebraska Medical Center, Omaha, USA, [3]Medical Faculty, University of Freiburg Medical Center, Freiburg, Germany, [4]Novo Nordisk A/S, Søborg, Denmark, [5]UT Southwestern Medical Center, Dallas, USA.

Background and aims: Semaglutide is a glucagon-like peptide-1 analogue for once-weekly treatment of type 2 diabetes (T2D). In the SUSTAIN 6 cardiovascular (CV) trial, subcutaneous semaglutide (0.5 mg and 1.0 mg) added to standard of care significantly reduced major adverse CV events (MACE: non-fatal myocardial infarction, non-fatal stroke or CV death) vs placebo over 2 years in subjects with T2D at high CV risk. This *post hoc* analysis assessed whether this CV risk reduction was consistent in male and female subjects.
Materials and methods: Endpoints analysed were MACE, the individual components of the MACE endpoint, first hospitalisation due to unstable angina, first hospitalisation due to heart failure, and first revascularisation. In total, 2,002 males and 1,295 females were randomised.
Results: MACE consistently occurred in fewer subjects with semaglutide vs placebo in both sexes, consistent with the overall study population ($p = 0.45$ for interaction): HR 0.68 (95% CI 0.50;0.92) in males, 0.84 (95% CI 0.54;1.31) in females and 0.74 (95% CI 0.58;0.95) in the total study population. Overall, this pattern was reflected across the individual MACE components, showing lower rates with semaglutide vs placebo regardless of sex, with the exception of CV mortality in female subjects (**Table**). In the overall trial population, there was no significant difference between semaglutide vs placebo for first hospitalisation due to unstable angina or heart failure: HR 0.82 (95% CI 0.47;1.44) and 1.11 (95% CI 0.77;1.61), respectively. These results were consistent in males (HR 0.66 [95% CI 0.33;1.34] and 1.18 [95% CI 0.75;1.86], respectively) and females (HR 1.17 [95% CI 0.45;3.03] and 0.93 [95% CI 0.49;1.76]) ($p = 0.35$ and $p = 0.55$ for interaction, respectively). Revascularisation was performed in significantly fewer subjects with semaglutide vs placebo (HR 0.65 [95% CI 0.50;0.86], $p = 0.003$). These results were consistent in males (HR 0.60 [95% CI 0.43;0.85], $p = 0.0035$) and females (HR 0.74 [95% CI 0.46;1.20], not statistically significant) ($p = 0.50$ for interaction). Adverse events were reported by similar proportions of males and females across treatments. The most frequent AEs reported were gastrointestinal, with higher rates in females than in males. The proportions of subjects prematurely discontinuing treatment due to AEs were comparable for males and females (**Table**).
Conclusion: The semaglutide-induced risk reduction in MACE and its components vs placebo seen in SUSTAIN 6 was consistent in both males and females. Numerically lower proportions of females than males reported MACE. The semaglutide treatment effect on the risk of secondary CV endpoints was also similar in both sexes.

Table. Major adverse cardiovascular events (MACE: non-fatal myocardial infarction, non-fatal stroke or cardiovascular death) and adverse events by sex in the SUSTAIN 6 trial

	Semaglutide (pooled 0.5 mg and 1.0 mg) n (%)	Placebo (pooled 0.5 mg and 1.0 mg) n (%)
CV outcomes		
MACE*		
Male	73 (7.2)¶	103 (10.4)¶
Female	35 (5.5)¶	43 (6.5)¶
Non-fatal MI*		
Male	32 (3.2)	48 (4.9)
Female	15 (2.4)	16 (2.4)
Non-fatal stroke*		
Male	18 (1.8)	27 (2.7)
Female	9 (1.4)	17 (2.6)
CV death*		
Male	30 (3.0)	34 (3.4)
Female	14 (2.2)	12 (1.8)
Other safety outcomes		
Any AE†		
Male	888 (88.2)	879 (89.1)
Female	566 (89.1)	574 (87.4)
Serious AE†		
Male	327 (32.5)	357 (36.2)
Female	177 (27.9)	217 (33.0)
Severe AE†		
Male	233 (23.1)	238 (24.1)
Female	137 (21.6)	128 (19.5)
AE leading to premature treatment discontinuation†		
Male	126 (12.5)	70 (7.1)
Female	88 (13.9)	40 (6.1)
Gastrointestinal AE†		
Male	488 (48.5)	316 (32.0)
Female	353 (55.6)	248 (37.7)

*In-trial analysis comprising events with onset on, or after, the day of randomisation and until end-of-trial (semaglutide males: n=1,013; semaglutide females: n=635; placebo males: n=989; placebo females: n=660); †On-treatment analysis comprising events with onset from the date of first dose to either the end-of-treatment follow-up visit, the date of last dose plus 42 days, the end-of-trial follow-up visit, or the date of withdrawal from trial, whichever came first (semaglutide males: n=1,007; semaglutide females: n=635; placebo males: n=987; placebo females: n=657); ¶Numbers are equal to 'first event', as subjects experiencing more than one component of the composite endpoint contributed to MACE once.
AE, adverse event; CV, cardiovascular; MACE, major adverse cardiovascular event; MI, myocardial infarction.

Clinical Trial Registration Number: NCT01720446
Supported by: Novo Nordisk A/S research support
Disclosure: **I. Hramiak:** Employment/Consultancy; Amgen Inc., Sanofi, Novo Nordisk Inc., Merck & Co., Janssen Pharmaceuticals Inc., Insulet Corp., Roche Pharma, GlaxoSmithKline, Eli Lilly and Co., Boehringer Ingelheim, AstraZeneca/Bristol-Myers Squibb. Grants; AstraZeneca/Bristol-Myers Squibb, Sanofi, Novo Nordisk Inc., Merck & Co., Medtronic, Lexicon Pharmaceuticals Inc., Janssen Pharmaceuticals Inc., GlaxoSmithKline plc., Eli Lilly and Co.. Lecture/other fees; AstraZeneca/Bristol-Myers Squibb, Sanofi, Novo Nordisk Inc., Merck & Co., Janssen Pharmaceuticals Inc.

735

Effect of liraglutide on ambulatory blood pressure in hypertensive patients with type 2 diabetes: randomised, double blind, placebo controlled trial

A. Liakos[1], K. Balampanis[2], E. Barmpa[3], E. Angeloudi[1], A. Katsoula[1], E. Athanasiadou[1], S. Gerou[4], V. Lampadiari[2], A. Bargiota[3], I. Avramidis[5], K. Kotsa[1], N. Tentolouris[2], E. Bekiari[1], A. Tsapas[1,6];
[1]Aristotle University Thessaloniki, Thessaloniki, Greece, [2]University of Athens, Athens, Greece, [3]University of Thessaly, Larisa, Greece, [4]Analysi Iatriki SA, Thessaloniki, Greece, [5]Papanikolaou General Hospital, Thessaloniki, Greece, [6]University of Oxford, Oxford, UK.

Background and aims: Arterial hypertension complicates most patients with type 2 diabetes further contributing to the increased cardiovascular risk. Liraglutide reduces incidence of cardiovascular events but the underlying mechanisms are yet poorly understood. We conducted an investigator-initiated, parallel group, randomised, double blind, multicentre trial assessing the effect of liraglutide on 24-h ambulatory blood pressure in patients with hypertension (pre- and stage 1 hypertension) and inadequately controlled type 2 diabetes (HbA_{1c} 7–10%).
Materials and methods: Eligible patients were on stable background antihyperglycemic therapy excluding insulin, glucagon-like peptide 1 receptor agonists and dipeptidyl-peptidase 4 inhibitors. Subjects were centrally randomized via a web-based system in a 1:1 ratio to receive either daily liraglutide 0.6 mg titrated to 1.2 mg after the first week or placebo for 5 weeks. Primary outcome was change in 24-h ambulatory systolic blood pressure (SBP). Secondary outcomes included change in ambulatory diastolic blood pressure (DBP) and heart rate as well as daytime and nighttime measurements. Analysis was based on the intention-to-treat principle.
Results: Of 87 subjects assessed for eligibility 62 patients (66% males) with mean age 60.2 years and BMI 33.8 kg/m^2 were allocated to liraglutide ($N = 31$) or placebo ($N = 31$). All participants received background therapy with metformin, whilst 35% were treated concomitantly with sulphonylurea and 15% with pioglitazone. Mean HbA_{1c} at baseline was 7.9% and mean duration of diabetes was 9.0 years. Compared with placebo liraglutide reduced 24-h SBP by 5.73 mmHg (95% CI −9.81 to −1.65) and had a neutral effect on 24-h DBP (mean difference −1.42 mmHg; 95% CI −4.25 to 1.40) whilst increasing 24-h heart rate by 6.16 beats/min (95% CI 3.25 to 9.07). Findings were consistent for daytime and nighttime measurements (Table). In each group two subjects discontinued treatment prematurely. Four patients (13%) in each arm experienced an adverse event. Of these, two patients in the placebo and one patient in the liraglutide arm discontinued the study drug due to an adverse event. No serious adverse events or deaths were reported.
Conclusion: Based on 24-h ambulatory measurements short term treatment with liraglutide had a favourable effect on systolic blood pressure whilst increasing heart rate.

Outcome	Change from baseline (SE)		Mean difference (95% CI)
	Liraglutide (N = 31)	Placebo (N = 31)	
24-h SBP, mmHg	–4.72 (1.43)	1.02 (1.43)	–5.73 (–9.81 to –1.65)
24-h DBP, mmHg	–0.58 (0.99)	0.84 (0.99)	–1.42 (–4.25 to 1.40)
24-h HR, beats/min	8.55 (1.03)	2.39 (1.03)	6.16 (3.25 to 9.07)
Daytime SBP, mmHg	–5.61 (1.47)	0.83 (1.47)	–6.43 (–10.63 to –2.24)
Daytime DBP, mmHg	–1.10 (1.02)	0.60 (1.02)	–1.70 (–4.60 to 1.20)
Daytime HR, mmHg	8.35 (1.06)	2.16 (1.06)	6.19 (3.19 to 9.18)
Nighttime SBP, mmHg	–1.67 (1.65)	1.77 (1.65)	–3.44 (–8.15 to 1.28)
Nighttime DBP, mmHg	–0.30 (1.07)	0.67 (1.07)	–0.97 (–4.03 to 2.09)
Nighttime HR, mmHg	9.25 (1.11)	3.09 (1.11)	6.17 (3.03 to 9.30)

SBP: systolic blood pressure, DBP, diastolic blood pressure, HR: heart rate.

Clinical Trial Registration Number: EudraCT 2013-002348-99
Supported by: Novo Nordisk
Disclosure: **A. Liakos:** None.

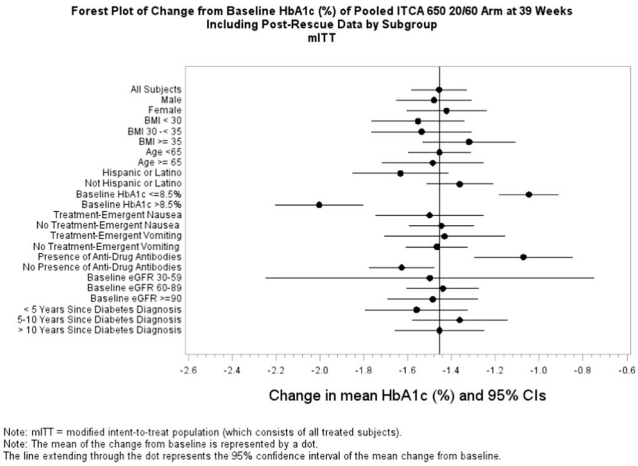
PS 059 On the efficacy of GLP1 receptor agonists

736

ITCA 650 provides consistent efficacy in type 2 diabetes irrespective of baseline characteristics: results of a pooled subgroup analysis

B. Schwartz, P. Prabhakar, L. Kjems, H. Huang, M. Baron;
Intarcia Therapeutics, Boston, USA.

Background and aims: ITCA 650 consists of a small titanium osmotic mini-pump that is subdermally placed in the abdominal wall during a brief in-office procedure. As an investigational product for the treatment of type 2 diabetes (T2D), ITCA 650 provides a continuous subcutaneous infusion of exenatide over 3 or 6 months.
Materials and methods: Pooled 39-week data from two double-blind, randomized, Phase 3 studies were used to evaluate the efficacy of ITCA 650 20/60 mcg/d in patients with T2D inadequately controlled by oral antidiabetic drugs (OADs). Results in the overall population ($N = 683$) and in subgroups according to age, gender, BMI, ethnicity, time since diagnosis, baseline HbA1c, GI adverse events, presence of anti-drug antibodies, and renal function are reported.
Results: As shown in the figure, the overall mean (standard deviation [SD]) reduction in HbA1c (%) was 1.5% (1.2) with clinically meaningful reductions in HbA1c consistently observed irrespective of age, gender, BMI, ethnicity, time from T2D diagnosis, eGFR and background OADs (data not shown). Patients with a higher baseline HbA1c had a greater response. Mean weight loss was 3.4 kg (SD 4.7), and 40.7% (95% CI 0.36–0.46) of patients achieved a composite endpoint of HbA1c/weight reduction of >0.5%/≥2 kg. Similar changes were likewise seen consistently across subgroups.
Conclusion: ITCA 650 demonstrated consistent efficacy across a wide spectrum of patients with T2D.



Note: mITT = modified intent-to-treat population (which consists of all treated subjects).
Note: The mean of the change from baseline is represented by a dot.
The line extending through the dot represents the 95% confidence interval of the mean change from baseline.

Supported by: Intarcia Therapeutics, Inc.
Disclosure: **B. Schwartz:** Employment/Consultancy; Intarcia Therapeutics.

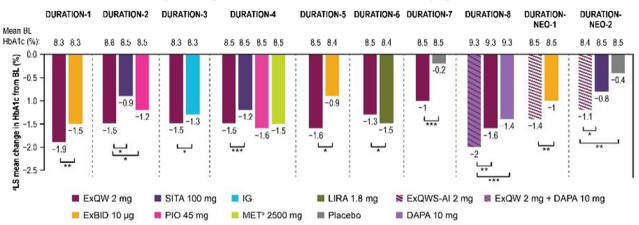
737

Efficacy and safety with exenatide once weekly: clinical trial results from 10 randomised trials (the DURATION programme)

O. Motawakel[1], P.S. Leigh[2], E. Hardy[1];
[1]AstraZeneca, Gaithersburg, USA, [2]AstraZeneca, Cambridge, UK.

Background and aims: Multiple clinical studies have investigated the glucagon-like peptide-1 receptor agonist (GLP-1RA) exenatide, a novel microsphere-based therapy once weekly administered s.c. (ExQW) or with an autoinjector with a miglyol diluent (ExQWS-AI), in patients with type 2 diabetes (T2D). This analysis summarises the efficacy of ExQW and ExQWS-AI across 10 comparator-controlled, phase 3, 24- to 30-week clinical studies from the DURATION clinical programme. The DURATION-1 study was extended to 7 years of follow-up; currently, this is the longest duration that any GLP-1RA has been studied in a clinical trial.
Materials and methods: Patients received ExQWS-AI or ExQW as monotherapy or in combination with other antidiabetic drugs (e.g. metformin, sulphonylurea, a thiazolidinedione [pioglitazone], dapagliflozin [DAPA] and insulin glargine). Efficacy and safety data were summarised from individual studies.
Results: In total, 2251 patients received ExQW 2 mg ($n = 1841$) or ExQWS-AI 2 mg ($n = 410$); 2870 patients received comparators (non-GLP-1RAs, $n = 1481$ [metformin, sitagliptin, pioglitazone, insulin glargine, and DAPA], ExBID [$n = 416$], liraglutide [$n = 450$], ExQW+DAPA [$n = 231$], or placebo [$n = 292$]). In the individual studies, the mean age of patients ranged from 52–58 years, and 48–65% were men. Mean duration of diabetes ranged from 5–11.3 years, except in a study of treatment-naïve patients (mean: 2.7 years). ExQW and ExQWS-AI reduced HbA1c in all trials from baseline to weeks 24–30 (mean change from baseline: −1.0% to −1.9%; **Figure**); proportions of patients achieving HbA1c ≤7.0% ranged from 27–77%. ExQW and ExQWS-AI resulted in fasting glucose reductions ranging from −0.7 to −2.5 mmol/L. Mean body weight reductions with ExQW and ExQWS-AI ranged from −1.0 to −3.7 kg. Efficacy has been observed in all patient subpopulations (e.g. by age, sex, baseline HbA1c, baseline body mass index, renal function and duration of diabetes) and diabetes therapy combinations studied to date. Overall, 67.5% and 64.1% of patients treated with ExQW or ExQWS-AI experienced ≥1 treatment-emergent adverse event (AE), respectively; 3.9% of patients discontinued treatment due to AEs, and 3.2% of patients had ≥1 serious AE with ExQW or ExQWS-AI. In patients receiving a comparator drug, 61.0% experienced ≥1 treatment-emergent AE. The most common AEs collectively with ExQW and ExQWS-AI were nausea, diarrhoea and vomiting, which occurred in 11.7%, 7.9%, and 3.6% of patients, respectively.
Conclusion: The 10 studies show consistent efficacy for ExQW (8 studies) and ExQWS-AI (2 studies) with HbA1c reductions of 1.0–1.9% at 24–30 weeks in patients with T2D with no new or unexpected safety findings in the integrated population. Consistent results were seen across clinical trials despite different patient populations, treatment settings, and medication combinations.

Figure. Change in HbA1c from baseline across the DURATION programme



*P<0.05; **P<0.01; ***P<0.001; †Direct comparisons of results across the DURATION studies cannot be made. Study designs and patient populations differed across the studies
‡MET could be increased up to 2500 mg based on glycaemic control. The proportion of patients on a stable dose of MET ≥2000 mg was 76%
BL, baseline; DAPA, dapagliflozin; ExBID, exenatide twice daily; ExQW, exenatide once weekly; ExQWS-AI, exenatide once weekly with an autoinjector; HbA1c, glycated haemoglobin; IG, insulin glargine; LIRA, liraglutide; LS, least square; MET, metformin; PIO, pioglitazone; SITA, sitagliptin

Supported by: Funding for this analysis was supported by AstraZeneca Pharmaceuticals LP.
Disclosure: **O. Motawakel:** Employment/Consultancy; AstraZeneca.

738

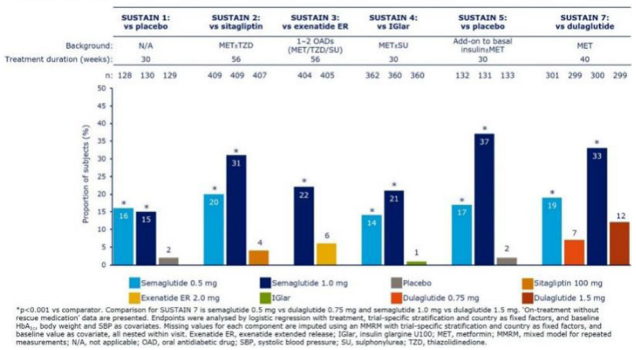
More subjects achieved composite reductions of ≥1% HbA_{1c}, ≥5% body weight and ≥5 mmHg SBP with semaglutide vs comparators across the SUSTAIN 1-5 and 7 trials

K.M. Dungan[1], V.R. Aroda[2,3], F.K. Knop[4], L.A. Leiter[5], N.L. Lausvig[6], S. Lindberg[6], J. Ferløv Schwensen[6], J.J. Meier[7];

[1]The Ohio State University, Columbus, USA, [2]Brigham and Women's Hospital, Boston, USA, [3]MedStar Health Research Institute, Hyattsville, USA, [4]Steno Diabetes Center Copenhagen, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark, [5]Li Ka Shing Knowledge Institute, St Michael's Hospital, University of Toronto, Toronto, Canada, [6]Novo Nordisk A/S, Søborg, Denmark, [7]Diabetes Division, St Josef Hospital, Ruhr-University Bochum, Bochum, Germany.

Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue for the once-weekly treatment of type 2 diabetes (T2D). Across the SUSTAIN clinical trial programme, subjects with T2D achieved greater reductions in three cardiovascular (CV) risk factors, HbA_{1c}, body weight (BW) and systolic blood pressure (SBP), with semaglutide vs placebo (SUSTAIN 1 and 5) or active comparators (sitagliptin [SUSTAIN 2], exenatide extended release [SUSTAIN 3], insulin glargine U100 [SUSTAIN 4] or dulaglutide [SUSTAIN 7]). The aim of this analysis was to evaluate to what extent subjects achieved clinically meaningful reductions in a composite of these three CV risk factors with semaglutide vs comparators in the SUSTAIN clinical trial programme.
Materials and methods: Six SUSTAIN trials (SUSTAIN 1–5 and 7; see figure for details) were assessed *post hoc* to determine the proportion of subjects who achieved clinically meaningful reductions in all three CV risk factors (composite endpoint: ≥1% decrease in HbA_{1c}, ≥5% BW loss, and ≥5 mmHg SBP reduction). Endpoints were analysed by logistic regression. Missing values for each component were imputed using a mixed model for repeated measurements.
Results: Across trials, mean baseline HbA_{1c}, BW and SBP ranges were 8.1–8.4%, 89.5–95.8 kg and 128.8–134.8 mmHg, respectively. Significantly more subjects achieved the composite endpoint with semaglutide (0.5 mg: 14–20%; 1.0 mg: 15–37%) vs comparators (1–12%; $p < 0.001$ for all comparisons; **Figure**). Evaluation of the two trials vs GLP-1 receptor agonists (GLP-1RAs) showed that the composite endpoint was achieved by a significantly greater proportion of subjects treated with semaglutide (0.5 mg: 19%; 1.0 mg: 22–33%) vs exenatide extended release 2.0 mg (6%; SUSTAIN 3) or dulaglutide (0.75 mg: 7%; 1.5 mg: 12%; SUSTAIN 7) ($p < 0.001$ for all comparisons; **Figure**).
Conclusion: With semaglutide, a significantly greater proportion of subjects achieved clinically meaningful improvements in the composite endpoint of HbA_{1c}, BW and SBP reductions vs comparators (including other GLP-1RAs), which may promote an improved overall CV risk profile with semaglutide vs comparators.

Figure. Proportion of subjects achieving the composite endpoint of ≥1% HbA_{1c}, ≥5% body weight and ≥5 mmHg SBP reduction across the SUSTAIN 1–5 and 7 trials



*p<0.001 vs comparator. Comparison for SUSTAIN 7 is semaglutide 0.5 mg vs dulaglutide 0.75 mg and semaglutide 1.0 mg vs dulaglutide 1.5 mg. 'On-treatment without rescue medication' data are presented. Endpoints were analysed by logistic regression with treatment, trial-specific stratification and country as fixed factors, and baseline HbA_{1c}, body weight and SBP as covariates. Missing values for each component are imputed using an MMRM with trial-specific stratification and country as fixed factors, and baseline value as covariate, all nested within visit. Exenatide ER, exenatide extended release; IGlar, insulin glargine U100; MET, metformin; MMRM, mixed model for repeated measurements; N/A, not applicable; OAD, oral antidiabetic drug; SBP, systolic blood pressure; SU, sulphonylurea; TZD, thiazolidinedione.

Clinical Trial Registration Number: NCT02054897; NCT01930188; NCT01885208; NCT02128932; NCT02305381; NCT02648204
Supported by: Novo Nordisk A/S research support
Disclosure: **K.M. Dungan:** Employment/Consultancy; GlaxoSmithKline plc., Sanofi-Aventis. Grants; AstraZeneca, GlaxoSmithKline plc., Novo Nordisk Inc., Sanofi-Aventis. Other; DKBmed (CME activity, funded by Novo Nordisk, Sanofi Aventis, Merck), Horizon (CME activity funded by Sanofi Aventis), Projects in knowledge (CME activity funded by Eli Lily), Rockpointe (CME activity funded by Astra Zeneca).

739

Efficacy and safety of ITCA 650, an injection-free GLP-1 RA, in patients with type 2 diabetes: a pooled analysis of phase 3 studies

M. Baron, L. Kjems, P. Prabhakar, B. Schwartz, H. Huang;
Intarcia Therapeutics, Inc., Boston, USA.

Background and aims: ITCA 650 consists of a small titanium osmotic mini-pump that is subdermally placed in the abdominal wall during a brief office procedure. As an investigational product for the treatment of type 2 diabetes (T2D), ITCA 650 provides a continuous subcutaneous infusion of exenatide over 3 or 6 months.
Materials and methods: An integrated analysis of efficacy and safety was conducted from 2 double-blind, randomized, Phase 3 studies, which evaluated pooled data with ITCA 650 20/60 mcg/d vs. placebo or sitagliptin for the treatment of patients with T2D, inadequately controlled on antidiabetic drugs. The efficacy endpoints were mean change from baseline at Week 39 for HbA1c, body weight, composite endpoints of HbA1c and weight loss, and proportion achieving HbA1c <7%. The incidence of treatment-emergent adverse events (TEAEs) was also reported.
Results: Significant and clinically meaningful improvements at 39 weeks were observed with ITCA 650 20/60 mcg/d for all these endpoints. The higher incidence of TEAEs with ITCA 650 vs. comparators was explained by the higher incidence of GI TEAEs. Similar proportions of patients in each group discontinued for TEAEs.
Conclusion: The results are consistent with results observed from individual Phase 3 studies, which demonstrated that ITCA 650 is effective for lowering HbA1c and weight, for achieving a composite of HbA1c/weight reduction and target HbA1c <7%, and is well tolerated.

Table. Baseline characteristic, outcomes, and adverse events from pooled analysis

	ITCA 20/60 mcg/d N=418	Placebo N=154	Sitagliptin 100 mg/d N=265
Age, years	55.1 ± 9.7	54.7 ± 9.1	54.6 ± 10.3
Baseline HbA1c, % [a]	8.5 ± 0.9	8.5 ± 0.8	8.7 ± 0.9
Baseline weight, kg [a]	94.2 ± 19.5	98.2 ± 21.9	92.4 ± 21.3
Background therapy			
Metformin only	326 (78%)	66 (42.9%)	265 (100%)
Metformin + sulfonylurea	65 (15.6%)	64 (41.6%)	1 (0.4%)
HbA1c, % [b]	-1.4 ± 0.06	0.05 ± 0.15	-0.9 ± 0.07
Weight, kg [b]	-3.5 ± 0.25	-1.6 ± 0.50	-1.1 ± 0.28
HbA1c/weight decreased >0.5%/≥2 kg [c]	156 (37.3%)	17 (11%)	43 (16.2%)
HbA1c/weight decreased >1%/≥3 kg [c]	115 (27.5%)	10 (6.5%)	26 (9.8%)
HbA1c <7% [c]	165 (39.5%)	13 (8.4%)	59 (22.3%)
Any treatment-emergent AE (≥ 5%)	264 (63.2%)	69 (44.8%)	133 (50.2%)
Nausea	131 (31.3%)	15 (9.7%)	36 (13.62%)
Vomiting	88 (21.1%)	3 (1.9%)	14 (5.3%)
Minor hypoglycemia	21 (5.0%)	4 (2.6%)	5 (1.9%)
Serious TEAEs	21 (5.0%)	5 (3.2%)	20 (7.5%)

Only within treatment group comparisons were performed. Between treatment group comparisons were not performed because placebo or sitagliptin was not used in both studies in this analysis.
Background therapy included various oral agents in FREEDOM-1 and metformin only in FREEDOM-2. Excluded post rescue data.
[a] Mean ± standard deviation
[b] LS mean ± standard error change at Week 39
[c] Percentage was based on all treated patients from each group. Patients with missing Week 39 values were considered as non-achievers.

Supported by: Intarcia Therapeutics, Inc.
Disclosure: **M. Baron:** Employment/Consultancy; Intarcia Therapeutics.

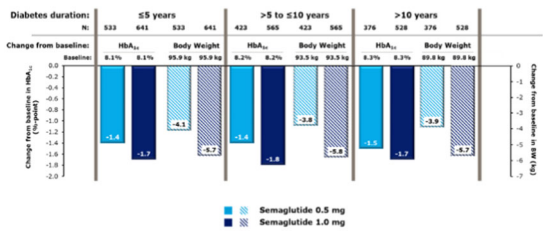
740

Consistent HbA_{1c} and body weight reduction with semaglutide independent of diabetes duration: SUSTAIN 1–5 and 7 patient-level meta-analysis

S. Madsbad[1], S.C. Bain[2], L. Chaykin[3], J. Lüdemann[4], J. Kjærulff Furberg[5], J. Rask Larsen[5], E. Yildirim[5], J. Rosenstock[6];
[1]Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark, [2]School of Medicine, Swansea University, Swansea, UK, [3]Meridien Research, Bradenton, USA, [4]Diabetes-Falkensee, Diabetes Centre and Centre for Clinical Studies, Falkensee, Germany, [5]Novo Nordisk A/S, Søborg, Denmark, [6]Dallas Diabetes Research Center at Medical City, Dallas, USA.

Background and aims: Achieving glycaemic targets is challenging. Over time, people with type 2 diabetes (T2D) have declining beta-cell function and may become less responsive to therapies. Semaglutide, a once-weekly glucagon-like peptide-1 (GLP-1) analogue for T2D treatment, showed superior reductions in HbA_{1c} and body weight in the SUSTAIN 1–5 and 7 clinical trials vs placebo or active comparators. This meta-analysis assessed the effect of semaglutide 0.5 mg and 1.0 mg on HbA_{1c} and body weight by baseline T2D duration.
Materials and methods: Efficacy and safety data from SUSTAIN 1–5 and 7 were pooled. For efficacy, 3,066 subjects receiving either semaglutide 0.5 mg or 1.0 mg were analysed by baseline T2D duration: ≤5 years (n = 1,174), >5 to ≤10 years (n = 988), >10 years (n = 904). Safety analyses by baseline T2D duration evaluated semaglutide 0.5 mg (n = 1332) and 1.0 mg (n = 1734) arms vs 2,032 subjects in the comparator arms: ≤5 years (n = 766), >5 to ≤10 years (n = 643), >10 years (n = 622). Patients were randomised and exposed to at least one dose of trial product. Proportions of subjects experiencing at least one adverse event (AE) were Cochran-Mantel-Haenszel adjusted.
Results: Semaglutide consistently reduced HbA_{1c} by 1.4–1.8 percentage-points from baseline to week 30 or 40 across subgroups by T2D duration (**Figure**). Reductions in body weight from baseline to week 30 or 40 were also consistent across subgroups (semaglutide 0.5 mg and 1.0 mg: ≤5 years, −4.1 and −5.7 kg; >5–≤ 10 years, −3.8 and −5.8 kg; >10 years, −3.9 and −5.7 kg; respectively). The proportions of subjects on semaglutide (both doses) reporting AEs were generally similar vs comparators: ≤5 years, 69.6 vs 65.9%; >5–≤ 10 years, 69.9 vs 70.8%; and >10 years, 73.5 vs 69.0%. Serious AEs were reported by 6.2 vs 6.6%, 7.2 vs 6.0% and 8.3 vs 6.3% of subjects, respectively. Nausea was reported by 20.5 vs 9.3%, 20.7 vs 9.1% and 20.1 vs 9.5%; and vomiting by 9.4 vs 4.5%, 7.2 vs 4.2% and 8.7 vs 4.3% of subjects receiving semaglutide vs comparators; premature treatment discontinuation due to AEs was reported by 7.7 vs 3.6%, 7.9 vs 3.6% and 8.5 vs 4.6% of subjects treated with semaglutide vs comparators.
Conclusion: Semaglutide consistently reduced HbA_{1c} and body weight, regardless of baseline T2D duration. The safety profile of semaglutide was as expected and appeared to be unaffected by diabetes duration.

Figure. Estimated change from baseline in HbA_{1c} and body weight to week 30 or 40 by diabetes duration in the SUSTAIN 1–5 and 7 clinical trials



On-treatment without rescue medication data were pooled on the basis of all subjects contributing to the full analysis sets in SUSTAIN 1–5 and 7. Data presented are estimated change from baseline to week 30 or 40 based on a meta-analysis across the six trials. These analyses were based on week 30 data for SUSTAIN 1 to 5, and on week 40 data for SUSTAIN 7 because the protocol did not include visits at week 30. BW, body weight.

Clinical Trial Registration Number: NCT02054897; NCT01930188; NCT01885208; NCT02128932; NCT02305381; NCT02648204
Supported by: Novo Nordisk A/S research support
Disclosure: **S. Madsbad:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novartis, Novo Nordisk, Sanofi Aventis. Grants; Novo Nordisk, Boehringer Ingelheim. Lecture/other fees; Novo Nordisk Inc., Sanofi Aventis, AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli Lilly, Merck Sharp & Dohme, Novartis.

741

Predictors of glucose-lowering response to treatment with glucagon-like peptide-1 receptor agonists in patients with diabetes and obesity
M.V. Amosova, O.Y. Gurova, V.V. Fadeev;
Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation, Moscow, Russian Federation.

Background and aims: Glucose-lowering response to glucagon-like peptide-1 receptor agonists (GLP-1 RA) varies significantly among patients with type 2 diabetes mellitus (T2DM). The mechanism of this variability has not been determined yet. On the ground of this study we would like to identify the most valued characteristics that have influence on treatment outcomes in patient with T2DM and obesity.
Materials and methods: We conducted a 24-week, prospective, open-label, randomized trial including 58 patients with T2DM and obesity (body mass index (BMI) 37,7 (35;42,1)), at the age from 35 to 71 years, receiving basal insulin (BI) + metformin+ one of the other glucose-lowering medication. Baseline glycosylated hemoglobin (HbA1c) was 9,5%(8,6;10,95%). Patients were randomized into two groups: 1) short-acting GLP-1 RA + BI + metformin; 2) long-acting GLP-1 RA + BI + metformin. The potential predictors that we analyzed in this study were type of eating behavior (according to the data from The Dutch Eating Behavior Questionnaire (DEBQ)), diabetes duration, age of the patient, C-peptide level, BMI, baseline level of HbA1c, fasting blood glucose (FPG) and postprandial glucose (PPG). We used the Spearman correlation analysis to assess association between this characteristics and glycemic response (HbA1c change 0 to 6 months).
Results: Glycemic response to GLP-1 RA was associated with diabetes duration, baseline levels of HbA1c, FPG, PPG and type of eating behavior. Diabetes duration: in patients with a history of diabetes more than 10 years, there was less reduction of HbA1c (−2.05% >10 years; −2.8%. <10 years, p = 0.02)), which, however, does not associate with the c-peptide level (p = 0.38). HbA1c: the most significant reduction was observed in patients with higher baseline HbA1c level (−3.2% (>10%) vs −1.8% (<10%) (p = 0.016). FPG: among patients with higher levels of FPG, long-acting GLP-1-RA were more effective than short-acting GLP-1-RA (−4.3 mmol/l vs −2.6 mmol/l, p = 0.039). In contrast, among patients with higher level of PPG the short-acting GLP-1-RA demonstrated greater glycemic control (−5.6 mmol/l vs −3.9 mmol/l, p = 0.002). BMI: there was no influence of the baseline BMI on the glycemic control. (p = 0.4). Eating behavior: in this cohort of patients, emotional (32%), and mixed eating model prevailed (45%). If this form of eating disorders was present the HbA1c and body weight decreased more significantly. In patients without eating disorders less effectiveness of the GLP-1-RA in reduction of HbA1c (−3.6% with disorders vs without −2.05%) and body mass (−16 kg with disorders vs without −4.5 kg) was noted.
Conclusion: In this study, we identified that certain characteristics such as diabetes duration, eating behavior, HbA1c, FPG and PPG baseline levels associated with an effective response to GLP-1-RA therapy. Identifying the predictors of a therapeutic response to the use of GLP-1-RA is the basis for rational personalization in the selection of the optimal medication. However, some aspects of this work require conducting of further research.
Disclosure: **M.V. Amosova:** None.

742

Comparison of the efficacy of insulin degludec/liraglutide (IDdegLira) and insulin glargine/lixisenatide (iGlarLixi) fix combinations: a meta-analysis
B.A. Domján[1], M.M. Svébis[1], B.D. Lovász[1], A.G. Tabák[1,2];
[1]Semmelweis University, Budapest, Hungary, [2]University College London, London, UK.

Background and aims: Fix combinations of glucagone-like peptide 1 (GLP-1) receptor agonists and basal insulins are emerging as feasible alternatives to basal-bolus insulin regimens according to recent literature. The efficacy of the 2 commercially available fix combinations of GLP-1 agonists and basal insulins (insulin degludec/liraglutide [IDegLira] and insulin glargine/lixisenatide [iGlarLixi]) has been evaluated in two meta-analysises that reached differing conclusions with the use of questionable methods. Thus our aim was to compare the efficacy of these 2 fix combinations by an indirect meta-analysis and meta-regression based on randomised controlled trials.
Materials and methods: We searched PubMed and ClinicalTrials.gov for randomised-controlled trials conducted with IDegLira and iGlarLixi published until 09/JAN/2018. Baseline characteristics of participants, active and comparator medications, mean differences between treatment arms in HbA1c, fasting glucose, and bodyweight, as well as the number of patients with hypoglicaemic events were recorded. Results were analysed using random effect model indirect meta-analysis, and indirect meta-regression adjusted for relevant baseline and treatment parameters.
Results: Altogether 9 randomised controlled trials fulfilled inclusion criteria (2 double-blind and 7 open-labelled). Mean age was 55–60 years (range), duration of diabetes was 6.3–12.1 years, mean HbA1c at the time of randomization 7.7–8.8%, 26–76% of the participants were male. According to the meta-regression with adjustment for relevant clinical parameters IDegLira treatment was associated with a 0.2% lower (95% CI −0.1–0.5%, $p = 0.17$) HbA1c-level (adjusted for the number of comparator medications and baseline bodyweight), a 0.24 mmol/l (95% CI −0.33–0.81 mmol/l; $p = 0.34$) lower fasting glucose (adjusted for the presence of basal insulin), and a 0.21 kg (95% CI −1.90–2.31 kg; $p = 0.83$) lower body weight (adjusted for the effect of comparator on body weight) compared to iGlarLixi. All these differences were statistically non-significant. IDegLira was associated with a non-significantly increased risk of hypoglycaemia (OR 1.29; 95% CI 0.66–2.53; $p = 0.39$) compared to iGlarLixi.
Conclusion: We were unable to prove a statistically significant difference in the efficacy of IDegLira and iGlarLixi based on the meta-analysis of published randomised controlled trials, although due to the wide confidence intervals clinically significant differences may still be present between these medications. Longer studies with direct comparisons are needed to more precisely compare the efficacy of IDegLira and iGlarLixi.
Disclosure: **B.A. Domján:** None.

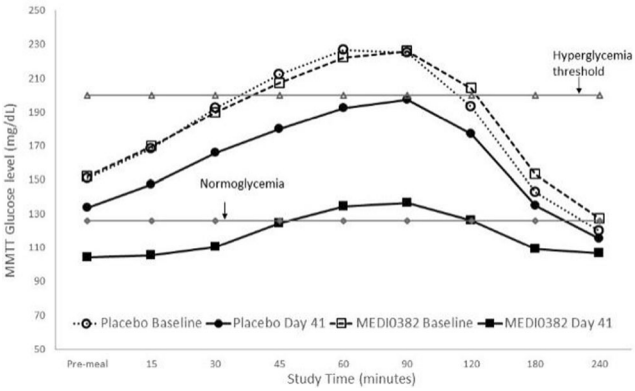
743

Robust glucose control and weight loss after 6 weeks of treatment with MEDI0382, a balanced GLP-1/Glucagon receptor dual agonist, in patients with type 2 diabetes

P. Ambery[1], M. Stumvoll[2], M. Posch[3], T. Heise[4], L. Plum-Moerschel[5], L.-F. Tsai[6], D. Robertson[1], M. Petrone[1], C. Rondinone[6], V. Parker[1], B. Hirshberg[6], L. Jermutus[1];
[1]MedImmune, Cambridge, UK, [2]University Hospital Leipzig, Leipzig, Germany, [3]Charité Research Organisation GmbH, Berlin, Germany, [4]Profil Institut, Neuss, Germany, [5]Profil Institut, Mainz, Germany, [6]MedImmune, Gaithersburg, USA.

Background and aims: MEDI0382 is under development for the treatment of type 2 diabetes mellitus and nonalcoholic steatohepatitis.
Materials and methods: In a double-blind study, 51 patients with type 2 diabetes and a body mass index of 27–40 kg/m^2 were randomized (1:1) to a daily subcutaneous dose of MEDI0382 200 μg or placebo.
Results: Based on an analysis of covariance model adjusting treatment and baseline values, MEDI0382 markedly reduced fasting glucose (change from baseline at day 41, −49.9 vs −19.2 mg/dL; $P < 0.0001$) and postprandial glucose in a mixed-meal tolerance test (percent change from baseline in glucose $AUC_{0\text{-}4h}$, −32.8 vs −10.2; $P < 0.0001$) (Figure) with no increase in hypoglycemia. HbA1c levels decreased −0.9% with MEDI0382 and −0.6% with placebo ($P = 0.0004$). Weight loss was 3.8 kg vs baseline (1.7 kg vs placebo; $P = 0.0008$); 92% of MEDI0382 patients lost >2 kg. Mean reduction in ambulatory systolic blood pressure was −4.2 for MEDI0382 vs −1.5 mmHg for placebo (P = ns). As with marketed GLP-1 analogs, a mean pulse increase of 6.8 bpm was seen for MEDI0382, vs a fall of 2.0 bpm for placebo ($P < 0.0001$). Treatment-related adverse events such as decreased appetite, vomiting, and headache were seen in more patients on MEDI0382 (20 [80.0%] vs 15 [57.7%]); none were grade >3 severity, 3 led to study discontinuation for MEDI0382 vs 1 for PBO, and 1 serious adverse event in the placebo arm.
Conclusion: Overall, MEDI0382 normalized fasting and postprandial blood glucose, significantly reduced body weight, and had an acceptable safety profile over 41 days of dosing in obese/overweight patients with type 2 diabetes mellitus.

Figure 1. Mean change from baseline in glucose AUC after MMT



Clinical Trial Registration Number: NCT02548585
Disclosure: **P. Ambery:** Employment/Consultancy; MedImmune. Stock/Shareholding; AstraZeneca.

PS 060 GLP1 receptor agonists: How good are they in real practice?

744

Effectiveness of the switch from insulin to incretin therapy in patients with long-lasting type 2 diabetes: a 6-month longitudinal, real-life study

R. Citarrella, M. Di Vittorio, A. Bonfiglio, S. Speciale, D. Ruggirello, G. Castellino, R. Chianetta, D. Nikolic, C. Mannina, G. Montalto;
DIBIMIS, University of Palermo, Italy, Palermo, Italy.

Background and aims: The effects beyond glycemic control including cardiovascular (CV) benefits of incretin-based therapies (IBTs), such as glucagon-like peptide 1 (GLP-1) receptor agonists, are well documented in type 2 diabetes mellitus (DMT2) treatment. However, several studies directly investigated the replacement of insulin by IBTs in diabetes care. Here we investigated glycemic and non-glycemic effects of GLP-1 receptor agonists compared to insulin therapy in order to reach the following objectives: 1) to demonstrate non-inferiority of IBT versus insulin in improving glycemic control and 2) to demonstrate an improvement in overall cardio-metabolic profile after the switch form insulin to GLP-1 receptor agonists.
Materials and methods: Sixty patients (32 men and 28 women; age: 61 ± 8 years) with long-lasting T2DM, treated over the 6-year period with basal-bolus insulin therapy in addition to oral hypoglycemic agents (OHA) and with poor adherence and were included in the present study. The study population was subdivided in 2 groups: 1) Group A ($n = 30$): switching from insulin to IBTs was performed (liraglutide was given to 25 patients at dose of 1.2 mg/day) and 2) Group B $n = 30$) included patients who continued and intensified the insulin therapy (average insulin dose 0.74 ± 0.31 U/kg per day). The Visceral Adiposity Index (VAI), a gender-specific mathematical model for assessing cardiometabolic risk associated with visceral obesity, was calculated. Statistical analysis was performed using Wilcoxon or the Mann-Whitney *U* test.
Results: The only statistically significant difference between 2 groups at baseline was found for triglyceride levels that was higher in group A ($p = 0.030$). After 6 months of the therapeutic switch in the group A statistically significant changes were found in all measured parameters, with the exception of total-, LDL-cholesterol and triglycerides (Table 1). In the group B, the only HbA1c changed significantly ($p = 0.004$). Comparing the 2 groups, after 6 months of follow-up from the therapeutic switch, a statistically significant difference was observed in reduction of HbA1c ($p < 0.001$), waist circumference ($p = 0.010$) and body weight ($p = 0.023$), in favor of Group A. The VAI reduced significantly ($p = 0.017$) only in the group A, as well as blood pressure (PAS $p = 0.003$ and PAD $p = 0.039$) and aminotransferases (AST $p = 0.004$ and ALT $p < 0.001$).
Conclusion: After 6-month of the therapeutic switch, GLP-1 receptor agonists were found to be more effective compared to insulin in improving glyco-metabolic compensation, promoting the reduction of anthropometric parameters. Overall, such effects may reduce progression of the complications of diabetes, including the risk of CV events and mortality rate as it has been shown in recent CVOTs.

Parameters	Mean (ds)		p-value
	Baseline	After 6 moths	
PAS (mmHg)	134.8 (15.3)	127.7 (14.1)	**0.003**
PAD (mmHg)	78.3 (11.8)	74.4 (11.9)	**0.039**
Body weight (kg)	95.8 (12.1)	86.7 (10.0)	**<0.001**
BMI (Kg/m^2)	36.3 (4.2)	32.6 (3.5)	**<0.001**
Waist circumference (cm)	115.0 (6.7)	107.6 (7.1)	**<0.001**
HbA1c (mmol/mol)	92.4 (6.0)	59.6 (8.2)	**<0.001**
Total colesterol (mmol/L)	4.54(1.29)	4.45(1.22)	0.463
HDL-C (mmol/L)	0.98(0.24)	1.11(0.19)	**0.007**
LDL-C (mmol/L)	2.57 (1.05)	2.51 (1.09)	0.609
TG (mmol/L)	2.15 (0.87)	1.82 (0.53)	0.123
AST (U/L)	27.4 (10.6)	21.4 (7.8)	**0.004**
ALT (U/L)	37.6 (12.5)	25.5 (11.2)	**<0.001**

Disclosure: **R. Citarrella:** None.

745

Comparative glycaemic effectiveness of dulaglutide vs liraglutide and exenatide once weekly in a US real-world setting

R. Mody[1], Q. Huang[2], M. Yu[3], H. Patel[1], R. Zhao[2], M. Grabner[2], L. Fernández Landó[1];
[1]Eli Lilly and Company, Indianapolis, USA, [2]HealthCore, Inc., Wilmington, USA, [3]Eli Lilly and Company, Toronto, Canada.

Background and aims: The objective of this retrospective observational study was to compare 6-month and 1-year real-world glycaemic effectiveness among patients initiating GLP-1 receptor agonists (GLP-1RA), dulaglutide (DULA) vs. liraglutide (LIRA) or DULA vs. exenatide QW (EQW), using US claims data from the HealthCore Integrated Research Database (HIRD®) between November 2014 and May 2016 (index date = earliest GLP-1RA fill date).
Materials and methods: Patients ≥18 years old with T2DM, no claim for any GLP-1RA in the 6 months pre-index period (baseline), continuous enrolment 6 months pre- and 1-year post-index, ≥1 $HbA1_c$ result pre-index and 1-year post-index were included. DULA users were propensity-matched 1:1 to LIRA (585 pairs) or EQW (422 pairs) users and the matched cohorts were balanced in baseline patient characteristics including mean $HbA1_c$.
Results: The mean age of all cohorts was 53 years and approximately 50% were males. Among the DULA vs. LIRA matched cohorts, 59% and 41% initiated on DULA 0.75 mg and 1.5 mg QW, respectively, and 44% and 56% initiated on LIRA 0.6/1.2 mg and 1.8 mg QD, respectively. The key effectiveness results are included in the table.
Conclusion: At both 6-month and 1-year post-index, patients initiating DULA experienced a greater reduction in $HbA1_c$ compared to LIRA or EQW initiators ($P < 0.05$). In all cohorts, patients adherent to their GLP-1RA treatment had greater reductions in $HbA1_c$ than non-adherent patients ($P < 0.05$).

Table: Real-world glycaemic effectiveness outcomes among GLP-1RA initiators

6-Month post-index outcomes[1], n	Matched DULA vs. LIRA cohorts		Matched DULA vs. EQW[2] cohorts	
	DULA 420	LIRA 433	DULA 307	EQW 296
$HbA1_c$ Outcomes, mean (SD) (mmol/mol)				
Pre-index $HbA1_c$ result[3]	71.69 (19.24)	71.25 (18.91)	72.23 (19.24)	70.92 (19.79)
Change in $HbA1_c$ from baseline	-12.02 (18.26)	-9.40 (17.27)*	-12.57 (18.47)	-10.06 (18.36)*
Change in $HbA1_c$ among adherent patients[4]	-14.10 (17.16)‡	-11.37 (16.29)‡	-14.65 (17.93)‡	-13.55 (17.16)‡
Change in $HbA1_c$ among non-adherent patients[5]	-8.20 (19.57)	-7.65 (17.93)	-8.31 (18.91)	-7.98 (18.80)
12-Month post-index outcomes[5], n	**DULA 585**	**LIRA 585**	**DULA 422**	**EQW 422**
$HbA1_c$ Outcomes, mean (SD) (mmol/mol)				
Pre-index $HbA1_c$ result[3]	72.45 (19.90)	71.91 (19.79)	73.00 (20.22)	73.66 (21.32)
Change in $HbA1_c$ from baseline	-10.71 (18.58)	-8.42 (18.80)*	-10.93 (19.24)	-8.42 (19.57)*
Change in $HbA1_c$ among adherent patients[6]	-13.66 (17.27)‡	-11.59 (18.58)‡	-14.43 (18.36)‡	-12.13 (14.76)‡
Change in $HbA1_c$ among non-adherent patients[6]	-7.43 (19.57)	-6.23 (18.58)	-6.56 (19.35)	-6.89 (21.10)

*Significant difference in mean $HbA1_c$ change between the matched cohorts with P-value <0.05.
‡Significant difference in mean $HbA1_c$ change between adherent and non-adherent patients within each cohort with P-value <0.05.
[1]Assessed among subgroup of patients with 6-month post-index $HbA1_c$ results (between index date + 93 days and index date + 228 days).
[2]The study included only exenatide QW pen users.
[3]Pre-index $HbA1_c$ results were obtained between index date - 183 days and index date + 14 days.
[4]Adherent patients were defined as those with proportion of days covered (PDC) ≥80% at 6-month post-index.
[5]Assessed among patients with 1-year post-index $HbA1_c$ results (between index date + 275 days and index date + 410 days).
[6]Adherent patients were defined as those with PDC ≥80% at 1-year post-index.

Supported by: Eli Lilly and Company
Disclosure: **R. Mody:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

746

Time to treatment intensification with GLP-1 receptor agonists for patients with type 2 diabetes in the UK: medical record review study

K. Norrbacka[1], D. Stein[2], L.S. Matza[3], J.B. Jordan[3], L.-E. García-Pérez[4], S.W. Hassan[4], K.S. Boye[4];
[1]Eli Lilly Finland, Helsinki, Finland, [2]Evidera, London, UK, [3]Evidera, Bethesda, USA, [4]Eli Lilly and Company, Indianapolis, USA.

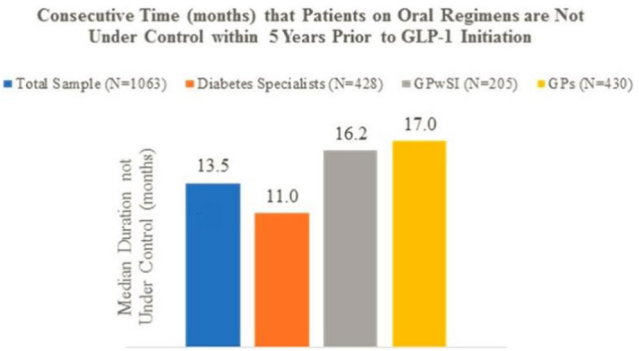
Background and aims: Patients with type 2 diabetes (T2D) who fail to meet recommended glycemic control targets are at increased risk of diabetes complications. The National Institute for Health and Care Excellence (NICE) guidelines recommend drug intensification in patients not able to maintain HbA1c levels ≤7.0% (53 mmol/mol). For patients who cannot maintain glycemic control on oral medication, one recommended option is to add an injectable glucagon-like peptide-1 receptor

agonist (GLP-1 RA) to their treatment regimen. This is the first known study to examine time to treatment intensification with GLP-1 RA, including the duration of time that patients did not maintain glycemic control with oral medication.

Materials and methods: This was a medical record review conducted in the UK via an online physician survey from Jul to Oct 2017. Participating physicians represented three medical specialties: endocrinologists/diabetologists (specialists), general practitioners (GPs) with special interest in diabetes (GPwSIs), and GPs with no special diabetes interest (GPs). Patients eligible to have their records reviewed were required to be ≥18 years of age, have a confirmed T2D diagnosis, and have newly initiated GLP-1 RA for T2D in the prior 6 months. All HbA1c within 5 years prior to GLP-1 RA initiation were collected. Duration of poor glycemic control since most recently added oral regimen (oral±injectable) was calculated based on consecutive HbA1c values >7.0% (53 mmol/mol) prior to GLP-1 RA initiation.

Results: 113 physicians (38.9% Specialists, 20.4% GPwSIs, 40.7% GPs) contributed data for 1096 patients (Specialists: 437, 39.9%, GPwSIs: 216, 19.7%, GPs: 443, 40.4%). Patient mean (SD) age at GLP-1 RA initiation was 55 (12), 607 were male (55.4%), and 782 were white/Caucasian (71.4%); mean (SD) BMI was 35.2 (6.0) and mean (SD) number of HbA1c assessments/year was 1.9 (0.8). Most common diabetes treatment regimen prior to GLP-1 RA was oral (918, 83.8%) followed by oral+injectable (145, 13.2%). Median time from T2DM diagnosis to GLP-1 RA initiation was 6.1 years. Median time from most recently added oral diabetes regimen (oral ± injectable) to GLP-1 RA initiation was 3.0 years; majority of patients on oral regimens had ≥1 uncontrolled HbA1c prior to GLP-1 RA initiation (1047, 98.5%). Median consecutive time patients on oral regimens were not under control prior to GLP-1 RA initiation was 13.5 months (Specialists: 11.0, GPwSIs: 16.2, GPs: 17.0 months) (Figure).

Conclusion: Results suggest that treatment intensification is often delayed despite consistently poor glycemic control for more than 12 months, contrary to treatment guideline recommendations. Findings from this study highlight that there may be T2D patients who would benefit from more rapid treatment intensification, thereby reducing the risk for many short and long-term health complications.



Disclosure: **K. Norrbacka:** Employment/Consultancy; Employee of Eli Lilly. Stock/Shareholding; Shareholder of Eli Lilly and Co.

747

Early impact of liraglutide in routine clinical use (ABCD nationwide liraglutide audit) on cardiovascular risk (UKPDS risk engine)

C. Walton[1], S. Kassim[2], R. Harper[3], P. McDonald[3], U. Brennan[3], J. Harding[3], T. Htay[4], R. Nayar[5], A. Pernet[6], S. Rowles[7], K. Adamson[8], K.Y. Thong[9], R.E.J. Ryder[10], ABCD nationwide liraglutide audit contributors;
[1]Hull Royal Infirmary, Hull, UK, [2]Northern HSC, Antrim, UK, [3]The Ulster Hospital, Dundonald, UK, [4]Kingston Hospital, London, UK, [5]City Hospitals, Sunderland, UK, [6]Kings College Hospital, London, UK, [7]Pennine Acute Hospitals, Manchester, UK, [8]St John's Hospital, Livingston, UK, [9]School of Medicine and Pharmacology, Perth, Australia, [10]City Hospital, Birmingham, UK.

Background and aims: Liraglutide has been shown to reduce cardiovascular outcomes in patients at high cardiovascular disease (CVD) risk (LEADER study). Uncertainty exists regarding the impact of liraglutide on CVD risk in routine clinical care. The United Kingdom Prospective Diabetes Study (UKPDS) CVD risk engine version 2.0 uses recognised risk factors to calculate future CVD risk. Our aim was to investigate the impact of liraglutide in routine use on 10 year CVD risk.

Materials and methods: We used data from the Association of British Clinical Diabetologists (ABCD) nationwide liraglutide audit which assesses liraglutide in routine clinical practice (6959 patients, 163 centres, 2009–2017). For this analysis we included all patients with all the factors utilised by the risk engine (age, duration of diabetes, ethnicity, systolic blood pressure, HbA1c, total cholesterol and HDL cholesterol) measured before and at the earliest return to clinic between 3 and 9 months after commencing liraglutide. As we did not have data on atrial fibrillation or smoking these were assumed to be absent for the purposes of the analysis.

Results: The table shows baseline characteristics of the 747 patients and the early impact of liraglutide treatment on CVD risk factors. There were highly significant falls in all parameters involved in CVD risk assessment other than HDL cholesterol which was unchanged. The UKPDS risk engine mean ± SD 10 year coronary heart disease (CHD) risk fell by 2.7 ± 7.6% from 18.7 ± 13.0% to 16.1 ± 11.6% ($p < 0.001$). 10 year fatal CHD risk fell by 0.3 ± 2.8% from 7.9 ± 8.7% to 7.6 ± 8.3% ($p < 0.001$). 10 year stroke risk fell by 0.94 ± 1.35% from 5.92 ± 4.27% to 4.98 ± 3.45% ($p = 0.003$). 10 year fatal stroke risk fell by 0.1 ± 0.7% from 1.2 ± 1.4% to 1.1 ± 1.3% ($p = 0.001$). Weight, which is not a factor utilised in the UKPDS risk engine was assessed in the 3535 patients in the audit with weight and BMI data during the same time interval. Weight fell by 2.8 ± 6.1 kg from 110.0 ± 22.3 to 107.9 ± 22.1 kg ($p < 0.001$), and BMI by 0.98 ± 2.2 kg/m^2 from 38.7 ± 7.0 to 37.8 ± 6.9 kg/m^2 ($p < 0.001$).

Conclusion: Starting liraglutide reduced 10-year CVD risk. These data suggest that liraglutide used in routine clinical care in 100 patients could prevent 3 events of CHD or stroke and save 2 or more lives over the next 10 years. As this represented the earliest assessment after commencement of liraglutide it is possible that the impact would be greater with longer follow up. The results are likely to be an underestimate as the UKPDS risk engine does not take into account BMI which is also reduced by liraglutide.

Parameter	Baseline	At 3-9 months	Difference	P value
Age (years)	56.6±10.3			
Sex (%male)	56.2			
Ethnicity: % White % Afro-Caribbean % Asian-Indian	89.2 2.9 7.9			
Diabetes Duration (Median (IQR) years)	9.0 (6.0-13.0)			
HbA1c (mmol/mol)	77.2±18.0	67.4±18.6	-9.8±17.9	<0.001
HbA1c (%)	9.2±1.6	8.3±1.7	-0.9±1.6	<0.001
Systolic Blood Pressure (mm Hg)	136.8±16.6	133.3±17.3	-3.5±17.7	<0.001
Serum total cholesterol (mmol/L)	4.22±1.57	3.97±1.01	0.25±1.45	<0.001
Serum HDL cholesterol (mmol/L)	1.10±0.32	1.12±0.79	-0.02±0.78	0.39
Weight (kg) (n=3535)	110.0±22.3	107.9±22.1	-2.8±6.1	<0.001
BMI (kg/m^2) (n=3535)	38.7±7.0	37.8±6.9	-0.98±2.2	<0.001

Table: Baseline characteristics of the 747 patients who returned to clinic between 3 and 9 months after starting liraglutide and the change in cardiovascular risk parameters at the return visit as mean±SD or median (interquartile range [IQR]). Weight and BMI measurements in 3535 patients during the same time interval. P-values reflect change from baseline.

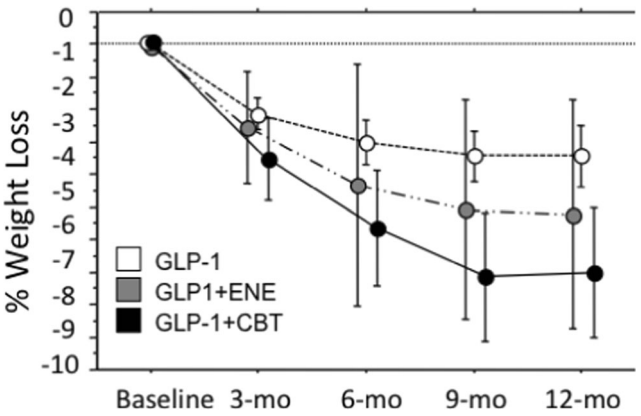
Disclosure: **C. Walton:** None.

748

COMBINATION study: COMbined Behavioural/INcretin Action sTudy In Obese/diabetic persoNs

L. Montesi[1], A. Mazzotti[1], L. Brodosi[1], M.T. Caletti[1], F.A. Barbanti[1], P. Di Bartolo[2], G. Marchesini[1];
[1]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy, [2]Diabetes Clinical Network, Romagna Local health Authority, Ravenna, Italy.

Background and aims: Cognitive-behavior therapy (CBT) and drug treatment show multiplicative effects in obesity treatment. We aimed to test the reinforcing effects of CBT in patients with type 2 diabetes mellitus (T2DM), newly-treated with glucagon-like peptide-1 receptor agonists (GLP1-RAs).
Materials and methods: The anthropometric and clinical data of 207 T2DM patients (93 F, 114 M; mean age 56 ± SD 10.3) who shifted to GLP-1RAs in the period 2013–6 were retrospectively analyzed and weight loss trajectories were reconstructed to determine the effects of new treatment in relation to the participation in structured lifestyle intervention programs. The primary outcome was weight loss (≥10% initial body weight); secondary endpoint was A1c target reach (<53 mmol/mol - 7%). According to our protocol, behavior therapy is offered to all cases first attending our institution. Behavioral treatments were carried out within 6 months of GLP-1RA treatment and were classified either as elementary nutritional education (ENE, 5 group sessions) or as more intensive lifestyle interventions (CBT treatment, 12 group sessions). 147 cases (68 F, 79 M) received liraglutide, 35 exenatide BID (14 F, 21 M), 20 exenatide-LAR (6 F, 12 M) and only 5 the most recent GLP-1RA, dulaglutide (2 F, 3 M). 151 case did not receive behavior therapy (CONT-Group A; 64 F, 87 M); 56 cases participated in educational programs during the pre-specified temporal window (Group B, divided into ENE - B1 ($n = 20$) and CBT - B2 ($n = 36$)).
Results: In the whole group, 38 cases (19%) reached the primary end-point (weight loss ≥10%) and 122 cases (61%) reached the secondary end-point (A1c <7%) at 12-month follow-up. Among the different sub-groups, weight loss was larger in Group B compared with Group A: after 6 months the average weight loss was −3.2 ± 4.4% in Group A and −6.4 ± 2.6% e −7.7 ± 5.9% in ENE and CBT, respectively. The trend was maintained after 12 months: −3.7 ± 6.0% in Group A, −5.6 ± 4.5% in ENE e −6.8 ± 7.4% in CBT ($P < 0{,}0001$). No significant differences among groups were measured in the A1c end-point ($P = 0.195$). At logistic regression analysis, the participation into CBT increased the probability of weight loss target reach (OR, 2.59; 95% CI, 1.24-5-41; $p = 0.011$). The secondary end point reach (HbA1c <7%) was associated with baseline metabolic control and the achievement of the primary end-point (OR, 3.47; 1.44–8.36). In both analyses BMI at GLP-1RA shifting, sex and age did not change the association with outcome reach.
Conclusion: The results suggest that initial GLP1-RA treatment may be effectively summed up to behavior therapy to enhance weight loss in T2DM. Initial treatment with GLP-1RAs may be a crucial point to implement effective, successful behavioral treatment.



Clinical Trial Registration Number: 182/2917/O/OssN
Supported by: Fondazione del Monte BO-RA (FdM/4714)
Disclosure: **L. Montesi:** Grants; Supported by grant from Fondazione del Monte di Bologna e Ravenna, 2016.

749

GLP-1 RA treatment: a benefit-risk analysis from a retrospective cohort study

E. Konstantara, A.P. McGovern, W. Hinton, R. Coyle, M. Feher, N. Munro, S. de Lusignan;
Department of Clinical and Experimental Medicine, University of Surrey, Guildford, UK.

Background and aims: Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are an established drug class of injectable non-insulin treatments for type 2 diabetes (T2D). Benefits include reductions in HbA_{1c} and weight, and low hypoglycaemia risk. A predominant limiting factor of widespread use of GLP-1 RAs is gastrointestinal side-effects. We aimed to identify which people are most likely to benefit from GLP-1 RAs with the lowest risk of adverse effects.
Materials and methods: We performed a retrospective cohort study using routine data from a primary care sentinel network in England (Royal College of General Practitioners Research and Surveillance Centre). People with T2D were identified if they had been prescribed GLP-1 RA therapy (Exenatide, Lixisenatide, Liraglutide, Albiglutide or Dulaglutide) between 1 January 2005 and 31 July 2016. Using regression modelling, factors associated with improvement in HbA_{1c}, weight reduction, presence of gastrointestinal side-effects (nausea, vomiting, and diarrhoea) were identified. Treatment discontinuations over 1 year from the first GLP-1 RA prescription were also recorded. Adjustments were made for; gender, age, ethnicity, socioeconomic status, BMI, duration of diabetes, and HbA_{1c} at initiation.
Results: From 144,427 people with T2D, 3.8% ($n = 5515$) were initiated on GLP-1 RA. Mean HbA_{1c} at GLP-1 RA initiation was 76.05 mmol/mol (SD 18.86) with mean HbA_{1c} improvement −4.99 mmol/mol (SD 18.90). Mean BMI at initiation was 37.98 kg/m^2 (SD 7.03) with mean BMI improvement −1.19 (SD 3.18). After adjusting for baseline HbA_{1c}, people 55+ years old had a greater reduction in HbA_{1c} at 1 year, compared to those <55 years (55–74 years: −2.63; 95% CI −4.25 to −1.02; 75+ years: −5.12; 95% CI −7.61 to −2.63). Females, compared to males, showed a greater HbA_{1c} reduction (−1.89 mmol/mol; 95% CI −3.21 to −0.56). People of black ethnicity showed a smaller reduction in HbA_{1c} at 1 year, compared to people of white ethnicity (6.54 mmol/mol; 95% CI 2.38 to 10.71); no other ethnic differences were identified. People who had diabetes for 10+ years had a smaller reduction in HbA_{1c}, compared to people who had diabetes for under a year (3.44 mmol/mol; 95% CI 1.23 to 5.65). Socioeconomic status and BMI were not associated with glycaemic improvement. Side effects were almost twice as frequently reported by females (OR 1.79; 95% CI 1.27 to 2.51). There were no other associations with reporting of adverse effects. Weight reduction was greater in those with the highest initial BMI (−0.13 kg/m^2; 95% CI −0.14 to −0.11). There were no other associations identified. Treatment discontinuation was less common in those who had T2D for 1–3 years (OR 0.49; 95% CI 0.37 to 0.67) and more common in those diagnosed 10+ years ago (OR 1.73; 95% CI 1.45 to 2.05) compared to those with T2D for 4–6 years. Treatment discontinuation was also less likely in those with a high HbA_{1c} at initiation (73.9+ mmol/mol) (OR 0.61; 95% CI 0.49 to 0.74) compared to those with a lower HbA_{1c} (47.6–57.4 mmol/mol); and more likely for those with a BMI ≥40 kg/m^2 (OR 1.54; 95% CI 1.21 to 1.97) compared to those with BMI 25.0–29.9 kg/m^2.
Conclusion: Older people and those with diabetes of short duration achieve the greatest glycaemic benefit from GLP1 RAs. Females also have greater HbA_{1c} reduction despite reporting more adverse effects. Weight loss appears to be consistent across all groups. These findings will help develop a targeted approach to GLP-1 RA prescribing in individuals with T2D.
Disclosure: **E. Konstantara:** None.

750

Lifetime cost-effectiveness simulation of exenatide once-weekly in type 2 diabetes: evidence from the EXSCEL trial

F. Becker[1], H. Dakin[1], S.D. Reed[2], Y. Li[2], J. Leal[1], S.M. Gustavson[3], B. Kartman[4], E. Wittbrodt[3], A.M. Gray[1], A.F. Hernandez[2], R.R. Holman[5]; [1]Health Economics Research Centre, Oxford, UK, [2]Duke Clinical Research Institute, Durham, USA, [3]AstraZeneca Research and Development, Gaithersburg, USA, [4]AstraZeneca, Mölndal, Sweden, [5]Diabetes Trials Unit, Oxford, UK.

Background and aims: The Exenatide Study of Cardiovascular Event Lowering (EXSCEL) assessed the effect of branded exenatide 2 mg once-weekly (EQW) vs placebo added to usual care in 14,752 patients with type 2 diabetes (T2D), with or without previous cardiovascular disease. This pragmatic, randomized, double-blind, placebo-controlled, event-driven trial demonstrated a statistically non-significant reduction in major adverse cardiovascular events and a nominally significant reduction in all-cause mortality with allocation to EQW. We assessed the lifetime cost-effectiveness of EQW added to usual care, compared with usual care alone.

Materials and methods: Medical resource use and EuroQol 5-Dimension (EQ-5D) data were collected throughout the study. Within-trial results were extrapolated to a lifetime horizon using the UKPDS Outcomes Model version 2 after accounting for missing within-trial data. Cost-effectiveness was evaluated separately for US and UK settings, with costs assessed from a healthcare perspective and outcomes measured by quality-adjusted life-years (QALYs). The base case analyses extrapolated the 9% annual discontinuation rate observed in the trial forward for the first 10 years of the extrapolated period. In the US setting, a 23.1% discount on the wholesale acquisition price of branded EQW was applied. Further analyses were performed using pre-specified patient sub-groups and assuming patients who were still receiving EQW at the end of the trial remained on branded EQW during lifetime.

Results: Branded EQW plus usual care was estimated to gain 0.151 QALYs ($p < 0.001$) over a lifetime horizon at an additional cost of USD34,410 ($p < 0.001$) per patient, compared with usual care in a US setting. The incremental cost-effectiveness ratio (ICER) was estimated as USD230,429/QALY (base case; Table). In a UK setting, the estimated net gain was 0.141 QALYs ($p < 0.001$) at an additional cost of GBP4,566 ($p < 0.001$) with an ICER of GBP32,782/QALY. The base case ICERs exceeded the standard cost-effectiveness thresholds of USD100,000 and GBP20,000 per QALY, respectively. However, the ICERs for different sub-groups were found to be considerably lower, with an ICER of USD88,608 for patients enrolled in US sites and an ICER of GBP16,319 for patients aged 65 years and older in the UK setting.

Conclusion: Branded EQW added to usual care was associated with greater QALY gain and additional costs compared with usual care alone during a lifetime. The base case ICERs exceeded standard cost-effectiveness thresholds. However, EQW was cost-effective in specific sub-groups of the trial population, such as patients enrolled in US sites and (in the UK setting) patients aged 65 years and older.

Table 1: Lifetime cost-effectiveness of exenatide once-weekly vs placebo – base case, sensitivity and sub-group analyses

	n	US			UK		
		Additional costs (SE) [USD]	*Additional QALYs (SE)*	*ICER (95% CI) [USD/QALY]*	*Additional costs (SE) [GBP]*	*Additional QALYs (SE)*	*ICER (95% CI) [GBP/QALY]*
Base case (accounting for discontinuation of EQW)	EQW: 7,356 Plac: 7,396	34,410 (616)	0.151 (0.018)	230,429 (173,110 to 287,749)	4,566 (252)	0.141 (0.017)	32,782 (24,580 to 40,984)
All patients remaining on treatment during lifetime horizon	EQW: 7,356 Plac: 7,396	41,955 (719)	0.162 (0.018)	262,895 (204,574 to 321,215)	6,328 (259)	0.150 (0.017)	42,596 (32,791 to 52,400)
Patients enrolled in US sites	EQW: 1,567 Plac: 1,597	35,151 (1,451)	0.402 (0.046)	88,608 (68,211 to 109,006)	#	#	#
Age ≥65 years	EQW: 2,964 Plac: 2,975	31,220 (953)	0.270 (0.028)	116,704 (93,734 to 139,675)	4,020 (413)	0.249 (0.025)	16,319 (11,895 to 20,742)
Patients with cardiovascular history at baseline	EQW: 5,394 Plac: 5,388	31,758 (747)	0.163 (0.020)	197,589 (150,833 to 244,346)	4,106 (319)	0.154 (0.018)	26,923 (19,899 to 33,947)

EQW: exenatide once-weekly, Plac: placebo, SE: standard error, USD: US dollars, GBP: British pound, QALY: quality-adjusted life-year; ICER: incremental cost-effectiveness ratio.
 # The sample size of patients enrolled in UK sites was too small to estimate robust results for this sub-group.

Clinical Trial Registration Number: NCT01144338
Supported by: AstraZeneca (Gaithersburg, MD, USA).
Disclosure: **F. Becker:** Grants; Amylin Pharmaceuticals (AstraZeneca, Gaithersburg, MD, USA).

PS 061 GLP1 receptor agonists: Do age and ethnicity matter?

751

Efficacy and safety of semaglutide in elderly subjects with type 2 diabetes: a post hoc analysis of the SUSTAIN 7 trial

V.R. Aroda[1,2], D. Sugimoto[3], D. Trachtenbarg[4], M. Warren[5], A. Navarria[6], G. Nayak[6], M. Abildlund Nielsen[6], V. Woo[7];
[1]Brigham and Women's Hospital, Boston, USA, [2]MedStar Health Research Institute, Hyattsville, USA, [3]Cedar Crosse Research Center, Chicago, USA, [4]University of Illinois College of Medicine at Peoria, Peoria, USA, [5]Physicians East, Greenville, USA, [6]Novo Nordisk A/S, Søborg, Denmark, [7]University of Manitoba, Winnipeg, Canada.

Background and aims: The SUSTAIN 7 trial investigated efficacy and safety of semaglutide, a glucagon-like peptide-1 analogue, vs dulaglutide in subjects with type 2 diabetes (T2D). The aim of this *post hoc* analysis was to compare the efficacy and safety of semaglutide and dulaglutide in elderly (≥65 years old) vs non-elderly (<65 years old) in the SUSTAIN 7 trial.
Materials and methods: Subjects with T2D were randomised to once-weekly subcutaneous semaglutide or dulaglutide for 40 weeks. As pre-specified in SUSTAIN 7, semaglutide 0.5 mg was compared with dulaglutide 0.75 mg; semaglutide 1.0 mg with dulaglutide 1.5 mg. For this analysis, subjects were stratified by age (≥65 and <65 years old). Post-baseline data were analysed using a mixed model for repeated measurements.
Results: This analysis comprised 1,199 subjects (260 elderly and 939 non-elderly; mean ages 69.3 and 51.9 years, respectively). Mean baseline HbA_{1c} and body weight were lower in elderly vs non-elderly subjects (**Table**). Across treatment arms, reductions from baseline were similar between elderly and non-elderly subjects for HbA_{1c} (interaction p value: $p > 0.05$) and body weight (interaction p value: $p > 0.05$). Reductions in HbA_{1c} and body weight were greater with semaglutide vs dulaglutide in all subgroups (**Table**). The proportions of subjects achieving HbA_{1c} <7.0% and ≤6.5% were higher in elderly than non-elderly subjects across all treatment arms, and were higher with semaglutide vs dulaglutide across subgroups (**Table**). This is consistent with lower baseline HbA_{1c} in elderly vs non-elderly subjects and similar reductions in HbA_{1c} between age groups. More elderly than non-elderly subjects reported adverse events (AEs) with semaglutide 1.0 mg and dulaglutide 1.5 mg. More elderly than non-elderly subjects reported serious AEs across all treatment arms except semaglutide 1.0 mg. Most AEs were mild to moderate in severity. A higher proportion of elderly than non-elderly subjects discontinued semaglutide 1.0 mg due to AEs. The proportion of subjects discontinuing treatment due to AEs in other treatment arms was similar between elderly and non-elderly subjects (**Table**).
Conclusion: In the SUSTAIN 7 trial, reductions in HbA_{1c} and body weight were comparable in both elderly and non-elderly subjects, and were greater with semaglutide vs dulaglutide across most subgroup comparisons. These improvements in glycaemic control in elderly subjects were not associated with a higher incidence of hypoglycaemia. The overall safety profile for semaglutide was in line with the SUSTAIN 1–5 trials and these results may help guide treatment in elderly patients with T2D.

Table. Changes in HbA_{1c} and body weight, and AEs, in elderly vs non-elderly subjects in SUSTAIN 7

		Semaglutide 0.5 mg		Dulaglutide 0.75 mg		Semaglutide 1.0 mg		Dulaglutide 1.5 mg	
		Elderly n=79	Non-elderly n=222	Elderly n=61	Non-elderly n=238	Elderly n=53	Non-elderly n=247	Elderly n=67	Non-elderly n=232
HbA_{1c}, %	Observed mean at BL	8.1	8.4	8.0	8.2	7.9	8.3	8.0	8.3
	Est. mean change from BL	−1.5	−1.5	−1.2	−1.1	−1.6	−1.8	−1.4	−1.4
	ETD* [95% CI];								
	Elderly	−0.33 [−0.65;−0.01]				−0.27 [−0.63;0.09]			
	Non-elderly	−0.43 [−0.61;−0.25]				−0.45 [−0.62;−0.28]			
Achieving target, %	HbA_{1c} <7%	77.2	65.3	57.4	50.8	84.9	77.3	77.6	63.4
	HbA_{1c} ≤6.5%	59.5	45.5	36.1	33.6	75.5	64.8	59.7	43.5
Body weight, kg	Observed mean at BL	92.4	97.8	92.1	96.5	87.4	97.2	90.2	94.3
	Est. mean change from BL	−4.9	−4.4	−2.6	−2.2	−6.7	−6.5	−3.8	−2.8
	ETD* [95% CI];								
	Elderly	−2.32 [−3.89;−0.74]				−2.92 [−4.67;−1.17]			
	Non-elderly	−2.26 [−3.12;−1.39]				−3.73 [−4.58;−2.87]			
Subjects reporting AEs, n (%)	Overall AEs	52 (65.8)	152 (68.5)	36 (59.0)	150 (63.0)	41 (77.4)	166 (67.2)	54 (80.6)	167 (72.0)
	Serious AEs	7 (8.9)	10 (4.5)	6 (9.8)	18 (7.6)	3 (5.7)	20 (8.1)	6 (9.0)	16 (6.9)
	Severe AEs	3 (3.8)	17 (7.7)	4 (6.6)	12 (5.0)	5 (9.4)	15 (6.1)	6 (9.0)	13 (5.6)
	Premature discontinuation due to AEs	7 (8.9)	17 (7.7)	2 (3.3)	12 (5.0)	10 (18.9)	19 (7.7)	4 (6.0)	16 (6.9)
	Gastrointestinal AEs	30 (38.0)	99 (44.6)	20 (32.8)	80 (33.6)	28 (52.8)	105 (42.5)	35 (52.2)	108 (46.6)
	Gallbladder disorders	0 (0)	2 (0.9)	0 (0)	4 (1.7)	0 (0)	4 (1.6)	3 (4.5)	5 (2.2)
	Hepatic disorders†	3 (3.8)	4 (1.8)	1 (1.6)	8 (3.4)	0 (0)	8 (3.2)	2 (3.0)	6 (2.6)
	Severe or BG-confirmed‡ symptomatic hypoglycaemia	0 (0)	2 (0.9)	2 (3.3)	1 (0.4)	1 (1.9)	4 (1.6)	0 (0)	5 (2.2)

*Semaglutide 0.5 mg vs dulaglutide 0.75 mg; semaglutide 1.0 mg vs dulaglutide 1.5 mg; †Hepatic disorders were defined by system organ class terms: hepatobiliary investigations, hepatobiliary disorders and hepatobiliary neoplasms; ‡BG <3.1 mmol/L (<56 mg/dL). For proportion of subjects achieving HbA_{1c} targets, on-treatment without rescue medication data with missing HbA_{1c} (%) data were imputed from an MMRM, with treatment and country as fixed factors and baseline value as covariate, all nested within visit. After imputation, continuous HbA_{1c} (%) data were dichotomised according to ADA and AACE criteria. Elderly ≥65 years old; non-elderly <65 years old. AACE, American Association of Clinical Endocrinologists; ADA, American Diabetes Association; AE, adverse event; BG, blood glucose; BL, baseline; CI, confidence interval; Est. estimated; ETD, estimated treatment difference; MMRM, mixed model for repeated measurements.

Clinical Trial Registration Number: NCT02648204
Supported by: Novo Nordisk A/S research support
Disclosure: **V.R. Aroda:** Employment/Consultancy; Adocia, Astra Zeneca, Janssen, Medscape, Novo Nordisk, Sanofi, Tufts. Grants; Amylin, Astra Zeneca, Boehringer Ingelheim, Calibra Medical, Eisai Inc., Elcelyx, GI Dynamics, GSK, N-Gene, Novo Nordisk, Sanofi, Takeda, Theracos Inc. Honorarium; IMNE, Novo Nordisk, Sanofi.

752

Efficacy and safety of lixisenatide as add-on in patients with type 2 diabetes aged ≥70 years uncontrolled on basal insulin in the GetGoal-O study

G. Dailey[1], T. Dex[2], M. Roberts[2], G. Meneilly[3];
[1]Scripps Whittier Diabetes Institute, La Jolla, USA, [2]Sanofi, Bridgewater, USA, [3]Department of Medicine, University of British Columbia, Vancouver, Canada.

Background and aims: Patients aged ≥70 years with type 2 diabetes (T2D) are often excluded from clinical trials. The GetGoal-O trial specifically enrolled elderly, nonfrail patients with T2D uncontrolled on their current therapy. This analysis assesses the efficacy and safety of lixisenatide as add-on therapy to basal insulin (BI) in these patients with or without renal insufficiency.
Materials and methods: A post hoc subgroup analysis was performed in nonfrail patients ≥70 years, randomized to receive once-daily 20 μg lixisenatide or placebo on a background of BI ± oral antidiabetic drugs for 24 weeks. Patients were stratified by background antidiabetic therapy and presence of renal insufficiency (defined as having an estimated glomerular filtration rate [eGFR] of 30≤eGFR<60 or eGFR ≥60 mL/min/1.73 m^2) at screening.
Results: In total, 108 patients met the inclusion criteria, of whom 36 had 30≤eGFR<60 mL/min/1.73 m^2. Average BI dose at baseline was 0.47 U/kg, indicating that most patients were approaching a point where they would require postprandial glucose control. Overall, patients randomized to lixisenatide, compared with placebo, had significantly greater reductions in HbA1c, 2-h postprandial glucose (2-h PPG), average 7-point self-monitored plasma glucose (SMPG), and weight. No significant differences between treatment arms were observed for change in fasting plasma glucose (FPG), eGFR, or daily BI dose by weight. Treatment-emergent adverse events (TEAEs) occurred more frequently in the lixisenatide

group. Incidence of documented symptomatic hypoglycemia (<60 mg/dL) was low in both groups: 5.7% of lixisenatide and 12.7% of placebo-treated patients. There were no instances of severe symptomatic hypoglycemia in either treatment group. Significantly more patients treated with lixisenatide achieved >0.5% reduction in HbA1c with no documented symptomatic hypoglycemia compared with the placebo group (P = 0.002) (Table). Although reduction in HbA1c also remained significantly greater in lixisenatide-treated patients regardless of baseline eGFR, the difference was slightly greater in those with renal insufficiency (−0.68% vs −0.55%). No significant differences for other efficacy outcomes were observed between patients stratified by baseline eGFR; insufficient numbers were available for a meaningful analysis of hypoglycemia or TEAEs.

Conclusion: This post hoc analysis suggests that adding lixisenatide in nonfrail patients with T2D aged ≥70 years uncontrolled with BI is efficacious and has a good safety profile. Furthermore, despite small expected increases in exposure and a very small sample size, this treatment approach suggests similar efficacy of lixisenatide in patients with mild-to-moderate renal insufficiency, with no dose adjustments required.

Table

	Placebo (N=55)	Lixisenatide (N=53)
Age, years	74.7±4.4	73.8±4.1
Duration of BI treatment, years	3.81±3.38	4.32±4.97
Daily dose of BI/weight, U/kg	0.48±0.31	0.47±0.23
HbA1c - Baseline, %	8.19±0.72	8.24±0.75
Week 24 (LOCF)	8.36±0.96	7.69±1.00
LS mean difference (SE), *P* value	**−0.68 (0.167); *P*=0.0001**	
2-h PPG - Baseline, mmol/L	15.60±4.39	15.49±4.24
Week 24 (LOCF)	14.80±4.29	9.93±4.20
LS mean difference (SE), *P* value	**−4.38 (0.925) *P*<0.0001**	
7-pt SMPG - Baseline, mmol/L	10.07±2.12	10.48±2.44
Week 24 (LOCF)	9.85±2.01	8.87±1.81
LS mean difference (SE), *P* value	**−0.97 (0.435), *P*=0.0290**	
Weight - Baseline, kg	82.05±18.80	79.23±12.80
Week 24 (LOCF)	82.20±19.34	77.70±13.17
LS mean difference (SE), *P* value	**−1.36 (0.525), *P*=0.0108**	
eGFR categories at screening[a], n (%)		
≥30–<60 mL/min/1.73m^2	19 (34.5)	17 (32.1)
≥60 mL/min/1.73m^2	36 (65.5)	36 (67.9)
Patients reaching HbA1c reduction >0.5% without documented symptomatic hypoglycemia[b], n (%)	8 (14.5)	22 (41.5)
% difference (95% CI)	**27.0 (10.75–43.17) *P*=0.0020**	
Any TEAE, n (%)	5 (9.1)	18 (34.0)
Diarrhea	4 (7.3)	5 (9.4)
Nausea	1 (1.8)	14 (26.4)

Data are shown as mean ± SD, unless indicated otherwise.
[a] Screening was done at Week −1; [b] Symptomatic hypoglycemia as defined by PG <3.3 mmol/L (60 mg/dL).

Clinical Trial Registration Number: NCT01798706

Disclosure: **G. Dailey:** Employment/Consultancy; Sanofi, Novo Nordisk. Grants; Sanofi, Novo Nordisk, Eli Lilly and Company. Honorarium; Sanofi, AstraZeneca.

753

Dulaglutide has favourable outcomes in elderly or renal impairment patients with type 2 diabetes

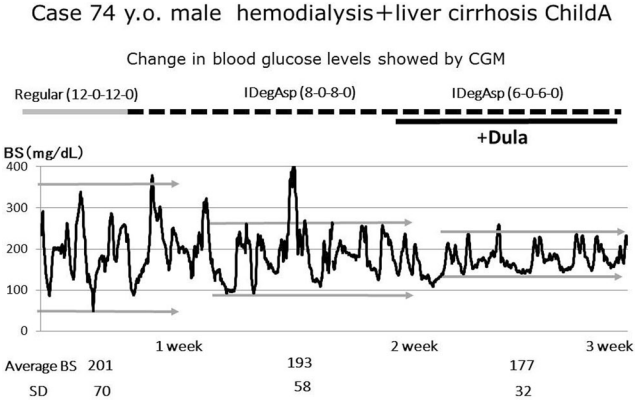
M. Kadoya, Y. Ueda, Y. Tahara, S. Kaneko;
Takatsuki Red Cross Hospital, Takatsuki, Osaka, Japan.

Background and aims: Japan is becoming a super-aged society. Additionally, dialysis is being performed in one out of 400 people. Therefore, safe, effective and convenient therapies for elderly or renal impairment type 2 diabetic patients (T2D) including those undergoing dialysis are needed. We investigated the long-term efficacy and safety of a new once weekly GLP1RAg dulaglutide (Dula) on glycemic control in the above.

Materials and methods: We conducted two different retrospective analyses of the efficacy and safety of Dula in T2D for 2 years according to; 1) subgroups stratified by ages (≤70 years old or >71 years old), 2) renal impairment including those undergoing dialysis**.**

Results: Regarding 1), Of a total of 360 T2D, 180 elderly T2D (>71 years old, 94 males, 78.2 ± 5.0 years old, disease duration of 7.6 ± 7.4 years, HbA1c 8.3 ± 1.8%, BMI 24.1 ± 4.1) and 180 younger T2D (≤70 years old, 113 males, 60.4 ± 9.7 years old, disease duration of 5.6 ± 5.6 years, HbA1c 7.9 ± 1.4%, BMI 25.5 ± 4.4) were newly administered Dula weekly. Elderly and younger T2D treated with Dula achieved 7.0 ± 0.9% and 7.2 ± 0.9% in HbA1c after 2 years respectively. 20 elderly T2D were injected with help from a carer due to cognitive impairment. Three patients discontinued Dula due to intolerance. Nocturnal hypoglycemia never occurred. Regarding 2), 33 patients with renal impairment (Impairment group) (69.6 ± 9.7 years old, HbA1c 7.7 ± 1.1%, BMI 23.8 ± 3.7, eGFR 39.7 ± 10.2 mL/min/1.73 m^2), 8 patients undergoing dialysis (Dialysis group) (6 hemodialysis and 2 peritoneal dialysis) and 1 patient who received a kidney transplant (Transplant group) were administered Dula weekly. The levels of HbA1c in Impairment group maintained <7% and their levels of eGFR remained as they were at the start of Dula therapy. Only one patient in Dialysis group discontinued Dula due to vomiting. In the remainder of Dialysis group and in Transplant group, there were no complications due to administration of Dula and good blood glucose levels were maintained.

Conclusion: Weekly GLP-1RAg provides more effective and safer glycemic control in elderly T2D with/without renal impairment irrespective of whether dialysis was being performed. In addition to relieving patient burden due to weekly injections, the administration of Dula might be expected to reduce the risk of hypoglycemia and lower the burden on carers in an aging society. It might also be expected to improve the QOL of patients due to a reduction in the overall burden of taking medicines (i.e. by switching from daily to weekly injections) and a reduction in the incidence of hypoglycemia. Carers' burdens in aiding elderly patients, compounded by a labor force shortage, will also be lessened. The net result could also be a reduction in medical costs.



Disclosure: **M. Kadoya:** None.

754

Effect of liraglutide on cardiovascular outcomes in elderly patients in the LEADER trial

M.P. Gilbert[1], S.C. Bain[2], E. Franek[3], E. Jodar-Gimeno[4], M.A. Nauck[5], R.E. Pratley[6], R. Réa[7], J. Saraiva[8], S. Rasmussen[9], K. Tornøe[9], B. von

Scholten[9], J. Buse[10], LEADER Publication Committee on behalf of the LEADER Trial Investigators;
[1]The Robert Larner, M.D. College of Medicine at The University of Vermont, South Burlington, USA, [2]Institute of Life Science, Swansea University, Swansea, UK, [3]Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, [4]Quirónsalud Madrid University Hospital, Madrid, Spain, [5]Diabetes Center Bochum-Hattingen, St. Josef-Hospital (Ruhr-Universität Bochum), Bochum, Germany, [6]Florida Hospital Translational Research Institute, Orlando, USA, [7]Clinical Hospital of the Federal University of Paraná (SEMPR), Curitiba, Brazil, [8]Pontifícia Universidade Católica de Campinas, Campinas, Brazil, [9]Novo Nordisk A/S, Søborg, Denmark, [10]University of North Carolina School of Medicine, Chapel Hill, USA.

Background and aims: The burden of chronic diseases such as type 2 diabetes (T2D) and associated comorbidities increases with ageing, making the elderly more vulnerable to potential side effects of medical treatment. Information about the effect of antihyperglycaemic therapy on cardiovascular (CV) events in the elderly is limited. Liraglutide reduced the risk of CV events in the LEADER trial compared with placebo, and this post hoc analysis assesses risk of CV events and all-cause mortality in elderly patients with T2D.
Materials and methods: In the LEADER trial, 9340 patients with T2D at high risk for CV events were randomised 1:1 to liraglutide or placebo, both on top of standard of care, and followed for up to 5 years. The primary composite outcome was defined as time to first occurrence of death from CV causes, non-fatal myocardial infarction (MI) or non-fatal stroke. Secondary outcomes included the expanded composite outcome and all-cause mortality. In this analysis, outcomes were assessed in patients aged ≥75 years.
Results: Among those aged ≥75 years, baseline characteristics were well matched between treatment arms ($N = 418$ for both treatment arms). Compared with placebo, liraglutide significantly reduced the risk of the primary composite outcome, expanded composite outcome, non-fatal MI, coronary revascularisation, death from any cause and death from non-CV causes (Table).
Conclusion: In the LEADER trial, liraglutide treatment reduced the risk of CV events and all-cause mortality in elderly patients with T2D.

Table: Risk of cardiovascular events in patients aged ≥75 years

Outcome	Liraglutide (N=418)		Placebo (N=418)		HR (95% CI)	*p*-value (test for HR=1)
	N (%)	Events/ 100 PYO	N (%)	Events/ 100 PYO		
Primary composite endpoint	77 (18.4)	4.9	106 (25.4)	7.0	0.69 (0.51–0.92)	0.012
Expanded composite endpoint*	108 (25.8)	6.9	140 (33.5)	9.2	0.73 (0.56–0.93)	0.013
CV death	34 (8.1)	2.2	42 (10.0)	2.8	0.78 (0.50–1.23)	0.291
Non-fatal MI	30 (7.2)	1.9	51 (12.2)	3.4	0.56 (0.36–0.88)	0.011
Non-fatal stroke	19 (4.5)	1.2	22 (5.3)	1.5	0.84 (0.45–1.55)	0.577
Hospitalisation for heart failure	28 (6.7)	1.8	42 (10.0)	2.8	0.64 (0.40–1.03)	0.067
Coronary revascularisation	32 (7.7)	2.1	48 (11.5)	3.2	0.64 (0.41–1.00)	0.049
Hospitalisation for unstable angina	11 (2.6)	0.7	9 (2.2)	0.6	1.20 (0.50–2.90)	0.684
All-cause death	60 (14.4)	3.9	83 (19.9)	5.5	0.70 (0.50–0.97)	0.035

HRs and p-values are from Cox proportional hazards model with treatment, subgroup and their interaction. *Expanded composite endpoint = primary composite endpoint plus coronary revascularisation, hospitalisation for unstable angina and for heart failure.
N, number of patients with an event; PYO, patient-years of observation

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **M.P. Gilbert:** Other; Funding: Novo Nordisk A/S.

755

Efficacy and safety of semaglutide in subjects with type 2 diabetes by race and ethnicity: a post hoc analysis of the SUSTAIN trials

C. Desouza[1], S. Garg[2], B. Cariou[3], J.K. Furberg[4], A. Navarria[4], G. Nayak[4], V. Fonseca[5];
[1]University of Nebraska Medical Center, Omaha, USA, [2]Barbara Davis Center for Diabetes, University of Colorado Denver, Denver, USA, [3]CHU de Nantes, L'Institut du Thorax, Department of Endocrinology, Nantes, France, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Tulane University Health Sciences Center, New Orleans, USA.

Background and aims: The SUSTAIN clinical trials investigated the effects of semaglutide, a glucagon-like peptide-1 analogue for the treatment of type 2 diabetes (T2D). This *post hoc* analysis compared efficacy and safety of semaglutide in race and ethnicity subgroups pooled from SUSTAIN 1–5 and 7.
Materials and methods: Subjects with T2D were randomised to once-weekly subcutaneous semaglutide 0.5 or 1.0 mg (only 1.0 mg in SUSTAIN 3) vs placebo (SUSTAIN 1 and 5; 30 weeks), sitagliptin (SUSTAIN 2; 56 weeks), exenatide extended release (SUSTAIN 3; 56 weeks), insulin glargine (SUSTAIN 4; 30 weeks) or dulaglutide (SUSTAIN 7; 40 weeks). Efficacy (change from baseline in HbA_{1c} and body weight) and safety (adverse event [AE]) data at week 30 (SUSTAIN 1–5) or 40 (SUSTAIN 7) were pooled and analysed by race (Asian, Black/African American, Caucasian, Other) and ethnicity (Hispanic, non-Hispanic).
Results: The analysis included 3,066 subjects who received semaglutide 0.5 or 1.0 mg, pooled from SUSTAIN 1–5 and 7. 'Other' race subgroup data are not reported due to low subject numbers ($n = 25$ [semaglutide 0.5 mg]; $n = 50$ [semaglutide 1.0 mg]). Estimated changes from baseline in HbA_{1c} and body weight by race and ethnicity are shown in the table. HbA_{1c} reductions ranged from 1.4 to 1.5% with semaglutide 0.5 mg. With semaglutide 1.0 mg, HbA_{1c} was reduced by 1.6–2.0%; Asians had greater reductions than Black/African Americans or Caucasians despite similar baseline HbA_{1c}. Body weight reductions in Caucasians were 4.2 and 6.0 kg with semaglutide 0.5 and 1.0 mg, respectively, and ranged from 3.3 to 5.3 kg in remaining race groups. Reductions were smallest in Asians, who also had the lowest baseline weight. Body weight reductions were greater in non-Hispanics vs Hispanics (4.2 vs 2.6 kg and 6.1 vs 4.1 kg with semaglutide 0.5 and 1.0 mg). The proportions of subjects experiencing AEs, gastrointestinal AEs and AEs leading to treatment discontinuation are shown in the table. The proportion of Caucasian, Black/African American and Asian subjects reporting serious AEs were 6.8, 5.1 and 4.1% with semaglutide 0.5 mg, and 7.7, 7.6 and 4.3% with semaglutide 1.0 mg. The proportion of Hispanics and non-Hispanics reporting serious AEs were 6.6 and 4.7% with semaglutide 0.5 mg, and 7.9 and 5.1% with semaglutide 1.0 mg.
Conclusion: Semaglutide provided clinically relevant reductions in HbA_{1c} (1.4–2.0%) and body weight (2.6–6.1 kg) in subjects with T2D across SUSTAIN 1–5 and 7 regardless of race and ethnicity. The safety profile in each subgroup was similar to that of all subjects in the SUSTAIN clinical trial programme, with slight variations in safety across race and ethnicity groups.

Table. Estimated change from baseline to 30 or 40 weeks in HbA_{1c} and body weight, and AEs by race and ethnicity subgroups across SUSTAIN 1–5 and 7

			Baseline	Number of subjects	Semaglutide 0.5 mg	Number of subjects	Semaglutide 1.0 mg
HbA_{1c} (%)	Race	Caucasian	8.2	982	−1.4 (0.03)	1,328	−1.7 (0.02)
		Asian	8.2	243	−1.5 (0.05)	232	−2.0 (0.05)
		Black/African American	8.3	82	−1.4 (0.09)	124	−1.6 (0.07)
	Ethnicity	Hispanic	8.3	208	−1.4 (0.06)	324	−1.7 (0.05)
		Non-Hispanic	8.2	1,124	−1.5 (0.03)	1,410	−1.8 (0.02)
Body weight (kg)	Race	Caucasian	97.0	982	−4.2 (0.14)	1,328	−6.0 (0.12)
		Asian	75.3	243	−3.3 (0.28)	232	−4.6 (0.29)
		Black/African American	94.9	82	−3.3 (0.48)	124	−5.3 (0.39)
	Ethnicity	Hispanic	88.1	208	−2.6 (0.31)	324	−4.1 (0.24)
		Non-Hispanic	94.4	1,124	−4.2 (0.13)	1,409	−6.1 (0.12)
AEs (%)	Race	Caucasian	-	982	69.0	1,328	69.8
		Asian	-	243	76.6	232	73.3
		Black/African American	-	82	63.4	124	67.4
	Ethnicity	Hispanic	-	208	67.7	324	62.0
		Non-Hispanic	-	1,124	70.5	1,410	72.5
GI AEs (%)	Race	Caucasian	-	982	39.4	1,328	40.6
		Asian	-	243	48.9	232	49.7
		Black/African American	-	82	32.8	124	32.9
	Ethnicity	Hispanic	-	208	42.5	324	37.0
		Non-Hispanic	-	1,124	40.3	1,410	42.5
AEs leading to PTD (%)	Race	Caucasian	-	982	6.4	1,328	8.0
		Asian	-	243	10.0	232	13.4
		Black/African American	-	82	5.1	124	6.9
	Ethnicity	Hispanic	-	208	6.0	324	7.7
		Non-Hispanic	-	1,124	7.0	1,410	8.8

Efficacy analyses included pooled 'on-treatment without rescue medication' data from all subjects contributing to the full analyses sets from SUSTAIN 1–5 and 7. Data from the 'Other' race subgroup are not included due to low subject numbers. These analyses were based on week 30 data for SUSTAIN 1–5, and week 40 data for SUSTAIN 7 (due to the visit schedule not including week 30). Presented HbA_{1c} and body weight data are estimated change from baseline (standard error of the estimate) based on a meta-analysis across the six trials. Safety summaries included pooled 'on-treatment' data from all subjects in the safety analysis sets. AE data are presented as proportion of subjects experiencing an AE. AE, adverse event; GI, gastrointestinal; PTD, premature treatment discontinuation.

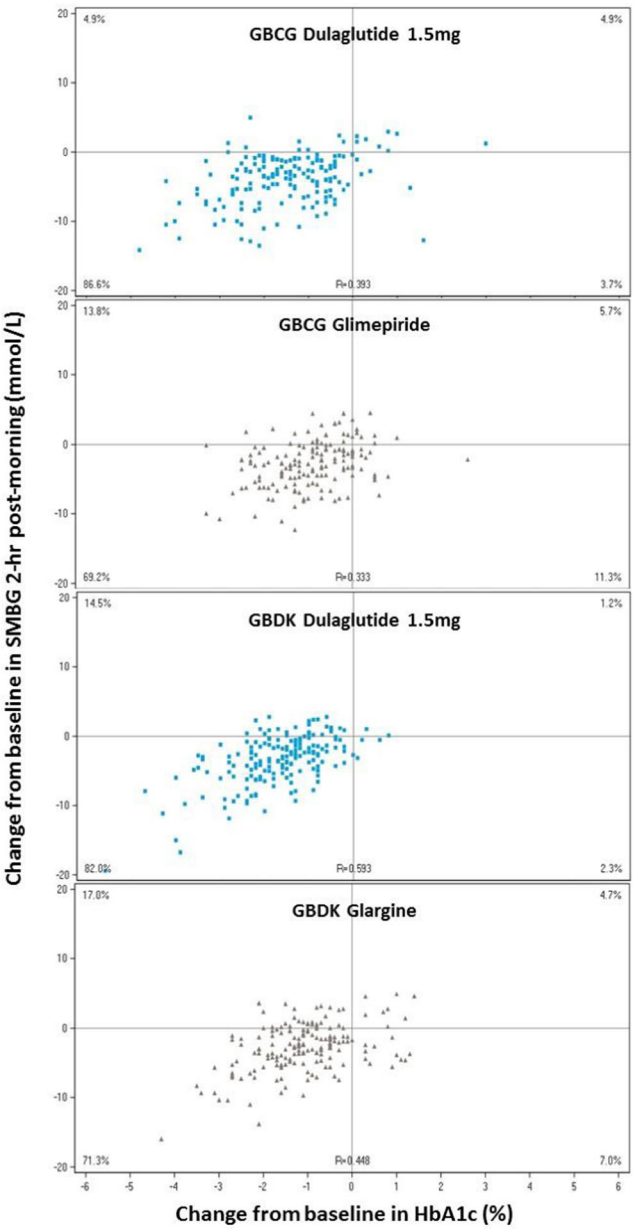
Clinical Trial Registration Number: NCT02054897, NCT01930188, NCT01885208, NCT02128932, NCT02305381, NCT02648204
Supported by: Novo Nordisk A/S research support
Disclosure: **C. Desouza:** Employment/Consultancy; Novo Nordisk. Grants; Janssen, NIH, Novo Nordisk, Sanofi, Theracos. Honorarium; None. Lecture/other fees; None. Non-financial support; Novo Nordisk (travel and writing support). Stock/Shareholding; None. Other; None.

756

Relationship between HbA_{1c} reduction and postprandial glucose change with once weekly dulaglutide in Chinese patients with type 2 diabetes

T. Hong[1], X. Liu[2], W. Zhao[2], F. Wang[2], L. Gu[2];
[1]Peking University Third Hospital, Beijing, [2]Lilly Suzhou Pharmaceutical Co. Ltd., Shanghai, China.

Background and aims: A post-hoc analysis was conducted on two randomized trials of dulaglutide, a once weekly GLP-1 receptor agonist to assess the relationship between HbA1c reduction and postprandial glucose (PPG) change in Chinese patients with type 2 diabetes mellitus (T2DM) after 26 weeks of treatment with dulaglutide 1.5 mg or 0.75 mg vs glimepiride or insulin glargine.
Materials and methods: Patients in the dulaglutide vs glimepiride study (GBCG, $n = 556$) were treatment-naive or discontinued from monotherapy with oral anti-diabetic drugs; those in the dulaglutide vs glargine study (GBDK, $n = 591$) continued on metformin and/or sulfonylurea. Analyses were conducted based on mixed-models repeated measures using modified intent-to-treat population, by trial rather than by pooling, due to difference in background therapies and baseline characteristics.
Results: The superiority of dulaglutide 1.5 mg vs. glimepiride or glargine was achieved in the two studies. In GBCG study, least-squares (LS) mean changes from baseline in HbA1c were −1.46%, −1.25%, and −0.92%; and the LS mean changes in PPG (2-hr post-morning meal, mmol/L) measured by SMBG was −4.07, −3.28, and −2.71 in dulaglutide 1.5 mg, 0.75 mg, and glimepiride groups, respectively. In GBDK study, LS mean changes from baseline in HbA1c were −1.67%, −1.31%, and -1.11%; and the LS mean changes in PPG was −3.41, −3.12, and −2.74 in dulaglutide 1.5 mg, 0.75 mg, and insulin glargine groups, respectively. In the two studies, 92%–97% and 86%–92% of patients treated with dulaglutide 1.5 mg and 0.75 mg, respectively, demonstrated HbA1c reduction, vs 83% and 88% with glimepiride and insulin glargine. Among the patients with HbA1c reduction, 82%–87% and 76%–80% of patients treated with dulaglutide 1.5 mg and 0.75 mg also experienced PPG reduction, vs 69% and 71% with glimepiride and glargine. Correlation was observed between HbA1c reduction and PPG change in dulaglutide 1.5 mg and its comparator in both GBCG ($R = 0.393$ for dulaglutide, $R = 0.333$ for glimepiride) and GBDK ($R = 0.593$ for dulaglutide, $R = 0.448$ for glargine) studies.
Conclusion: Dulaglutide is an effective treatment option for Chinese patients with T2DM, resulting in significant HbA1c reduction. In addition, the association between HbA1c reduction and PPG change further supports a strong effect of dulaglutide on PPG, besides the known effect on fasting glucose.



Clinical Trial Registration Number: NCT01644500, NCT01648582
Disclosure: **T. Hong:** None.

757

Safety and efficacy of lixisenatide vs sulfonylurea added to basal insulin in patients with type 2 diabetes who fast during Ramadan (LixiRam): a randomised controlled trial

M.M. Hassanein[1], K. Hafidh[2], N. Shehadeh[3], S. Azar[4], W. Hanif[5], H. Li[6], K. Djaballah[7], R. Sahay[8];
[1]Dubai Hospital, Dubai, United Arab Emirates, [2]Rashid Hospital, Dubai, United Arab Emirates, [3]Rambam Medical Center, Haifa, Israel, [4]American University of Beirut Medical Center, Beirut, Lebanon, [5]University Hospital Birmingham, Birmingham, UK, [6]Sanofi-Aventis (China), Beijing, China, [7]Sanofi, Paris, France, [8]Osmania Medical College, Hyderabad, India.

Background and aims: Some type 2 diabetes (T2D) people who fast during Ramadan are at increased risk of hypoglycaemia. This is more so for those treated with insulin. Lixisenatide (Lixi) is a once-daily prandial glucagon-like peptide-1 receptor agonist (GLP-1 RA) with a relatively low risk of hypoglycaemia. This is the first randomized controlled trial (RCT) comparing the efficacy and safety of Lixi vs sulphonylurea (SU), both combined with basal insulin (BI), in T2D people fasting during Ramadan.
Materials and methods: Adults with T2D, diagnosed for ≥1 year with insufficient glycaemic control with BI + SU intending to fast during Ramadan, were randomized 1:1 to receive BI + SC Lixi or oral SU at optimal doses in this phase IV, 12–22-week trial. Primary endpoint: % of people with ≥1 documented symptomatic hypoglycaemia event (plasma glucose ≤70 mg/dL; 3.9 mmol/L) during the Ramadan fast. Secondary endpoints included HbA_{1c}, weight, BI dose, and safety.
Results: In total, 184 people were randomized (Lixi [$n = 92$]; SU [$n = 92$]; safety population). Primary endpoint: for Lixi vs SU, respectively, 3.3% (3/91) vs 8.9% (8/90) of people had ≥1 documented symptomatic hypoglycaemia event (intent-to-treat population [$n = 181$]; Lixi [$n = 91$]; SU [$n = 90$]; OR: 0.34; 95% CI: 0.09, 1.35) during Ramadan. Secondary safety: for Lixi vs SU, respectively the % of people with any hypoglycaemia event was 3.3% vs 15.2% (OR: 0.17 [95% CI: 0.05, 0.61]; difference: −0.12 [95% CI: −0.20, −0.04]) during the pre-Ramadan period and 4.3% vs 17.4% (OR: 0.22 [95% CI: 0.07, 0.68]; difference: −0.13 [95% CI: −0.22, −0.04]), during Ramadan. For Lixi vs SU, respectively, no severe hypoglycaemia was reported during pre-Ramadan, and 0% vs 1.1% during Ramadan. Total people with any hypoglycaemia during the entire observation period were 15.8% overall with 5.4% vs 26.1% (OR: 0.16 [95% CI: 0.06, 0.44]) for Lixi vs SU, respectively. Secondary efficacy: least squares mean (standard error) absolute change from baseline to post-Ramadan for Lixi vs SU, respectively: HbA_{1c} (%), −0.43 (0.11) vs −0.49 (0.11); weight (kg), −2.1 (0.27) vs −1.4 (0.27); and BI dose (units), 2.44 (0.63) vs 3.24 (0.64). Any treatment-emergent adverse events (TEAEs) for Lixi vs SU, respectively, were 17.4% vs 16.3% during Ramadan; no treatment-emergent serious adverse events were reported and TEAEs leading to discontinuation were 1.1% vs 0%. No deaths were reported.
Conclusion: In this first RCT exploring treatment with a GLP-1 RA + BI in T2D people fasting during Ramadan, the % of people experiencing ≥1 symptomatic hypoglycaemia event during Ramadan was numerically lower with Lixi. Any hypoglycaemia was clinically and statistically significantly lower in Lixi added to BI compared to SU added to BI. HbA_{1c} and weight fell despite a slight increase in BI dose in both arms. No specific safety concerns were raised.
Clinical Trial Registration Number: NCT02941367
Supported by: Sanofi
Disclosure: **M.M. Hassanein:** Employment/Consultancy; Sanofi, Boehringer Ingelheim, Novo Nordisk. Lecture/other fees; Sanofi, Novo Nordisk, Lilly, MSD, Janssen, Lifescan BI.

758

Physicians' intention and actual pattern of treatment in drug-naive patients with type 2 diabetes in the real-world setting in Japan

H. Murayama[1], K. Imai[1], M. Bauer[2], M. Odawara[3];
[1]Medical Division, Novartis Pharma K.K, Tokyo, Japan, [2]Real World Data Analytics, Novartis Global Service Center, Dublin, Ireland, [3]Department of Diabetes, Endocrinology, Metabolism, and Rheumatology, Tokyo Medical University, Tokyo, Japan.

Background and aims: The Japanese guidelines for type 2 diabetes mellitus (T2DM) don't define tailored first-line therapy. Few reports show physicians' intentions for treatment choice and real-world treatment patterns of anti-diabetes drugs (AD) for drug-naive patients. We conducted a web-survey and database analysis to address these evidence gaps.
Materials and methods: We conducted a multiple choice question web-survey to physicians across eight regions in Japan. The primary endpoint was treatment factors and patient characteristics that influenced treatment choice for drug-naive patients. We also conducted a secondary data analysis of patients diagnosed with T2DM from October 2012 to September 2016 in the Medical Data Vision database. The primary endpoint was proportion of T2DM patients receiving each type of AD as first-line therapy. Secondary endpoints were features of drug-naive T2DM patients treated with the first and second most frequently used AD and requiring additional T2DM treatment during 180 days post-outpatient first-line (O-1L) treatment initiation.
Results: A total of 491 physicians participated in the web survey. Dipeptidyl peptidase-4 inhibitors (DPP-4is) were the most preferred first-line AD by both diabetes specialists and non-specialists, followed by metformin (54% and 39% of total ADs, respectively). Regression analysis revealed the dominant factor for choice of DPP-4is over metformin was its ease of use in patients with renal impairment, while metformin was chosen over DPP-4is for improved insulin resistance and low cost. The key patient characteristics driving the choice of DPP-4is over metformin as first-line AD and treatment intensification by physicians were similar for both groups. Post prandial glucose and renal function for DPP-4is; age, BMI, insulin resistance, and renal function for metformin.In the database analysis, 224,761 drug-naïve patients received O-1L T2DM treatment: 58,933 (26.2%) and 16,029 (7.1%) patients were treated with DPP-4is or metformin, respectively; 78,709 (35.0%) or 20,877 (9.3%) patients were treated with AD-combination therapy and any insulin therapy, respectively. Regression analysis revealed higher body mass index (OR: 0.90), diabetic retinopathy (OR: 0.74), liver disease (OR: 0.96) and higher HbA1c level (OR: 0.83) were patient characteristics influenced treatment choice of metformin over DPP-4is whereas male (OR: 1.10), age (OR: 1.06), renal disease (OR: 4.20), coronary heart disease and stroke (OR: 2.22) and clinic visits (OR: 1.31) influenced choice of DPP-4is over metformin. Male (OR: 1.03), diabetic retinopathy (OR: 1.33), diabetic neuropathy (OR: 1.05). diabetic nephropathy (OR: 1.08), higher baseline HbA1c level (OR: 1.45) and clinic visits (OR: 1.15) were positively associated with receiving additional T2DM treatment during 180 days post index date, while age (OR: 0.98), liver disease (OR: 0.88), renal disease (OR: 0.94) and coronary heart disease and stroke (OR: 0.73) were negatively associated.
Conclusion: We found similarities and differences between physicians' intention and actual prescription of drugs among drug-naive T2DM patients in Japan. These findings may help establish a treatment algorithm for T2DM, which may be especially useful for non-DM specialists.
Supported by: Novartis Pharma
Disclosure: **H. Murayama:** Employment/Consultancy; Novartis Pharma K.K.

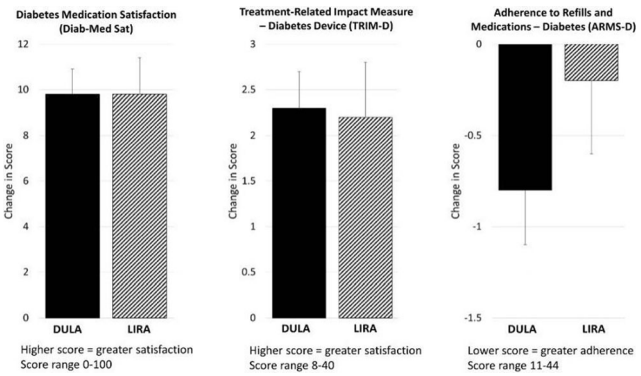
PS 062 Incretin-based therapies: adherence and tolerability

759

Patient reported outcomes following initiation of glucagon-like peptide-1 receptor agonists (GLP-1 RA) in patients with type 2 diabetes: PROGRESS-DIABETES study

R.E. Brown, A. Abitbol, H.S. Bajaj, H. Khandwala, R. Goldenberg, S. Abdel-Salam, R. Aronson;
LMC Diabetes & Endocrinology, Toronto, Canada.

Background and aims: Patient-reported outcomes (PRO's) can offer a unique patient perspective on the effectiveness of a therapy. The objective of this study was to better understand the influence of GLP-1 RA on patient reported outcomes in patients with type 2 diabetes (T2D) in a real-world clinical setting.
Materials and methods: This prospective, observational cohort study evaluates PRO's in patients initiating GLP-1 RA therapy in a diabetes specialist practice in Canada. Patients with T2D initiating GLP-1 RA therapy as part of their usual treatment approach were enrolled from Ontario-based LMC Diabetes & Endocrinology clinics. Patients completed questionnaires assessing diabetes medication satisfaction (Diab-Med Sat), diabetes device satisfaction (TRIM-D Diabetes), and diabetes medication adherence (ARMS-D) at baseline and at follow-up (3–6 months). Standard clinical outcomes were collected.
Results: Of the subjects who have enrolled into this study, 186 initiated once-weekly dulaglutide (DULA), 119 initiated once-daily liraglutide (LIRA), and 24 switched from LIRA to DULA. At baseline, PRO's and clinical measures were similar between cohorts (mean age 54.7 ± 9.7 years; mean HbA1c 8.4 ± 1.4%; mean body weight 99.3 ± 23.2 kg). In this interim analysis of completers to date who did not prematurely discontinue therapy (mean follow up 3.9 months), DULA completers (N = 135) and LIRA completers (N = 78) had similar improvements in the diabetes medication satisfaction and device satisfaction scores. There was a trend for DULA to have a greater improvement in diabetes medication adherence scores (Figure). To-date, DULA subjects had a 1.0 ± 1.1% reduction in HbA1c and 2.1 ± 3.0 kg reduction in weight. The LIRA subjects showed similar trends (0.9 ± 1.1% reduction in HbA1c and 2.8 ± 3.0 kg reduction in weight).
Conclusion: Interim analysis of this real-world, specialist-led registry of patients initiating GLP-1 RA therapy showed similar improvements in PRO's and clinical outcomes, with a trend for greater improvement in medication adherence in the DULA cohort compared to the LIRA cohort.



Supported by: Eli Lilly Canada
Disclosure: **R.E. Brown:** Grants; This study was funded by Eli Lilly Canada.

760

Dulaglutide has higher adherence and persistence than liraglutide and exenatide QW: 1-year follow-up from US real-world data

L. Fernández Landó[1], R. Mody[1], Q. Huang[2], M. Yu[3], R. Zhao[2], H. Patel[1], M. Grabner[2];
[1]Eli Lilly and Company, Indianapolis, USA, [2]HealthCore, Inc., Wilmington, USA, [3]Eli Lilly and Company, Toronto, Canada.

Background and aims: The objective of this retrospective real-world observational study was to compare 1-year adherence and persistence among patients initiating GLP-1 receptor agonists (GLP-1RA), dulaglutide (DULA) vs. liraglutide (LIRA) or DULA vs. exenatide QW (EQW) in the US, using claims data between November 2014 and May 2016 (index date = earliest GLP-1RA fill date) from the HealthCore Integrated Research Database (HIRD®).
Materials and methods: Patients ≥18 years old with T2DM, no claim for index drug in the 6 months pre-index period and continuous enrolment 6 months pre- and 1-year post-index were included. DULA users were propensity-matched 1:1 to LIRA (2,427 pairs) or EQW (1,808 pairs) users, and the matched cohorts were balanced in baseline characteristics.
Results: The mean age was 54 years with around 52% males. The key adherence and persistence outcomes are included in the table. At 1 year, DULA users were more likely to be adherent (Proportion of Days Covered [PDC] ≥80%) than LIRA (odds ratio [OR] = 1.76, 95% CI = [1.56, 1.99]) or EQW users (OR = 2.31, 95% CI = [2.00, 2.66]). Cox regression showed that DULA users were less likely to discontinue therapy than LIRA (hazard ratio [HR] = 0.75, 95% CI = [0.69, 0.81]) or EQW users (HR = 0.58, 95% CI = [0.53, 0.63]).
Conclusion: At 1-year follow-up, patients initiating DULA had higher medication adherence, and were more persistent to their treatment compared to patients initiating either LIRA or EQW.

Table: 1-Year Follow-up Adherence and Persistence Outcomes Among GLP-1 RA Initiators

	Matched DULA vs. LIRA Cohorts			Matched DULA vs. EQW[1] Cohorts		
	DULA	LIRA	P-value[2]	DULA	EQW[1]	P-value[2]
	N=2,427	N=2,427		N=1,808	N=1,808	
Proportion of Days Covered (PDC), mean (SD) in %	67.3% (32.1)	59.5% (32.6)	<0.001	66.8% (32.2)	51.3% (34.6)	<0.001
Adherence (PDC ≥80%), %	51.2%	38.2%	<0.001	50.7%	31.9%	<0.001
Patients who discontinued therapy, %	51.1%	62.0%	<0.001	51.1%	70.9%	<0.001
Persistence[3], mean (SD) days	251.9 (135.7)	217.3 (143.0)	<0.001	250.5 (136.8)	191.6 (139.3)	<0.001

[1]The study included only exenatide QW pen users.
[2]P-values for categorical variables were obtained using Chi-square tests; P-values for continuous variables were obtained using Wilcoxon rank sum tests.
[3]The number of days of continuous index GLP-1 RA therapy since initiation, allowing for a maximum gap (between fills) of 45 days. Patients who were censored at the end of the 1-year follow-up period were included.

Supported by: Eli Lilly and Company
Disclosure: **L. Fernández Landó:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company stockholder.

761

Semaglutide improves health-related quality of life vs placebo when added to standard-of care in patients with type 2 diabetes at high risk (SUSTAIN 6)

E. Jódar[1,2], W. Polonsky[3], R. Réa[4], M. Warren[5], T. Vilsbøll[6,7], J. Håkan-Bloch[8], H. Vrazic[8], S. Lindberg[8], S.C. Bain[9];
[1]Hospital Universitario Quirón Salud Madrid, Madrid, Spain, [2]Facultad de Ciencias de la Salud, Universidad Europea de Madrid, Madrid, Spain, [3]University of California, San Diego, USA, [4]Federal University of Paraná, Paraná, Brazil, [5]Physicians East, PA, Greenville, USA, [6]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [7]University of Copenhagen, Copenhagen, Denmark, [8]Novo Nordisk A/S, Søborg, Denmark, [9]School of Medicine, Swansea, UK.

Background and aims: The Short Form-36 health survey, version 2 (SF-36v2®) is a validated, widely used tool to assess health-related quality of

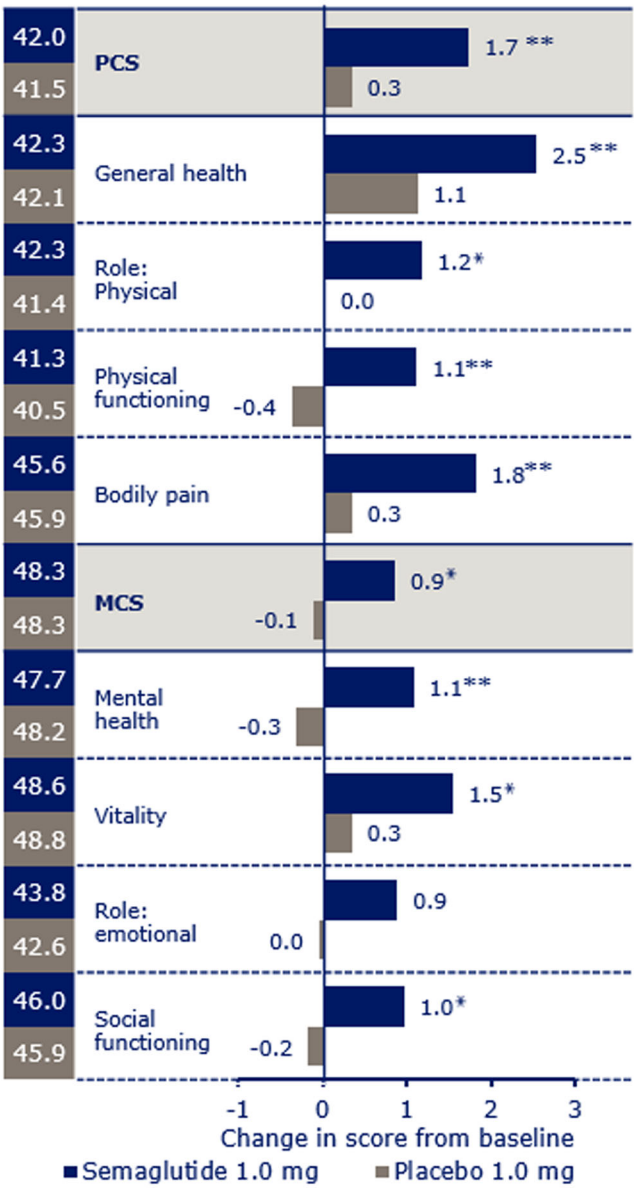
life (HRQoL) in patients with many different diseases. Research has shown that a 1-point increase in the physical component summary (PCS) score of the SF-36v2® is associated with clinical benefits, including reduced risk of mortality and hospitalisation. Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue for the treatment of type 2 diabetes (T2D). In SUSTAIN 6, a 2-year cardiovascular (CV) outcomes trial ($N = 3297$), semaglutide significantly reduced the risk of the primary outcome (CV death, non-fatal myocardial infarction or non-fatal stroke) vs placebo, when added to standard of care, in patients with T2D at high CV risk. This analysis examined the effect of semaglutide vs placebo on SF-36v2® in SUSTAIN 6.

Materials and methods: SF-36v2® was assessed from baseline to Week 104. A *post hoc* analysis evaluated SF-36v2® scores in patients who had a primary outcome event ($n = 254$) vs those who did not ($n = 3043$).

Results: At baseline, all domain scores were similar across treatment groups. Changes from baseline to Week 104 in PCS (1.74 vs 0.35) and mental component summary (MCS) (0.86 vs −0.11) scores were significantly greater for semaglutide 1.0 mg vs placebo 1.0 mg ($p = 0.0004$ and $p = 0.0489$, respectively; **Figure**) in the overall study population; semaglutide 1.0 mg showed significant improvements across all subcategories, except 'role emotional', vs placebo 1.0 mg (**Figure**). For PCS and MCS scores with semaglutide 0.5 mg vs placebo 0.5 mg, a similar trend was observed, although this did not reach significance; of the associated domains, only improvement in 'general health' was significant ($p = 0.0350$). There was a negative effect on change from baseline in PCS and MCS scores in patients who had a primary outcome event vs those who did not (PCS: −0.36 vs 0.81, $p = 0.0460$; MCS: −1.44 vs 0.29, $p = 0.0170$).

Conclusion: In SUSTAIN 6, after 104 weeks, HRQoL was consistently improved with semaglutide 1.0 mg vs placebo 1.0 mg, added to standard of care, in patients at high CV risk. A similar, non-significant, trend was observed for the low dose comparison. HRQoL was improved in patients who did not have a primary outcome event vs those who did.

Figure: Change from baseline in SF-36v2® PCS and MCS scores, and their associated domain scores, after 104 weeks in the SUSTAIN 6 trial



*p<0.05; **p<0.005. MCS, mental component summary; PCS, physical component summary; Short Form-36 health survey, version 2®.

Clinical Trial Registration Number: NCT01720446
Supported by: Novo Nordisk A/S research support
Disclosure: **E. Jódar:** Employment/Consultancy; Novo Nordisk, AstraZeneca, Lilly. Grants; Novo Nordisk, AstraZeneca, GSK, Janssen, Lilly, MSD. Lecture/other fees; Novo Nordisk, AstraZeneca, MSD, Lilly.

762

Exploring two dose regimens of ITCA 650 to switch from stable liraglutide therapy in patients with type 2 diabetes

N. Rasouli[1], J. Rosenstock[2], S. Nakhle[3], B. Schwartz[4], P. Prabhakar[4], S. Kruger[4], H. Huang[4], M. Baron[4];
[1]University of Colorado School of Medicine, Aurora, [2]Dallas Diabetes Research Center at Medical City, Dallas, [3]Southern Hills Hospital and Medical Center, Las Vegas, [4]Intarcia Therapeutics Inc., Boston, USA.

Background and aims: ITCA 650 is an investigational titanium osmotic mini pump that is subdermally placed in the abdominal wall during a brief office procedure to continuously deliver exenatide over 3 and 6-month periods and has the potential to improve medication adherence because patients do not self-administer.
Materials and methods: In this 26 week, open-label phase 3b study, 136 patients with T2D receiving liraglutide (1.2 to 1.8 mg/d) and metformin (≥1000 mg/d) were randomized to either the standard ITCA 650 dose regimen used in phase 3 trials of 20 mcg/d for 13 weeks followed by a 60 mcg/d maintenance dose (for 13 weeks in this study) or starting directly with the maintenance dose of 60 mcg/d for 26 weeks. The last injection of liraglutide occurred 2 days prior to randomization. The primary endpoint compared the incidence of nausea (N) and vomiting (V) between the two dose regimens.
Results: Switching from liraglutide to either dose regimen of ITCA 650 had a similar incidence of transient mild to moderate N/V (Table). Of note, 3 sites, which recruited 25% of the study population, accounted for 47% and 68% of the total N/V seen in the study. 4 patients discontinued due to GI AEs. Glycemic control remained stable in both groups. At Week 26, significant weight reduction was observed in both groups ($p < 0.05$ vs. baseline).
Conclusion: Patients on stable liraglutide therapy can be switched to ITCA 650 60 mcg/d without the need for up-titration from a lower dose.

Table. Baseline Characteristics and Clinical Outcomes*

Parameter	ITCA 650 20/60 mcg/d N=67	ITCA 650 60 mcg/d N=68
Baseline Characteristics		
Age, years[a]	60.0 ± 10.1	58.7 ± 9.7
Weight, kg[a]	95.9 ± 19.2	92.8 ± 18.4
Body mass index, kg/m^2 [a]	33.9 ± 5.5	33.1 ± 5.6
Baseline HbA1c (%)[a]	7.0 ± 1.1	6.9 ± 1.0
Duration of diabetes, years[a]	10.7 ± 6.6	10.0 ± 6.8
Duration of liraglutide use, days[a]	751 ± 663	589 ± 575
Liraglutide dose, mg/d[a]	1.6 ± 0.3	1.6 ± 0.3
Metformin dose, mg/d[a]	1716 ± 428	1687 ± 483
Clinical Outcomes		
LS Mean (SE) change HbA1c (%) at Week 26	-0.13 (0.10) p=0.241 [b]	0.05 (0.11)
LS Mean (SE) change in weight, kg at Week 26	-1.9 (0.51) p=0.237 [b]	-1.0 (0.52)
Nausea, n (%)	18 (26.5%) p=0.731 [c]	16 (23.9%)
Vomiting, n (%)	11 (16.2%) p=0.503 [c]	8 (11.9%)

* 136 patients were randomized, but 135 were treated.
[a] Mean ± standard deviation
[b] t-test for difference between treatment groups
[c] Cochran-Mantel-Haenszel chi-square test
LS = Least squares

Clinical Trial Registration Number: NCT02638805
Supported by: Intarcia Therapeutics, Inc.
Disclosure: **N. Rasouli:** Grants; Intarcia Therapeutics, Inc.

763

Evaluating clinical outcomes of changing type 2 diabetes patients from other DPP-4 inhibitor therapy to alogliptin in a primary care setting

W.D. Strain[1], P. McEwan[2], H. Howitt[3], S.B. Meadowcroft[3];
[1]Diabetes and Vascular Research Centre University of Exeter Medical School, Exeter, [2]Health Economics & Outcomes Research Ltd, Cardiff, [3]Takeda UK Ltd, High Wycombe, UK.

Background and aims: Guidelines increasingly recommend considering cost-effectiveness when choosing agents within a class of drugs. As a result, several UK regions initiated a within-therapy class switch policy, aiming to switch dipeptidyl peptidase-4 inhibitors (DPP-4i) with a higher tariff to alogliptin. Clearly such policy is only appropriate if it can be verified that glycaemic control is not attenuated when switching therapies. We aimed to assess the impact of switching from a higher tariff DPP-4i to alogliptin on glycaemic control.
Materials and methods: We worked with six clinical commissioning groups (CCGs) that had recommended to its primary care teams an active switch program for all patients on DPP-4 inhibitors to alogliptin based on the potential savings of at least £6,518 per 100 patient years treatment. Using the primary care electronic records, we performed a retrospective, observational cohort study using individual patient-level routine data collated from before and after the switch. Only people prescribed DPP-4i according to licensed indications were included in this analysis. Index was defined as the point that the switch took place. Unadjusted mean HbA1c values were compared to assess changes in HbA1c from the most recent value prior to index to the first HbA1c recorded ≥2.5 months after index.
Results: Data were available for 865 people with Type 2 diabetes mellitus (T2DM) switched from a DPP-4i (mainly sitagliptin) to alogliptin. The mean age was 64.35 years (SD = 11.19) and 63.7% were male. Pre-index mean (SD) HbA1c was 8.44% (1.52) at median of 58 days pre-switch (interquartile range (IQR) 137–21). HbA1c remained effectively unchanged at 8.42% (1.62) at 167 days (IQR 112–245) post-index. There was little difference in the distribution of individual HbA1c values pre and post switch (figure 1). At 6 months, the majority of people (80.8%) remained on alogliptin; a small proportion (4.1%) reverted to an alternative DPP-4i, whilst only 4.5% of people underwent other changes to their glycaemic medication.
Conclusion: A policy of switching from a higher tariff DPP-4i to alogliptin was well tolerated, as evidenced by a high persistence of therapy and was not associated with any deterioration in glycaemic control over a 6-month period. Such policy has the potential to generate significant cost savings without detriment to the diabetic control of people with T2DM.

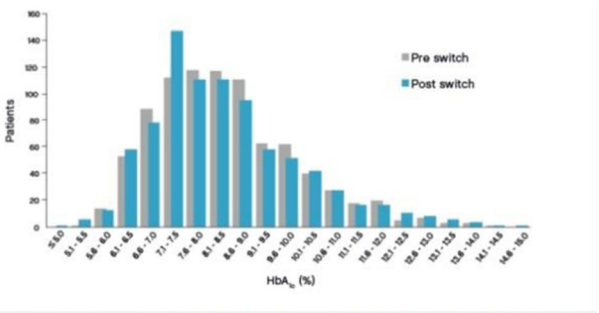


Figure 1. HbA1c levels pre- and post- switch to alogliptin from other DPP-4 inhibitors at 6 months post switch

Supported by: Takeda UK Ltd
Disclosure: **W.D. Strain:** Honorarium; Takeda UK Ltd.

764

Comparison of the incidence of hospital admissions for severe hypoglycaemia in type 2 diabetes patients treated with different dipeptidyl peptidase-4 inhibitors

C.-M. Chang[1], J. Wang[2], K.-L. Chien[2], J.-F. Chen[1];
[1]Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, [2]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei City, Taiwan.

Background and aims: Dipeptidyl peptidase-4 inhibitors (DPP4Is) have been shown to provide a wide spectrum of glycemic *effects* with lower

risk of hypoglycemia in patients with type 2 diabetes mellitus (T2DM). We aimed to compare the incidence of hospital admissions for hypoglycemia in patients with T2DM exposed to the combinations of oral anti-hyperglycemic drugs (OADs) with DPP4Is versus without DPP4Is, and to examine whether a class-effect or difference for DPP4Is on the incidence of hospitalization for hypoglycemia exist.

Materials and methods: This study was based on the *National Health Insurance* (NHI) program, which is a compulsory healthcare insurance system in Taiwan. All patients newly diagnosed with diabetes mellitus (DM) after 2008, when DPP4I was first approved in Taiwan, and before July 2013 were included. Patients with a history of hypoglycemia were excluded. In dataset 1, we investigated the correlation between DPP4Is exposure and the incidence of hospitalization for hypoglycemia. The participants were stratified into four different groups by the numbers of OAD types, and DPP4Is users and non-users were included in each group. In dataset 2, we investigated the incidence of hospitalization for hypoglycemia between the 3 types of DPP4Is. We calculated the incidence rate by dividing the numbers of hospitalization for hypoglycemia events by the total follow-up person-years (events per 1,000 person-years) within each group. Univariate and multivariate regression analyses were subsequently utilized to estimate the HRs and 95% CIs based on Cox proportional hazards model in order to assess the risk of hospitalization for hypoglycemia due to DPPI4s use. The other OADs users (dataset 1) or sitagliptin users (dataset 2) served as the reference. The full models was adjusted for age, sex, residence, comorbidities, and medications.

Results: A total 2,036,531 DM patients were identified between 2007 and 2013. 680,992 and 303,890 DM patients were enrolled to dataset 1 and dataset 2 respectively. We observed DPP4I user was associated with significant lower incidence of hospitalization for hypoglycemia compared to non-DPP4I user among using two and three types OAD patients (HR 0.65; 95% CI, 0.54 to 0.78; HR 0.79; 95% CI, 0.72 to 0.88, respectively).After adjusting for age, gender, comorbidities, and medications, we found the significant lower risk of hospitalization for hypoglycemia in DPP4Is group among using one, two and three types OAD population (HR 0.26; 95% CI, 0.14 to 0.51; HR 0.46; 95% CI, 0.36 to 0.55; HR 0.73; 95% CI, 0.66 to 0.81, separately).Compared with Sitagliptin, Vildagliptin and Saxagliptin had significant lower incidence of hospitalization for hypoglycemia (HR 0.69; 95% CI, 0.61 to 0.78; HR 0.82; 95% CI, 0.73 to 0.92, respectively). The results were consistent after adjusting for confounding factors (vildagliptin vs sitagliptin: HR 0.69; 95% CI, 0.61 to 0.78; saxagliptin vs sitagliptin: HR 0.78; 95% CI, 0.69 to 0.87).

Conclusion: Incidence of hospital admissions for severe hypoglycemia was significantly less in T2DM patients exposed to DPP4Is, when treated with combination therapies of no more than three types OADs. Within DPP4I class, compared with sitagliptin, vildagliptin and saxagliptin may provide lower risk of hospitalization for hypoglycemia.

Disclosure: **C. Chang:** None.

765

Minimal contribution of nausea or vomiting to superior semaglutide-mediated weight loss vs exenatide and dulaglutide in type 2 diabetes

I. Lingvay[1], R. de la Rosa[2], M. Marre[3], M. Nauck[4], V. Woo[5], T. Hansen[6], J.R. Larsen[6], E. Yildirim[6], J. Wilding[7];
[1]University of Texas Southwestern Medical Center, Dallas, USA, [2]Four Rivers Clinical Research, Paducah, USA, [3]Hôpital Bichat-Claude Bernard, Paris, France, [4]Diabetes Center Bochum-Hattingen, St. Josef-Hospital, Ruhr-University, Bochum, Germany, [5]University of Manitoba, Winnipeg, Canada, [6]Novo Nordisk A/S, Søborg, Denmark, [7]University of Liverpool, Liverpool, UK.

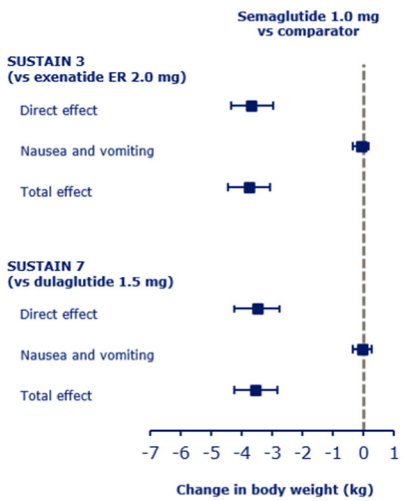
Background and aims: Semaglutide, a glucagon-like peptide-1 (GLP-1) analogue for the treatment of type 2 diabetes (T2D), showed superior HbA_{1c} and body weight reductions vs comparators in the SUSTAIN clinical trials. Gastrointestinal adverse events are common with GLP-1 receptor agonists (GLP-1RAs). A previous analysis showed the contribution of nausea or vomiting to the superior body weight loss with semaglutide in SUSTAIN 1–5 was small. This *post hoc* analysis assessed the contribution of nausea or vomiting to the greater weight loss with semaglutide vs once-weekly GLP-1RAs, exenatide extended-release (ER) and dulaglutide, in SUSTAIN 3 and 7.

Materials and methods: Subjects with T2D were randomised to once-weekly semaglutide 1.0 mg vs exenatide ER 2.0 mg in SUSTAIN 3 or once-weekly semaglutide 1.0 mg vs dulaglutide 1.5 mg and semaglutide 0.5 mg vs dulaglutide 0.75 mg in SUSTAIN 7. Subjects were grouped based on the occurrence of nausea or vomiting. A mediation analysis was done to determine how much of the estimated treatment differences (ETDs) in weight loss was due to direct effects of semaglutide vs nausea or vomiting.

Results: In SUSTAIN 3 ($N = 813$), proportions of subjects experiencing nausea or vomiting were 24.0 (semaglutide) vs 14.1% (exenatide ER). In SUSTAIN 7 ($N = 1201$), proportions were 24.0 vs 23.1% (semaglutide 1.0 mg vs dulaglutide 1.5 mg), and 25.2 vs 16.1% (semaglutide 0.5 mg vs dulaglutide 0.75 mg). In SUSTAIN 3, mean changes in body weight from baseline were −6.9 (semaglutide) and −2.7 kg (exenatide ER) in subjects with nausea or vomiting vs −5.4 and −1.6 kg in those without. In SUSTAIN 7, mean changes were −7.6 vs −3.9 kg (semaglutide 1.0 mg vs dulaglutide 1.5 mg) and −5.4 vs −3.3 kg (semaglutide 0.5 mg vs dulaglutide 0.75 mg) in those with nausea or vomiting, and −6.2 vs −2.7 kg (semaglutide 1.0 mg vs dulaglutide 1.5 mg) and −4.3 vs −2.1 kg (semaglutide 0.5 mg vs dulaglutide 0.75 mg) in those without. In SUSTAIN 3 and 7 (high-dose comparison), ETDs [95% CI] for body weight reductions favoured semaglutide and the contribution of nausea or vomiting was small (**Figure**). Similarly, the SUSTAIN 7 low-dose comparison led to a significant ETD [95% CI] for body weight reduction (−2.3 [−2.91; −1.59]); 0.03 kg of this difference was due to nausea or vomiting.

Conclusion: In SUSTAIN 3 and 7, body weight reductions were significantly greater with semaglutide vs exenatide ER and dulaglutide in subjects with and without nausea or vomiting. Nausea or vomiting contributed minimally to the superior weight reductions, thus treatment differences were largely mediated by direct effects of semaglutide.

Figure. Mediation analysis of direct (due to treatment) and indirect (due to nausea or vomiting) effects on weight loss for subjects treated with semaglutide 1.0 mg in SUSTAIN 3 and 7.



Data are ETD [95% CI] for the change from baseline in body weight based on the full analysis set using 'on-treatment without rescue medication' data. Post-baseline responses were analysed using a mixed model for repeated measurements that included the interaction of treatment and any nausea or vomiting. ETD, estimated treatment difference.

Clinical Trial Registration Number: NCT01885208, NCT02648204
Supported by: Novo Nordisk A/S research support

Disclosure: **I. Lingvay:** Employment/Consultancy; Novo Nordisk (paid to UTSW), AstraZeneca, Sanofi, Lilly. Grants; Novo Nordisk, Novartis, GI Dynamics, Pfizer, Merck. Honorarium; None. Lecture/other fees; None. Non-financial support; None. Stock/Shareholding; None. Other; AstraZeneca, Sanofi, Boehringer Ingelheim, Novo Nordisk, Lilly, Intarcia, TARGET Pharma (editorial support and travel; paid to UTSW).

766

Changes in serum calcitonin concentrations and incidence of adjudicated medullary thyroid carcinoma in the EXenatide Study of Cardiovascular Event Lowering (EXSCEL)

B. Katona[1], M.A. Bethel[2], R. Patel[2], V.P. Thompson[3], S.M. Gustavson[1], P. Ohman[1], N. Iqbal[1], J.B. Buse[4], A.F. Hernandez[3], R.R. Holman[2], for the EXSCEL Study Group;
[1]AstraZeneca, Gaithersburg, USA, [2]Diabetes Trials Unit, Oxford, UK, [3]Duke Clinical Research Institute, Durham, USA, [4]University of North Carolina School of Medicine, Chapel Hill, USA.

Background and aims: Serum calcitonin concentration is used as a tumour marker for neoplasia of the thyroid C-cells, including medullary thyroid carcinoma (MTC), which has been associated with glucagon-like peptide-1 (GLP-1) receptor activation in pre-clinical studies. EXSCEL was a multinational, randomised, double-blinded, pragmatic cardiovascular outcomes trial evaluating exenatide, a once-weekly GLP-1 receptor agonist, versus placebo on the background of usual care in type 2 diabetes. We report changes in serum calcitonin concentrations in exenatide- and placebo-treated participants and incidence of MTC in EXSCEL over a median 3.2-year follow-up period.
Materials and methods: Participants (n = 14,752) in EXSCEL were randomised 1:1 to exenatide 2 mg once-weekly or placebo. Serum calcitonin concentration was measured in participants at baseline (ineligible for trial if >40 ng/L) and annually throughout follow-up (trial medication discontinued if ≥50 ng/L). A repeated measures mixed model analysis was performed, including serum calcitonin concentrations only from participants with a baseline and at least one post-baseline value. Median serum calcitonin concentration for treatment groups was calculated at baseline and at yearly intervals thereafter. Thyroid malignancies, including MTC, were collected prospectively throughout the trial and adjudicated using pre-specified criteria by an independent committee, blinded to treatment assignment.
Results: In the intention-to-treat population at baseline, 52 (30 exenatide, 22 placebo) participants had a serum calcitonin concentration >40 ng/L, and during follow-up 23 participants (15 exenatide, 8 placebo) had a serum calcitonin concentration ≥50 ng/L (excluding those with serum calcitonin concentration >40 ng/L at baseline). Median (IQR) baseline serum calcitonin concentration was 1.7 ng/L (1.7, 4.3) in the exenatide group and 1.7 ng/L (1.7, 4.2) in the placebo group. Serum calcitonin concentrations were unchanged over 36 months in both groups, with a median (IQR) change from baseline of 0.0 (−0.4, 0.0) in the exenatide group and 0.0 (−0.5, 0.0) in the placebo group. Confirmed MTC occurred in 3 participants (2 exenatide, 1 placebo), all of whom had elevated serum calcitonin concentrations at baseline (413, 422 and 655 ng/L).
Conclusion: Treatment with exenatide 2 mg once-weekly did not result in an increase in serum calcitonin concentrations, with no evidence of a difference in serum calcitonin concentrations between exenatide- and placebo-treated participants over a median 3.2-year follow-up period. All 3 confirmed cases of MTC in EXSCEL occurred in participants who had an elevated serum calcitonin at baseline, prior to administration of trial medication.
Clinical Trial Registration Number: NCT01144338
Supported by: AstraZeneca (Gaithersburg, MD)
Disclosure: **B. Katona:** Employment/Consultancy; AstraZeneca.

PS 063 DPP4 inhibitors: new regiments and new comparisons

767

A Comparative head-to-head study of the effect Of three different DPP-4 Inhibitors (CODI24) during 24h, in metformin-treated type 2 diabetes individuals

W. Alsalim[1], O. Göransson[2], B. Ahrén[1];
[1]Department of Clinical Sciences, Lund University, Lund, [2]Department of Experimental Medical Science, Lund University, Lund, Sweden.

Background and aims: Control of glycemia in treatment of type 2 diabetes (T2D) involves normalization of fasting and post-prandial blood glucose. It is known that DPP-4 inhibitors (DPP-4i) have effects on both fasting and postprandial glucose, but the extent of the glucose effect over the entire day after DPP-4 inhibition and whether DPP-4i differ are not known. Such comprehensive knowledge is important for clinical decision, particularly since discrepancies between DPP-4i are possible due to differences in chemical structure, mode of enzyme inhibition and half-life. In this study, we therefore compared the effect of three different DPP-4i (sitagliptin (SITA), vildagliptin (VILDA), saxagliptin (SAXA)) versus placebo (PBO) on glucose and insulin levels over an entire day with controlled and standardized meal ingestion (breakfast, lunch and dinner) in metformin-treated and well controlled T2D subjects.
Materials and methods: After an overnight fast, twenty-four metformin-treated T2D subjects (12 male, 12 female, mean age 63 yrs, BMI 31.0 kg/m^2 , HbA1c 44.7 mmol/mol = 6%) underwent four tests in random order ingesting either SITA; 100 mg, VILDA; 50 mg, SAXA; 10 mg, or PBO followed by ingestion of a standardized breakfast (525 kcal), lunch (780 kcal) and dinner (560 kcal) at specific time points. SITA and SAXA were ingested 30 min before breakfast while VILDA was ingested 30 min before breakfast and dinner. Blood samples were taken for analysis of glucose and insulin; their suprabasal 180 min areas under the curve (AUC) were calculated.
Results: SITA, VILDA, and SAXA suppressed the rise in glucose levels after all three meals (Fig. 1A), compared to the PBO, and the $AUC_{glucose180min}$ were significantly reduced by DPP-4i (SITA; 29, 41 and 22%, VILDA; 24, 34 and 24%, SAXA; 22, 25 and 16% after breakfast, lunch and dinner respectively (Fig. 1B). The fasting blood glucose levels the day after ingestion of DPP-4i did not differ significantly compared to PBO. The insulin levels increased after meal ingestion without significant differences between DPP-4i and PBO (Fig. 1C, D). There were no significant differences in glucose or insulin levels between the three DPP-4i.
Conclusion: Based on these data we conclude that in metformin-treated and well controlled T2D subjects; 1) DPP-4i reduced postprandial glucose levels, compared to placebo, 2) DPP-4i had the same effect after breakfast, lunch and dinner, 3) insulin levels increased after each meal with no significant difference between placebo and DPP-4i, and 4) there was no significant difference between the three DPP-4is. Therefore, we suggest that different DPP-4i have the same glucose-reducing effect over the day. Moreover, DPP-4 inhibition is associated with unchanged insulin levels compared to PBO, in spite of lower postprandial glycemia, as a sign of improved beta-cell function.

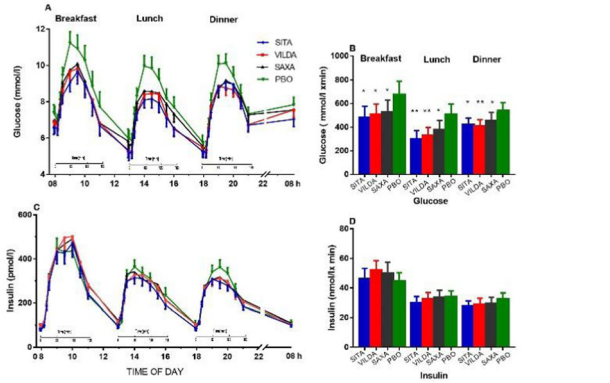


Figure 1. Plasma levels and suprabasal area under the curve (180 min) of glucose (A,B) and insulin (C,D) after ingestion of breakfast (525kcal), lunch (780kcal) and dinner (560kcal) with sitagliptin (SITA;100mg), vildagliptin (VILDA; 50mg b.i.d), saxagliptin (SAXA; 10mg) and placebo (PBO) in well controlled and metformin treated T2D subjects. Mean±SEM are shown. SITA, VILDA and SAXA compared to BPO; *$p \leq 0.05$ and **$p \leq 0.01$.

Clinical Trial Registration Number: EudraCT; 2013-005570-22
Supported by: Region skåne and VR
Disclosure: **W. Alsalim:** None.

768

Comparative efficacy of saxagliptin combination therapy with acarbose, or gliclazide modified release, or metformin in drug naive patients with type 2 diabetes

X.P. Chen[1], H.M. Li[2], H.Y. Kuang[3], L. Chen[4], J.H. Ma[5], Q. Zhang[6], T.R. Pan[7], J. He[8], W.Y. Yang[1];
[1]China–Japan Friendship Hospital, Beijing, China, [2]China Meitan General Hospital, Beijing, China, [3]The First Affiliated Hospital of Harbin Medical University, Harbin, China, [4]Qilu Hospital of Shandong University, Jinan, China, [5]Nanjing First Hospital Affiliated to Nanjing Medical University, Nanjing, China, [6]The First Affiliated Hospital of Anhui Medical University, Hefei, China, [7]The Second Hospital of Anhui Medical University, Hefei, China, [8]Tulane University, New Orleans, USA.

Background and aims: Newly diagnosed type 2 diabetic patients whose HbA1c more than 9% associated with hyperglycemia symptoms can implement short term intensive insulin therapy by AACE and Chinese guideline. Initiate metformin-based combination therapy for newly diagnosed T2DM with high HbA1c and no hyperglycemia symptoms is recommended in AACE guideline. However, less information exists about dipeptidyl peptidase-4 inhibitor based dual therapy in these patients. This is a 24-week, multicenter, randomized, open-label, parallel group study to assess the efficacy and safety of saxagliptin initial combination with acarbose or gliclazide modified release (MR), or metformin therapy in drug naïve patients with T2DM.
Materials and methods: 648 subjects aged ≥18 and ≤80 years old were enrolled in the study and mean HbA1c was 9.2%. Patients were randomized to saxagliptin plus acarbose ($n = 216$), or saxagliptin plus gliclazide MR ($n = 216$), or saxagliptin plus metformin ($n = 216$). Saxagliptin was given 5 mg once daily during entire trial and the trial included 4-week drug dose titration period. Acarbose was titrated up to 300 mg/d, metformin was up to 2000 mg/d, and gliclazide MR was up to maximum tolerated dose over 4-week.
Results: 583 patients started the intended study drug. HbA1c reduction at week 24 was −2.63% in the acarbose group, −2.77% in the gliclazide MR group and −2.92% in the metformin group. The proportion of patients with HbA1c less than 7.0% was 74.4% (acarbose), 80.3% (gliclazide MR) and 84.9% (metformin) at 24 weeks. The proportion of patients with HbA1c of 6.5% or less was 60.3% (acarbose), 63.6% (gliclazide MR) and 73.1% (metformin) at 24 weeks. At week 24, the HbA1c reduction difference was 0.29 (95% CI 0.07 to 0.52, $p = 0.1748$) for Acarbose, 0.15 (95% CI −0.07 to 0.37, $p = 0.0134$) for Gliclazide MR, compared to metformin, respectively. The mean change in body weight from baseline to endpoint in treatment groups was −1.5 kg for acrabose, 1.0 kg for gliclazide MR and −1.6 kg for metformin, respectively. Hypoglycemic events, almost all are mild, were reported during treatment period in acrabose (0.38%), gliclazide MR (1.42%), and metformin (0.68%).
Conclusion: The findings of this study provides evidence that remarkable efficacy and safe profile of initial saxagliptin combination therapy with acarbose, or gliclazide MR, or metformin in drug naïve Chinese T2DM patients with high HbA1c level. In addition to insulin intensive treatment, saxagliptin-based combination therapy is also one of optimal options for drug naive T2DM patients with high HbA1c level.
Clinical Trial Registration Number: ChiCTR-IPR-14005716
Supported by: AstraZeneca China
Disclosure: **X.P. Chen:** None.

769

Glycaemic efficacy and safety of linagliptin compared to basal bolus insulin regimen in non-cardiac surgical patients with type 2 diabetes: Linagliptin-Surgery trial

G. Umpierrez[1], P. Vellanki[1], N. rasouli[2], D. Baldwin[3], S. Alexanian[4], I. Anzola[1], M. Urrutia[1], P. Gomez[1], S. Cardona[1], H. Wang[1], F. Pasquel[1];
[1]Emory University School of Medicine, Atlanta, [2]Medicine, University of Colorado, Denver, [3]Rush University, Chicago, [4]Medicine, Boston Universty, Boston, USA.

Background and aims: Effective hospital and post-discharge regimens are needed for the management of general surgery patients with type 2 diabetes. We compared the safety and efficacy of linagliptin with a basal-bolus insulin regimen in the hospital, and determine the efficacy of an HbA1c-based algorithm to guide post-discharge diabetes therapy.
Materials and methods: This prospective, multicenter open-label study enrolled non-cardiac surgery patients with type 2 diabetes, blood glucose (BG) between 7.8–22.2 mmol/l and treated with diet, oral agents, or insulin at a total daily dose (TDD) ≤0.5 units/kg. Subjects were randomized to either linagliptin 5 mg daily ($n = 128$) or basal-bolus insulin regimen ($n = 122$). Insulin treated subjects were started at a TDD of 0.4 U/kg/day or 0.5 U/kg/day for randomization BG between 7.8 11.1 mmol/l or >11.1–22.1 mmol/l, respectively. If GFR <45 ml/min/1.73 m^2, TDD was reduced by 50%. Both groups received correction doses of rapid-acting insulin for BG >7.8 mmol/l before meals or every 6 hours if NPO. The discharge algorithm was based on admission HbA1c. If HbA1c <7% (<53 mmol/mol), 221 subjects were discharged on linagliptin or preadmission oral agents; patients with HbA1c 7% and 9% and those >9% were discharged on linagliptin with glargine at 50% or 80% of the hospital daily dose, respectively. The primary outcome was difference in mean hospital daily BG between groups. Secondary outcomes were inpatient hypoglycaemia and changes in HbA1c at 3 months after discharge.
Results: Difference in mean daily hospital BG between groups was 0.6 mmol/l. Linagliptin resulted in fewer hypoglycemic events (1.6% vs.11%, $p = 0.001$, relative risk reduction of 86%) and in lower number of daily insulin injections (2.0 ± 3.3 vs 3.1 ± 3.3, $p < 0.001$) compared to basal-bolus. In patients with a randomization BG <11.1 mmo/l (observed in 63% of overall cohort), linagliptin resulted in similar mean daily BG (8.9 ± 2.3 vs. 8.7 ± 2.3 mmol/l, $p = 0.43$); however, patients with BG ≥11.1 mmol/l treated with linagliptin had higher BG compared to basal-bolus (10.9 ± 2.6 vs. 9.2 ± 2.2 mmo/l, $p < 0.001$). There were no differences in length of hospital stay or in the rate of perioperative complications between treatment groups. Three months after discharge, HbA1c decreased from $7.9 \pm 2.0\%$ to $7.1 \pm 1.5\%$, $p < 0.0001$ using the discharge algorithm.
Conclusion: Linagliptin is an effective alternative to basal-bolus insulin regimen in general surgery patients with type 2 diabetes with mild to

moderate hyperglycemia. Our results indicate that in patients with BG <11.1 mmol/l, treatment with linagliptin resulted in similar improvement in glycemic control and in significantly lower rates of hypoglycemia compared to the basal-bolus insulin regimen. However, basal-bolus insulin regimen was superior to linagliptin in patients with BG ≥11.1 mmol/l. The proposed HbA1c-based hospital discharge algorithm with the use of linagliptin, with or without basal insulin, showed efficacious glycemic control after discharge.

Clinical Trial Registration Number: NCT02004366

Supported by: Boehringer Ingelheim, NIH

Disclosure: **G. Umpierrez:** Grants; American Diabetes Association.

770

Efficacy and safety of continuing sitagliptin when initiating insulin therapy in subjects with type 2 diabetes

R. Roussel[1], S. Duran-Garcia[2], Y. Zhang[3], S. Shah[3], C. Darmiento[3], R.R. Shankar[3], E.A. O'Neill[3], G.T. Golm[3], R.L.H. Lam[3], I. Gantz[3], K.D. Kaufman[3], S.S. Engel[3];

[1]Groupe Hospitalier Bichat, Paris, France, [2]Valme University Hospital Medical School, Seville, Spain, [3]Merck & Co., Inc., Kenilworth, USA.

Background and aims: DPP-4 inhibitors (DPP4is) are often discontinued with initiation of insulin therapy but the impact of this discontinuation on efficacy and hypoglycemia has not been studied. In this double-blind trial the safety and efficacy of initiating insulin while continuing sitagliptin (SITA) was evaluated.

Materials and methods: Eligible patients had inadequately controlled T2DM on metformin (MET, ≥1500 mg/day) in dual or triple combination therapy with a DPP-4i and/or sulfonylurea. Those on MET + SITA (100 mg/day) directly entered the trial; all others were switched to MET + SITA and stabilized during a run-in period. Subjects were randomized to continuing SITA or discontinuing SITA and switching to matching placebo, with both groups initiating insulin (LANTUS®), which was titrated based on fasting glucose.

Results: 746 subjects (mean HbA1c = 72.6 mmol/mol [8.8%], mean disease duration of 10.6 years) were randomized. After 30 weeks, continuing SITA was superior to discontinuing SITA in reducing HbA1c ($p < 0.001$). Patients who continued SITA had a lower event rate of documented symptomatic hypoglycemia (blood glucose ≤3.9 mmol/L) and daily insulin dose compared to patients who discontinued SITA. Summary adverse event measures and change in body weight (Week 30) were similar in the 2 treatment groups.

Conclusion: With the initiation of insulin therapy, continuation of SITA resulted in superior glycemic efficacy and less documented symptomatic hypoglycemia.

Change from Baseline to Week 30 in HbA1c, mmol/mol (%)[a]		
Treatment	**LS Mean (95% CI)**	**Difference in LS Means (95% CI)**
SITA, *n*=373	-20.5 (-21.6, -19.4)	-5.0 (-6.4, -3.7)
	(-1.88 [-1.98, -1.78])	(-0.46 [-0.58, -0.34])
PBO, *n*=370	-15.5 (-16.6, -14.4)	----
	(-1.42 [-1.52, -1.32])	----
Event Rate of Documented Symptomatic Hypoglycemia (Blood Glucose ≤3.9 mmol/L), events/patient-year[b,c]		
Treatment	**Event Rate (95% CI)**	**Event Rate Ratio (95% CI)**
SITA, *n*=371	1.55 (1.22, 1.96)	0.73 (0.54, 0.98)
PBO, *n*=370	2.12 (1.70, 2.66)	----
Total Daily Insulin Dose (Units) at Week 30[a,d]		
Treatment	**LS Mean (95% CI)**	**Difference in LS Means (95% CI)**
SITA, *n*=365	53.2 (48.5, 58.0)	-8.0 (-14.6, -1.5)
PBO, *n*=367	61.3 (56.5, 66.0)	----

[a]Analyzed using a longitudinal data analysis model.
[b]Analyzed using a negative binominal regression model.
[c]Two subjects (both in the sitagliptin group) were not included in the analysis due to a missing value of a model covariate (race).
[d]11 subjects did not have post baseline insulin dose data.

Clinical Trial Registration Number: NCT02738879

Supported by: Merck & Co., Inc.

Disclosure: **R. Roussel:** Honorarium; Sanofi, MSD, Janssen, Eli Lilly, Kayentis, Astra Zeneca, Boehringer Ingelheim, Novo Nordisk.

771

Efficacy and safety of linagliptin and metformin combination compared with metformin alone on stratified approach of type 2 diabetes treatment

Y. Shi[1], S.V.D. Walt[2], L. Miao[3];

[1]Department of Endocrinology and Metabolism, Changzheng Hospital, Shanghai, China, [2]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [3]Lilly (Shanghai) Management Co.,Ltd., Shanghai, China.

Background and aims: To evaluate the efficacy and safety of linagliptin and metformin combination versus metformin alone according to different baseline HbA1c level and individualized HbA1c target.

Materials and methods: Two phase 3 clinical trials evaluating efficacy and safety of initiated linagliptin/metformin combination or monotherapy in T2DM were included in this *post hoc* analysis. Patients were drug-naïve or pretreated with 1 OAD.The trials comprised a washout period (for patients pretreated with OAD) followed by placebo run-in period (all).Then, patients received 24 weeks of treatment period. For patients randomized to metformin 1000 mg bid, two weeks titration was applied with titration from 500 to 1000 mg bid. Pool I included all patients who randomized to metformin 500 mg bid (Met500), metformin 1000 mg bid (Met1000), linagliptin 2.5 mg + metformin 500 mg bid (Lina2.5/Met500) and linagliptin 2.5 mg + metformin 1000 mg bid (Lina2.5/Met1000). Pool II involved drug-naïve patients from Pool I.

Results: In pool I, 289 patients received Met500[mean HbA1c (SD) 8.68 (0.98)%], 291 received Met1000 [8.57 (0.93)%],290 received Lina2.5/Met500 [8.68 (0.94)%] and 290 received Lina2.5/Met1000 [8.71 (1.02)%]. At 24 week, in the category of baseline HbA1c <7.5%, there was no statistical difference between groups [lina2.5/Met500 vs Met500, lina2.5/Met1000 vs Met1000, $P > 0.05$ for both] on the proportion of patients who achieved HbA1c <7.0%. However, in the group of Lina2.5/Met1000, the proportion of patients who achieved HbA1c ≤6.5% was significantly higher than Met1000. For baseline HbA1c ≥7.5–9.0% population, a higher proportion of patients on initial dual therapy achieved HbA1c <7.0% or ≤6.5%,compared with monotherapy at 24 week. In the baseline HbA1c >9.0% population, the proportion of patients achieved HbA1c <7.0% or ≤6.5% in Lina2.5/Met1000 were highest at 24 week. The proportion of patients achieved HbA1c <7.0% in Lina2.5/Met1000 is significantly higher than Met1000 ($P = 0.0174$), but the proportion of patients achieved HbA1c ≤6.5% was non-significantly different with Met1000. In Met500, Met1000, Lina2.5/Met500 and Lina2.5/Met1000, 51.6%, 54.6%, 50.3% and 53.4% of patients experienced AEs. In pool II, we observed similar results.

Conclusion: For the patients whose baseline HbA1c is <7.5%, if the individualized glucose target is 7.0%, monotherapy or dual therapy are both appropriate. However, if the target is 6.5%, physicians can select Lina2.5/Met1000. For the patients whose baseline HbA1c level is ≥7.5–9.0%, regardless of the individualized HbA1c target is 6.5% or 7.0%, Lina2.5/Met1000 can make more patients achieve glucose target. For the patients whose baseline HbA1c level is >9.0%, if the individualized HbA1c target is 7.0%, Lina2.5/Met1000 maybe the best choice. However, if the target is 6.5%, triple-therapy may be needed.

Table1: At 24 week, the proportion of patients achieving HbA1c target in different baseline HbA1c categories

Baseline HbA1c level	Treatment group	n	At 24 week, the proportion of patients achieved HbA1c<7.0% (%)	P value (lina2.5/Met500 vs Met500)	P value (lina2.5/Met1000 vs Met1000)	At 24 week, the proportion of patients achieved HbA1c≤6.5% (%)	P value (lina2.5/Met500 vs Met500)	P value (lina2.5/Met1000 vs Met1000)
<7.5%	Met500	18	88.2	0.8817		64.7	0.6747	
	Lina2.5/Met500	20	88.2			75		
	Met1000	26	76.2		0.0572	57.7		0.0045
	Lina2.5/Met1000	26	95.7			84.6		
≥7.5%-9.0%	Met500	171	38	0.0018		25.1	0.0004	
	Lina2.5/Met500	161	52.8			41.0		
	Met1000	163	52.8		0.0008	36.8		0.004
	Lina2.5/Met1000	151	70.9			53.0		
>9.0%	Met500	96	18.8	0.0004		11.5	0.0009	
	Lina2.5/Met500	82	42.9			31.6		
	Met1000	98	35.4		0.0174	25.6		0.1056
	Lina2.5/Met1000	104	51.0			35.6		

Disclosure: **Y. Shi:** None.

772

Early initiation of sitagliptin during metformin up-titration in treatment of patients with type 2 diabetes

J.P. Frias[1], Z. Zimmer[2], R.L.H. Lam[2], G. Amorin[2], C. Ntabadde[2], C. Iredale[2], E.A. O'Neill[2], S.S. Engel[2], K.D. Kaufman[2], H. Makimura[2], M.F. Crutchlow[2];
[1]National Research Institute, Los Angeles, [2]Merck & Co., Inc., Kenilworth, USA.

Background and aims: Metformin (MET) is widely used as the first-line antihyperglycemic agent (AHA) for patients with T2DM; it is usually initiated at a low dose and up-titrated based on tolerability and glycemic response. For many patients not at glycemic goal on a sub-maximal MET dose, MET dose maximization does not result in attainment of HbA1c goal. To better understand the optimal thresholds for early addition of a second-line AHA, the safety and efficacy of MET maximization with simultaneous addition of sitagliptin (SITA) were compared to MET maximization alone in participants not at HbA1c goal on a sub-maximal dose of MET.
Materials and methods: Participants at baseline had inadequate glycemic control on no AHA or on monotherapy with MET at 1000 mg/day or on another AHA. Prior to randomization, all participants continued or transitioned to MET 1000 mg/day for a 6–10 week stabilization period, followed by a 2-week single-blind placebo (PBO) run-in. Those with HbA1c 58 to 97 mmol/mol [7.5 to 11.0%] prior to the run-in were eligible for randomization. At randomization, participants were assigned (1:1) to SITA 100 mg/day or matching PBO. The MET dose was to be increased from 1000 mg/day to 2000 mg/day by study Week 2 and continued through Week 20. Primary objectives were to compare the effect of up-titration of MET with and without addition of SITA on reduction from baseline in HbA1c after 20 weeks of treatment and to assess the overall safety and tolerability of these regimens. Secondary objectives included the effects of treatment on the percentage of patients at the HbA1c goal of <53 mmol/mol (<7%) and reduction from baseline in fasting plasma glucose (FPG) after 20 weeks.
Results: Treatment groups were well balanced at baseline ($n = 229$/group, mean HbA1c = 71.1 mmol/mol [8.7%], mean FPG = 10.2 mmol/L). At Week 20, LS mean changes from baseline HbA1c were greater with SITA vs. PBO (Table), $p < 0.001$. At Week 20, the HbA1c goal of <53 mmol/mol (<7.0%) was more likely to be met with SITA than with PBO overall (Table) (Relative Risk 1.7, $p = 0.001$), and in the pre-specified subgroup with baseline HbA1c ≥69 mmol/mol (8.5%) (Table; Relative Risk 2.4, $p = 0.026$). At Week 20, LS mean changes from baseline in FPG were greater with SITA vs. PBO, $p = 0.002$. Both treatment regimens were well tolerated, with no notable between-group differences in safety or tolerability.
Conclusion: In this study of T2DM patients with inadequate glycemic control on MET 1000 mg/day, early initiation of SITA, simultaneous with MET dose maximization, was well tolerated and increased HbA1c goal attainment. These data support that initiation of SITA concomitantly with MET up-titration may be a preferred treatment-intensification strategy for many T2DM patients not at HbA1c goal on a sub-maximal dose of MET.

At Week 20	MET maximization + SITA	MET maximization + PBO
Change from baseline in HbA1c, mmol/mol (%)	-12.1 (-1.1)	-7.6 (-0.7)
Between group difference (95% CI)		
mmol/mol	-4.5 (-6.5, -2.5)	----
%	-0.4 (-0.6, -0.2)	----
Patients at HbA1c goal <53 mmol/mol (<7%), %		
Overall population	28.8	16.6
Subgroup baseline HbA1c >69 mmol/mol (8.5%)	15.6	5.7
Change from baseline in FPG, mmol/L(mg/dL)	-1.6 (-29.3)	-0.9 (-16.9)
Between group difference (95% CI)		
mmol/L	-0.7 (-1.1, -0.3)	----
mg/dL	-12.4 (-20.2, -4.6)	----

Clinical Trial Registration Number: NCT02791490
Supported by: Merck & Co., Inc.
Disclosure: **J.P. Frias:** Grants; AbbVie, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, IONIS, Janssen, Johnson & Johnson, Ligand, Merck, Mylan, Novartis, Novo Nordisk, Pfizer, Sanofi, Theracos, vTv Therapeutics.

773

Efficacy and safety of saxagliptin and glimepiride in patients with type 2 diabetes inadequately controlled with metformin monotherapy: results from the SPECIFY study

T. Gu, L. Zhu, Q. Niu, C. Li, Y. Bi, D. Zhu;
Department of Endocrinology, Nanjing Drum Tower Hospital affiliated to Nanjing University Medical School, Nanjing, China.

Background and aims: There is no consensus on the preference of oral anti-diabetic agents when metformin fails in Type 2 diabetes (T2D). In this 48 weeks, multicentre, open-label, randomized, parallel trial, we aimed to compare the efficacy and safety profile of saxagliptin with glimepiride in T2D patients uncontrolled with metformin monotherapy.
Materials and methods: Patients were 1:1 randomized to saxagliptin (5 mg daily) add-on to metformin or glimepiride (initial with 1 mg, titrated 1 mg if fasting blood glucose >6.1 mmol/L till 6 mg daily) add-on to metformin. The primary endpoint was the achievement of HbA1c <7.0%, without hypoglycemia and weight gain (defined as plasma glucose <3.9 mmol/L with or without symptoms and weight gain <3.0%) after 48 weeks treatment.
Results: Of 388 patients randomized, 325 (83.8%) patients completed the study (163 in saxagliptin and 162 in glimepiride). The patients' mean (±SD) age was 53.3 ± 9.4 years, mean T2D duration was 5.0 ± 4.3 years, mean HbA1c was 8.0 ± 0.7% and mean BMI was 25.5 ± 2.5 kg/m^2, in the full analysis set. Over 48 weeks, greater proportion of patients achieved the primary endpoint with saxagliptin plus metformin versus glimepiride (mean dose 2.5 mg/day) plus metformin (43.3% in saxagliptin *versus* 31.3% in glimepiride; odds ratio 1.38, 95%CI 1.05, 1.82; $p = 0.019$). Mean reduction in HbA1c was similar in two treatment groups at Week 48 (−0.94% in saxagliptin *versus* −0.98% in glimepiride, $p = 0.439$). Bodyweight decreased with saxagliptin plus metformin (−0.7 ± 2.6 kg), but increased with glimepiride plus metformin (0.9 ± 2.8 kg) from similar mean baseline values (69.8 ± 9.6 kg *versus* 69.9 ± 10.1 kg); the treatment difference was −1.6 kg ($p < 0.001$). The proportion of patients experiencing hypoglycemia was much less with saxagliptin plus metformin (3.1% in saxagliptin *versus* 12.8% in glimepiride, $p < 0.001$). Furthermore, subgroup analysis demonstrated that the proportion of patients with baseline HbA1c levels <8.0% or T2D duration <5 years achieving the composite primary endpoint was significantly higher with saxagliptin, compared with glimepiride (54.5% in saxagliptin *versus* 39.3% in glimepiride, $p = 0.042$; and 54.0% in saxagliptin *versus* 37.1% in glimepiride, $p = 0.021$, respectively).
Conclusion: This study provides evidence that saxagliptin is similar to glimepiride in efficacy, with reduced body weight and a significantly lower risk of hypoglycemia in patients with T2D inadequately controlled with metformin monotherapy, especially in patients with moderate hyperglycemia and relatively shorter diabetes duration.
Clinical Trial Registration Number: NCT02280486
Supported by: AstraZeneca
Disclosure: **T. Gu:** None.

774

Safety and efficacy of sitagliptin compared with dapagliflozin in patients with type 2 diabetes, mild renal impairment, and inadequate glycaemic control on metformin ± a sulfonylurea

A. Raji[1], R. Scott[2], J. Morgan[1], Z. Zimmer[1], R.L.H. Lam[1], E.A. O'Neill[1], K.D. Kaufman[1], S.S. Engel[1];
[1]Merck & Co., Inc., Kenilworth, USA, [2]Christchurch Hospital, Christchurch, New Zealand.

Background and aims: While choice of AHAs may be modified in patients with T2D and moderate or severe renal insufficiency, this is generally not the case in patients with mild renal insufficiency. Clinical trial data focused on this population, which represents ~40% of patients with T2D, are lacking. In a randomized, double-blind, active comparator-controlled clinical trial, the safety and efficacy of adding sitagliptin (SITA) (100 mg qd) or dapagliflozin (DAPA) (10 mg qd) to treatment of patients with eGFR ≥60 and <90 mL/min/1.73 m^2 and HbA1c ≥53 and ≤80 mmol/mol (≥7.0% and ≤9.5%) while on metformin ± a sulfonylurea were assessed.
Materials and methods: The primary efficacy endpoint was change from baseline HbA1c at Week 24 (analyzed with a longitudinal data analysis model), with a primary hypothesis of non-inferiority of SITA to DAPA based on the prespecified criterion of the upper bound of the between-treatment difference 95% CI (SITA minus DAPA) <0.3%; if the upper bound was <0.0%, SITA would be declared superior.
Results: Treatment groups were well balanced at baseline ($n = 307$ and 306, mean HbA1c, mmol/mol, [%] = 60.9 [7.7] and 61.2 [7.8], mean eGFR, mL/min/1.73 m^2 = 79.4 and 76.9 for SITA and DAPA, respectively). At Week 24, LS mean changes from baseline HbA1c, mmol/mol (%) were −5.6 (−0.5) (SITA) and −3.9 (−0.4) (DAPA); between-group difference (95%CI) = −1.7 (−2.9, −0.5) (−0.2 [−0.3, −0.0]), $p = 0.006$, confirming both non-inferiority and superiority of SITA vs. DAPA. The pre-specified analysis of 2 hr post-prandial glycemic excursion showed no significant difference between groups. The HbA1c goal of <53 mmol/mol (<7%) was met by 43% (SITA) and 27% (DAPA) of patients. Treatments were well tolerated; there were significantly fewer patients with drug-related adverse events (AEs) with SITA than with DAPA, but summary AE profiles were otherwise similar.
Conclusion: SITA treatment over 24 weeks resulted in greater glycemic efficacy and greater % of patients at HbA1c goal than DAPA in patients with T2D and mild renal impairment who were inadequately controlled on metformin ± a sulfonylurea.
Clinical Trial Registration Number: NCT02532855
Supported by: Merck & Co., Inc.
Disclosure: **A. Raji:** Employment/Consultancy; Merck & Co., Inc. Stock/Shareholding; Merck & Co., Inc.

775

Efficacy of dapagliflozin plus saxagliptin vs insulin glargine at 52 weeks in patients with type 2 diabetes inadequately controlled by metformin with or without sulfonylurea

T. Vilsbøll[1], E. Ekholm[2], E. Johnsson[2], R. Garcia-Sanchez[3], N. Dronamraju[3], S.A. Jabbour[4], M. Lind[5];
[1]Steno Diabetes Center, Copenhagen, Denmark, [2]AstraZeneca, Mölndal, Sweden, [3]AstraZeneca, Gaithersburg, USA, [4]Thomas Jefferson University, Philadelphia, USA, [5]University of Gothenburg, Gothenburg, Sweden.

Background and aims: Metformin (MET) is recommended as a first-line treatment of type 2 diabetes (T2D). Many patients (pts) on MET continue to demonstrate inadequate glycaemic control. Combination agents may provide better glycaemic control in pts with HbA_{1c} ≥64 mmol/mol (≥8.0%). Although insulin therapy is effective in pts on MET, it may lead to hypoglycaemia and body weight gain. We recently showed in a randomized trial over 24 weeks (w) that co-administration of dapagliflozin (DAPA) and saxagliptin (SAXA) was non-inferior to insulin glargine (INS) in achieving HbA_{1c} reductions, with a reduced risk of body weight gain and hypoglycaemia in pts with T2D inadequately controlled on MET ± sulfonylurea (SU). Here the efficacy and tolerability of DAPA+SAXA vs INS up to 52w with regards to HbA_{1c}, body weight and hypoglycaemia are compared.
Materials and methods: In this international, multicentre, randomised, parallel-group, phase 3, 24w (short-term) study with a 28w extension (long-term), pts ≥18y with T2D (HbA_{1c} 64–108 mmol/mol or 8.0–12.0%) on MET ≥1500 mg/day ±SU ≥50% maximal dose received DAPA 10 mg/day + SAXA 5 mg/day or INS 100 units/mL/day. Fasting plasma glucose goal for INS titration was ≤100 mg/dL (5.5 mmol/L). Week 52 endpoints: Change in HbA_{1c} and body weight, and proportion of pts achieving therapeutic glycaemic response without hypoglycaemia (plasma glucose ≤70 mg/dL) were considered exploratory.
Results: Overall, 1,163 pts were enrolled, 707 entered the short-term phase, 643 received treatment, 600 (306, DAPA+SAXA; 294, INS) entered the long-term phase and 557 (284, DAPA+SAXA; 273, INS) completed treatment. Mean (SD) HbA_{1c} (%) at baseline was comparable for the 2 treatment groups: DAPA+SAXA, 9.04 (1.023) and INS, 9.04 (1.054); other patient characteristics were balanced. Number of pts requiring rescue medication during treatment was 76 (23.5%) and 97 (30.4%) in DAPA+SAXA and INS groups, respectively. Mean total daily INS dose at 52w was 37.9 units. At 52w, the adjusted mean (SE) reduction in HbA_{1c} (%) was greater in pts on DAPA+SAXA [−1.51 (0.07)] vs INS [−1.26 (0.07)] (**Table**). There was a reduction in total body weight expressed as adjusted mean (SE) change from baseline with DAPA+SAXA [−1.83 (0.27) kg] vs weight gain with INS [+2.75 (0.28) kg] (**Table**). More pts on DAPA+SAXA (17.6%) vs INS (9.1%) achieved HbA_{1c} <53 mmol/mol (7.0%) without hypoglycaemia than INS.
Conclusion: Oral treatment with DAPA+SAXA improved glycaemic control and reduced mean body weight vs INS 52w after treatment initiation. A larger proportion of pts on DAPA+SAXA achieved therapeutic glycaemic control without hypoglycaemia vs INS. DAPA+SAXA can be a useful add-on to MET and MET+SU in T2D pts with inadequately controlled glycaemia.

Table: Exploratory endpoints

	Mean (SD)	Mean (SD)	Mean change from baseline (SD)	LS mean (SE)	95% CI	LS mean (SE)	95% CI[b]	*p*-value
Treatment group	Baseline HbA_{1c} (%)	HbA_{1c} (%) at Week 52		Adjusted change in HbA_{1c} (%) from baseline[a]		Difference in HbA_{1c} (%) from INS[a]		
DAPA+SAXA (N=324)	9.04[†] (1.02)	6.97[=] (0.65)	-1.82 (0.98)	-1.51 (0.07)	(-1.64, -1.38)	-0.25 (0.1)	(-0.44, -0.06)	0.009
INS (N=319)	9.04[¶] (1.05)	7.04[¶¶] (0.83)	-1.70 (1.14)	-1.26 (0.07)	(-1.40, -1.13)	-	-	-
	Baseline weight (kg)	Weight at Week 52 (kg)		Adjusted change in weight from baseline[a] (kg)		Difference in weight from INS[a] (kg)		
DAPA+SAXA (N=324)	89.93[‡] (17.70)	87.08[=] (16.37)	-2.19 (4.10)	-1.83 (0.27)	(-2.35, -1.31)	-4.58 (0.38)	(-5.33, -3.83)	<0.001
INS (N=319)	89.36[§] (18.38)	91.05[§§] (18.26)	2.57 (4.46)	2.75 (0.28)	(2.21, 3.29)	-	-	-
Proportion of patients achieving therapeutic glycaemic response (HbA_{1c} <7%) without hypoglycaemia at Week 52								
	n/N[c]	Percent	Adjusted percent[d], (95% CI)			Odds ratio vs INS[d] (95% CI), *p*-value		
DAPA+SAXA (N=324)	57/324	17.6	15.2, (11.55, 19.77)			2.3 (1.41, 3.81), <0.001		
INS (N=319)	29/319	9.1	7.2, (4.79, 10.59)			-		

n=[†]319; [‡]212; [§]312; [¶¶]177; [§§]313; [¶¶]178; [a]Obtained from an MMRM. Covariance structure was modelled using unstructured covariance matrix; [b]An upper CI bound <0.30% implies noninferiority of DAPA+SAXA vs INS; [c]Pts with unknown status at 52w and pts rescued before 52w were treated as non-responders; [d]Logistic regression method with adjustment for baseline HbA_{1c} and randomisation stratification factor (MET ± SU).

Clinical Trial Registration Number: NCT02551874
Supported by: AstraZeneca Pharmaceuticals LP.
Disclosure: **T. Vilsbøll:** None.

PS 064 Incretin-based therapies: new mechanistic insights

776

The reduction in postprandial glucose by sitagliptin is related to the rate of gastric emptying in type 2 diabetes

K.L. Jones[1], J.E. Stevens[2], M. Buttfield[3], T. Wu[1], S. Hatzinikolas[1], H. Pham[1], K. Lange[1], C.K. Rayner[1], M. Horowitz[1];
[1]Adelaide Medical School, University of Adelaide, Adelaide, [2]School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, [3]School of Health Science, University of South Australia, Adelaide, Australia.

Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors are used widely, and for the main part, empirically in the management of type 2 diabetes (T2DM) and reduce fasting and postprandial glucose. The rate of gastric emptying (GE), which exhibits a substantial inter-individual variation in health and T2DM, is a major determinant of postprandial glycaemic excursions and the stimulation of glucagon-like peptide-1 (GLP-1). The latter relationship is non-linear e.g. the GLP-1 response is disproportionately greater when glucose delivery to the small intestine is 4 kcal/min when compared to 1–2 kcal/min. Therefore, while the effect of DPP-4 inhibitors on GE, if any, is modest, it is intuitively likely that postprandial glucose-lowering by DPP-4 inhibitors will be greater in T2DM patients with relatively more rapid GE. We have evaluated the relationship between the effect of sitagliptin on postprandial glucose and the rate of GE in T2DM.
Materials and methods: Fourteen subjects with T2DM (9M, 5F; age: 67.8 ± 1.5 yr; BMI: 31.2 ± 0.9 kg/m^2; duration of known diabetes: 4.2 ± 0.9 yr; HbA_1c: 6.35 ± 0.2%), managed by diet ± metformin, underwent concurrent measurements of GE and plasma glucose for 240 min after a mashed potato meal (368.5 kcal) labelled with 20 MBq ^{99m}Tc-Calcium Phytate, on two separate occasions. Participants received sitagliptin (100 mg) or placebo in randomised, double-blind, crossover fashion on 2 consecutive days; the last dose was given 60 min before the meal. Data are mean values ± SEM.
Results: There was no difference in the 50% GE time (T50) between the 2 days (sitagliptin: 71.5 ± 3.9 vs placebo: 71.1 ± 4.3 min). Sitagliptin had no effect on plasma glucose immediately before the meal ($t = -5$ min) (sitagliptin: 6.7 ± 0.4 vs placebo: 6.7 ± 0.3 mmol/L). The overall postprandial plasma glucose response was reduced by sitagliptin ($iAUC^{0–240}$ min: sitagliptin: 548 ± 79 vs placebo: 700 ± 96 mmol/L*min; $P < 0.005$) with a reduction in peak plasma glucose (sitagliptin: 11.4 ± 0.7 vs placebo: 12.7 ± 0.6 mmol/L; $P < 0.01$). The plasma glucose at 60 min was inversely related to the T50 on placebo ($r = -0.62$, $P = 0.01$), but not on sitagliptin ($r = -0.35$, $P = 0.22$). The magnitude of postprandial glucose-lowering by sitagliptin (change in $iAUC^{0–240}$ between placebo and sitagliptin) was related directly to the rate of GE (expressed as kcal/min) on placebo ($r = 0.68$, $P = 0.008$; *Figure*).
Conclusion: In T2DM, the acute effect of sitagliptin to reduce postprandial glucose is related to the baseline rate of GE, probably reflecting relative postprandial increases in both glucose and GLP-1. DPP-4 inhibitors may, accordingly, have greater therapeutic efficacy in T2DM patients with faster GE, providing a potential rationale for routine measurement of GE prior to the initiation of treatment.

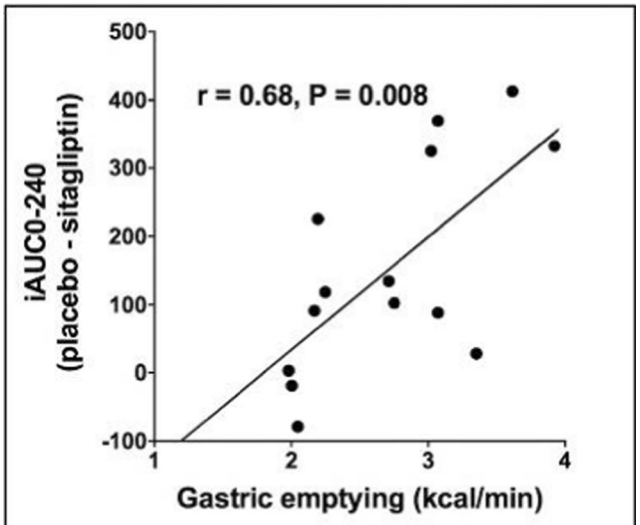


Figure: Relationship between the change in $iAUC^{0-240}$ min for plasma glucose from placebo to sitagliptin and the rate of GE (kcal/min) of a mashed potato meal on placebo in T2DM (n=14).

Clinical Trial Registration Number: NCT02324010
Supported by: Study supported by a RAH project grant; drug supplied by MSD
Disclosure: **K.L. Jones:** Grants; This study was supported by a clinical project grant from the Royal Adelaide Hospital Research Foundation. Non-financial support; Study medication was provided by MSD.

777

Effects of DPP-4 inhibitors on BNP, GLP-1, NPY, SP and global longitudinal strain measurements in type 2 diabetes patients

S. Güllü[1], E. Asfuroğlu Kalkan[1], B.I. Aydogan[1], S. Canlar[1], A. Gökçay Canpolat[1], I. Dinçer[2];
[1]Department of Endocrinology and Metabolism, Ankara University Faculty of Medicine, Ankara, [2]Department of Cardiology, Ankara University Faculty of Medicine, Ankara, Turkey.

Background and aims: Previously, a significant relationship between saxagliptin treatment and increased rate of hospitalization for congestive heart failure was reported. Echocardiographic global longitudinal strain (GLS) is a new and effective method in detecting myocardial damage and subclinical cardiac dysfunction even when left ventricular ejection fraction (LVEF) is normal. We aimed to investigate effects of vildagliptin and saxagliptin on brain natriuretic peptide (BNP), neuropeptide Y, substance P (SP), glucagon like peptide-1 (GLP-1) levels and left ventricular global longitudinal strain (GLS), assessed by 3-dimensional speckle tracking echocardiography in uncontrolled type 2 Diabetes Mellitus (T2DM).
Materials and methods: Thirty seven inadequately controlled type 2 DM (HbA1c ≥7.5%) patients who were recently prescribed to either vildagliptin 50 mg BID ($n = 21$) or saxagliptin 5 mg QD ($n = 16$) and completed the study period were included in this study. Exclusion criteria were; 1-Patients who received insulin (recent/previous), 2-Patients with known heart failure and/or coronary heart disease and/or hypertension. Informed consent forms were obtained from all patients and study was approved by Ethical Committee. Body mass index, HbA1c, Hb, creatinine, ALT, AST,BNP, NPY, SP, GLP-1 levels were measured at

admission, 1st and 3rd months of treatment. Global longitudinal strain (GLS) was measured at admission and 3rd month.

Results: Age, BMI, gender, HbA1c, ALT, AST and lipid profiles were not different between treatment groups. In whole group, AST, ALT and HbA1c levels decreased significantly at 3rd month of treatment ($p < 0.001$, $p = 0.002$, $p = 0.004$ respectively) while BNP and NPY values increased significantly ($p < 0.001$, 0.004; respectively). In the vildagliptin group, BNP and NPY values increased significantly at 3rd month of treatment (median = 10.06 (5.22–36) vs. 20.54 (6.4–60.89), $p = 0.020$ and 9.6 (4.3–27.7) vs. 17 (3.92–42.1), $p = 0.047$, respectively). In the saxagliptin group only BNP levels increased significantly (median = 8.5 (3.71–44) vs. 18.38 (2.36–50), $p = 0.015$). In both treatment groups; SP,GLP-1 levels and GLS measurements did not change significantly during follow-up period. Basal and 3rd month mean GLS were -16.34 ± 2.83 vs. -15.82 ± 2.40 in vildagliptin group and -16.41 ± 2.80 vs. -16.72 ± 2.83 in saxagliptin groups, respectively. All measured parameters were similar at the 1.st and 3rd. months of treatment. None of the patients required hospitalization for heart failure during follow-up.

Conclusion: The current study demonstrated that treatment with DPP-4 inhibitors, saxagliptin and vildagliptin, was associated with increased levels of BNP and NPY levels. However no evidence of subclinical myocardial damage or cardiac dysfunction could be detected by GLS measurements assessed by 3-dimensional speckle tracking echocardiography after three months of therapy. Since our study population had no previous clinical cardiac disorders, increases in BNP and NPY levels with these two DPP4 inhibitors can be considered as a safety signal. In order to clarify this, further studies including patients with known heart problems are needed.

Supported by: Ankara University Scientific Research Support

Disclosure: **S. Güllü:** None.

778

Effects of MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, on pancreatic and incretin hormones

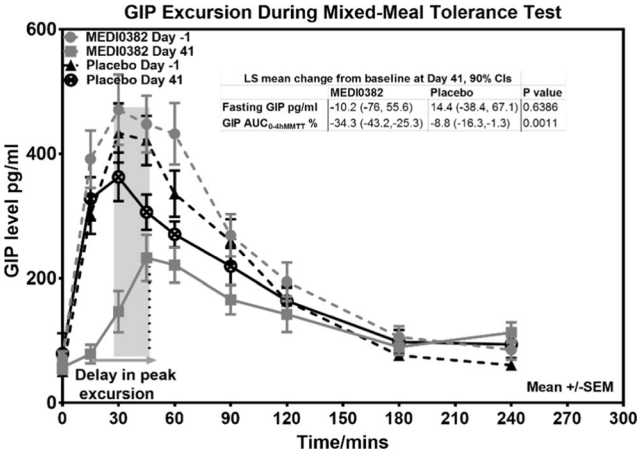
L. Jermutus[1], L.-F. Tsai[2], D. Robertson[1], M. Petrone[1], C. Rondinone[2], H. Boaz[2], P. Ambery[1], V. Parker[1];
[1]MedImmune, Cambridge, UK, [2]MedImmune, Gaithersburg, USA.

Background and aims: MEDI0382, a glucagon-like peptide 1 (GLP-1)/glucagon dual-receptor agonist, is being developed for the treatment of type 2 diabetes mellitus and nonalcoholic steatohepatitis. GLP-1 receptor agonists promote glucose-dependent insulin release, suppress glucagon, and increase or have minimal effect on glucose-dependent insulinotropic polypeptide (GIP); however, knowledge of the effect on GLP-1 is limited. We characterized fasting and postprandial hormone profiles associated with MEDI0382 therapy.

Materials and methods: In a phase 2a study, 51 subjects with type 2 diabetes and a body mass index of 27–40 kg/m^{2}received MEDI0382 (uptitrated to 200 μg) or placebo daily for 41 days. Glucose, insulin, glucagon, GIP, and GLP-1 were measured at baseline (day -1) and day 41 during a liquid mixed-meal tolerance test.

Results: MEDI0382 significantly reduced fasting glucose (−49.9 vs −19.2 mg/dL for placebo; $P < 0.0001$) and glucose $AUC_{0\text{-}4h}$ (−32.8 vs −10.2% for placebo; $P < 0.0001$) in association with increased fasting insulin (2.2 mU/L vs −3.9 mU/L for placebo; $P = 0.0164$), but postprandial insulin AUC was unchanged. In contrast, fasting and postprandial endogenous glucagon, GIP (Figure), and active and inactive GLP-1 were suppressed, and a delay in their kinetics was observed after MEDI0382 therapy.

Conclusion: The results suggest that MEDI0382 is insulinotropic and might also cause delayed gastric emptying. The effects of MEDI0382 on GIP have not been observed with GLP-1 receptor monoagonists and may represent a footprint of dual GLP-1/glucagon receptor agonism in this complex hormone signaling network.



Clinical Trial Registration Number: NCT02548585

Disclosure: **L. Jermutus:** Employment/Consultancy; MedImmune. Stock/Shareholding; AstraZeneca.

779

Comparison of therapy in which incretin action compatibility was considered in basal insulin therapy using continuous glucose monitor: randomised triple crossover study

S. Takeishi, H. Tsuboi, S. Takekoshi;
General Inuyama Chuo Hospital, Inuyama-city, Japan.

Background and aims: We investigated combinations of agents with incretin action used together with basal insulin to ascertain which was the most compatible combination. Vildagliptin can reduce postprandial and fasting glucose levels properly; metformin enhances the effect of long-acting insulin and endogenous insulin secreted from vildagliptin; and miglitol is effective at reducing postprandial glucose levels. We examined the following combinations using a continuous glucose monitor: vildagliptin 100 mg (V) +metformin 500 mg (M), V+miglitol 150 mg (α) or M+α.

Materials and methods: We calculated a required sample size of 30. 30 type 2 diabetic patients were randomly allocated to three groups. On admission, Group 1: Patients took V+M (VM) and glucose level were stabilized (not exceeding 180 mg/dL for 3 days) by insulin glargine 300 U/ml (Glargine300); next, patients wore a continuous glucose monitoring (FreeStyle Libre Pro) device and glycemic variability (GV) was evaluated on days 3 and 4; VM was then switched to V+α (Vα), and GV was evaluated on days 8 and 9; finally, Vα was switched to M+α (Mα) and GV was evaluated on days 13 and 14. Group 2: Patients took in the order of Vα, Mα, and VM, following the same regimen. Group 3, Patients took in the order of, Mα, VM and Vα, following the same regimen. V and M was taken at 08:00 and 18:00, α was taken at 08:00, 12:00, and 18:00. Glargine300 (the same dose as during the FreeStyle Libre Pro measurement period) was injected at 08:00. Data collected on the second evaluation day were analysed (mean amplitude of glycaemic excursion (MAGE), mean of daily differences (MODD) and average daily risk range (ADRR): on all evaluation days). Test meals were given.

Results: 24-h percentage of time in target range (70–140 mg/dL), 24-h mean absolute glucose (MAG), 24-h glycemic variability percentage, mean glucose level (24-h, 00:00–06:00), coefficient of variation (24-h, 00:00–06:00, 08:00–24:00), and high blood glucose index were significantly lower in patients on VM, Vα, and Mα, in that order (Friedman's test). The difference between groups (Δ = VM − Vα) in MAG significantly correlated to body mass index (BMI) ($r = -0.38$, $p = 0.04$). ΔMAGE also significantly correlated to BMI ($r = -0.4$, $p = 0.03$). Mean absolute relative difference compared to 9-point self-monitoring of blood glucose (SMBG) in patients on VM, Vα, and Mα was 10.2%,

10.0%, and 9.7%, respectively. FreeStyle Libre Pro values significantly correlated to SMBG values before and after breakfast, before and after lunch, before and after dinner, at bedtime and at 04:00 (9-point SMBG time) in patients on VM, Vα, and Mα ($r = 0.68$~0.95, $p < 0.0001$). FreeStyle Libre Pro values and SMBG values were not significantly different at 9-point SMBG time in patients on VM, Vα, and Mα.

Conclusion: Vildagliptin and metformin is the most compatible combination to improve GV in basal insulin therapy.

	a: Vildagliptin 100 mg + metformin 500 mg	b: Vildagliptin 100 mg + miglitol 150 mg	c: Metformin 500 mg + miglitol 150 mg	p	p (a vs. b)	p (b vs. c)	p (a vs. c)
24-h percentage of time in target range (70–140 mg/dL), %	61.5 (49.3-77.1)	49.5 (36.8-68.3)	42.2 (25.8-52.9)	<0.0001	0.03	0.0003	<0.0001
24-h mean absolute glucose (MAG), mg/dL	24.5 (20.5-27.9)	24.6 (18.1-30.2)	25.3 (21.0-30.3)	<0.0001	0.03	0.03	<0.0001
24-h glycemic variability percentage (GVP), %	12.5 (9.1-16.8)	12.6 (7.1-18.0)	13.7 (9.7-17.4)	<0.0001	0.049	0.049	<0.0001
24-h mean glucose level, mg/dL	114.1 (97.5-132.9)	125.8 (110.6-153.8)	135.5 (112.4-168.3)	<0.0001	0.02	0.02	<0.0001
00:00 to 6:00 mean glucose level, mg/dL	81.6 (68.4-93.6)	99.6 (87.0-116.2)	104.3 (87.7-145.0)	<0.0001	0.0002	0.01	<0.0001
08:00 to 24:00 mean glucose level, mg/dL	128.6 (110.4-151.4)	143.9 (124.9-169.0)	151.5 (125.8-200.6)	<0.0001	0.01	0.36	<0.0001
24-h standard deviation (SD), mg/dL	29.5 (23.2-45.1)	34.2 (26.6-42.1)	40.0 (29.9-52.6)	<0.0001	0.008	0.03	<0.0001
00:00 to 6:00 SD, mg/dL	8.1 (5.3-12.1)	11.3 (8.5-16.5)	16.2 (12.5-23.5)	<0.0001	0.0006	0.002	<0.0001
08:00 to 24:00 SD, mg/dL	27.4 (18.1-35.6)	31.0 (24.6-34.2)	31.6 (29.1-36.7)	0.001	0.59	0.04	0.002
24-h coefficient of variation (CV), %	22.9 (17.9-32.5)	23.6 (18.8-30.2)	29.6 (24.0-36.1)	<0.0001	0.03	0.008	<0.0001
00:00 to 6:00 CV, %	7.9 (6.3-11.1)	11.9 (8.9-16.1)	16.9 (12.6-19.6)	<0.0001	0.0006	0.0006	<0.0001
08:00 to 24:00 CV, %	18.0 (12.8-21.4)	19.8 (17.5-22.1)	21.8 (18.9-24.6)	<0.0001	0.03	0.008	<0.0001
Mean amplitude of glycemic excursion (MAGE), mg/dL	45.6 (33.6-61.4)	49.3 (37.1-55.3)	55.3 (45.2-69.4)	<0.0001	0.01	0.04	<0.0001
Mean of daily difference (MODD), mg/dL	18.3 (14.2-26.8)	19.2 (15.4-28.1)	17.3 (12.2-24.8)	0.6	1	0.7	0.66
Average daily risk range (ADRR)	12.5 (7.0-20.4)	18.2 (10.5-28.5)	23.2 (16.1-35.5)	<0.0001	0.008	0.01	<0.0001
High blood glucose index (HBGI)	2.8 (1.2-5.6)	3.2 (1.8-6.8)	4.7 (2.7-10.6)	<0.0001	0.03	0.0005	<0.0001
Highest postprandial glucose level within 3 hours after each meal, mg/dL							
breakfast	166.0 (139.0-201.8)	187.5 (144.8-217.5)	188.5 (158.0-229.8)	0.001	0.3	0.1	0.001
lunch	156.5 (120.3-206.0)	168.5 (128.5-223.8)	179.5 (148.3-245.0)	0.001	0.04	0.59	0.002
supper	171.0 (141.5-192.5)	174.0 (146.5-211.8)	181.5 (158.5-237.8)	0.009	0.49	0.19	0.009
24-h area over the glucose curve (AOC) (<70 mg/dL), mg * min/dL	0 (0-1912.5)	0 (0-16.9)	0 (0-1541.3)	0.14	0.29	0.19	0.97

Clinical Trial Registration Number: UMIN000028988
Disclosure: **S. Takeishi:** None.

780

Urinary proteomics may unmask the renal potential of the dipeptidyl peptidase (DPP)-4 inhibitor linagliptin in patients with diabetic kidney disease (DKD)

J. Siwy[1], H. Mischak[1], T. Klein[2], M. von Eynatten[3];
[1]mosaiques-diagnostics GmbH, Hannover, [2]Boehringer Ingelheim, Biberach, [3]Boehringer Ingelheim, Ingelheim, Germany.

Background and aims: DKD is a serious complication of hyperglycaemia and novel treatments to preserve renal function are needed. DPP-4 inhibitors are commonly used glucose-lowering drugs in type 2 diabetes (T2D) patients. Among these, linagliptin (LINA) has previously been suggested to have renal potential independent of its effects on hyperglycaemia. We aimed to characterise the urinary proteomic profile (UPP) before and after treatment with LINA in T2D patients at early stages of DKD.

Materials and methods: Samples were derived from the previously reported MARLINA-T2D™ trial. In brief, T2D patients ($n = 360$) with prevalent albuminuria (urinary albumin creatinine ratio [UACR] ≥30 mg/g) despite stable background renin-angiotensin system blockade were randomised 1:1 to receive double-blind, oral treatment with LINA 5 mg or placebo for 24 weeks. Urine samples were collected at baseline and after treatment. Capillary electrophoresis coupled with mass spectrometry was performed to assess UPP and a previously developed CKD273 score.

Results: Urine samples were available for 88.8% of the study population ($n = 320$). At baseline, the CKD273 score showed a strong correlation with UACR (rho = 0.54) and estimated glomerular filtration rate (eGFR) (rho = −0.41), respectively (both $p < 0.0001$). In addition, baseline CKD273 ($p = 0.027$) and baseline eGFR ($p = 0.015$), but not baseline UACR, were associated with eGFR decline per year in the placebo group. LINA treatment was not associated with a significant overall difference in eGFR compared to placebo at study end. However, after stratification of the study population according to low and high risk for renal disease progression (based on CKD273 score) we identified numerically less renal function loss in high risk patients with LINA (median GFR: 90.0 ml/min/1.73 m² at study end) as compared to placebo (median GFR: 65.0 ml/min/1.73 m² at study end; $p = 0.06$). No differences in eGFR at study end between the LINA and placebo groups were observed in the low risk group. We further compared the UPP before and after treatment with LINA ($n = 164$). We identified 993 peptides potentially affected by LINA and sequenced 314. The majority of sequenced peptides were increased after LINA. This finding was expected based on the protease feature of DPP-4. Consequently, out of the 95 most altered peptides, 53 had the amino acid proline in the penultimate position. This sequence is compatible with the DPP-4 active site, known to cleave X-proline dipeptides from the N-terminus. Therefore, the UPP after LINA was enriched for peptides containing the N-terminal X-proline, and consequently peptides lacking the DPP-4 signature dipeptide were decreased. We were able to identify such pairs of peptides. Finally, we found a significant ($p < 0.05$) negative correlation between the urinary concentration increase with reduced plasma DPP-4 activity for 20 peptides.

Conclusion: This sub-study of the MARLINA-T2D™ trial confirms previous evidence of the CKD273 score to be associated with markers of DKD. A novel finding was that CKD273 could not only identify patients at high risk for DKD progression but may also be indicative for the potential of LINA to slow renal function loss. Furthermore, our comprehensive assessment of the UPP revealed a significant impact of LINA on urinary peptides with a pattern reflective of the specific protease fingerprint of the DPP-4 enzyme.

Clinical Trial Registration Number: NCT01792518
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **J. Siwy:** Non-financial support; Boehringer Ingelheim.

781

DPP-4 inhibitor teneligliptin attenuates high glucose induced beta cell dysfunction via cAMP-Sirt1 pathway

S. Elumalai[1], U. Karunakaran[2], J. Moon[2], K. Won[2];
[1]Institute of Medical Science, Yeungnam University College of Medicine, Buk-gu, Daegu, [2]Department of Internal Medicine, Yeungnam University College of Medicine, Buk-gu, Daegu, Republic of Korea.

Background and aims: Teneligliptin, a newer DPP-4 inhibitor provides a narrative clinical efficacy and oral tolerability with a unique chemical structure amongst currently available DPP-4 inhibitors. A very recent study shows that DPP-4 is expressed in human pancreatic beta cells and there is no study about the effect of Teneligliptin in the pancreatic beta cells. Herein we investigated the direct effect of Teneligliptin, on beta-cell function and survival in response to high glucose conditions.

Materials and methods: We subjected INS-cells and human 1.1b4 pancreatic beta cells to high glucose (30mM) for 48 hours in the absence or presence of DPP-4 inhibitor Teneligliptin. Insulin secretion was assessed by Millipore ELISA Kit. P66Shc phosphorylation and mitochondrial translocation was measured by western blot and immunofluorescent analysis. Reactive oxygen species were measured by using DCFDA. Apoptosis was determined by TUNEL In-Situ cell death detection kit. The protein expression level of GLP1R, Sirt1, JNK and cleaved caspase-3 signaling and SERCA2B ubiquitination in response to high glucose was assessed by western blot analysis.

Results: Exposure of INS-1 cells or human 1.1b4 pancreatic beta cells to high glucose (30mM) downregulated GLP1R expression and induce beta cell apoptosis. Interestingly, Teneligliptin treatment stabilized GLP1R protein and increased the intracellular cAMP production ($p < 0.005$) and potentiate glucose stimulated insulin secretion (GSIS) ($p < 0.05$). In parallel, Teneligliptin treatment correlated with the up-regulation of Sirt1 expression. Further, Teneligliptin treatment significantly decreased the

high glucose induced reactive oxygen species (ROS) production ($p < 0.05$) and reduce the JNK mediated p66Shc serine 36 phosphorylation and its mitochondrial translocation and cleaved caspase-3 activation. Moreover, Teneligliptin counteracted the high glucose induced ubiquitination of SERCA2b and lowers the ER stress markers. Interestingly, cAMP pathway inhibition by H89 (PKA inhibitor) blocked the teneligliptin protective effects against the high glucose induced beta cell apoptosis.

Conclusion: Teneligliptin stabilizes GLP1R protein and increasing the cAMP dependent antioxidant response (Sirt1 activation) and its downstream signaling lead to β-cell function (GSIS) and survival under high glucose conditions. Teneligliptin ameliorates high glucose-induced endoplasmic reticulum stress by reducing the ubiquitination of SERCA2b. Collectively, our results unveil a direct effect of Teneligliptin on beta-cell function and survival.

Supported by: National Research Foundation of Korea (NRF)

Disclosure: **S. Elumalai:** None.

782

DPP-4 inhibitor reduces the risk of developing hypertrophic scars and keloids in diabetic patients: analysis using the National Database of Health Insurance Claims of Japan

H. Suwanai[1], R. Watanabe[2], M. Sato[1], H. Matsumura[1];
[1]Tokyo Medical University, Tokyo, Japan, [2]Kanagawa University of Human Services, Kanagawa, Japan.

Background and aims: Hypertrophic scars and keloids, abnormalities due to hyperplasia of collagen fibers, often occur in surgical wounds, but their exact cause and preventive measures are unknown. The administration of dipeptidyl peptidase-4 (DPP-4) inhibitors to humans is expected to suppress fibrosis in wounds and minimise hypertrophic scar and keloid formation. This study aimed to verify the suppressive effect of DPP-4 inhibitors on the formation of hypertrophic scars and keloids in diabetic patients using data from the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB).

Materials and methods: We extracted data from NDB between April 2013 and March 2015 and performed retrospective cohort study. NDB includes approximately 3.3 billion claims. Patients who underwent median sternotomy in April 2014 were included in the study based on their claimed surgical codes. Exclusion criteria included median sternotomy during the preceding or subsequent 1-year period, "scars" or "keloids" within 1 year prior to the procedure, and death during the study period. Subjects who were prescribeDPPP-4 inhibitors between April 2013 and March 2014 comprised the treatment group; subjects who were not prescribed or administereDPPP-4 inhibitors during that period comprised the non-treatment group. Subjects included 5430 patients throughout Japan (3509 men: mean age, 65.1 years; 1921 women: mean age, 66.0 years).

Results: Of the 446 subjects who were treated with DPP-4 inhibitors within 1 year before the procedure, six (1.35%) developed either hypertrophic scars or keloids within 1 year of the procedure. Of the 4984 subjects who were not treated, 152 (3.05%) were at significantly lower risk for developing hypertrophic scars and keloids (risk ratio, 0.696; $p = 0.04$). Logistic regression analysis was performed to adjust for confounding factors, with history of hypertrophic scar formation as the explained variable anDPPP-4 inhibitor treatment, age, sex, diabetes mellitus status, and steroid treatment as explanatory variables. Treatment with DPP-4 inhibitors minimised the development of hypertrophic scars and keloids, although it was not statistically significant (treatment odds ratio, 0.512; $p = 0.12$).

Conclusion: Treatment with DPP-4 inhibitors in diabetic patients minimised the risk of hypertrophic scars and keloids in humans. These results prove a cause-and-effect relationship and are highly generalisable to Japanese people.

Disclosure: **H. Suwanai:** None.

783

Liraglutide treatment in obese diabetic patients modulates gut microbiota

S. Moffa[1], V. Tesori[2], V.A. Sun[1], T. Mezza[1], C.M.A. Cefalo[1], F. Impronta[1], F. Cinti[1], G.P. Sorice[1], F. Scaldaferri[2], G. Cammarota[2], A. Gasbarrini[2], A. Giaccari[1];
[1]Center for Endocrine and Metabolic Diseases, Policlinico Gemelli, Rome, [2]Gastroenterology Division and Internal Medicine Institute, Policlinico Gemelli, Rome, Italy.

Background and aims: Liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, acts by stimulating glucose-dependent insulin secretion and by slowing gastric emptying. Recent studies in mouse models have shown that Liraglutide is able to modulate the composition of the intestinal microbiota suggesting that this may be one of the factors favoring the drug-induced weight loss. We here aimed to observationally evaluate the effect of Liraglutide on gut microbiota composition in obese diabetic humans.

Materials and methods: Eight obese diabetic subjects (age 61.7 ± 10.5; HbA1C 8.1 ± 1.4%; weight: 102.6 ± 11.9 kg), candidates to receive GLP1-analogs therapy, were included in the study. All subjects underwent, before (B) and after 6 weeks of treatment (PT) with Liraglutide 1.2 mg, metabolic evaluation including BMI, glucose, HbA1c and LDL-cholesterol. Lactulose Breath Test (LBT) was performed to characterize gut microbiota, measuring hydrogen (H2) and methane (CH4) production; while gastric emptying time (t/2) was assessed by Octanoic Acid Breath Test.

Results: All subjects experienced a significant weight loss (4% of the basal body weight; BMI B: 35.7 ± 3.3 kg/m^2, BMI PT: 34.9 ± 2.8 kg/m^2, $p = 0{,}03$), as well as a significant reduction of fasting plasma glucose (FPG) (FPG B: 153.7 ± 34.3 mg/dl, FPG PT: 135 ± 37.3 mg/dl $p = 0.02$), Hb1Ac (HbA1C B: 8.1 ± 1.4%, HbA1C PT: 7.2 ± 0.9% $p = 0.004$) and LDL-cholesterol (LDL B: 84.8 ± 42.1 mg/dl; LDL PT: 67.3 ± 26.6 mg/dl, $p = 0.005$). Methane and Hydrogen production (AUC) were significantly reduced by Liraglutide in the post-treatment group (H2 B: 7140 ± 12.5 ppm, H2 PT: 1831.8 ± 1130 ppm, $p = 0.0008$; CH4 B: 1663.5 ± 161.6, CH4 PT: 600 ± 366 ppm, $p = 0.001$); moreover, the LBT revealed that Liraglutide is able to significantly slow down the Oro-cecal Transit Time (OTT). As expected, gastric emptying was significantly reduced after Liraglutide treatment (t/2 B: 49.7 ± 12.5 min, t/2 PT: 88 ± 13.4 min $p = 0.002$).

Conclusion: These results confirm the effect of Liraglutide in delay gastric emptying and show also the effect in reducing the OTT. We, here, show that Liraglutide also induces a reduction in the intestinal gases production, which is an indirect measure of changes in gut microbiota. In conclusion, we highlight the close relationship between glucose lowering effect of Liraglutide and the intestinal microbiota composition, suggesting that the treatment effect may be potentially mediated by changes in gut microbiota, which could in turn represent a new therapeutic target for treatment of type 2 diabetes.

Disclosure: **S. Moffa:** None.

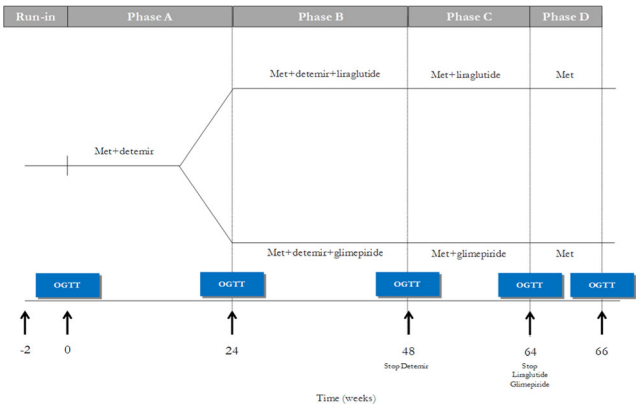
PS 065 Beta cell function and response to incretin-based therapies

784

Liraglutide and glimepiride in type 2 diabetic patients with failure to oral hypoglycaemic agents: effects on beta cell function

E. Salutini[1], S. Del Guerra[2], S. Barone[2], G. de Gennaro[2], S. Del Prato[2], C. Bianchi[3];
[1]Hospital of Pistoia, Pistoia, [2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, [3]University Hospital of Pisa, Pisa, Italy.

Background and aims: GLP-1 analogues have been claimed to prevent beta-cell loss and ensure a more sustained glycaemic control. However, whether this is related to duration of diabetes is presently unclear. We, therefore, compared the effect of liraglutide as compared to a sulphonylurea in sustaining beta-cell function in patients with Type 2 diabetes (T2DM) and secondary failure to oral agents after restoration of strict glycaemic control with insulin.
Materials and methods: Fifteen type 2 diabetic patients (age 66 ± 1 yrs; BMI 29.9 ± 1.5 Kg/m^2; mean ± SE) with poor glycaemic control (HbA1c 8.2 ± 0.2%) with metformin+sulfonylurea therapy were randomly assigned to liraglutide (LIR; $n = 8$) or glimepiride (GLI; $n = 7$) after the restoration of strict glycaemic control with basal insulin (Figure). Fasting and OGTT (75 g) plasma glucose, and insulin, were measured at entry and after completion of each of the 4-phases of intervention.
Results: After 24 wk insulin therapy HbA1c improved in both groups (LIR 7.5 ± 0.2 vs. GLI 7.5 ± 0.2; $p > 0.05$ vs. basal). LIR (1.4 ± 0.2 mg/day) or GLI (3.8 ± 0.4 mg/day) add-on therapy further lowered HbA1c (LIR 6.4 ± 0.1 vs. GLI 6.7 ± 0.2; $p > 0.05$). Insulin withdrawal was associated with only marginal and not different HbA1c increase (LIR 6.8 ± 0.2 vs. GLI 7.1 ± 0.2) with no changes 2 wks after LIR and GLI discontinuation (LIR 6.8 ± 0.2 vs. GLI 7.1 ± 0.3). With LIR the AUCins/AUCglucose increased at wk48 (basal 0.05 ± 0.01; wk48: 0.15 ± 0.04) and remained higher after insulin discontinuation (0.14 ± 0.04) and after LIR withdrawal (0.10 ± 0.03; all $p < 0.05$ vs. basal). GLI also was associated with an increase in the AUC ratio that was significant only after insulin discontinuation (Basal 0.03 ± 0.01; 48wk: 0.10 ± 0.05; 64wk 0.06 ± 0.01, $p > 0.05$ vs basal; 66wk 0.05 ± 0.01). HOMA-IR was not significantly affected in both treatment arms. Disposition index (DI) was calculated as the Insulinogenic Index adjusted by HOMA-IR x 100. In both arms, DI improved after initial insulin treatment (Basal: 0.3 ± 0.2; wk24, LIR 1.6 ± 0.2, GLI 3.9 ± 1.5) to return to baseline in the ensuing time points.
Conclusion: In T2DM patients with failure to oral agents restoration of glycaemic control with insulin is associated with an improvement of beta-cell function that seems to persist to a better extent with continuation of glucose lowering therapy with liraglutide than with glimepiride.



Supported by: Novo Nordisk
Disclosure: **E. Salutini:** None.

785

HbA_{1c} target attainment in patients with type 2 diabetes receiving iGlarLixi who reach postprandial glucose and fasting plasma glucose targets in the LixiLan-L trial

J. Davidson[1], J. Chao[2], A. Saremi[3], T. Dex[3], L. Leiter[4];
[1]Touchstone Diabetes Center, Dallas, USA, [2]Xinyi, Inc., Bridgewater, USA, [3]Sanofi US, Inc., Bridgewater, USA, [4]St. Michael's Hospital, Li Ka Shing Knowledge Institute, Toronto, Canada.

Background and aims: Treatments that reduce HbA_{1C} levels often do not reflect improvements in both fasting and postprandial hyperglycaemia.
Materials and methods: Data from 731 patients with type 2 diabetes uncontrolled on basal insulin ± oral antidiabetes drugs in the LixiLan-L trial were used to investigate the association between achieving fasting plasma glucose (FPG)/postprandial glucose (PPG) targets and HbA_{1C} target attainment after 30 weeks of treatment with a titratable once-daily fixed-ratio combination of basal insulin and lixisenatide (iGlarLixi; $n = 366$), or insulin glargine (iGlar; $n = 365$). Outcomes were HbA_{1C} target attainment, HbA_{1C} change from baseline, and mean HbA_{1C} at Week 30 in patients achieving both FPG and PPG targets, FPG target only, PPG target only, or neither, using American Diabetes Association (ADA) glycaemic targets.
Results: The proportion of patients reaching PPG only, or both FPG and PPG targets, was numerically higher for iGlarLixi, while the proportion reaching FPG target only was numerically higher for iGlar **(Table)**. iGlarLixi-treated patients reaching both FPG and PPG targets, or FPG target only, showed statistically significant greater HbA_{1C} changes from baseline, lower end-of-trial HbA_{1C}, and a higher proportion reaching HbA_{1C} target than iGlar-treated patients **(Table)**. Despite a numerically higher proportion of patients reaching FPG target only in the iGlar arm, HbA_{1C} outcomes were in favour of patients receiving iGlarLixi.
Conclusion: In conclusion, the complementary actions of iGlarLixi on both fasting and postprandial hyperglycaemia were associated with better HbA_{1C} target attainment compared with iGlar alone, which mainly targets fasting hyperglycaemia.

Table: FPG and PPG target attainment, mean HbA_{1C} at week 30, HbA_{1C} change from baseline, and HbA_{1C} target attainment in iGlarLixi- or iGlar-treated patients according to ADA targets (2-hour PPG <180 mg/dL; FPG <130 mg/dL; HbA_{1C} <7.0%).

		Glycaemic Targets			
		FPG and PPG at target	*FPG only at target*	*PPG only at target*	*Neither at target*
Proportion of patients reaching FPG and/or PPG targets (%)	iGlarLixi	42 (n = 152)	21 (n = 75)	11 (n = 39)	27 (n = 100)
	iGlar	14 (n = 51)	49 (n = 180)	2 (n = 7)	35 (n = 127)
HbA_{1c} change from baseline (%)	iGlarLixi	-1.4 (n = 151)	-1.2 (n = 74)	-1.0 (n = 39)	-0.6 (n = 82)
	iGlar	-1.0** (n = 51)	-0.7** (n = 179)	-1.1 (n = 7)	-0.2 (n = 118)
Mean HbA_{1c} at week 30 (%)	iGlarLixi	6.6 (n = 151)	6.9 (n = 74)	7.0 (n = 39)	7.6 (n = 82)
	iGlar	7.0** (n = 51)	7.4** (n = 179)	7.2 (n = 7)	7.8 (n = 118)
Proportion of patients reaching HbA_{1c} target (%)	iGlarLixi	75 (n = 114)	59 (n = 114)	59 (n = 23)	20 (n = 20)
	iGlar	59* (n = 30)	34** (n = 62)	14* (n = 1)	12 (n = 15)

* $P < 0.05$, ** $P < 0.01$ compared to iGlarLixi.

Clinical Trial Registration Number: NCT02058160
Supported by: Study and editorial support (provided by Excerpta Medica) funded by Sanofi
Disclosure: **J. Davidson:** Employment/Consultancy; Amgen Inc., Aspire Bariatrics, AstraZeneca, Boston Therapeutics, Eli Lilly, Intarcia, Janssen, Merck, Novo Nordisk, Sanofi, Valeritas.

786

Propensity-matched patient-level comparison of iGlarLixi and basal-bolus regimen in patients with type 2 diabetes

J. Meier[1], J. Anderson[2], C. Wysham[3], F.J. Tinahones[4], A. Saremi[5], P. Stella[5], M. Liu[6], A.G. Tabak[7];
[1]St Josef Hospital, Ruhr-University Bochum, Bochum, Germany, [2]The Frist Clinic, Nashville, USA, [3]Rockwood Clinic, Spokane, USA, [4]University of Malaga, Malaga, Spain, [5]Sanofi, Bridgewater, USA, [6]BDM Consulting, Somerset, USA, [7]Semmelweis University Faculty of Medicine, Budapest, Hungary.

Background and aims: Given the progressive nature of type 2 diabetes (T2D), glycaemic control is expected to become insufficient with basal insulin-supported oral therapy (BOT) in T2D and intensification with

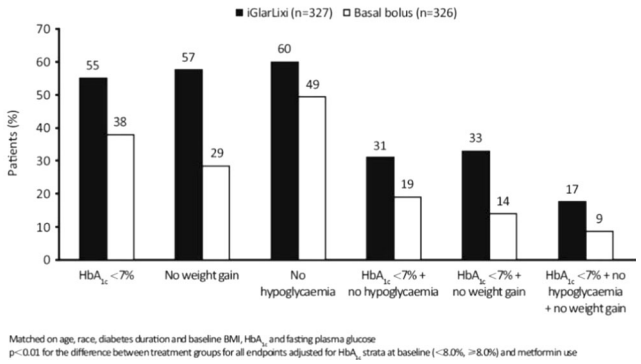
prandial insulin may be required. However, the high injection burden, weight gain, increased risk of hypoglycaemia, and the complexity of basal-bolus (BB) regimens are barriers in real-world practice. iGlarLixi, a once-daily titratable fixed-ratio combination of insulin glargine 100 U/mL and the glucagon-like peptide-1 receptor agonist lixisenatide, may offer a simple alternative to BB regimens to provide glycaemic control while mitigating weight gain and hypoglycaemia in patients with T2D.

Materials and methods: In this post hoc analysis, patients randomized to iGlarLixi in the LixiLan-L trial were matched (using propensity scores based on the covariates of age, race, diabetes duration, baseline BMI, HbA_{1c} and fasting plasma glucose) to patients randomized to BB insulin in the GetGoal Duo-2 trial, yielding 328 pairs. In addition, in a sensitivity analysis, 192 pairs were matched with baseline basal insulin dose added to the pool of matched covariates. Changes from baseline were compared by mixed-effect models using repeat measurements with treatment groups, randomization strata of HbA_{1c} (<8.0% or ≥8.0%) at screening, metformin use (yes/no), and country as fixed effects; and visit, baseline*visit interaction, and treatment*visit interaction as covariates.

Results: In the primary analysis (Figure), greater proportions of patients in the iGlarLixi group reached the individual endpoints of HbA_{1c} <7%, no weight gain and no hypoglycaemia, as well as composite endpoints (this was also the case in patients further matched for baseline insulin dose). In addition, a greater reduction in HbA_{1c} (least squares [LS] mean −0.28% [standard error (SE) 0.06]) was achieved in the iGlarLixi group versus the BB group ($p < 0.0001$). The LS mean (SE) differences in change from baseline total insulin dose (BB insulin vs iGlarLixi) were 3.31 (1.48) U and 5.08 (1.34) U in patients unmatched and matched for baseline dose, respectively ($p < 0.05$).

Conclusion: iGlarLixi offers an effective alternative to BB insulin, with lower rates of hypoglycaemia and weight gain in patients with T2D inadequately controlled with a BOT at a moderate basal insulin dose.

Figure. Proportion of patients reaching individual and combined outcomes



Matched on age, race, diabetes duration and baseline BMI, HbA_{1c} and fasting plasma glucose
$p<0.01$ for the difference between treatment groups for all endpoints adjusted for HbA_{1c} strata at baseline (<8.0%, ≥8.0%) and metformin use

Clinical Trial Registration Number: NCT02058160, NCT01768559
Supported by: Sanofi
Disclosure: **J. Meier:** Grants; Boehringer-Ingelheim, MSD, Novo Nordisk, Sanofi. Lecture/other fees; Astra Zeneca, Boehringer-Ingelheim, Eli Lilly, MSD, Novo Nordisk, Sanofi-Aventis, Servier. Other; Board Member/Advisory Panel: Astra Zeneca, Boehringer-Ingelheim, Eli Lilly, MSD, Novo Nordisk, Sanofi, Servier.

787

Impact of type 2 diabetes duration on response to iGlarLixi vs iGlar: a subanalysis of LixiLan-L

L. Blonde[1], L. Berard[2], A. Saremi[3], Y. Huang[4], V.R. Aroda[5], D. Raccah[6]; [1]Frank Riddick Diabetes Institute, Ochsner Medical Center, New Orleans, USA, [2]Wellness Institute Seven Oaks General Hospital, Winnipeg, Canada, [3]Sanofi, Bridgewater, USA, [4]BDM Consulting, Somerset, USA, [5]Brigham and Women's Hospital, Boston, USA, [6]University Hospital Sainte-Marguerite, Marseilles, France.

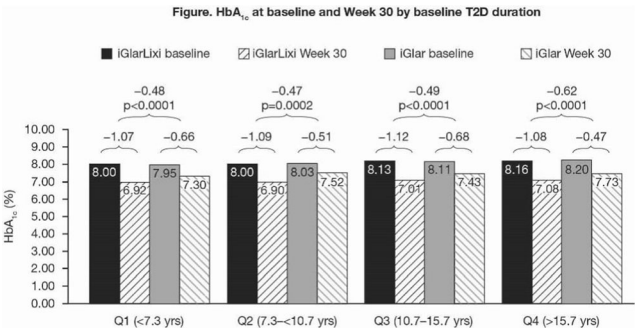
Background and aims: Glucagon-like peptide-1 receptor agonists such as lixisenatide have insulin-independent effects, which may allow benefit for patients with a longer duration of type 2 diabetes (T2D) and greater loss of β-cell function.

Materials and methods: We assessed the effects of insulin glargine U100 (iGlar) vs fixed-ratio iGlar plus lixisenatide (iGlarLixi) by T2D duration in the LixiLan-L trial ($N = 736$). Changes in glycated haemoglobin (HbA_{1c}), weight, and insulin dose from baseline to Week 30, as well as hypoglycaemia rates, were analysed in patients divided into quartiles by recorded baseline T2D duration (<7.3, 7.3–<10.7, 10.7-15.7, and >15.7 yrs). Patients were also grouped by both duration and baseline insulin dose, both of which may relate inversely to β-cell function.

Results: Baseline HbA_{1c} was higher in longer-duration quartiles but similar with iGlarLixi vs iGlar. iGlarLixi reduced HbA_{1c} more vs iGlar across all duration quartiles (Figure). The difference was greatest in patients in the longest duration quartile (least squares mean difference [standard error], −0.62 [0.13]; $p < 0.0001$). Mean (SD) weight change across quartiles ranged from −0.95 (3.50) to −0.11 (3.65) with iGlarLixi and from 0.43 (2.81) to 1.13 (2.83) with iGlar ($p < 0.0001$, 0.0053, <0.0001, and 0.3281 for between-group differences, in lowest to highest duration quartiles). In both treatment groups, patients in the shortest duration quartile had the greatest mean insulin dose change from baseline to Week 30 (11.91 and 14.36 U for iGlarLixi and iGlar, respectively, versus 8.84 and 8.90, respectively, in the longest duration quartile). The difference in hypoglycaemia (iGlarLixi vs iGlar) was greatest in patients in the longest duration quartile (3.3 vs 6.9 events/patient-yr; $p < 0.0001$). In patients grouped by T2D duration and insulin dose, those with both long duration (≥15.7 yrs) and high dose (≥42 U) showed the greatest difference in HbA_{1c} lowering with iGlarLixi vs iGlar.

Conclusion: In LixiLan-L, iGlarLixi lowered HbA_{1c} more vs iGlar regardless of T2D duration, with the greatest difference in those with the longest duration.

Figure. HbA_{1c} at baseline and Week 30 by baseline T2D duration



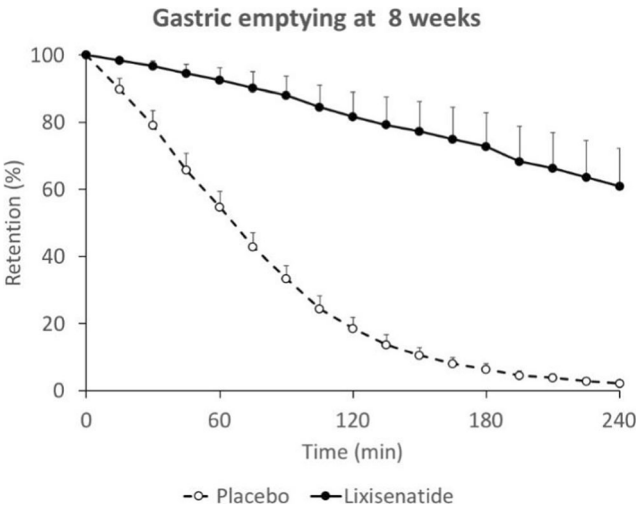
Clinical Trial Registration Number: NCT02058160
Supported by: Sanofi
Disclosure: **L. Blonde:** Employment/Consultancy; AstraZeneca, GlaxoSmithKline, Intarcia Therapeutics, Inc., Janssen Pharmaceuticals, Inc., Merck & Co., Inc., Novo Nordisk, Sanofi. Grants; AstraZeneca, Janssen Pharmaceuticals, Inc., Lexicon Pharmaceuticals, Inc., Merck & Co., Novo Nordisk, and Sanofi. Lecture/other fees; AstraZeneca, Janssen Pharmaceuticals, Inc., Merck & Co., Novo Nordisk, Sanofi.

788

Effects of sustained treatment with lixisenatide on gastric emptying and glucose metabolism in type 2 diabetes

C.K. Rayner[1,2], L.E. Watson[1], L.K. Phillips[1,2], M.J. Bound[1], J. Grivell[1], T. Wu[1,2], K.L. Jones[1], M. Horowitz[1,2], E. Ferrannini[3], D. Trico[3], S. Frascerra[3], A. Mari[4], A. Natali[3];
[1]University of Adelaide, Adelaide, Australia, [2]Royal Adelaide Hospital, Adelaide, Australia, [3]University of Pisa, Pisa, Italy, [4]Institute of Neuroscience, Padua, Italy.

Background and aims: Slowing of gastric emptying is a key mechanism by which 'short-acting' glucagon-like peptide-1 receptor agonists (GLP-1RAs) lower postprandial glycaemia in type 2 diabetes (T2DM). We examined the effects of sustained use of a standard clinical dose of lixisenatide (20mcg daily) on gastric emptying and postprandial glucose metabolism, measured using the 'gold standard' techniques of scintigraphy and double glucose tracers, in patients with T2DM.
Materials and methods: 18 metformin-treated T2DM patients (age 67 ± 1 years, HbA1c 7.1 ± 0.1%) consumed a 75 g glucose drink labelled with ^{99m}Tc-calcium phytate and [U-^{13}C]glucose during IV infusion of 6,6-[$^{2}H_2$]glucose. Gastric emptying (scintigraphy) and glucose fluxes were monitored for the next 240min. Patients were then randomised to receive 8 weeks daily lixisenatide (titrated up to 20mcg in the first 2 weeks) or placebo in a double blind parallel design, before the 75 g oral glucose study was repeated. Data are presented as mean values ± SEM.
Results: Gastric emptying did not differ between the groups at baseline, or after 8 weeks of placebo, but was markedly slower after 8 weeks of lixisenatide when compared to both baseline and placebo ($P < 0.0001$, figure). Compared to placebo, lixisenatide was associated with a much lower (i) incremental area under the postprandial glucose curve (349 ± 155 vs 1389 ± 82 mmol.L^{-1}.min), (ii) area under the [U-^{13}C] glucose curve (465 ± 165 vs 1516 ± 95), and (iii) rate of appearance of [U-^{13}C] glucose (all $P < 0.0005$). In patients receiving lixisenatide, the decrements in both blood glucose and plasma [U-^{13}C] glucose at 90 and 120 min, compared to baseline, were strongly related to the reduction in the proportion of oral glucose emptied from the stomach at each time point (r = 0.94 and 0.77 for blood glucose, $r = 0.86$ and 0.78 for plasma [U-^{13}C] glucose, all $P < 0.05$).
Conclusion: 8 weeks treatment with lixisenatide in T2DM is associated with sustained slowing of gastric emptying and marked reductions in postprandial glycaemia and appearance of ingested glucose. 'Short-acting' GLP-1 RAs therefore represent an effective long-term therapy for specifically targeting postprandial glucose excursions.



Clinical Trial Registration Number: ACTRN12616001059459
Supported by: This investigator initiated study received funding from Sanofi
Disclosure: **C.K. Rayner:** Grants; This study is supported by Sanofi Australia. Sanofi have had no involvement in the design and/or development of the study and will have no role in the collection, analysis, and interpretation of data.

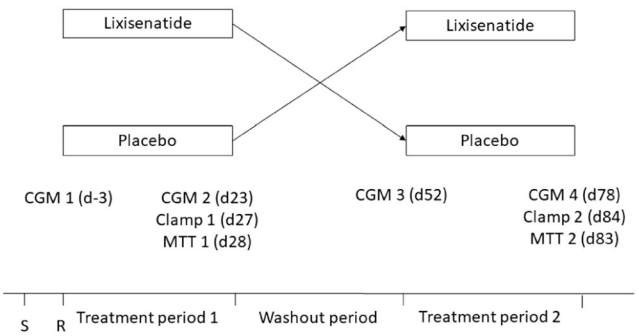
789

The effect of lixisenatide on post-prandial blood glucose and glucagon in type 1 diabetes

C. Ballav[1], A. Dhere[1], O. Agbaje[2], I. Kennedy[2], R.R. Holman[2,3], K.R. Owen[1,3];
[1]Oxford Centre for Diabetes Endocrinology and Metabolism, Oxford, [2]Diabetes Trial Unit, Oxford, [3]Oxford Biomedical Research Centre, Oxford, UK.

Background and aims: The glucagon-like peptide-1 receptor agonist, Lixisenatide (Lix), suppresses glucagon and reduces hyperglycaemia in type 2 diabetes. We studied the effect of Lix on post-prandial blood glucose (PPBG) and glucagon in type 1 diabetes (T1D).
Materials and methods: In a double-blinded, placebo-controlled, cross-over study, 25 patients with T1D (13 females, mean ± SE HbA_{1C} 65.6 ± 8.1 mmol/mol, BMI 27.0 ± 3.6 Kg/m^2) received treatment in random order with Lix and placebo (Plac) in addition to their usual insulin therapy for four weeks, with a four week washout period in between. Participants had continuous glucose monitoring (CGM) for at least 3 days before and at the end of each treatment period, as well as post treatment standard mixed meal tests (MMT; Fortisip 360 Kcal) and hyperinsulinaemic hypoglycaemic clamps (target glucose 2.5 mmol/L). (See Figure). The primary outcome was defined as the proportion of CGM readings in the range 4 to 10 mmol/L during the 3-hour post-prandial period.
Results: The mean ± SE percentage of PPBG CGM readings in range was similar before and after treatment and for each meal for Lix compared with Plac (breakfast 45.4 ± 6.0 vs. 44.3 ± 6.0, $p = 0.9$, lunch 45.5 ± 5.8 *vs.* 50.6 ± 5.3, p = 0.6, and dinner 43.0 ± 6.7 *vs.* 47.7 ± 5.6, $p = 0.6$). Mean HbA1c did not change during treatment periods and was similar between Lix and Plac (64.7 ± 1.6 *vs.* 64.1 ± 1.6 mmol/mol, $p = 0.3$). The overall daily prandial insulin dose post-treatment was significantly less after Lix compared with Plac (−0.7 ± 0.6 *vs.* +2.4 ± 0.7 units/day, $p = 0.004$), but the total insulin dose was not different between treatments. The post MMT mean ± SE 120 minute glucose area under the curve (AUC) was lower with Lix compared with Plac (392.0 ± 167.7 *vs.* 628.1 ± 132.5 mmol/L × min, $p < 0.001$), as was the corresponding glucagon AUC (140.0 ± 110.0 *vs.* 304.2 ± 148.2 nmol/L × min, $p < 0.001$). Glucagon values at a blood glucose level of 2.4 mmol/L during the hypoglycaemic clamp, were similar for Lix compared with Plac (3.1 ± 3.7 *vs.* 2.6 ± 1.7 nmol/L, p = 0.7). Mean adrenaline, noradrenaline, cortisol and pancreatic polypeptide values did not differ during the clamp between Lix and Plac.
Conclusion: Lixisenatide suppresses glucagon and may reduce post prandial glycaemia without compromising counter-regulatory responses during hypoglycaemia in T1D.

Figure 1. Trial design (S: screening, R: Randomisation, d: day)



Clinical Trial Registration Number: ISRCTN no. 00290196
Supported by: Funding for this Investigator Sponsored research was provided by Sanofi
Disclosure: **C. Ballav:** None.

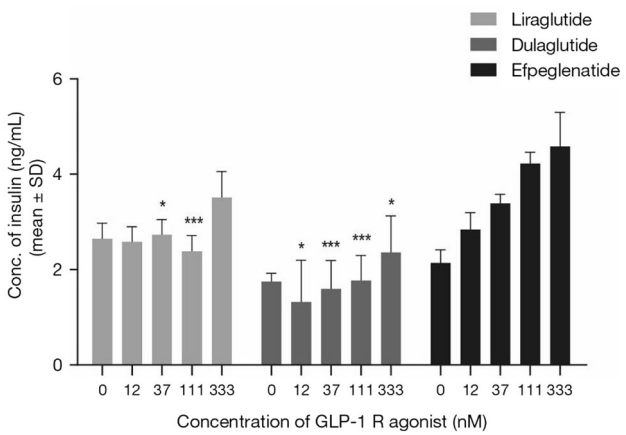
790

In vitro studies to evaluate the receptor kinetics of efpeglenatide versus other glucagon-like peptide-1 receptor (GLP-1 R) agonists

I. Choi[1], M. Moon[1], M. Trautmann[2], M. Hompesch[2], C. Sorli[3];
[1]Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea, [2]ProSciento, Inc., Chula Vista, USA, [3]Sanofi, Bridgewater, USA.

Background and aims: Efpeglenatide is a long-acting GLP-1 R agonist in development for the treatment of type 2 diabetes. Efpeglenatide's effects on the GLP-1 R suggest that it is a superagonist: a ligand that leads to greater maximal signalling and stimulation compared with the endogenous ligand.
Materials and methods: *In vitro* studies evaluated the superagonistic effects of efpeglenatide and compared them to the effects of liraglutide (lira) and dulaglutide (dula) on GLP-1 R-binding kinetics and internalization, and cell signalling/desensitization.
Results: Efpeglenatide had a higher dissociation constant vs lira or dula (360.7 vs 58.7 and 39.4 nmol/L, respectively) indicating a lower binding affinity for GLP-1 R (~6.1 fold vs lira, ~9.2 vs dula) and faster dissociation vs lira (~3.6 fold; $p < 0.001$) or dula (~2.9 fold; $p < 0.001$). At 100 nM, efpeglenatide led to less receptor internalization vs lira or dula (% of initial internalized receptor: 140% vs 1191% and 714%, respectively; $p < 0.001$ each) and lower proportions of receptor lost from the cell surface (32% vs 74% and 69%; $p < 0.001$). After pretreatment for 4 h, efpeglenatide led to significantly greater insulinotropic activity vs lira or dula (Figure). After pretreatment for 24 h, significantly greater accumulation of cyclic AMP was observed with efpeglenatide vs lira or dula.
Conclusion: The superagonistic effect of efpeglenatide on the GLP-1 R may be due to its specific binding characteristics, allowing more cell-surface receptor availability for intracellular signalling. The clinical relevance of these findings should be assessed further.

Figure. Reduced *in vitro* GLP-1 R desensitization with efpeglenatide vs other GLP-1 RAs



Cells were pretreated with 10 nM GLP-1 R agonist for 4 h and insulin release was measured after retreatment with 0–333 nM GLP-1 R agonist for 1 h
*p<0.05, **p<0.01, ***p<0.001, vs efpeglenatide by one-way ANOVA test at each concentration
ANOVA, analysis of variance

Supported by: Hanmi
Disclosure: **I. Choi:** Employment/Consultancy; Hanmi Pharm Co.

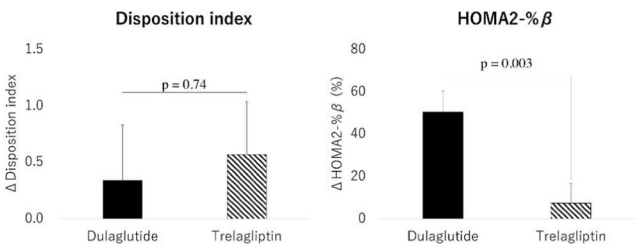
791

Effects of dulaglutide and trelagliptin on beta cell function in patients with type 2 diabetes: a randomised controlled study: DUET-beta study

Y. Kondo[1], S. Satoh[2], Y. Terauchi[1];
[1]Department of Endocrinology and Metabolism, Graduate School of Medicine, Yokohama City University, Yokohama, [2]Department of Endocrinology and Metabolism, Chigasaki Municipal Hospital, Chigasaki, Japan.

Background and aims: Some studies with glucagon-like peptide 1 receptor agonists (GLP-1-RA) and dipeptidyl peptidase-4 inhibitors (DPP-4-I) have suggested positive effects on the beta-cells. But the direct comparison between weekly GLP-1-RA: dulaglutide (Dula) and weekly DPP-4-I: trelagliptin (Trela) effects on beta-cell function has not yet been clarified. Therefore, we compared the effects of Dula and Trela on beta-cell function in patients with type 2 diabetes (T2D) in open-label, parallel-group, randomized controlled trial.
Materials and methods: In this trial, subjects received Dula 0.75 mg/week or Trela 100 mg/week for 24 weeks. Beta-cell function was assessed by glucagon stimulation test (GST) based disposition index (DI = area under the curve of C-peptide during 6 min GST ÷ HOMA2-IR). The primary endpoint was the difference between the two groups of change in DI over the 24-weeks treatment period. Body composition was also assessed by bioelectrical impedance method.
Results: Fifty metformin ± basal insulin-treated patients with T2D were randomized to Dula or Trela. Forty-eight patients completed 24 weeks of weekly administration of Dula ($n = 23$) or Trela ($n = 25$). The primary outcome of change in DI during 24 weeks was not different between both groups (Dula: +0.34 ± 0.49, Trela: +0.57 ± 0.47, $p = 0.74$). However, change in HOMA2-%β was higher in Dula group than Trela group (Dula: +50.6 ± 9.9% vs. Trela: +7.5 ± 9.5%, $p = 0.003$). HbA1c was decreased in greater extent with Dula group (Dula: −0.77 ± 0.07%, Trela: −0.57 ± 0.07%, $p = 0.04$). Severe hypoglycemia was not observed in both groups. Body weight was reduced more in Dula group than Trela group (Dula: −1.4 ± 0.3 kg vs. Trela: −0.3 ± 0.3 kg, $p = 0.02$). Body fat mass was reduced more in Dula group than Trela group (Dula: −1.2 ± 0.3 kg vs. Trela: −0.3 ± 0.2 kg, $p = 0.02$). However, change in skeletal muscle mass did not differ between the groups (Dula: −0.2 ± 0.1 kg vs. Trela: −0.1 ± 0.1 kg, $p = 0.66$). Dula did not reduce skeletal muscle mass from baseline (−0.2 ± 0.1 kg, $p = 0.31$).
Conclusion: The effects of Dula and Trela on beta-cell function were not different in GST-based DI. However, Dula increased HOMA2-%β level more than Trela. Dula reduced body fat mass without skeletal muscle mass loss.

Figure 1. Change in indices of beta-cell function



Clinical Trial Registration Number: UMIN-CTR 000024164
Disclosure: **Y. Kondo:** None.

792

Rescue therapy with linagliptin to improve glucose metabolism and pancretic beta cell function in patients with prediabetes with no response to metformin

S. Salazar-Lopez[1], M. Alvarez-Canales[1], D. Farfan[1], L. Jimenez-Ceja[1], M. Montes de Oca[1], F. Angulo-Romero[1], M. Reyes-Escogido[1], E. Durán-Pérez[2], A. Aguilar-García[1], R. Guardado-Mendoza[1,2];
[1]Metabolic Research Laboratory, University of Guanajuato, Leon, Guanajuato, [2]Hospital Regional de Alta Especialidad del Bajío, Leon, Guanajuato, Mexico.

Background and aims: Patients with prediabetes have a high risk to develop T2DM. Lifestyle modifications and metformin are therapeutic options in these patients with limited results. The goal of this work was to evaluate the effect of adding linagliptin to metformin on glucose metabolism and beta cell function in patients with prediabetes with no response to metformin alone during the previous 12 months
Materials and methods: Patients with impaired glucose tolerance (IGT) were previously on metformin therapy 1700 mg/day during 12 months plus a lifestyle program and those that showed no improvement on glucose metabolism were selected for the present study; they had a metabolic evaluation including oral glucose tolerance test (OGTT) with insulin measurements, body composition, lipid profile and HbA1c, and were randomized to: *i) linagliptin 2.5 mg/metformin 850 mg twice daily (**LM group**, n = 15)*, or *ii) continue with metformin alone 850 mg twice daily* (***M group***, $n = 10$), with monthly follow-up and a 6 month metabolic evaluation. Insulin sensitivity and pancreatic beta cell function were calculated from the OGTT. The protocol was approved by the Ethical Committee. Inter and intragroup differences were analyzed with a T test.
Results: All patients had IGT at the beginning of the study, and there were not basal differences in age (53 vs 50 y) and body weight (75 vs 75 kg) between the LM and M group. After 6 months of treatment, the LM group had better improvements in weight (−2.0 vs −0.26 kg, p 0.096), glucose during the OGTT at 0′(−12 vs 0.3 mg/dl, p 0.013), 30′(−36 vs −5 mg/dl, p 0.056), 60′(−57 vs −7 mg/dl, p 0.012), 90′(−43 vs −8 mg/dl, p 0.020) and 120′(−46 vs −5 mg/dl, p 0.011), and $AUCglucOGTT_{0\text{-}120}$ −4675 vs −711 mg/dl/120 min, p 0.015, HbA1c (−0.20 vs 0.26%, p 0.003), insulin sensitivity (Matsuda index +2.0 vs −1.4, p 0.008), and beta cell function (Disposition index +0.77 vs −0.12, p 0.001, and Oral disposition index +0.11 vs −0.01, p 0.036).
Conclusion: Adding linagliptin to metformin, together with a lifestyle program, improved better glucose metabolism, insulin sensitivity, insulin secretion and beta cell function in patients with IGT previously treated and not improved with metformin alone for 12 months. This could be a useful preventive strategy in patients with prediabetes with no response to metformin and a high risk of T2DM
Supported by: Hospital Regional de Alta Especialidad del Bajío
Disclosure: **S. Salazar-Lopez:** None.

PS 066 Multiple facets of continuous glucose monitoring

793

Real-time decoding of endogenous islet algorithms and their use in a type 1 diabetes simulator

M. Raoux[1], M. Jaffredo[1], L. Olçomendy[2], A. Pirog[2], B. Catargi[3], S. Renaud[2], J. Lang[1];
[1]CNRS UMR 5248, CBMN, Univ. Bordeaux, Bordeaux, [2]CNRS UMR 5218, IMS, Bordeaux INP, Univ. Bordeaux, Bordeaux, [3]Hôpital Saint-André, Bordeaux University Hospital, Bordeaux, France.

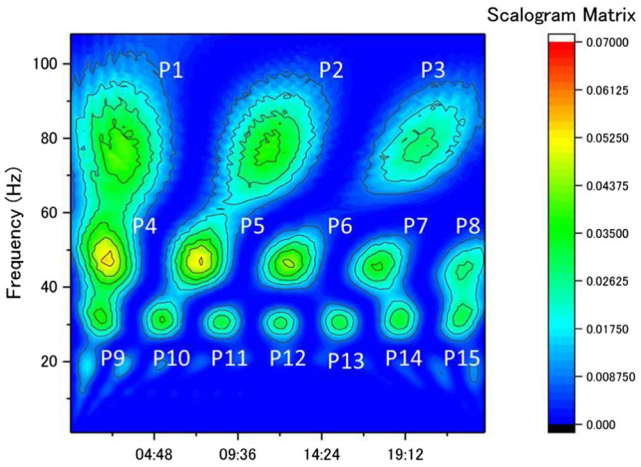
Background and aims: Pancreatic islets continuously sense and process multiple nutritional and neuro-hormonal inputs by "endogenous algorithms" to precisely adapt insulin secretion dynamics. New non-invasive and high-resolution sensors are needed to decipher these natural algorithms. Electrical signals are the first readout integrating multiple physiological inputs and they can be monitored extracellularly with multi-electrode arrays (MEAs). Whereas action potentials (APs) translate single-cell activity, the so-called slow potentials (SPs) reflect physiologically important coupling among islet β cells. Here we asked whether both signals can (i) be recorded simultaneously, (ii) be analyzed online and in real-time (iii) and can qualify as signals to drive an insulin pump during continuous monitoring.
Materials and methods: Intact mouse or human islets were cultured on MEAs in which the electrodes were covered with an electroactive polymer, PEDOT:CNT. Electrical signals were recorded with a high sampling rate (10,000 Hz) and analyzed i) offline with the commercial software MC_Rack (Multichannel Systems) and ii) online and in real-time on custom digital integrated circuits (Field-Programmable Gate Array, FPGA). To test their capacity driving an insulin pump, data were introduced via a Python program in the UVA/Padova T1DM Simulator, an FDA-approved model of T1D patients, in a three-meal 24 h scenario.
Results: Mouse islets were exposed to physiological glucose elevation (3–8.2 mM; 1h30). Electrodes covered with the polymer detected SPs and APs with a significant amelioration in comparison with classical non-polymer electrodes (42.5 ± 3.7 vs $69.8 \pm 6.3\%$ of electrodes with detectable SPs and APs; $p < 0.05$; $N = 7$ independent preparations). In this setting, our integrated circuit was able for the first time to automatically and simultaneously analyze in real-time (<40 μs) three relevant electrical parameters: SPs' frequency and amplitude as well as APs' frequency. The FPGA-based hardware linked to MEAs clearly detected a biphasic electric profile of islet activation with the same efficiency as offline analysis by commercial software ($n = 70$ electrodes; $N = 3$). Long-term monitoring with the set-up showed signal stability for at least up to 25 days. The system also detected and analyzed simultaneously SPs and APs generated by human islets ($N = 6$ pancreata). Biosensor data were modeled and introduced in the whole human body UVA/Padova T1DM Simulator. The introduction of the biphasic electrical component resulted in glycemic profiles in these in-silico T1D patients similar to those observed in healthy subjects and without the use of further algorithms. Furthermore, this approach was exempt of hypoglycemic incident and maximal post-prandial glucose levels were similar to healthy subjects.
Conclusion: Electrical activity of islets as recorded by MEAs parallels known secretion patterns. The in-silico validation shows that the device qualifies as efficient CGM system for the control of insulin delivery in closed-loop configurations. The bio-electronic sensor developed here will also serve to further decode islet's "endogenous algorithms" in response to physiological combinations of nutrients and hormones.
Supported by: French ANR, Région Nouvelle Aquitaine, FEDER
Disclosure: **M. Raoux:** None.

794

New insight into evaluation of continuous glucose monitoring index using continuous wavelet transformation

Y. Nakamura[1], S. Furukawa[2];
[1]Specified Clinic of Soyokaze CardioVascular Medicine and Diabetes Care, Matsuyama, [2]Department of Epidemiology and Preventive Medicine, Ehime University Graduate School of Medicine, Toon, Japan.

Background and aims: Continuous glucose monitoring (CGM) has recently become available to select a therapeutic strategy for poorly controlled diabetes. However, CGM is not yet a gold standard marker since many glucose data are manipulated and daily glucose value changes occur due to feeding behavior etc. Continuous wavelet transformation (CWT) which extracts valid information from time and frequency domains, is used in the cardiology field. We hypothesized that CWT could contribute to sourcing glucose changes based on patient background, as such change would be converted to a waveform.
Materials and methods: 126 sets of daily glucose data were evaluated in 7 type 2 diabetic patients (T2DM) (59 ± 5 years) and 3 type 1 patients (T1DM) (54 ± 13 years). Data were obtained from subcutaneous tissue every 15 min over 2 weeks. If data were lost due to sensor error, that day's data were excluded. Complete data were assessed via CWT with Morlet wavelets ($n = 7$) as the mother waveform. The CGM sensor was also attached to 10 healthy volunteers (60 ± 14 years). Daily glucose data showing levels of 69–146 mg/dl were used as controls.
Results: CGM data were obtained from 116 diabetics, 23 controls. Hemoglobin A1c was 9.8 ± 0.9% in T2DM, 8.9 ± 0.4% in T1DM and 5.4 ± 0.1% in control. Average glucose levels were 212 ± 76 mg/dl, 156 ± 71 mg/dl, 101 ± 14 mg/dl, respectively ($p < 0.0001$). Maximum glucose levels in T2DM were higher than for T1DM (333 ± 77 mg/dl vs 274 ± 72 mg/dl, $p < 0.0001$). In contrast, minimum data showed no significant difference (72 ± 34 mg/dl vs 117 ± 34 mg/dl, NS). Variations in daily glucose changes in T1DM and T2DM were also similar. CWT was visualized as a contour map with x-axis; time, y-axis; frequency and z-axis; scalogram (Fig). A high frequency (75 ± 6 Hz) wave (HW)(P1) at midnight disappeared in T1DM and T2DM, though it was present in all controls (22%, 9%, 0%, respectively, $p = 0.0251$). Evening HW (P3) in T1DM and T2DM was also significantly decreased compared to control (24%, 8%, 0%, respectively, $p = 0.00649$). Power values of the scalogram obtained from CWT were lower than in control (P1;0.047 ± 0.012, 0.049 ± 0.008, 0.057 ± 0.005, respectively, $p = 0.0001$, P2; 0.053 ± 0.009, 0.054 ± 0.008, 0.064 ± 0.004, respectively, $p < 0.0001$, P3; 0.049 ± 0.008, 0.048 ± 0.009, 0.058 ± 0.004, respectively, $p < 0.0001$). Low frequency small waves (LW) (30 ± 2 Hz) such as P9–P12 emerged in T2DM from midnight to noon (14% in P19, $p = 0.00859$, 19% in P10, $p = 0.003$, 20% in P11, $p = 0.0118$, 19% in P12, $p = 0.0353$). LW appeared in the afternoon area and night time showed no significant differences among groups. Also, super-high frequency (98 ± 8 Hz) waves were amplified in 8 maps (22%) in T1DM, 6 maps (8%) in T2DM but not in control ($p = 0.0167$).
Conclusion: CWT differentiated specificity of each group at a point distinct from that of previous markers. This method may contribute to selection of therapeutic strategies such as choice and combination of drugs or evaluation of pathogenesis.



Disclosure: **Y. Nakamura:** None.

795

Avoidance of glucose excursions with predictive alerts in the Guardian™ Connect CGM system: real-world paediatric data

O. Cohen[1], S. Abraham[2], C. McMahon[2], P. Agrawal[2];
[1]Medtronic, Tolochenaz, Switzerland, [2]Medtronic, Northridge, USA.

Background and aims: The Guardian™ Connect continuous glucose monitoring (CGM) system allows users to view sensor glucose (SG) data directly on a smartphone. The Guardian™ Connect system has built-in glucose threshold alerts to notify users about low and high glucose excursions. The system also includes predictive glucose threshold alerts that notify users 10–60 minutes before a low or high glucose excursion. The real-world rates of alerts and outcomes of Guardian™ Connect system users, from January 2, 2017-March 13, 2018, were evaluated. These results were compared to a control dataset where alerts were disabled.
Materials and methods: De-identified CareLink™ sensor data from 1,183 children ≤15 years old with >5 days of sensor data were analyzed. Excursions were defined as ≥3 consecutive SG values beyond the preset low threshold range (2.2–21.6 mmol/L [40–390 mg/dL]) and high threshold range (2.8–22.2 mmol/L [50–400 mg/dL]). The low and high SG thresholds for control were marked during the periods when no alerts were enabled and when the SG level crossed 4.0 mmol/L (73 mg/dL) and 12.9 mmol/L (233 mg/dL), respectively. Both levels were based on the median low and high SG threshold settings of the users. The low and high predictive alerts for control were marked when the SG level would have crossed the low SG threshold and high SG threshold (17.5 minutes and 12.5 minutes before the user-established median settings, respectively). For analyses, the window of evaluation for excursion start times was within 60 minutes following an alert and the excursion duration following an alert was segmented into: avoided, ≤20 min, 20–60 min, and >60 min.
Results: The table shows the percentage of each alert resulting in an excursion, which are stratified per excursion duration. Users who received predictive alerts avoided 59% of low events and 37% of high events. The percentage point improvement for excursions avoided was 31% and 28% following predicted low and predicted high alerts, respectively, compared to control. The percentage point reduction for excursions >60 min was 14% and 25% following predicted low and predicted high alerts, respectively, compared to control.
Conclusion: Guardian™ Connect CGM system users who enabled predictive alerts avoided more than 59% and 37% of predicted low and high events, respectively. Predictive alerts are a useful technology for users with diabetes to keep SG levels within target range.

		Cohort	Low SG Excursion		High SG Excursion	
			Predictive Alerts Enabled	Alerts Not Enabled	Predictive Alerts Enabled	Alerts Not Enabled
Time to Excursion Resolution		Total Alert Count	301246	9693	134453	115170
	Avoided	Excursion Count	176469	2683	49384	10932
		% of Total Alerts	59%	28%	37%	9%
	≤20 min	Excursion Count	54967	2534	14694	13302
		% of Total Alerts	18%	26%	11%	12%
	20-60 min	Excursion Count	52493	2504	23340	21729
		% of Total Alerts	17%	26%	17%	19%
	>60 min	Excursion Count	17317	1972	47035	69207
		% of Total Alerts	6%	20%	35%	60%

Disclosure: **O. Cohen:** None.

796

Accuracy assessment of the WaveForm Cascade CGM system versus FreeStyle Libre over 14 days

M. Rebec[1], E. Anderson[1], R. Dutt-Ballerstadt[1], A. Haidar[2], A. Singh[2], A. Janez[3];
[1]WaveForm Technologies, Inc, Wilsonville, USA, [2]BioMedical Engieneering Department, Mc Gill University, Montreal, Canada, [3]Department of Endocrinology, Diabetes and Metabolic Diseases, University of Ljubljana, Ljubljana, Slovenia.

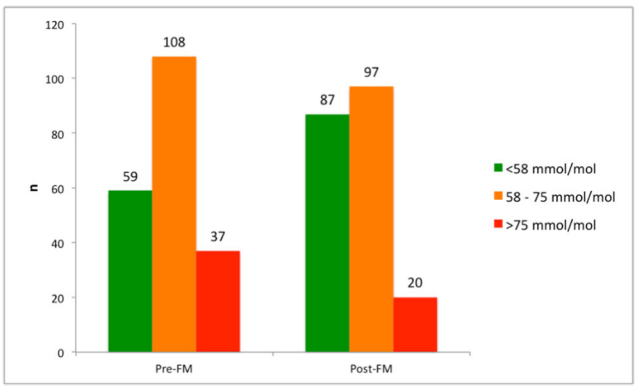
Background and aims: WaveForm is finalizing the development of the Cascade CGM system. The Cascade CGM device features trocar-free insertion and will be launched with 14 day transdermal sensor that is based on a variant of an amperometric GOx-based technology. Completing the algorithm and validating the clinical performance of the device is the final development stage. We are reporting the preliminary analysis of 14-day clinical study that was used to evaluate the accuracy of the Abbott FreeStyle Libre and the Cascade CGM.
Materials and methods: The clinical study that was used to evaluate the two CGM systems included 10 subjects with type 1 and 2 diabetes. There were five in-clinic days (1, 4, 7, 10 and 14) that were used to assess the performance of the CGM's accuracies. Each subject wore two Cascade CGM devices in the abdominal area and one Abbott FreeStyle Libre sensor on the back of the upper arm over 14 days. YSI glucose measurements were performed on plasma from venous blood sampled every 15 minutes during the 12 hour in-clinic days. The overall MARD and MAD calculation for the Cascade CGM and Abbott devices based on a comparison to paired YSI values at the same time points. The Cascade CGM values were obtained by prospectively applying an advanced algorithm to data generated during the study.
Results: Head-to-head MARD comparison between the WaveForm and Abbott FreeStyle Libre sensor over 14 days showed that MARD for the Cascade CGM was11.6 vs 14.0% for the FreeStyle Libre. Consensus error grid analysis for Cascade device showed that 99.7% of data points were in zone A and B, with the remaining 0.3% in zone C.
Conclusion: Overall performance of the Cascade CGM device over 14 days meets the clinical expectations for a potentially non-adjunctive commercial CGM. Direct comparison with the FreeStyle Libre shows that the WaveForm device met and in a number of parameters surpassed the performance of the FreeStyle Libre. The Cascade CGM will be launched as 14-day wear CGM system.
Clinical Trial Registration Number: TD-17-012 (UP14CGM)
Disclosure: **M. Rebec:** Employment/Consultancy; Employee of Agamatrix, Inc.

797

Flash Glucose Monitoring is associated with improved glycaemic control and quality of life in people with type 1 diabetes: a large 'real-world' assessment

F.W. Gibb, R.H. Stimson, N.N. Zammitt, A.R. Dover;
Edinburgh Centre for Endocrinology & Diabetes, Edinburgh, UK.

Background and aims: Flash Glucose Monitoring (FM) has been associated with reduced hypoglycaemia and, in uncontrolled studies, with improvements in HbA1c in type 1 diabetes. NHS (National Health Service) funded FM was recently introduced in our centre. We sought to assess the 'real world' impact of FM in a University hospital diabetes clinic upon HbA1c and patient reported outcomes.
Materials and methods: 2211 people with T1 diabetes were invited to attend an education event leading to NHS-funded FM prescription in February 2018. 646 (29.2%) attended and commenced NHS funded FM. 240 (37.2%) reported previous self-funded use of FM. SCI-Diabetes (national diabetes register) was interrogated to obtain pre- and post-FM HbA1c in previous 'self-funders'. Deprivation was assessed by Scottish Index of Multiple Deprivation 2016 (SIMD). Attendees were invited to complete a questionnaire (36.4% response rate) assessing the impact of FM, including on parameters from the diabetes distress scale (DDS).
Results: Self-funded FM was associated with a median 3 mmol/mol fall in HbA1c, $p < 0.001$ ($n = 204$, median 10 month follow-up [IQR 5–11]). The reduction was greater in those with >75% FM use (−4.5 vs. −2 mmol/mol, $p = 0.01$) and in those above 58 mmol/mol prior to FM use (−4 vs. 0 mmol/mol, $p < 0.001$). Overall, the proportion of people achieving target HbA1c (<58 mmol/mol) rose from 28.9% to 42.6% (figure). 35.3% of our total clinic population in the least deprived quintile commenced NHS-funded FM compared to only 17.5% of those in the most deprived quintile ($p < 0.001$). 37.8% of those with an HbA1c <58 mmol/mol commenced NHS-funded FM compared to only 26.4% of those above target ($p < 0.001$). NHS funded FM use was also greater in women ($p < 0.001$) and younger individuals ($p < 0.001$). User satisfaction was high: the median response (out of 10) was 10 for the question 'How useful has the Libre been in helping control your diabetes?' Emotional burden elements of the DDS were 'unchanged' in 37.4%, 'less of a problem' in 41.9% 'much less of a problem' in 14.6%. Regimen related distress elements were 'unchanged' in 25.2%, 'less of a problem' in 39.5% and 'much less of a problem' in 29.8%.
Conclusion: Self-funded FM was associated with clinically meaningful improvements in HbA1c, particularly in those with higher baseline HbA1c and those using the system regularly. Self-funded and NHS-funded FM is still disproportionately used in more affluent individuals and those with lower HbA1c at baseline. Greater efforts are required to ensure equity of access to diabetes technologies. Patient reported outcomes are overwhelmingly positive - the impact of FM on quality of life should not be underestimated.



Disclosure: **F.W. Gibb:** None.

798

Occurrence of severe hypoglycaemic events in the future: analysis of CGM data of the HypoDE study

N. Hermanns[1], D. Ehrmann[1], G. Freckmann[2], D. Waldenmaier[2], S. Pleus[2], G. Faber-Heinemann[3], L. Heinemann[3];
[1]Research Institute of the Diabetes Academy Bad Mergentheim (FIDAM), Bad Mergentheim, [2]IDT-Institut für Diabetes-Technologie, Forschungs- und Entwicklungsgesellschaft mbH an der Universität Ulm, Ulm, [3]Science-Consulting in Diabetes GmbH, Düsseldorf, Germany.

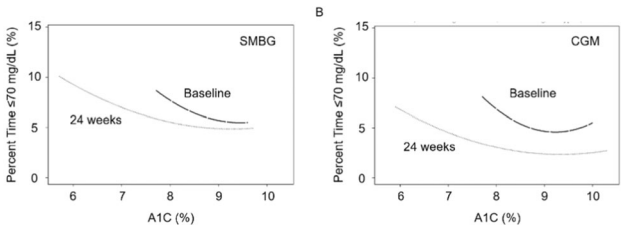
Background and aims: Besides many other outcome parameters, rtCGM systems allow an estimation of low glucose events (LGE). In addition to the international consensus statement on CGM data, more empirical evidence is needed to evaluate which rtCGM parameter is the best predictor of the occurrence of severe hypoglycaemic events in the future (SH; defined as events that need third party assistance for recovery). Therefore, we analysed data of the control group of the HypoDE study, a randomised controlled trial that has shown that rtCGM had a beneficial impact on the occurrence of LGE (<55 mg/dl) in patients with type 1 diabetes on multiple daily injections (MDI) and hypoglycaemia problems. We analysed the ability of different rtCGM outcome parameters at baseline to predict the occurrence of SH during therapy- and follow-up-phase (T/F-phase) of the HypoDE-study.
Materials and methods: Participants in the control group wore a masked rtCGM system for 4 weeks in the baseline phase. In the consecutive 26 weeks of the T/F-phase, they continued with blood glucose measurements. The rtCGM data of 66 participants of the control group (age 47 ± 10 years, diabetes duration 21 ± 13 years, HbA1c 7.4 ± 1.0%, 61% with SH during the previous year) were analysed. The area under the Receiver Operating Characteristics (ROC) curves of different rtCGM parameters were used to compare the predictive performance of these parameters.
Results: During the T/F-phase, 39 episodes of SH were observed. At least one episode of SH occurred in 14 of the 66 participants (21.2%). The area under the ROC curve for the percentage of glucose values ≤70 mg/dl was 0.69 (95% CI 0.54–0.85, $p = 0.027$), for ≤55 mg/dl 0.68 (95% CI 0.52–0.85, $p = 0.034$), for ≤45 mg/dl 0.66 (95% CI 0.49–0.84, $p = 0.060$), for time-in-range 0.59 (95% CI 0.44–0.75, $p = 0.293$), for coefficient of variation 0.67 (95% CI 0.49–0.84, $p = 0.056$), for standard deviation 0.51 (95% CI 0.36–0.67, $p = 0.863$), for mean sensor glucose 0.70 (95% CI 0.55–0.86, $p = 0.022$), and for low blood glucose index 0.70 (95% CI 0.55–0.86, $p = 0.022$).
Conclusion: The overall predictive power of rtCGM data for future SH was low. However, the best and significant predictors were percentage ≤70 mg/dl or ≤55 mg/dl, the low glucose index and mean glucose. Lower cut-off values for LGE like ≤45 mg/dl showed a slight deterioration of the predictive performance. Coefficient of variation showed a borderline significance for the prediction of future SH. A systematic analysis of rtCGM while in use and an increase in the number of LGE might be used as a warning signal for people with diabetes to prevent occurrence of SH.
Clinical Trial Registration Number: NCT02671968
Supported by: Dexcom Inc, San Diego
Disclosure: **N. Hermanns:** Employment/Consultancy; Abbott, Sanofi, Lilly. Grants; Berlin Chemie, Roche, Abbott, Dexcom. Honorarium; Berlin Chemie, Dexcom, Abbott.

799

The relationship between HbA_{1c} and hypoglycaemia in the Diamond trial

N. Oliver[1], M. Reddy[1], M. Gimenez[2], V. Moscardo[3];
[1]Endocrinology and Metabolism, Imperial College, London, UK, [2]Endocrinology Department, Hospital Clinic, Barcelona, Spain, [3]Universitat Politecnica de Valencia, Valencia, Spain.

Background and aims: Intensification of insulin therapy and self-monitoring of blood glucose (SMBG) with the aim of reducing glycated hemoglobin (A1C) is classically associated with increased rates of hypoglycemia for patients with type 1 diabetes (T1D). Real-time awareness of sensor glucose (SG) values from continuous glucose monitoring (CGM) systems helps patients with diabetes reduce A1C and avoid hypoglycemia. However, the relationship between A1C and hypoglycemia during CGM use has not been assessed. We retrospectively analyzed data from a large trial, which introduced CGM to subjects with T1D using multiple daily injections (MDI), to determine the relationship between A1C and percent time in hypoglycemia (≤70 mg/dL).
Materials and methods: The DIAMOND clinical trial compared usual care (SMBG) to CGM in subjects with diabetes who were using MDI and who had A1C levels between 7.5% to 10.0%. Data from subjects with T1D completing Phase 1 ($n = 105$ and 53 in the CGM and control groups, respectively) were used for the current analysis. The percentages of sensor glucose (SG) values ≤70 mg/dL (≤3.9 mmol/L) ("%≤70") during the initial and final weeks of the 24-week study were calculated for each subject and compared to corresponding baseline and 24-week A1C values. Logistic regression was performed to determine any potential confounding factor that may have affected differences between the treatment groups. Multivariate regression analysis was performed to adjust for the confounders and assess the treatment effect on %≤70.
Results: Those subjects who had lower baseline A1C spent as much as 8% of the time in hypoglycemia (≤70 mg/dL) (Panels A and B), supporting earlier observations that low A1C is associated with high risk of hypoglycemia. Subjects who were randomized to usual care (SMBG) and those randomized to CGM demonstrated improved A1C after 24 weeks (Panels A and B). At both baseline and week 24, subjects with the lowest A1C values had the highest rates of hypoglycemia (Panels A and B). However, regardless of A1C, subjects randomized to CGM demonstrated lower %≤70 than those randomized to SMBG (Panel B). Moreover, the association between decreasing A1C and increasing exposure to SG values ≤70 mg/dL was attenuated for subjects in the CGM group after 24 weeks of treatment (Panel B). By week 24, some subjects randomized to CGM were able to reach A1C values ~6% while simultaneously reducing their exposure to hypoglycemia (Panel B).
Conclusion: Historically, attempts to achieve near-normal glucose concentrations during aggressive diabetes treatment have come at the expense of increased risk for hypoglycemia. Data shown here demonstrate that CGM allows safe intensification of MDI therapy and achievement of optimal A1C levels while attenuating hypoglycemia. These data also suggest that it is possible that features unique to CGM, such as programmable alerts and alarms, allow patients to take appropriate and timely measures to avoid hypoglycemia.



Clinical Trial Registration Number: NCT02282397
Disclosure: **N. Oliver:** None.

800

A new formula to compute eA1c from 3-months average interstitial glucose measured by FreeStyle Libre in patients with type 1 diabetes

P. Oriot[1], M.P. Hermans[2];
[1]Mouscron Hospital Center, Mouscron, [2]Cliniques universitaires St-Luc, Brussels, Belgium.

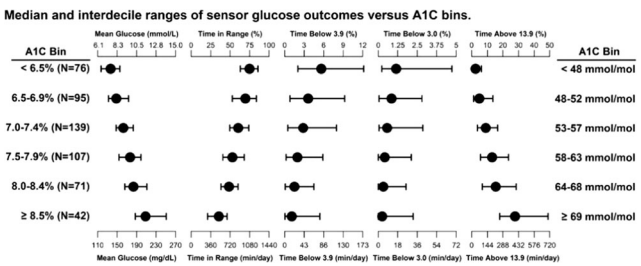
Background and aims: The FreeStyle Libre sensor automatically captures interstitial glucose levels several times an hour and, provided that the rate of scanned results is sufficient (≥70% of the studied period), it is possible to predict a corresponding value of HbA1c over a similar period of glycaemic exposure (3 months) based on the following equation: eA1c (%) = (mean glucose (mg/dL) + 46.7)/28.7. The actual performance of mean interstitial glucose compared to the measured HbA1c in discriminating patients according to their glycaemic levels is not known. Due to methodological limitations it has become increasingly needed to have a new and validated equation that is not skewed in predicting HbA1c obtained from FreeStyle Libre sensor's recordings.
Materials and methods: The Discriminant Ratio (DR) method was used to compare 3-months average interstitial glucose ($_{3\text{-months}}$AIG; FreeStyle Libre) vs. HbA1c measured in the laboratory during the same period to rank diabetic patients according to their glycaemic exposure. The DR is the ratio of the underlying between-subject standard deviation (SD) to the within-subject SD, and takes into account the variation between subjects, the within-subject biological variation, and the analytical variation. The DRs were calculated over two consecutive 3-months periods in 153 T1DM patients with a sensor scanning frequency ≥70%. The correlation coefficients between the 2 periods were adjusted in order to include an estimate of the underlying correlation, since standard coefficients, due to the presence of within-subject variation, underestimate the true correlation between tests (attenuation). Finally, an unbiased estimation of the linear relationship between $_{3\text{-months}}$AIG and measured HbA1c was established.
Results: Duplicates values of $_{3\text{-months}}$AIG and HbA1c for the 2 consecutive periods of 90 days were 1.84 (0.34) g/L and 1.82 (0.33) g/L for $_{3\text{-months}}$AIG, *vs* 7.76 (0.86)% and 7.84 (0.88)% for HbA1c, respectively. The DR of $_{3\text{-months}}$AIG was 2.34 (2.5%–97.5% CIs: 2.06–2.68), and that of HbA1c was 1.83 (2.5%–97.5% CIs: 1.59–2.12). The difference between DRs was significant (p 0.0137), showing superior intrinsic discriminatory power of $_{3\text{-months}}$AIG. Both interstitial glucose and HbA1c were strictly correlated, with an unadjusted Pearson coefficient between methods of 0.80, rising up to 1.00 following adjustment for the attenuation. The DR method allowed for deriving a novel equation estimate of HbA1c (eA1c) from $_{3\text{-months}}$AIG from the slope and intercept of the unbiased linear relationship between the two measures: **eA1c** *(%)* **= 2.431** x $_{\textbf{3 months}}$ **AIG** *(g/L)* **+ 3.348** or **eA1c** *(%)* **= 0.438** x $_{\textbf{3 months}}$ **AIG** *(mmol/L)* **+ 3.348**
Conclusion: Average interstitial glucose over 3 months ($_{3\text{-months}}$AIG) provides significantly higher discrimination than measured HbA1c over the same period to rank patients with T1DM according to their glycaemic levels. The DR method provides a novel, unbiased equation to calculate HbA1c from $_{3\text{-months}}$AIG values.
Disclosure: **P. Oriot:** None.

801

Associations between HbA_{1C} and continuous glucose monitoring-derived glycaemic parameters

T.C. Walker, P. Calhoun, T.K. Johnson, J.B. Welsh, D.A. Price;
Dexcom, Inc., San Diego, USA.

Background and aims: Although A_{1C} is strongly associated with the risk of vascular complications in diabetes, a wide range of mean glucose concentrations and glucose profiles can be associated with any given A_{1C} level. Continuous glucose monitoring (CGM) systems can accurately detect and characterize a wider range of glucose characteristics and behaviors; however, consensus goals for many CGM-derived parameters are lacking. We sought to associate several narrow A_{1C} ranges with CGM-derived parameters.
Materials and methods: CGM and A_{1C} data from 4 clinical trials were analyzed: DIAMOND Phase 1 (N = 104), DIAMOND Phase 2 (N = 69 completers of DIAMOND Phase 1), REPLACE-BG (N = 216), and HypoDE (N = 141). Each study lasted ≥24 weeks, used current-generation CGM systems (Dexcom, Inc.), and included comparisons of central-lab end-of-study A_{1C} to CGM data during the preceding 3 months. Data from 530 adults with diabetes (455 with T1D and 75 with T2D; 279 using multiple daily injections and 251 using insulin pumps). Time in range (TIR) was defined as the percentage of sensor glucose (SG) values in the 3.9–10.0 mmol/L (70–180 mg/dL) range, inclusive, and expressed as either a percentage or as minutes/day (assuming 1 SG value = 5 min).
Results: The Figure shows population median and interdecile ranges for mean SG, TIR, time <3.9, <3.0, and >13.9 mmol/L for six A_{1C} bins. Higher A_{1C} bins were associated with higher mean SG and lower TIR values; lower A_{1C} bins were associated with lower mean SG and higher TIR values. For subjects with A_{1C} values <7.0%, median TIR was 72%, with 90% of subjects having TIR >57%. For subjects with A_{1C} ≥8.0%, median TIR was 44%, with 90% of the subjects having TIR <59%. Of the subjects with TIR >60%, 2.7% had an A_{1C} of >8.0% and 55.8% had an A_{1C} of <7.0%. Of the subjects with TIR >70%, 0.7% had an A_{1C} of >8.0% and 75.4% had an A_{1C} of <7.0%. Median time with SG <3.0 mmol/L was <20 min/day for all A_{1C} bins, but median time with SG >13.9 mmol/L increased from 36 min/day for those in the lowest A_{1C} bin to 400 min/day for those in the highest A_{1C} bin.
Conclusion: In CGM users, low A_{1C} values can be achieved with minimal additional exposure to hypoglycaemia. The observed associations between CGM-derived glycaemic parameters and specific A_{1C} ranges may help clinicians and patients establish appropriate TIR goals and guide therapy intensification efforts.



Clinical Trial Registration Number: NCT02282397, NCT02258373, NCT02671968
Disclosure: **T.C. Walker:** Employment/Consultancy; Dexcom, Inc.

PS 067 Artificial insulin delivery and insulin pump therapy

802

The artificial pancreas ski camp: real-time monitoring and glucose control in youth with type 1 diabetes

D.R. Chernavvsky, M. DeBoer, M. Breton, J. Pinnata, B. Kovatchev; Center for Diabetes Technology, University of Virginia, Charlottesville, USA.

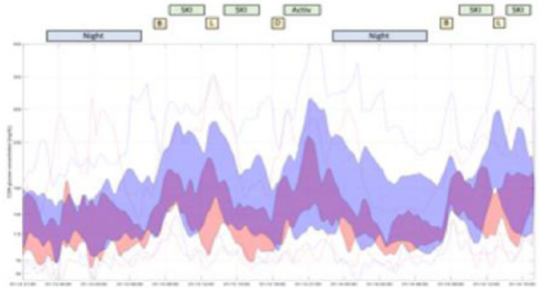
Background and aims: In Type 1 Diabetes (T1D) prolonged moderate/intense physical activity (PA) may result in hypoglycemia and is generally hard to manage. Winter-sport activities add external factors, e.g. cold and altitude, which make them most challenging to patients, health-care providers, and diabetes technologies such as insulin pumps, continuous glucose monitoring (CGM), and the emerging Artificial Pancreas (AP) systems. While AP has been shown to improve glycemic control during and after exercise, AP systems have been tested during structured winter-sport studies only once - in our recently reported 2016 skiing camp trial. Building on this previous experience, we now present the testing of a new system - the Tandem X2 with Control IQ Technology - in a series of ski studies in the winter of 2018. The premise of these studies is that the challenges of skiing provide great environment to test the AP in extreme real-life conditions.

Materials and methods: A sequence of three winter camp trials at ski resorts in Virginia, Colorado and California enrolled $N = 48$ children, ages 6–18, $N = 24$, $N = 12$, and $N = 12$ at each of these sites, respectively. All participants are randomized to the Tandem X2 Insulin Pump with Control IQ Technology (AP group) vs. sensor augmented pump (SAP control group). This new system consists of a G6 CGM (Dexcom, Inc.) and an X2 pump with embedded Control IQ algorithm (Tandem Inc. and Typezero, Inc.), which is identical to the AP algorithm originally developed at the University of Virginia (UVA) and tested in a number of UVA studies. The AP and SAP groups were matched by age and HbA1c. The studies continued for 2 days and on each day the participants had 3 hours of morning and 3 hours of afternoon skiing with instructors. For added safety, all subjects (AP and SAP) were monitored by a physician 24/7 using Dexcom Share G5 CGM. At the time of this writing, the results from the first ski camp in Virginia are ready and presented here; the study in Colorado is completed, and the study in California is scheduled for April 8–10.

Results: The participants in the first camp were teenagers ages 13–18 (14 males). Compared to SAP, glycemic control was significantly better in the AP group, including: percent time between 3.9–10mmol/L of 55.4% (SAP) vs 73.1% (AP), $p = 0.032$; percent above 10 mmol/L and above 13.9 mmol/L of 41%(SAP) vs 21.6% (AP), $p = 0.026$ and 14.8% (SAP) vs 5.6% (AP), $p = 0.02$; mean blood glucose of 9.4 mmol/L (SAP) vs 7.8 mmol/L (AP), $p = 0.03$, and no increase in hypoglycemic events on AP. Hyperglycemia post meals was reduced on AP, thus reducing glucose variability (Figure 1).

Conclusion: During its first winter/ski camp, the new X2 Insulin Pump with embedded Control IQ AP algorithm improved significantly the glycemic control in children with T1D, without adverse events, and with overwhelmingly positive patient feedback.

Figure 1: interquartile plots: Sensor-augmented pump (blue); Tandem X2 with Control IQ (purple)



Clinical Trial Registration Number: NCT03369067

Supported by: Tandem Diabetes

Disclosure: **D.R. Chernavvsky:** Other; Tandem Diabetes awarded a grant to run the ski camps in Colorado and Stanford.

803

Real-world use of the MiniMed™ 670G system by patients with type 1 or type 2 diabetes

S.W. Lee, J. Shin, X. Chen, M.-H. Nguyen, T.L. Cordero, F.R. Kaufman; Medtronic, Northridge, USA.

Background and aims: The MiniMed™ 670G system with the SmartGuard™ Auto Mode feature automatically adjusts basal insulin delivery through its hybrid closed-loop algorithm. A retrospective analysis of glycemia in patients self-reported to have type 1 diabetes (T1D) or type 2 diabetes (T2D) during real-world use of the MiniMed™ 670G system (available in the US) was performed.

Materials and methods: Patients with insulin-dependent diabetes using the MiniMed™ 670G system and with ≥3 months of CareLink™ software data (insulin utilization, continuous glucose monitoring [CGM], etc.) were selected. Glycemic control during baseline Manual Mode was compared to that after Auto Mode was enabled and evaluated by diabetes type. All data were de-identified and analyzed in aggregate. Change from baseline data were analyzed using a paired t-test or Wilcoxon signed-rank test. Analyzed endpoints included the mean percentage of time in target glucose range (TIR, 70–180 mg/dL [3.9–10 mmol/L]), hypoglycemic ranges (<50, <54, and <70 mg/dL [<2.8, <3.0, and <3.9 mmol/L]), and hyperglycemic ranges (>180, >250 and >350 mg/dL [>10, >13.9, >19.4 mmol/L]).

Results: For T1D patients ($N = 1833$), the baseline mean ± SD (median, min-max) of age was 45.8 ± 16.5 (49.0, 5.0–84.0) years and that of total daily dose of insulin (TDD) was 45.3 ± 26.1 (38.4, 6–231.3) units. For T2D patients ($N = 58$), the baseline mean ± SD (median, min-max) of age was 54.6 ± 9.0 (55.5, 31.0–72.0) years and that of TDD was 68.7 ± 43.0 (57.6, 28.3–224.7) units. The table shows the mean percentage of glucose values in the different SG ranges for both groups of patients using the MiniMed™ 670G system. A significant increase in TIR and a significant reduction in time >180 mg/dL (10mmol/L) were observed compared to baseline ($p < 0.001$, for both).

Conclusion: An analysis of real-world CGM data demonstrated that use of the MiniMed™ 670G system by patients with T1D and T2D was associated with a significant increase in TIR and a significant reduction in SG values in hyperglycemic ranges. Improved overall glycemia observed with the MiniMed™ 670G system was not associated with increased hypoglycemia. These findings suggest that automated basal insulin delivery with the MiniMed™ 670G system can help manage real-world patients with either T1D or T2D.

Table. Percentage of glucose values across sensor glucose ranges for each group.

	Patients with T1D (N=1833)			Patients with T2D (N=58)		
	Manual Mode (Baseline)	Auto Mode	p value	Manual Mode (Baseline)	Auto Mode	p value
<50 mg/dL (<2.8 mmol/L)	0.22±0.38 (0.07, 0.00-0.28)	0.19±0.29 (0.10, 0.03-0.23)	0.050[a]	0.09±0.20 (0.00, 0.00-0.07)	0.08±0.14 (0.02, 0.00-0.06)	0.219[a]
<54 mg/dL (<3 mmol/L)	0.40±0.62 (0.17, 0.00-0.52)	0.35±0.47 (0.21, 0.07-0.43)	0.978[a]	0.16±0.32 (0.00, 0.00-0.11)	0.16±0.25 (0.04, 0.02-0.15)	0.175[a]
<70 mg/dL (<3.9 mmol/L)	2.19±2.27 (1.50, 0.59-3.01)	1.88±1.68 (1.47, 0.75-2.43)	<0.001[a]	1.23±1.85 (0.42, 0.14-1.52)	1.03±1.12 (0.68, 0.23-1.66)	0.508[a]
70 ≤ SG ≤ 180 (3.9-10 mmol/L)	65.25±14.78 (66.02, 55.97-75.78)	73.47±9.40 (74.13, 67.78-79.66)	<0.001[a]	70.74±17.35 (69.43, 61.65-84.57)	76.44±11.06 (76.24, 69.93-85.15)	<0.001
>180 mg/dL (>10 mmol/L)	32.56±15.52 (31.74, 21.45-41.94)	24.65±9.58 (23.92, 18.08-30.27)	<0.001[a]	28.03±17.55 (29.79, 14.52-37.50)	22.53±10.78 (22.32, 14.39-28.60)	<0.001
>250 mg/dL (>13.9 mmol/L)	8.17±7.64 (5.99, 2.52-11.47)	5.13±4.41 (4.02, 2.15-6.90)	<0.001[a]	6.20±7.88 (3.97, 0.71-8.11)	4.27±4.61 (3.20, 0.84-5.90)	0.015[a]
>350 mg/dL (>19.4 mmol/L)	0.50±0.98 (0.06, 0.00-0.62)	0.29±0.57 (0.11, 0.02-0.32)	<0.001[a]	0.45±1.06 (0.00, 0.00-0.40)	0.24±0.64 (0.05, 0.00-0.21)	0.099[a]

All values are shown as means±SD (median, IQR)
[a]Wilcoxon signed-rank test

Disclosure: **S.W. Lee:** Employment/Consultancy; Medtronic. Stock/Shareholding; Medtronic.

804

Comparing patch vs pen bolus insulin delivery in type 2 diabetes using continuous glucose monitoring metrics and profiles

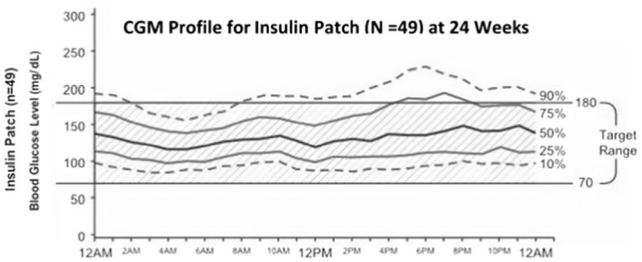
M.L. Johnson[1], D.M. Dreon[2], B.L. Levy[2], S. Richter[1], D. Mullen[1], R.M. Bergenstal[1];
[1]International Diabetes Center, Park Nicollet, Minneapolis, [2]Calibra Medical, Wayne, USA.

Background and aims: Adults with type 2 diabetes ($n = 97$, HbA_{1c} ≥7.5% [58 mmol/mol] on basal insulin) performed continuous glucose monitoring in a sub-study of a large randomized, controlled trial ($n = 278$) to evaluate initiating mealtime insulin (aspart) with a wearable bolus insulin delivery patch (Patch) vs an insulin pen (Pen) using a self-monitoring blood glucose-based titration algorithm. The patch was applied at least once every 3 days and delivered subcutaneous bolus insulin in 2-U increments per manual click.

Materials and methods: Blinded continuous glucose monitoring was conducted by a sub-set of subjects (50 in each of the treatment arms) for one week during the baseline period and for one week prior to Week 24. Subjects recorded 3 days of 7-point self-monitoring blood glucose and insulin doses during the week that continuous glucose monitoring was conducted.

Results: There was a significant improvement ($p < 0.0001$) in HbA_{1c} and all continuous glucose monitoring metrics at Week 24, but no difference between groups. HbA_{1c}, % ± SD, in patch vs pen arms at baseline was 8.6 ± 0.9 (70 ± 9.8 mmol/mol) vs 8.8 ± 1.0 (73 ± 10.9 mmol/mol) and at Week 24 was 6.8 ± 1.0 (51 ± 10.9 mmol/mol) vs 6.7 ± 0.8 (50 ± 8.7 mmol/mol). Continuous glucose monitoring, mg/dl ± SD, average glucose in patch vs pen at baseline was 188.9 ± 40.9 (10.5 ± 2.3 mmol/l) vs 200.3 ± 41.4 (11.1 ± 2.3 mmol/l) and at Week 24 was 142.4 ± 31.4 (7.9 ± 1.7 mmol/l) vs 140.4 ± 28.3 (7.8 ± 1.6 mmol/l). Continuous glucose monitoring time in range 70–180 mg/dl (4.0–10.0 mmol/l) (% time ± SD) in patch vs pen at baseline was 48.4 ± 25.2 vs 42.4 ± 23.8 and at Week 24 was 74.1 ± 18.7 vs 75.2 ± 16.1. Continuous glucose monitoring >180 mg/dl (>10.0 mmol/l) (% time ± SD) in patch vs pen at baseline was 50.4 ± 26.1 vs 56.7 ± 24.8 and at Week 24 was 21.1 ± 19.9 vs 19.7 ± 17.5. Continuous glucose monitoring >250 mg/dl (13.9 mmol/l) (% time ± SD) in patch vs pen at baseline was 18.3 ± 18.3 vs 23.4 ± 21.8 and at Week 24 was 5.6 ± 9.7 vs 4.6 ± 8.3. Low glucose as measured by continuous glucose monitoring <70 mg/dl (≤3.9 mmol/l) (% time ± SD) in patch vs pen at baseline was 1.2 ± 2.4 vs 0.9 ± 3.2 and at Week 24 was 4.7 ± 5.2 vs 5.1 ± 5.8. Very low glucose as measured by continuous glucose monitoring <54 mg/dl (<3.0 mmol/l) (% time ± SD) in patch vs pen at baseline was 0.2 ± 0.7 vs 0.2 ± 1.1 and at Week 24 was 1.1 ± 2.0 vs 1.2 ± 2.0. Continuous glucose monitoring profiles (Figure) demonstrate it is possible to optimize basal-bolus therapy, dramatically increasing time-in-target range 70–180 mg/dl (4.0–10.0 mmol/l) with minimal significant hypoglycemia <54 mg/dl (<3.0 mmol/l) while achieving a flat bedtime to morning glucose profile.

Conclusion: This is one of the first trials to demonstrate how continuous glucose monitoring in type 2 diabetes can provide a more clinically relevant comparison of different approaches to optimizing glucose management.



Clinical Trial Registration Number: NCT02542631
Supported by: The study was funded by Calibra Medical
Disclosure: **M.L. Johnson:** Other; Research support by NIDDK, Medtronic, DexCom, Novo, Abbott, Hygieia, Johnson & Johnson.

805

Assessment of infusion set survival of the newly developed coated Lantern catheter in type 1 diabetes by glucose clamp technique

J.K. Mader[1], A. Ajsic[1], M.C. Krasser[1], R. Juliussen[2], P.K. Schondorff[2], M. Heschel[2], T. Pöttler[1], D. Schwarzenbacher[1], T. Augustin[3], T.R. Pieber[1], G. Treiber[1];
[1]Department of Internal Medicine/Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria, [2]ConvaTec, Lejre, Denmark, [3]HEALTH, Joanneum Research ForschungsGmbH, Graz, Austria.

Background and aims: The catheter-tissue interface is the bottle neck of insulin pump therapy (CSII). Currently infusion sets shall be changed every 2–3 days to avoid lipohypertrophy, fluctuations in insulin absorption and occlusion. Patients would prefer an extended wear time if stable insulin absorption could be achieved. The novel coated catheter featuring Lantern Technology shall utilize anti-inflammatory effect and allow more stable insulin delivery via slots in the shaft of the soft cannula, even if kinking or clotting occurs. The aim of the present study was to investigate clinical performance of the coated Lantern catheter in 16 patients with type 1 diabetes using CSII over a period of up to 7 days.

Materials and methods: A combined design comprising inpatient (euglycaemic clamps on days 1, 4 and 7) and outpatient phases (insulin pump therapy over 7 days) is chosen to allow assessment of performance and survival time of the coated Lantern catheter. 16 c-peptide negative patients (age 44.2 ± 15.4 years, BMI 24.5 ± 2.3 kg/m^2, HbA1c 55 ± 8 mmol/mol, diabetes duration 20 ± 9 years) completed the 7-day study period.

Results: Geometric means of maximum glucose infusion rates (GIR) were similar for days 1, 4 and 7 (6.1 ± 1.5, 7.2 ± 1.3, 5.8 ± 1.4; $p = 0.14$). Time to reach 50% of the maximum GIR were similar over time (31.5 ± 1.6 min vs. 29.3 ± 1.3 min vs. 27.3 ± 1.3 min for days 1, 4 and 7 respectively; $p = 0.51$). Area under the log-transformed GIR curve did not significantly differ similar for the first 2 hours between days (343.7 ± 1.5 vs. 421.3 ± 1.6 vs. 350.6 ± 1.8; $p = 0.14$; days 1, 4 and 7, respectively); however, there was a trend towards reduced area under the GIR curve over 8 hours over time (874.2 ± 1.4 vs. 744.5 ± 1.7 vs. 509.2 ± 2.0; days 1, 4 and 7, respectively; $p < 0.05$). During outpatient care no severe hypoglycaemic event or ketoacidosis occurred.

Conclusion: The novel coated Lantern catheter could be safely used over an extended wear-time of 7 days. There was a trend towards reduced insulin action over time. The findings need to be confirmed in a larger scale trial under routine conditions.

Clinical Trial Registration Number: DRKS00013263
Supported by: ConvaTec
Disclosure: **J.K. Mader:** None.

806

An assessment on insulin pump precision for artificial pancreas efficiency

S. Girardot[1], F. Mousin[1], S. Hardy[1], J.-P. Riveline[2];
[1]Medico-technical, Explor by Air Liquide Healthcare, Gentilly, [2]Centre Universitaire du diabète et de ses complications, Hôpital Lariboisière, Paris, France.

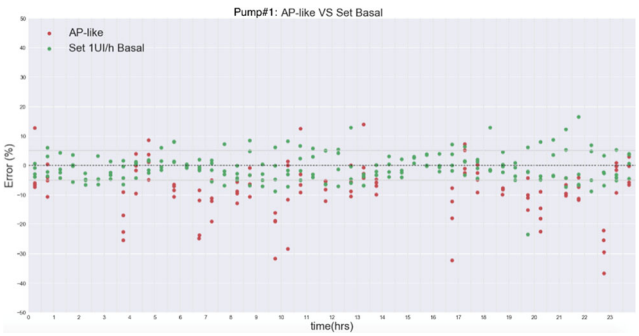
Background and aims: Insulin pump is nowadays one of the trendiest treatment for type 1 diabetes and is about to become artificial pancreas (AP) major component. However, glycemic control among patients remain unpredictable. Insulin pump accuracy has not been so well studied

so far, especially in a context of a closed-loop system. A leading edge direct flow rate measurement method has been used to compare insulin pump accuracy of 24 h-tests with set basal rate and an AP-like way of delivering.

Materials and methods: Insulin pump tubing is connected to a Bronkhorst BL100: a micro flowmeter based on inertial movement technology. Downstream tubing beyond the instrument is then connected to a micro precision weight scale (XPE 56, Mettler Toledo) which records weight of delivered insulin over the time. This second lecture helps as a measurement control method of the direct flow meter. Instantaneous flow rate recorded by the direct flow is analysed to study performances of insulin pump delivery. Two insulin pump (pump#1: $n = 4$, pump#2: $n = 6$) from the market have been compared regarding their delivering precision while set on two different modes: *Set basal rate mode:* Insulin pump is programmed to deliver at 1UIh basal rate for the whole test. *AP-like mode:* Insulin pump is programmed to get as close as possible to an artificial pancreas way of deliver. Each test last 24-hours and targeted insulin volume has been compared with actual delivered volume for each 30-minutes time intervals. A mean absolute relative difference (MARD) indicator has been used to illustrate overall performances.

Results: Test results suggest differences of performances between pumps and between delivering mode for the same pump. Pump#1 delivering errors are significantly higher for AP-like delivering mode (MARD: 9.64%) rather than for a set 1 UI/h basal rate (MARD: 3.76%) ($p = 3.08.10^{-19}$). Pump#2 accuracy is generally much lower than pump#1. 1 UI/h basal rate set mode for pump#2 remain more accurate (MARD: 14.07%) than AP-like delivering mode (MARD: 36.02%) ($p = 0.01$).

Conclusion: As one of the main artificial pancreas component, insulin pump might drive to unexpected clinical or technical limitation. Beyond obvious clinical consequences, feeding AP control algorithm with inaccurate pump delivering data is likely to be a lack for overall system efficiency.



Disclosure: **S. Girardot:** None.

807

Fully closed-loop glucose control in haemodialysis patients with type 2 diabetes

L. Bally[1], H. Thabit[2], S. Hartnell[3], E. Andereggen[1], Y. Ruan[4], M.E. Wilinska[4], M.L. Evans[4,3], M. Semmo[1], B. Vogt[1], A.P. Coll[4,3], C. Stettler[1], R. Hovorka[4];
[1]Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, [2]Manchester University Hospitals NHS Foundation Trust, Manchester, UK, [3]Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK, [4]Wellcome Trust–MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK.

Background and aims: The management of individuals with diabetes undergoing haemodialysis remains challenging for both patients and healthcare providers.

Materials and methods: Automated fully closed-loop (CL) insulin delivery system without meal-bolusing was evaluated in adult inpatients with insulin-treated type 2 diabetes undergoing haemodialysis. Nineteen participants were randomised to either CL-directed s/c insulin delivery ($n = 10$) or conventional s/c insulin therapy as per local practice with masked continuous glucose monitoring ($n = 9$) for up to 15-days. Participants consumed self-selected hospital meals and were matched for age (72 ± 7 vs. 67 ± 9yrs; CL vs. control), HbA1c (7.4 ± 1.1 vs. 7.2 ± 1.7%) and BMI (31.1 ± 6.1 vs. 33.1 ± 6.5 kg/m^2). During CL, participant's usual insulin and sulphonylurea therapy were withheld.

Results: In an intention to treat analysis, proportion of time when sensor glucose was in target range was 38.6 percentage points (95% confidence interval [CI] 25.9 to 51.2; $p < 0.001$; primary end point; table 1) greater during CL compared to control. CL significantly decreased time spent above target by 40.5 percentage points (95% CI 22.0 to 59.1; $p < 0.001$) without increasing total daily insulin delivered ($p = 0.51$). Mean glucose and standard deviation of sensor glucose were significantly reduced by 3.2 (0.8) mmol/l ($p = 0.001$) and 1.2 (0.4) mmol/l ($p = 0.009$) during CL compared to control, whilst area under the curve below 3.0mmol/l was not different ($p = 0.91$). No episodes of severe hypoglycaemia or hyperglycaemia with ketonaemia occurred during either study period. Study duration was 7.6 and 7.4 days in the closed-loop and control group, respectively ($p = 0.88$). No severe hypoglycaemia or serious adverse events occurred in either group.

Conclusion: Fully CL without meal-bolusing in haemodialysis patients with insulin-treated type 2 diabetes improves glucose control without increasing the risk of hypoglycaemia, and may be a promising treatment modality for this vulnerable population.

	Closed-loop insulin delivery (n=10)	*Conventional insulin therapy (n=9)*	*p*
Time spent at sensor glucose levels (%)			
5.6 to 10.0 mmol/l	67.8±11.9	29.3±14.3	<0.001
>10.0 mmol/l	20.3±9.3	60.8±26.1	<0.001
<5.6 mmol/l	11.8±5.2	9.9±13.7	0.68
<3.0 mmol/l	0.0 (0.0, 0.1)	0.0 (0.0, 0.4)	0.84
Mean sensor glucose (mmol/l)	8.1±0.5	11.3±2.4	0.001
SD of sensor glucose (mmol/l)	2.3±0.5	3.5±1.1	0.009
Total daily insulin (U)	40.8±24.2	47.7±20.7	0.51

Data are mean ± SD or median (IQR).

Clinical Trial Registration Number: NCT01774565
Supported by: Diabetes UK, CTU Research Grant Bern University Hospital
Disclosure: **L. Bally:** None.

808

A comparative effectiveness analysis of real-world use of the MiniMed™640G and MiniMed™ 670G systems

J. Shin, S. Huang, X. Chen, M.-H. Nguyen, T.L. Cordero, S.W. Lee, F.R. Kaufman;
Medtronic, Northridge, USA.

Background and aims: The MiniMed™ 640G system with the SmartGuard™ predictive low glucose management (PLGM) *Suspend before Low* feature suspends insulin delivery in advance of sensor glucose (SG) levels reaching a preset low SG limit, and resumes insulin delivery after SG levels recover. The MiniMed™ 670G system with the SmartGuard™ Auto Mode feature automatically adjusts basal insulin delivery through its hybrid closed-loop algorithm. A retrospective analysis of glycemia during real-world use of the MiniMed™ 640G system

(available outside the US) versus use of the MiniMed™ 670G system (available in the US) was performed.

Materials and methods: Patients with insulin-dependent diabetes using the MiniMed™ 640G system or the MiniMed™ 670G system and with ≥3 months of CareLink™ software data (insulin utilization, continuous glucose monitoring [CGM], etc.), were matched based on a logistic regression model with demographic information that included age, total daily dose of insulin (TDD), and sex. All data were de-identified and analyzed in aggregate. Propensity score of matched patients from each group (MiniMed™ 640G system versus MiniMed™ 670G system) was determined and data were compared using a two-sample t-test or Wilcoxon rank-sum test. Analyzed endpoints included the mean percentage of time in target glucose range (TIR, 70–180 mg/dL [3.9–10 mmol/L]), hypoglycemic ranges (<50, <54, and <70 mg/dL [<2.8, <3.0, and <3.9 mmol/L]), and hyperglycemic ranges (>180, >250, >300 and >350 mg/dL [>10, >13.9, >16.7, >19.4 mmol/L]).

Results: Demographics of patients (N = 1514/group, 45% male/group) were equivalent for both groups. The mean ± SD (median, min-max) of age was 40.7 ± 17.2 (42.0, 4.0–77.0) years, and that of TDD was 41.5 ± 17.3 (38.3, 5.8–152.9) units. The table shows percentage of glucose values in the different SG ranges for both systems. For patients using the MiniMed™ 670G system compared to those using the MiniMed™ 640G system, a significant increase (+9.5%) in TIR and a significant reduction (−9.2%) in time >180 mg/dL (>10 mmol/L) were observed ($p < 0.001$, for both). There was no difference in the mean percentage of time in hypoglycemic ranges.

Conclusion: An analysis of real-world CGM data demonstrated that use of the MiniMed™ 670G system was associated with a significant increase in TIR and a significant reduction in SG values in the hyperglycemic ranges compared to use of the MiniMed™ 640G system. Improved overall glycemia observed with the MiniMed™ 670G system was not associated with an increase in hypoglycemia. These real-world findings indicate that automated basal insulin delivery with the MiniMed™ 670G system provides significant clinical benefit in glucose management.

Table. Percentage of glucose values across sensor glucose ranges for each system.

Glucose Range	MiniMed™ 640G System	MiniMed™ 670G System
<50 mg/dL (<2.8 mmol/L)	0.26 ± 0.43	0.23 ± 0.32
<54 mg/dL (<3 mmol/L)	0.46 ± 0.70	0.42 ± 0.53
<70 mg/dL (<3.9 mmol/L)	2.38 ± 2.42	2.15 ± 1.86
70 ≤ SG ≤ 180 (3.9-10 mmol/L)	**62.98 ± 13.97**	**72.44 ± 9.56**
>180 mg/dL (>10 mmol/L)	34.64 ± 14.99	25.41 ± 9.84
>250 mg/dL (>13.9 mmol/L)	9.50 ± 7.90	5.60 ± 4.74
>300 mg/dL (>16.7 mmol/L)	2.97 ± 3.55	1.54 ± 1.99
>350 mg/dL (>19.4 mmol/L)	0.71 ± 1.12	0.34 ± 0.63
All values are shown as mean±SD N=1514/group		

Disclosure: **J. Shin:** Employment/Consultancy; Medtronic. Stock/Shareholding; Medtronic.

809

Optimising basal-bolus therapy in type 2 diabetes: a randomised, controlled trial comparing bolus insulin delivery using an insulin patch vs an insulin pen

S. Ramtoola[1], V.R. Aroda[2], T.S. Bailey[3], R.L. Brazg[4], D.M. Dreon[5], J.P. Frias[6], D.C. Klonoff[7], D.F. Kruger[8], B.L. Levy[5], M. Peyrot[9], J. Rosenstock[10], P. Serusclat[11], R.S. Weinstock[12], V. Zraick[5], R.M. Bergenstal[13];

[1]East Lancashire Hospitals NHS Trust, Blackburn, UK, [2]Medstar Health Research Institute, Hyattsville, USA, [3]AMCR Institute Inc., Escondido, USA, [4]Rainier Clinical Research Center, Renton, USA, [5]Calibra Medical, Wayne, USA, [6]National Research Institute, Los Angeles, USA, [7]Diabetes Research Institute, San Mateo, USA, [8]Henry Ford Health System, Detroit, USA, [9]Loyola University Maryland, Baltimore, USA, [10]Dallas Diabetes Research Center at Medical City, Dallas, USA, [11]Groupe Hospitalier Mutualiste Les Portes du Sud, Vénissieux, France, [12]SUNY Upstate Medical University, Syracuse, USA, [13]International Diabetes Center, Park Nicollet, Minneapolis, USA.

Background and aims: This multicenter randomized, controlled trial compared efficacy, safety, and subject-reported outcomes for adults with type 2 diabetes on basal insulin (HbA_{1c} 7.5–11% [58–97 mmol/mol]) initiating mealtime insulin (aspart) with a wearable bolus insulin delivery patch (Patch) vs an insulin pen (Pen). The Patch was applied at least every 3 days and delivered subcutaneous bolus insulin in 2-U increments per manual click.

Materials and methods: Subjects (n = 278, mean age: 59 years, mean diabetes duration: 15 years) receiving 0.52 U/kg glargine on average, were randomized to Patch (n = 139) or Pen (n = 139). Baseline glargine dose was divided 1:1 into basal:bolus. Using a pattern-control logbook, subjects adjusted basal and bolus insulin weekly based on fasting and pre-meal glucose targets.

Results: Change in HbA_{1c} from baseline to Week 24 (primary endpoint) for Patch was non-inferior ($p < 0.0001$) to Pen (least squares mean change ± SEM: Patch, −1.7 ± 0.1% [−19 ± 1.0 mmol/mol] vs Pen, −1.6 ± 0.1% [−17 ± 1.0 mmol/mol]); this reduction was significant ($p < 0.0001$) in both groups. HbA_{1c} improvement was maintained at 44 weeks (Figure). At Week 24, 63% of Patch users and 56% of Pen users achieved HbA_{1c} ≤7.0% (≤53 mmol/mol) (OR, 1.3; SEM, 0.25; 95% CI, 0.81, 2.14; p = 0.26). The proportions of Patch and Pen users who achieved HbA1c ≤7.0% (≤53 mmol/mol) at Week 44 rose to 65% and 63%, respectively (OR, 1.2; SEM, 0.28; 95% CI, 0.64, 1.93; p = 0.71). CV of 7-point self-monitoring blood glucose decreased significantly more with Patch compared to Pen; change from baseline to Week 44 was −1.2 ± 0.8% and 1.4 ± 0.8%, respectively (difference, −2.6 ± 1.1%; 95% CI, −4.8, −0.4; p = 0.022). Subjects in Patch and Pen arms, respectively, reported high adherence (mean ± SEM, %) to their insulin regimens at Week 24 (79 ± 18% vs 78 ± 16%; p = 0.70) and Week 44 (81 ± 15% vs 81 ± 17%; p = 0.78). There were no significant differences in adverse events, including hypoglycemia (3 severe episodes/group). There were significantly greater improvements in subject-reported outcomes for Patch vs Pen.

Conclusion: Bolus insulin with Patch or Pen and dosing algorithms improved HbA_{1c} with better experience and preference for Patch.

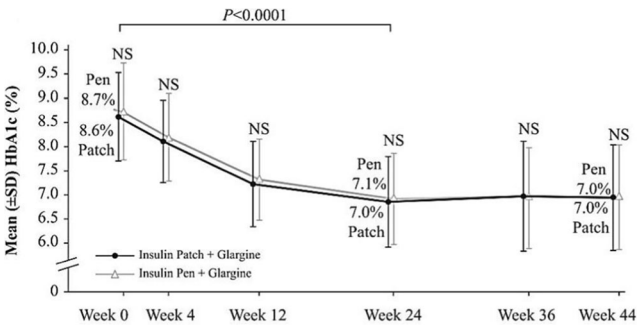


Figure. HbA_{1c} over time from baseline to Week 44 in Patch vs Pen users

Clinical Trial Registration Number: NCT02542631

Supported by: The study was funded by Calibra Medical

Disclosure: **S. Ramtoola:** Other; Participated in other clinical trials sponsored by Johnson & Johnson.

810

Insulin pump therapy with simple infusion scheme in insufficiently-controlled patients with type 2 diabetes on intensive injection therapy: a real life study

H.W. de Valk[1], B. Silvius[1], M.J. Kramer[2], BIDON-study group;
[1]Internal Medicine, University Medical Center Utrecht, Utrecht, [2]Internal Medicine, Free University Medical Center, Amsterdam, Netherlands.

Background and aims: A considerable part of patients with type 2 diabetes mellitus on intensive (4 times daily) insulin injection therapy (MDI) do not reach adequate glycemic control. A recent randomized controlled, 26-week clinical trial has shown that continuous subcutaneous insulin infusion (CSII) using a simple infusion scheme, improves glycemic control. This scheme means a single basal infusion rate and a three times daily roughly similar meal-time insulin bolus. The aim of this study was to assess the effectiveness of this simple insulin regime with CSII in obtaining better glycemic control in a real-life setting.
Materials and methods: Patients with type 2 diabetes with HbA1c ≥64 mmol/mol on MDI (at least 4 times daily) with an insulin requirement between 0.7 and 2.2 U/day. were eligible Insulin infusion scheme was calculated as follows: hourly basal insulin rate was 50% of the total daily dose (TDD) divided by 24 and prandial insulin (thrice daily) was calculated by dividing 50% of TDD by 3. Small changes to basal rate and prandial insulin dose were allowed. Trial duration was 6 months. Results at 6 months were compared with those at baseline with (non-)parametric paired tests.
Results: Sixty-seven patients were analyzed. Mean age: 61.3 ± 0.9 years, 42% female; duration of disease: 16.0 ± 7.6 years; weight: 107.4 ± 21.9 kg; BMI: 35.8 ± 6.9 kg/m^2, baseline insulin dose: 135.5 ± 62.3 U/day (large SD due to a few high doses). Macro-angiopathy 28.3%, proliferative retinopathy 13%, renal disease 18%. Mean HbA1c fell from 80.1 ± 15.2 to 62.4 ± 10.0 mmol/mol ($p < 0.001$). Mean change in HbA1c −17.7 ± 2.1 mmol/mol. The main improvement was already visible at three months (mean HbA1c 65.3 ± 1.4 mmol/mol). An improvement in HbA1c at 6 months was seen in 64 patients (96%); in 56 patients, HbA1c fell at least 5 mmol/mol (84%). There were 3 outliers (δHbA1c >−50 mmol/mol (65, 88, 95 mmol/mol)). After explorative exclusion, the change in the remaining 64 patients was 78.0 ± 11.8 to 63.0 ± 9.7 mmol/mol ($p < 0.001$); mean change −15.0 ± 1.5 mmol/mol. The mean change in HbA1c in the randomized trial was 12 mmol/l; the current result of 15 mmol/mol was even slightly better ($p = 0.01$, one sample T-test). In these 64 patients, total cholesterol decreased slightly (4.33 ± 0.13 to 4.21 ± 0.13 mmol/l, $p < 0.05$) with no changes in the other lipid parameters or blood pressure. Body weight increased from 106.2 ± 19.5 to 109.3 ± 20.61 kg ($p < 0.001$), although 20% actually lost weight. Mean weight increase was significantly higher than in the randomized trial (3.3 vs 1.5 kg, $p < 0.001$).
Conclusion: This real-life study shows that introducing CSII in patients with moderately- to poorly-controlled type 2 diabetes on intensive insulin therapy in a real-life setting, leads to a major improvement of glycemic control in most patients. Results are at least comparable to the ones obtained in the prior randomised clinical trial. Glycemic improvement is already visible after 3 months on CSII. Weight increased significantly suggesting that intensified targeted nutritional therapy is crucial with this profound, insulin-driven improvement in glycemic control.
Supported by: Medtronic
Disclosure: **H.W. de Valk:** None.

PS 068 Faster acting insulins: state of the art

811

BioChaperone technology enables the development of pramlintide-prandial insulin combinations

R. Soula, G. Meiffren, A. Geissler, Y. Meyer, A. Ranson, C. Fortier, O. Soula, B. Alluis, R. Charvet;
Adocia, Lyon, France.

Background and aims: Pramlintide is currently used on top of mealtime insulin therapy by T1D or T2D patients to achieve a better control of post-prandial glucose excursion. Indeed, pramlintide affects the rate of post-prandial glucose appearance by slowing down gastric emptying, reducing postprandial glucagon secretion and modulating satiety, which affects caloric intake. Nevertheless, the use of pramlintide is currently limited as it cannot be combined in a single formulation with prandial insulin due to pH incompatibility, which results in high burden of additional injections. In this work, BioChaperone technology was used to develop a stable co-formulation of pramlintide and human insulin (BC Pram Ins) at neutral pH.
Materials and methods: BC Pram Ins physical stability was evaluated by visual inspection and micro-flow imaging. For chemical stability, recovery was measured by reversed-phase HPLC while high molecular weight species were measured by size-exclusion HPLC. A preliminary in-use pump stability was performed using a commercially available insulin pump system at 37°C. Pharmacokinetics data were obtained by single subcutaneous administration of the desired formulation at doses of 0.1875 U/kg insulin and 1.125 μg/kg pramlintide to fasted healthy pigs.
Results: BC Pram Ins formulation is physically and chemically stable for at least 6 weeks at 30°C and 9 weeks at 25°C. The physical and chemical stability was similar to that of commercial Humulin® and Symlin®. Under simulated in-use pump conditions at 37°C, BC Pram Ins formulation shows physical and chemical stability for at least 1 week, with insulin and pramlintide recoveries higher than 95% and a formulation free of particles. Following a single subcutaneous administration to fasted healthy pigs, BC Pram Ins results in slower absorption of pramlintide (LSM ratio [95%C|] $\Delta AUC_{Pram0\text{-}30min}$: 0.45 [0.20; 1.05]), while the late exposure to pramlintide is higher ($\Delta AUC_{Pram60\text{-}180min}$: 2.65 [1.44; 4.90]) compared to the separate injections of human insulin and pramlintide.
Conclusion: The *in-vitro* and preclinical pharmacodynamic properties of BC Pram Ins support its clinical development as an improved treatment alternative for postprandial glycemic control in patients with T1D and T2D.
Disclosure: **R. Soula:** Employment/Consultancy; Adocia. Stock/Shareholding; Adocia.

812

Glycaemic control with fast-acting insulin aspart according to dose adjustment method in type 1 diabetes: a post hoc analysis of onset 8

T.R. Pieber[1], J. Buse[2], L. Rose[3], A. Gorst-Rasmussen[4], T.W. Boesgaard[4], K. Buchholtz[4], O. Mosenzon[5];
[1]Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria, [2]University of North Carolina School of Medicine, Chapel Hill, USA, [3]Institute of Diabetes Research, Münster, Germany, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Diabetes Unit, Hadassah Hebrew University Hospital, Jerusalem, Israel.

Background and aims: Insulin dosing based on carbohydrate counting is the gold standard for improving glycaemic control in type 1 diabetes (T1D). It has previously been suggested that for patients using carbohydrate counting, fast-acting insulin aspart (faster aspart) may offer improved glycaemic control vs insulin aspart (IAsp). This *post hoc* analysis of a large phase 3 trial aimed to further assess the impact of dose adjustment methodology (DAM) in relation to the efficacy and safety of mealtime faster aspart in T1D.

Materials and methods: onset 8 was a 26-week, multicentre, treat-to-target trial in which adults with T1D were randomised to double-blind mealtime faster aspart or IAsp, or open-label post-meal faster aspart, each with insulin degludec. Subjects with previous experience continued carbohydrate counting (baseline characteristics of the mealtime faster aspart [$n = 142$] vs IAsp [$n = 136$] arms: male, 51.4 vs 52.2%; age, 42.3 vs 43.9 years; duration of diabetes, 19.4 vs 18.0 years; HbA_{1c}, 7.5 vs 7.4%), while remaining subjects used a simple bolus algorithm (baseline characteristics of the mealtime faster aspart [$n = 200$] vs IAsp [$n = 206$] arms: male, 55.5 vs 52.4%; age, 40.9 vs 38.7 years; duration of diabetes, 16.3 vs 15.9 years; HbA_{1c}, both 7.4%). In this *post hoc* analysis, endpoints for mealtime faster aspart and IAsp were analysed based on statistical models including a treatment arm-by-DAM interaction term.

Results: HbA_{1c} reduction was similar in both mealtime treatment arms (Table), and non-inferiority of faster aspart was confirmed in the onset 8 population. The estimated treatment difference (95% CI) (faster aspart – IAsp) for change from baseline in HbA_{1c} was −0.14% (−0.28;0.003) in the carbohydrate counting group and 0.06% (−0.06;0.18) in the bolus algorithm group. Rates of severe or blood glucose-confirmed hypoglycaemia (<3.1 mmol/L [56 mg/dL]), and total daily and daily bolus insulin doses were similar between faster aspart and IAsp across DAM groups. No clinically relevant differences were observed in change in body weight between treatments with either DAM. No other safety issues were reported.

Conclusion: Regardless of bolus adjustment method, faster aspart was as effective as IAsp in terms of HbA_{1c} reduction. This analysis is consistent with previous findings and suggests a tendency for improved glycaemic control with faster aspart vs IAsp in patients with T1D using carbohydrate counting, with similar insulin dose and weight gain and without an increased risk of hypoglycaemia.

Table. Endpoints after 26 weeks of mealtime faster aspart vs IAsp for all subjects and by dose adjustment methodology.

		Faster aspart	IAsp	Estimated treatment difference (faster aspart – IAsp), [95% CI]
Change from baseline HbA_{1c} (%)*	All subjects	−0.12	−0.10	−0.02 [−0.11;0.07]
	Carbohydrate counting	−0.16	−0.02	−0.14 [−0.28;0.003]
	Bolus algorithm	−0.09	−0.15	0.06 [−0.06;0.18]
Change from baseline body weight (kg)*	All subjects	1.43	1.24	0.19 [−0.22;0.60]
	Carbohydrate counting	0.76	1.09	−0.33 [−0.97;0.32]
	Bolus algorithm	1.91	1.39	0.51 [−0.02;1.05]
		Faster aspart	**IAsp**	**Estimated treatment ratio (faster aspart/IAsp), [95% CI]**
Total daily insulin dose (U/kg)†	All subjects	0.72	0.74	0.98 [0.92;1.03]
	Carbohydrate counting	0.66	0.66	1.00 [0.91;1.09]
	Bolus algorithm	0.76	0.79	0.96 [0.90;1.04]
Daily insulin bolus dose (U/kg)†	All subjects	0.37	0.39	0.97 [0.90;1.04]
	Carbohydrate counting	0.33	0.34	0.95 [0.85;1.07]
	Bolus algorithm	0.41	0.42	0.98 [0.89;1.07]
Severe or BG-confirmed hypoglycaemic episodes/PYE‡	All subjects	33.72	40.00	0.84 [0.70;1.01]
	Carbohydrate counting	32.96	40.69	0.81 [0.61;1.08]
	Bolus algorithm	34.28	39.56	0.87 [0.68;1.10]

*Change from baseline is analysed using a mixed effects model for repeated measures.
†Doses are analysed on a log-scale using a mixed effects model for repeated measures, with back-transformation to express results as dose ratios.
‡Hypoglycaemia rates are analysed using a negative binomial model for hypoglycaemic episode count with a log link function, and the logarithm of the exposure time as offset.
For all endpoints, estimates by treatment are based on LS mean values.
BG-confirmed: plasma glucose value <3.1 mmol/L (56 mg/dL)
BG, blood glucose; CI, confidence interval; faster aspart, fast-acting insulin aspart; IAsp, insulin aspart; LS, least squares; PYE, patient-year of exposure.

Clinical Trial Registration Number: NCT02500706
Supported by: Novo Nordisk
Disclosure: **T.R. Pieber:** Employment/Consultancy; Arecor, AstraZeneca, Eli Lilly, Novo Nordisk, Sanofi [consultant]; CBmed [CSO]. Grants; Novo Nordisk and AstraZeneca [research support paid directly to Medical University of Graz].

813

Improved post-prandial blood glucose excursions with Technosphere inhaled insulin compared to aspart in adult patients with type 1 diabetes: STAT study intention to treat analysis

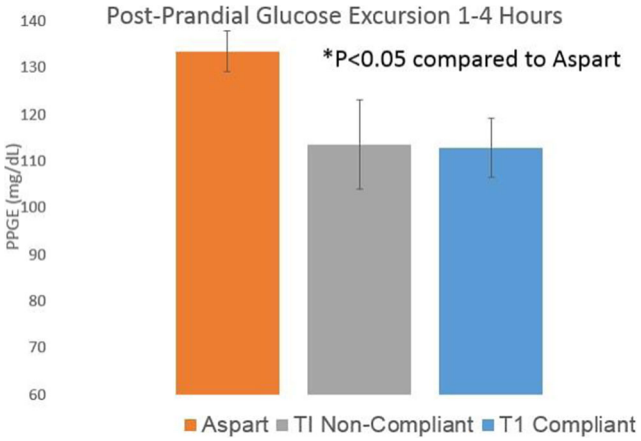
H.K. Akturk[1], J.K. Snell-Bergeon[1], E.A. Beck[1], L.J. Klaff[2], B. Bode[3], A. Peters[4], T.S. Bailey[5], S.K. Garg[1];
[1]University of Colorado, Aurora, [2]Rainier Clinical Research Center, Renton, [3]Atlanta Diabetes Associates, Atlanta, [4]USC Westside Center for Diabetes, Beverly Hills, [5]AMCR Clinic, Escondido, USA.

Background and aims: Post-prandial hyperglycemia remains a significant clinical concern; in great part due to the lack of very rapidly acting prandial insulin therapy. Over the past 2 decades, multiple efforts resulted in the development of faster-acting insulin analogs and inhaled insulin for use in clinical care. In this investigator-led, collaborative open-label randomized pilot clinical trial we evaluated the efficacy of Technosphere Insulin (TI) for lowering post-prandial blood glucose (PPBG) and post-prandial glucose excursion (PPGE) in a 4-week treatment period.

Materials and methods: Sixty patients with T1D on multiple daily injections were randomized in a multi-center study, stratified by A1c values (≤8% or >8%) to the control arm using aspart ($n = 34$) vs TI group ($n = 26$). Two patients in the TI arm discontinued from the study, and 2 had inadequate CGM data for analysis. Patients in the TI arm were instructed per protocol to take insulin doses pre-meal, and at 1 and 2 hours after meals based on observed PPBG values. Compliance with TI use was based on using TI per protocol pre-meal and at 1- and 2-hours post-meal, based on PPBG measures. Patients with at least 80% compliance were included in the TI-compliant per protocol group ($n = 15$), and seven TI patients were evaluated in the per protocol non-compliant group. Baseline characteristics of the study group were compared to the randomization group using a student's t-test, and CGM data from the study group was analyzed using linear regression models. Primary outcomes were CGM glucose percentage time in range (70–180 mg/dL) and PPGE 1–4 hours after meals.

Results: Groups had similar baseline characteristics, including age, sex, HbA1c, bolus insulin dose, and FEV1. Mean CGM glucose, glycemic variability (glucose SD/CV), time in range (70–180 mg/dL), and time in hyper- (>180 mg/dL) or hypoglycemia (<70, <60, or <50 mg/dL) were similar between groups. PPGE was significantly lower in the TI group than in the aspart group (Figure). The PPBG at 1 hour was lower in the TI group (mean ± SE PPBG difference −31.7 ± 6.6 mg/dL, $p < 0.0001$) and was numerically lower at 2 hours (mean ± SE PPBG −13.0 ± 7.1 mg/dL, $p = 0.07$) with no difference at 3 and 4 hours. The TI group increased their bolus insulin dose to mean ± SD of 47.8 ± 23.9 U/day compared to the aspart group (mean ± SD =23.0 ± 9.8 U/day; $p < 0.0001$) during the initial week of the study. There was no difference in HbA1c by study group at either screening or at the study end.

Conclusion: This data demonstrates that Technosphere Insulin when administered at meal time and supplemented (if needed) at appropriate post-meal intervals, significantly improved PPBG at 1- and 2-h after meals and decreased PPGE when compared with insulin aspart in adult patients with T1D using MDI.



Clinical Trial Registration Number: NCT03143816
Supported by: Mannkind Corp.
Disclosure: **H.K. Akturk:** Grants; Mannkind Co.

814

Ultra rapid lispro (URLi) reduces postprandial glucose excursions vs lispro in patients with type 2 diabetes at multiple meal-to-dose timing intervals

C. Kapitza[1], J. Leohr[2], R. Liu[2], S.R. Reddy[2], M.A. Dellva[2], M. Matzopoulos[3], M.P. Knadler[2], M.T. Loh[4], T.A. Hardy[2], C. Kazda[2];
[1]Profil Institut fur Stoffwechselforschung GmbH, Nuess, Germany, [2]Eli Lilly and Company, Indianapolis, USA, [3]Eli Lilly and Company, Surrey, UK, [4]Lilly-NUS Centre Clin Pharm Pte Ltd, Singapore, Singapore.

Background and aims: Ultra Rapid Lispro (URLi; LY900014), a novel ultra-rapid mealtime insulin in Phase 3 development, is shown to reduce postprandial glucose after subcutaneous injection.
Materials and methods: This 2-part, randomised, double-blind, Phase 1b study evaluated differences in PK and PD between URLi and lispro (Humalog®) in 30 patients with T2D. Part A used a 6-period crossover design to assess safety and compare PK and postprandial glucose response to solid mixed meal tolerance tests (MMTT) with the same, individualised doses of URLi or lispro at different injection-to-mealtime intervals (-15, 0 & +15 min). Part B evaluated safety, PK and PD during 2 wks of multiple daily dosing (immediately before a meal) in a parallel design. Patients were stabilised overnight to a fasting blood glucose level of 7 mmol/L before the MMTT procedure.
Results: In Part A, URLi reduced glucose excursions (assessed as change in area under the concentration curve vs. time [ΔAUC]) vs. lispro during the first 2 hrs (ΔAUC0-2 h) and entire 5 hrs (ΔAUC0-5 h) of the MMTT regardless of dose timing (Figure). URLi reduced ΔAUC0-2 h by 37% ($p = 0.014$), 47% ($p < 0.0001$), and 4% (p = NS) and ΔAUC0-5 h by 49% ($p = 0.049$), 105% ($p < 0.0001$), and 29% ($p = 0.076$) vs. lispro at −15, 0 and +15 min (significance level = 0.1). The PK and PD profiles for URLi and lispro were sustained after 2 wks of outpatient dosing (Part B). More hypoglycaemic events occurred with URLi during MMTTs but these were mild and mostly asymptomatic. Only a few events occurred in either group during 2 wks of outpatient dosing, with no differences between treatments. Local tolerability was similar between treatments.
Conclusion: These results provide preliminary evidence that URLi may improve postprandial glucose control in T2D.

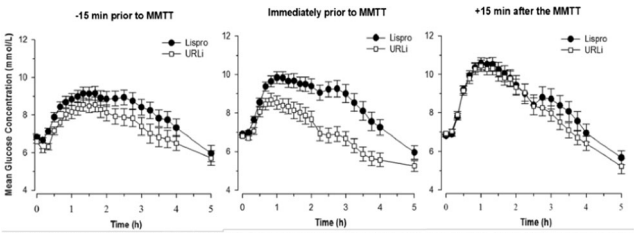


Figure: Mean glucose concentration (±SE) versus time when dosed 15 min before (left), immediately prior (middle), and 15 min post-test meal (right) by treatment following a single dose (Part A).

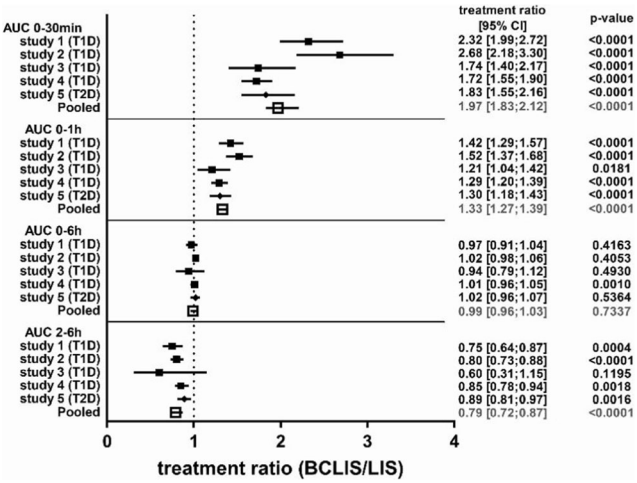
Clinical Trial Registration Number: NCT02703337
Disclosure: **C. Kapitza:** Employment/Consultancy; Profil Institut fur Stoffwechselforschung GmbH.

815

Pooled analysis of clinical trials investigating the pharmacokinetics (PK) of ultra-rapid insulin BioChaperone Lispro vs lispro in subjects with type 1 and type 2 diabetes

T. Heise[1], A. Ranson[2], M. Gaudier[2], O. Soula[2], B. Alluis[2], E. Zijlstra[1], S. Glezer[2], G. Meiffren[2];
[1]Profil, Neuss, Germany, [2]Adocia, Lyon, France.

Background and aims: BioChaperone Lispro (BCLIS) is an ultra-rapid insulin lispro formulation designed to accelerate the time-action profile versus conventional short-acting insulin analogs.
Materials and methods: PK characteristics of single doses of BCLIS and insulin lispro (LIS) were characterized in four randomized, double-blind, crossover studies in altogether 112 patients with type 1 diabetes and 51 people with type 2 diabetes who received BCLIS and LIS (0.2 U/kg in studies 1 & 2, individualized doses in studies 3 & 4) subcutaneously by syringe.
Results: Insulin absorption was consistently faster with BCLIS than with LIS as indicated by reaching early half-maximum insulin levels (early $t_{50\%max}$) 10.0 (95% confidence interval [−14.3; −5.8]) and time to maximum levels (t_{max}) 8.4 [−9.6; −7.2] min earlier ($p < 0.0001$ for both comparisons). Early insulin exposure was significantly greater for BCLIS for up to 2 hours after administration (figure). BCLIS also showed faster offset of exposure, with a 22.3 [−28.8; −15.7] min earlier time to late half-maximum insulin levels (late $t_{50\%max}$) ($p < 0.0001$) and a 24% lower late exposure ($AUC_{2\text{-}6h}$; figure). Total exposure ($AUC_{0\text{-}6h}$) was similar for both formulations in all studies (treatment ratio in pooled analysis 0.99 [0.95; 1.03], p = NS).
Conclusion: BCLIS consistently shows faster onset and offset of exposure than conventional LIS in both patients with type 1 diabetes and people with type 2 diabetes.



Supported by: Adocia
Disclosure: **T. Heise:** Grants; Adocia.

816

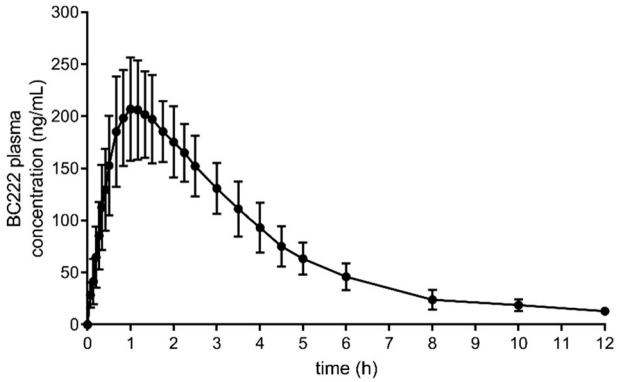
BioChaperone 222, the new excipient enabling the ultra-rapid BioChaperone Lispro formulation, is completely absorbed and rapidly excreted after subcutaneous injection

O. Soula[1], L. Plum-Mörschel[2], E. Anastassiadis[2], G. Andersen[3], S. Glezer[1], A. Ranson[1], B. Alluis[1], J. Correia[1], C. Seroussi[1], M. Gaudier[1], G. Meiffren[1];
[1]Adocia, Lyon, France, [2]Profil, Mainz, Germany, [3]Profil, Neuss, Germany.

Background and aims: BioChaperone 222 (BC222) is an oligosaccharide grafted with anionic charges and amino acid moieties designed to speed up the absorption of insulin lispro in the BioChaperone Lispro (BCLIS) formulation. Non-clinical data indicated that BC222 is rapidly excreted unchanged by the kidney.
Materials and methods: This open label clinical trial conducted in 12 healthy volunteers [mean ± SD age 31.4 ± 9.5 y; weight: 95.3 ± 16.8 kg;

BMI 26.6 ± 4.1 kg/m²] investigated the PK properties and safety and tolerability of a single subcutaneous (s.c.) dose of BC222 (equal to the BC222 content in 1.0 U/kg BCLIS) using validated liquid chromatography mass spectrometry assays for BC222 in plasma and urine samples.
Results: BC222 was rapidly absorbed, with a median plasma Tmax of 1.2 h (figure). BC222 was rapidly cleared from the blood with a mean terminal half-life of 2.07 (±0.52) h reaching baseline levels 10–12 h after administration. Urinary BC222 concentrations reached 95.4 ± 4.7% of the injected dose within 12 h post-dosing and were as high as 97 ± 5% of the total dose after 48 h indicating that BC222 was completely absorbed from the s.c. tissue into the blood and quickly cleared via the urine. BC222 was well tolerated and safe with only two mild adverse events occurring in the whole study.
Conclusion: BC222 is completely absorbed after s.c. injection and rapidly excreted by the kidneys.

Figure: Mean ± SD BC222 plasma concentration over time after subcutaneous administration



Clinical Trial Registration Number: EudraCT 2016-000937-29
Supported by: Adocia
Disclosure: **O. Soula:** Employment/Consultancy; Adocia. Stock/Shareholding; Adocia.

817

Ultra rapid lispro (URLi) shows faster insulin absorption vs lispro during insulin pump (CSII) use in patients with type 1 diabetes

C. Kazda[1], J. Leohr[2], R. Liu[2], T.A. Hardy[2], S. Reddy[2], S.P.C. Chua[3], X. Guo[2], U. Hovelmann[4], C. Kapitza[4];
[1]Lilly France, Neuilly-sur-Seine, France, [2]Eli Lilly and Company, Indianapolis, USA, [3]Lilly-NUS Centre for Clinical Pharmacology Pte Ltd, Singapore, Singapore, [4]Profil Institut für Stoffwechselforschung GmbH, Nuess, Germany.

Background and aims: Ultra Rapid Lispro (URLi; LY900014), a novel ultra-rapid mealtime insulin in Phase 3 development, is shown to reduce postprandial glucose after subcutaneous injection.
Materials and methods: This study evaluated the pharmacokinetics and pharmacodynamics (PD) of URLi via CSII. In a double-blind, randomised cross-over study, 24 adult patients with T1D received URLi or lispro (Humalog®) for 3 days. Mixed meal tolerance tests (MMTT) were conducted on Days 1 and 3 after catheter insertion using a standard (1.5 U/min) single-wave bolus with the same, individualised doses.
Results: URLi showed faster insulin lispro absorption on both days compared to lispro. URLi reduced time to early half-maximal drug concentration by 37% (−8.5 min) and 32% (−5.3 min) compared to lispro on Days 1 and 3 (both $p < 0.0001$). Area under the insulin lispro concentration time curve (AUC) for the first 15 min was >50% higher than lispro after dosing with URLi on Days 1 and 3 ($p < 0.005$). URLi reduced 1-h postprandial glucose excursion of the MMTT by 45% on Day 1 (p = NS) and 47% on Day 3 ($p = 0.059$) compared with lispro (Figure). Accelerated absorption with URLi was associated with trends toward lower postprandial glucose excursion for the entire MMTT (57% and 20% reductions in ΔAUC[0–5 h] on Days 1 and 3; both NS). The study was not powered for PD assessment which may contribute to the lack of statistical significance. No differences were seen in the number or severity of hypoglycaemic events or local tolerability between URLi and lispro.
Conclusion: URLi demonstrated accelerated absorption and a trend toward improved postprandial glucose control in T1D subjects using CSII.

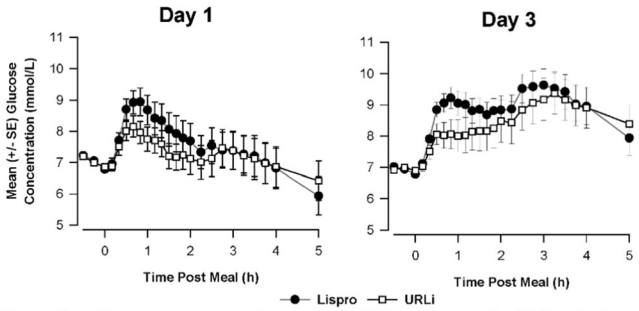


Figure Mean plasma glucose concentration over time for URLi and Lispro on Day 1 (left) vs Day 3 (right).

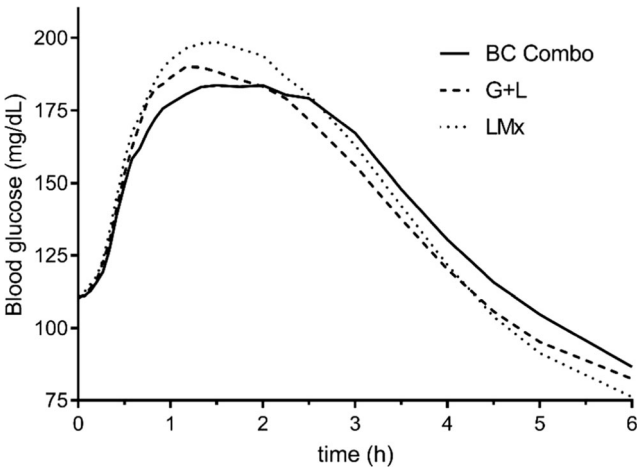
Clinical Trial Registration Number: NCT03056456
Disclosure: **C. Kazda:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

818

Better postprandial glucose control with BioChaperone Combo than with lispro Mix25 or separate glargine and lispro (G+L) administrations in subjects with type 2 diabetes

T. Herbrand[1], L. Plum-Mörschel[2], C. Mégret[3], T. Heise[1], V. Vacher[3], E. Anastassiadis[2], O. Klein[1], M. Gaudier[3], O. Soula[3], S. Glezer[3], B. Alluis[3], G. Meiffren[3];
[1]Profil, Neuss, Germany, [2]Profil, Mainz, Germany, [3]Adocia, Lyon, France.

Background and aims: BioChaperone Combo (BC Combo) is a co-formulation of prandial insulin lispro (25%) and basal insulin glargine (75%) with a rapid "prandial" insulin component and prolonged flat "basal" component compared to lispro Mix25 (LMx). In this study the effects of BC Combo on postprandial glucose control were investigated vs LMx and G+L.
Materials and methods: Thirty-nine people with type 2 diabetes (mean ± SD age 60.8 ± 7.5 years and HbA1c 7.97 ± 0.6%) were randomised to receive the three insulin combinations immediately before a standardised solid meal test (MMT, 20% protein, 30% fat, 50% carbohydrates) in a double-blind, double-dummy, cross-over design. The individual insulin dose was the same for each visit day (mean 0.62 U/kg).
Results: BC Combo demonstrated improved postprandial glucose control in people with type 2 diabetes compared to LMx (Figure, reduction in the area of postprandial blood glucose excursions ($\Delta AUC_{BG,0\text{-}2h}$) by 18%, $p = 0.0009$) and G+L (reduction in $\Delta AUC_{BG,0\text{-}2h}$ of 10%, $p = 0.0450$). The proportion of subjects experiencing documented symptomatic hypoglycaemic events (plasma glucose <70 mg/mL) over 24 h was numerically lower with BC Combo (15.8%) vs LMx (32.4%) and G+L (21.6%). The total insulin PK profile of BC Combo showed a faster time to insulin peak and a lower exposure in the late prandial phase (2–6 h) than LMx and G+L.
Conclusion: BC Combo demonstrated superior postprandial glucose control with numerically fewer subjects experiencing symptomatic hypoglycaemia compared to both Lispro Mix 25 and separate injections of glargine and lispro.



Clinical Trial Registration Number: NCT02915250
Supported by: Adocia
Disclosure: **T. Herbrand:** None.

PS 069 Approaches to insulin titration

819

Efficacy of advanced carbohydrate counting and automated insulin bolus calculators in type 2 diabetes: the BolusCal2 study, an open-label, randomised controlled trial

M.B. Christensen[1], N. Serifovski[1], A.M.H. Herz[1], S. Schmidt[1], P. Gaede[2], E. Hommel[3], L. Raimond[3], A. Gotfredsen[1], K. Nørgaard[1,3]; [1]Dept. of Endocrinology, Hvidovre Hospital, [2]Dept. of Endocrinology, Slagelse Hospital, [3]Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: Carbohydrate counting and use of an automated bolus calculator can reduce HbA1C in type 1 diabetes but this approach has never been tested in type 2 diabetes. We aimed to evaluate the efficacy of advanced carbohydrate counting and use of an automated bolus calculator (ABC) compared with mental insulin bolus calculation (MC) in persons with type 2 diabetes on basal-bolus insulin therapy.
Materials and methods: A 24-week open-label, randomized controlled study was conducted in persons with type 2 diabetes treated with basal-bolus insulin. All participants had HbA1C ≥58 mmol/mol, were GAD antibody negative, naïve to carbohydrate counting and bolus calculation and were treated with basal and bolus insulin ≥6 months. 79 participants (mean age 62.5 ± 9.6 yrs, mean HbA1C 72 ± 11 mmol/L, mean diabetes duration 18.7 ± 7.6 yrs, mean BMI 33 ± 6 kg/m^2, mean fasting c-peptide 656 ± 486 pmol/L) were randomized 1:1 into two groups. ABC group received training in advanced carbohydrate counting and use of an automated bolus calculator. MC group received training in advanced carbohydrate counting and mental calculation of insulin bolus (without a bolus calculator). Participants wore blinded continuous glucose monitor (CGM) for 6 days at baseline and at study end. Primary endpoint was change in HbA1C. Secondary endpoints were time spent in glycemic ranges and frequency of severe hypoglycemia. Data is reported for 64 participants having completed the study at present.
Results: Baseline HbA1C was similar in the ABC group and in the MC group (71 ± 11 vs 72 ± 12 mmol/mol). At 24 weeks HbA1C had decreased by 8.1 mmol/mol ($P \leq 0.001$) in the ABC group and by 8.0 mmol/mol ($P \leq 0.001$) in the MC group. There was no difference in change in HbA1C between groups at study end ($P = 0.96$). CGM data showed that time in euglycemic range (sensor glucose (SG) 4.0–10.0 mmol/L) increased significantly from baseline to study end in the ABC group (57.7% to 66.7%, $P = 0.03$) in contrast to the MC group (59.7% to 63.3%, $P = 0.22$), but there was no significant difference in increase between the two groups ($P = 0.52$). Time in hyperglycemia (SG >10 mmol/L) tended to decrease in the ABC group (40.7% to 32.0%, $P = 0.051$) but was unchanged in the MC group (38.3% to 35.6%, $P = 0.29$) with no difference between groups ($P = 0.52$). Time in hypoglycemia (SG ≤3.9 mmol/mol) decreased insignificantly in both groups (ABC: 1.7% to 1.4%, $P = 0.26$; MC: 2.0% to 1.1%, $P = 0.08$) with no difference between groups ($P = 0.67$). Coefficient of variance (CV) decreased in both groups (ABC: 29.1% to 26.7%, $P = 0.03$; MC: 29.4% to 26.1%, $P = 0.004$) without any difference in decrease between groups ($P = 0.87$). There were no episodes of severe hypoglycemia in any of the groups.
Conclusion: Advanced carbohydrate counting and insulin bolus calculation is an efficient, low-cost tool to reduce HbA1C in persons with basal-bolus insulin treated type 2 diabetes. Similar effect was seen with use of an automated bolus calculator and with use of mental bolus calculation. Blinded CGM revealed decreased glycemic variability with both options, whereas only the group using automated bolus calculation increased their time in euglycemic range.
Clinical Trial Registration Number: NCT02887898
Supported by: Roche a/s
Disclosure: **M.B. Christensen:** Grants; Unrestricted grant from Roche a/s.

820

Therapy adjustments of patients with type 1 diabetes on multiple daily injections with an increased risk for hypoglycaemia using real-time continuous glucose monitoring (rtCGM)

D. Waldenmaier[1], G. Freckmann[1], L. Heinemann[2], N. Hermanns[3], S. Pleus[1], C. Haug[1];
[1]Institut für Diabetes-Technologie, Forschungs- und Entwicklungsgesellschaft mbH an der Universität Ulm, Ulm, [2]Science-Consulting in Diabetes GmbH, Düsseldorf, [3]Forschungsinstitut der Diabetes-Akademie Bad Mergentheim, Bad Mergentheim, Germany.

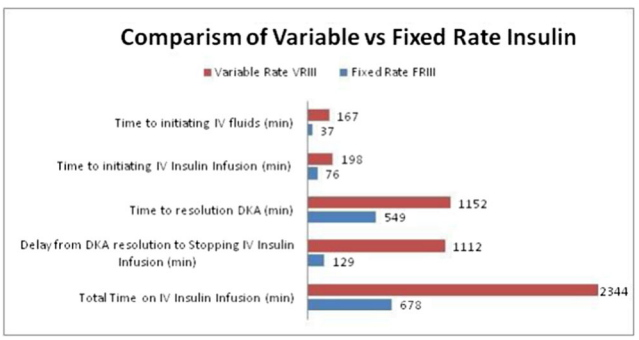
Background and aims: There is limited knowledge about how persons with diabetes modify their insulin therapy in daily life based on real-time continuous glucose monitoring (rtCGM) data. The HypoDE study recently demonstrated that the use of an rtCGM system reduces the occurrence of hypoglycaemic events (<55 mg/dl) in patients with type 1 diabetes on multiple daily injections with an increased risk for hypoglycaemia. The present analysis investigated how the patients adjusted their therapeutic decisions.
Materials and methods: Study participants were randomized to rtCGM use (CGM group) or self-monitoring of blood glucose (control group) for 26 weeks. CGM group subjects were trained in interpretation and use of CGM data for therapeutic adjustments. Treating physicians in both groups modified therapies based on available data. During the baseline phase (before randomization) and at the end of the study (follow-up phase), subjects documented every insulin dose and meal intake for 7 days each in a diary. For each subject, changes in daily means of insulin dose and carbohydrate (CHO) intake between the two phases were calculated. Rescue CHO for prevention or treatment of hypoglycaemic events were identified, and based on current glucose values a patient-specific rescue CHO intake threshold was determined.
Results: Diaries for both study phases were available from 96% of the participants (70 CGM group, 65 control group). These subjects were representative for the whole HypoDE population regarding demographic data and occurrence of hypoglycaemic events. CGM group subjects showed a larger reduction of hypoglycaemic events (−1.4 ± 2.3 events per 7 days, $p > 0.001$) from baseline to follow-up phase than subjects in the control group. Based on the subjects' records in their diaries, no changes in insulin doses (total daily dose CGM group: 0.6 ± 0.3 U/kg/day to 0.6 ± 0.4 U/kg/day, control group: 0.6 ± 0.2 U/kg/day to 0.6 ± 0.2 U/kg/day), nor insulin distribution were observed in any of the two study groups. However, CGM subjects, but not control subjects, reported a moderate increase in their blood glucose target value from 120 ± 16 mg/dl to 126 ± 20 mg/dl ($p = 0.015$). In addition, they reported an increased injection meal interval (from 10–11 min to 13–15 min). There was no significant change in daily CHO intake, but CGM group subjects reported more frequent CHO intake during nights of the follow-up phase (from 0.2 ± 0.2 to 0.3 ± 0.3 meals/night, $p = 0.001$). Considering only rescue CHO, a more frequent intake among subjects in the CGM group was observed (from 0.8 ± 0.6 to 1.0 ± 0.8 intakes/day vs. 1.1 ± 0.8 to 0.9 ± 0.8, $p = 0.008$), especially during the night. However, as CGM group subjects reduced the average size of single rescue CHO intakes, only a small difference in total daily rescue CHO amount was observed. The mean rescue CHO intake threshold determined for the CGM group was raised from 71 ± 13 to 79 ± 14 mg/dl ($p < 0.001$) whereas it remained unchanged in the control group.
Conclusion: Participants of the HypoDE study were able to reduce the occurrence of hypoglycaemic events by usage of rtCGM without major changes to their insulin therapy. Instead, they were more active in preventing hypoglycaemia by consuming rescue CHO more often and earlier.
Clinical Trial Registration Number: NCT02671968
Supported by: Dexcom Inc.
Disclosure: **D. Waldenmaier:** Grants; Dexcom Inc.

821

To compare the effectiveness of intravenous variable rate insulin compared to fixed rate weight based insulin infusion on the resolution of diabetic ketoacidosis

Y. Yap, T. Molyneux, C. Gadd, V. Stewart, S. Hammersley;
Department of Endocrine and Diabetes, Leighton Hospital, Crewe, UK.

Background and aims: The latest 2013 Joint British Diabetes Societies Inpatient Care Group guidance (JBDS) has recommended a weight-based fixed rate insulin (FRIII) approach for the treatment of Diabetic ketoacidosis (DKA) as opposed to the variable rate insulin infusion (VRIII) published in 2010. The current evidence for this change is limited. The aim of this study is to review the real world effects on how this change in the rate of insulin delivery affects the time to resolution of DKA and its secondary impact on the length of inpatient stay (LOS).
Materials and methods: A retrospective case note analysis was conducted on patients admitted with DKA treated with a VRIII over 6 months from August 2016 to February 2017. Following implementation of a FRIII protocol a second 6 month period was studied from July 2017 to January 2018. Data gathered included patient demographics, HbA1c, admission blood pH and capillary blood glucose and level of care required. We measured time to resolution of DKA, number of hypoglycaemic episodes on treatment, length of inpatient stay, total time of insulin treatment and time from resolution to insulin cessation.
Results: Baseline demographics for VRIII versus FRIII were: number of patients 21:20 with a male to female ratio of 8:13 and 9:11 respectively. The mean age was 42:45 years old with an average HbA1c of 100:91mmol/mol respectively. The mean pH was 7.14:7.01, mean serum bicarbonate of 11.45:12mmol/L and mean capillary blood glucose 29:28mmol/l respectively. In the VRIII cohort 3 patients required critical care admission versus none in the FRIII group. There were 6 hypoglycaemia episodes on VRIII and 4 episodes on FRIII. There were 3 patients (1 in VRIII and 2 in the FRIII group) that had an extended LOS unrelated to DKA therefore the median value was taken as the measure of LOS. This was 5.5 days in the VRIII group compared with 3 days in the FRIII group.
Conclusion: In conclusion, the FRIII in combination with a treatment protocol achieved a significantly reduced length of time to initiation of treatment (intravenous insulin and intravenous fluids) and improved time to resolution of DKA. Total time on insulin was reduced. Importantly there were less hypoglycaemic episodes and reduced LOS. Our real world data supports the use of FRIII in line with guidelines from JBDS.



Disclosure: **Y. Yap:** None.

822

Smartphone triggered diabetes self-management education and support in insulin treated type 2 diabetes patients: results of the randomised TRIGGER study

A. Boels[1], R.C. Vos[1], L.-T. Dijkhorst-Oei[2,3], G.E.H. Rutten[1];

[1]University Medical Center Utrecht, Utrecht, [2]Meander Medical Center, Amersfoort, [3]VU University Medical Center, Amsterdam, Netherlands.

Background and aims: Innovative and cost-effective interventions to promote diabetes self-management education and support (DSME/S) are needed. We evaluated the (cost-)effectiveness of DSME/S delivered via a smartphone application in individuals with type 2 diabetes mellitus (T2DM) on insulin therapy.
Materials and methods: We conducted a non-blinded two-arm multicentre randomised clinical superiority trial with parallel-groups and equal randomisation (1:1). In total, 5 hospitals and 66 general practices in the Netherlands included 230 individuals with T2DM aged 40–70 years, on insulin therapy since ≥3 months, with HbA1c >53 mmol/mol (>7%). The intervention group received unidirectional messages with DSME/S on dietary habits, physical activity, hypoglycaemia prevention and glucose variability. Messages were sent at different times of the day, 2 or 6 times per week for 6 to 9 months. Participants could choose their preferred topics, frequency and the duration of the intervention. The control group received usual care. The primary study endpoint was the HbA1c level after 6 months follow-up. Secondary endpoints were body mass index, waist circumference, blood pressure, insulin dose, lipid profile, self-management (assessed with Summary of Diabetes Self-Care Activities), health status (assessed with EQ-5D), diabetes-dependent quality of life (assessed with Audit of Diabetes Dependent Quality of Life), and diabetes treatment satisfaction (assessed with Diabetes Treatment Satisfaction Questionnaire). Here we present the results of the complete case analyses of the first 150 participants who completed the study. At the EASD conference we will be in the position to present the final results of all 230 participants. We used the intention-to-treat principle and general linear models, adjusted for baseline value, baseline insulin dose, age, sex, and duration of diabetes to analyse the data.
Results: There were no differences in baseline characteristics between the control and intervention group: mean age was 58.6 years (intervention group) versus 59.8 years (control group), and the baseline HbA1c was 64.7 mmol/mol (intervention group) versus 66.5 mmol/mol (control group). After 6 months of follow-up, the mean HbA1c in the intervention group was 61.7 mmol/mol (95% CI 58.9–64.5), compared to 65.7 mmol/mol (95% confidence interval (CI) 62.9–68.6) in the control group, $p = 0.051$ ($n = 150$). After adjustment, the mean HbA1c levels became 63.1 mmol/mol (95% CI 61.0–65.2) in the intervention group, versus 64.9 mmol/mol (95% CI 62.8–67.0) in the control group, $p = 0.230$ ($n = 139$). After 6 months, the mean number of days on which participants performed foot care was higher in the control group, adjusted: 1.3 days (95% CI 0.9–1.7) in the intervention group versus 1.9 days (1.5–2.3) in the control group, $p = 0.027$ ($n = 125$). There were no statistically significant effects on other secondary outcomes.
Conclusion: The preliminary results of this trial, which is one of the larger ones in this field worldwide, show modestly promising, yet inconclusive results on the effectiveness of smartphone triggered diabetes self-management education and support in insulin treated T2DM patients. Analyses of all cases will provide final evidence on its effectiveness.
Clinical Trial Registration Number: Dutch Trial Register NTR5515
Supported by: unrestricted research grant from Sanofi-Aventis
Disclosure: **A. Boels:** Grants; unrestricted research grant from Sanofi-Aventis.

823

Baseline nocturnal glucose change as a predictor of treatment effect of bolus intensification in insulin-treated type 2 diabetes

K. Salvesen-Sykes[1], A.L. Peters[2], M. Piletic[3], J. Snyder[1], K. Bowering[4]; [1]Novo Nordisk Inc., Plainsboro, USA, [2]Keck School of Medicine of the University of Southern California, Los Angeles, USA, [3]General Hospital, Novo Mesto, Slovenia, [4]Division of Endocrinology and Metabolism, University of Alberta, Edmonton, Canada.

Background and aims: onset 3 (a phase 3, 18-week, randomised controlled trial) demonstrated that fast-acting insulin aspart (faster aspart) in a basal-bolus therapy (BBT) was superior in reducing HbA_{1c} and controlling postprandial glucose (PPG) excursions compared with basal-only therapy (BOT) in type 2 diabetes (T2D). This *post hoc* analysis of onset 3 explored the utility of PPG increments, nocturnal change (NC) and HbA_{1c} for predicting response to bolus insulin intensification.
Materials and methods: Analyses were performed on 236 randomised subjects receiving metformin: 116 received faster aspart BBT; 120 received BOT. Subjects were grouped according to three baseline parameters: HbA_{1c}, PPG increments (calculated as the difference between self-measured plasma glucose 2-h post-meal and pre-meal recorded in a 7-point profile over 3 days), and NC (calculated as the mean of two measurements of the difference between the bedtime glucose measurement and the fasting glucose measurement taken the following morning). Analyses were performed on the end-of-trial treatment differences (BBT versus BOT) between 'high' and 'low' baseline values of each parameter, defined as being above or below the median value at baseline (HbA_{1c}, 7.8% [61.7 mmol/mol]; PPG increment, 2.42 mmol/L [43.6 mg/dL]; NC, 3.11 mmol/L [56 mg/dL]).
Results: At baseline, there was a positive correlation between PPG increment and NC ($r = 0.66$). The changes in mean HbA_{1c} and mean PPG increment at end-of-trial were consistently in favour of faster aspart across all subgroups, and there were significantly larger treatment differences in subjects with high (versus low) baseline NC and PPG increment values (Table). This was not the case for baseline HbA_{1c}, which showed significantly larger treatment differences for change in end-of-trial PPG increments but not for change in end-of-trial HbA_{1c}. There were no significant differences in the treatment rate ratios of severe or blood glucose-confirmed hypoglycaemia between high subgroups or between low subgroups. Those in the high baseline PPG increment and NC subgroups had higher absolute rates of hypoglycaemia.
Conclusion: Within the limitations of a *post hoc* analysis, these data suggest that both mean PPG increment and NC may be useful for identifying subgroups of patients with T2D that would more likely benefit from bolus intensification; NC may perform as well as mean PPG increment while being easier to measure.

Table. Change in HbA_{1c} and mean PPG increment from baseline to end-of-trial stratified by HbA_{1c}, PPG increment, and nocturnal change at baseline.

Baseline value		Δ HbA_{1c}			
		Faster aspart + basal	Basal-only insulin therapy	ETD [95% CI]*	*p*-value
HbA_{1c} (%)	≤7.80 >7.80	−1.36 −0.98	−0.47 0.04	−0.88 [−1.20, −0.56] −1.03 [−1.34, −0.71]	0.5328
Nocturnal change (mmol/L)	≤3.11 >3.11	−0.92 −1.42	−0.34 −0.03	−0.58 [−0.91, −0.26] −1.40 [−1.72, −1.07]	0.0006**
PPG increment (mmol/L)	≤2.42 >2.42	−0.98 −1.41	−0.31 −0.14	−0.68 [−1.00, −0.35] −1.27 [−1.59, −0.96]	0.0099**
		Δ mean PPG increment			
HbA_{1c} (%)	≤7.80 >7.80	−1.23 −1.99	−0.74 −0.25	−0.49 [−1.00, 0.02] −1.74 [−2.24, −1.24]	0.0007**
Nocturnal change (mmol/L)	≤3.11 >3.11	−1.29 −1.87	−0.99 0.02	−0.30 [−0.81, 0.21] −1.88 [−2.39, −1.38]	<0.0001**
PPG increment (mmol/L)	≤2.42 >2.42	−1.46 −1.80	−0.78 −0.22	−0.68 [−1.19, −0.16] −1.59 [−2.10, −1.08]	0.0141**

*In favour of faster aspart; **statistically significant change to treatment by subgroup interaction values ($p<0.05$). CI, confidence interval; ETD, estimated treatment difference; faster aspart, fast-acting insulin aspart; PPG, postprandial glucose; T2D, type 2 diabetes.

Clinical Trial Registration Number: NCT01850615
Supported by: Novo Nordisk
Disclosure: **K. Salvesen-Sykes:** Employment/Consultancy; Novo Nordisk Inc. Stock/Shareholding; Novo Nordisk Inc.

824

Achievement of individual HbA_{1c} targets with self- vs physician-led titration of newly or recently initiated basal insulin in type 2 diabetes: DUNE real-world study results

L. Berard[1], D. Mauricio[2], K. Khunti[3], D.R. Franco[4], J. Westerbacka[5], C. Candelas[6], V. Pilorget[6], R. Perfetti[7], L. Meneghini[8];
[1]Winnipeg Regional Health Authority Health Sciences Centre, Winnipeg, Canada, [2]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [3]Diabetes Research Centre, University of Leicester, Leicester, UK, [4]CPClin Clinical Research Center, São Paulo, Brazil, [5]Sanofi, Paris, France, [6]Sanofi, Chilly-Mazarin, France, [7]Sanofi, Bridgewater, USA, [8]University of Texas Southwestern Medical Center and Parkland Health & Hospital System, Dallas, USA.

Background and aims: The Diabetes Unmet Need with basal insulin Evaluation (DUNE) study aimed to assess the impact of symptomatic hypoglycaemia on individualised HbA_{1c} target achievement. In this analysis, the achievement of individualised HbA_{1c} targets at 12 weeks with self- vs physician-led insulin titration was evaluated.
Materials and methods: DUNE was a 12-week, prospective, observational study of 3139 adults with type 2 diabetes (T2DM), newly (at enrolment) or recently (<12 months) initiated on basal insulin (BI).
Results: Irrespective of titration method, the proportion of participants who achieved their individualised HbA_{1c} target at week 12 was <30% in both the newly and recently initiated BI groups (**Table**). For both titration groups, there were comparable improvements in fasting plasma glucose from baseline and similar incidence of hypoglycaemia. Increase in mean total daily BI dose for self- vs physician-led titration in the recently initiated BI group was 4.85 vs 4.91 U, respectively, while in newly initiated individuals it was 9.68 vs 7.77 U, respectively. Most participants who were self-titrating did so every 1–3 days, compared with no more frequently than every week for most participants who had physician-led titration. In all groups, the most frequently utilised dose increment was 2 U.
Conclusion: The inability of most participants to achieve their target HbA_{1c} in a real-world scenario, irrespective of the method of titration, warrants further investigation.

Table. Comparison of physician-led and self-titration in participants newly (at enrolment) or recently (<12 months) initiated on basal insulin

	Newly initiated (N=1716)[a]		Recently initiated (N=1423)[b]	
	Self-titration (n=1094)	Physician-led titration (n=622)	Self-titration (n=896)	Physician-led titration (n=527)
Participants who achieved individualised HbA_{1c} target at week 12, %	28.3	27.2	26.0	28.3
FPG change from baseline to week 12, mmol/l	−3.4 (3.7)	−3.3 (3.9)	−1.3 (3.0)	−1.3 (3.6)
BI dose change from baseline, U	9.68 (13.03)	7.77 (11.11)	4.85 (7.90)	4.91 (9.58)
Any symptomatic hypoglycaemic event during study, n (%)	157 (14.4)	86 (13.8)	159 (17.8)	101 (19.2)
Any documented symptomatic (≤3.9 mmol/l) hypoglycaemic event, n (%)	123 (11.3)	60 (9.7)	129 (14.5)	81 (15.6)
Any documented symptomatic (<3.0 mmol/l) hypoglycaemic event, n (%)	47 (4.3)	22 (3.6)	35 (3.9)	32 (6.2)
Frequency of titration, n (%)				
Every 1 to 3 days	724 (66.2)	45 (7.4)	461 (51.5)	27 (5.5)
Every 4 to 6 days	100 (9.1)	7 (1.2)	72 (8.0)	3 (0.6)
Every 1 week	241 (22.0)	304 (50.2)	297 (33.2)	196 (39.7)
> Every 1 week	29 (2.7)	249 (41.2)	65 (7.3)	268 (54.2)

Data are mean (standard deviation) unless otherwise indicated.
[a]1715 (99.9%) newly initiated participants were set individualised HbA_{1c} targets.
[b]1416 (99.5%) recently initiated participants were set individualised HbA_{1c} targets.
BI, basal insulin; FPG, fasting plasma glucose

Clinical Trial Registration Number: OBS13780
Supported by: Sanofi
Disclosure: **L. Berard:** Employment/Consultancy; Eli Lilly; Sanofi; Novo Nordisk Lifescan; Abbott; BD; MontMed: Merck; Janssen: AstraZeneca; Boehringer Ingelheim. Grants; MontMed. Honorarium; Eli Lilly; Sanofi; Novo Nordisk Lifescan; Abbott; BD; Merck; Janssen; AstraZeneca. Lecture/other fees; Eli Lilly; Sanofi; Novo Nordisk Lifescan; Abbott; BD; Merck; Janssen; AstraZeneca.

825

Improved efficacy with self- vs physician-led titration of insulin glargine 300 or 100 U/ml in type 2 diabetes: comparison of TAKE CONTROL, AT.LANTUS and ATLAS

M. Davies[1], E. Boelle-Le Corfec[2], M. Bonnemaire[2], M. Odawara[3], L. Popescu[4], J. Sieber[5], D. Russell-Jones[6], K. Strojek[7], N. Tentolouris[8];
[1]Diabetes Research Centre, University of Leicester, University Hospitals of Leicester NHS Trust, Leicester, Leicestershire, UK, [2]Sanofi, Paris, France, [3]Tokyo Medical University, Tokyo, Japan, [4]Global R&D Operations, Sanofi, Bucharest, Romania, [5]Sanofi, Frankfurt, Germany, [6]Department of Diabetes and Endocrinology, Royal Surrey County Hospital, Guildford, UK, [7]Department of Internal Diseases, Diabetology and Cardiometabolic Diseases SMDZ, Zabrze, Silesian Medical University, Katowice, Poland, [8]First Department of Internal Medicine, Medical School, National and Kapodistrian University of Athens, Laiko General Hospital, Athens, Greece.

Background and aims: Empowering individuals to self-titrate their basal insulin may help to reduce rates of sub-optimal titration often observed in clinical practice and help more people achieve HbA_{1c} goals. This analysis examined clinical outcomes with insulin glargine 300 U/mL (Gla-300) or 100 U/mL (Gla-100) following self- or physician-led titration.
Materials and methods: Results from the recently completed TAKE CONTROL study, which evaluated self-titration and physician-led titration of Gla-300 in people with T2DM, were compared with similar studies (AT.LANTUS and ATLAS) of Gla-100. All 3 studies were 24-week, multicentre, randomized, open-label, parallel-group studies.
Results: Fasting blood glucose targets were 4.4–7.2 mmol/L (80–130 mg/dL) in TAKE CONTROL, ≤5.5 mmol/L (≤100 mg/dL) in AT.LANTUS and ≤6.1 mmol/L (≤110 mg/dL) in ATLAS. Mean baseline HbA_{1c} was between 8.4 and 8.9% (**Table**). Self-titration statistically significantly lowered mean HbA_{1c} by 0.97 to 1.40% versus physician-led titration (0.84 to 1.25%; $p < 0.05$ for all 3 studies). The incidence of severe hypoglycaemia and other safety outcomes was similar between titration arms in all 3 studies (**Table**). Both Gla-300 and Gla-100 were effective in improving glycaemic control.
Conclusion: Self-titration with Gla-300 resulted in significantly improved glycaemic control versus physician-led titration, as demonstrated previously with Gla-100, without increased hypoglycaemia.

Table. Comparison of self-titration and physician-led titration with Gla-300 and Gla-100

	TAKE CONTROL (24 weeks)		AT.LANTUS (24 weeks)		ATLAS (24 weeks)	
	Self-titration Gla-300 N=314	Physician-led titration Gla-300 N=317	Self-titration Gla-100 N=2,273	Physician-led titration Gla-100 N=2,315	Self-titration Gla-100 N=275	Physician-led titration Gla-100 N=277
T2DM population	Insulin naïve and previously treated Europe		Insulin naïve and previously treated Europe, South America, Asia, Africa/Middle East		Insulin naïve Asia	
Mean diabetes duration, years	12.9 (7.2)	12.8 (6.9)	12.3 (7.0)	12.3 (7.3)	10.3 (6.9)	9.1 (5.3)
Baseline HbA_{1c}, %	8.4 (0.9)	8.4 (0.9)	8.9 (1.3)	8.9 (1.3)	8.7 (1.0)	8.8 (1.1)
Final HbA_{1c} [change from baseline], %	7.4 (1.0) [−0.97*]	7.6 (0.9) [−0.84]	7.7 (1.2) [−1.22*]	7.9 (1.2) [−1.08]	7.3 (0.9) [−1.40*]	7.5 (1.0) [−1.25]
HbA_{1c}, LS mean difference self- vs physician-led titration	−0.13 (−0.26 to −0.00)		–		−0.15 (−0.29 to −0.00)	
Total basal insulin dose per day, U Baseline/end of treatment/mean change from baseline	24.1/39.7/15.6	25.7/36.9/11.2	23.5/45.0/21.6	22.3/41.0/18.7	8.2[a]/28.9/–	8.1[a]/22.2/–
Incidence of severe/symptomatic/nocturnal hypoglycemia, %	0.6/26.3/8.0	0.3/25.6/11.4	1.1/29.7/4.1	0.9/26.3/3.2	0.7/36.0/16.4	0.7/25.6/6.5
Any treatment-emergent AE/serious treatment-emergent AE	33.7/3.2	34.5/3.8	48.0/5.1	49.4/5.1	35.0/3.3	32.1/1.8

Data are mean (standard deviation) unless otherwise indicated.
*Statistically significantly different from physician-led titration.
[a]Starting dose
AE, adverse event; T2DM, type 2 diabetes

Clinical Trial Registration Number: EudraCT 2015-001626-42
Supported by: Sanofi
Disclosure: **M. Davies:** Employment/Consultancy; Acted as consultant for Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca and Janssen. Grants; Received grants in support of investigator and investigator initiated trials from Novo Nordisk, Sanofi-Aventis, Lilly, Boehringer Ingelheim and Janssen. Honorarium; Fees for advisory board member from Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca, Janssen and Servier. Lecture/other fees; Speaker fees from Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca, Janssen, Mitsubishi Tanabe Pharma Corporation and Takeda Pharmaceuticals International In.

826

Daytime and nocturnal glucose checking and hypoglycaemia patterns from real-world flash continuous glucose monitoring use

Y. Xu[1], H. Pryor[2], E. Budiman[1], T. Dunn[1];
[1]Abbott Diabetes Care, Alameda, USA, [2]Abbott Diabetes Care, Witney, UK.

Background and aims: Previous analyses of real-world data have shown that frequent use of flash continuous glucose monitoring (FreeStyle Libre™) is associated with lower average glucose level and shorter time in hypoglycaemia. We studied the patterns of checking glucose during the day and night and time in hypoglycaemia.
Materials and methods: Users scan the sensor with the reader to collect current glucose, glucose trend, and up to 8 hours of glucose readings, which are automatically stored every 15 minutes. When the readers are connected to the desktop software using an internet ready PC, de-identified 90-day reader data is uploaded to a database. De-identified data from September 2014 to September 2017 of all sensors with at least 120 hours of operation were analysed from 202,179 readers and 1.4 million sensors worldwide (84% from Europe). Scan rate per reader was determined and two sets of twenty equally sized rank-ordered groups, categorised by day and night scan frequencies, were evaluated. Time in significant hypoglycaemia (≤3.0 mmol/L [54 mg/dL]) and number of scans during the day (06:00–23:00) and during the night (23:00–06:00) were calculated.
Results: The median (IQR) night time and day time hourly checking rates are 0.22 (0.13, 0.35) and 0.61 (0.40, 0.92), respectively. Higher rates of glucose checking at night time are associated with shorter night time hypoglycaemia duration (15.3 to 9.6 minutes or 3.6% to 2.3% of time per night, p value <0.001) and lower estimated A1c (eA1c) (62 to 54 mmol/mol [7.8% to 7.1%], p value <0.001). Higher rates of glucose checking during the day are associated with lower eA1c (68 to 51 mmol/mol [8.4% to 6.8%], p value <0.001) and shorter daytime hypoglycaemia duration (19.1 to 11.7 minutes or 1.9% to 1.1% of time per day, p value <0.001) when average eA1c level is lower than 62 mmol/mol (7.8%).
Conclusion: Increased glucose testing at night time is associated with decreased eA1c level and lower night time hypoglycaemia duration. Increased glucose testing at daytime is associated with decreased eA1c level. Daytime hypoglycaemia duration reduction does not occur until eA1c level drops below 62 mmol/mol (7.8%), possibly due to glucose reduction being the priority for those with eA1clevel higher than 62 mmol/mol (7.8%).
Disclosure: **Y. Xu:** Employment/Consultancy; Abbott Diabetes Care.

827

Use of supportive tools and education enables self-titration with insulin glargine 300 or 100 U/ml in type 2 diabetes: results from TAKE CONTROL, INNOVATE and AUTOMATIX

M. Bonnemaire[1], M. Kvapil[2], H. Goyeau[3], N. Papanas[4], L. Popescu[5], B. Schultes[6], J. Sieber[7], L. Smircic Duvnjak[8];
[1]Global Diabetes Division, Sanofi, Paris, France, [2]Department of Internal Medicine, Second Faculty of Medicine, Charles University, Prague, Czech Republic, [3]Sanofi, Chilly-Mazarin, France, [4]Diabetes Centre, Second Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece, [5]Global R&D Operations, Sanofi, Bucharest, Romania, [6]eSwiss Medical & Surgical Center, Department of Internal Medicine, Endocrinology, Diabetes, & Metabolism, St. Gallen, Switzerland, [7]Global Diabetes Division, Sanofi, Frankfurt, Germany, [8]University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Vuk Vrhovac, School of Medicine, University of Zagreb, Zagreb, Croatia.

Background and aims: Supporting people with T2DM with education and titration tools may empower them to optimize basal insulin self-titration in order to reach glycaemic targets. This analysis aimed to examine the impact of titration tools and education to support individuals to self-titrate insulin glargine 300 U/mL (Gla-300) or 100 U/mL (Gla-100).
Materials and methods: Three multicentre, randomized, open-label, parallel-group studies have investigated self-titration (supported by either a paper algorithm, web tool or device) vs physician-led titration in people with T2DM, switching to or initiating Gla-300 (TAKE CONTROL and AUTOMATIX) or Gla-100 (INNOVATE). Fasting blood glucose targets for self-titration were 4.4–7.2 mmol/L (80–130 mg/dL) in TAKE CONTROL and 5.0–7.2 mmol/L (90–130 mg/dL) in both INNOVATE and AUTOMATIX.
Results: Self-titration resulted in greater or similar mean HbA_{1c} reductions from baseline vs physician-led titration (**Table**). By using self-titration with Gla-300, more people with T2DM achieved fasting glucose targets without confirmed or severe hypoglycaemia vs physician-led titration (TAKE CONTROL and AUTOMATIX). Severe hypoglycaemia and other safety outcomes were similar between titration arms in all 3 studies.
Conclusion: Self-titration with Gla-300 and Gla-100 using either a paper or device-based algorithm resulted in improved or similar reductions in HbA_{1c}, without an increased risk of hypoglycaemia or safety concerns.

Table. Comparison of self-titration and physician-led titration with Gla-300 and Gla-100

	TAKE CONTROL (24 weeks)		INNOVATE (12 weeks)		AUTOMATIX (16 weeks)	
	Self-titration Gla-300 N=314	Physician-led titration Gla-300 N=317	Self-titration Gla-100 N=72	Physician-led titration Gla-100 N=67	Self-titration Gla-300 N=75	Physician-led titration Gla-300 N=76
T2DM population	Insulin naïve and previously treated 10 European countries		Insulin naïve and previously treated 10 European countries		Insulin naïve and previously treated Canada	
Self-titration support tools provided	Paper algorithm		Paper algorithm		Self-titration web tool (MyStar WebCoach®)	
Mean diabetes duration, years	12.9 (7.2)	12.8 (6.9)	11.1 (6.0)	12.9 (7.5)	12.2 (7.2)	12.3 (6.4)
Baseline HbA_{1c}, %	8.4 (0.9)	8.4 (0.9)	8.8 (1.3)	8.8 (1.4)	8.8 (1.0)	8.6 (0.8)
Final HbA_{1c}, % [change from baseline]	7.4 (1.0) [−0.97*][c]	7.6 (0.9) [−0.84*]	NA (NA) [−1.0][b]	NA (NA) [−1.1][b]	7.6 (1.1) [−1.12*]	7.6 (0.9) [−1.07*]
Total basal insulin dose per day, U baseline/end of study/mean change from baseline	24.1/39.7/15.6	25.7/36.9/11.2	26.2/41.8/NA	28.5/42.6/NA	27.8/49.3/22.0	26.6/42.4/15.9
Reached fasting SMPG target without hypoglycaemia: severe/confirmed or severe/any, %	NA/67.5[d]/NA	NA/58.4[d]/NA	15[d]/NA/47[a]	41[d]/NA/51[a]	45.9[f]/34.3[f]/NA	36.8[f]/14.5[f]/NA
Incidence of severe/documented (≤3.9 mmol/L) symptomatic/nocturnal hypoglycaemia, %	0.6/23.1/8.0	0.3/22.8/11.4	0/20/6	0/30/12	0/13.3/10.7	1.3/13.2/14.5
Any TEAE/serious TEAE, %	33.7/3.2	34.5/3.8	NA/0	NA/0	42.7/2.7	38.2/3.9

Data are mean (standard deviation) unless otherwise indicated. *Statistically significantly different from physician titration. [a]Difference from baseline is least squares mean change. [b]Composite endpoint: reached median fasting pre-breakfast SMPG (target 4.4–7.2 mmol/L) without experiencing any confirmed (≤3.0 mmol/L) or severe hypoglycaemia event during the 24-week on-treatment period. [c]Primary composite: at least 4 out of 7 FPGs in the range of 5.0–7.2 mmol/L (inclusive); mean FPG for 3 consecutive prior FPGs before the end of 12 weeks in the range of 5.0–7.2 mmol/L; no severe hypoglycaemia reported during the 7–10 day period prior. [d]Alternative composite: mean of last 5 FPG within the last 2 weeks prior to the visits, with no hypoglycaemia. [e]Primary endpoint: mean of the last 5 available SMPG values within the 2 weeks before the end of 16 weeks of treatment in the range of 5.0–7.2 mmol/L; no severe hypoglycaemia event during the 16 weeks of treatment. [f]Secondary endpoint: mean of the last 5 available SMPG values within the 2 weeks before the end of 16 weeks of treatment in the range of 5.0–7.2 mmol/L; no confirmed (≤3.9 mmol/L) or severe hypoglycaemia event during the 16 weeks of treatment. TEAE, treatment-emergent adverse event; NA, not available

Clinical Trial Registration Number: EudraCT 2015-001626-42
Supported by: Sanofi
Disclosure: **M. Bonnemaire:** Employment/Consultancy; Sanofi. Stock/Shareholding; Sanofi.

PS 070 Clinical outcomes in insulin treated patients

828

The relationship between urinary albumin excretion, cardiovascular outcomes and total mortality among large cohort of insulin-treated patients with type 2 diabetes

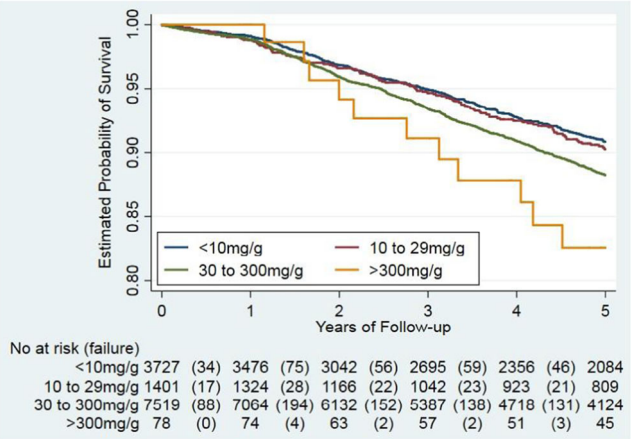
R. Donnelly, U. Anyanwagu, I. Idris;
Royal Derby Hospital Centre, University of Nottingham, Derby, UK.

Background and aims: Albuminuria is a recognised diagnostic and prognostic marker of chronic kidney disease (CKD) and cardiovascular (CV) risk but the precise relationship between increments in urinary albumin-creatinine ratio (ACR) and CV outcomes and mortality among insulin treated patients with Type 2 Diabetes (T2D) in routine clinical care is unclear.

Materials and methods: We investigated data for insulin users with T2D from UK general practices between 2007 and 2014. Urinary ACR at the time of insulin initiation was measured and categorised as: <10 mg/g; 10 to 29 mg/g; 30 to 300 mg/g; and >300 mg/g. Patients were followed up for 5 years or the earliest occurrence of all-cause mortality, non-fatal myocardial infarction (MI) or stroke. Cox proportional hazard models were fitted to estimate the risk of a composite of these events.

Results: A total of 12,725 patients with T2DM (mean age: 58.6 ± 13.8 years, mean HbA1c: 8.7 ± 1.8) initiating insulin therapy between 2007 and 2014 met the inclusion criteria. The adjusted risk of a 3-point composite of all-cause mortality, non-fatal MI and stroke is shown in Figure 1. Compared to patients whose ACR levels at insulin initiation were below 10 mg/g, the adjusted risk of the 3-point composite endpoint was 7%, 30% and 93% higher in those with ACR levels between 10–29 mg/g; 30–300 mg/g and >300 mg/g, respectively, after a follow up period of 5 years. ACR category on its own did not predict risk of all-cause mortality.

Conclusion: This study shows that in patients with T2D on insulin therapy increased urinary ACR is independently associated with an increased risk of major adverse CV events and all-cause mortality.



Disclosure: **R. Donnelly:** Lecture/other fees; Novo Nordisk, Eli Lilly, Astra Zeneca, MSD, Jansen, Sanofi Aventis.

829

The association between insulin initiation and adverse outcomes after hospital discharge: a population-based cohort study

L.L. Lipscombe[1,2], Z. Lysy[1,2], H.D. Fischer[3], K. Fung[3], V. Giannakeas[2,3], C.M. Bell[4];
[1]Medicine, University of Toronto, Toronto, [2]Medicine, Women's College Hospital, Toronto, [3]Institute for Clinical Evaluative Sciences, Toronto, [4]Medicine, Sinai Health System, Toronto, Canada.

Background and aims: Transitional care after discharge from a hospitalization may be inadequate for older patients with diabetes, particularly for those started on insulin therapy in hospital. These patients may thus be at increased risk for serious adverse events after discharge. The aims of this study were to quantify the incidence of death and return to hospital after discharge for older hospitalized patients prescribed anti-hyperglycemic agents, and to compare risk of these adverse events between patients prescribed new insulin therapy versus oral hypoglycemic agents (OHAs).

Materials and methods: This retrospective population-based cohort study in Ontario, Canada included persons aged 66 and over discharged from a hospitalization between April 1st 2004 and November 30th 2013, and dispensed a prescription for insulin and/or an OHA within 7 days of discharge.Prescriptions were categorized as new insulin (no insulin before admission), prevalent insulin (prescribed insulin before admission and at discharge), new OHA(s) (no OHA or insulin before admission), and prevalent OHA [prescribed OHA(s) before admission and at discharge] as the referent category. The primary and secondary outcomes were deaths and a return to hospital (emergency visits or hospital admissions) respectively within 30 days of discharge.

Results: Of 104,525 patients, 9.2% were initiated on insulin, 4.1% died and 26.2% had a return to hospital within 30 days. Deaths occurred in 7.14% of new insulin users, 4.86% of prevalent insulin users, 3.25% of new OHA users, and 3.45% of prevalent OHA users. Rates of return to hospital were 28.1% among new insulin users, 29.8% among prevalent insulin users, 25.1% among new OHA users, and 25.0% among prevalent OHA users. After adjustment for covariates, new insulin users had a significantly higher 30-day risk of death (adjusted hazard ratio, aHR 1.52, 95% confidence interval, CI 1.39 to 1.67) and return to hospital (aHR 1.16, 95% CI 1.11 to 1.21) than prevalent OHA users. Findings were similar for hospital visits for hypo/hyperglycemia (see Table).

Conclusion: Older hospitalized patients discharged on new insulin therapy have a high rate of death and return to hospital after discharge, and their risk is significantly higher than prevalent OHA-treated patients. These findings highlight a need for better discharge planning and transitional care for hospitalized patients treated with insulin. Further inquiry should determine appropriate interventions to reduce adverse outcomes after insulin initiation in older hospitalized patients, so that the benefits of effective diabetes management while in hospital are maintained when patients leave the hospital.

Outcomes	Prevalent OHA (referent) N=62,018	New insulin N=9,592	Prevalent insulin N=25,203	New OHA N=7,712
All-cause mortality, 30 days				
Events, N(%)[a]	2,137 (3.45%)	685 (7.14%)	1,224 (4.86%)	251 (3.25%)
Out of hospital deaths, N(%)	1,026 (48.0%)	412 (60.1%)	638 (52.1%)	100 (39.8%)
In-hospital deaths, N(%)	1,111 (52.0%)	273 (39.9%)	586 (47.9%)	151 (60.2%)
unadjusted HR (95% CI)	1.00	2.12 (1.94 -2.31)	1.42 (1.32 -1.52)	0.94 (0.83 -1.08)
adjusted HR (95% CI)[c]	1.00	1.52 (1.39 -1.67)	1.11 (1.03 -1.20)	1.23 (1.08 -1.40)
Return to hospital (any ED visit or hospital admission), 30 days				
Events, N(%)[a]	15,386 (25.0%)	2,644 (28.1%)	7,425 (29.8%)	1,927 (25.1%)
ED visits only, N(%)	7,828 (50.9%)	1,322 (50.0%)	3,636 (49.0%)	1,049 (54.4%)
Re-admissions, N(%)	7,558 (49.1%)	1,322 (50.0%)	3,789 (51.1%)	878 (45.6%)
unadjusted HR (95% CI)	1.00	1.14 (1.10 -1.19)	1.22 (1.19 -1.25)	1.00 (0.96 -1.05)
adjusted HR (95% CI)[c]	1.00	1.16 (1.11 -1.21)	1.14 (1.11 -1.18)	1.04 (1.00 -1.10)
Return to hospital (ED visit or hospital admission for hypo/hyperglycemia), 30 days				
Events, N(%)[a]	302 (0.49%)	113 (1.21%)	289 (1.17%)	63 (0.82%)
unadjusted HR (95% CI)	1.00	2.47 (1.99 -3.06)	2.37 (2.02 -2.79)	1.68 (1.28 -2.20)
adjusted HR (95% CI)[c]	1.00	2.40 (1.92 -3.00)	2.31 (1.95 -2.73)	1.68 (1.28 -2.21)

Supported by: CIHR
Disclosure: **L.L. Lipscombe:** None.

830

Durability of improved patient-reported outcomes in type 2 diabetes patients treated with dapagliflozin plus saxagliptin vs insulin glargine

M.A. Testa[1], J.F. Hayes[2], M. Lind[3], T. Vilsbøll[4], S. Jabbour[5], E. Ekholm[6], E. Johnsson[6], D.C. Simonson[7];
[1]Harvard T. H. Chan School of Public Health, Boston, USA, [2]Phase V Technologies, Wellesley Hills, USA, [3]University of Gothenburg, Gothenburg, Sweden, [4]Steno Diabetes Center, Copenhagen, Denmark, [5]Thomas Jefferson University, Philadelphia, USA, [6]AstraZeneca, Mölndal, Sweden, [7]Endocrinology, Brigham and Women's Hospital, Boston, USA.

Background and aims: Consensus statements on the management of type 2 diabetes (T2D) now recommend personalizing treatment decisions by considering patient-centered behavioral, psychological and emotional factors. We previously reported that T2D patients inadequately controlled on metformin reported more favorable patient-reported outcomes (PRO) with dapagliflozin plus saxagliptin add-on therapy (DAPA+SAXA, $n = 324$) versus insulin glargine add-on therapy (INS, $n = 319$) after 24 weeks during an international, randomized, non-inferiority trial. To evaluate the durability of these effects, we analyzed data through 52 weeks of treatment.
Materials and methods: A1C, weight, and PRO questionnaires were obtained at baseline and weeks 12, 24 and 52. Linear mixed models and correlation analyses were used for testing per protocol 52-week completers not requiring rescue medication (addition of insulin or other glucose-lowering agent) and all completers. Treatment satisfaction included 71 questions measuring overall satisfaction, with subscales of efficacy, flexibility, side effects, convenience, burden, preference, social, general satisfaction, pain, hassle, and interference. Quality-of-life (QOL) measures included 165 items with scales in the physical, mental, social, cognitive, symptoms, weight concern and interference, body image, sexual and functional health domains.
Results: Baseline data were: 54.0% male; 80.4% white; 60.7% working ≥3 days/week; age 55.5 ± 9.6 years; A1C 9.0 ± 1.0%; BMI 32.2 ± 5.3 kg/m^2; diabetes duration 9.4 ± 6.3 years. Satisfaction scales, weight concern and perceived health favored DAPA+SAXA at both weeks 24 and 52 (see Table). Body image and anxiety favored DAPA+SAXA more at week 52 as weight loss and concern continued to improve for DAPA+SAXA. Actual weight loss at 52-weeks was associated with improvements in weight concern ($p < 0.001$) and body image ($p < 0.001$). Greater weight concern and weight-related symptom interference were also associated with decreases in QOL and satisfaction scales ($p < 0.05$ to $p < 0.001$).
Conclusion: At 52 weeks, compared to INS, DAPA+SAXA had better QOL and satisfaction outcomes due to improved perceived health, less weight concern and anxiety, improved body image and greater regimen acceptance. The treatment differences at week 24 were sustained through week 52 providing confirmatory evidence that differential treatment impact on satisfaction and QOL may persist without attenuation due to adaptation or coping. Treatment choices should address both the short and long-term psychological and behavioral effects of diabetes treatment regimens.

Change from Baseline (Day 1) in A1C and PRO at Weeks 24 and 52 [LS Mean (SE)]

	24 Weeks[1]			52 Weeks[1,2]		
Endpoint	**DAPA+SAXA**	**INS**	**P-value**	**DAPA+SAXA**	**INS**	**P-value**
HbA1c (%)	−1.67 (0.06)	−1.54 (0.06)	0.118	-1.51 (0.07)	-1.26 (0.07)	0.0009
Overall Satisfaction	10.14 (0.63)	6.19 (0.64)	<0.0001	9.35 (0.67)	6.03 (0.68)	0.0006
- Regimen Acceptance	7.00 (0.72)	2.28 (0.73)	<0.0001	7.28 (0.76)	3.06 (0.77)	0.0001
-- Burden	7.70 (0.85)	2.48 (0.86)	<0.0001	6.86 (0.92)	3.15 (0.93)	0.0049
-- Convenience	8.01 (0.89)	2.95 (0.91)	<0.0001	7.19 (0.94)	2.10 (0.94)	0.0001
-- Flexibility	9.91 (1.01)	4.69 (1.02)	<0.001	11.11 (1.07)	7.30 (1.09)	0.0130
-- Hassle	5.92 (0.94)	1.83 (0.95)	0.002	5.93 (1.00)	2.35 (1.01)	0.0121
-- Interference	6.45 (0.98)	2.75 (1.00)	0.009	8.74 (0.98)	3.35 (0.99)	0.0001
-- Social	4.32 (0.83)	0.62 (0.85)	0.002	4.88 (0.88)	0.88 (0.90)	0.0015
-- Pain	4.41 (0.94)	−1.93 (0.96)	<0.0001	3.83 (0.96)	-1.44 (0.97)	0.0001
-- Side Effects	6.06 (0.99)	0.17 (1.00)	<0.0001	4.70 (1.12)	-0.69 (1.13)	0.0008
Weight Concern	17.68 (2.46)	10.38 (2.50)	0.038	15.61 (2.73)	2.69 (2.75)	0.0009
Body Image	-6.29 (2.06)	-1.26 (2.08)	0.0865	-7.82 (2.19)	2.57 (2.21)	0.0009
Perceived Health	0.64 (0.09)	0.36 (0.09)	0.0258	0.63 (0.09)	0.31 (0.09)	0.0140
Anxiety	16.77 (4.15)	6.84 (4.22)	0.0940	20.57 (4.91)	3.81 (4.97)	0.0168

[1] Positive change in PRO scales indicates improvement, except for Body Image, where a negative change is more favorable.
[2] Means shown for completers not requiring rescue medication. Results for all completers analyses were similar.

Clinical Trial Registration Number: NCT02551874
Supported by: AstraZeneca
Disclosure: **M.A. Testa:** None.

831

Effects of dulaglutide vs glargine in patients with different baseline glycaemic patterns (high/low fasting or high/low postprandial glucose): AWARD-2 post hoc analysis

F. Giorgino[1], M. Yu[2], A. Haupt[2], Z. Milicevic[2], L.-E. García-Pérez[2];
[1]University of Bari Aldo Moro, Bari, Italy, [2]Eli Lilly and Company, Indianapolis, USA.

Background and aims: Insulin glargine exerts its action primarily through a decrease in fasting plasma glucose (FPG), whereas dulaglutide, a once weekly glucagon-like peptide-1 receptor agonist, targets both FPG and postprandial glucose (PPG). This post hoc analysis of the AWARD-2 study assessed the efficacy of dulaglutide vs glargine in patients with type 2 diabetes with different glycaemic patterns at baseline (BL) determined by self-monitoring of blood glucose (fasting glucose [FG] vs PPG).
Materials and methods: Patients were categorized into 4 groups based on combinations of low and high FG and PPG, with median BL values of FG (8.38 mmol/L) and PPG (10.10 mmol/L) used as thresholds for low and high, respectively. Analyses were conducted using analysis of covariance.
Results: Dulaglutide showed a statistically significantly greater reduction in HbA_{1c} vs glargine for all subgroups, except for low FG/high PPG where the numerical difference favoured dulaglutide, but did not reach statistical significance (Table). Total hypoglycaemia was consistently lower for dulaglutide vs glargine in all subgroups.
Conclusion: Dulaglutide showed efficacy on HbA_{1c} reductions across different BL glycaemic patterns vs glargine, indicating a clinical benefit of targeting both FG and PPG, regardless of BL glycaemic phenotype.

Variable	Low FG, Low PPG		Low FG, High PPG		High FG, Low PPG		High FG, High PPG	
	DU 1.5	GLAR	DU 1.5	GLAR	DU 1.5	GLAR	DU 1.5	GLAR
	n=82	n=101	n=30	n=27	n=35	n=28	n=108	n=93
HbA1c, mmol/mol								
BL	57.37 (6.67)	58.46 (7.65)	63.93 (8.31)	62.83 (9.07)	63.93 (7.32)	65.02 (9.07)	73.76 (10.82)	72.67 (9.29)
Change from BL at week 52	-6.56 (1.20)*¤	-2.19 (1.09)	-9.84 (1.57)*	-5.47 (2.08)*	-10.93 (1.86)*¤	-5.47 (2.08)*	-15.30 (1.09)*¤	-9.84 (1.09)*
FPG, mmol/L								
BL	7.46 (1.90)	7.88 (2.24)	7.78 (1.63)	8.33 (1.95)	9.65 (2.47)	8.64 (2.24)	10.68 (2.674)	10.61 (2.63)
Change from BL at week 52	-0.40 (0.30)	-0.94 (0.27)*	-1.02 (0.47)*	-1.55 (0.51)*	-1.61 (0.46)*	-1.46 (0.52)*	-2.68 (0.27)*	-2.71 (0.29)*
FG, mmol/L								
BL	7.02 (0.81)	7.10 (0.85)	7.51 (0.75)	7.53 (0.76)	9.30 (0.64)	9.42 (0.81)	10.93 (2.00)	10.75 (1.67)
Change from BL at week 52	-0.44 (0.20)*	-0.12 (0.17)	-0.48 (0.29)	0.09 (0.33)	-2.00 (0.29)*	-1.84 (0.33)*	-3.28 (0.17)*	-3.27 (0.19)*
PPG, mmol/L								
BL	8.16 (1.16)	8.38 (1.11)	11.49 (1.23)	11.52 (1.14)	9.11 (0.73)	8.99 (1.02)	12.95 (2.34)	12.86 (1.86)
Change from BL at week 52	-0.49 (0.23)*	-0.18 (0.21)	-2.52 (0.35)*	-1.55 (0.39)*	-1.06 (0.34)*	-0.94 (0.39)*	-3.73 (0.21)*	-3.47 (0.22)*
Weight, kg								
BL	81.8 (18.53)	87.4 (18.98)	83.2 (17.99)	85.7 (24.48)	90.4 (20.50)	83.9 (19.22)	86.9 (16.53)	89.0 (18.68)
Change from BL at week 52	-1.7 (0.39)*¤	1.2 (0.35)*	-1.6 (0.60)*¤	2.5 (0.67)*	-1.6 (0.60)*¤	0.7 (0.68)	-1.8 (0.35)*¤	2.0 (0.37)*
Total hypoglycaemia episodes								
Week 52, n (%)	51 (62.2)	71 (70.3)	20 (57.1)	24 (82.8)	16 (45.7)	18 (64.3)	54 (52.4)	58 (63.7)
GLAR dose, units								
Week 52, mean (SD)	–	24.22 (20.35)	–	32.76 (39.20)	–	24.57 (11.96)	–	32.29 (19.27)

Values are presented as mean (SD) for BL, least-squares mean (SE) for change from BL at week 52, unless otherwise indicated. *p<0.05 change from BL; ¤p<0.01 for change from BL; #p<0.05 for comparison with GLAR; ##p<0.01 for comparison with GLAR.
Abbreviations: BL, baseline; DU, dulaglutide; FG, fasting glucose (SMBG); FPG, fasting plasma glucose; GLAR, glargine; PPG, postprandial glucose (SMBG); SD, standard deviation; SE, standard error; SMBG, self-monitoring of blood glucose.

Clinical Trial Registration Number: NCT01075282
Disclosure: **F. Giorgino:** Employment/Consultancy; Boehringer Ingelheim; Lifescan; Merck Sharp & Dohme; Sanofi, AstraZeneca, Medimmune, Roche Diabetes Care. Other; Advisory Boards: AstraZeneca; Eli Lilly; Novo Nordisk; Roche Diabetes Care; Research Support: Eli Lilly; Lifescan, Takeda.

832

Exploring clinical outcomes in diverse populations with uncontrolled type 2 diabetes switching to insulin Gla-300: first-stage analysis of the pooled European Gla-300 studies (REALI)

N. Freemantle[1], P. Gourdy[2], D. Mauricio[3], D. Müller-Wieland[4], R.C. Bonadonna[5], L. Pedrazzini[6], G. Bigot[7], C. Mauquoi[8], M. Bonnemaire[6];
[1]University College London, London, UK, [2]Toulouse University Hospital, Toulouse, France, [3]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [4]University Hospital Aachen, Aachen, Germany, [5]University of Parma and AOU of Parma, Parma, Italy, [6]Global

Diabetes, Sanofi, Paris, France, [7]IVIDATA, Paris, France, [8]IDDI, Louvain-la-Neuve, Belgium.

Background and aims: The REALI project is a large database aimed to collect data from over 10,000 people with type 2 and type 1 diabetes mellitus (T2DM, T1DM) uncontrolled with antidiabetic therapy and switched to/initiated on insulin glargine 300 U/ml (Gla-300), providing a pre-defined common core data set from different European Gla-300 studies with diverse objectives and inclusion criteria. We used this platform to perform proof-of-concept analysis on the first three completed, available, interventional and observational studies to evaluate robustness and diversity of Gla-300 efficacy in insulin-naïve and insulin-pre-treated patients.

Materials and methods: The three studies investigated efficacy and safety of Gla-300: Take Control ($N = 631$) was a 24-week interventional, randomised 1:1, 2-arm, controlled study investigating patient- versus physician-managed titration of Gla-300; BOT PLUS Neo ($N = 1523$) and TOP-II ($N = 1216$) were 12-month prospective non-interventional single-arm studies. This first analysis of pooled data examined baseline characteristics, 24-week efficacy endpoints of glucose control, severe hypoglycaemia and body weight in insulin-naïve and insulin-pre-treated T2DM subpopulations using a statistical descriptive and generalised model-based approach.

Results: (Table 1.) Overall, 92.8% of patients across all studies received different insulin regimens before switch to Gla-300 and 7.2% of patients from only Take Control were insulin-naïve. Patient demographics and baseline characteristics are shown. Measurements were performed from baseline to week 24 following Gla-300 initiation. HbA_{1c} LS mean change estimate was −1.5% (95% CI −1.6 to −1.4) in insulin-naïve patients, with 37.3% being at HbA_{1c} target (<7% [53 mmol/mol]). HbA_{1c} LS mean change estimate was −0.5% (95% CI −0.6 to −0.50) in insulin-pre-treated patients, with 20.1% at HbA_{1c} target. Mean (SD) change in basal insulin daily dose was 14.00 (16.2) U/day with mean (SD) body weight gain of 1.2 (2.5) kg for insulin-naïve patients, and 5.7 (14.2) U/day and loss of 0.03 (4.5) kg for insulin-pre-treated patients. Severe hypoglycaemic events were rare.

Conclusion: Switching to/starting on Gla-300 achieved improved glycaemic control and was associated with modest weight change among insulin-naïve patients and prior insulin-treated patients. This first analysis indicates that pooling of diverse populations with uncontrolled T2DM is a powerful approach to investigate the effectiveness of Gla-300 and potentially to predict different clinical outcomes in patient subpopulations.

Table 1. Description of baseline clinical characteristics and outcomes in insulin-naïve and insulin-pre-treated patients over the 24-week treatment with Gla-300 period.

Included	Insulin-naïve patients (N=242)	Insulin-pre-treated patients (N=3,128)	Total (N=3,370)
Age (years) [N]	239	3,011	3,250
Mean (SD)	63.7 (8.4)	64.6 (10.2)	64.6 (10.1)
BMI [N]	242	1,944	2,186
<30 kg/m², n (%)	91 (37.6)	693 (35.6)	784 (35.9)
≥30 kg/m², n (%)	151 (62.4)	1,251 (64.4)	1,402 (64.1)
Duration of diabetes [N]	242	1,759	2,001
<10 years, n (%)	100 (41.3)	704 (40.0)	804 (40.2)
≥10 years, n (%)	142 (58.7)	1,055 (60.0)	1197 (59.8)
Baseline HbA_{1c} [N]	242	2,694	2,936
Mean (%) (SD)	8.8 (0.98)	8.4 (0.93)	8.4 (0.94)
Treated	**Insulin-naïve patients (N=240)**	**Insulin-pre-treated patients (N=3,014)**	**Total (N=3,254)**
Change of HbA_{1c} (%)* [N]	233	2,078	2,311
LS means % (SE)	−1.5 (0.05)	−0.5 (0.02)	−0.7 (0.02)
95% CI	[−1.6; −1.4]	[−0.6; −0.5]	[−0.7; −0.6]
Patients at target (HbA_{1c}<7%) [N]	233	2,304	2,537
n (%)	87 (37.3)	462 (20.1)	549 (21.6)
Severe hypo[‡] [N]	240	3,014	3,254
Any time of the day, n (%)	3 (1.3)	7 (0.2)	10 (0.3)
Nocturnal, n (%)	0 (0.0)	4 (0.1)	4 (0.1)
Change of BI daily dose (U/day)* [N]	235	2,300	2,535
Mean (SD)	14.0 (16.2)	5.7 (14.2)	6.5 (14.6)
Change of body weight (kg)* [N]	182	1,242	1,424
Mean (SD)	1.2 (2.5)	−0.03 (4.5)	0.13 (4.3)

*Change from baseline to week 24; [‡]Number of patients with at least one event
BI, basal insulin; BMI, body mass index; CI, confidence interval; HbA_{1c}, glycated haemoglobin; hypo, hypoglycaemia; LS, least squares; SD, standard deviation; SE, standard error

Disclosure: **N. Freemantle:** None.

833

Lower pharmacokinetic and pharmacodynamic within-day variability of individual clinical doses of insulin glargine 300 U/ml vs glargine 100 U/ml in type 1 diabetes

C.G. Fanelli, P. Lucidi, P. Candeloro, P. Cioli, A. Marinelli Andreoli, G.B. Bolli, F. Porcellati;
University of Perugia, Perugia, Italy.

Background and aims: Previous studies have examined variability of pharmacokinetics (PK, plasma insulin concentration) and pharmacodynamics (PD, glucose infusion rate, GIR) of basal insulins at steady-state (SS) and at fixed doses in all subjects studied. To establish within-day PK/PD variability of individual, different, doses of insulin Glargine 300 U/ml (Gla-300) vs Glargine 100 U/ml (Gla-100) that people with T1DM use in real life.

Materials and methods: Eighteen T1DMs [age 40 ± 11 years, diabetes duration 26 ± 12 years, BMI 23.4 ± 2.1 kg/m^2, A1C 7.2 ± 0.5% (55 ± 6 mmol/mol)] were studied after 3 month treatment with Gla-300 and Gla-100 titrated to fasting euglycemia, with a 24 h euglycemic clamp (randomized, crossover). The individual basal insulin doses that subjects used (0.35 ± 0.08 Gla-300, 0.28 ± 0.07 Gla-100, U/kg) were injected s.c. in the clamp study.

Results: Prior to clamp, glycemic control was comparable with Gla-300 and Gla-100. In the clamp, the individual doses of Gla-300 and Gla-100 resulted in 24 h PK/PD bioequivalence, but in lower variability indices of PK/PD with Gla-300 vs Gla-100 (Table).

Conclusion: At clinical, individual doses used by T1DMs in real life, Gla-300 has lower PK/PD within-day variability vs Gla-100. These results may explain the lower glycemic variability and lower risk for hypoglycaemia reported in clinical studies with Gla-300 vs Gla-100.

Pharmacokinetic and pharmacodynamic variables after subcutaneous injection of individual doses of insulin Gla-300 and insulin Gla-100 in subjects with type 1 diabetes in steady state.

	0.35 U/kg	0.28 U/Kg	Gla-300/Gla-100 ratio
	Gla-300	Gla-100	Point estimate[a] (90% CI)
PK parameters			
Swing [b]	1.0 (0.7;1.4)	3.1 82.3; 4.2)	0.33 (0.26; 0.43)
Fluctuation [c] (µU·ml^{-1})	0.65 (0.5;0.8)	1.20 (1.1;1.4)	0.54 (0.45; 0.64)
Fluctuation [c] (%)	6.0 (4.1; 8.6)	12.0 (8.5;16)	0.51 (0.45; 0.64)
Delta INS [d] (µU·ml^{-1})	7.1 (6; 8.3)	12.3 (10,15)	0.57 (0.49; 0.67)
PD parameters			
Fluctuation[c] [mg·kg^{-1}·min^{-1}]	0.61 (0.5; 0.8)	0.73 (0.6;0.9)	0.83 (0.74;0.93)
Fluctuation [c] (%)	82 (65; 106)	102 (84;124)	0.81 (0.70; 0.94)

Data are geometric mean (95% CI)* [a]Point estimates of treatment ratios with 90% confidence intervals (CIs) were calculated using a linear mixed effects model based on log-transformed data and re-transformations. [b]Swing: [(FIRI C_{max} - FIRI C_{min}) / FIRI C_{min}]; [c]Fluctuation (F24): [(FIRI C_{max}-FIRI C_{min} / C_{avg})]. Fluctuation (%): [100% x (F24 / FIRI C_{avg})]. [d]Delta INS: (FIRI C_{max} - FIRI C_{min})

Clinical Trial Registration Number: EudraCT 2015-002135-17
Supported by: Partially supported by Sanofi
Disclosure: **C.G. Fanelli:** Honorarium; Sanofi Menarini. Lecture/other fees; Sanofi, Menarini.

834

Long-acting insulin analogues versus human isophane insulin for type 2 diabetes: update of a Cochrane review

J. Engler[1], K. Horvath[2,2], T. Semlitsch[2], K. Jeitler[3,2], A. Berghold[3], A. Siebenhofer[2,1];
[1]Institute for General Practice, Goethe University, Frankfurt, Germany, [2]Institute of General Practice and Evidence-Based Health Services Research, Medical University of Graz, Graz, Austria, [3]Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria.

Background and aims: In persons with type 2 diabetes mellitus (T2DM) insulin treatment is frequently performed by administering basal insulin.

Common adverse effects are hypoglycaemia and weight gain, which can be responsible for difficulties in achieving lower HbA1c. Long-acting insulin-analogues have been developed to minimise side effects and allowing for better blood glucose control. To assess the effects of long-term treatment with long-acting insulin analogues compared to human isophane insulin (NPH insulin) in adult persons with T2DM.

Materials and methods: Systematic review of randomized controlled trials lasting 24 weeks or longer.

Results: We identified 16 studies comparing insulin glargine and 8 studies comparing insulin detemir to NPH insulin. All trials had an unclear or high risk of bias for several Domains. Treatment with insulin glargine compared to NPH insulin treatment showed an OR for severe hypoglycaemia of 0.65 (95% CI 0.48 to 0.87); $p = 0.004$; 14 trials; very low quality evidence. The OR for serious hypoglycaemia (severe event fulfilling at least one criterion for a serious adverse event) was 0.73 (95% CI 0.50 to 1.07); $p = 0.11$; 10 trials; low quality evidence. Treatment with glargine reduced the incidence of confirmed and confirmed nocturnal hypoglycaemia. Differences in the mean change of HbA1c were not statistically significant; very low quality of evidence. Treatment with insulin detemir compared to NPH insulin showed an OR for severe hypoglycaemia of 0.37 (95% CI 0.15 to 0.92); $P = 0.03$; 5 trials; very low-quality evidence. The OR for serious hypoglycaemia was 0.16 (95% CI 0.04 to 0.61); $P = 0.007$; 5 trials; very low-quality evidence. Treatment with detemir also reduced the incidence of confirmed and confirmed nocturnal hypoglycaemia. Differences in the mean change of HbA1c were not statistically significant; very low quality of evidence. Information on diabetes-related complications or health-related quality of life was insufficient in almost all trials. For those outcomes for which some data were available, no meaningful differences were found. The incidence of adverse events was comparable for persons treated with glargine, or detemir, and persons treated with NPH.

Conclusion: Treatment with insulin glargine or insulin detemir resulted in fewer participants experiencing severe, overall and nocturnal hypoglycaemia. The effects on HbA1c were comparable. Low-quality evidence and trial designs that did not conform with current clinical practice meant it remains unclear if the same effects will be observed in daily clinical practice.

Supported by: Federal Ministry of Education and Research 01KG1707
Disclosure: **J. Engler:** Grants; Federal Ministry of Education and Research.

PS 071 Combination therapy with ultra-long-acting insulin

835

Insulin degludec/insulin aspart (IDegAsp) twice daily (BID) vs biphasic insulin aspart 30 (BIAsp 30) BID: a randomised trial in Chinese patients with type 2 diabetes

J. Ma[1], W. Yang[2], T. Hong[3], M. Liu[4], H. Miao[5], Y. Peng[6], C. Wang[7], X. Xu[8], T. Yang[9], W. Liu[10], A. Moeller Nielsen[11], L. Pan[10], Z. Weigang[12]; [1]Nanjing First Hospital, Nanjing, China, [2]China-Japan Friendship Hospital, Beijing, China, [3]Peking University Third Hospital, Beijing, China, [4]Tianjin Medical University General Hospital, Tianjin, China, [5]Nanjing Medical School, 2nd Affiliated Hospital, Nanjing, China, [6]Shanghai First People's Hospital, Shanghai, China, [7]First Affiliated Hospital of Anhui Medical University, Hefei City, China, [8]Fuzhou General Hospital of Nanjing Military Command, Nanjing, China, [9]The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [10]Novo Nordisk, Beijing, China, [11]Novo Nordisk, Søborg, Denmark, [12]Peking Union Medical College Hospital, Beijing, China.

Background and aims: IDegAsp is the first coformulation of long-acting basal (degludec) and bolus (IAsp) insulin with no need for re-suspension. This 26-week, phase 3, open-label, treat-to-target, 2:1, randomised trial assessed the efficacy and safety of IDegAsp BID vs. BIAsp 30 BID ±metformin in Chinese adults ($N = 541$) with type 2 diabetes inadequately controlled on pre-/self-mix or basal insulin ±metformin.

Materials and methods: Hierarchical testing was used with non-inferiority of HbA_{1c} change from baseline to Week 26 as the primary endpoint and superiority for secondary endpoints.

Results: Non-inferiority of HbA_{1c} change from baseline to Week 26 and statistical superiority of IDegAsp BID vs. BIAsp 30 BID for change in fasting plasma glucose, nocturnal (00:01–05:59 hours inclusive) confirmed hypoglycaemic and confirmed hypoglycaemic episodes (severe or plasma glucose <56 mg/dL with or without symptoms) was confirmed (Table). Significantly more patients reached HbA_{1c} <7% without confirmed hypoglycaemia with IDegAsp BID vs. BIAsp 30 BID by Week 26. Daily insulin dose (U/kg [SD]) was lower in patients receiving IDegAsp BID vs. BIAsp 30 BID at Week 26 (0.78 [0.35] vs. 0.95 [0.35]). No new safety signals were identified.

Conclusion: These results demonstrate the efficacy and safety of IDegAsp in Chinese patients with type 2 diabetes, confirming results from other international trials comparing the two treatment modalities.

	IDegAsp BID (n=360)			BIAsp 30 BID (n=181)			Conclusion* (LS Mean Treatment contrast [95% CI])
	Baseline	End of trial (LOV)	Mean change	Baseline	End of trial (LOV)	Mean change	
Mean HbA_{1c} (% [SD])	8.31 [0.76]	6.95 [0.77]	−1.37 [0.94]	8.33 [0.77]	7.01 [0.72]	−1.32 [0.81]	Non-inferiority (−0.08 [−0.20; 0.05], p<0.0001)
Mean FPG (mg/dL [SD])	163.42 [39.90]	109.40 [31.30]	−53.91 [46.68]	163.43 [44.82]	134.80 [36.15]	−28.25 [46.47]	Superiority (−1.42 [−1.74; −1.10], p<0.0001)
Nocturnal confirmed hypoglycaemia†	34.86			61.02			Superiority (0.53 [0.33; 0.87], p=0.0056)
Confirmed hypoglycaemia†	237.16			412.16			Superiority (0.57 [0.42; 0.77], p=0.0001)
Mean body weight (kg [SD])	68.41 [11.53]	71.22 [11.97]	2.81 [2.56]	69.47 [12.38]	71.73 [12.44]	2.26 [2.70]	Test process stopped (0.61 [0.15; 1.08]) p=0.9954)
HbA_{1c} <7% without confirmed hypoglycaemia‡ (%)	42.4			26.4			Inconclusive (2.22 [1.47; 3.35], p<0.0001)

†Rate (number of events divided by PYE multiplied by 100) for total treatment period. ‡A patient who meets the HbA_{1c} target (<7%) at end of trial without confirmed hypoglycaemia during the last 12 weeks of treatment or within 7 days after the last randomised treatment. The endpoint is only defined for patients who have been exposed for at least 12 treatment weeks.*p-values are from the 1-sided test for non-inferiority and superiority. BIAsp 30, biphasic insulin aspart 30; BID, twice daily; FPG, fasting plasma glucose; IDegAsp, insulin degludec/insulin aspart; LOV, last observed value; LS, Least squares; PYE, patient years of exposure

Clinical Trial Registration Number: NCT02762578
Supported by: Novo Nordisk
Disclosure: **J. Ma:** None.

836

Similar glycaemic control and less nocturnal hypoglycaemia with intensification of IDegAsp QD or BID vs glargine U100 QD + IAsp 1-3 in adults with type 2 diabetes

Y. Gupta[1], K. Astamirova[2], E. Fita[3], T. Demir[4], A. Haggag[5], D. Roula[6], B.A. Bak[3], A. Møller Nielsen[3], A. Tsimikas[7];
[1]All India Institute Of Medical Science, Delhi, India, [2]Saint-Petersburg Territorial Diabetic Centre, Saint-Petersburg, Russian Federation, [3]Novo Nordisk A/S, Søborg, Denmark, [4]Dokuz Eylül Üniversity, İzmir, Turkey, [5]Anaheim Clinical Trials, Anaheim, USA, [6]Salah Boubnider University, Constantine, Algeria, [7]Scripps Whittier Diabetes Institute, San Diego, USA.

Background and aims: If intensification of basal insulin is needed, addition of bolus or fixed ratio insulin therapies are recommended treatment options. No head-to-head trials compare IDegAsp once daily (QD) with a "basal plus" insulin regimen, or intensification of IDegAsp QD to twice daily (BID). This study aimed to compare: 1) IDegAsp QD vs insulin glargine 100 units/mL (glargine U100) QD + insulin aspart (IAsp) QD after 26 weeks (w); 2) optional stepwise intensification (26–38 w) of IDegAsp QD/BID vs. glargine U100 + IAsp QD/BD/TID.
Materials and methods: A 38-week, randomised, open-label, treat-to-target trial in adults with type 2 diabetes (T2D), treated with basal insulin ±oral anti-diabetes drugs (OADs) in need of intensification (HbA_{1c} 7–10%). Patients could be intensified (at physician discretion + patient consent) at w 26 + 32 if HbA_{1c} off target (≥7%) in previous week. Randomisation: 1:1 to IDegAsp (w 0–26: IDegAsp QD with largest meal; w 27–38: IDegAsp QD/BID with largest meals), or glargine U100 + IAsp (w 0–26: glargine U100 QD + IAsp QD with largest meal; w 27–38: glargine U100 QD + IAsp QD/BID/TID at main meals). Both groups: ±OADs throughout. Self-measured plasma glucose titration targets: IDegAsp and glargine U100, 4–5 mmol/L; IAsp, 4–6 mmol/L. Hypoglycaemia evaluation included severe (ADA defined) or blood glucose-confirmed (<3.1 mmol/L) symptomatic episodes. Primary endpoint: change HbA_{1c} (%; w 0–26), non-inferiority margin: 0.4%.
Results: Across treatment groups, baseline characteristics and safety profiles 0–38 w were comparable. For w 0–26 + 0–38 data, see Table. Both groups had similar change in HbA_{1c} (%; w 0–26) (mean [SD]: IDegAsp QD, −1.1 [0.9]; glargine U100 QD + IAsp QD, −1.1 [0.8]) and the estimated treatment difference: 0.07% (95% CI −0.06; 0.21) confirmed non-inferiority. After 38 w, changes in HbA_{1c} remained similar. Achievement of HbA_{1c} <7%, mean FPG and mean post-prandial increments were similar across groups at w 26 and 38. At week 38, more patients achieved HbA_{1c} <7% without hypoglycaemic episodes on-treatment with IDegAsp (22.5%) vs glargine U100 + IAsp (21.1%). The hypoglycaemia profile favoured IDegAsp, driven by significantly fewer nocturnal (occurring 00.01–05.59) episodes.
Conclusion: IDegAsp QD and BID are simple, effective treatment intensification options in T2D compared with basal plus or full basal bolus therapy, achieving similar glycaemic control, with fewer nocturnal hypoglycaemia episodes, lower insulin dose and fewer injections.

	Initiation period (0–26 weeks)			Baseline–end of treatment (0–38 weeks)		
	IDegAsp (N=267)	Glargine U100 +IAsp (N=265)	ETD or ETR† (95% CI)	IDegAsp (N=267)	Glargine U100 +IAsp (N=265)	ETD or ETR† (95% CI)
Change from baseline HbA_{1c} (%), mean (SD)	-1.1 (0.9)	-1.1 (0.8)	ETD 0.07 (-0.06; 0.21)	-1.3 (0.8)	-1.2 (0.8)	ETD 0.09 (-0.04; 0.22)
Proportion of HbA_{1c} responders (<7%), %	44.9	45.3	ETR 1.07 (0.74; 1.54)	52.1	52.8	ETR 0.95 (0.66; 1.38)
Overall hypoglycaemia,‡ mean rate (events/100 PYE)	258.5	296.1	ETR 0.90 (0.67; 1.22)	287.1	343.3	ETR 0.86 (0.65; 1.14)
Nocturnal hypoglycaemia,‡ mean rate (events/100 PYE)	47.9	92.9	ETR 0.55 (0.34; 0.90)	60.4	101.4	ETR 0.61 (0.40; 0.93)
Total daily insulin dose (Units), mean (SD)	70.9 (41.5)	79.4 (37.7)	Not tested	83.4 (51.3)	89.3 (43.1)	Not tested
Change from baseline FPG (mmol/L), mean (SD)	-2.3 (2.9)	-2.3 (3.3)	ETD 0.04 (-0.34; 0.42)	-2.7 (3.0)	-2.3 (3.1)	ETD -0.24 (-0.60; 0.13)
Change from baseline SMPG post-prandial increment (mmol/L), mean (SD)	-0.6 (2.6)	-0.5 (2.3)	ETD 0.10 (-0.23; 0.43)	-1.0 (2.2)	-1.1 (2.5)	ETD 0.30 (-0.01; 0.62)
Total number of injections/ day, mean (SD)	1 (0.00)	2 (0.00)	Not tested	1.62 (0.49)	2.85 (0.87)	Not tested
Daily insulin dose ratio: IDegAsp (Units) / glargine U100+IAsp (Units)	0.89 at Week 26		Not tested	0.93 at Week 38		Not tested

Data are observed values except ETD and ETR (estimated values, as prespecified in the protocol) and number of injections 0–26 weeks (per protocol). †ETD are IDegAsp – glargine U100+IAsp; ETR are IDegAsp / glargine U100+IAsp. ‡Hypoglycaemia included severe (ADA definition) or blood glucose-confirmed (<3.1 mmol/L) symptomatic episodes, nocturnal episodes occurred 0.01–05.59 (both inclusive). ETD, estimated treatment difference; ETR, estimated treatment ratio; FPG, fasting plasma glucose; IDegAsp, insulin degludec/aspart fixed ratio combination once or twice daily; Glargine U100+IAsp, insulin glargine 100 units/mL once daily + insulin aspart once-, twice- or three-times daily; OAD, oral anti-diabetes drug; PYE, patient years of exposure; SMPG, self-measured plasma glucose.

Clinical Trial Registration Number: NCT02906917
Supported by: Novo Nordisk A/S
Disclosure: **Y. Gupta:** Grants; Department of Science and Technology, India, Indian Council of Medical Research, India, Site Principal Investigator for Novo Nordisk sponsored clinical trials.

837

Effects of IDegLira (insulin degludec/liraglutide) in patients with poorly controlled type 2 diabetes with HbA_{1c} >9%: analyses from the DUAL programme

S. Bain[1], J. Frias[2], D. Gouet[3], R. Takács[4], T. Jia[5], P. Örsy[5], D. Sugimoto[6];
[1]Swansea University, Swansea, UK, [2]National Research Institute, Los Angeles, USA, [3]La Rochelle Hospital, La Rochelle, France, [4]University of Szeged, Szeged, Hungary, [5]Novo Nordisk A/S, Søborg, Denmark, [6]Cedar Crosse Research Center, Chicago, USA.

Background and aims: Despite a variety of treatment options for type 2 diabetes (T2D), more than half of patients do not achieve glycaemic control.
Materials and methods: In a *post hoc* analysis of the DUAL I (oral antidiabetic drugs [OADs]), II, V and VII (basal insulin + OADs) trials, we evaluated patients with an HbA_{1c} >9% at baseline to determine the impact of IDegLira on their glycaemic control. The definition of hypoglycaemia was: unable to self-treat and/or plasma glucose [PG] <3.1 mmol/L (DUAL I, II and V); unable to self-treat or PG <3.1 mmol/L with hypoglycaemia symptoms (DUAL VII).
Results: Within each DUAL trial, baseline characteristics for patients with HbA_{1c} >9% were similar for all treatment groups. In DUAL I, II and V, treatment with IDegLira resulted in greater reductions in HbA_{1c} from baseline, versus comparators of basal insulin or liraglutide, leading to lower HbA_{1c} at end of trial (EOT). In DUAL VII, reduction in HbA_{1c} from baseline and HbA_{1c} at EOT were comparable for IDegLira and

insulin glargine U100 (100 U/mL) + insulin aspart (≤4 times/day). At EOT, the composite endpoint of HbA_{1c} <7% without hypoglycaemia was achieved by a greater proportion of patients treated with IDegLira than with comparators.

Conclusion: Even in patients with T2D with HbA_{1c} >9%, IDegLira treatment achieved glycaemic control with a high proportion of patients achieving HbA_{1c} <7% and clinically important composite endpoints of HbA_{1c} <7% without hypoglycaemia and/or weight gain.

Change in HbA_{1c}, HbA_{1c} at EOT and % patients achieving composite endpoints at EOT for patient group with baseline HbA_{1c} >9% from DUAL I, II, V and VII

	DUAL I			DUAL II		DUAL V		DUAL VII	
	IDegLira	Degludec	Liraglutide	IDegLira	Degludec†	IDegLira	IGlar U100	IDegLira	IGlar U100 + IAsp
N	190	107	86	62	84	71	52	37	46
Mean Δ HbA_{1c}, %	–2.7	–2.0	–1.9	–2.5	–1.2	–2.6	–1.8	–2.3	–2.2
Mean HbA_{1c} at EOT, %	6.8	7.6	7.7	7.2	8.3	6.9	7.8	7.1	7.3
% achieving HbA_{1c} <7%	61.6	41.1	34.9	45.2	16.7	56.3	23.1	43.2	45.7
% achieving HbA_{1c} <7% w/o hypoglycaemia*	46.3	26.2	33.7	37.1	14.3	42.3	11.5	38.2	22.0
% achieving HbA_{1c} <7% w/o hypoglycaemia* or weight gain	20.0	8.4	32.6	29.0	6.0	26.8	1.9	23.5	2.4

*In the last 12 weeks of treatment. †In DUAL II, maximum allowed dose of degludec was 50 U. Δ, change; EOT, end of trial; HbA_{1c}, glycated haemoglobin; IAsp, insulin aspart; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; w/o, without.

Clinical Trial Registration Number: DUAL I: NCT01336023; DUAL II: NCT01392573; DUAL V: NCT01952145; DUAL VII: NCT02420262
Supported by: Novo Nordisk
Disclosure: **S. Bain:** Grants; Novo Nordisk.

838

Lower day-to-day fasting self-measured plasma glucose (SMPG) variability with insulin degludec/liraglutide (IDegLira) vs insulin glargine 100 units/ml (IGlar U100)

E. Jaeckel[1], A. Doshi[2], P. Garcia-Hernandez[3], J.F. Merino Torres[4], M. Rodacki[5], S. Eggert[6], R. Grøn[6], I. Lingvay[7];
[1]Hannover Medical School, Hannover, Germany, [2]PrimeCare Medical Group, Houston, USA, [3]Hospital Universitario Dr José Eleuterio González, Monterrey, Mexico, [4]University Hospital La Fe, Valencia, Spain, [5]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [6]Novo Nordisk A/S, Søborg, Denmark, [7]UT Southwestern Medical Center, Dallas, USA.

Background and aims: Diabetes therapy aims for stable glycaemic control and minimal hypoglycaemia risk, which has previously been linked to day-to-day variability. In a *post-hoc* analysis, day-to-day fasting glycaemic variability with IDegLira was compared with IGlar U100, in DUAL V (IDegLira vs. IGlar U100) and VII (IDegLira vs IGlar U100 and bolus insulin aspart).

Materials and methods: Daily fasting SMPG values were used to calculate a weekly measure of day-to-day fasting glycaemic variability measurement for each patient by calculating the variance of the daily log-transformed SMPG value. These weekly variances were analysed on log-scale. Based on the weekly variances we calculated an overall variability measure and divided the population into tertiles indicating (high, low, medium) variability. Overall day-to-day variability group (High, Medium, Low) was analysed in multinomial model using a cumulative logit link function. The model included treatment as a fixed factor. Hypoglycaemia was defined as subject unable to treat themselves and/or have a recorded PG <3.1 mmol/L, in DUAL VII the subjects also had to have symptoms consistent with hypoglycaemia. Odds ratios were based on modelling the probability of being in a lower variability group.

Results: Proportional odds were statistically significant favouring IDegLira (Table). More subjects treated with IDegLira had lower overall day-to-day fasting glucose variability vs. IGlar 100 (Table). Hypoglycaemia rates were reduced with lower variability with IDegLira vs. comparators in all tertiles (Table). In DUAL V (IDegLira vs IGlar U100) and DUAL VII (IDegLira vs IGlar U100 plus bolus insulin aspart) respectively, statistical analysis of weekly variances showed a 32% ($p < 0.0001$) and 23% ($p = 0.001$) lower day-to-day fasting glycaemic variability.

Conclusion: Based on fasting SMPG values, there was lower day-to-day variability with IDegLira, possibly contributing to the lower hypoglycaemia rate, compared with IGlar U100 in DUAL V and VII.

Table: Patients grouped according to tertiles of overall day-to-day SMPG variability by treatment

Treatment arm	Low variability		Medium variability		High variability		Probability of subjects having lower variability	
	Proportion of patients, %	Observed hypoglycaemia rates, events/PYE	Proportion of patients, %	Observed hypoglycaemia rates, events/PYE	Proportion of patients, %	Observed hypoglycaemia rates, events/PYE	IDegLira/IGlar U100 OR [95% CI]	*P*-value
IDegLira, DUAL V	38.1	0.40	38.5	1.61	23.0	6.47	1.99 [1.46; 2.72]	<0.0001
IGlar U100, DUAL V	28.3	0.75	28.3	1.99	43.4	9.95		
IDegLira, DUAL VII	36.5	0.23	35.7	0.94	27.0	2.45	1.55 [1.12; 2.14]	0.0085
IGlar U100 +IAsp, DUAL VII	29.1	1.35	30.7	4.59	38.6	16.16		

IAsp, insulin aspart; IDegLira, insulin degludec/liraglutide combination; IGlar U100, insulin glargine 100 units/mL; OR, odds ratio; PYE, patient-year of exposure; SMPG, self-measure plasma glucose.

Clinical Trial Registration Number: NCT01952145 and NCT02420262
Supported by: Novo Nordisk A/S
Disclosure: **E. Jaeckel:** Grants; Research grants: NovoNordisk, Novartis, Gilead, Roche, Miltenyi Biotech, Biotest, Wacker Chemie, Fresenius, Additional research grants: DFG, BMBF, EU, JDRF, VW-Stiftung. Honorarium; Advisory board: NovoNordisk, Lilly, AstraZeneca, Boehringer, MSD, Janssen, Roche, Novartis, Board member: NovoNordisk, Lilly, Speaker's bureau: NovoNordisk, Lilly, AstraZeneca, Boehringer, MSD, Janssen, Roche, Novartis.

839

Patient-reported outcomes for insulin degludec/liraglutide vs insulin glargine as add-on to sodium-glucose co-transporter-2 inhibitor in type 2 diabetes: DUAL IX trial

M. Brod[1], L. Billings[2], R. Busch[3], S. Harris[4], C. Morales Portillo[5], R. Sahay[6], A. Busk[7], S. Eggert[7], N. Halladin[7], A. Philis-Tsimikas[8];
[1]The Brod Group, Mill Valley, USA, [2]NorthShore University HealthSystem, Evanston, USA, [3]Albany Medical Center, New York, USA, [4]Western University, London, Canada, [5]Hospital Virgen Macarena, Seville, Spain, [6]Osmania Medical College, Hyderabad, India, [7]Novo Nordisk A/S, Søborg, Denmark, [8]Scripps Whittier Diabetes Institute, San Diego, USA.

Background and aims: In DUAL IX, a 26-week, phase 3b, treat-to-target, open-label trial, patients with uncontrolled type 2 diabetes on sodium-glucose co-transporter-2 inhibitor ± oral antidiabetic drugs (N = 420) were randomised 1:1 to once-daily insulin degludec/liraglutide (IDegLira) or insulin glargine 100 units/mL (IGlar U100) add-on therapy. IDegLira was superior to IGlar U100 for HbA_{1c} (1.9 vs 1.7% reduction), body weight (0.0 vs 2.0 kg gain) and hypoglycaemia rate (58% lower with IDegLira).
Materials and methods: Patient-reported outcomes were measured at baseline and week 26 with the 5-domain Treatment Related Impact Measure - Diabetes (TRIM-D) questionnaire, with higher scores indicating better outcomes.
Results: After 26 weeks, improvements were significantly greater with IDegLira vs IGlar U100 in total TRIM-D, treatment burden domain and especially the diabetes management domain (Table), including 4 of the 5 individual items (estimated treatment ratio [95% CI]): help you control your diabetes: 2.17 [1.47; 3.21], $p < 0.0001$; help you avoid hyperglycaemia: 1.95 [1.32; 2.87], $p = 0.0007$; help you avoid hypoglycaemia: 1.62 [1.12; 2.36], $p = 0.0105$; help you manage your weight: 2.44 [1.69; 3.52], $p < 0.0001$; help you prevent feeling tired/lack of energy: 1.36 [0.95; 1.96], $p = 0.0945$.
Conclusion: Treatment with IDegLira vs IGlar U100 resulted in better clinical and treatment management outcomes.

Table: Change from baseline to week 26 in TRIM-D domain scores – DUAL IX trial

	Baseline score[1] (SD)		Change (SD) from baseline at week 26		ETD [95% CI]	*p*-value
	IDegLira	IGlar U100	IDegLira	IGlar U100		
Total TRIM-D	75.8 (12.4)	75.3 (12.7)	8.3 (12.6)	5.4 (11.7)	2.78 [0.83; 4.73]	**0.0052**
Treatment burden	70.6 (19.0)	70.7 (18.9)	7.8 (20.7)	4.0 (19.7)	3.18 [0.12; 6.24]	**0.0414**
Daily life	85.3 (15.4)	83.1 (16.7)	2.5 (17.3)	2.9 (17.0)	0.85 [–1.96; 3.66]	0.5546
Diabetes management	56.9 (20.1)	56.3 (20.9)	17.4 (20.8)	9.3 (23.6)	7.27 [3.98; 10.57]	**<0.0001**
Compliance	80.6 (16.5)	82.5 (16.8)	8.8 (17.4)	6.2 (17.4)	1.00 [–1.62; 3.62]	0.4564
Psychological health	83.3 (15.2)	82.1 (16.2)	6.4 (15.4)	5.2 (14.9)	1.64 [–0.74; 4.03]	0.1767

[1]All scores were based on a 0–100 scale with higher values representing better outcomes.
ETD, estimated treatment difference; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; TRIM-D, Treatment Related Impact Measure – Diabetes.

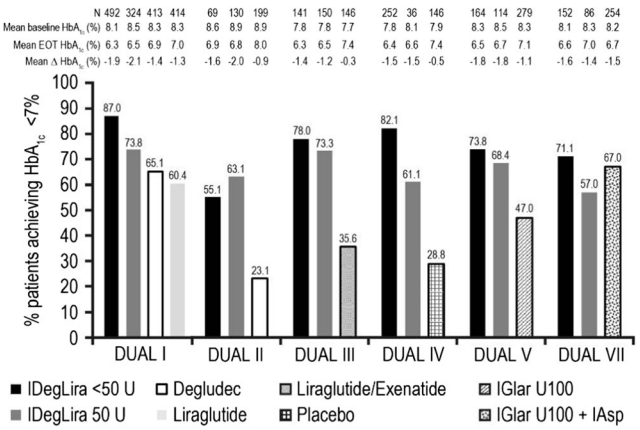
Clinical Trial Registration Number: NCT02773368
Supported by: Novo Nordisk A/S
Disclosure: **M. Brod:** Employment/Consultancy; Novo Nordisk.

840

Patients with type 2 diabetes on the maximum dose of insulin degludec/liraglutide (IDegLira) achieve glycaemic target: analyses from the DUAL programme

L. Meneghini[1], S. Linjawi[2], P. Serusclat[3], T. Vilsbøll[4], B.F. Agner[5], T. Hansen[5], L.A. Leiter[6];
[1]UT Southwestern Medical Center, Dallas, USA, [2]Coffs Diabetes and Endocrine Centre, Coffs Harbour, Australia, [3]Clinique Portes du Sud, Vénissieux, France, [4]Gentofte Hospital, Hellerup, Denmark, [5]Novo Nordisk A/S, Søborg, Denmark, [6]University of Toronto, Toronto, Canada.

Background and aims: The efficacy and safety of IDegLira has been established in the DUAL clinical development programme.
Materials and methods: This *post hoc* analysis evaluated glycaemic control in the subgroup of patients titrated to the maximum approved IDegLira dose of 50 dose steps/units (U) (50 U insulin degludec +1.8 mg liraglutide), from trials that evaluated IDegLira versus other comparators (DUAL I-V and VII). For DUAL I-V, missing data were imputed using last observation carried forward.
Results: In all DUAL trials, baseline HbA_{1c} was similar between IDegLira and comparator arms. In DUAL I-V, regardless of end-of-trial (EOT) doses (50 or <50 U), more patients on IDegLira achieved the American Diabetes Association target of HbA_{1c} <7% versus monotherapy of basal insulin, glucagon-like peptide-1 receptor agonist, or placebo comparators (Figure). In DUAL VII, compared with basal-bolus insulin therapy (insulin glargine 100 U/mL + insulin aspart ≤4 times daily [mean total daily insulin dose of 84 U at EOT]), the percentage of patients achieving an HbA_{1c} <7% was greater for patients on <50 U of IDegLira and lower for patients at 50 U of IDegLira at EOT. For patients at 50 U and <50 U of IDegLira, the mean change in HbA_{1c} at EOT was numerically greater than or similar to comparators for all trials.
Conclusion: A high proportion of patients receiving the maximum approved dose of IDegLira are able to achieve good glycaemic control.



Δ, change; EOT, end of trial; HbA_{1c}, glycated haemoglobin; IAsp, insulin aspart; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; U, units.

Clinical Trial Registration Number: DUAL I, NCT01336023; DUAL II, NCT01392573; DUAL III, NCT01676116; DUAL IV, NCT01618162; DUAL V, NCT01952145; DUAL VII, NCT02420262
Supported by: Novo Nordisk
Disclosure: **L. Meneghini:** Employment/Consultancy; Novo Nordisk. Lecture/other fees; Novo Nordisk.

841

IDegLira improves glycaemic control in subjects with type 2 diabetes uncontrolled on basal insulin without deterioration despite discontinuing pre-trial sulphonylurea

A. Janez[1], R. Silver[2], T. Vilsbøll[3], R. Grøn[4], N. Halladin[4], P. Őrsy[4], S. Harris[5];
[1]University Medical Center Ljubljana, Ljubljana, Slovenia, [2]Southern New Hampshire Medical Center, Nashua, USA, [3]Steno Diabetes Center, University of Copenhagen, Copenhagen, Denmark, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Western University, London, Canada.

Background and aims: As combining sulphonylurea (SU) and insulin can elevate the risk of hypoglycaemia, prescribers often reduce SU dose or stop SUs altogether when initiating insulin. This can lead to a deterioration of glycaemic control. The DUAL II trial compared the efficacy and safety of insulin degludec/liraglutide fixed-ratio combination (IDegLira) versus insulin degludec (degludec), (starting doses 16 U, max doses 50 U), both plus metformin (met), in subjects with poor

glycaemic control previously treated with met ± SU/glinides and basal insulin (20–40 U). This sub-group analysis compared clinical findings in subjects discontinuing SU (pre-trial SU users) to those not taking SU pre-trial (non-SU users).

Materials and methods: Change from baseline in HbA_{1c}, fasting plasma glucose (FPG) and body weight, and end of trial (EOT) insulin dose after 26 weeks of treatment were analysed with an analysis of covariance (ANCOVA) model with region, pre-trial use of SU at screening, randomised treatment and interaction between pre-trial use of SU and randomised treatment as fixed factors, and baseline value as covariate (and baseline HbA_{1c} for insulin dose). Treatment-emergent confirmed hypoglycaemia was analysed using a negative binomial regression model with a log link and the logarithm of the time period in which a hypoglycaemic episode is considered treatment emergent as offset and the same fixed effects as the ANCOVA model. Missing data were imputed using last observation carried forward.

Results: IDegLira resulted in greater reductions in HbA_{1c}, FPG and body weight from baseline and lower rates of hypoglycaemia (Table) compared with degludec in both pre-trial SU users and non-SU users. Minor differences were seen in EOT insulin doses. Treatment effect was consistent between the two groups, with no statistically significant interaction between randomised treatment and SU use for all endpoints. As insulin dose was reduced at randomisation from a mean of 27–32 U to 16 U and pre-trial SU stopped, a non-clinically relevant increase in mean self-measured fasting plasma glucose (SMPG) was seen in weeks 0–3 in both arms in the pre-trial SU users. This had returned to baseline by week 4, with a general decrease continuing until the EOT. Mean SMPG decreased from week 0 until EOT with IDegLira in the non-SU users group.

Conclusion: In subjects who reduced their insulin dose and discontinued SU at IDegLira initiation, no clinically relevant deterioration in glycaemic control was seen. For all endpoints analysed, regardless of SU use pre-trial, IDegLira showed better results in all metabolic parameters versus degludec (both with a max dose of 50 U). The clinical findings were consistent between pre-trial SU users and non-SU users.

Efficacy in DUAL II by pre-trial SU use category

	Pre-trial SU users			Non-SU users			Test for treatment by subgroup interaction, p-value
	IDegLira* (N=99)	Degludec* (N=99)	ETD/ERR [95% CI]	IDegLira* (N=100)	Degludec* (N=100)	ETD/ERR [95% CI]	
Baseline HbA_{1c}, %	8.7 (0.7)	8.9 (0.7)	–	8.8 (0.7)	8.8 (0.8)	–	–
Δ HbA_{1c}, %	-1.7 (1.2)	-0.6 (1.1)	ETD: -1.13 [-1.41; -0.84]	-2.1 (1.0)	-1.2 (1.2)	ETD: -0.94 [-1.23; -0.66]	0.3828
Δ FPG, mmol/L	-3.3 (3.2)	-2.4 (3.3)	ETD: -0.94 [-1.59; -0.30]	-3.7 (2.7)	-2.8 (3.3)	ETD: -0.52 [-1.17; 0.13]	0.3618
Baseline weight, kg	96.5 (22.8)	93.9 (20.2)	–	94.3 (15.4)	93.2 (19.8)	–	–
Δ Body weight, kg	-3.1 (3.6)	-0.3 (2.6)	ETD: -2.77 [-3.75; -1.79]	-2.3 (3.8)	0.3 (4.0)	ETD: -2.41 [-3.39; -1.43]	0.6137
Daily insulin dose at screening, U	27.5 (7.0)	26.8 (7.1)	–	30.6 (8.1)	31.5 (7.5)	–	–
EOT daily insulin dose*, U	43.9 (10.2)	44.9 (9.3)	ETD: -0.80 [-3.39; 1.79]	45.8 (8.5)	44.9 (9.7)	ETD: 1.09 [-1.52; 3.69]	0.3132
Hypo events/PYE	1.7	3.0	ERR: 0.56 [0.26; 1.22]	1.4	2.3	ERR: 0.88 [0.41; 1.92]	0.4221

Data are mean (SD) unless otherwise stated. *Maximum 50 U IDegLira and Degludec; CI, confidence interval; EOT, end of trial; ETD, estimated treatment difference; ERR, estimated rate ratio; FPG, fasting plasma glucose; IDegLira, insulin degludec/liraglutide combination; Degludec, insulin degludec; hypo, unable to self-treat and/or blood glucose <3.1 mmol/L; non-SU users, subjects not taking SU pre-trial; pre-trial SU users, subjects discontinuing SU; PYE, patient-year of exposure; U, units.

Clinical Trial Registration Number: NCT01392573
Supported by: Novo Nordisk A/S
Disclosure: **A. Janez:** Honorarium; AstraZeneca, Boehringer Ingelheim, Eli Lilly, MSD, Sanofi, Novo Nordisk.

842

Simplification of complex insulin regimens with preserving good glycaemic control in type 2 diabetes

Z. Taybani[1], B. Botyik[1], M. Katkó[2];
[1]1. Department of Endocrinology, Békés County Central Hospital, Dr. Rethy Pal Member Hospital, Bekescsaba, [2]Division of Endocrinology, University of Debrecen, Faculty of Internal Medicine, Debrecen, Hungary.

Background and aims: Type 2 diabetic patients presenting with severe hyperglycemia are often put on multiple daily insulin injections (MDI). If glucose toxicity resolves, the regimen may potentially be simplified, but there are no specific guidelines regarding this and a lot of patients are left on MDI. We aimed to examine prospectively the safety and efficacy of switching from MDI to once daily IDegLira, a fixed-ratio combination of insulin degludec and liraglutide, in relatively well controlled (HbA1c <7.5%) subjects with type 2 diabetes using low total daily insulin dose (TDD).

Materials and methods: 48 adults with type 2 diabetes (mean ± SD: age 65 ± 8.6 years, HbA1c 6.48 ± 0.65%, BMI 32.28 ± 6.77 kg/m^2, body weight 90.25 ± 18.83 kg, TDD 40.9 ± 11.1 units, duration of diabetes 12.3 ± 8.1 years) treated with MDI ± metformin were enrolled in our study. Previous insulins were stopped and once daily IDegLira was started. IDegLira was titrated every 3 days with 2 dose steps (each dose steps contains 1 unit of insulin degludec and 0.036 mg of liraglutide) by the patients to achieve a self-measured pre-breakfast plasma glucose contrentration of <6 mmol/l.

Results: After 95.8 days of average follow-up HbA1c, body weight and BMI decreased significantly. Mean HbA1c changed by −0.25% to 6.23 ± 0.61% ($p < 0.001$), body weight changed by −2.63 kg to 87.62 ± 17.78 kg ($p < 0.001$), and BMI changed to 31.34 ± 6.38 kg/m^2 ($p < 0.001$). At the end of the follow-up mean dose of IDegLira was 20.1 dose steps. IDegLira±metformin combination therapy was safe and generally well tolerated. During the month before baseline visit 26 patients (54%), while during the follow-up only 5 (10.4%) patients had at least one documented (self-measured plasma glucose <3.9 mmol/L) or symptomatic hypoglycemia.

Conclusion: In everyday clinical practice switching from low dose MDI to IDegLira in patients with well-controlled type 2 diabetes is safe, may induce weight loss and result in similar or better glycemic control. Simplifying complex treatment regimens may improve adherence and quality of life.

Disclosure: **Z. Taybani:** None.

PS 072 Clinical and patient reported outcomes

843

User- and health care provider-reported outcomes for a wearable bolus insulin delivery patch

B.L. Levy[1], R.M. Bergenstal[2], D.M. Dreon[1], V. Zraick[1], V.R. Aroda[3], T.S. Bailey[4], R.L. Brazg[5], J.P. Frias[6], D.C. Klonoff[7], D.F. Kruger[8], S. Ramtoola[9], J. Rosenstock[10], P. Serusclat[11], R.S. Weinstock[12], M. Peyrot[13];
[1]Calibra Medical, Wayne, USA, [2]International Diabetes Center, Park Nicollet, Minneapolis, USA, [3]Medstar Health Research Institute, Hyattsville, USA, [4]AMCR Institute Inc., Escondido, USA, [5]Rainier Clinical Research Center, Renton, USA, [6]National Research Institute, Los Angeles, USA, [7]Diabetes Research Institute, San Mateo, USA, [8]Henry Ford Health System, Detroit, MI, USA, [9]East Lancashire Hospitals NHS Trust, Blackburn, UK, [10]Dallas Diabetes Research Center at Medical City, Dallas, USA, [11]Groupe Hospitalier Mutualiste Les Portes du Sud, Vénissieux, France, [12]SUNY Upstate Medical University, Syracuse, USA, [13]Loyola University Maryland, Baltimore, USA.

Background and aims: This multi-center randomized, controlled trial compared efficacy, safety, and User- /Health Care Provider-reported outcomes for adults with type 2 diabetes (HbA_{1c}: 7.5–11% [58–97 mmol/mol]) on basal insulin initiating mealtime insulin (aspart) with a wearable bolus insulin delivery patch (Patch, $n = 139$) vs an insulin pen (Pen, $n = 139$). Patch was applied at least every 3 days and delivered subcutaneous bolus insulin in 2-U increments per manual click.
Materials and methods: Study duration was 48 weeks with cross-over at Week 44; 88% of Patch Users and 86% of Pen Users completed Week 24 assessments; 77% and 76%, respectively, completed Week 48 assessments. Treating Health Care Providers ($n = 89$) rated their experience with Patch at Week 24.
Results: Change in HbA_{1c} from baseline to Week 24 (primary endpoint) was significant ($p < 0.0001$) in both groups (least squares mean change ± SEM: Patch, $-1.7 \pm 0.1\%$ [-19 ± 1.0 mmol/mol] vs Pen, $-1.6 \pm 0.1\%$ [-17 ± 1.0 mmol/mol]). Change in User satisfaction (measured by the Insulin Delivery System Rating Questionnaire) at Week 24 favored Patch over Pen for all measures; comparisons for convenience and overall satisfaction were significant ($p < 0.01$). Change in User quality of life (measured by the Diabetes-Specific Quality of Life Survey) at Week 24 favored Patch over Pen for 6 of 7 measures; comparisons for daily functions and diet restrictions were significant ($p < 0.05$). Comparisons of User experience ratings at Week 24 favored Patch over Pen for all 11 items; 7 of those showed a significant difference (Table). A User preference survey (Week 48) indicated a significant preference for Patch over Pen in those who used Patch for 44 weeks and those who crossed over to Patch for 4 weeks ($p < 0.0001$); 69% in Patch group wanted to switch from Pen to Patch or had no preference (10%). Health Care Provider questionnaire ratings in favor of Patch at Week 24 ranged from 67% to 85% ($p < 0.0001$). Additionally, 74% rated training for Patch use as "easy" and 89% reported that it took ≤30 minutes; 91% of Health Care Providers preferred Patch over Pen for initiating mealtime insulin ($p < 0.0001$).
Conclusion: The Patch is a viable alternative to Pen for mealtime insulin; both Users and Health Care Providers preferred patch to pen.

User experience survey, week 24 % favorable	Patch (*n*=123) % (95% CI)	Pen (*n*=109) % (95% CI)	Odds Ratio (95% CI)	*p* value (patch vs pen)
Dosed without attracting attention	93.5 (89.8, 97.2)	68.8 (61.5, 76.1)	6.5 (2.9, 14.8)	*p*<0.0001
Taking meal time insulin was painless	90.2 (85.8, 94.6)	70.4 (63.1, 77.6)	3.9 (1.9, 8.0)	*p*=0.0002
Could do things on the spur of the moment	87.0 (82.0, 92.0)	66.7 (59.2, 74.1)	3.3 (1.7, 6.5)	*p*=0.0003
Always had meal time insulin with me	91.2 (86.8, 95.5)	72.5 (65.3, 79.8)	3.9 (1.8, 8.5)	*p*=0.0006
Felt comfortable using it socially	89.3 (84.7, 93.9)	71.0 (63.8, 78.2)	3.4 (1.7, 7.0)	*p*=0.0007
Taking mealtime insulin was easy	92.7 (88.8, 96.5)	78.0 (71.5, 84.5)	3.6 (1.6, 8.1)	*p*=0.002
Recommend for meal time insulin	91.0 (86.7, 95.2)	78.9 (72.5, 85.3)	2.7 (1.2, 5.8)	*p*=0.01
Better relationship with my HCP	74.6 (68.1, 81.1)	66.1 (58.6, 73.5)	1.5 (0.9, 2.7)	*p*=0.16
Confident that dosed correctly	91.9 (87.8, 95.9)	89.9 (85.2, 94.7)	1.3 (0.5, 3.1)	*p*=0.60
Engaged with managing my diabetes	90.1 (85.6, 94.6)	88.9 (83.9, 93.9)	1.1 (0.5, 2.6)	*p*=0.77
Made improvements to my diabetes	87.0 (82.0, 92.0)	86.2 (80.8, 91.7)	1.1 (0.5, 2.3)	*p*=0.87

Clinical Trial Registration Number: NCT02542631
Supported by: The study was funded by Calibra Medical
Disclosure: **B.L. Levy:** Employment/Consultancy; Full-time employee of Calibra Medical.

844

Fast improvement of glycaemic control during transition of young adults with type 1 diabetes

A. Maurizi, S. Pieralice, R. Del Toro, D. Tuccinardi, A. Lauria Pantano, C. Guglielmi, E. Maddaloni, E. Fioriti, S. Manfrini, P. Pozzilli;
University Campus Bio-Medico, Rome, Italy.

Background and aims: Transition from paediatric to adult diabetes clinics for type 1 diabetes (T1D) patients represents a critical phase often characterized by worsening effects on diabetes outcomes (attendance visit, metabolic control and diabetes related complications). Aim of this study was to evaluate metabolic control of 122 T1D patients [82/40 M/F, mean (SD), age 25.1 ± 5.7 years, disease duration 17.2 ± 8.1 years, HbA1c $7.9\% \pm 1.4$] at time of transition from paediatric clinics to our adult diabetes center.
Materials and methods: At baseline, 102 patients were on multiple daily insulin injections (MDI) and 20 subjects on continuous subcutaneous insulin infusion (CSII). The transition process was performed in a specific "transition clinic" according to the protocol of the Consensus Statement of the American Academy of Paediatrics, American Diabetes Association, Academy of Family Physicians and the American College of Physicians.
Results: Results after 3 and 6 months follow-up showed a significant improvement in metabolic control with reduction of HbA1c in the whole population [Δ HbA1c 0–3 months: −0.3% ($p < 0.05$), Δ HbA1c 0–6 months: −0.5% ($p < 0.02$)] as well as in the different age groups [15–20, 21–30 ($p < 0.05$, $p < 0.001$, respectively)]. At baseline females showed a worst metabolic control compared to male patients (HbA1c: $8.3\% \pm 1.5$ vs. $7.6\% \pm 1.1$, $p = 0.005$) and this result was confirmed at the end of the study period (HbA1c: $7.9\% \pm 1.0$ vs. $7.1\% \pm 0.8$, $p < 0.001$). After 6 months since transition 27% of patients were on CSII compared to 16% of patients in the pre-transfer period, however improvement of metabolic control was independent of CSII use.
Conclusion: In conclusion, our data showed that transition from paediatric to an adult diabetes center promotes a very fast reduction of HbA1c

detectable after 3 months of follow-up only. This unexpected improvement is likely due not only to increased use of technology but to an appropriate clinical setting for emerging adults as recommended by some scientific societies.
Disclosure: **A. Maurizi:** None.

845

Impairment of cognitive function in newly diagnosed type 2 diabetes, but not in type 1 diabetes

T. van Gemert[1,2], W. Woelwer[3], K.S. Weber[1,4], A. Hoyer[4,5], K. Strassburger[4,5], N.T. Bohnau[1,4], M.A. Brueggen[1,4], K. Ovelgoenne[1,4], E.-M. Goessmann[1,4], V. Burkart[1,4], J. Szendroedi[1,2], M. Roden[1,2], K. Muessig[1,2], GDS Group;
[1]Institute for Clinical Diabetology, German Diabetes Center at Heinrich Heine University, Leibniz Institute for Diabetes Research, Duesseldorf, [2]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Duesseldorf, [3]Department of Psychiatry and Psychotherapy, Medical Faculty, Heinrich Heine University, Duesseldorf, [4]German Center for Diabetes Research (DZD), München-Neuherberg, [5]Institute for Biometrics and Epidemiology, German Diabetes Center at Heinrich Heine University, Leibniz Institute for Diabetes Research, Duesseldorf, Germany.

Background and aims: Diabetes associates with higher risk of cognitive decline and dementia. We hypothesized that cognitive function is already impaired during the early course of diabetes.
Materials and methods: A cross-sectional analysis within the German Diabetes Study included patients within the first year after diagnosis of type 1 (n [%male] = 82[57], age 35 ± 10 years, body mass index (BMI) 25.3 ± 3.8 kg/m²) or type 2 diabetes (n = 119[64], 52 ± 9 years, 32.0 ± 5.7 kg/m²), metabolically healthy persons (n = 42[83], 49 ± 12 years, 29.0 ± 6.1 kg/m²) as well as individuals five years after study inclusion with type 1 (n = 45[60], 41 ± 13 years, 25.1 ± 3.0 kg/m²) or type 2 diabetes (n = 65[63], 59 ± 10 years, 32.1 ± 5.5 kg/m²). They all underwent comprehensive metabolic phenotyping and testing of different domains of cognitive function. Cognition test outcomes were compared between groups using linear regression models with age, sex and crystallized intelligence as independent variables. Furthermore, within the groups, associations between cognitive function and age, sex, BMI, insulin sensitivity, high-sensitivity C-reactive protein, hemoglobin A1c (HbA1c) and crystallized intelligence were analysed using linear regression.
Results: In participants with newly diagnosed diabetes, verbal memory was poorer in patients with type 2 diabetes (ß[mean difference] = −0.49, p = 0.03), but not in patients with type 1 diabetes (β = −0.37, p = 0.16) when compared to healthy persons. Patients with type 2 diabetes examined at five years after diagnosis, also showed lower verbal memory than patients with type 1 diabetes (β = −0.81, p = 0.01). In addition to crystallized intelligence, a higher BMI among individuals with newly diagnosed type 2 and male sex among individuals with newly diagnosed type 1 diabetes were associated with impaired verbal memory (all p < 0.05).
Conclusion: Verbal memory is already impaired within the first year after diagnosis of type 2 diabetes, and associates with higher body mass.
Clinical Trial Registration Number: NCT01055093
Supported by: BMBF, DZD
Disclosure: **T. van Gemert:** None.

846

Individuals with stable glucose levels show improved longitudinal glycaemic outcomes: a worldwide observational study

R.A. Ajjan[1], S.R. Jangam[2], Y. Xu[2], G. Hayter[2], T. Dunn[2];
[1]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK, [2]Abbott Diabetes Care, Alameda, USA.

Background and aims: Previous analyses of real-world data have shown that testing using flash glucose monitoring (FreeStyle Libre™ system) is associated with improved longitudinal glycemic outcomes amongst individuals at high risk of hyperglycemia or hypoglycemia. Our aim was to understand the relationship between glucose variability (GV) and long-term hypoglycemia using a longitudinal, observational real world study.
Materials and methods: De-identified glucose results were collected from individuals using the Freestyle Libre system and data from 6802 individuals were analyzed. We investigated the effects of testing patterns on GV and analyzed the relationship between hyperglycemia, hypoglycemia and GV in a longitudinal, observational study design. Individuals were divided into groups of high and low GV using coefficient of variation over a period of 6 months (total of 12 sensors). Based on recent consensus, a coefficient of variation of 36% was used as the threshold. Hyperglycemia (time above 240 mg/dL) and hypoglycemia (time at or below 54 mg/dL) were then analyzed during the 6 months of the study. Similar analysis was repeated after including only individuals at high risk of hypoglycemia.
Results: It was observed that increased testing with the system was associated with lower glucose variability. Coefficient of variation decreased from 41.6% to 35.3% (p < 0.001) from the lowest to highest testing frequency groups (4 and 35 scans/day, respectively). It was observed that overall, individuals with low GV spent 81% less time in hypoglycemia across 6 months compared to individuals with high GV (9.37 ± 0.65 and 48.11 ± 1.28 min/day, respectively; p < 0.001). The effect of GV on time spent in hyperglycaemia was also significant, with 38% reduction (1.36 h/day) comparing individuals having low and high GV (2.23 ± 0.09 and 3.59 ± 0.14 h/day, respectively; p < 0.001). Further analysis of individuals at high risk of hypoglycemia showed that those with low GV had, over the study period, more reduction of time spent in hypoglycemia (63%; 69 ± 71 min/day to 26 ± 41 min/day, p < 0.001) compared to those with high GV (23%; 91 ± 65 min/day to 70 ± 72 min/day, p < 0.001).
Conclusion: Increased testing is associated with decreased GV in the real world. High GV shows a strong relationship with hypoglycaemia and hyperglycaemia in a large real world sample population. Low GV is associated with more effective reduction in time spent in hypoglycemia in higher risk individuals.

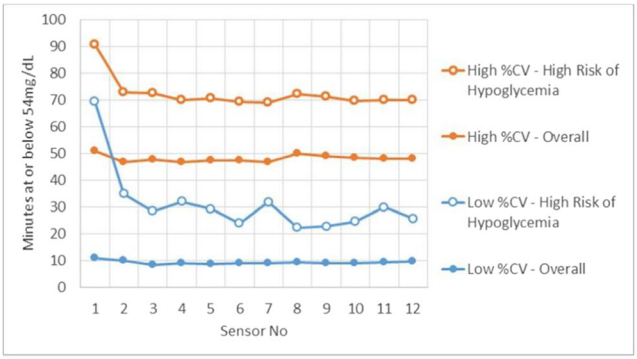


Figure 1. Time spent at or below 54mg/dL over 6 months. The plots show the differences in hypoglycemia outcomes over the long-term in individuals who have high and low variability.

Supported by: All work was funded by Abbott Diabetes Care.
Disclosure: **R.A. Ajjan:** None.

847

Upper extremity impairments in type 1 diabetes is strongly related to low health related quality of life

K. Gutefeldt[1], C.A. Hedman[1], I.S.M. Thyberg[2], M. Bachrach-Lindström[3], H.J. Arnqvist[4], A. Spångeus[1];

[1]Department of Endocrinology, Department of Medical and Health Sciences, Linköping, [2]Department of Rheumatology, Department of Clinical and Experimental Medicine, Linköping, [3]Division of Nursing Sciences, Department of Medical and Health Sciences, Linköping, [4]Department of Endocrinology, Department of Clinical and Experimental Medicine, Linköping, Sweden.

Background and aims: Upper extremity disability is much more common in patients with diabetes compared to non-diabetic subjects. The aim was to compare health related quality of life (HRQL) in type 1 diabetes (T1DM) with non-diabetic controls and to explore HRQL to presence of upper extremity impairments in T1DM patients.
Materials and methods: In a cross-sectional, population based study patients with T1DM, onset before 35 years of age, duration ≥20 years, <67 years old and matched controls were invited to participate. The participants fulfilled a postal questionnaire including HRQL by the Short Form 36 (SF-36) and study specific questions regarding upper extremity impairments affecting shoulder, hands and fingers. Results are presented as mean ± SD.
Results: A total of 773 patients, age 50 ± 10 years, diabetes duration 35 ± 10 years and 708 controls, age 54 ± 9 years were recruited. Patients reported significantly lower HRQL compared to controls, and the difference was most evident in the subscales general health 59 ± 26 vs 74 ± 22, vitality 55 ± 26 vs 67 ± 24 and bodily pain 64 ± 27 vs 74 ± 25, p value <0.001. Women reported lower HRQL than men in both groups. Patients experiencing shoulder pain and stiffness scored lower in all 8 subscales of SF-36 in comparison with shoulder asymptomatic patients. This was most evident for role physical 55 ± 42 vs 82 ± 31, bodily pain 46 ± 22 vs 75 ± 24, general health 47 ± 24 vs 67 ± 23 and vitality 43 ± 25 vs 63 ± 24, p value <0.001.
Conclusion: T1DM was associated with a lower HRQL compared to non-diabetic controls. Furthermore, in T1DM presence of upper extremity impairments was strongly related to low HRQL. Recognition of upper extremity impairments and their relation to low HRQL are clinically important and early preventive strategies as well as therapeutic and rehabilitative interventions are needed.
Supported by: FORSS, Forsknings och stiftelseförvaltningen, Region Östergötland
Disclosure: **K. Gutefeldt:** None.

848

Quality of life in diabetes: influence of glycaemic control and other associated psychological variables

M. Carreira[1], M.S. Ruiz de Adana[2,3], M. Domínguez-López[2,3], M.T. Anarte[1];
[1]Department of Personality, Assessment and Psychological Treatment. Instituto de Investigación Biomédica de Málaga (IBIMA), University of Málaga, Malaga, [2]Clinical Management Unit of Endocrinology and Nutrition, Instituto de Investigación Biomédica de Málaga (IBIMA), Regional University Hospital of Málaga, University of Málaga, Malaga, [3]CIBERDEM, Málaga, Spain.

Background and aims: Diabetes has been associated with a poorer quality of life than the general population. In this case, the aim is to evaluate the quality of life in a population with diabetes and its relationship with biomedical and psychological variables depending on the type of diabetes (type 1 and type 2).
Materials and methods: The sample of this study consists of 259 people with type 1 diabetes, 116 people with type 2 diabetes and 182 people from the general population. The biomedical variables were collected in medical consultation (revision). Additionally, a psychological assessment of the following variables was carried out with the indicated instruments: quality of life, evaluated through the Health Questionnaire (SF-12) and the Diabetes Quality of Life (DQOL); depression (Structured Clinical Interview for DSM-IV Axis I Disorders: SCID-1); distress related to diabetes (Diabetic Distress Scale: DDS) and fear of hypoglycemia (Fear of Hypoglycemia Questionnaire: FH-15). To analyze the differences between groups was used the Student's t. Analyzes were performed with the SPSS 15.0 program.
Results: In this study, people with diabetes had worse scores in the Physical SF-12 (PCS) with respect to the general population (p = 0.001). No differences were found in the Mental Scale (MCS) of the SF-12. In addition, people with type 1 diabetes had worse quality of life than people with type 2 diabetes in PCS (p = 0.002) and MCS (p = 0.047) scales. There are also worse results in people with type 1 diabetes on the DQOL scale (p < 0.001). People with diabetes and poor glycemic control (HbA1c >7%) obtained worse scores in the DQOL (type 1: p = 0.026; type 2: p = 0.015). It is observed that the quality of life of people who present complications is worse than in people without complications in both types of diabetes (type 1: p = 0.006, type 2: p = 0.023). Patients with depression and diabetes had worse quality of life (DQOL) than patients without depression and diabetes (type 1: p < 0.001, type 2: p < 0.001). Regarding the fear of hypoglycemia, worse scores were obtained in those people with fear of hypoglycemia (type 1: p < 0.001, type 2: p = 0.007). With respect to distress related to diabetes, people with distress presented worse quality of life (type 1: p < 0.001, type 2: p < 0.001) in both types of diabetes.
Conclusion: In this study, people with diabetes have a poorer quality of life than the general population, with people with type 1 diabetes getting the worst results. On the other hand, it has been found that poor glycemic control and the presence of diabetes complications worsen the quality of life of the person with diabetes in both types, as well as having depression, fear of hypoglycemia or distress related with diabetes. These results highlight the importance of these variables in obtaining an adequate quality of life of the subject with diabetes.
Disclosure: **M. Carreira:** None.

849

No deterioration in quality of life, treatment satisfaction and wellbeing over 6 years of follow up in people with recently diagnosed type 2 diabetes

H.S. Oldershaw[1], R.A. Oram[1,2], J. Dennis[1], R.C. Andrews[1];
[1]University of Exeter Medical School, Exeter, [2]NIHR Exeter Clinical Research Facility, Exeter, UK.

Background and aims: People living with Type 2 Diabetes (T2DM) report poorer quality of life than those without the disease. It is not clear whether there is a fall in quality of life due to being diagnosed with T2DM or whether this falls over time due to living with the disease. Some studies have shown quality of life declines initially at diagnosis but then returns to its previous level. The American Diabetes Prevention Program (DPP) found quality of life declined over 6 years of follow up. In this study we aimed to assess quality of life, treatment satisfaction and wellbeing in a cohort with recently diagnosed T2DM in the United Kingdom over 6 years.
Materials and methods: The cohort we used were participants enrolled in the Early ACtivity In Diabetes Trial. It was a randomised controlled trial in southwest England in adults aged 30–80 years in whom T2DM had been diagnosed 5–8 months previously. Participants were randomised to a 1 year diet intervention, a 1 year diet and activity intervention, or usual care in a 5:5:2 ratio. At the end of the year participants' care was returned to their general practitioner and they were seen annually for a further 5 years. Patient-reported measures (PRMs) were recorded at 0, 6, 12, 24, 36, 48, 60, 72 months. Health Status was assessed using the EuroQol - 5 Dimensions (EQ-5D) and Brief Illness Perception Questionnaire (BIPQ). Treatment Satisfaction was assessed using the Diabetes Treatment Satisfaction Questionnaire (DTSQ). Self-esteem was assessed using Rosenberg's Self-Esteem Scale (RSES). Life satisfaction was assessed using Diener's Satisfaction with Life Scale (SWLS). We compared scores at baseline and end of follow up and additionally

assessed change in score with a linear mixed effects model with time in years and baseline score as fixed effects and participants as random effects to allow for clustering of results within individuals.

Results: Baseline cohort characteristics were: 64% male, median age 61 years, median time since diagnosis 189 days, mean Hba1c 6.7%, mean BMI 31.7. At baseline 582 of 596 (98%) participants completed all the questionnaires. This fell across the study with 517 (87%) retained at year 1 and 276 (46%) at year 6. Assessing mean (sd) change from baseline score, treatment satisfaction (max score 36) was modestly increased by 0.4 (5.9). Self-esteem (max score 30) and life satisfaction (max score 35) also increased slightly by 0.3 (2.5) and 0.2 (5.5) respectively. Health status declined by −0.05 (0.2) for EQ-5D Index (max score 10) and −1.4 (17.0) for EQ-5D Visual Analogue Scale (VAS) (max score 100). Illness perception (max score 80) was also reduced by −0.4 (7.6). Analysing the change in scores over time there was a very modest decrease in EQ-5D Index (−0.008, $p < 0.001$), EQ-5D VAS (−0.66, $p < 0.001$), BIPQ (−0.23, $p = 0.001$), DTSQ (−0.43, $p < 0.001$), and RSES (0.05, $p = 0.009$). SWLS did not decrease over the study period (−0.03, $p = 0.5$). The trial arm participants were assigned to during the first year did not have an effect on any PRMs.

Conclusion: Quality of life, treatment satisfaction and wellbeing are stable in patients recently diagnosed with T2DM over a 6 year period. Whether people received a diet or diet and activity intervention or usual care made no difference to these measures over the 6 years. Further research is needed to clarify what happens to these measures in the first months after diagnosis.

Clinical Trial Registration Number: ISRCTN92162869
Supported by: Diabetes UK & UK Dep. of Health
Disclosure: **H.S. Oldershaw:** None.

850

Patients' and clinicians' preferences on outcomes and medication attributes for type 2 diabetes: a mixed methods study

T. Karagiannis[1], I. Avgerinos[1], M. Toumpalidou[1], G. Dimitriadis[2], A. Bargiota[3], N. Papanas[4], I. Avramidis[1], A. Tentolouris[2], K. Kitsios[1], S. Giannakopoulos[1], S. Alexiadis[1], T. Chatziadamidou[1], A. Liakos[1], K. Malandris[1], A. Tsapas[1,5];
[1]Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]National and Kapodistrian University of Athens, Athens, Greece, [3]University of Thessaly, Larissa, Greece, [4]Democritus University of Thrace, Alexandroupolis, Greece, [5]Harris Manchester College, Oxford, UK.

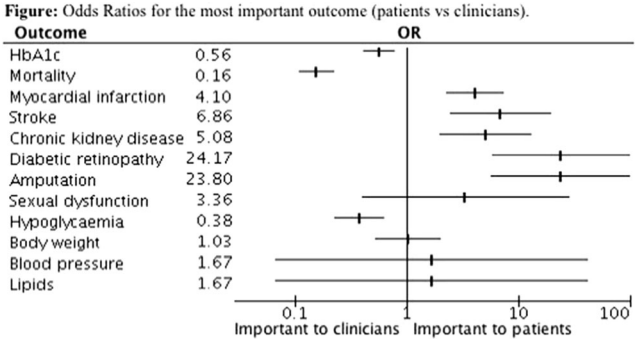
Background and aims: It is unclear whether clinicians' perceptions are in agreement with patients' preferences regarding outcomes for type 2 diabetes. We conducted a 2-phase mixed methods study to explore both patients' and clinicians' preferences for outcomes and medication attributes for type 2 diabetes.

Materials and methods: We conducted a qualitative study using 6 focus groups (33 patients) and used thematic analysis to identify patient important outcomes. These findings were then used to design a survey, which was administered to 656 patients with type 2 diabetes and 363 clinicians (215 diabetologists, 118 general practitioners and 30 endocrinologists). It included three questions aiming at eliciting preferences about which diabetes-related outcomes and drug attributes are considered most important when choosing among antidiabetic medications. We used descriptive statistics and calculated odds ratios (ORs) to compare patients' preferences with those of clinicians.

Results: When asked to choose 5 among 12 outcomes, the 2 most frequently endorsed outcomes were prevention of diabetic retinopathy (68.6%) and myocardial infarction (66.5%) for patients ($n = 656$), and low incidence of hypoglycaemia (79.9%) and HbA_{1c} reduction (78.5%) for clinicians ($n = 363$). 576 patients and 320 clinicians answered a question about their top choice among the 5 selected outcomes. Patients were significantly more likely than clinicians to rate prevention of diabetic retinopathy (OR 24.17; 95% CI 5.89 to 99.09), amputation (OR 23.8; 95% CI 5.8 to 97.62), stroke, chronic kidney disease and myocardial infarction as the most important outcome, while clinicians were more concerned about prevention of all-cause mortality (OR 0.16; 95% CI 0.11 to 0.22), hypoglycaemic risk (OR 0.38; 95% CI 0.23 to 0.63) and effect on HbA_{1c} (Figure). Regarding specific drug attributes, patients were less concerned than clinicians about drug cost (OR 0.16; 95% CI 0.11 to 0.23) and considered oral (as opposed to injectable) mode of administration (OR 2.56; 95% CI 1.85 to 3.54) and need for less frequent glucose self-monitoring (OR 1.64; 95% CI 1.14 to 2.37) more important.

Conclusion: Patients and clinicians differ in their perceptions of the relative importance of various diabetes-related outcomes, with patients being more concerned on incidence of macrovascular and microvascular endpoints that can directly affect their quality of life. These concerns and preferences should be discussed during the clinical encounter when deciding on an optimal treatment plan for type 2 diabetes.

Figure: Odds Ratios for the most important outcome (patients vs clinicians).



Supported by: EFSD award supported by AstraZeneca
Disclosure: **T. Karagiannis:** None.

851

Glycaemia as a risk factor for falls in the hospital population

C. Berra[1], E. Azzolini[1], F. De Fazio[1], M. Mirani[1], G. Favacchio[1], M. Albini[1], F. Folli[2];
[1]Humanitas Research Hospital, Rozzano, [2]Università di Milano, Milano, Italy.

Background and aims: Patient's falls are adverse events in the hospital population, with consequences on worsen prognosis, increased in-patient time and economic costs. Hyperglycemia has been associated with increased morbidity and mortality in hospitalized patients, increased glycemic variability during hospitalization was found to be associated with increased mortality on long-term follow up in non-critically ill patients. Hypoglycemia is associated with increased risk of falls in diabetic patients in outpatients setting. Our aim was to investigate the role of hypoglycemia (glucose <70 mg/dl) or hyperglycemia (glucose >200 mg/dl), or the combination of both, as independent risk factors for falls in a hospitalized population

Materials and methods: A retrospective analysis of patients admitted in Humanitas Research Hospital from January 2015 to December 2016 was performed. All capillary glucose values in fall population and non-fall population were analyzed. Hypoglycemia was defined as value of capillary blood glucose <70 mg/dl during the hospital stay, while hyperglycemia was defined as value of capillary blood glucose more than 200 mg/dl. Fall was defined as an unexpected and unintentional event in which the person came to rest on the ground, floor or lower level. Events were registered in the central network system of the hospital and was available for analysis (EMRS). The obtained data were subjected to multivariate analysis matched for sex, age, admission to hospital (from emergency care or elective) surgical or medical admission and discharge diagnosis

Results: We analyzed medical records of 57411 patients, 759 had a fall during the hospital stay. mean age was 60.7, 52.7% male, 62.4% had medical while 37.6% had surgical discharge diagnosis. 13065 subjects were admitted from emergency room (23.7%). By employing the Charlson index 11.7% had score = 2 while 6.6% scored 1 and 81.7% scored 0. In the group which did not have any capillary glucose measurements ($N = 43627$) there were 374 falls (0.8%). In the group which had at least one capillary glucose measurement ($N = 13410$) there were 385 falls (2.9%) ($p < 0.001$). The presence of one glycemic value out of the range (>200 mg/dl and <70 mg/dl) had an odd ratio for risk of falls of 1.76 (confidence interval 1.42–2.19; $p < 0.001$). Subjects treated with hypoglycemic agents had an odd ratio of 2.97 (confidence interval 2.54–3.49; $p < 0.001$). Insulin treatment (4682 subjects) was more significantly correlated with the risk of falls (odd ratio 3.03; confidence interval 2.53–3.63; $p < 0.001$). After multivariate analysis for sex, age, admission to hospital (from ER/elective) surgical or medical and discharge diagnosis, the presence of at least one glycemic value out of the range confers an odd-ratio for fall of 1.5 (confidence interval between 1.20 and 1.86; $p < 0.001$)

Conclusion: Our data indicate that subjects undergoing capillary blood glucose monitoring falls more than those in which this control is not necessary, independently from diagnosis of diabetes. A strong correlation between hypoglycemia and hyperglycemia with the risk of falling was found. We also found a significant correlation between the number of value lower than 70 mg/dl or over 200 mg/dl in the same subject with the risk for falling, to suggest that also glucose variability could play an important role

Disclosure: **C. Berra:** None.

PS 073 Diabetes control around the world

852

Poor glycaemic control in people with type 1 and type 2 diabetes: results from the International Diabetes Management Practices Study (IDMPS)

F. Lavalle[1], P. Aschner[2], J.J. Gagliardino[3], H. Ilkova[4], A. Ramachandran[5], G. Kaddaha[6], J.C. Mbanya[7], M. Shestakova[8], J.-M. Chantelot[9], J.C.N. Chan[10];

[1]Facultad de Medicina de la Universidad Autónoma de Nuevo León, Monterrey, Mexico, [2]Javeriana University School of Medicine and San Ignacio University Hospital, Bogotá, Colombia, [3]CENEXA, Center of Experimental and Applied Endocrinology (La Plata National University – La Plata National Scientific and Technical Research Council), La Plata, Argentina, [4]Istanbul University, Cerrahpasa Medical Faculty, Department of Internal Medicine, Division of Endocrinology, Metabolism and Diabetes, Istanbul, Turkey, [5]India Diabetes Research Foundation, Dr. A. Ramachandran's Diabetes Hospitals, Chennai, India, [6]Government of Dubai, Dubai Health Authority, Dubai, United Arab Emirates, [7]Biotechnology Center, Doctoral School of Life Sciences, Health and Environment, and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, [8]Endocrinology Research Center, Moscow, Russian Federation, [9]Sanofi, Paris, France, [10]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, Hong Kong SAR, China.

Background and aims: Patient knowledge and self-management are important factors in attaining glycaemic goals. We collected physician-reported data on the achievement of glycaemic control and the challenges that people with diabetes face in achieving glycaemic targets in the developing world.

Materials and methods: The IDMPS is a global observational survey on the management and patterns of care of people with T1D and T2D. Participants were enrolled between 2016 and 2017 in 24 countries across Africa, the Middle East, South Asia and Eurasia.

Results: In people with T1D ($N = 2000$; mean [SD] age 34.0 [12.3] years; 48.8% male), HbA_{1c} levels targeted by physicians were <7% in 45.3% and 7–7.5% in 44.6% of participants. Overall, glycaemic control was poor, with 28.3% of participants attaining their target HbA_{1c} levels and an HbA_{1c}level of <7% (indicative of good control) achieved by only 21.8%. Fear of hypoglycaemia, lack of insulin titration, and cost were the most common reasons given for not attaining glycaemic goals. In people with T2D ($N = 6283$; 57.2 [11.1] years; 47.8% male), 30.1% achieved HbA_{1c} <7%. In those treated with insulin alone or insulin plus oral glucose-lowering drugs (OGLDs), the most common reasons for not attaining glycaemic goals were lack of insulin titration (40.8% and 38.6%, respectively), lack of experience with insulin dosing (40.1% and 34.5%), and lack of education (both 35.0%). The majority (~80%) of insulin users had a glucometer; however, there was a limited number of daily glucose measurements (median 2). In addition, <50% of people with T2D on insulin self-adjusted their dose.

Conclusion: The proportion of people with HbA_{1c} <7% is low. The lack of self-monitoring of blood glucose and of self-adjustment of insulin calls for improved patient education regarding use of glucometers and titration of insulin.

Table. Glycaemic status, insulin management and physician-reported reasons for non-achievement of glycaemic target of people with T1D and T2D

	T1D N=2000*	T2D, OGLD + insulin N=1936	T2D, insulin alone N=660
HbA_{1c} <7 %, %	21.8	14.2	20.7
Plasma glucose (mean ± SD), mmol/l (mg/dl)			
FPG	8.3 ± 3.7 (150.1 ± 66.2)	8.6 ± 3.3 (154.6 ± 58.6)	8.7 ± 3.7 (157.4 ± 66.9)
PPG†	10.3 ± 4.0 (184.8 ± 72.3)	11.3 ± 4.1 (202.8 ± 73.3)	11.2 ± 4.4 (201.9 ± 80.0)
Glucometer			
Yes, %	89.1	82.5	78.7
Number of tests per day (median), n	2	2	2
SMBG and self-adjustment of insulin (yes), %	72.8	45.0	47.3
Reasons for non-achievement of HbA_{1c} goal targeted by the physician, %			
Lack of titration of insulin	38.9	38.6	40.8
Discontinuation of insulin	11.5	9.8	10.2
Lack of experience in the insulin dosing	29.1	34.5	40.1
Lack of support	19.3	20.6	22.7
Lack of diabetes education	26.4	35.0	35.0
Cost of medicine and strips	30.2	26.2	32.9
Weight gain	12.5	20.7	17.9
Fear of hypoglycaemia	40.8	25.1	26.2
Occurrence of hypoglycaemia	22.1	6.5	13.7

*N=1396 for reasons for non-achievement. †at last measurement. FPG, fasting plasma glucose; OGLD, oral glucose-lowering drug; PPG, postprandial plasma glucose; SMBG, self-monitoring of blood glucose

Supported by: Sanofi
Disclosure: **F. Lavalle:** None.

853

Socioeconomic inequalities in glycaemic control in people with newly diagnosed type 1 and type 2 diabetes

E. Jacobs[1,2], K. Strassburger[1,2], A. Icks[2,3], O. Kuss[1,2], V. Burkhart[2,4], J. Szendrödi[2,4], K. Müssig[2,4], W. Rathmann[1,2], M. Roden[2,4], and the GDS Group;
[1]Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ), Leibniz Centre for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, [2]German Center for Diabetes Research (DZD), München-Neuherberg, [3]Institute for Health Service Research and Health Economics, German Diabetes Center (DDZ), Leibniz Centre for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, [4]Institute for Clinical Diabetology, German Diabetes Center (DDZ), Leibniz Centre for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Background and aims: There is a lack of studies on the association between socioeconomic status and glycemic control in recently diagnosed diabetes and in people with type 1 diabetes. The aim was to investigate whether low socioeconomic status (SES) is associated with increased HbA1c and fasting blood glucose levels in newly diagnosed type 1 and type 2 diabetes.
Materials and methods: In the prospective German Diabetes Study, people with type 1 ($n = 221$, mean age 36.3 ± 10.6 years) and type 2 diabetes ($n = 469$, mean age 53.0 ± 9.9 years) underwent detailed metabolic characterization within the first year after diagnosis. SES was documented using a standardized German questionnaire (Robert Koch Institute). Associations between SES with HbA1c and fasting blood glucose were assessed using multivariable linear regression analyses. Regression models were fitted using the overall metric SES score (3–21 points) and dichotomized SES Score, in which the SES score was divided in three status groups (low SES: 20%, medium SES: 60% and high SES: 20% of the participants). People in the lowest SES group (<10.8 points) were compared to people in higher SES groups (≥10.8 points). Further independent variables included were age, sex, BMI, smoking status, nationality, diabetes duration, marital status, physical activity, pre-existing conditions (heart, lung, liver), high-sensitivity C-reactive protein, C-peptide secretion (IVGTT), glucose-lowering therapy, antihypertensive and lipid-lowering therapy, process of care indicators (diabetes passport, diabetes education courses, type of health insurance), mental health and diabetes-related emotional distress (SF-36, PAID). All analyses were stratified by diabetes type.
Results: Overall, people with newly diagnosed diabetes had good glycemic control (type 1 diabetes: mean HbA1c 6.7 ± 1.2%, type 2 diabetes: 6.4 ± 0.9%). The multivariate analyses revealed that a higher SES score was inversely associated with lower HbA1c (β-coefficient: −0.113; $p = 0.002$) and with lower fasting blood glucose (β-coefficient: −3.727; $p = 0.011$) in recently diagnosed type 1 diabetes. In the analyses using the dichotomized SES, people with type 1 diabetes and low SES compared to medium and high SES had 1% higher HbA1c (β-coefficient: 0.977; $p = 0.0010$) and 36 mg/dl higher fasting blood glucose (β-coefficient: 35.755; $p = 0.0023$). In type 2 diabetes, no associations of SES with glycemic markers were observed.
Conclusion: The results indicate that socioeconomic inequalities in glycemic control already exist during the first year after manifestation of type 1 diabetes, but not in type 2 diabetes. Whether these inequalities persist after several years and are associated with micro- and macrovascular complications require further investigation in the future.
Clinical Trial Registration Number: NCT01055093
Supported by: DZD e.V.
Disclosure: **E. Jacobs:** Grants; German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.).

854

Global patterns of cardiovascular risk factor control in patients with type 2 diabetes: insights from the global DISCOVER study programme

M.B. Gomes[1], K.K. Patel[2], B. Charbonnel[3], H. Chen[4], J. Cid-Ruzafa[5], P. Fenici[6], N. Hammar[7], L. Ji[8], K.F. Kennedy[2], S. Pocok[9], M.V. Shestakova[10], I. Shimomura[11], H. Watada[12];
[1]State University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Saint Luke's Mid America Heart Institute, Kansas City, USA, [3]University of Nantes, Nantes, France, [4]AstraZeneca, Gaithersburg, USA, [5]Evidera, Barcelona, Spain, [6]AstraZeneca, Cambridge, UK, [7]AstraZeneca Gothenburg, Mölndal, Sweden, [8]Peking University People's Hospital, Beijing, China, [9]London School of Hygiene and Tropical Medicine, London, UK, [10]Endocrinology Research Center, Diabetes Institute, Moscow, Russian Federation, [11]Osaka University, Osaka, Japan, [12]Juntendo University, Tokyo, Japan.

Background and aims: Guidelines recommend optimal control of cardiovascular (CV) risk factors such as blood pressure (BP), lipids and smoking in addition to glycaemic control to reduce the risk of vascular complications in patients with type 2 diabetes (T2D). However, the extent of CV risk factor control in patients with T2D globally is not well-known. DISCOVER is a 3-year, observational study programme of patients with T2D initiating a second-line glucose-lowering therapy across 38 countries. Here, we assessed the level of CV risk factor control at baseline in patients from 35 countries.
Materials and methods: Optimal CV risk factor management at baseline was defined as control of the following risk factors: 1) Systolic BP <140 mmHg for all patients; 2) statin prescription in patients over 40 years old, high-intensity statin for those with atherosclerotic CV disease (ASCVD); 3) non-smoking status; 4) treatment with angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEi/ARB) in patients with hypertension (HTN)/albuminuria; and 5) secondary ASCVD prevention with low-dose aspirin (ASA) in patients with ASCVD. Global and country specific rates of individual and combined risk factor control were calculated.
Results: For the 15,636 patients included in the analysis, 54.1% were male, mean age was 57.1 (SD = 12.0) years, mean BMI was 29.0 (SD = 5.9) kg/m^2, median duration of T2D was 4.1 (IQR: 1.9–7.9) years. A total of 1779 (11.7%) patients had ASCVD, 7982 (51.1%) had HTN and 582

(4.2%) had albuminuria. Among patients for whom data were available, BP was controlled in 67.9% (10 157/14 968); statin treatment was prescribed in 41.7% (5994/14 370); 83.6% 13 065/14 636) were not smoking; ACEi/ARB treatment was prescribed in 54.0% (5489/10 172), and ASA for secondary prevention was prescribed in 52.8% (940/1179) of patients with ASCVD. Of 14 553 patients with three or more risk factors, 6007 (41.3%) had optimal control of at least three risk factors with variability across countries and regions (Table).

Conclusion: Comprehensive control of CV risk factors was not achieved in most DISCOVER patients, with wide variability across countries. Better strategies are needed to provide consistent and comprehensive CV risk factor control in patients with T2D to improve long-term outcomes.

Table. Proportions of DISCOVER patients with controlled CV risk factors, overall and by WHO region.

	Total (n = 15 636)	Africa (n = 812)	Americas (n = 2002)	South East Asia (n = 3360)	Europe (n = 3123)	Eastern Med. (n = 2182)	Western Pacific (n = 4157)
SBP < 140 mmHg	67.9%	70.0%	70.3%	75.1%	57.9%	62.9%	71.9%
Statin treatment	41.7%	47.9%	43.9%	48.5%	41.9%	47.9%	35.6%
Non-smoking status	83.6%	90.0%	89.2%	96.6%	79.6%	85.5%	78.7%
ACEi/ARB for HTN/ albuminuria	54.0%	53.6%	60.8%	48.6%	60.7%	56.1%	45.9%
Secondary prevention with ASA for ASCVD	52.8%	76.4%	45.3%	41.3%	51.7%	65.6%	48.2%
Optimal control of at least three CV risk factors[a]	41.3%	45.8%	46.3%	50.3%	39.6%	41.3%	33.2%

[a]Proportions adjusted for age, sex, history of ASCVD and duration of T2D. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ASA, aspirin; ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; CV, cardiovascular; HTN, hypertension; Med., Mediterranean; SBP, systolic blood pressure; T2D, type 2 diabetes; WHO, World Health Organization.

Clinical Trial Registration Number: NCT02322762
Supported by: AstraZeneca
Disclosure: **M.B. Gomes:** Honorarium; Astra, Merck-Serono.

855

Cross-sectional study of annual glycaemic control between 2003 and 2015 in primary care: management of type 2 diabetic patients in the Nordic countries

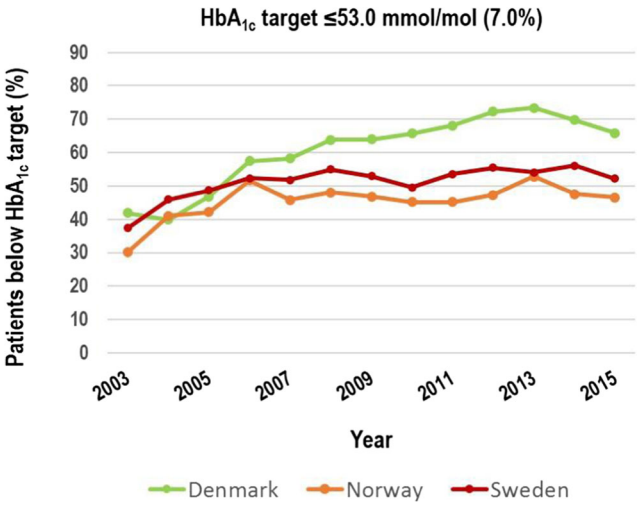
S.T. Knudsen[1], J. Bodegard[2], K.I. Birkeland[3], K. Furuseth[4], M. Thuresson[5], A. Lindh[6], P.M. Nilsson[7], M. Alvarsson[8], M.E. Jørgensen[9], J. Søndergaard[10], F. Persson[9];
[1]MEA, Department of Endocrinology and Diabetes, Aarhus University Hospital, Aarhus, Denmark, [2]AstraZeneca Nordic-Baltic, Oslo, Norway, [3]University of Oslo, Oslo, Norway, [4]Solli Klinikk, Jessheim, Norway, [5]Statisticon AB, Uppsala, Sweden, [6]Åkersberga, Åkersberga, Sweden, [7]Lunds University, Lund, Sweden, [8]Karolinska University Hospital, Stockholm, Sweden, [9]Steno Diabetes Center Copenhagen, Gentofte, Denmark, [10]University of Southern Denmark, Odense, Denmark.

Background and aims: The Nordic countries have nationwide public primary health care systems. Although guidelines argue for treatment intensification at uncontrolled HbA_{1c}, there are country specific differences in T2D treatment guidelines and this might be reflected in differences in glycaemic control. The aim of this study was to describe the annual proportion of patients successfully below HbA_{1c} target levels of 47.5 mmol/mol (DCCT 6.5%), 53 (7.0%) and 58.5 (7.5%) during 12-years using data from Denmark, Norway and Sweden.

Materials and methods: Electronic medical record data were extracted from 60 primary care clinics in Denmark, Norway and Sweden comprising all patients having a diabetes diagnosis and/or prescription of any glucose lowering drug during 2003–2015. Patients with T1D and gestational diabetes were excluded. The study used a cross-sectional method analysing the HbA_{1c} annually from 2003 to 2015.

Results: In 2015, a total of 20,183 T2D patients were identified in Denmark (3909), Norway (3706) and Sweden (12,568). Mean age 66, 65 and 70 years; females 45, 45 and 42%; previous myocardial infarction 8, 8 and 10%; and chronic kidney disease 6, 10 and 7%, respectively. Use of newer GLDs (DPP-4i, GLP-1RA and SGLT-2i) was highest in Norway, followed by Denmark and lastly Sweden. In 2015, the proportion of T2D patients below HbA_{1c} 47.5 mmol/mol was 42%, 29% and 27% in Denmark, Norway and SE respectively (Figure showing for target 53 mmol/mol). Denmark also showed similar beneficial patterns for HbA_{1c} targets below 53.0 and 58.5 mmol/mol compared with the other countries (Figure). Also, the proportion of patients with HbA_{1c} above 70.0 mmol/mol was lower in Denmark, in 2015 9.9% vs 13.4% and 15.0% in Norway and Sweden respectively. From 2003 to 2015, we found that Norway and Sweden had an initial improvement of patients below targets over the first - while remaining unchanged over the next years, whereas Denmark has showed continuous improvement during the whole observation period.

Conclusion: Despite similar demographics and health care systems in three Nordic countries, we have shown marked better glycaemic control in Denmark as compared to Norway and Sweden. This may indicate a more proactive disease management in the general practices included for this observational study, and also point out potential areas of improvement in the management of type 2 diabetes patients particularly in Norway and Sweden.



Supported by: AstraZeneca Nordic-Baltic
Disclosure: **S.T. Knudsen:** None.

856

The influences of ethnicity on the quality of type 2 diabetes care in Norwegian general practice

A.T. Tran[1], B. Gjelsvik[1], T.J. Berg[2], K. Nøkleby[1], J.G. Cooper[3,4], Å. Bakke[3,5], T. Claudi[6], S. Sandberg[4,5], G. Thue[4,5], A.K. Jenum[1];
[1]General Practice Research Unit, Department of General Practice, University of Oslo, Oslo, [2]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, [3]Department of Medicine, Stavanger University Hospital, Stavanger, [4]Norwegian Quality Improvement of Laboratory Examinations, Bergen, [5]Department of Global Public Health and Primary Care, University of Bergen, Bergen, [6]Nordlandssykehuset, Bodø, Norway.

Background and aims: The population in Norway has become multiethnic during the last decades. Ethnic minority groups have a higher prevalence of type 2 diabetes (T2DM), and treatment may be more complicated. We therefore aimed to explore the influence of ethnicity on the quality of type 2 diabetes care in general practice.

Materials and methods: Data on 10 164 patients with T2DM cared for by 282 general practitioners (GP) were extracted from electronic medical records in 2014. Ethnicity was based on country of birth and categorized as follows: Westerners, East Europeans, East Asians, South Asians,

Middle East/North Africa and Horn of Africa. Multilevel regression models with random effects at GP practice level were used to estimate the ethnic differences with Westerners as reference group adjusted for patient factors (age, gender, diabetes duration and education) and GP factors (gender, specialist status, years working as GP in Norway).

Results: Diabetes was diagnosed 6–12 years earlier in patients from ethnic minority groups (Table 1). Only minor differences in processes of care between patient groups were observed. Glucose-lowering agents were prescribed more frequently to all minority groups compared with Westerners (>76% vs. 66%, $p \leq 0.05$). Nevertheless, mean HbA_{1c} was higher in minority groups (East Europeans: 7.60%, South Asians: 7.29%, Middle East/North Africa: 7.21%) than in Westerners (6.98%, $p \leq 0.01$). A significantly lower proportion of East Europeans, South Asians and patients from Middle East/North Africa achieved the treatment target ($HbA_{1c} \leq 7.0\%$) compared with Westerners. Similarly, a larger proportion of these groups had HbA_{1c} >8.5% than Westerners. Anti-hypertensive agents were prescribed less frequently to patients from South Asia, Middle East/North Africa and Horn of Africa compared with Westerners (<51% vs. 71%, $p \leq 0.001$). However, mean blood pressure was lower in these minority groups (South Asians: 131/75 mmHg, Middle East/North Africa: 133/76 mmHg, Horn of Africa: 132/74 mmHg) than in Westerners (136/79 mmHg, $p \leq 0.05$) and a larger proportion of patients achieved the treatment target of ≤135/80 mmHg compared with Westerners (>62% vs. 48%, $p \leq 0.05$).

Conclusion: Age at the time of diagnosis of T2DM was 6–12 years younger in minority groups compared with Westerners. Many minority patients had worse glycaemic control, indicating a need for special attention to compliance, tight follow-up and more shared care between GPs and endocrinologists.

Table 1: Patient characteristics, process of care, medication and achievement of treatment target in different ethnic groups

	Parameter	Westerners n=8497	East Europeans n=184	East Asians n=218	South Asians n=799	MENA n=340	Horn of Africa n=126
Patient characteristics	Age, median (years)	67	60	57	57	53	50
	Age at diagnosis, median (years)	58	52	50	46	45	46
Performed process of care (%)	HbA_{1c}	90	87	89	91	88	84
	Blood pressure	88	85	88	88	86	81
	LDL	68	66	70	72	70	61*
	U-albumin	32	29	40	30	33	35
	Eye examination	63	49	60	50	44	57
	Foot examination	32	21	20	19	19*	23
Medication (%)	Glucose-lowering	66	77*	77*	79**	76*	78*
	Anti-hypertensive	71	60	57	51***	44***	29***
	Lipid-lowering	58	56	50	54	48*	26***
Achievement of treatment target (%)	$HbA_{1c} \leq 7.0\%$	64	46***	58	48***	51**	63
	BP ≤ 130/80 mmHg	48	49	60*	67***	62**	66**
	LDL ≤2.5/1.8 mmol/L	51	46	62*	58	59*	71**
	All three target	13	13	20*	16	17	25**
Poor glycaemic control (%)	$HbA_{1c} > 8.5\%$	8	18***	10	18***	18***	10

*p≤ 0.05, **p≤ 0.01, ***p≤ 0.001. MENA: Middle East/North Africa.

Supported by: ExtraFoundation Health and Rehabilitation

Disclosure: **A.T. Tran:** Grants; ExtraFoundation Health and Rehabilitation and Norwegian Women's Public Health Association.

857

Management and achievement of glycaemic goal in people with diabetes in Africa: results from the International Diabetes Management Practices Study (IDMPS)

J.C. Mbanya[1], P. Aschner[2], J.J. Gagliardino[3], H. Ilkova[4], F. Lavalle[5], A. Ramachandran[6], G. Kaddaha[7], M. Shestakova[8], J.-M. Chantelot[9], J.C.N. Chan[10];

[1]Biotechnology Center, Doctoral School of Life Sciences, Health and Environment, and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, [2]Javeriana University School of Medicine and San Ignacio University Hospital, Bogotá, Colombia, [3]CENEXA, Center of Experimental and Applied Endocrinology (La Plata National University – La Plata National Scientific and Technical Research Council), La Plata, Argentina, [4]Istanbul University, Cerrahpasa Medical Faculty, Department of Internal Medicine, Division of Endocrinology, Metabolism and Diabetes, Istanbul, Turkey, [5]Facultad de Medicina de la Universidad Autónoma de Nuevo León, Monterrey, Mexico, [6]India Diabetes Research Foundation, Dr. A. Ramachandran's Diabetes Hospitals, Chennai, India, [7]Government of Dubai, Dubai Health Authority, Dubai, United Arab Emirates, [8]Endocrinology Research Center, Moscow, Russian Federation, [9]Sanofi, Paris, France, [10]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, Hong Kong SAR, China.

Background and aims: By 2045, Africa is predicted to experience a >150% increase in the proportion of people with diabetes; however, there is a lack of data to support clinical practice and healthcare planning. Here we investigate current management practices in people with diabetes in Africa.

Materials and methods: The IDMPS is an observational survey on the management of people with type 1 (T1D) and type 2 (T2D) diabetes in the developing world. Cross-sectional results were generated using data collected in 2015–2016 from 12 African countries. To identify factors related to glycaemic control, a logistic regression model per region was performed, entering factors significant at the 10% level from the univariate analysis. OR with 95% CI were estimated for each significant predictor. A backward selection procedure identified independent predictive factors significant at the 5% level.

Results: Premixed human insulins were the most common formulation (**Table**). Most participants received some diabetes education, but only 14% received a structured course. In people with T1D and T2D, hospitalisations in the past 12 months (OR [95% CI] 2.3 [1.2, 4.2] and 2.7 [1.3, 5.4], respectively), unhealthy diet/lack of exercise (OR 7.1 [4.3, 11.8] and 4.5 [2.7, 7.5]) and lack of self-management (OR 2.5 [1.5, 4.19] and 1.7 [1.0, 2.7]) were significantly associated with an increased risk of not achieving glycaemic control. Only people with T1D were negatively impacted by poor diabetes education (OR 2.7 [1.2, 6.3]) and longer disease duration (OR 1.9 [1.2, 2.9]).

Conclusion: Characteristics of people with diabetes in Africa are similar to those observed in other regions, except for the preferred use of premixed human insulin. Predictors of poor glycaemic control indicate the need for improved patient education.

Table 1. Baseline demographics and disease characteristics in people with T1D and T2D in Africa*

	T1D n=788	T2D OGLD n=1497	T2D insulin only n=240	T2D OGLD + insulin n=638
Baseline demographics/disease characteristics				
Baseline demographics				
Age (mean ± SD), years	33.9 ± 12.5	57.4 ± 11.0	59.8 ± 11.0	58.2± 10.4
Gender, male, %	46.8	50.5	48.8	46.1
BMI/disease duration				
BMI, (mean ± SD), kg/m²	24.8 ± 4.6	29.2 ± 5.5	28.2 ± 5.3	30.7 ± 5.8
Duration (mean ± SD), years	12.5 ± 10.1	7.6 ± 6.2	13.8 ± 9.4	12.8 ± 8.0
Blood pressure/lipid profile				
High blood pressure, %†	16.5	59.5	65.3	72.7
Abnormal lipid profile, %†	19.7	52.9	57.4	67.7
HbA_{1c}				
Mean ± SD, %	8.7 ± 2.0	7.7 ± 1.9	8.6 ± 2.4	8.7 ± 1.9
<7 %, %	18.3	40.5	26.6	16.2
Glucose levels				
FPG (mean ± SD), mmol/l (mg/dl)	9.1 ± 4.5 (163.8 ± 80.2)	8.0 ± 3.0 (144.3 ± 54.5)	8.9 ± 4.1 (160.1 ± 72.9)	8.7 ± 3.6 (156.3 ± 65.0)
PPG (mean ± SD), mmol/l (mg/dl)†	11.3 ± 4.8 (203.6 ± 86.1)	10.5 ± 4.0 (188.5 ± 71.6)	11.8 ± 4.7 (211.5 ± 84.8)	11.3 ± 4.8 (203.1 ± 85.6)
Vascular complications				
≥1 microvascular complication, %	38.7	34.2	60.8	53.4
≥1 macrovascular complication, %	4.1	8.3	22.4	15.1
Patient education and follow-up				
Membership of a diabetes association or peer support group, %	13.8	8.8	9.3	11.8
Use of diabetes-related websites, %	45.5	22.0	19.4	23.9
Insulin use				
Premixed insulin, %	46.5	N/A	67.4	42.8
Human insulin, %	75.6	N/A	72.0	53.8

*Data collected from Algeria, Cameroon, Côte d'Ivoire, Democratic Republic of the Congo, Egypt, Kenya, Madagascar, Morocco, Nigeria, Senegal, South Africa and Tunisia; †as reported by the physician; ‡at last measurement. FPG, fasting plasma glucose; N/A, not applicable; OGLD, oral glucose-lowering drug; PPG, postprandial plasma glucose

Supported by: Sanofi

Disclosure: **J.C. Mbanya:** Employment/Consultancy; GlaxoSmithKline plc. Lecture/other fees; Sanofi, Serview, Novo Nordisk A/S.

858

Improved diabetic outcomes through a new value based commissioned service in England 2014–2017

J. Llewelyn, C. Neely, Y. Kyaw, M. Spring;
Surrey Downs Diabetes Service, Kingston upon Thames, UK.

Background and aims: National Health Service (NHS) diabetes related costs are £10 billion per annum. Compliance with NICE recommended standards of diabetes care including annual care processes and treatment targets for HbA1c, BP and lipids have been shown to be associated with lower morbidity and mortality. Data from the 2012–13 National Diabetes Audit for England and Wales (NDA) showed the Surrey Downs region, (population 300,000) did not meet NICE quality thresholds for diabetes standards with large variability between localities. As part of a major structural NHS reorganisation, a new single integrated diabetes service focused on the regional needs of people with diabetes was commissioned (Surrey Downs Diabetes Service, SDDS) with the aim of improving diabetic outcomes in a cost efficient manner.
Materials and methods: Working with 31 primary care practices a team of consultant diabetologists, primary care specialist physicians, diabetic specialist nurses (DSN), dieticians, podiatrists, clinical psychologists and dedicated administrative staff led by a senior SDN provided the service. A new tier of secondary patient care was introduced with 4 mulit-disciplinary community based 'hospital style' outpatient clinics called hubs. A dedicated SDN was attached to each practice to provide specialist support for complex patients, education of all healthcare professionals (HCPs) involved in diabetes care (including community and nursing home staff), patient education and community based structured education programmes. All consultations were recorded using the same electronic systems already in use by the primary care practices so were instantly available and good communication between all HCPs providing diabetic care was strongly encouraged. A new Hypoglycaemic Pathway was incorporated with the regional ambulance service informing SSDS of every hypoglycaemic event attended. The DSN attached to the practice of the patient would contact them and review their diabetic care.
Results: Between 2013 and 2017 the number of practices in the region participating in the NDA increased from 21 to 30 and the total number of diabetic patients registered who had recorded annual care processes and treatment targets rose from 6919 to 12,360. By 2017 recorded HbA1c (95.3% type 2, 85% type 1), BP(96% type 2, 87.9% type 1) and cholesterol (92.5% type 2,81.6% type1) was increased to align with the national average. In type 2 patients 72.3% had an HbA1c <58 mmol/mol (66% 2013), 71.2% BP <140/80 mmHg (68.3%) and 41,8% cholesterol<4 mmol/L(39.1%). 33.9% type 1 patients had an HbA1c <58 mmol/l (27.1% 2013), 79%BP <140/80 mmHg (75.2%) and 28.1% chol<4(29.5%). Local variability between practices diminished although 4 practices remained below the national average for at least one care process. The number of hospital admissions for diabetes related causes decreased from 681 to 573 with an associated cost reduction of £845,456 per annum. The annual cost of the SDDS was £744,623. The number of notifications of severe hypoglycaemia decreased from 63(2015) to 25.
Conclusion: More patient focused and community based diabetes services designed according to regional needs require large organisational change but are cost efficient and result in higher completion of annual care processes, improved attainment of treatment targets, particularly in type 2 patients and lower diabetes related hospital admissions.
Disclosure: **J. Llewelyn:** None.

859

Screening patients of type 2 diabetes for probable hypoglycaemia using standford hypoglycaemia questionnaire in outpatient settings in north India

R. Awasthi, K. Chandra, A. Pande, M. Gupta, A. Tewari, V. Agarwal, N. Gupta, S. Chaubey, S. Chowdhary, S. Ansari, Lucknow Diabetes Study Group;
Lucknow Diabetes Study Group, Lucknow, India.

Background and aims: As the current consensus recommends stricter control of glucose levels, hypoglycemia is emerging as major clinical problem in subjects with diabetes on anti diabetic medications. Co-morbidities further complicate this issue. Occurrence of hypoglycemia is known to increase the mortality and morbidity.The present study was aimed to identify the incidence of unreported hypoglycemia in subjects with type 2 diabetes (T2D), on medication, screened using Stanford Hypoglycemia Questionnaire (SHQ). Further analysis was done to see the risk factors associated with high scores.
Materials and methods: This is a multicenter observational study on consecutive subjects attending 10 diabetes care centers across Lucknow, North India. Inclusion criteria were known subjects of T2D, literate, age >18 years, on at least one anti-diabetic agent for >1 month and not doing regular self-monitoring of blood glucose (SMBG). SHQ was translated into Hindi from English for use. Hindi version of SHQ was self-administered to included subjects after written informed consent during routine follow-up visit to their physician. A Score of 1–3 was taken mild, 4–5 moderate and 6–7 severe risk of hypoglycemia.
Results: From August 2017 to April 2018, 1200 patients were included. Of these 47% were males. Mean age was 53.9 (±10.83) years. Among them 46.7% were on sulphonylurea, 16.5% were on pioglitazone, 81.09% on metformin, 7.6% on DPP4i, 2.07% on SGLT2i. Mean SHQ score was 2.04 (±1.59). Incidence of probable hypoglycemia was mild 60.6%, moderate 16.99% and severe 1.82%, with highest proportion among those on sulphonylurea (mean 2.22 (±1.68) $p < 0.004$). On univariate analysis, probable hypoglycemia was statistically significantly higher in older individual ($P < 0.008$), raised Diastolic BP ($P < 0.0001$), and with one or more chronic complications of T2D (retinopathy (mean SHQ score of 2.71, $P < 0.005$), nephropathy (mean SHQ score of 2.40, $P = 0.026$), neuropathy (mean SHQ score of 2.48, $P < 0.001$) or history suggestive of coronary artery disease (mean SHQ score of 2.66, $P = 0.013$). Hypoglycemia was significantly more common in those with previous history of hypoglycemia (mean SHQ score of 2.37 (±1.57), $P < 0.0001$.
Conclusion: Since incidence of probable hypoglycemia is high in T2D not doing SMBG, those at risk must be identified for appropriate management. For this SHQ was found to be simple and cost effective screening tool in resource crunched Indian setup and can be used in outpatient settings.
Disclosure: **R. Awasthi:** None.

PS 074 Cost effectiveness in diabetes therapies

860

Population based diabetes screening is associated with less insulin therapy compared to usual care

R.C. Vos[1], H. den Ouden[1], L.A. Daamen[1], H.J.G. Bilo[2], P. Denig[3], G.E.H. Rutten[1];
[1]Julius Center of Health Sciences and Primary Care, Department of General Practice, Univercity Medical Center Utrecht, Utrecht, [2]Department of Internal Medicine, Diabetes Center Isala, Zwolle, [3]Clinical Pharmacy and Pharmacology, University Medical Center Groningen, Groningen, Netherlands.

Background and aims: The number of people with type 2 diabetes (T2DM) in need of insulin might be lower if they were diagnosed by population-based screening, i.e. about three years earlier than during care-as-usual. Dutch care-as-usual implies opportunistic screening in people with a high risk of T2DM during surgery hours in primary care. We compared insulin prescription and glycemic control 10 years after population based screening of T2DM with data from people with known diabetes duration of 7 and 10 years after opportunistic screening.
Materials and methods: People from three cohorts were included, index year 2014: 391 with 10 years screen-detected diabetes duration (ADDITION-NL study), 4473 with 7 years and 2660 with 10 years diabetes diagnosis (GIANTT/ZODIAC primary care databases). Treatment guidelines since 2009 were comparable. Insulin prescription and HbA1c were compared using logistic and linear regression analysis, adjusted for possible confounders (age, sex, BMI, LDL-cholesterol, HbA1c/glucose lowering medication, smoking).
Results: People with screen-detected T2DM were older (71.4 vs 68.4 and 69.7 years, $p < 0.01$) and more often male; 53.8% vs 50.5% in the 7 years and 47.8% in the 10 years diabetes cohorts respectively (Table). Insulin prescription in these groups was: 10.5%, 14.7% and 18.9%. People diagnosed 7 and 10 years before during care-as-usual had 1.5 (95%CI 1.0–2.1) and 1.9 (95%CI 1.3–2.7) higher adjusted odds for insulin prescription than those with screen-detected T2DM. The mean difference in HbA1c levels between people with screen-detected T2DM diagnosis and those diagnosed during care-as-usual 7 and 10 years before was 1.6 mmol/mol (95% CI 0.5–2.7) and 1.8 mmol/mol (95% CI 0.7–2.9) higher for the latter two, after adjustment.
Conclusion: Population based diabetes screening is associated with good glycemic control on the long-term and less need for insulin compared to usual care.

Table: Subject characteristics of the three cohorts ADDITION-NL, 7 and 10 years diabetes diagnosis during care-as-usual

	ADDITION-NL (n=391)	7 years diabetes diagnosis (n= 4473)	10 years diabetes diagnosis (n= 2660)	F-test / Chi-square	P-value
Age (years)	71.6 (5.3)	68.4 (11)	69.7 (10.6)	26.2	<0.01
Sex, male (n,%)	268 (53.8)	2266 (50.5)	1272 (47.8)	8.2	0.02
Smoking, yes (n,%)	55 (14.4)	706 (16.4)	407 (15.9)	1.1	0.58
BMI (kg/m^2)	30.4 (6.7)	29.8 (5.3)	29.4 (5.2)	6.5	<0.01
HbA1c (mmol/mol)	49.8 (9.9)	51.8 (10.1)	52.8 (10.4)	18.6	<0.01
SBP (mmHg)	135 (15.4)	138 (16.1)	138 (16.6)	5.1	<0.01
DBP (mmHg)	74 (9.5)	77 (9.2)	76.1 (9.1)	15.1	<0.01
LDL (mmol/L)	2,1 (0.9)	2.5 (0.9)	2.5 (0.9)	32.3	<0.01
Glucose lowering medication				40.1	<0.01
Lifestyle only	72 (18.4)	795 (17.7)	380 (14.3)		
Oral medication	278 (71.1)	3033 (67.5)	1776 (66.7)		
Insulin +/- oral medication	41 (10.5)	661 (14.8)	504 (19)		

Disclosure: **R.C. Vos:** None.

861

Impact of V-Go versus multiple daily injections on glycaemic control, insulin utilisation and diabetes medication costs among individuals with type 2 diabetes

A. Raval[1], M. Nguyen[2], S. Zhou[1], M. Grabner[1], J. Barron[1], R. Quimbo[1];
[1]Healthcore, Inc., Wilmington, [2]Valeritas, Inc., Bridgewater, USA.

Background and aims: Individuals with type 2 diabetes mellitus (T2DM) requiring multiple daily injections (MDI) of insulin face challenges with compliance, discomfort, and cost. The V-Go® Wearable Insulin Delivery Device may improve the insulin delivery experience and outcomes for such population. A few studies examined the relative effectiveness of V-Go in terms of clinical and economic outcomes in comparison to MDI. This study examined the effectiveness of V-Go in terms of glycemic control, insulin utilization and diabetes medication costs compared to MDI among individuals with T2DM in the United States.
Materials and methods: A retrospective cohort study was performed using a commercial administrative claims-database between 07/01/2011–07/31/2017. Study cohorts included individuals with T2DM aged 21–80 years either newly initiating V-Go or using MDI for basal-bolus insulin with prior exposure to basal ± bolus or premixed insulin. The main sample included individuals with ≥1 glycosylated hemoglobin (HbA1c) laboratory result during the baseline (6 months) and follow-up periods (3–13 months). Insulin utilization and diabetes medication costs were examined during baseline and second-half of 1-year follow-up (months 7–12) among those with continuous health plan coverage. V-Go and MDI users were 1:1 matched on baseline insulin exposure, HbA1c level, and other characteristics. T-test, McNemar test and Chi-square test were used to compare follow-up outcomes. A sensitivity analysis was conducted to examine insulin utilization and diabetes medication costs among a larger sample of individuals with or without HbA1c results.
Results: Matched cohorts included 118 well-balanced pairs for the main analysis (mean age: 56 years; mean baseline HbA1c: 9.2%) and 585 well-balanced pairs for the sensitivity analysis (mean age: 57 years). In the main analysis, both cohorts experienced improvements in glycemic control during follow-up relative to baseline (% with HbA1c ≤9%, pre/post: V-Go 49/69, $p < 0.001$; MDI 50/60, $p = 0.046$). With similar baseline number of insulin Rx fills and diabetes-related medication costs, V-Go users had fewer insulin Rx fills (mean change from baseline: −0.8 vs. +1.8 fills, $p < 0.001$), a 21% decline in insulin total daily dose (TDD) (mean change TDD in IU: −29.2 vs. +5.8, $p < 0.001$), and a lower increase in diabetes-related medication costs (mean change in 2016 USD: $341 vs. $1,628, $p = 0.012$) as compared to MDI users, during the last 6 months of follow-up. Consistent with the main analysis, V-Go users also had fewer insulin Rx fills (mean change from baseline: −0.4 vs. +1.8 fills, $p < 0.001$), a 17.5% decline in TDD (mean change TDD in IU: −23.9 vs. +6.0, $p < 0.001$), and a lower increase in diabetes-related medication costs (mean change in 2016 USD: $506 vs. $1129, $p < 0.001$) in the larger sensitivity analysis sample.
Conclusion: This study is the first to report clinical and economic outcomes associated with V-Go use up to a one-year follow-up period. V-Go therapy was associated with improved glycemic control, and with less total daily insulin requirement and lower diabetes medication costs compared to MDI. V-Go therapy represents an opportunity to improve quality clinical measures more efficiently and less costly among a T2DM population requiring basal-bolus insulin treatment.
Supported by: Study funding provided by Valeritas, Inc.
Disclosure: **A. Raval:** Employment/Consultancy; I am an employee of Healthcore, Inc. Healthcore, Inc. received the fundings to conduct this research study from the Valeritas, Inc.

862

Long-term cost-effectiveness of sitagliptin and SGLT2i combination therapy for the management of type 2 diabetes in the UK

M. Pawaskar[1], P. Bilir[2], A. Graber-Naidich[2], C. Gonzalez[1], S. Rajpathak[1], G. Davies[1];
[1]Merck & Co., Inc, Kenilworth, [2]QuintilesIMS, San Francisco, USA.

Background and aims: Clinical benefits of dipeptidyl peptidase-4 inhibitors (DPP-4i) and sodium-glucose co-transporter 2 inhibitors (SGLT2i) combination therapy have been demonstrated through clinical trials; however, there is limited understanding of economic benefits of sequential use of these therapies relative to generic therapies such as NPH insulin. This study evaluated the long-term cost-effectiveness of a treatment strategy involving intensification with SGLT2is (pathway 1) compared to NPH insulin (pathway 2) as a 3rd line therapy in type 2 diabetes (T2D) patients not at goal on metformin (1st line) and sitagliptin (2nd line) therapy in the UK.

Materials and methods: Cost-effectiveness analysis was performed using the validated QuintilesIMS CORE Diabetes Model from a UK payer perspective. Clinical and economic outcomes were modeled over a lifetime for a cohort of T2D patients who fail to achieve glycemic goal on metformin monotherapy. Treatment effect data were obtained from randomized clinical trials and economic data such as direct medical costs (e.g., medications, diabetes management, adverse events, and complications) were obtained from multiple published sources. Several scenario analyses including changes in clinical parameters were performed to assess the robustness of base case results.

Results: Pathway 1 increased total life years (13.49 vs. 13.37, respectively) and quality-adjusted life years (QALY) (9.40 and 9.22, respectively) compared to pathway 2. Although drug costs in pathway 1 were higher than pathway 2, they were offset by decreases in diabetes-related complications and body mass index, leading to lower total direct medical costs for pathway 1 (£25,747 vs £26,095). Thus, pathway 1 dominates pathway 2, as it is both more effective (measured in terms of quality adjusted life years) and less costly. Results from scenario analyses assessing changes in treatment effect, hypoglycemia rate, body mass index, cardiovascular protective effect of SGLT2i (using EMPA-REG, CANVAS, CDV-REAL data) consistently showed that pathway 1 was either dominant (incremental cost effectiveness ratio (ICER) <$0/QALY) or cost-effective per the National Institute for Health and Clinical Excellence's cost-effectiveness threshold of <£30,000/QALY [(ICER <£3,000/QALY for patients with lower baseline HbA1c (7%) or older age (65+ years)].

Conclusion: Among patients not at goal on metformin and sitagliptin therapy, treatment intensification with SGLT2i is likely to be cost effective compared to intensification with NPH insulin as a 3rd line therapy for management of T2D in the UK.

Table: cost effectiveness analysis of two treatment pathways for management of type 2 diabetes

	Pathway 1 (Includes metformin+sitagliptin+SGLT2is)	Pathway 2 (Includes metformin+sitagliptin +NPH insulin)
Life years gained	13.49	13.37
Quality adjusted life years	9.40	9.22
Total medical costs	£ 25,747.38	£ 26,095.18
Incremental cost effectiveness ratio	Pathway 1 dominates Pathway 2	

Supported by: Merck Inc

Disclosure: **M. Pawaskar:** Employment/Consultancy; Merck Inc. Stock/Shareholding; Merck Inc.

863

Cost-effectiveness analysis of empagliflozin in comparison to standard of care, sitagliptin and saxagliptin based on cardiovascular outcomes trials

M. Ramos[1], V. Foos[1], A. Ustyugova[2], N. Hau[3], P. Gandhi[4], M. Lamotte[1];
[1]HEOR, IQVIA, Zaventem, Belgium, [2]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [3]Boehringer Ingelheim Ltd, Bracknell, UK, [4]Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, USA.

Background and aims: Over the recent years, several cardiovascular outcome trials (CVOTs) have been published with the glucose lowering drugs. In the EMPA-REG OUTCOME trial, empagliflozin (SGLT-2) + standard of care (SoC) was compared to SoC (placebo arm in the trial) in patients with type 2 diabetes mellitus (T2DM) and established cardiovascular disease (CVD). The TECOS and SAVOR-TIMI 53 CVOT trials for sitagliptin and saxagliptin (both DPP-4 inhibitors), respectively, were designed similarly. This study assessed the cost-effectiveness of empagliflozin + SoC in comparison to SoC, sitagliptin + SoC and saxagliptin + SoC in patients with T2DM and established CVD based on the data from the respective CVOTs.

Materials and methods: The IQVIA Core Diabetes Model (CDM), a well-established model, was calibrated to reproduce the outcomes from the EMPA-REG OUTCOME trial, both for empagliflozin + SoC and SoC. Baseline characteristics and observed effects on physiological parameters (HbA1c, BMI, blood pressure, lipids) were used as inputs. Network meta-analysis (NMA) provided the relative risks for cardiovascular outcomes with empagliflozin versus sitagliptin and saxagliptin. The CDM was then calibrated to reproduce sitagliptin and saxagliptin trials' outcomes. The effects of the CVOTs were applied for 3 years (the median observation time in the EMPA-REG OUTCOME trial) after which, the UKPDS 82 risk equations predicted events based on HbA1c and other physiological parameters. After the first 3 years, HbA1c progression for all arms was projected based on the progression in the EMPA-REG OUTCOME trial. Given the long diabetes history patients had, basal bolus rescue therapy was assumed whenever modeled individuals exceeded an HbA1c threshold of 8.5%. The analyses were performed from the UK National Health Service (NHS) perspective. UK unit costs of complications and quality of life data were taken from literature. The drug costs were from the British National Formulary and Monthly Index of Medical Specialities. Annual discounting rates of 3.5% were applied.

Results: CDM lifetime projections including CVOT calibration in the initial 3 years demonstrated 9.215, 9.614, 9.235 and 9.169 life years, 5.306, 5.582, 5.342 and 5.297 quality-adjusted life years (QALY), and 44,086 GBP, 45,452 GBP, 44,399 GBP and 44,338 GBP total lifetime costs for SoC, empagliflozin, sitagliptin and saxagliptin, respectively. The incremental cost-effectiveness ratio (ICER) of empagliflozin versus SoC, sitagliptin + SoC and saxagliptin + SoC were 4,952GBP/QALY, 4,389GBP/QALY and 3,911GBP/QALY, respectively. The probabilistic sensitivity analyses demonstrated that empagliflozin is cost-effective at a willingness to pay threshold of 20,000GBP/QALY in 90%, 89% and 64% of cases, when compared to SoC, sitagliptin + SoC and saxagliptin + SoC, respectively. Running these analyses with a time horizon of 5 years showed the same trend.

Conclusion: Based on the results of the EMPA-REG OUTCOME, TECOS and SAVOR-TIMI 53 studies, the cost effectiveness analyses suggest that empagliflozin + SoC is cost-effective treatment alternative to SoC, sitagliptin + SoC and saxagliptin + SoC from the UK NHS perspective.

Supported by: IQVIA received consulting honoraria from Boehringer Ingelheim.

Disclosure: **M. Ramos:** Employment/Consultancy; IQVIA, my employer received consultancy fees for the work performed.

864

Impact of exenatide on medical costs and health utilities in type 2 diabetes: experience from EXSCEL

S.D. Reed[1], Y. Li[1], H. Dakin[2], F. Becker[2], J. Leal[2], S.M. Gustavson[3], B. Kartman[4], E. Wittbrodt[3], R.J. Mentz[1], N.J. Pagidipati[1], M.A. Bethel[5], A.M. Gray[2], R.R. Holman[5], A.F. Hernandez[1];
[1]Duke Clinical Research Institute, Durham, USA, [2]Oxford Health Economic Research Centre, Oxford, UK, [3]AstraZeneca, Gaithersburg, USA, [4]AstraZeneca, Molndal, Sweden, [5]Diabetes Trials Unit, Oxford, UK.

Background and aims: The Exenatide Study of Cardiovascular Event Lowering (EXSCEL) demonstrated a numerical, but not statistically significant, reduction in major adverse cardiovascular events and a nominally significant improvement in all-cause mortality in 14,752 patients with type 2 diabetes (T2D), with or without previous cardiovascular disease, randomized 1:1 to exenatide 2 mg once-weekly (EQW) or placebo added to usual care. Those allocated EQW experienced significantly greater reductions in glycated hemoglobin, body weight, LDL-cholesterol and systolic blood pressure compared with placebo. Medical resource use and EQ-5D data were collected throughout the study.
Materials and methods: Medical resources were valued from US and UK perspectives using Medicare payments and wholesale acquisition costs (WAC) for concomitant medications with a 23.1% discount for EQW in the US analysis, and using the English National Schedule of Reference Costs and Prescription Cost Analysis database for the UK analysis. EQ-5D-5L and EQ-5D-3L responses were mapped to 3-level health utilities using both US and UK tariffs. Hierarchical generalized linear models were used to compare medical resource use, costs and health utilities with specific error distributions and link functions.
Results: Mean follow-up was 3.3 years. Mean number of hospitalizations per patient were similar in both groups (EQW 0.83 vs. placebo 0.84; $p = 0.31$), as were annual hospitalization rates, ranging from 0.24–0.29 per person-year from year 1 to year 5. The mean cumulative number of inpatient days over the trial follow-up period was 0.41 days lower in the EQW group than the placebo group (7.05 days vs. 7.46 days respectively; relative rate ratio 0.91; $p = 0.05$). Inpatient and outpatient costs were similar between treatment groups when US or UK costs were assigned. Although EQW-treated patients incurred lower costs for concomitant diabetic and non-diabetic medications, and in overall total costs excluding study medication, inclusion of EQW costs led to higher total mean costs in the EQW arm (Table). There were no significant differences observed in US or UK EQ-5D health utilities between groups throughout the follow-up period.
Conclusion: Similar hospitalization rates and health utilities were reported across time with EQW, compared with placebo. However, mean cumulative inpatient days were significantly reduced with EQW, compared with placebo. Total costs were significantly lower in the EQW group when the cost of study medication was excluded, but were significantly greater when the cost of branded EQW was included in the analysis.

Table: Medical costs over trial period in exenatide and placebo groups, using US and UK unit costs (mean, SD)

	US analysis ($)			UK analysis (£)		
	Exenatide (N=7,356)	Placebo (N=7,396)	Mean cost ratio p-value	Exenatide (N=7,356)	Placebo (N=7,396)	Mean cost ratio p-value
Inpatient costs	9,654 (25,051)	10,078 (26,016)	0.92, p=0.10	5,021 (14,028)	5,204 (13,929)	0.92, p=0.51
Outpatient costs	2,156 (1,872)	2,139 (1,910)	1.01, p=0.68	1,313 (1,231)	1,293 (1,215)	1.01, p=0.74
Total non-study medication costs	17,098 (15,992)	18,698 (16,899)	0.91, p≤.01	2,457 (2,558)	2,708 (2,913)	0.91, p≤0.01
Diabetic medication costs	13,882 (14,592)	15,445 (15,295)	0.89, p≤.01	1,640 (1,618)	1,823 (1,687)	0.89, p≤0.01
Other medication costs	3,216 (3,610)	3,252 (3,876)	0.99, p=0.39	817 (1,676)	885 (2,019)	0.96, p=0.03
Study medication: exenatide 2mg once-weekly	13,790 (8,374)	--	--	2,084 (1,254)	--	--
Total costs (excluding study medication)	28,907 (32,600)	30,914 (34,089)	0.92, p=0.02	8,790 (15,024)	9,204 (14,970)	0.93, p=0.02
Total costs	42,697 (34,355)	30,914 (34,089)	1.39, p≤.01	10,874 (15,136)	9,204 (14,970)	1.18, p≤.01

Clinical Trial Registration Number: NCT01144338
Supported by: AstraZeneca (Gaithersburg, MD)
Disclosure: **S.D. Reed:** Grants; Merck & Co., Inc., AstraZeneca, Sanofi US, Janssen Pharmaceuticals, Inc.

865

No availability, no uptake - exploring the reasons behind low uptake of insulin pump therapy in Irish adults with type 1 diabetes: findings from a national survey

K.A. Gajewska[1], S. Sreenan[2,3], K.E. Bennett[4], J. Gajewski[1], R. Biesma[1];
[1]Department of Epidemiology and Public Health, Royal College of Surgeons in Ireland, Dublin, [2]Department of Diabetes and Endocrinology, Royal College of Surgeons in Ireland, Dublin, [3]3U Diabetes Consortium, 3U Partnership, Dublin, [4]Division of Population Health Sciences, Royal College of Surgeons in Ireland, Dublin, Ireland.

Background and aims: Insulin pump therapy is fully covered for all patients with diabetes in Ireland by the Long-Term Illness Scheme and users are not subject to any out-of-pocket expenses. Despite this, the uptake of insulin pumps in Irish patients with Type 1 diabetes is lower than the European average (9% vs. 15%). It is hypothesised that the low uptake of insulin pumps may be related to availability of clinics offering insulin pumps and the staff trained in insulin pump therapy. The aim of this study is to investigate the availability of insulin pump therapy in adults with Type 1 diabetes in diabetes clinics in Ireland.
Materials and methods: We undertook a cross-sectional, national survey of all diabetes clinics ($n = 50$) offering services to adult patients with Type 1 diabetes in Ireland. The data were collected from private and public clinics through an online questionnaire and by phone, according to participants' preference. One relevant healthcare professional was asked to complete the questionnaire on behalf of the clinic.
Results: Forty-seven diabetes clinics (34 public and 13 private) took part in the study (94% response rate/100% in public clinics). The survey was completed mainly by nurses (49% cases) and endocrinologists (47%). Of all patients with Type 1 diabetes ($n = 22321$), 9.7% were using insulin pumps. One third (32%) of all clinics did not offer any services for patients on pumps. The main reasons for the lack of such services were: absence of a diabetes nurse specialist (60%) or dietician (67%) trained in insulin pump therapy and high current workload (53%). 45% of the clinics in Ireland reported availability of insulin pump therapy and offered training to commence it (37% of public hospitals, 54% of private), but only 12.5% of patients in these clinics were on pumps. Large public clinics ($n >$ median of 315 patients) had higher insulin pump uptake (number of insulin pump users divided by the number of all patients with Type 1 diabetes) then smaller clinics (9% vs. 2%, $p = 0.01$). Less than one percent (0.42%) of patients in clinics offering training to commence insulin pump therapy received such training in the last 12 months ($n = 179$), what gives the average of 9 patients trained per clinic/year. There was no correlation between the number of patients trained to commence an insulin pump therapy and current workload, estimated by the number of patients per healthcare professionals' Whole-Time Equivalent (WTE). The size of the clinic and the number of healthcare professionals' WTE per clinic were the only significant factors associated with frequency of insulin pump training in the last 12 months prior to survey completion.
Conclusion: The low uptake of insulin pump therapy (less than 10% of adults with Type 1 diabetes) may be influenced by the low availability of insulin pump training in diabetes clinics. More studies to explore the determinants of the access to insulin pump therapy and poor availability of insulin pump-related services in Ireland are needed to complement the evidence.
Supported by: This research was funded by the HRB SPHeRE/2013/1, Ireland
Disclosure: **K.A. Gajewska:** Grants; Health Research Board is a sponsor of my SPHeRE PhD Programme and a stipend.

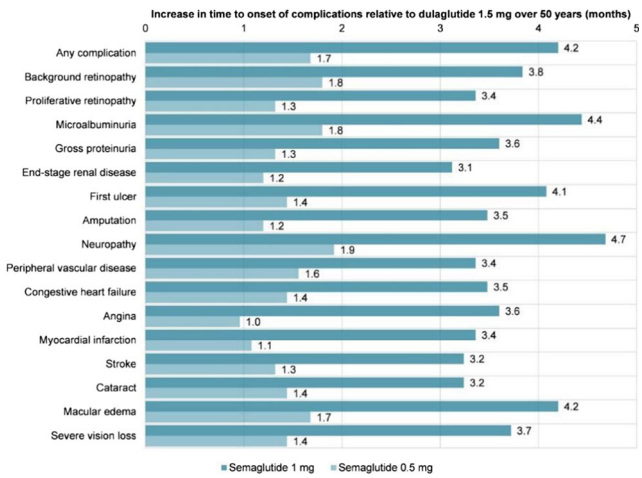
866

Once-weekly semaglutide provides better health outcomes compared to dulaglutide as dual therapy in the treatment of type 2 diabetes: a cost-effectiveness analysis

C.K. Tikkanen[1], P. Johansen[2], B. Hunt[3], S. Malkin[3], R.F. Pollock[3];

[1]Novo Nordisk Scandinavia AB, Copenhagen, Denmark, [2]Novo Nordisk A/S, Søborg, Denmark, [3]Ossian Health Economics and Communications GmbH, Basel, Switzerland.

Background and aims: Once-weekly semaglutide (semaglutide) is a novel glucagon-like peptide 1 (GLP-1) analogue for the treatment of type 2 diabetes (T2D). The safety and efficacy of semaglutide has been compared with GLP-1 receptor agonist dulaglutide in SUSTAIN 7, a 40-week randomized phase 3b trial in subjects with T2D inadequately controlled with metformin. Semaglutide 0.5 mg and 1 mg lowered HbA_{1c} by 1.5% and 1.8%, respectively, compared to 1.4% for dulaglutide 1.5 mg. Similarly, semaglutide 0.5 mg and 1 mg reduced body weight by 4.6 kg and 6.5 kg, respectively, compared to 3.0 kg for dulaglutide 1.5 mg. The aim of the study was to use computer simulation modelling to project long-term health and economic outcomes with semaglutide 0.5 mg and 1 mg versus dulaglutide 1.5 mg from a Danish healthcare payer perspective.
Materials and methods: Long-term projections of the incidence of complications, costs, and impact on health-related quality of life, were made using the IQVIA CORE Diabetes Model over a 50-year time horizon. Baseline patient characteristics, initial treatment effects, and hypoglycaemic event rates were sourced from the trial. A 3 year treatment period was assumed with semaglutide and dulaglutide after which patients switched to basal insulin rescue therapy. Following switch, a natural HbA_{1c} progression was modelled based on the United Kingdom Prospective Diabetes Study Outcomes Model equation. Treatment and complication costs were captured in 2017 Danish Krone (DKK), and future costs and outcomes were discounted at 3% *per annum*. Extensive one-way and probabilistic sensitivity analyses were conducted around key model assumptions.
Results: Treatment with semaglutide 0.5 mg and 1 mg resulted in increased time to onset of complications relative to dulaglutide 1.5 mg (**Figure**), and reduced incidence of complications over a 50 year time horizon. Mean time to onset of any complication increased by 1.7 and 4.2 months with semaglutide 0.5 mg and 1 mg, respectively. This improved quality-adjusted life expectancy for patients treated with semaglutide 0.5 mg and 1 mg by 0.06 and 0.14 quality-adjusted life years (QALYs), respectively, compared to dulaglutide 1.5 mg. Semaglutide 1 mg was projected to be cost saving relative to dulaglutide 1.5 mg, while the 0.5 mg dose increased total costs by DKK 2,123, resulting in an incremental cost-utility ratio of DKK 37,435 per QALY gained.
Conclusion: This study showed that both doses of semaglutide would result in reductions in cumulative incidence and increased time to onset of complications of T2D relative to dulaglutide 1.5 mg. In patients with T2D inadequately controlled on metformin, treatment with semaglutide 0.5 mg would be cost-effective, while semaglutide 1 mg represented a dominant (i.e. more efficacious and less costly) treatment option relative to dulaglutide 1.5 mg.



Supported by: This study was funded by Novo Nordisk A/S
Disclosure: **C.K. Tikkanen:** Employment/Consultancy; Employment: Novo Nordisk.

867

The importance of incorporating cardio-protective effects of once-weekly semaglutide in estimates of health benefits for patients with type 2 diabetes

L.M. Evans[1], P. Johansen[2], H. Vrazic[2], A. Pitcher[3], E. Falla[3];
[1]Department of Diabetes, University Hospital Llandough, Cardiff, UK, [2]Novo Nordisk A/S, Søborg, Denmark, [3]IQVIA, London, UK.

Background and aims: Economic modelling of type 2 diabetes (T2D) relies on sets of risk equations (e.g., United Kingdom Prospective Diabetes Study [UKPDS] 68 and 82) to predict cardiovascular (CV) events and mortality as mediated through changes in risk factors (e.g., HbA_{1c}). Recent CV outcomes trials (CVOTs) have shown significant benefits in events over a short time period (between 2–4 years), along with beneficial changes in risk factors such as blood pressure, HbA_{1c}, and weight. The contributions of these changes to the observed outcomes within these CVOTs are unclear. In patients with high CV risk, the SUSTAIN 6 trial of once-weekly semaglutide+standard of care (SoC) reported, amongst other findings, a statistically significant reduction in non-fatal stroke (HR = 0.61; 95% confidence interval 0.38–0.99) after 104 weeks versus placebo+SoC. We sought to establish the contribution of risk factor changes to the observed stroke (non-fatal and fatal) outcome, by evaluating the performance of traditional risk equations in predicting outcome benefits seen with once-weekly semaglutide. As a secondary objective, the impact of incorporating the stroke benefit on estimated long term health benefits was assessed.
Materials and methods: The IQVIA Core Diabetes Model was populated with patient characteristics and risk factor changes (e.g., HbA_{1c}, blood pressure, body-mass index, lipids) for once-weekly semaglutide+SoC and placebo+SoC, as observed in the trial. The model was then run for a two-year time horizon to assess the predictive accuracy of the UKPDS 68 and UKPDS 82 risk equations on the observed stroke (non-fatal and fatal) benefit, as mediated through changes in the risk factors. Secondly, the model was calibrated to preserve the relative risk (RR) ratio of the observed cumulative incidence of stroke between the treatment arms (after taking into account the benefits generated by the risk equations). This was achieved by an iterative process by applying a stroke RR to the once-weekly semaglutide arm only, using the TREND function in Excel® until the predicted RR ratio matched the trial-observed RR ratio. Finally, results were extrapolated over 50 years.
Results: The UKPDS 68 and 82 equations were able to predict 64% and 50%, respectively, of the observed 2-year stroke risk reduction, suggesting a drug-mechanistic effect other than observed through changes in the risk factors. Calibration results indicate that a drug-specific RR of stroke of 0.83 (17% reduction) and 0.75 (25% reduction) applied to once-weekly semaglutide was required to preserve the RR ratio of the observed cumulative incidence, using UKPDS 68 and UKPDS 82, respectively. Results of extrapolation increased quality-adjusted life-years (QALYs) by 0.18 and life-years (LYs) by 0.29 with once-weekly semaglutide+SoC vs placebo+SoC when using UKPDS 68 equations. Using UKPDS 82 equations increased QALYs by 0.11 and LYs by 0.16. QALYs and LYs were slightly increased when the stroke benefit were taken into account, compared to not accounting for it.
Conclusion: Results of this analysis highlight the importance of considering recent evidence on CV benefits for economic modelling, and may have implications to future approaches assessing the long-term economic impact of new therapies in T2D in this era of multiple CVOTs.
Supported by: This study was funded by Novo Nordisk A/S

Disclosure: **L.M. Evans:** Employment/Consultancy; Consultancy: Novo Nordisk, Consultancy: Novartis, Consultancy: MSD, Consultancy: MundiPharama, Consultancy: Jansen. Grants; Novo Nordisk. Lecture/other fees; BI, Janssen, MundiPharma, Novartis, Novo Nordisk, Takeda.

868

Real world practice level data analysis confirms link between variability within blood glucose monitoring strip and glycosylated haemoglobin in type 1 diabetes

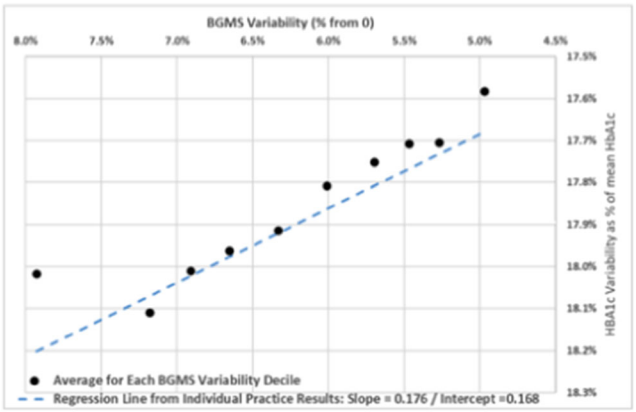
M. Stedman[1], M. Lunt[2], M. Livingston[3], A. Fryer[4], G. Moreno[5], S. Anderson[6], R. Gadsby[7], I. Laing[8], R. Young[9], A. Heald[2,8];
[1]RES Consortium, Andover, UK, [2]University of Manchester, Manchester, UK, [3]Walsall Manor Hospital, Walsall, UK, [4]Keele University, Keele, UK, [5]High Speciality Regional Hospital of Ixtapaluca, Mexico, Mexico, [6]Institute of Cardiovascular Sciences, University of Manchester, Manchester, UK, [7]Warwick Medical School, University of Warwick, Wiltshire, UK, [8]Department of Diabetes and Endocrinology, Salford Royal Hospital, Salford, UK, [9]National Diabetes Audit, Leeds, UK.

Background and aims: Minimising blood glucose variation is key to optimising health outcomes for people with diabetes. Our aim was to see if we could quantify the impact of Blood Glucose Monitoring Strips variability (BGMSV) at GP practice level on the variability of reported glycated haemoglobin (HbA1cV) levels published in the National Diabetes Audit, and from that estimate the impact on Blood Glucose Variability (BGV)

Materials and methods: The overall GP Practice BGMSV was calculated from the quantity of main types of BGMS being prescribed combined with the published accuracy, as % results within ±% bands from reference value for the selected strip type. An estimated HbA1c mean and variability (HbA1cV) was calculated for each practice year from % results within HbA1c bands published in the National Diabetes Audit for Type 1 diabetes (T1DM). The regression coefficient between the BGMSV and HbA1cV was calculated. To allow for the aggregation of estimated 3 tests/day over 13 weeks (i.e. 300 samples) of actual Blood Glucose values up to the HbA1c, we multiplied HbA1cV coefficient by √300 to estimate an empirical value for the impact of BGMSV on BGV.

Results: 4,524 practice years with 159,700 T1DM patient years where accuracy data was available for more than 80% of strips prescribed were included, with overall BGMSV 6.5% and HbA1c mean of 66.9 mmol/mol (8.3%) with variability of 13 mmol/mol equal to 19% of the mean. At a GP practice level, BGMSV and HbA1cV as % of mean HbA1c (in other words the spread of HbA1c) were closely related with a regression coefficient of 0.176, p value <0.001 (see Figure). After correction for aggregation the equivalent BGV correlation factor was calculated at 3. The comparable figure previously found in an in-silico study was 2.7. Applying this factor for BGMS to the national ISO accepted standard where 95% results must be ≤±15% from reference, revealed that for BG, 95% results would ≤±45% from the reference value. So for a patient with BG target @10mmol/l using ISO standard strips, on 1/20 occasions (average 1/week) their actual blood glucose value could be >±4.5 mmol/l from target, compared to the best performing BGMS with BG >±2.2 mmol/l from reference on 1/20 occasions.

Conclusion: Use of more variable/less accurate BGMS is associated both theoretically and in practice with a larger variability in measured BG and HbA1c, with implications for patient confidence in their day to day monitoring experience. Our results suggest clear advantages of best in class accuracy BGMS.



Relation at GP practice Level between BGMS variability and HbA1c variability taken as % of the mean HbA1c for that practice. The points reflect the average within the deciles of practices sorted by BGMS variability. The regression over all practice years gave r^2=0.003 and p value <0.001.

Disclosure: **M. Stedman:** None.

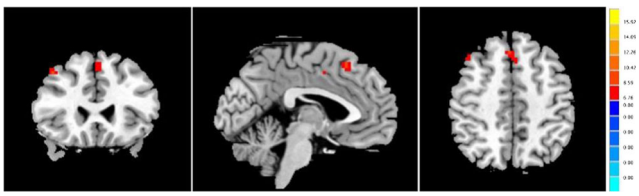
PS 075 Psychosocial aspects in diabetes

869

Changed amygdala functional network in type 2 diabetes with major depression disorder

L. Gao;
Southeast University, Nanjing, China.

Background and aims: Patients with type 2 diabetes (T2D) showed significantly increased risk for various psychological disorders, including major depression disorder (MDD), which results from altered connectivity of brain functional network. Previous investigations have suggested the importance of amygdala functional network (AFC) in the pathophysiology process of MDD, however, its neurobiology underlying the diabetes-related mood disorder is poorly understood. The present study aimed to reveal the AFC alteration related to the T2D-MDD interaction via functional MRI approach.
Materials and methods: A total of 163 participants were enrolled, 78 of which were patients with T2D. For the 78 patients with T2D, 37 of them were diagnosed as MDD and the remaining patients had no signs of mood disorder. For the 85 participants without T2D, 37 of them were patients with MDD and the remaining participants had no signs of mood disorder. All the participants went through the clinical evaluations and functional MRI scans. To explore the influence of MDD-T2D interaction on the AFC connectivity, a mixed analysis of covariance (ANCOVA) with T2D status (2 levels: T2D and non-T2D) and MDD status (2 levels: MDD and non-MDD) as fixed factors was performed, followed by post-hoc analyses. Further, the partial correlations analyses were applied to explore the behavior significance of the brain regions with T2D × MDD interactions.
Results: The interactive effects of T2D-MDD on the left AFC connectivity were mainly detected within right middle frontal gyrus (RMFG, $P < 0.01$, corrected by Monte Carlo simulation). Post-hoc analyses suggested T2D patients with MDD showed the significantly decreased connectivity within RMFG compared to the other three groups (all $P < 0.01$). Further, MDD subjects without T2D showed significantly decreased connectivity compared to healthy control ($P = 0.015$). Finally, revealed by the correlations analyses, functional connectivity within AFC showed significantly positive association with HAMD scores for participants with MDD (rho = 0.66, $P = 0.007$).
Conclusion: The functional MRI provided new approach to understand the underlying mechanisms of the MDD for T2D patients, and improving the AFC function may be a promising method to relieve the T2D-related mood disorder.



Disclosure: **L. Gao:** None.

870

Depression as an independent predictor for type 2 diabetes incidence in adults: result from Qingdao Diabetes Prevention programme

F. Ning[1], D. Zhang[2], L. Zhang[3], Z. Pang[1], Q. Qiao[4], Standing for Qingdao Diabetes Prevention Program;
[1]Qingdao Centers for Disease Control and Prevention, Qingdao, China, [2]Huangdao Centers for Disease Control and Prevention, Qingdao, China, [3]Qingdao Endocrine and Diabetes Hospital, Qingdao, China, [4]University of Helsinki, Helsinki, Finland.

Background and aims: Cross-sectional studies evidenced the bidirectional associations between depression syndrome and type 2 diabetes in adults. The current study will evaluate depression predicted risk for onset of type 2 diabetes in a longitudinal study in China.
Materials and methods: The 2006 World Health Organization diagnostic criteria and Zung self-assessment score were employed to identify type 2 diabetes and depression syndrome at baseline, respectively. A total of 3581 participants aged 35 to 74 years at baseline randomly sampling from Qingdao Diabetes Prevention Program were collected development of type 2 diabetes followed for 4 years. Multivariate Logistic regression was employed to assess their relationship between depression syndrome and type 2 diabetes, adjusting for age, sex, body mass index, residential area, serum triglycerides, hypertension, family history of diabetes, marital status, education level, income level, physical activity, occupation, smoking and drinking status.
Results: During a 4-year follow up, 305 cases of incident type 2 diabetes was identified, with cumulative incidence of diabetes of 8.52%. At baseline, individuals with depression were positively associated with fasting plasma glucose, women, lower education and lower frequency of metabolic equivalent ($p < 0.01$ for all comparisons). The significant relative risks and 95% confidence interval of depression for onset of type 2 diabetes was 1.52(1.05–2.21) after adjustment for conventional risk factors. Sensitive analysis showed that depression syndrome was significantly associated with type 2 diabetes in women, but moderate in men. The corresponding figures were 1.82(1.14–2.91) and 1.12(0.58–2.17), respectively.
Conclusion: The relationship between depression syndrome and type 2 diabetes incidence is partly explained by health behavior and socioeconomic status. Early identification and lifestyle intervention should prevent residents from risk of depression and type 2 diabetes in China.
Clinical Trial Registration Number: NCT01053195
Supported by: World Diabetes Foundation, China Postdoctoral Foundation
Disclosure: **F. Ning:** None.

871

Depression in type 1 diabetes was associated with high levels of circulating galectin-3

E.O. Melin[1,2], J. Dereke[3], M. Thunander[1,2], M. Hillman[3];
[1]Lund University, Department of Clinical Sciences, Section Endocrinology and Diabetes, Lund, [2]Research and Development, Region Kronoberg, Växjö, [3]Lund University, Department of Clinical Sciences, Diabetes Research Laboratory, Lund, Sweden.

Background and aims: Neuroinflammatory responses are implicated in depression. Galectin-3 is a soluble β-galactoside binding lectin involved in several inflammatory processes, to our knowledge not previously investigated in patients with depression or with type 1 diabetes (T1D). Depression and galectin-3 are both associated with Alzheimer's disease, increased cardiovascular and all-cause mortality. Our hypothesis was that depression is associated with high galectin-3. The aim was to explore whether depression in patients with T1D was associated with high galectin-3, controlling for metabolic variables, s-creatinine, life style factors, medication, and cardiovascular complications.
Materials and methods: Cross-sectional study including 283 T1D patients (56% men, age 18–59 years, diabetes duration ≥1 year), consecutively recruited at regular follow up visits. Depression was assessed by Hospital Anxiety and Depression Scale-depression subscale; blood samples, anthropometrics and blood pressure were collected; data was collected from medical records and the Swedish National Diabetes Registry. Galectin-3 ≥2.562 μg/l, corresponding to the 85th percentile, was defined as high galectin-3.
Results: Median (quartile$_1$, quartile$_3$) galectin-3 (μg/l) was 1.3 (0.8, 2.9) for the 30 depressed patients, and 0.9 (0.5, 1.6) for the 253 non-depressed, $P = 0.009$. Multiple logistic regression analysis (Backward Wald) showed

that depression was associated with high galectin-3 in all the 283 patients (Adjusted odds ratio (AOR) 3.5), in the 161 men (AOR 3.4), and in the 122 women (AOR 3.9). HbA1c, s-lipids, s-creatinine, blood pressure, obesity, smoking, physical inactivity, cardiovascular complications, and drugs (antidepressants, antihypertensive, lipid lowering, and oral antidiabetic drugs) were not associated with high galectin-3.
Conclusion: This is the first study to show an association between depression and galectin-3. Depression was the only explored parameter associated with high circulating galectin-3 levels in the 283 T1D patients. High galectin-3 levels might contribute to the increased risk for Alzheimer's disease, cardiovascular and all-cause mortality observed in persons with depression.
Supported by: RaD, RK
Disclosure: **E.O. Melin:** None.

872

Illness perception moderates the relationship between diabetes distress and depressive symptoms

A.S. Mocan[1,2], A. Nouwen[3], D.E. Dumitraş[4], S.S. Iancu[1], A.S. Baban[2]; [1]Center for Diabetes, Nutrition and Metabolic Diseases, County Emergency Hospital, Cluj-Napoca, Romania, [2]Babes-Bolyai University, Cluj-Napoca, Romania, [3]Middlesex University, London, UK, [4]University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania.

Background and aims: Depression is twice as common in type 2 diabetes than in general population. Both diabetes distress and illness perception play a role in the development of depression, but their exact contribution is not clear. The Common Sense Self-Regulation Model of Illness Perception posits that people have different representations and understanding of their illness impacting on self-care behaviors and emotional outcome. The aim of the present study was to assess the role of illness perceptions in the relationship between diabetes distress and depressive symptoms and whether they mediate or moderate this relationship.
Materials and methods: A sample of 431 people with type 2 diabetes were enrolled in the study. Participants completed the Illness Perception Questionnaire- Revised, Diabetes Distress Scale and Beck Depression Inventory-II. Negative events were analyzed as one single variable, life stress. Linear multiple regression analysis was used to determine the association between depressive symptoms and socio-demographic, clinical and illness perception dimensions. Then mediation and moderation equations were established based on the significant associated dimensions of illness perception to depressive symptoms. Last, hierarchical multiple linear regression was used to test the moderation when the following predictors groups were included: socio-demographic (Model 1), clinical characteristics (Model 2), life stress (Model 3), illness perception, diabetes distress and moderation (Model 4), and previous depression (Model 5).
Results: Linear multiple regression showed that of the illness perception dimensions, only perceived illness consequences were significantly associated with depressive symptoms ($\beta = 0.08$, $p = 0.02$) and were further examined. Illness consequences did not significantly mediate the relationship between diabetes distress and depressive symptoms but was a moderator between the two ($\beta = 0.2$, $p = 0.01$). The final model of the hierarchical multiple linear regression showed that illness consequences did not moderate the distress-depressive symptoms relationship ($p = 0.06$) but were independently associated to depressive symptoms ($\beta = 0.2$, $p < 0.001$) along with education ($\beta = -1.4$, $p = 0.002$), diabetes distress ($\beta = 3.1$, $p = 0.001$) and previous depression ($\beta = 5.7$, $p = 0.001$).
Conclusion: The results imply that for people with type 2 diabetes, perceived illness consequences moderated the relationship between diabetes distress and depressive symptoms only in the absence of previous depression. Psychological interventions targeting people's perceptions about the consequences of diabetes might have a positive effect on future depressive symptoms.
Disclosure: **A.S. Mocan:** None.

873

Psychological burden and self-reported foot care among participants with or without diabetic foot ulcer

P.T. Murphy[1], B. McGuire[1], S. Conneely[1], J. Bogue[1], M. Pilch[1], A. O'Loughlin[2], S. Dinneen[2], A. Doherty[2], A. Liew[2]; [1]School of Psychology, National University of Ireland, Galway, Galway, [2]School of Medicine, National University of Ireland, Galway, Galway, Ireland.

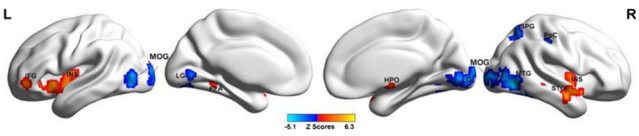
Background and aims: The role of personality, mood, diabetes-related distress, illness perception and self-reported foot care on the risk of diabetic foot ulcer (DFU) is currently unclear. In this study, we aimed to assess the differences in the personality, mood, diabetes-related distress, illness perception and self-reported foot care between participants with DFU versus those without DFU. We hypothesised that there is a significant difference in these psychological and self-care variables between participants with DFU versus those without DFU.
Materials and methods: Consecutive participants with diabetes mellitus (DM) attending Galway University Hospitals were invited to participate in this study. Following informed consent, structured interviews were conducted using standard questionnaires to simultaneously examine aspects of personality (Type D Scale-14 [DS14] and Standardised Assessment of Personality-Abbreviated Scale [SAPAS]), mood (Patient Health Questionnaire [PHQ-9]), diabetes-related distress (Problem Areas in Diabetes Scale-5 [PAID-5]), illness perceptions (Brief Illness Perceptions Questionnaire [BIPQ]; questions relating to symptoms, perceived control and understanding of diabetes) and self-reported foot care (The Summary of Diabetes Self-Care Activities Measure [SDSCA]). Continuous variables were compared using t-tests with Levene's test for equality of variances. Categorical variables were tested using Chi-squared or Fisher's exact test where appropriate.
Results: One hundred and twenty participants completed the questionnaires. There was no significant difference in gender, occupational history, type of DM, HbA1c among participants with or without DFU. Participants with DFU were significantly older (65.5 ± 9.2 years vs 59.5 ± 16.9 years; $p < 0.05$), had a significantly higher BMI (34.8 ± 7.6 kg/m^2 vs 29.6 ± 7.4 kg/m^2; $p < 0.01$), longer duration of DM (18.6 ± 12.1 years vs 11.9 ± 8.3 years; $p < 0.01$) as well as, more likely to be on insulin therapy (73.7% vs 35.6%; $p < 0.05$) and have concomitant microvascular complications (26.3% vs 14.9%; $p < 0.001$). As expected, participants with DFU perceived more symptoms of diabetes, as compared with those without DFU (4.00 ± 4.44 vs 2.36 ± 2.46; $p = 0.007$). Intriguingly, there was no statistical difference between participants with or without DFU in relation to the other illness perceptions examined, namely, personal control over diabetes or understanding diabetes. Similarly, there was no significant difference in personality, mood, diabetes-related distress, and self-reported foot care between the two groups. Post-hoc analysis of participants who were on insulin therapy showed no significant difference in the results for all questionnaires between those with or without DFU.
Conclusion: Apart from experiencing more symptoms of diabetes and, contrary to expectations, there was no significant difference in personality, mood, diabetes-related distress, other illness perceptions, and self-reported foot care between participants with DFU versus those without DFU.
Disclosure: **P.T. Murphy:** None.

874

Mediating role of ventral striatum network in the relationship between the diabetes risk multilocus genetic profile and major depressive disorder

C. He;
Department of Neurology, Affiliated ZhongDa Hospital, School of Medicine, Southeast University, Nanjing, China.

Background and aims: Patients with type 2 diabetes mellitus (T2DM) showed significantly increased risk for major depressive disorder (MDD), which result in injuries of brain structure and function. But how T2DM and MDD are pathologically connected is poorly understood. Substantially, multiple genetic loci of diabetes risk (DR) gene has been suggested to associated with depressive symptom in MDD. However, neural mechanism underlying the polygenic effects involving in DR-pathways on depression remains unclear. We aimed to investigate the neural mechanism underlying the polygenic effects in DR-pathways on the ventral striatum (VS) network in MDD using imaging genetic approach.
Materials and methods: Fifty-nine MDD patients and 37 cognitively normal (CN) subject were recruited and completed the resting-state functional MRI scan. Four loci included in the genetic profile were carefully selected based on previous research. Multivariate linear regression analysis was employed to investigate the effects of disease and multilocus genetic profile scores (MGPS) on the ventral striatum functional connectivity (VSFC) network.
Results: DR-MGPS was widely association within VSFC network, mainly in inferior frontal cortex, occipital cortex, insular, hypothalamus, and superior temporal gyrus. The partial correlation analysis revealed that the DR-MGPS was negatively correlated with depressive symptom ($r = -0.355$, $P = 0.039$). The pattern of DR-MGPS effects in fronto-striatal pathway was opposite in MDD patients compared with CN subjects. More importantly, the VS-mPFC (medial prefrontal cortex) connectivity mediates the association between DR-MGPS and anxious depression traits in MDD patients.
Conclusion: DR multilocus genetic profile makes a considerable contribution to anxious depression in MDD patients. These findings extended our understanding about bidirectional associations between T2DM and depression and give us a new insight into the pathophysiology of polygenic effects underlying the brain network in comorbid T2DM and MDD patient.



Clinical Trial Registration Number: 2017ZDKYSB117
Disclosure: **C. He:** None.

875

Perceptions of diabetes control among physicians and people with type 2 diabetes on basal insulin in Italy

K. Vaccaro[1], S. Frontoni[2], M. Comaschi[3], G. Lastoria[4], A. Nicolucci[5]; [1]CENSIS, Rome, [2]Tor Vergata University, Rome, [3]GVM Care & Research, Rapallo (GE), [4]NovoNordisk SpA, Rome, [5]Center for Outcomes Research and Clinical Epidemiology, Pescara, Italy.

Background and aims: There is limited understanding of how patients perceive diabetes control and whether it differs from physicians' perceptions. The purpose of the study was to investigate how physicians and patients with type 2 diabetes (T2D) using basal insulin perceive diabetes control.
Materials and methods: A web survey of 250 adults with T2D treated with basal insulin and 100 physicians was conducted in Italy.
Results: In defining control, physicians were more likely than patients ($p < 0.05$) to indicate that HbA1c (94% vs. 69%), frequency/severity of hypoglycaemia (93% vs. 52%) and hyperglycemia (79% vs. 61%), adherence to insulin therapy (82% vs. 69%), complications from diabetes (91% vs. 68%), and following a healthy diet (82% vs. 66%) were important. Patients were more likely than physicians to report that other factors were important, including energy levels (58% vs. 38%) and how the patient feels emotionally (56% vs. 43%). Physicians saw more obstacles making control difficult compared to patients ($p < 0.05$), including medicine side effects (63% vs. 32%), complicated schedule for taking insulin (55% vs. 26%), other health issues (60% vs. 36%), alcohol intake (46% vs. 26%), life crises (60% vs. 44%), lack of education from healthcare professionals (47% vs. 31%), diabetes doctor not understanding patient's individual situation (47% vs. 34%), work schedules (47% vs. 34%), and not having a regular daily routine (46% vs. 34%). Patients with uncontrolled diabetes were significantly more likely than those with controlled diabetes to report as barriers to control a wide array of different factors. The largest differences in perceptions of obstacles making diabetes control very/extremely difficult were: people around patient not understanding diabetes (38.0% vs. 12.3%; $p < 0.0001$), other health issues (43.6% vs. 18.8%; $p < 0.0001$), food cravings (64.2% vs. 41.3%; $p = 0.001$), not having a regular daily routine (40.9% vs. 19.4%; $p = 0.001$), skipping insulin doses (35.4% vs. 14.7%; $p = 0.002$) and alcohol intake (31.7% vs. 11.5%; $p = 0.002$). Overall, 14.4% of respondents reported that their diabetes physician had recommended adding bolus insulin therapy to their current basal insulin regimen. The most frequently reported reasons for not taking bolus insulin therapy currently, despite physician recommendation, were not wanting to add more injections (44.4%), being worried it would be too complicated (44.4%), and "doctor agreed I could wait" (44.4%).
Conclusion: Physicians and patients with T2D differ in how they define control and the obstacles they perceive. Raising the awareness of physicians about how patients' perceptions of control may differ from theirs could help improve diabetes management.
Supported by: NovoNordisk SpA, Rome, Italy
Disclosure: **K. Vaccaro:** None.

876

A machine learning algorithm can identify clusters of patients with favourable glycaemic outcomes in a pooled European Gla-300 studies (REALI): Novel signposts for clinicians?

M. Rollot[1], M. Bonnemaire[2], C. Brulle-Wohlhueter[2], L. Pedrazzini[2], E. Boëlle-Le Corfec[2], G. Bigot[3], M. Didac[4], R. Bonadonna[5], P. Gourdy[6], D. Müller-Wieland[7], O. Hacman[1], A. Chiorean[1], N. Freemantle[8]; [1]Quinten, Paris, France, [2]Sanofi, Paris, France, [3]IVIDATA, Paris, France, [4]Hospital Universitari Germans Trias i Pujol, Barcelona, Spain, [5]University of Parma School of Medicine and AOU of Parma, Parma, Italy, [6]CHU Toulouse Hospital, Toulouse, France, [7]University Clinic of the RWTH Aachen, Aachen, Germany, [8]University College London, London, UK.

Background and aims: Detecting consistent patterns of interest can be performed using data-driven subgroup discovery algorithms. These may be instrumental in exploiting large healthcare databases and enabling a patient-level and data-driven analysis aiming at identifying patient clusters for clinicians. The REALI project, a pool of a large database collecting data from more than 10,000 people with type 2 and type 1 diabetes mellitus uncontrolled with antidiabetic therapy and initiated on Gla-300 from different European Gla-300 studies, is an appropriate source of data for such an analysis. The objective of the present work was to explore patient-related variables and to identify clusters of patients who: 1. achieved HbA_{1c} drop ≥0.5% from baseline to end of study (EoS); 2. experienced hypoglycemia event during the study (HEOS), and 3. achieved the combined outcome of HbA_{1c} <7% at EoS without HEOS.
Materials and methods: Q-finder, a subgroup discovery algorithm, was first applied to a single study (Take Control) from REALI pooled database. Q-finder is a proprietary non-parametric supervised learning algorithm working with no particular assumption regarding distributions of the outcome or explanatory variables. This algorithm explores the space of explanatory variables to identify areas where the outcome specified for

the exploration shows higher or lower concentration than average. The output is a set of patient clusters, defined as combinations of variables and thresholds which characterize subpopulations with significantly higher/lower probability of experiencing an outcome of interest. All results were tested for their significance in models adjusted for confounding factors and taking into account multiple testing (Bonferroni's correction).
Results: Take Control was a 24-week interventional study of Gla-300 efficacy and safety, including 631 patients. Thousands of queries were performed by the algorithm, and 32 were found statistically significant after Bonferroni's adjustment in models including confounding factors. Among the clusters that were generated, patients with higher probability of having an HbA_{1c} reduction ≥0.5% at EoS were those with no previous insulin exposure (234 patients [pts], RR = 1.6, $p < 10^{-6}$), and those with a baseline HbA_{1c} value ≥7.9% (421 pts, RR = 1.5, $p < 10^{-10}$). Additionally, our findings suggest that patients who received a pre-hypoglycemia average insulin dose ≤33.3 U were at greater risk of HEOS (363 pts, RR = 1.7, $p < 10^{-5}$); moreover, patients with a duration of disease ≤12 years and a baseline alkaline phosphatase value ≤64 U/L were more likely to achieve the combined outcome of HbA_{1c} <7% at EoS without HEOS (127 pts, RR = 2.2, $p < 10^{-6}$).
Conclusion: Our analysis is a new promising and powerful tool, which provides simple and efficient criteria for the clinician to identify clusters of patients in whom the intervention of Gla-300 is most efficacious and safe. This approach will be applied on additional REALI datasets.
Disclosure: **M. Rollot:** None.

PS 076 Education and patient/provider perceptions

877

An interest of people with diabetes and their relatives in diabetes social media communities

L. Chernilova[1], M. Dunicheva[2], E. Patrakeeva[3], J. Zakharchenko[2], E. Shilova[4];
[1]City outpatient clinic 117, St. Petersburg, [2]City center of family planing and reproduction, St. Petersburg, [3]First Pavlov Medical University, St. Petersburg, [4]Almazov National Medical Research Centre, St. Petersburg, Russian Federation.

Background and aims: To study the interest of people with diabetes and their relatives in information about diabetes in social media. To assess the impact of previous diabetes education experience on interest in information about diabetes in social media.
Materials and methods: Participants of the diabetic community Diabet.Connect in social networks Vkontakte and Instagram were asked to fill in a special anonymous questionnaire containing questions about duration and level of diabetes control, their interest in diabetes specialized groups in social media and their previous experience of obtaining knowledge about diabetes. We used ANOVA method and linear regression model for statistical analyses.
Results: 591 people took part in the survey, 37.6% of them were relatives of people with diabetes. The majority of participants (53.5%) were 26–40 years old, 85.5% were women, 71.5% had higher education. According to the received data, the less the relatives of people with diabetes had been satisfied with previous diabetes education experience, the more they tended to devote time to searching for correct information on the Internet ($p = 0.002$). Whereas there was no correlation between these two indices for the actual people with diabetes ($p = 0.234$). For people with diabetes there was no correlation between the value of HbA1c and the interest in diabetic social media resources ($p = 0.97$). While relatives of people with diabetes had a positive feedback between the level of interest in diabetic social media and the level of HbA1c their loved ones ($p = 0.024$).The longer the patient had a duration of diabetes, the less his family and friends tended to seek information about diabetes in social media ($p = 0.000$). Dependence of interest in information in social media on the the diabetes duration for the people with diabetes was not detected ($p = 0.168$). Respondents under the age of 18 were significantly less likely to recommend diabetic communities to other people than respondents over 18 years old. At the same time gender had no influence on the willingness to recommend in any of the age groups.
Conclusion: According to the received data, diabetes communities in social media can be a very important source of information about diabetes, not only for the patients with diabetes themselves, but also for their relatives.
Disclosure: **L. Chernilova:** None.

878

Key components and mechanisms in the integration of self-management education in routine care of people with type 2 diabetes

C. Huber[1], C. Montreuil[2], A. Forbes[1];
[1]Florence Nightingale Faculty of Nursing, Midwifery & Palliative Care, King's College London, London, UK, [2]Department of Cardiovascular Prevention, Outpatient Clinic, University of Lausanne, Lausanne, Switzerland.

Background and aims: National and international guidelines recommend self-management education as a key element of care. However, only a third of people with type 2 diabetes participate in self-management education, suggesting that such programmes are insufficiently integrated in patient care processes. The aim of this study was to

understand how integration at the patient, healthcare professionals (HCPs) and system levels influences the experience of and participation in self-management education. A theoretically and empirically grounded model of integration and its relationship to self-management care delivery helps inform the design of future self-management education programmes.

Materials and methods: A case study research design was used to consider the study aim. Three self-management education programmes were purposively sampled from a quality improvement initiative. The research involved observations, semi-structured in-depth interviews and documentary analysis using NVivo 11 for data management. 20 people with type 2 diabetes and 36 HCPs participated in in-depth interviews; 88 hours of direct programme observations; and 14 programme documents were analysed. The data were triangulated and analysed thematically using a narrative approach; then synthesised across the cases using complex adaptive systems theory to guide the synthesis.

Results: Five main themes were identified representing key components of integration in self-management education: 1. Personalised care - HCPs support individual patient activities but variably implement this in care delivery; 2. Inter-professional work - HCPs may more consistently encourage self-management when they collaboratively consider and develop their understanding supported in interdisciplinary training; 3. Communal resources - self-management education is shaped by the local structures and evident in the supplied resources that direct the programme access and execution; 4. Logical programme guidance - the theoretical programme framework that philosophically guides the self-management delivery but may be differently understood or inconsistently implemented by HCPs; and 5. Programme regulators - the guidelines and recommendations that frame the implementation of the programme in routine practice and permit evaluation in the context. Four key mechanisms were identified to activate the components. These were the extent to which the participants: a) identified with the condition and activity to prioritise self-management, b) experienced social support to learn from sharing practice, c) co-created interactions to build an equal relationship, and d) accepted the paradigm of care to develop a common understanding of self-management. The mechanisms interacted within and across the components of integration. These data were used to develop a model of integrated person-centred self-management education.

Conclusion: This study has identified components and mechanisms that influence the integration of self-management education in routine care at the individual, professional and system level to understand the uptake of such programmes. The findings of the model indicate that multiple strategies are required to enhance programme integration and these strategies may impact on the adoption of individual self-management behaviours.

Supported by: Unconditional grant from the Nursing Science Foundation Switzerland

Disclosure: **C. Huber:** Grants; Nursing Science Foundation Switzerland.

879

Comparison of two therapeutic education methods in diabetic patients: a randomised controlled trial

N. Lanasri[1], N.W. Nibouche[1], L. Atif[2], L. Makhlouf[1], F. Zeraoulia[1], F. Hansal[1], L. Benaoua[1], K. Zouai[3], S. Ouadah[3], K. Messous[1], A. Chibane[4], A. Biad[1];
[1]Internal Medicine Service, Ain-taya Hospital, [2]Epidemiology Service, Blida Hospital, [3]Biochemistry Laboratory, Ain-taya Hospital, Algeria.

Background and aims: Because of its effectiveness on glycemic control and quality of life in diabetic patients, a strategy of Therapeutic Education of Patient (TEP) is being settled in Algeria. However, the TEP practices are heterogeneous and disparate. The aim of this study was to compare the impact on the bioclinic and behavioral aspects of 2 TEP methods in diabetic patients.

Materials and methods: A randomized controlled trial was performed to compare the 2 structured methods of TEP. Type 2 diabetic adult patients who had never benefited of therapeutic education were included and randomized in 2 groups: (i) patients who received a TEP program during a 5-day hospitalization and (ii) patients who received an ambulatory TEP program (1 weekly session during 2 weeks). Both programs had the same content, the same educational method, the same overall time, and were delivered by the same team. Outcomes included psycho-social and bioloclinic criteria, were measured at 0, 3 and 12 months, and compared between the 2 diabetic patients groups. HbA1c was used as major biological criteria. The overall quality of life score, evaluated by the ADDQOL questionnaire, was used as major psycho-social criteria.

Results: A total of 200 type 2 diabetic patients were included and randomized: 97 in the outpatient group and 103 in the weekly hospitalization group. Patients' characteristics were not statistically different between the 2 randomized groups. The 2 methods of TEP were effective on almost outcomes criteria. An average reduction (mean ± standard deviation) of HbA1c of 1.6% ± 1.8% was observed at 12 months in the outpatient group versus 1.9% ± 2.0% in the weekly hospitalization group. The difference is not statistically significant. The overall quality of life score increased during 12 month from 3,6/6 to 5,0 /6 in the outpatient group versus 3,5/6 to 4,9 /6 in the weekly hospitalization group. The difference is not statistically significant between the two groups.

Conclusion: The two studied TEP methods have proved effective. Because of its efficiency, the ambulatory TEP program should be recommended for diabetic patients.

Disclosure: **N. Lanasri:** None.

880

Effectiveness evaluation of diabetes Ramadan conversation map intervention for managing type 2 diabetes during Ramadan

E. Srulovici[1,2], M. Leventer-Roberts[2], M. Hoshen[2], A. Bachrach[2], M. Rotem[3], E. Shadmi[1], C. Key[3], B. Feldman[2];
[1]Nursing, University of Haifa, Haifa, [2]Clalit Research Institute, Clalit Healthcare Services, Tel Aviv, [3]Community Nursing Division, Clalit Healthcare Services, Tel Aviv, Israel.

Background and aims: Some individuals with diabetes fast during Ramadan, which has the potential to increase the risk of adverse outcomes, such as hypoglycemia, in high or moderate risk groups. The Diabetes Ramadan Conversation Map (Ramadan Map) is a self-management education group-based intervention for Muslims with type 2 diabetes specifically addressing management during Ramadan. The aim of this study was to evaluate the effectiveness of the Ramadan Map intervention among the participants using short-term clinical outcomes and healthcare utilization.

Materials and methods: This was a retrospective rolling cohort study of members of Clalit Health Services with type 2 diabetes who participated in a Ramadan Map intervention between 2014 and 2017. We used a difference-in-differences (self-comparison) design to examine the association of the Ramadan Map intervention with laboratory test results (HbA1c, glucose, LDL, HDL, and triglycerides) and healthcare utilization outcomes (primary care clinic visits and hospital admissions) comparing changes in outcomes in the six month pre- and post -intervention periods. The differences in the outcomes in the intervention year were compared to the differences in the outcomes the previous year pre- and post-Ramadan. Mixed Model Linear and random effects model with square root transformation regressions were used to estimate adjusted outcomes as appropriate.

Results: There were 1,732 members of Clalit who were included in the study. These cohort members experienced a reduction of 8.61 mg/dL in their glucose levels ($p = 0.005$) and a 0.34% decline in their HbA1c levels ($p < 0.001$) following the Diabetes Ramadan Conversation Map participation. Patient characteristics that were positively associated with this reduction in glucose levels included being 75 years or older, having diabetes for less than 10 years, and having high oral medication adherence. In a sub-group analysis of participants with pre-intervention levels

of HbA1c >7%, participants experienced a reduction of 17.02 mg/dL ($p < 0.001$) in their glucose levels and 0.63% ($p < 0.001$) in their HbA1c levels. They also experienced a reduction of 4.83 mg/dL in their LDL level ($p = 0.007$) and 0.21 fewer primary care visits ($p < 0.001$).

Conclusion: Following participation in the Diabetes Ramadan Conversation Map, Clalit members had lower glucose and HbA1c levels. The effect of participation was more pronounced among those whose baseline HbA1c was considered uncontrolled (>7%). This has important global health impact for the millions of people with type 2 diabetes for whom Ramadan can pose a challenge in disease control.

Table 1: Difference-in-Differences Analysis of Multivariable Mixed Linear Regression Models Comparing Differences Pre-And Post- Ramadan in the Intervention Year to the Year Prior

	Total study population			Sub-group of population with HbA1c >7.0%		
Outcome	Estimate	Std. Error	p-value	Estimate	Std. Error	p-value
Glucose levels						
Ramadan Map (ref- a year prior)	7.90	2.25	<0.001	14.03	2.99	<0.001
Time (ref- pre)	4.44	2.19	0.043	9.17	2.81	0.001
Ramadan Map* Time	-8.61	3.08	0.005	-17.02	4.03	<0.001
HbA1c levels						
Ramadan Map (ref- a year prior)	0.28	0.05	<0.001	0.43	0.06	<0.001
Time (ref- pre)	0.32	0.05	<0.001	0.49	0.06	<0.001
Ramadan Map* Time	-0.34	0.06	<0.001	-0.63	0.08	<0.001
LDL levels						
Ramadan Map (ref- a year prior)	4.48	1.07	<0.001	6.26	1.36	<0.001
Time (ref- pre)	2.92	1.02	0.004	4.39	1.26	0.001
Ramadan Map* Time	-2.69	1.43	0.060	-4.83	1.80	0.007
Primary care visits						
Ramadan Map (ref- a year prior)				0.21	0.03	<0.001
Time (ref- pre)				-0.04	0.03	0.298
Ramadan Map* Time				-0.21	0.05	<0.001

All regression models are adjusted for sex, age group, socio-economic status, diabetes duration, body mass index, smoking status, insulin use, and medication adherence.
Abbreviations: DID, difference-in-differences; LDL cholesterol, low-density lipoprotein cholesterol.

Supported by: Eli Lilly and company
Disclosure: **E. Srulovici:** None.

881

Treatment of type 2 diabetes: learning from patients' preferences

G. Marchesini, the Italian DCE Study Group;
Metabolic Diseases and Clinical Dietetics, University of Bologna, Italy.

Background and aims: T2DM patients' preferences are not adequately considered by diabetologists, rarely checking patients' adherence. A critical issue is the passage from oral to injectable therapy, perceived as a non-return step, marking disease progression , associated with insulin use, blood glucose monitoring and complications. This is no longer the case with GLP-1 receptor agonists (RA). We aimed to determine patients' preferences whenever treatment intensification is needed to achieve a satisfactory metabolic control.

Materials and methods: We tested preferences using the Delphi method and the procedures of Discrete Choice Experiment (DCE). We considered the following variables, covering the peculiar aspects of most recent antidiabetic drugs: administration route (oral *vs.* injectable), timing (daily *vs.*weekly), type of device (single-dose, disposable *vs.* multidose, to be adjusted), effects on body weight (neutral *vs.* weight loss), possibility of adverse events (AEs): nausea and genito-urinary infections (UTI)(no risk *vs.* high risk for both AEs). According to these 6 variables, 22 possible scenarios were built (excluding impossible, dominant and dominated scenarios), transferred into 192 paired choices. These scenarios were proposed to 491 T2DM patients, naïve to injectable treatment (8 paired choices/patient) and to 171 cases treated by GLP-1RA (12 paired choices/patient). Patients were invited to select their preferences in any paired choice in the event that current treatment might require treatment intensification, independent of their actual metabolic control. The new proposed treatments were supposed to be equally effective.

Results: The two groups were well balanced as to BMI (mean, 29.5 and 29.7 kg/m^2, respectively), and age (66 and 64). In the overall sample, every attribute had a significant effect on patients' choice. While preferences expressed according to dosing frequency, risk of nausea and risk of UTIs were similar across groups (naïve vs. non-naïve), age (>65 vs. ≤65), sex (males *vs.* females) and BMI (>28 *vs.* ≤28), two interactions were highly significant ($p < 0.01$): i) Type of delivery*Group, and ii) Weight change*BMI class, i.e., the preferred type of delivery was different according to previous experience with injectable GLP-1RAs, whereas weight loss was only significant in the presence of obesity (BMI ≥30 kg/m^2). Overall, the route of administration and type of delivery remained the most important attribute accounting for 1/3 of patients' preferences; the risk of UTI, nausea and dose frequency followed, each accounting for approx. 20% of preferences, and a small fraction was left to weight loss (6%). In a random effects logit regression model, patients' preferences were significantly modulated by the combination of different attributes, ranging from above 80% for the most preferred ones to only about 15% for the lowest. However, being naïve or non-naïve significantly affected the ranking of preferences, as indicated by Medications*Group interaction (Wald chi-square, 87.0; df, 22; $p < 0.001$). The first three preferred medications (all injectable) were the same in both groups, but the order markedly differed for other scenarios, with preferences shifted towards injectable medications in non-naïve.

Conclusion: Previous experience with injectable GLP-1RAs favored patients' willingness to accept injectable treatment, particularly when coupled with a ready-to-use device and weekly dosing. However, this treatment was the preferred one also in the total sample, independently of treatment history.

Disclosure: **G. Marchesini:** Non-financial support; Eli Lilly.

882

Using structured self-monitoring of blood glucose to improve diabetes knowledge: the SMBG study

S.N. Parsons, S.D. Luzio, D.R. Owens, SMBG Study Group;
Diabetes Research Unit Cymru, Swansea University, Swansea, UK.

Background and aims: The SMBG Study was a 12 month, multi-centre, randomised controlled trial conducted in people with established (>1 year) type 2 diabetes not on insulin therapy, with poor glycaemic control (HbA1c ≥58 to ≤119 mmol/mol or ≥7.5% ≤13%). The primary aim of the study was to determine whether HbA1c was significantly different at 12 months comparing those undertaking SMBG and a control group. The study also assessed patient reported outcomes such as diabetes specific knowledge.

Materials and methods: Participants ($n = 446$) were recruited into one of three groups; Group 1 (G1, $n = 151$), a control group receiving usual diabetes care; Group 2 (G2, $n = 147$), undertook structured SMBG with clinical review every 3 months; Group 3 (G3, $n = 148$), undertook structured SMBG with additional monthly TeleCare support from a trained study nurse. All participants received the same standardised diabetes education before randomisation. Participants in both SMBG groups (G2 & G3) and all healthcare professionals involved in the study received standardised training on SMBG technique, glycaemic pattern recognition and the use of management algorithms. The testing regimen consisted of paired testing pre and 2 hours post breakfast and main meal, 2 days each week plus a 7 point profile for 3 days the week prior to the 3 monthly study visit. In addition to clinical measurements, at each study visit quality of life measures were also administered along with questionnaires to gauge attitudes towards SMBG and measure diabetes knowledge at the beginning and end of the study.

Results: As previously reported there was a statistically significant reduction in mean HbA1c at 12 months in all three groups which was significantly improved in those undertaking SMBG versus the control group with a mean reduction in HbA1c of 0·82%/8·91 mmol/mol (95% CI −1·09 to −0·54/−11·97 to −5·85, $p \leq 0.0001$). The difference between

the two SMBG groups was not significant. At baseline diabetes knowledge, as measured by the ADKnowl questionnaire was similar across the 3 groups with a mean overall score of 65.5%. Knowledge deficits identified included the symptoms and treatments of hypoglycaemia and the effect of alcohol and particular food groups on blood glucose levels. At 12 months, diabetes knowledge had improved overall for those who completed the study (Table 1). Greater improvement in overall knowledge was seen in the SMBG groups and in particular the questions concerning the effects of exercise and food on blood glucose levels. Questions regarding hypoglycaemia and its treatment scored low across all groups at both baseline (47.7%) and at 12 months (56%).

Conclusion: Structured SMBG has been shown to be a valuable tool to improve HbA1c. In this study we have demonstrated that it can also improve overall diabetes knowledge and in particular, significantly improve specific knowledge regarding the effects of food and exercise on blood glucose levels.

Table 1: Diabetes Knowledge as measured by the ADKnowl Questionnaire

	All Participants			Control (Group 1)			SMBG Alone (Group 2)			SMBG+TeleCare (Group 3)		
	Baseline N=446	12 mths N=322	P-value	Baseline N=151	12 mths N=116	P-value	Baseline N=147	12 mths N=98	P-value	Baseline N=148	12 mths N=108	P-value
All Questions	65.5 (25.1)	71.1 (23.6)	0.056	64.7 (25.2)	69.2 (24.0)	0.273	66.2 (25.2)	73.2 (23.6)	0.178	65.5 (25.3)	73.2 (23.8)	0.178
Q7: Effect of exercise on BG *	69.4 (9.6)	80.4 (9.6)	**0.001**	66.9 (10.7)	70.1 (11.8)	0.380	67.7 (10.0)	84.6 (9.3)	**0.004**	73.6 (8.2)	87.7 (7.5)	**0.009**
Q8: Effect of food on BG**	52.4 (27.5)	62.0 (26.3)	**0.008**	51.2 (26.8)	56.8 (25.4)	0.239	53.1 (28.6)	64.1 (26.9)	0.076	52.9 (27.5)	65.7 (28.3)	**0.042**

All values are mean % (SD) correct answers

* Mean values of 3 questions ** Mean values of 8 questions

Clinical Trial Registration Number: ISRCTN21390608
Supported by: EFSD Lifescan Programme. Roche Diabetes Care GmbH equipment & unrestricted
Disclosure: **S.N. Parsons:** Grants; EFSD Lifescan, Roche Diabetes Care GmbH equipment and unrestricted grant.

883

Seasonal trends in HbA_{1c} level in adult patients with type 1 diabetes treated with personal insulin pumps

B. Matejko, B. Kieć-Wilk, S. Mrozińska, T. Klupa, M. Malecki;
Jagiellonian University Medical College, Krakow, Poland.

Background and aims: The DCCT and other studies showed that the variability in HbA1c added to mean HbA1c increases the risk of the development of complications of diabetes. There were some earlier reports showing a seasonal variability in the HbA1c level in a pediatric population.

Materials and methods: We evaluated seasonal HbA1c changes over a period of 9 years (2009–2017) in 453 adults with type 1 diabetes (T1DM, 61% women) treated with personal insulin pumps. HbA1c was measured at a tertiary care university hospital on the Bio-Rad D10 hemoglobin testing system. Differences between groups (12 groups for 12 months and six for every consecutive two months) were assessed using the Kruskal-Wallis and post hoc tests.

Results: Patients median age was 24 years [range 18–80 years], median BMI 22.9 kg/m^2 [15.6–43.7 kg/m^2] , median diabetes duration 12 years [1–40 years] and median duration on personal insulin pump 6 years [0–18 years]. A total of 1, 438 HbA1c measurements were analyzed. Median HbA(1c) level for the whole study period was 7.25% [55.7 mmol/mol] (range 4.8–12.8% [29–116.4 mmol/mol]). There were seasonal differences in HbA1c over 12 months ($p = 0.02$): The lowest HbA1c was observed in summer (July, 6.8% [50.8 mmol/mol) and the highest in winter months (from 7.1% [54.1 mmol/mol] in January to 7.3% [56.3 mmol/mol] in February). HbA1c was lower in July than in February ($p = 0.03$). After combining two consecutive months in one group seasonality of HbA1c values was still observed ($p = 0.008$). Median HbA1c in July/August (6.9% [51.9 mmol/mol]) was lower than in January/February (7.2% [55.2 mmol/mol], $p = 0.01$) and in November/December groups (7.3% [56.3 mmol/mol], $p = 0.02$).

Conclusion: To our knowledge this is the first report concerning HbA1c seasonal fluctuations in well controlled cohort of adult T1DM patients treated with insulin pumps. Seasonal changes of HbA1c levels (peak in summer months, drop in winter months) in such a group of patients should be considered in patient education, diabetes management and epidemiological interpretation.

Disclosure: **B. Matejko:** None.

884

Factors predictive of HbA_{1c} in insulin pump users in Galway University Hospital

C.E.H. Fang[1,2], E. O'Sullivan[1,2];
[1]Centre for Diabetes, Endocrinology and Metabolism, Galway University Hospitals, Galway, [2]School of Medicine National University of Ireland Galway, Galway, Ireland.

Background and aims: Insulin pump therapy in Type 1 Diabetes Mellitus (T1D) patients has been shown to improve glycaemic control, reduce hypoglycaemic episodes and improve quality of life compared to multiple dose injection therapy (MDI). However less improvement in outcomes in adults with T1D using pump therapy compared to those using MDI was shown when both received structured education (SE). It is not always possible for patients to access SE and there are no studies showing whether SE prior to starting insulin pump therapy leads to better outcomes. The purpose of this study was to first, determine if pump therapy was beneficial in reducing HbA1c for adult T1D patients managed in diabetes clinic in Galway University Hospital (GUH). Second, to determine factors predictive of HbA1c such as SE.

Materials and methods: This was a retrospective cohort study on patients with T1D who started insulin pump from January 2014 to December 2017 in UHG. Data was gathered from the DIAMOND database and analysed with Student's t-tests.

Results: 76 patients were started on insulin pump therapy from 2014 to 2017. 4 patients did not have documented pre-pump HbA1c on the DIAMOND system and were excluded. Of the 72 patients, there were 41 females and 31 males, mean age 41.5 ± 12.4 years (range 16–69), average duration of diabetes 22.6 ± 12.0 years (range 10–55). Average age at start of insulin pump was 39 ± 12 years. Average number of clinic visits per year before pump was 1.6 and after pump was 1.4. Baseline HbA1c average 67.1 ± 16.6 mmol/mol (range 59–126). 25 patients were on continuous glucose monitoring pumps. 60 patients underwent a SE programme (DAFNE). Overall, at each time point; from before starting pump therapy, to 3 months, 6 months, 12 months and 24 months, there was a trend toward reduction in HbA1c. There was no significant difference between the HbA1c of patients who had SE and those that did not at baseline or at any of the follow-up time points. Patients who started pump therapy with a high HbA1c (≥75 mmol/mol) had a significant reduction in HbA1c at all time points. However the change in HbA1c for patients who started at a lower HbA1c (≤53 mmol/mol and 54–74 mmol/mol) was not significant.

Conclusion: In the cohort of insulin pump users, there was no difference in HbA1c for those who had SE versus no SE, suggesting that for selected patients who cannot access this, it is reasonable to start insulin pump therapy after one to one educational sessions. For those starting on insulin pump with a high HbA1c (≥75 mmol/mol) there was a significant reduction in HbA1c but not for those staring with a lower HbA1c. Selected

patients who have not attended a SE programme but have had 1:1 education from diabetes educators can benefit from pump therapy, as they may have very elevated HbA1c. Other factors such as sex, age at diagnosis, duration of diabetes and number of clinic visits per year were not shown to be significant.

HbA1c	PrePump	3 months	6 months	12 months	24 months
mean	67.14	68.19	65.72	64.08	63.69
SD	16.60	15.35	16.18	11.55	11.13
n	72	36	32	38	26
p value		0.4	0.3	0.2	0.2

Disclosure: **C.E.H. Fang:** None.

PS 077 Therapeutic adherence and satisfaction

885

Outpatients' wait times and healthcare professionals' communication behaviours may influence on treatment satisfaction and the intention to drop out

S. Sato[1], T. Tosaki[1], C. Oshiro[1], A. Inagaki[2], M. Kondo[3], S. Tsunekawa[3], Y. Kato[3], H. Kamiya[3], J. Nakamura[3];
[1]Tosaki Clinic for Diabetes and Endocrinology, Nagoya, [2]Diabetes and Endocrinology, Japanese Red Cross Nagoya Daini Hospital, Nagoya, [3]Division of Diabetes, Aichi Medical University School of Medicine, Nagakute, Japan.

Background and aims: We studied influences of perceptions of patients' wait times and communication behaviors of healthcare professionals on treatment satisfaction and the intention to drop out in diabetes outpatients.
Materials and methods: A questionnaire was sent to 888 outpatients with diabetes or impaired glucose tolerance who visited our clinic in April 2016. We also measured time spent in clinics.
Results: Totally, 759 patients responded to the questionnaire; 717 valid responses were obtained for statistical analyses. In the group with the intention to drop out, many replied that they felt that "the wait time from physical examination to accounting" was long; this association was significant ($P = 0.009$, χ^2 test). Communication behaviors of healthcare professionals were also associated with the presence of the intention to drop out. Among patients who intended to drop out, many replied negatively to the following items: "explanation of test results and treatment strategy," "explanation of caution in daily life," "clear and understandable responses to questions," and "communicative atmosphere." Among those who did not intend to drop out, fewer responded negatively to those items. The associations were all significant ($P < 0.001$, $P < 0.005$, $P = 0.001$, and $P = 0.002$, respectively, χ^2 test). Median scores of the first factor of the Diabetes Treatment Satisfaction Questionnaire (DTSQ) were lower in those who intended to drop out than in those who did not (52 vs 632 patients); the DTSQ first factor score was significantly associated with the presence of the intention to drop out ($P < 0.001$, Mann-Whitney U test). Receiver operating characteristic curve analysis revealed the DTSQ score as a significant predictor of the presence of the intention to drop out (area under the curve = 0.746, $P < 0.001$), with an optimal cut-off of 22.5 (sensitivity 70.6%, specificity 67.3%). We compared perceptions about wait times and healthcare professionals' communication behaviors between patients with scores above and below the cut-off. Among less-satisfied patients, many replied that the "wait times (from blood collection to physical examination, from physical examination to accounting, and total)" were long and replied negatively to the items of "explanation about test results and treatment strategy," "caution in daily life," "clear and understandable response to question," and "communicative atmosphere"; here, all associations were significant. Time spent in clinics was also significantly associated with the "perception of wait time (total)."
Conclusion: The DTSQ first factor score cut-off in this study was consistent with the value reported by Saisho et al among 139 patients. However, the findings of this study in a larger sample size suggest that improved treatment satisfaction may reduce incidences of drop out. Thus, improved wait times and better communication behavior from healthcare professions may improve patient satisfaction and reduce the intention to drop out and possibly the dropout rate.
Disclosure: **S. Sato:** None.

886

A novel liquid chromatography tandem mass spectrometry method detects high non-adherence rates in people with diabetes

P. Gupta[1], A.A. Mohamed[1], P. Patel[1], A. Burns[1], M. Saeed[1], D. Lane[1], S. Seidu[2], K. Khunti[2];
[1]Chemical Pathology and Metabolic Diseases, University Hospitals of Leicester NHS Trust, Leicester, [2]Diabetes Care Centre, University of Leicester, Leicester, UK.

Background and aims: Type 2 diabetes mellitus (T2DM) is a complex chronic disease associated with life-threatening complications. Poor glycaemic control in people with T2DM is associated with higher morbidity and mortality. It has been shown that up to one-third of people with T2DM fail to derive optimal benefit from therapy because of medication non-adherence. Few practical tools exist to accurately and routinely detect non-adherence to therapy. We have set up a unique and robust biochemical method that can detect 60 most common cardiovascular medications in a spot urine sample.
Aim To determine the prevalence of non-adherence to antidiabetic, antihypertensive and lipid-lowering medications in people with T2DM in the primary care settings.
Materials and methods: 256 patients from six different general practice (GP) surgeries in Leicestershire, UK agreed to aliquots from urine samples collected for routine albumin-creatinine ratio (ACR) analysis to be assessed for adherence to antidiabetic, antihypertensive and lipid-lowering drug intake using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Basic demographic information (age, sex, GP surgery) and biochemical laboratory values for ACR, HbA1c and lipid profile were retrieved retrospectively from laboratory database. Data on the number and doses of prescribed medications were collected from the patients' prescription lists.
Results: The clinical and biochemical characteristics of the patients are shown in table 1. Overall, 27% patients ($N = 69$) were completely or partially non-adherent to antidiabetic, antihypertensive and/or lipid lowering medications (total non-adherence 5.5%, partial non-adherence 21.5%). The highest rate of non-adherence was for statins (21%, $N = 42$ of 198 analysed drugs) while non-adherence to antidiabetic medications was 8% ($N = 27$ of 337 analysed drugs). After adjusting for age and sex using ANCOVA analysis, mean of ACR, HbA1c and lipid profiles were statistically higher for the non-adherent patients compared to adherent patients [Table 1].
Conclusion: Our first of its kind study shows that non-adherence to antidiabetics, anti-hypertensives and lipid-lowering therapy is common in patients with diabetes in primary care settings. Markers of glycaemic and lipid control as well as renal function were significantly worse in non-adherent patients compared to adherent patients. LC-MS/MS urine analysis could be used to objectively detect non-adherence in primary care and could be used to inform clinical decisions of treatment alteration and improve patient outcomes.

Table 1 General demographics, clinical and biochemical characteristics of T2DM patients

	All patients	Adherent	Any non-adherence	Total non-adherent	Difference (P value)*	Difference (P value) †
N (%)	256	187 (73%)	69 (27%)	14 (5.5%)		
Age (years ± SD)	59.93 ± 12.43	60.33 ± 12.38	58.83 ± 12.59	46.07 ± 12.02	1.50 (0.391)	
Sex – Male N (%)	140 (54.7%)	105 (56.1%)	35 (50.7%)	7 (50%)	-	-
Average number of prescribed medications	4 (3-6)	4 (2-6)	5 (3-6)	3 (2-4)	-1 (0.262)	1 (0.187)
Average number of screened medications	3 (2-4)	3 (2-4)	4 (3-6)	2 (1-3)	-1 (0.043)	1 (0.323)
Average number of detected medications	3 (2-4)	3 (2-4)	2 (1-4)	0	1 (0.026)	3 (0.005)
ACR (mg/mmol creat)	7.89 ± 25.08	4.46 ± 10.95	16.87 ± 43.34	20.55 ±44.35	-11.69 (0.002)	-16.10 (0.001)
HbA1c (mmol/mol)	54.95 ± 14.39	53.12 ± 10.95	59.96 ± 20.40	62.10 ± 31.64	-6.52 (0.001)	-5.10 (0.193)
Total Cholesterol (mmol/L)	3.95 ± 0.99	3.85 ± 0.68	4.27 ± 1.52	5.52 ± 2.90	-0.40 (0.004)	-1.54 (<0.001)
LDL Cholesterol (mmol/L)	2.07 ± 0.59	2.02 ± 0.53	2.23 ± 0.73	2.61 ± 0.87	-0.21 (0.014)	-0.375 (0.039)
HDL Cholesterol (mmol/L)	1.40 ± 2.56	1.33 ± 1.28	1.84 ± 5.12	1.00 ± 0.24	-0.45 (0.482)	0.18 (0.630)
TC:HDL ratio	3.46 ± 0.98	3.33 ± 0.89	3.83 ± 1.13	4.97 ± 1.19	-0.51 (<0.001)	-1.37 (<0.001)

* Difference in mean or median between total adherent and any (total and partial) non-adherent patients.
† Difference in mean or median between total adherent and total non-adherent patients.
*† Independent t-test was used to compare means of ages. ANCOVA analysis was used to adjust for age and sex when comparing the means of ACR, HbA1c and lipid profile (difference is based on estimated marginal means). Mann-Whitney U test was used to compare the median of number of drugs prescribed, screened for and detected.

Clinical Trial Registration Number: UHL registration number: 7766e
Disclosure: **P. Gupta:** None.

887

Patient-level predictors of delay in insulin initiation and periods of insulin discontinuation among adults with type 2 diabetes

L. Fisher[1], W. Polonsky[2], D. Hessler[1], J.I. Ivanova[3], U. Desai[4], D. Cao[5], F.J. Snoek[6], M. Perez-Nieves[5];
[1]University of California, San Francisco, San Francisco, USA, [2]Behavioral Diabetes Institute, San Diego, USA, [3]Analysis Group, Inc., New York, USA, [4]Analysis Group, Inc., Boston, MA, USA, [5]Eli Lilly and Company, Indianapolis, USA, [6]VU University Medical Center, Amsterdam, Netherlands.

Background and aims: About 30% of adults with type 2 diabetes (T2D) who are encouraged to initiate basal insulin (BI) by their health care professionals (HCP) initially refuse to do so, and after initiation, many patients use BI only intermittently. This study examined whether key factors, including patient demographics, T2D history and patients' observations of the impact of insulin on family/friends, were associated with delays in BI initiation and problematic BI persistence over time (i.e., having BI discontinuation for $\geq$7 days since BI initiation).

Materials and methods: 594 T2D adults across seven countries (Brazil, Canada, Germany, Japan, Spain, United Kingdom, United States), who indicated initial reluctance to begin BI but eventually agreed to do so, participated (mean age = 53.3 [SD = 11.3], 56.7% male). Two BI use behaviors were assessed: 1) immediate initiation when recommended initially vs. delay; and 2) BI interruption (i.e., discontinuation for ≥7 days) since BI initiation. Examined patient factors included: demographics, T2D history, and patients' perceptions of impact of insulin on overall health and mood in insulin-using family/friends.

Results: In models controlling for patient characteristics and length of time using BI, and adjusting for clustering by country, a delay in beginning BI was significantly ($p < 0.05$) associated with: short duration of T2D (odds ratio [OR] = 0.95), ≥1 severe hypoglycemic episode prior to BI initiation (OR = 1.87) and the belief that insulin use in family/friends had led to their poorer overall health over time. (OR = 1.27). Similarly, interruptions in BI use over time were significantly associated with: younger age (OR = 0.96), higher BMI (OR 25–29.9 kg/m^2 = 0.50; ≥30 kg/m^2 = 0.39), prior use of injectables (OR = 0.38), ≥1 severe hypoglycemic episode prior to BI initiation (OR = 2.55), and the belief that prior insulin use in family/friends had led to poorer overall mood over time (OR = 0.26).

Conclusion: We find that younger age, previous use of injectables, previous severe hypoglycemic episodes, fewer years with T2D, and perceived experiences of family/friends' BI use are significantly associated with delay in BI initiation or problematic BI persistence over time. To enhance a smooth transition to BI and to help maintain BI use over time, HCPs need to consider patients' T2D history and their observations and interpretations of the effects of BI use in others.

Supported by: Eli Lilly and Company

Disclosure: **L. Fisher:** Other; Research support provided by Eli Lilly and Company.

888

On Time: an innovative online discussion tool to overcome barriers to insulin initiation

P. Filteau[1], J. Gilbert[2], G. MacNeill[3], E. Cooke[4], M. Vallis[5], M. Groleau[6], P. Javadi[7], C. Lebovics[6];
[1]Family Doctor, St-Marc-des-carrieres, Canada, [2]Sunnybrook Health Sciences Centre, Toronto, Canada, [3]Mount Sinai Hospital, Toronto, Canada, [4]Elaine Cooke Consulting, Maple Ridge, Canada, [5]Dalhousie University, Halifax, Canada, [6]Sanofi Canada, Laval, Canada, [7]Sanofi, Paris, France.

Background and aims: The On Time education program is an innovative, online point-of-care tool designed to uncover and address barriers to insulin initiation, and to facilitate timely insulin initiation, when appropriate, in insulin-naïve individuals with type 2 diabetes (T2D).

Materials and methods: A total of 195 health care professionals (HCPs) completed online profiles of 1025 insulin-naïve individuals with T2D currently treated with non-insulin antihyperglycemic agents (NIAHAs) and with HbA_{1c} levels above the Diabetes Canada target (for most individuals ≤7%). After having completed the discussion tool and questionnaires, participating HCPs were asked to evaluate the program, tool, and questionnaires.

Results: Mean age of participants was 61.2 years; 55% were male; mean duration of diabetes was 10.5 years. For the majority of participants (70%) the recommended HbA_{1c} target was ≤7.0%. On average, participants were prescribed 2.6 NIAHAs, mainly metformin and dipeptidyl peptidase-4 inhibitors. Prior to using the On Time discussion tool, only 23% of individuals with diabetes were judged as likely (16%) or extremely likely (7%) willing to initiate insulin. The leading barriers to initiating insulin were apprehension toward needles/injections (59%), belief that insulin was complicated (56%), and psychological insulin resistance (45%). After using the On Time discussion tool, participants' perceived willingness to initiate insulin increased (likely: 34%, extremely likely: 28%). Initiation of insulin was planned in 77% of participating individuals. The evaluation questionnaire was completed by 149 HCPs (76.4%), and showed that the discussion tool was perceived to help HCPs address insulin-related barriers (82%), positively impacted their practice (79%), and improved their approach when discussing insulin initiation with individuals with diabetes (76%).

Conclusion: Identifying the barriers to initiating insulin and providing educational interventions to address them may help to improve insulin acceptance.

Supported by: Sanofi

Disclosure: **P. Filteau:** Grants; Merck, Pfizer, Sanofi Aventis, Novo Nordisk, Astra Zeneca, Janssen. Honorarium; Merck, Pfizer, Sanofi Aventis, Novo Nordisk, Bristol Myers Squibb, Boehringer Ingelheim, Life Scan, Eli Lilly, Astra Zeneca, Bayer, Abbott, Janssen.

889

Attitudes among adults with type 2 diabetes affecting insulin initiation and discontinuation

M. Perez-Nieves[1], W. Polonsky[2,3], L. Fisher[3], F. Snoek[4,5], I. Hadjiyianni[6], D. Cao[1], J. Ivanova[7], U. Desai[8], D. Hessler[3];
[1]Eli Lilly and Company, Indianapolis, USA, [2]Behavioral Diabetes Institute, San Diego, USA, [3]University of California, San Francisco, USA, [4]VU University Medical Center, Amsterdam, Netherlands, [5]Academic Medical Center, Amsterdam, Netherlands, [6]Eli Lilly and Company, Bad Homburg, Germany, [7]Analysis Group, Inc, New York, USA, [8]Analysis Group, Inc., Boston, USA.

Background and aims: Approximately 30% of patients with type 2 diabetes (T2D) are reluctant to initiate basal insulin when recommended by their healthcare provider (HCP). Even after initiation, many patients use insulin intermittently. Examining patients' attitudes towards insulin and their links to subsequent use can aid HCPs in improving insulin uptake and adherence.

Materials and methods: 594 T2D adults across seven countries who indicated initial reluctance to begin basal insulin but eventually agreed to do so [mean age = 53.3 (SD = 11.3), 56.7% male], participated in a survey as part of the EMOTION (AccEpting Insulin TreatMent for Reluctant PeOple with Type 2 DIabetes Mellitus - A GlObal Study to IdeNtify Effective Strategies) study. In addition to self-reported insulin behavior (immediate initiation vs. delay, discontinued use for ≥7 days), attitudes towards insulin immediately prior to initiating insulin were captured using a modified version of the Insulin Treatment Appraisal Scale (mITAS).

Results: An exploratory factor analysis of the 21 negatively worded mITAS items (1 = strongly disagree to 5 = strongly agree) yielded four factors: "concerns about injections" ($M = 3.4$, SD = 1.0), "failed diabetes management" ($M = 3.8$, SD = 0.8), "increased disease severity" ($M = 3.0$, SD = 0.9), and "concerns about side effects" (i.e., weight gain, hypoglycemia) ($M = 3.4$, SD = 0.8). In models controlling for patient characteristics and clustering by country, higher scores (more negative appraisal) in all four mITAS factors were linked with greater likelihood of delaying insulin initiation (ORs: 1.34 to 1.73; all $p < 0.05$). Only "concerns about side effects" was associated with discontinuation and inversely related (OR = 0.60, $p < 0.05$). Overall, higher mITAS scores were associated with being female, younger, more recently diagnosed, and without previous experience with injectable medications.

Conclusion: Negative patient appraisals of insulin are linked with delays in their insulin initiation in T2D; while other factors may be more impactful for predicting discontinuation. These patient attitudes should be considered when discussing insulin initiation.

Disclosure: **M. Perez-Nieves:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

890

The impact of cardiovascular disease family history on drug adherence in patients with hypertension and type 2 diabetes

B.D. Schaan[1,2], L.G. Bottino[1], G.H. Telo[1];
[1]Universidade Federal do Rio Grande do Sul, Porto Alegre, [2]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Background and aims: The impact of family history for some diseases such as breast cancer has been associated with better personal health care and treatments adherence. For chronic diseases, however, literature is still not clear. The aim of this study was to investigate the impact of cardiovascular disease (CVD) family history and diabetes family history on the adherence to treatment in patients with hypertension and type 2 diabetes.
Materials and methods: We conducted a cross-sectional study in a tertiary hospital in Southern Brazil. We included patients with type 2 diabetes, hypertension and age <65 years old. Trained researchers collected all clinical and laboratory data. Family history for CVD was defined as having a first-degree relative affected by CVD at age 60 or younger, and family history for diabetes was defined as having a first-degree relative diagnosed with diabetes. The Morisky Questionnaire, which is a 4-item survey designed to evaluate drug adherence, was used in this study. Based on the answers (yes/no), patients were classified as adherent when all the answers were "no", and as non-adherent when one or more "yes" answers were provided. Statistical analyses were performed using SPSS version 18.0; continuous variables were analyzed using the Student t-test; categorical variables were analyzed using the chi-square test. We used 5% as the cutoff of *P* value. This study report followed the STROBE guideline.
Results: The study population was obtained from a consecutive sample of 2342 patients screened, from which 302 were randomly selected. Mean age was 57.2 ± 6.1 years old; 65% were female and 50% were obese (body mass index ≥30.0 kg/m^2). The mean HbA1c was 8.0% (range, 6.9–9.6%), and only 29% of patients had an HbA1c ≤7.0%, which is considered the goal for most adults with diabetes. Mean systolic blood pressure was 142.4 ± 17.8 mmHg, and 27% of patients had a well-controlled blood pressure (systolic <130 mmHg). A hundred and forty-two patients (47%) were classified as non-adherent; 93 (31%) had a family history of CVD and 237 (79%) had a family history of diabetes. Patients with family history for CVD were more frequently classified as adherents than those without CVD family history (63% vs. 49%; p = 0.035). However, this same pattern was not seen in patients with family history for diabetes (50% vs. 64%; p = 0.059). Patients with family history for CVD more likely reported "no" to the Morisky Questionnaire item *"Sometimes, do you neglect to take medication?"* (75% *vs.* 62%; p = 0.041). The other Morisky items reports were not different between patients with and without family history for CVD or diabetes.
Conclusion: Our results showed that patients with family history of CVD had a better drug adherence. This is in agreement with the literature that shows that patients with family history of aggressive and disabling diseases, such as myocardial infarction and stroke, tend to be more adherent to drug therapy. Having diabetes in their family did not change the way in which patients faced their disease, possibly because the relationship between diabetes and complications was not so clear for most patients.
Supported by: FIPE-HCPA; PIBIC-UFRGS
Disclosure: **B.D. Schaan:** None.

891

Storage conditions of insulin in domestic refrigerators and carried by patients: insulin is often stored outside recommended temperature range

K. Braune[1], L.A. Kraemer[2], A. Zayani[2], J. Weinstein[2], L. Heinemann[3];
[1]Department of Paediatric Endocrinology and Diabetes, Charité - Universitaetsmedizin Berlin, Berlin, [2]MedAngel BV, Nijmegen, Netherlands, [3]Science & Co, Düsseldorf, Germany.

Background and aims: Not much is known about how patients with diabetes store their insulin in daily life. Objective of our study was to monitor temperature of refrigerated and carried insulin in industrialized countries to investigate how often storage conditions do not meet the manufacturers' recommendations regarding temperature range.
Materials and methods: Patients (n = 338; 46% located in the US, 41% in the EU) put a total number of 400 temperature loggers (MedAngel ONE, Netherlands) next to their insulin into their refrigerator or diabetes bag. Temperature was measured every 3 min (up to 480 times per day). Measurements were automatically sent to an app and stored in a protected online database. Whenever temperature exceeded the recommended range (2–8°C for refrigerated insulin, 2–30°C when opened or carried as a spare), the user was notified by an alarm. Data was collected from Nov 2016 to Feb 2018 with an average protocol length of 49 days.
Results: A total number of 400 temperature logs from individual sensors were analyzed (230 for refrigerated, 170 for carried insulin). Deviations were found in 315 (78.8%) logs (230 (100%) refrigerated, 85 (50%) carried). For refrigerated insulin, temperature recorded by an average sensor was out of the 2–8°C range for 11.31% of the time (10.10%–13.10%; 2h43min per day) with an average deviation of 3.68K (SD 5.02K). For carried insulin, temperatures were out of 2–30°C range 0.54% of the time (0.48%–0.64%; 8min per day) with an average deviation of 1.11K (SD 1.24K). 16.5% of sensors measured temperatures below 0°C (57 for refrigerated, 9 for carried insulin).
Conclusion: Long-term storage conditions of insulin are known to have an impact on its blood-glucose lowering effect. These observational data showed that in a significant number of cases insulin was exposed to temperatures outside the recommended range, especially when refrigerated. Thus, domestic refrigerators may pose an underestimated risk for insulin quality. The extent of how temperature deviations in storage affect insulin efficacy and patient outcomes needs further systematic Investigation.
Disclosure: **K. Braune:** None.

892

Do patients with type 1 diabetes and type 2 diabetes understand the medical terms related to their disease?

N. Novoselova[1], A. Mosikian[1], O. Martyanova[2], E. Patrakeeva[3], A. Zalevskaya[3];
[1]Almazov National Medical Research Centre, St. Petersburg, [2]Outpatient clinic № 27, St. Petersburg, [3]Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russian Federation.

Background and aims: 1) To evaluate how patients with diabetes understand the meaning of medical terms related to their disease. 2) To determine social and demographic factors which influence on medical terms understanding. 3) To analyze the impact of medical terms understanding on HbA_{1c}level.
Materials and methods: We asked the group of endocrinologists about frequently used T1D and T2D related terms. Then we created questionnaires based on specialists' answers. The questionnaire for T1D patients included 10 terms: hypoglycemia, glycated hemoglobin, diabetic retinopathy, diabetic nephropathy, diabetic polyneuropathy, diabetic ketoacidosis, bolus insulin, basal insulin, glycemic index, insulin sensitivity factor. The questionnaire for T2D patients included: hyperglycemia, insulin resistance, hypertension, glycated hemoglobin, diabetic polyneuropathy, lipid profile, hypoglycemia, obesity, body mass index, diabetic nephropathy. Then T1D and T2D patients explained the meaning of each term that they understand. The definition accuracy was independently scaled from 0 to 10 by 3 endocrinologists with a «0» is for «completely wrong» and «10» - for «completely correct». Information about gender, age, duration of diabetes, level of education, the last HbA_{1c} was also collected. Patients with a history of mental disorders were excluded. To analyze the results we used Wilcoxon test and linear regression model.

Results: 89 patients with T1D (27% men, HbA_{1c} (mean ± SD) 7.95 ± 1.77%) and 86 patients with T2D (27% men, HbA_{1c} (mean ± SD) 8.11 ± 1.91%) were included in the study. T1D patients received a greater overall score for understanding the terms than T2D patients ($p < 0.0001$) - 57.84 ± 22.66 and 39.33 ± 22.02 from 100, respectively. 38 (42.7%) T1D participants reported that they know all 10 terms, but only 15 (16.8%) respondents understand terms correctly. The greatest frequency of misunderstanding was for insulin sensitivity factor, diabetic polyneuropathy and glycemic index. In T2D group, 9 (10.5%) patients answered yes for all terms, but really know terms only 2 (2.3%) participants. Less than one third patients with T2D knew what means insulin resistance, lipid profile, diabetic polyneuropathy and body mass index. In both groups, the total score of the terms knowledge did not correlate with the HbA_{1c} level ($p = 0.70$ and $p = 0.32$ for T1D and T2D group, respectively). Older women with T1D received a lower overall score in assessing knowledge of medical terms ($p = 0.011$). However, T2D patients of different age received the same score ($p = 0.324$). In T1D group, we revealed tendency to increase an overall score for understanding the terms with increasing duration of diabetes, but it was not statistically significant ($p = 0.18$). The level of education did not influence understanding the terms in both groups.
Conclusion: Our study showed that most patients do not understand the meaning of medical terms, which their doctors use. However, terms understanding does not influence on glycemic control. Medical doctors should use simpler explanations while they consult their patients.
Disclosure: **N. Novoselova:** None.

PS 078 Hypoglycaemia rates with basal insulin

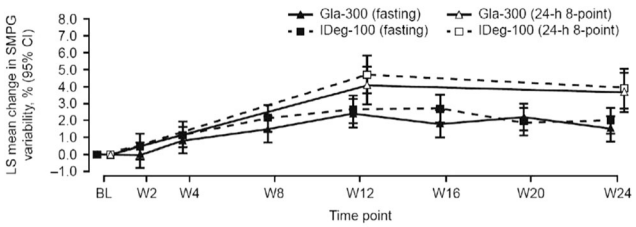
893

Similar variability of fasting and 24-hr self-measured plasma glucose with Gla-300 vs IDeg-100 in insulin-naive adults with type 2 diabetes: the randomised BRIGHT trial

R. Ritzel[1], A. Cheng[2], Z. Bosnyak[3], E. Boëlle-Le Corfec[3], A.M.G. Cali[4], X. Wang[5], J. Frias[6], R. Roussel[7], G.B. Bolli[8];
[1]Klinikum Schwabing and Klinikum Bogenhausen, Städtisches Klinikum München GmbH, Munich, Germany, [2]Department of Medicine, University of Toronto, Toronto, Canada, [3]Sanofi, Paris, France, [4]Sanofi, Tokyo, Japan, [5]Sanofi, Beijing, China, [6]National Research Institute, Los Angeles, USA, [7]INSERM, UMR_S 1138, Centre de Recherche des Cordeliers, Paris, France, [8]Perugia University Medical School, Perugia, Italy.

Background and aims: BRIGHT investigated the efficacy and safety of insulin glargine 300 U/ml (Gla-300) and insulin degludec 100 U/ml (IDeg-100) in insulin-naïve participants with uncontrolled type 2 diabetes (T2DM). The primary objective (non-inferiority of Gla-300 vs IDeg-100 in HbA_{1c}change from baseline to week 24) was met. This analysis examined a secondary endpoint: change in variability of fasting and 24-h self-measured plasma glucose (SMPG).
Materials and methods: BRIGHT was an open-label, randomised, parallel-group, 24-week study. Participants were randomised to Gla-300 or IDeg-100, titrated to a target fasting SMPG of 4.4–5.6 mmol/l.
Results: Eight-point SMPG profiles were similar for both groups at week 24. Mean baseline coefficient of variation (CV) of ≥3 fasting SMPG measurements over 7 days was 13.73% and 14.63% for Gla-300 and IDeg-100, respectively. Change in fasting SMPG variability (standard error [SE]) to week 24 was 1.49% (0.39) and 1.97% (0.39) for Gla-300 and IDeg-100, respectively (least squares [LS] mean difference [95% CI] −0.48 [−1.49 to 0.53]) (**Figure**). Mean baseline CVs for 8-point profiles (24-h SMPG) were 22.60% and 23.41% for Gla-300 and IDeg-100. Mean change in 24-h SMPG variability (SE) was 3.70% (0.59) and 3.95% (0.60) for Gla-300 and IDeg-100 at week 24 (LS mean difference −0.25 [−1.72 to 1.22]).
Conclusion: Gla-300 and IDeg-100 had similar variability of fasting and 24-h SMPG over the 24-week treatment period in BRIGHT.

Figure. LS mean change in the variability of fasting SMPG and 24-h 8-point SMPG



Variability was assessed by the CV calculated over at least 3 SMPG measurements during the 7 days preceding the given visit. For all patients receiving rescue medication during the 24-week period, only the post-baseline fasting SMPG or 24-h SMPG measurements before rescue and during the 24-week on-treatment period are presented. BL, baseline;
CI, confidence interval; CV, coefficient of variation; LS, least squares; SMPG, self-measured plasma glucose; W, week

Clinical Trial Registration Number: NCT02738151
Supported by: Sanofi
Disclosure: **R. Ritzel:** Employment/Consultancy; Novo Nordisk, Sanofi, Servier. Lecture/other fees; Astra Zeneca, Novartis, MSD, Berlin-Chemie, Boehringer Ingelheim, Novo Nordisk, Sanofi, Lilly, Servier, Medscape.

894

Switching to insulin glargine 300 U/ml in patients with type 2 diabetes on basal insulin supported oral therapy (BOT) improves glycaemic control

J. Seufert[1], A. Fritsche[2], H. Anderten[3], K. Pegelow[4], S. Pscherer[5], M. Pfohl[6]; [1]Department of Internal Medicine II, University Hospital, Medical Faculty, University of Freiburg, Freiburg, [2]Department of Medicine IV, University Hospital, Tuebingen, [3]Joint Practice Dres. Anderten-Krok & Partner, Hildesheim, [4]Sanofi-Aventis Deutschland GmbH, Berlin, [5]Department of Internal Medicine III, Sophien- und Hufeland-Klinikum, Weimar, [6]Department of Internal Medicine I, Evang. Bethesda-Krankenhaus, Duisburg, Germany.

Background and aims: The prospective, single-arm, observational TOP-2 study investigated the effects of switching patients (pts) with type 2 diabetes mellitus (T2DM) in Germany (n = 1,662), Austria (n = 103) and Switzerland (n = 100), uncontrolled (HbA_{1c} 7.5–10%) on BOT with other basal insulins (BI), to insulin glargine 300 U/mL (Gla-300) in primary care.

Materials and methods: Primary endpoint was the proportion of pts achieving fasting plasma glucose (FPG) values of ≤110 mg/dL after 6 and 12 months of Gla-300 treatment, respectively. Secondary endpoints included changes over time in HbA_{1c}, FPG, body weight (BW) and insulin dose, hypoglycaemia incidence and safety. Here we report the results of pts recruited at German sites with 12 month results available (n = 679).

Results: At baseline, mean (±SD) age was 64.7 ± 10.3 years, mean BMI 32.0 ± 5.7 kg/m², 56.3% of pts were male, and 54.8% were obese (BMI ≥30 kg/m²). Mean T2DM duration was 11.2 ± 6.8 years. Mean HbA_{1c} was 8.23 ± 0.8%, and mean FPG was 172.7 ± 43.9 mg/dL. The mean pre-specified individual HbA_{1c} target was 7.0 ± 0.4%. Pre-switch BI was predominantly insulin glargine 100 U/mL (Gla-100; 49.2%), and most commonly used oral therapy was metformin ± DPP-4 inhibitors (46.4%). At 12 months, the primary endpoint of an FPG ≤110 mg/dL was achieved by 27.0% of pts, and 43.2% reached their individual HbA_{1c} targets. FPG target achievement was highest in previous Gla-100 pts (29.3%), and HbA_{1c} target achievement was highest in previous insulin detemir pts (55.3%). Hypoglycaemia incidence was low with a trend of >70% reduction of nocturnal hypoglycaemia incidence (Table 1), despite an HbA_{1c} reduction of 0.81 ± 0.99%. BW remained stable. Mean BI doses from baseline slightly increased from 28.1 ± 15.4 U/d (0.30 ± 0.16 U/kg*d) to 33.6 ± 19.6 U/d (0.36 ± 0.19 U/kg*d) at 12 months.

Conclusion: Switching the BI in a BOT regimen to Gla-300 allowed not well controlled pts with T2DM to reduce HbA_{1c} by 0.81% with less nocturnal hypoglycaemia, minor BI dose changes, and weight maintenance in primary care.

Table 1 Hypoglycaemia endpoints and basal insulin dose (full analysis set (FAS); n=679)

Hypoglycaemia (hypo.) endpoints	Incidence before start of Gla-300# [N (%)]	N	Incidence after start of Gla-300## [N (%)]	N	Hypoglycaemia rate§ N (95% CI)]	N
Symptomatic hypo.	10 (1.5%)	679	8 (1.2%)	679	0.06 (0.04, 0.08)	679
Confirmed (SMBG ≤70 mg/dL) symptomatic hypo.	8 (1.2%)	679	6 (0.9%)	679	0.05 (0.03, 0.07)	679
Nocturnal (10pm – 6am) confirmed symptomatic hypo.	9 (1.3%)	679	2 (0.3%)	679	0.01 (0.00, 0.02)	679
Severe confirmed (SMBG ≤56 mg/dL) or symptomatic (assistance required) hypo.	1 (0.1%)	679	1 (0.1%)	679	0.00 (0.00, 0.01)	679
Severe nocturnal confirmed or symptomatic (assistance required) hypo.	1 (0.1%)	679	1 (0.1%)	679	0.00 (0.00, 0.01)	679
BI dose [U/d] BI dose / kg BW [U/kg*d]	**Last dose previous insulin [mean±SD]**	**N**	**Gla-300 starting dose [mean±SD]**	**N**	**Gla-300 12 mo dose [mean±SD]**	**N**
Insulin glargine 100 U/mL	29.8±15.7 0.33±0.18	326 313	30.6±15.2 0.33±0.16	329 315	35.3±19.9* 0.38±0.19*	324 318
Insulin degludec	33.5±22.9 0.35±0.20	49 48	34.5±23.1 0.36±0.20	49 48	37.5±27.0 0.38±0.23	49 48
Insulin detemir	27.4±12.5 0.31±0.14	85 81	25.4±10.7 0.28±0.12	86 82	32.3±15.2* 0.35±0.15*	84 81
NPH insulin	25.7±14.8 0.27±0.15	136 136	23.9±12.8 0.25±0.13	135 135	29.3±16.0* 0.31±0.17*	134 134

CI, confidence interval; FAS, full analysis set; FPG, fasting plasma glucose; mo, month; SD, standard deviation; SMBG, self measured blood glucose; * p<0.0001; # Incidence within 12 weeks before start of Gla-300; ## Incidence within last 12 weeks 12 months after start of Gla-300; § Calculated as events/patient year for month 0-12 for all patients with treatment duration and number of hypoglycaemia available.

Supported by: Sanofi-Aventis Deutschland GmbH

Disclosure: **J. Seufert:** Employment/Consultancy; Boehringer Ingelheim GmbH, Janssen Pharmaceuticals, Inc., GI Dynamics Inc., Novo Nordisk A/S, Sanofi-Aventis Deutschland GmbH, Sanofi. Grants; Boehringer Ingelheim GmbH, GI Dynamics, Inc., GlaxoSmithKline plc., Intarcia Therapeutics, Inc., Ipsen Biopharmaceuticals, Inc., Janssen Pharmaceuticals, Inc., Novartis Pharmaceuticals, Inc., Novo Nordisk A/S, Sanofi-Aventis Deutschland GmbH, Ypsomed AG. Lecture/other fees; Astrazeneca, Berlin-Chemie AG, Boehringer Ingelheim GmbH, Janssen Pharmaceuticals, Inc., Eli Lilly and Company, Merck Sharp & Dohme Corp., Novartis Pharmaceuticals Corp., Novo Nordisk A/S, Sanofi-Aventis Deutschland GmbH.

895

Basal insulin initiation on the top of metformin improves glycaemic control and safety in Chinese insulin-naive patients with type 2 diabetes

H. Zhang[1], P. Zhang[1], Y. Luo[2], D. Zhu[1], X. Li[1], J. Ji[1], L. Ji[2]; [1]The George Institute for Global Health at Peking University Health Science Center, Beijing, [2]Department of Endocrinology and Metabolis, Peking University People's Hospital, Beijing, China.

Background and aims: Metformin has been recommended to be combined with basal insulin (BI) when initiating BI treatment by current guidelines. The real-world use as well as the effectiveness and safety of BI initiation on the top of metformin was evaluated in this study.

Materials and methods: For this 6-month, prospective, real-world study, we recruited 16,341 insulin-naïve patients with T2DM uncontrolled (HbA1c ≥7%) on OADs, who initiated BI treatment at physician's discretion and patient's willingness. The effectiveness and safety of metformin-based BI were evaluated by using intent-to-treat (ITT) and per-protocol (PP) analysis separately. The ITT population constituted all patients who successfully completed the 6-month visit, according to whether metformin was prescribed at baseline, irrespective changes of subsequent therapy during follow-up. The PP population constituted patients who kept BI with or without metformin during follow-up as that at baseline. Propensity score adjustment was used to balance covariates of baseline characteristics and process factors during follow-up between two groups.

Results: A total of 7,736 (47.3%) were prescribed metformin in addition to BI, accounting for about half of all patients initiating BI. In ITT analysis, control rates of both HbA1c ≤6.5% and HbA1c <7.0% were significantly higher in metformin-based BI group (26.7% and 40.1%) than those in BI without metformin group (23.6% and 37.2%) ($P < 0.0001$, $P = 0.0009$), whereas the former was associated with lower insulin dose at 6 months (23.0 vs 23.6 IU/day, $P < 0.0001$) and dose increment from baseline to 6 months (−0.3 vs 0.9 IU/day, $P < 0.0001$) compared with the latter; the former showed a lower incidence of the total minor hypoglycemia compared with the latter (24.9 vs 29.6 times/person/year, $P < 0.0001$). These results in PP analysis were concordant with those in ITT analysis. In PP analysis, initiating BI with metformin was significantly associated with greater reduction in HbA1c (−2.4 vs −2.3% $P = 0.0289$) and less weight gain (−0.2 vs 0.1 kg $P = 0.0001$) compared with BI without metformin, whereas these differences between two groups were not significant in ITT analysis.

Conclusion: Metformin-based BI combination therapy in real-world practice was associated with better glycemic control, fewer hypoglycemia events, less weight gain and smaller insulin dose, which corroborates current guidelines that recommend combination regimen of metformin and BI.

Table. Propensity sore regression results on effectiveness and safety of BI plus metformin after 6 months (data are presented as percentage or mean [95% CI])

Variables	No metformin	Metformin	P value
ITT population			
HbA1c level (%)			
Mean level at 6 months	7.48 (7.42, 7.55)	7.46 (7.39, 7.52)	0.2660
Change from baseline	-2.28 (-2.37, -2.19)	-2.32 (-2.42 ,-2.23)	0.2290
Control rate of HbA1c (%)			
≤6.5%	23.57 (21.64, 25.61)	26.67 (24.59, 28.86)	<.0001
<7.0%	37.22 (34.91, 39.59)	40.05 (37.68, 42.47)	0.0009
Weight (kg)			
Mean level at 6 months	71.14 (70.70, 71.57)	71.23 (70.80, 71.67)	0.5638
Change from baseline	0.23 (0.10, 0.36)	0.19 (0.06, 0.33)	0.4712
Severe hypoglycemia (times/person/year)	5.63 (4.66, 6.810)	5.29 (4.28, 6.54)	0.5462
Minor hypoglycemia (times/person/year)	29.59 (28.94, 30.25)	24.91 (24.31, 25.51)	<.0001
Insulin dose			
Insulin dose at v3 (IU/day)	23.57 (23.27, 23.87)	22.96 (22.66, 23.26)	<.0001
Change from v1 to v3 (IU/day)	0.92 (0.50, 1.33)	-0.25 (-0.67, 0.17)	<.0001
PP population			
HbA1c level (%)			
Mean level at 6 months	7.27 (7.21, 7.33)	7.20 (7.14, 7.26)	0.0292
Change from baseline	-2.30 (-2.39,-2.20)	-2.40 (-2.49, -2.31)	0.0289
Control rate of HbA1c (%)			
≤6.5%	28.09 (25.92, 30.37)	32.89 (30.68, 35.18)	<.0001
<7.0%	44.85 (42.41, 47.32)	49.12 (46.79,51.45)	0.0005
Weight (kg)			
Mean level at 6 months	72.27 (71.83, 72.71)	72.93 (72.52, 73.35)	0.0029
Change from baseline	0.09 (-0.04, 0.23)	-0.16 (-0.28, -0.04)	0.0001
Severe hypoglycemia (times/person/year)	11.49 (8.25, 16.02)	7.730 (5.84, 10.23)	0.0335
Minor hypoglycemia (times/person/year)	25.70 (24.92, 26.52)	24.30 (23.53, 25.08)	0.0030
Insulin dose			
Insulin dose at v3 (IU/day)	28.66 (28.38, 28.94)	27.93(27.66, 28.20)	<.0001
Change from v1 to v3 (IU/day)	5.13 (4.80, 5.46)	3.41 (3.09, 3.73)	<.0001

Clinical Trial Registration Number: NCT01859598
Supported by: Sanofi-Aventis (Shanghai, China)
Disclosure: **H. Zhang:** Grants; Sanofi-Aventis (Shanghai, China).

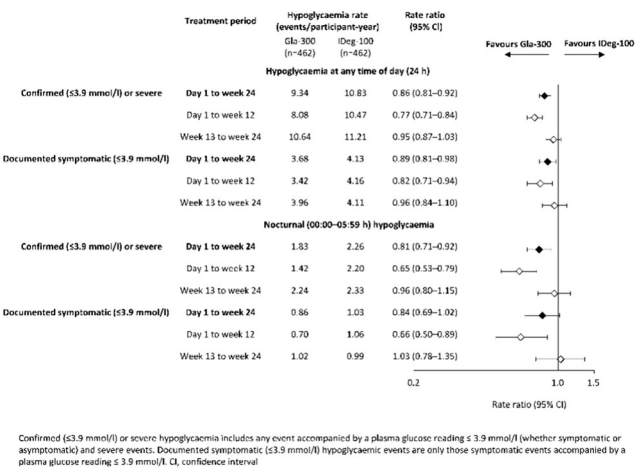
896

Lower hypoglycaemia rates with insulin glargine 300 U/ml vs insulin degludec 100 U/ml in insulin-naive adults with type 2 diabetes: the BRIGHT randomised trial

G.B. Bolli[1], A. Cheng[2], Z. Bosnyak[3], E. Boëlle-Le Corfec[3], A.M.G. Cali[4], X. Wang[5], J. Frias[6], R. Roussel[7], J. Rosenstock[8];
[1]Perugia University Medical School, Perugia, Italy, [2]Division of Endocrinology and Metabolism, University of Toronto, Toronto, Canada, [3]Sanofi, Paris, France, [4]Sanofi, Tokyo, Japan, [5]Sanofi, Beijing, China, [6]National Research Institute, Los Angeles, USA, [7]INSERM, UMR_S 1138, Centre de Recherche des Cordeliers, Paris, France, [8]Dallas Diabetes Research Center at Medical City, Dallas, USA.

Background and aims: BRIGHT is the first head-to-head trial of insulin glargine 300 U/ml (Gla-300) and insulin degludec 100 U/ml (IDeg-100) in type 2 diabetes (T2DM). Non-inferiority of Gla-300 vs IDeg-100 was demonstrated for the primary endpoint (HbA_{1c} change, baseline to week 24). Here we analysed hypoglycaemia across the full study, and during the titration and maintenance periods.
Materials and methods: BRIGHT was a 24-week open-label, treat-to-target trial that investigated the efficacy and safety of Gla-300 vs IDeg-100 in insulin-naïve people with T2DM, inadequately controlled on oral antihyperglycaemic drugs (OADs) ± glucagon-like peptide-1 receptor agonists (GLP-1 RAs). In this analysis, hypoglycaemia was examined by treatment period; day 1 to week 24 (full study period), day 1 to week 12 (titration period) and week 13 to week 24 (maintenance period).
Results: Incidence of confirmed (≤3.9 mmol/l) or severe hypoglycaemia at any time of day (24 h) was lower with Gla-300 vs IDeg-100 (OR 0.74 [95% CI: 0.57 to 0.97]) during the titration period but was similar at any time of day (24 h) or at night (00:00–05:59 h) during the maintenance and full study periods. Annualised rates of hypoglycaemia (**Figure**) were lower with Gla-300 vs IDeg-100 during the full study period, particularly over the titration period, despite higher final daily doses of Gla-300 vs IDeg-100 (0.54 and 0.43 U/kg from starting doses 0.2 U/kg and 10 U/day [0.12 U/kg], respectively).
Conclusion: Gla-300 resulted in similar incidence but lower rates of hypoglycaemia, particularly during the titration period, with comparable glycaemic control vs IDeg-100 in insulin-naïve people with T2DM on OADs ± GLP-1 RAs.

Figure. Annualised rates of hypoglycaemia with Gla-300 vs IDeg-100, by study period



Confirmed (≤3.9 mmol/l) or severe hypoglycaemia includes any event accompanied by a plasma glucose reading ≤ 3.9 mmol/l (whether symptomatic or asymptomatic) and severe events. Documented symptomatic (≤3.9 mmol/l) hypoglycaemic events are only those symptomatic events accompanied by a plasma glucose reading ≤ 3.9 mmol/l. CI, confidence interval

Clinical Trial Registration Number: NCT02738151
Supported by: Sanofi
Disclosure: **G.B. Bolli:** Honorarium; Sanofi, Menarini. Lecture/other fees; Sanofi.

897

The risk of total hypoglycaemia in patients with type 2 diabetes self-titrating insulin glargine U-100

J. Kiljanski, E. Spaepen, C. Harris;
Eli Lilly and Company, Indianapolis, USA.

Background and aims: Insulin glargine (IGlar) U-100 has been established as a standard treatment for patients with Type 2 diabetes (T2D) in need of basal insulin. Results of the ELEMENT trials, which compared LY IGlar and Lantus®, showed that patients were able to improve their glycaemic control by self-titrating IGlar U-100 with a simple self-titration algorithm to a fasting blood glucose (BG) target of 5.56 mmol/L. In spite of a very low incidence of severe hypoglycaemia in the ELEMENT trials (<1%), insulin titration can expose patients to potential harm. We aimed to evaluate if level of glycaemic control, age, or being insulin naïve affected the risk of hypoglycaemia (defined by BG <3 mmol/L) during IGlar self-titration.
Materials and methods: We performed exploratory analyses of pooled treatment groups, statistically equivalent according to clinical study results published earlier, in each of 2 Phase 3 studies of LY IGlar vs. Lantus®: ELEMENT-2 (double-blind) and ELEMENT-5 (open label). Hypoglycaemia at Weeks 8 and 12 (titration phase) and at Week 24 (maintenance phase) was analysed by category of HbA1c (<7%, 7–8.5%, >8.5%), and subgroups of age (<65, ≥65 yrs) and previous insulin use (naïve or not). Analyses used were ANOVA for baseline analyses and ANCOVA or MMRM for post-baseline analyses, with a significance level of $\alpha = 0.05$.
Results: In ELEMENT-2 ($N = 756$), 50.0% of patients were male, 28.3% ≥65 yrs of age, 60.4% insulin naïve, and 83.3% on sulfonylureas; baseline HbA1c was 8.33% ± 1.08%. In ELEMENT-5 ($N = 493$), 52.1% were male, 21.3% ≥65 yrs of age, 45.2% insulin naïve, and 84.0% on sulfonylureas; baseline HbA1c was 8.61% ± 1.06%. In ELEMENT-2, there

were no differences in rate or incidence of total hypoglycaemia (BG <3 mmol/L) among HbA1c categories throughout the study. In ELEMENT-5, patients with HbA1c >8.5% had lower rates and incidences of hypoglycaemia throughout the study compared to those in the lower HbA1c category. In both studies, patients ≥65 yrs of age had lower baseline HbA1c, smaller increases in dose with the same frequency of hypoglycaemia compared to patients <65 yrs of age, with no differences in HbA1c post-baseline. The rates and incidences of total hypoglycaemia were similar between naïve patients and patients previously on basal insulin, across all levels of glycaemic control. Hypoglycaemia rates were generally similar during titration phase and maintenance phase for all HbA1c categories and subgroups.

Conclusion: As shown in the ELEMENT trials, patients with T2D can significantly improve glycaemic control by adopting a simple self-titration algorithm. Here we support the safety of this method for most patients. The risk of total hypoglycaemia (BG <3 mmol/L) was similar among new users and prior users of basal insulin who started insulin titration with the algorithm. The risk of hypoglycaemia was not higher among older patients. As with any insulin treatment, hypoglycaemic risk increases as HbA1c approaches 7%; therefore, mitigation of hypoglycaemic risk is prudent as HbA1c improves.

Total Hypoglycaemia, Blood Glucose <3 mmol/L

	Age		Prior insulin use		HbA1c at Wk 12		
	<65 yrs	≥65 yrs	Naïve	Not naive	<7%	7-8.5%	>8.5%
ELEMENT-2 (N=756)							
N for subgroups	n=537	n=212	n=451	n=298	n=288	n=367	n=45
HbA1c, baseline, % HbA1c, Wk 12, %	8.43 ± 0.05 7.18 ± 0.05	8.07 ± 0.07 7.23 ± 0.07	8.46 ± 0.05 7.01 ± 0.05	8.13 ± 0.06 7.48 ± 0.06	7.81 ± 0.06 6.48 ± 0.03	8.61 ± 0.05 7.54 ± 0.03	9.48 ± 0.14 9.27 ± 0.07
Rate (events/ patient/yr)	2.73 ± 0.30	3.27 ± 0.50	2.94 ± 0.34	2.76 ± 0.38	3.03 ± 0.41	2.78 ± 0.36	2.92 ± 0.89
RR (95% CI)	1.19 (0.88, 1.62)		0.94 (0.70, 1.25)			0.92 (0.68, 1.25)*	0.96 (0.51, 1.82)*
Incidence, n (%)	226 (42.1)	95 (44.8)	199 (44.1)	122 (40.9)	141 (49.0)	158 (43.1)	15 (33.3)
ELEMENT-5 (N=493)							
N for subgroups	n=386	n=103	n=221	n=268	n=149	n=256	n=56
HbA1c, baseline, % HbA1c, Wk 12, %	8.65 ± 0.05 7.42 ± 0.04	8.41 ± 0.10 7.59 ± 0.08	8.82 ± 0.07 7.14 ± 0.06	8.43 ± 0.06 7.71 ± 0.05	8.09 ± 0.08 6.53 ± 0.04	8.70 ± 0.06 7.64 ± 0.03	9.58 ± 0.13 9.12 ± 0.06
Rate (events/ patient/yr)	0.94 ± 0.16	2.09 ± 0.89	0.89 ± 0.18	1.37 ± 0.34	1.58 ± 0.34	1.04 ± 0.25	0.35 ± 0.15
RR (95% CI)	2.24 (0.98, 5.10)		1.53 (0.88, 2.69)			0.66 (0.40, 1.07)*	0.22 (0.08, 0.60)*
Incidence, n (%)	113 (29.3)	33 (32.0)	58 (26.2)	88 (32.8)	62 (41.6)	71 (27.7)	9 (16.1)

Least square means ± SE unless otherwise indicated. BG=blood glucose; CI=confidence interval; RR=relative rate *compared to the <7% HbA1c reference category.

Clinical Trial Registration Number: NCT01421459, NCT02302716
Disclosure: **J. Kiljanski:** Employment/Consultancy; Lilly. Stock/ Shareholding; Lilly.

898

Improved or comparable efficacy without increased hypoglycaemia with self- vs physician-led titration of insulin glargine 300 U/ml in age groups <65 or ≥65 years: TAKE CONTROL

K. Strojek[1], G. Bigot[2], M. Bonnemaire[3], E. Delgado[4,5], V. Donicova[6], M. Kvapil[7], N. Papanas[8], L. Popescu[9], R. Ritzel[10], B. Schultes[11], L. Smircic Duvnjak[12];
[1]Department of Internal Diseases, Diabetology and Cardiometabolic Diseases SMDZ, Zabrze, Silesian Medical University, Zabrze, Poland, [2]IVIDATA, Levallois-Perret, France, [3]Global Diabetes Division, Sanofi, Paris, France, [4]Department of Medicine, University of Oviedo, Oviedo, Spain, [5]Endocrinology and Nutrition Service, Hospital Universitario Central de Asturias, Oviedo, Spain, [6]Outpatient Department for Internal Medicine and Diabetology, Medical Faculty of PJ Safarik University Teaching Affiliate, Kosice, Slovakia, [7]Department of Internal Medicine, Second Faculty of Medicine, Charles University, Prague, Czech Republic, [8]Diabetes Centre, Second Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece, [9]Global R&D Operations, Sanofi, Bucharest, Romania, [10]Klinikum Schwabing and Klinikum Bogenhausen, Städtisches Klinikum München GmbH, Munich, Germany, [11]eSwiss Medical & Surgical Center, Department of Internal Medicine, Endocrinology, Diabetes, & Metabolism, St. Gallen, Switzerland, [12]University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Vuk Vrhovac, Croatia.

Background and aims: TAKE CONTROL, a 24-week, multicentre, randomized, open-label, parallel-group study, aimed to evaluate self- vs physician-led titration of insulin glargine 300 U/mL (Gla-300) in people with T2DM.
Materials and methods: Efficacy and safety outcomes in people <65 years vs ≥65 years were assessed.
Results: In each age subgroup, baseline HbA_{1c} and fasting SMPG were comparable between self- vs physician-led titration (**Table**); in the older and younger groups, HbA_{1c} ($p = 0.46$ and $p = 0.29$) and fasting SMPG ($p = 0.56$ and $p = 0.15$) reductions were similar between self- vs physician-led titration. Attainment of the fasting SMPG target (4.4–7.2 mmol/L) without confirmed (<3.0 mmol/L) or severe hypoglycaemia was slightly higher in older people; differences in self- vs physician-led titration were not significant in older or younger groups ($p = 0.19$ and $p = 0.05$). Incidences of hypoglycaemia at any time of day (24 h) appeared slightly higher in the ≥65 years, physician-led group. There was no evidence of heterogeneity of effect of self- vs physician-led titration on hypoglycaemia ($p > 0.05$). Adverse events were similar in each group.
Conclusion: Self-titration of Gla-300 resulted in similar glycaemic target achievement to physician-led titration, without an increased risk of hypoglycaemia, irrespective of age. There was a trend for more people to reach fasting SMPG targets without hypoglycaemia with self- vs physician-led titration, including those ≥65 years.

Table. Change in HbA_{1c} and fasting SMPG, and risk ratio for confirmed or severe hypoglycaemia with self- and physician-led insulin titration stratified by age

Parameter	<65 years (N=329)		≥65 years (N=302)	
	Self-titration (N=155)	Physician-led titration (N=174)	Self-titration (N=159)	Physician-led titration (N=143)
Duration of diabetes at baseline, years	10.7 (5.7)	11.5 (5.9)	15.0 (7.8)	14.4 (7.6)
Duration of previous basal insulin treatment at baseline, years	2.9 (2.8)	3.3 (3.7)	3.9 (5.2)	3.9 (4.5)
Baseline HbA_{1c}, %	8.48 (0.93)	8.45 (0.89)	8.32 (0.85)	8.39 (0.95)
HbA_{1c} change from baseline to week 24, %	−1.02 (1.13)	−0.90 (0.97)	−0.89 (0.99)	−0.79 (1.14)
Baseline fasting SMPG[a], mmol/L	8.6 (2.2)	8.4 (2.0)	8.2 (2.0)	8.1 (2.1)
Fasting SMPG[a] change from baseline to week 24, mmol/L	−1.8 (2.2)	−1.5 (2.1)	−1.7 (1.9)	−1.6 (2.2)
Attainment of the fasting SMPG target (4.4–7.2 mmol/L) without confirmed (<3.0 mmol/L) or severe hypoglycaemia, %	67.4	56.4	75.2	68.1
Incidence of confirmed (≤3.9 mmol/L) or severe hypoglycaemia at any time of day (24 h), n (%)	50 (32.5)	51 (29.3)	54 (34.2)	57 (40.1)
Risk ratio self- vs physician-led titration [95% CI]	1.11 [0.81 to 1.53]		65–75 years: 0.85 [0.61 to 1.18] ≥75 years[b]: 0.93 [0.52 to 1.68]	
Incidence of confirmed (3.0 mmol/L) or severe hypoglycaemia at any time of day (24 h), n (%)	11 (7.1)	12 (6.9)	12 (7.6)	13 (9.2)
Risk ratio self- vs physician-led titration [95% CI]	1.05 [0.48 to 2.29]		65–75 years: 0.94 [0.42 to 2.12] ≥75 years[b]: 0.31 [0.03 to 2.85]	

Values are mean (SD) unless otherwise indicated
[a]Fasting pre-breakfast SMPG values are derived weekly using the median value of fasting pre-breakfast SMPGs collected during the 7-day period from the day following the first treatment injection
[b]Self-titration: n=34; physician-led titration: n=34
CI, confidence interval; SD, standard deviation; SMPG, self-monitored plasma glucose

Clinical Trial Registration Number: EudraCT: 2015-001626-42
Supported by: Sanofi
Disclosure: **K. Strojek:** Lecture/other fees; Sanofi Aventis, Novo Nordisk, Servier, AstraZeneca, Eli Lilly, MSD, Boehringer Ingelheim, Krka. Other; Clinical sponsored by AstraZeneca, Pfizer, Sanofi Aventis, Novo Nordisk, Amgen.

899

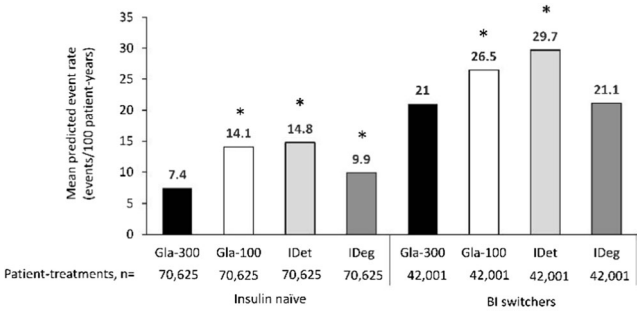
Similar or lower severe hypoglycaemia rates with Gla-300 vs Gla-100, IDet and IDeg in 112,626 people with type 2 diabetes: predictive modelling using real-world data: Lightning

R. Berria[1], J.H. Pettus[2], T. Bailey[3], F.L. Zhou[1], Z. Bosnyak[4], J. Westerbacka[4], J. Jimenez[1], I. Hramiak[5], L. Meneghini[6,7];

[1]Sanofi, Bridgewater, USA, [2]University of California, San Diego, USA, [3]AMCR Institute, Escondido, USA, [4]Sanofi, Paris, France, [5]St Joseph's Health Care, London, Canada, [6]University of Texas Southwestern Medical Center, Dallas, USA, [7]Parkland Health & Hospital System, Dallas, USA.

Background and aims: Using real-world US electronic health record (EHR) data, representative of the general population and real-life practice, the Lightning study aimed to predict hypoglycaemia rates in people with type 2 diabetes (T2DM) prescribed basal insulin (BI) analogues.
Materials and methods: Hypoglycaemic events during BI treatment, as recorded by physicians in EHR, including patients initiating BI ("insulin-naïve") and switching between BIs ("switchers"), were collected between 01 April 2015 and 31 March 2017. Based on real-world data, a predictive model (implementing machine learning and controlling for >160 baseline demographic and clinical variables) was developed and validated for each drug-specific cohort (patients treated with a specific BI).
Results: The models predict significantly lower rates of severe hypoglycaemia in real-world use with insulin glargine 300 U/ml (Gla-300) vs insulin glargine 100 U/ml (Gla-100) or insulin detemir (IDet), irrespective of prior insulin use. In insulin-naïve patients, lower rates of severe hypoglycaemia are predicted with Gla-300 vs insulin degludec (IDeg) ($p < 0.05$), an unexpected finding that warrants further investigation, whereas comparable rates are shown in switchers (**Figure**).
Conclusion: Using real-world data, these predictive modelling results show similar or significantly lower severe hypoglycaemia rates with Gla-300 vs other BIs in people with T2DM.

Figure. Estimated rates of severe hypoglycaemia



Severe hypoglycaemia was defined by: ICD 9/10 codes (either codes specifically for severe hypoglycaemia, or those where hypoglycaemia was the reason for care or occurred on the same day as an emergency department visit/inpatient admission); or blood glucose <3.0 mmol/l; or intramuscular glucagon administration; or mention of hypoglycaemia in patient notes with a descriptor denoting severity or on the same day as an emergency department visit/inpatient admission. BI, basal insulin; ICD, International Classification of Diseases; Patient-treatment, the period during which a patient is using a BI (used as the unit of analysis)
*p<0.05 Gla-300 versus BI comparator

Supported by: Sanofi
Disclosure: **R. Berria:** Employment/Consultancy; Sanofi. Stock/Shareholding; Sanofi.

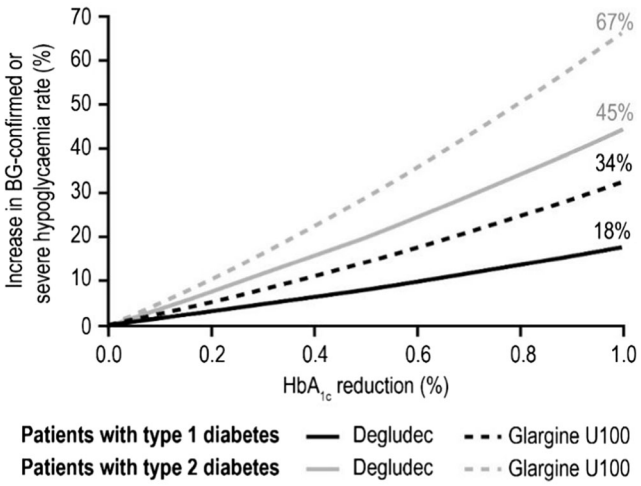
900

Relationship between HbA_{1c} and hypoglycaemia risk in individual patients comparing insulin degludec with insulin glargine U100

U. Pedersen-Bjergaard[1], A. Philis-Tsimikas[2], W. Lane[3], C. Wysham[4], L. Bardtrum[5], S. Østoft[5], S. Heller[6];
[1]Nordsjællands University Hospital Hillerød, Hillerød, Denmark, [2]Scripps Whittier Diabetes Institute, San Diego, USA, [3]Mountain Diabetes and Endocrine Center, Asheville, USA, [4]Rockwood Clinic, Spokane, USA, [5]Novo Nordisk A/S, Søborg, Denmark, [6]University of Sheffield, Sheffield, UK.

Background and aims: Targeting a lower HbA_{1c} may increase the hypoglycaemia risk in patients with diabetes. We investigated the relationship between HbA_{1c} and hypoglycaemia risk on an individual level.
Materials and methods: This *post hoc* analysis used data from two double-blind, randomised, treat-to-target, two-period (32 weeks each) crossover trials of insulin degludec (degludec) vs. insulin glargine 100 units/mL (glargine U100) in patients with type 1 (T1D; SWITCH 1, $n = 501$) or type 2 diabetes (T2D; SWITCH 2, $n = 721$).
Results: For each patient at each visit, HbA_{1c} was linked with the number of hypoglycaemic events (blood glucose-confirmed [<3.1 mmol/L] with symptoms or severe [third-party assistance]) since last visit. A 1% (10.9 mmol/mol) HbA_{1c} reduction led to an 18% (degludec) and 34% (glargine U100) increased risk of hypoglycaemia in T1D, and 45% (degludec) and 67% (glargine U100) increased risk in T2D (Figure). Assuming an 11% (T1D) and 30% (T2D) reduction in hypoglycaemia risk, as seen in the SWITCH trials, this can be translated into a 0.61% (T1D) and 0.67% (T2D) HbA_{1c} reduction with degludec, with no increase in hypoglycaemia risk vs. glargine U100.
Conclusion: Lowering HbA_{1c} led to a higher hypoglycaemia risk; however, the lower incremental hypoglycaemia risk with degludec vs. glargine U100 may allow for a lower HbA_{1c} target in both T1D and T2D with degludec than with glargine U100 in clinical practice, when hypoglycaemia is a limiting factor for glycaemic control.

Figure. Proportional increase in rate of BG-confirmed or severe hypoglycaemia resulting from a 1% (10.9 mmol/mol) HbA_{1c} reduction with degludec and glargine U100, based on the SWITCH trials



Hypoglycaemia was defined as BG-confirmed (3.1 mmol/L) symptomatic or severe (requiring third party assistance) events.
BG, blood glucose; Glargine U100, insulin glargine 100 units/mL.

Clinical Trial Registration Number: SWITCH 1: NCT02034513; SWITCH 2: NCT02030600
Supported by: Novo Nordisk
Disclosure: **U. Pedersen-Bjergaard:** Grants; Novo Nordisk. Lecture/other fees; Novo Nordisk.

PS 079 Clinical pathophysiology of insulin and hypoglycaemia

901

Early menopause and primary ovarian failure are associated with increased risk of type 2 diabetes: a systematic review and meta-analysis

P. Anagnostis[1,2], K. Christou[3,2], A.-M. Artzouchaltzi[3,2], S.A. Paschou[4], M. Potoupnis[3,5], E. Tsiridis[3,5], I. Lambrinoudaki[6], D.G. Goulis[1,7];
[1]Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, [2]Division of Endocrinology, Police Medical Centre of Thessaloniki, Thessaloniki, [3]Centre of Orthopedic and Regenerative Medicine Research (CORE), Aristotle University of Thessaloniki, Thessaloniki, [4]Division of Endocrinology and Diabetes, "Aghia Sophia" Hospital, Medical School, National and Kapodistrian University of Athens, Athens, [5]Academic Orthopaedic Unit, General Hospital Papageorgiou, Aristotle Univeristy of Thessaloniki, Thessaloniki, [6]Second Department of Obstetrics and Gynecology, National and Kapodistrian University of Athens, Athens, [7]Centre of Orthopedic and Regenerative Medicine Research (CORE), Aristotle Univeristy of Thessaloniki, Thessaloniki, Greece.

Background and aims: Menopausal transition has been associated with a derangement of glucose metabolism. However, it is not known if early menopause (EM, defined as age of menopause <45 years) or primary ovarian failure (POF, defined as age of menopause <40 years) are associated with increased risk of type 2 diabetes mellitus (T2DM). The aim of this study was to systematically investigate and meta-analyze the best evidence regarding the association of menopausal age with the risk of developing T2DM.
Materials and methods: A comprehensive search was conducted in three databases (PubMed, CENTRAL, Scopus) for English papers (up to January 31st, 2018). Random effects model was used for data synthesis. Data were expressed as odds ratio (OR) with 95% confidence intervals (CI) and were combined by the inverse variance method. The I^2 index was employed to indicate heterogeneity; publication bias was inspected by Funnel plots and tested by the Egger's test.
Results: The initial search provided 1,851 studies. After excluding duplicates and after critical appraisal, 13 studies were included in the qualitative and quantitative analysis, providing a total of 191,762 postmenopausal women, with 21,664 cases of T2DM. Both women with EM and POF were at higher risk for developing T2DM compared with those with normal age at menopause (45–55 years) [OR 1.446 (95% CI 1.004–2.084, $p < 0.048$) and 1.134 (95% CI 1.033–1.244, $p < 0.008$), respectively. When women with EM were compared with those of menopausal age >45 years, a greater T2DM risk was detected for the former group (OR 1.121, 95% CI 1.018–1.234, $p < 0.02$).
Conclusion: This is the first meta-analysis showing that both EM and POF are associated with increased risk of T2DM.
Disclosure: **P. Anagnostis:** None.

902

Left ventricular systolic and diastolic function in type 1 diabetic adults with different glucose control

K. Moshenets, N. Pertseva;
Endocrinology, SE "Dnipropetrovsk medical academy of Health Ministry of Ukraine", Dnipro, Ukraine.

Background and aims: To investigate cardiac function in adult patients with type 1 diabetes mellitus (T1DM) depending on glucose control and hypoglycemia
Materials and methods: The study involved 60 patients with T1DM, including 25 men (41%), women - 35 (59%). The average age of patients - 30,1 (22.0; 39.0) yrs., disease duration 11.0 (5.0; 18.0) yrs., BMI 22.01 (20.10; 25.56) kg/m^2. All patients used basic-bolus insulin therapy with daily dose of Units 45 (33; 58). C-peptide, HbA1c, blood creatinine, glomerular filtration rate (GFR) CKD-EPI, first morning urinary albumin excretion (UAE) were determined for verification and evaluation of control. Blood glucose levels were conducted by CGMS (Continuous Glucose Monitoring System). 10 sex- and age-matched healthy controls were included. Systolic and diastolic function were evaluated by standard conventional transthoracic echocardiography and tissue Doppler echocardiography. Patients were divided to groups according to HbA1c and hypoglycemia. Group 1 had HbA1c ≤7.0%, group 2 had HbA1c >7.0%, and subgroups: A-without hypoglycemia, B-with hypoglycemia
Results: The groups were similar according to sex, age, BMI and frequency of hypoglycemia. Calculation of results depending on the duration T1DM showed an increase in left ventricular mass index (LVMI) ($r = 0.31$ $p = 0.02$), and decrease in GFR ($r = -0.40$ $p = 0.01$). Diabetic patients had such significantly higher conventional echocardiography parameters compared to healthy control: left ventricular mass 142.5 (126.5; 168.0) g vs. 121 (115.5; 128.5) g, $p = 0.008$; left ventricular posterior wall thickness (LVPWT) 0.98 (0.94; 1.06) sm vs. 0.91 (0.87; 0.93) sm, $p = 0.02$; interventricular septal excursion (IVSE) 1.24 (1.18; 1.39) sm vs 0.97 (0.86; 1.13) sm, $p < 0.001$; left ventricular posterior wall excursion (LVPWE) 1.34 (1.23; 1.4) sm vs. 1.16 (1.05; 1.25) sm $p = 0.004$. Daily insulin dose correlated with LVMI ($r = 0.31$ $p = 0.02$), IVSE ($r = 0.33$ $p = 0.03$) and LVPWE ($r = 0.36$ $p = 0.04$). In 1 group correlation analyses demonstrated a positive correlation between UAE and LVPWE ($r = 0.59$ $p = 0.03$), IVSE ($r = 0.60$, $p = 0.03$), relative wall thickness (RWT) ($r = 0.58$ $p = 0.02$), and negative correlation with HbA1c ($r = -0.52$ $p = 0.02$). In 2 group UAE correlated with left ventricular end-diastolic volume (EDV) ($r = 0.34$ $p = 0.01$) and stroke volume correlated with GFR ($r = 0.47$ $p = 0.02$) The presence of hypoglycemic episodes affected increasing LVPWE ($r = 0.34$ $p = 0.03$; left ventricular (LV) end-systolic volume ($r = 0.41$ $p = 0.035$) and decreasing ejection fraction ($r = -0.32$ $p = 0.001$). Diastolic dysfunction with impaired relaxation (grade I) had 34.62% diabetic patients: 34.62% in group 1 compared to 36.11% in group 2, $p = 0.04$
Conclusion: Patients with T1DM have increased risk of cardiovascular disease due to early development of left ventricular hypertrophy (LVH). Poor glucose control (HbA1c >7%) is associated with systolic and diastolic dysfunction. Hypoglycemia adversely affects LV myocardial structure and function regardless of HbA1c level with early changes of LV function. LVPWT, IVSE, LVPWE are the independent predictors of cardiovascular events and cardiovascular mortality which progress more rapidly due to hypoglycemia. The low risk of hypoglycemia is the priority task to prevent progression of cardiovascular complications and predict adverse cardiac events in patients with T1DM
Disclosure: **K. Moshenets:** None.

903

The role of hyperglycaemia in the appearance of tachyarrhythmia in patients with type 2 diabetes, paroxysmal atrial fibrillation (pAfib) and ischaemic stroke

A. Sianni[1], I. Matsoukis[2], T. Athanasopoulou[1], A. Ganotopoulou[3], K. Kanellopoulou[4], X. Triantafillopoulou[1], L. Skorda[5], A. Masgala[1];
[1]Department of Internal Medicine, "Konstantopouleio" - General Hospital of Nea Ionia-Patision, Athens, [2]Department of Cardiology, "Korgialenio-Benakio" - General Hospital of Athens, Athens, [3]Department of Internal Medicine, "Tzaneio" - General Hospital of Pireaus, Athens, [4]Department of Cardiology, "Konstantopouleio" - General Hospital of Nea Ionia-Patision, Athens, [5]Department of Internal Medicine, "Korgialenio-Benakio" - General Hospital of Athens, Athens, Greece.

Background and aims: To study the frequency of hypoglycemia and its correlation with the events of tachyarrhythmia in patients with DMT2 (on

treatment with insulin or oral medication), pAfib (receiving anticoagulation) and ischemic stroke.
Materials and methods: The study duration was 36 months and included 278 patients with DMT2 that had pAfib and ischemic stroke. All patients visited for routine health screening examination or with minor diseases the departments of internal medicine of 3 general hospitals. All participants had ECGs, cerebral computed tomography and a Holter monitoring of rhythm in order to establish the diagnosis of pAfib and previous ischemic stroke. Fasting blood glucose and postprandial glucose (2 hrs after meal) were measured. An ECG was performed to all participants every 3 months and when they visited the collaborating hospitals due to a feeling of high pulses (tachyarrhythmias).
Results: 166 patients (60%) out of 278 suffered from tachyarrhythmias in the context of pAfib. The remaining 112 patients (40%) did not have similar episodes of tachyarrytmia. From blood glucose measurements, there were 133 patients (80%) from the first group that had 10–18 episodes of hypoglycemia (2–4 episodes per month). From the second group that did not show any tachyarrhythmias, only 14 patients had 8–10 episodes of hypoglycemia (0–2 episodes per month). The difference between the two groups was statistically significant ($p = 0.04$)
Conclusion: Hypoglycemia increases the risk of tachyarrhythmia (estimated 20% increase) in the context of pAfib in patients with DMT2 and ischemic stroke. Avoiding hypoglycemia must be a main goal when treating patients with DMT2 especially in those patients with pAfib and ischemic stroke.
Disclosure: **A. Sianni:** None.

904

Acute hypoglycaemia results in increased activation of responses to an inflammatory challenge induced 48 hours later in human subjects

A. Iqbal[1], L. Prince[1], P. Novodvorsky[1], A. Bernjak[1], M. Thomas[1,2], L. Birch[1], D. Lambert[1], L. Kay[1], F. Wright[1], R. Jacques[1], R. Storey[1], R. McCrimmon[3], S. Francis[1], I. Sabroe[1], S. Heller[1];
[1]University of Sheffield, Sheffield, [2]University of Birmingham, Birmingham, [3]University of Dundee, Dundee, UK.

Background and aims: Hypoglycaemia is emerging as a risk factor for cardiovascular (CV) morbidity and mortality in diabetes but the precise mechanisms are unclear. The innate immune system is critical to the aetiology of CV events. Because CV events do not appear to occur during the hypoglycaemic episode *per se*, we sought to examine the hypothesis that hypoglycaemia may prime the innate immune system, leading to a more marked inflammatory response to a subsequent inflammatory challenge.
Materials and methods: In a novel *in vivo* human experimental model, we combined an established hypoglycaemic stimulus with a classical systemic stimulus of the innate immune system (lipopolysaccharide challenge). Twenty-four healthy volunteers underwent either a hyperinsulinaemic-hypoglycaemic (2.5 mmol/l) ($n = 8$), euglycaemic (6.0 mmol/l) ($n = 8$) or sham-saline clamp ($n = 8$) (normoglycaemic conditions). To determine if hypoglycaemia primed innate immune responses, all participants then received a low-dose (0.3 ng/kg) intravenous endotoxin challenge 48 hours later. Using flow cytometry, we studied total white blood cell and subset kinetics and determined monocyte activation by measuring CD11b expression.
Results: Compared to euglycaemia, both hypoglycaemia and sham-saline were associated with greater leucocyte mobilisation in response to endotoxin ($P < 0.05$, Fig 1). There was a trend towards a higher total monocyte count in the hypoglycaemia group 4 hours post endotoxin compared to euglycaemia (mean ± SEM cells/μL: 0.56 ± 0.11 vs 0.37 ± 0.06; $P = 0.08$). Monocyte CD11b expression at 4 and 6 hours following endotoxin was significantly higher compared to baseline in all groups ($P < 0.001$). The percentage of total monocytes that were positive for CD11b expression was higher in hypoglycaemia versus euglycaemia at 2 hours post endotoxin (mean ± SEM %: 94.10 ± 1.11 vs 86.02 ± 4.64; $P < 0.01$). Adrenaline levels were not significantly different between study groups 6 hours following endotoxin (mean ± SEM nmol/L hypoglycaemia: 0.15 ± 0.04 vs euglycaemia: 0.06 ± 0.01 vs sham-saline 0.09 ± 0.01; $P > 0.05$).
Conclusion: We show that a single episode of hypoglycaemia compared to euglycaemia invokes a stronger proinflammatory response to endotoxin up to 2 days later. Further, the drivers for differential leucocyte mobilisation to endotoxin are not due to between group differences in catecholamines. This suggests hypoglycaemia may prime the innate immune system leading to a more profound inflammatory response to a subsequent inflammatory stimulus, which may in turn explain the increased CV risk associated with hypoglycaemia in diabetes.

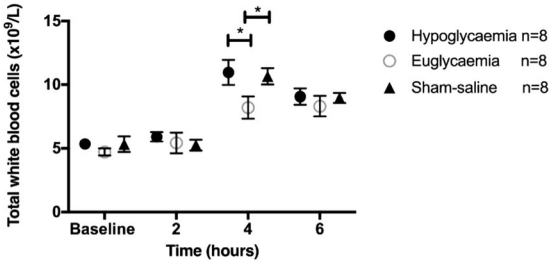


Figure 1: Peripheral total white blood cell (WBC) kinetics post endotoxin challenge. Total number of WBCs 2, 4 and 6 hours following low dose (0.3 ng/kg) intravenous endotoxin challenge in participants that underwent hypoglycaemia, euglycaemia or a sham-saline clamp 48 hours earlier. Data are mean (SEM), *p<0.05, P-values are provided for comparison between study groups.

Supported by: MRC Clinical Research Training Fellowship Award to Dr Ahmed Iqbal
Disclosure: **A. Iqbal:** Grants; Medical Research Council Clinical Research Training Fellowship.

905

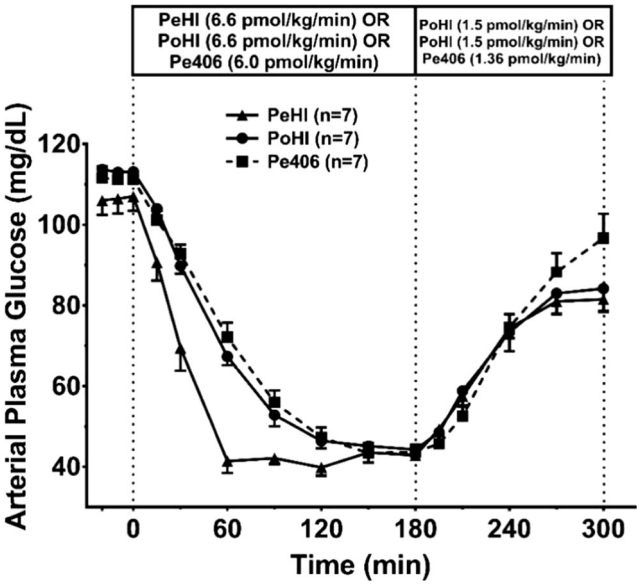
Peripheral infusion of a hepato-preferential insulin analogue mimics the hypoglycaemia-sparing effect of portal vein human insulin infusion in dogs

G. Kraft[1], M. Scott[1], M. Smith[1], J. Hastings[1], C.L. Brand[2], C. Fledelius[2], P. Madsen[2], T. Kjeldsen[2], S. Hostrup[2], E. Nishimura[2], A.D. Cherrington[1], J.M. Gregory[1];
[1]Vanderbilt University, Nashville, USA, [2]Novo Nordisk, Copenhagen, Denmark.

Background and aims: We previously quantified the hypoglycemia-sparing effect of portal vs peripheral human insulin delivery. As insulin administered via the sub-cutaneous (s.c.) route distributes like peripheral insulin delivery, an acylated insulin analog (INS-406) was engineered to distribute more like portal insulin when delivered s.c. In the present work we hypothesized that a bioequivalent infusion of the hepato-preferential INS-406 into a peripheral vein could achieve a protective effect against hypoglycemia, similar to that of portal human insulin delivery.
Materials and methods: In conscious dogs, human insulin (HI) was infused into either the portal vein (PoHI, $n = 7$) or a peripheral vein (PeHI, $n = 7$) for 180 min at a rate 4.4x basal (6.6 pmol/kg/min). INS-406 (Pe406, $n = 7$) was infused into a peripheral vein at 6.0 pmol/kg/min, a rate determined to bring about the same rate of fall in plasma glucose in the first 60 min as PoHI (before stimulating counter-regulatory hormones). Somatostatin was infused to inhibit glucagon secretion so it could be kept at a basal level, mimicking the diminished α-cell response seen in type 1 diabetes (T1DM). A 120 min recovery period followed the 180 min infusion period. In the PeHI and PoHI groups, insulin was infused portally and reduced to the basal secretion rate and in Pe406 the insulin infusion was reduced by the same proportion and given peripherally.

Results: Glucose fell quickly with PeHI, reaching 41 ± 3 mg/dL at 60 min, but it fell more slowly with PoHI and Pe406 (67 ± 2, $p < 0.01$ vs PeHI and 72.1 ± 4 mg/dL $p < 0.01$ vs PeHI, respectively at 60 min) (see figure). The hypoglycemic nadir occurred at 60 min with PeHI (41 ± 3 mg/dl) vs. 120 min with PoHI (45 ± 1 md/dl) and Pe406 (45 ± 1 mg/dl). IV glucose infusion was needed in four PeHI dogs to prevent severe hypoglycemia but no dogs in the other groups required glucose. ΔAUC (infusion period - basal) for epinephrine in PeHI, PoHI and Pe406 was 204 ± 21, 95 ± 29 ($p < 0.01$ vs PeHI), and 139 ± 21 ng/mL/180 min ($p = 0.04$ vs PeHI), respectively. Glucose production (mg/kg/min) was least suppressed from baseline in PeHI (0.79 ± 0.33) and equivalently but further suppressed in PoHI and Pe406 (1.16 ± 0.21 and 1.18 ± 0.17, respectively). Peak glucose utilization (mg/kg/min) was highest in PeHI (4.94 ± 0.17) and less in PoHI (3.58 ± 0.58, $p = 0.03$ vs PeHI) and Pe406 (3.26 ± 0.08, $p < 0.01$ vs PeHI).

Conclusion: We conclude that peripheral infusion of the hepatopreferential analog, INS-406, can achieve a metabolic profile that closely mimics that seen with portal insulin delivery and would be protective against hypoglycemia compared with peripheral insulin infusion. It is therefore hypothesized that hypoglycemia-sparing effects may be apparent with eventual s.c. treatment with INS-406 compared to conventional insulin therapy.



Supported by: NIH F32DK100114-01A1
Disclosure: **G. Kraft:** None.

906

Improved skeletal muscle energy metabolism relates to the recovery of beta cell function by early insulin intensive therapy in drug naive type 2 diabetes

W. Tang[1], Y. Bi[1], H. Wang[2], B. Zhang[2], D. Zhu[1];
[1]Department of Endocrinology, Drum Tower Hospital Affiliated to Nanjing University Medical School, Nanjing, [2]Department of Radiology, Drum Tower Hospital Affiliated to Nanjing University Medical School, Nanjing, China.

Background and aims: Early insulin intensive therapy protracts glycaemic remission in patients with newly diagnosed type 2 diabetes (T2DM), while the underlying mechanism remains to be elucidated. Recent studies have shown that an impaired skeletal muscle mitochondrial function is associated with the development of insulin resistance and type 2 diabetes mellitus. Thus, we aimed to characterize the contribution of mitochondrial activity to insulin-induced glycaemic control.

Materials and methods: Drug-naïve T2DM patients were recruited to receive continuous subcutaneous insulin infusion with an insulin pump. Treatment was stopped after normoglycaemia was maintained for 7 days. Muscular phosphocreatine (PCr) flux was acquired by ^{31}P magnetic resonance spectroscopy before and after insulin therapy. The total acquisition time consisted of 2 min of rest, 10 min of plantar flexion exercise and 10 min of recovery. The rates of adenosine triphosphate (ATP) synthesis in gastrocnemius were calculated as ΔPCr/Δtime during the first 10s after cessation of exercise. The study protocol was carried out in compliance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Dum Tower Hospital affiliated to Nanjing University.

Results: A total of 20 patients enrolled in the study, with mean age of 40.0 years, mean body mass index (BMI) of 24.3 kg/m^2 and mean HbA1c of 11.2%. β-cell function (represented by HOMA2-B) and systematic insulin sensitivity (represented by HOMA2-IR) improved significantly after intensive insulin intervention. In addition, the relative changes of postprandial C peptide increased along with the improvement of muscular ATP synthesis efficacy after adjustments of age and BMI ($r = 0.620$, $p = 0.006$). Participants who achieved higher relative changes of postprandial C peptide had approximately 2-fold increase in the rates of ATP synthesis and performed lower HbA1c (6.3 ± 0.4 versus 7.6 ± 0.4, $p = 0.044$) after 3-months follow-up.

Conclusion: These data firstly suggested that improved muscular mitochondrial ATP synthesis might play a role in the promising effects of insulin on glycaemic control in newly diagnosed type 2 diabetes.

Supported by: National Natural Science Foundation of China
Disclosure: **W. Tang:** Grants; National Natural Science Foundation of China.

907

Circulating dipeptidyl peptidase-4 activity is decreased by short-term intensive insulin therapy in patients with newly diagnosed type 2 diabetes

L. Liehua, W. Ke, H. Li, J. Liu, X. He, J. Chen, Y. Li;
Endocrinology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

Background and aims: Short-term intensive insulin therapy is shown to induce recovery of β cell function and subsequent glycemic remission in patients with newly diagnosed type 2 diabetes, but the detailed mechanism is not fully understood. Activity of dipeptidyl peptidase-4 (DPP-4) is an important regulatory factors of β cell function as it cleaves incretin hormones. Whether DPP-4 plays a role in the mechanism of restoration of β cell function remains to be clarified. This study analyzed the changed of DPP-4 activity during short-term intensive insulin therapy and its association with metabolic parameters.

Materials and methods: In this study we enrolled 51 patients with newly diagnosed type 2 diabetes who had never accepted any anti-hyperglycemic agents. Baseline blood samples were collected for measurements of lipid profiles, alanine transaminase (ALT) , aspartate transaminase (AST), γ-glutamyltransferase (GGT), free fatty acid (FFA), HbA1c and fasting and post-prandial plasma glucose. Fasting DPP-4 activity was determined using Gly-Pro-p-nitroanilineas substrate with the release of p-nitroaniline monitored at 405 nm. An intravenous tolerance test is performed for assessing acute insulin response (AIR). Afterwards, short-term intensive insulin therapy using insulin pump was given to all patients with near-normoglycemia (fasting blood glucose 4.4–6.1 mmol/L, 2 h post-prandial blood glucose 4.4–7.8 mmol/L) achieved and maintained for 2 weeks. Then insulin infusion was stopped and baseline measurements were repeated in the next morning.

Results: After the therapy, fasting plasma glucose (11.5 ± 3.3 mmol/L vs 6.6 ± 1.2 mmol/L, $P < 0.001$), post-prandial plasma glucose (17.7 ± 6.8 mmol/L vs 9.4 ± 2.4 mmol/L, $P < 0.001$) ALT(37.0 ± 30.3 U/L vs 29.1 ± 20.8 U/L), GGT (49.2 ± 40.3 U/L vs 30.1 ± 24.5 U/L) , FFA (672.4 ± 230.2 μmol/L vs 571.8 ± 209.6 μmol/L, $P < 0.02$) and HOMA IR (3.5 ± 2.4 vs 2.4 ± 1.4, $P = 0.001$) were all significantly decreased, while AIR (−61.5 ± 26.4 pmol/L min vs 388.0 ± 72.3 pmol/L min, $P < 0.001$) and HOMA B (22.1 ± 20.0 vs 54.7 ± 33.8 , $P < 0.001$) were elevated. Serum DPP-4 significantly decreased from 36.1 ± 8.4 nmol/min/ml to 31.0 ± 7.2 nmol/min/ml ($P < 0.001$). At baseline, Circulating DPP-4 activity was associated with ALT ($r = 0.30$, $P = 0.03$), AST ($r = 0.32$, $P = 0.02$), GGT ($r = 0.36$, $P = 0.01$) and FFA ($r = 0.41$, $P = 0.01$); similar association of DPP-4 with liver enzymes was found after the therapy (for ALT, $r = 0.34$, $P = 0.02$; for AST, $r = 0.29$, $P = 0.04$; for GGT, $r = 0.42$, $P = 0.003$). Decrement of DPP-4 was only associated with reduction of FFA ($r = 0.50$, $P < 0.001$). No significant association was found between DPP-4 and blood glucose, HbA1c, lipid profiles, HOMA IR, HOMA B, AIR and body-weight at baseline or after the therapy.
Conclusion: Circulating DPP-4 activity is significantly decreased after short-term intensive insulin therapy. Moreover, circulating DPP-4 activity was only associated with liver enzymes and FFA other than blood glucose, HOMA IR or β-cell function indices. This fact implied that circulating DPP-4 activity is a potential indicator of metabolic dysfunction in liver and adipose tissue, instead of glucose homeostasis.
Clinical Trial Registration Number: NCT01471808
Disclosure: **L. Liehua:** None.

908

Intraperitoneal insulin does not result in less systemic oxidative stress

H. van Goor[1], A. Pasch[2], N. Kleefstra[3], S.J.J. Logtenberg[4], K.H. Groenier[5], T.M. Vriesendorp[5], F. Waanders[6], H.J.G. Bilo[5,7], P.R. van Dijk[5,7];
[1]Department of Pathology and Medical Biology, Division of Pathology, University of Groningen, Groningen, Netherlands, [2]Department of Biomedical Research, University of Bern, Bern, Switzerland, [3]Langerhans Medical Research Group, Ommen, Netherlands, [4]Internal Medicine, Diakonessenhuis, Utrecht, Netherlands, [5]Diabetes Centre, Isala, Zwolle, Netherlands, [6]Internal Medicine, Isala, Zwolle, Netherlands, [7]Internal Medicine, University of Groningen, Groningen, Netherlands.

Background and aims: Continuous intraperitoneal insulin infusion (CIPII) is a last-resort treatment option for patients with type 1 diabetes mellitus (T1DM) who fail to achieve glycemic control with subcutaneous (SC) insulin administration. In animal experiments CIPII was accompanied by less oxidative stress as compared to SC insulin administration. Data concerning humans are lacking. As thiols (R-SH; compounds with free sulfhydryl groups) are readily oxidized by reactive oxygen and sulfur species, their circulating concentrations directly reflect systemic redox status. We hypothesized that in patients with T1DM CIPII lead to higher R-SH concentrations as compared to SC insulin administration.
Materials and methods: This study is a post-hoc analysis of a prospective, observational case-control multicentre study. Age and gender matched patients were treated with either CIPII or SC insulin using multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII). Measurements were performed at the start and end of a 26-week interval. Differences between IP and SC groups averaged over the study period and in time were estimated using a general linear model. Multivariate regression analysis was performed with the mean score over the study period. A total of 180 patients with a mean age of 50 (12) years, BMI of 26 (5) kg/m^2, diabetes duration of 24 [17, 35] years and HbA1c of 64 (10) mmol/mol were analysed. Of these patients 39 were treated with IP and 141 with SC insulin (67 MDI and 74 CSII).
Results: For all patients, the estimated geometric mean R-SH concentrations increased with 21 μM (95%CI 13 to 28) from 250 μM (95%CI 245 to 256) at visit 1 to 271 μM (95%CI 266 to 276) at visit 2. Among patients treated with CIPII this increase was 18 μM (95%CI 5 to 32), from 254 μM (95%CI 244 to 264) at visit 1 to 272 μM (95%CI 263 to 282) at visit 2. And it was 23 μM (95%CI 15 to 30), 246 (95%CI 240 to 251) to 269 μM (95%CI 264 to 274), among SC treated patients. R-SH concentrations among patients treated with CIPII were similar as compared to patients treated with SC insulin: 263 μM (95%CI 256 to 270) versus 257 μM (95%CI 254 to 261), difference: 6 μM (95%CI −1 to 13). In multivariate regression analysis, R-SH concentrations were significantly associated with BMI and total insulin dose while gender, HbA1c, LDL cholesterol and route of insulin administration were not.
Conclusion: This in the fist study in human T1DM patients to demonstrate that the route of insulin administration (CIPII or SC) does not significantly influence the systemic redox status.
Supported by: Isala Innovatie en Wetenschapsfonds
Disclosure: **H. van Goor:** None.

PS 080 Causes and consequences of hypoglycaemia

909

Moderate to vigorous physical activity is not associated with increased hypoglycaemia or glycaemic variability in individuals with type 1 diabetes

G.S. Taylor[1], R.J. Stefanetti[2], S. Cassidy[1], A.J. Flatt[1], A.S. Bashir[1], M.D. Campbell[3], E.J. Stevenson[1], J.A. Shaw[1], D.J. West[1];
[1]Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, [2]Institute of Neuroscience, Newcastle University, Newcastle Upon Tyne, [3]Institute for Sport, Physical Activity & Leisure, Leeds Beckett University, Leeds, UK.

Background and aims: Regular physical activity (PA) is recommended for individuals with Type 1 diabetes (T1D) as it can improve a range of cardiovascular risk factors. PA levels are generally lower in T1D compared to the wider population, with disrupted glucose control, particularly hypoglycaemia, suggested to be a barrier to people achieving the recommended 150 minutes of moderate to vigorous activity (MVPA) per week. At present there is limited data that has objectively measured PA levels alongside free-living glucose control. The aim of this study was to explore PA in individuals with T1D, and determine if the amount of MVPA is associated with measures of glycaemic control.
Materials and methods: 47 participants with T1D (M/F 27/20, age 40 ± 11 years, HbA1c 57.2 ± 9.1 mmol/mol^{-1}, BMI 25.3 ± 3.4 kg/m^2 and diabetes duration 21 ± 11 years) attended the laboratory for fitting of a blinded interstitial Continuous Glucose Monitor (CGM) and physical activity accelerometer. The devices were worn for 7 days. ≥3 valid daily blood glucose calibrations with a correlation >0.79 were required for the day's CGM data to be accepted. Time spent in range was calculated as a percentage of total time. Accelerometer data were processed in R using R-package GGIR with the threshold for moderate activity or greater set as ≥100 mg. 18 hours of daily wear time was considered an acceptable threshold for data inclusion and only moderate and vigorous activity bouts of ≥10 minutes duration were analysed. Data were reported as mean ± SD and analysed by an independent sample t-test and Pearson's correlation with significance accepted at $p \leq 0.05$.
Results: Participants achieved 26.8 ± 20.7 minutes of MVPA a day with no difference between genders ($p = 0.872$). 24 (51%) of the participants completed >150 minutes of MVPA across 7 days, with 4 participants completing 0 minutes. No correlations were found between amount of MVPA and mean glucose ($r = -0.210$ $p = 0.166$), HbA1c ($r = 0.045$, $p = 0.770$), or any CGM glycaemic parameters: time <3 mmol/l $r = -0.038$, $p = 0.805$; >13.9 mmol/l $r = -0.154$, $p = 0.313$; time in range 3.9–10 mmol/l $r = 0.207$, $p = 0.172$; outside range <3.9 and >10 mmol/l $r = -0.207$, $p = 0.172$. There was no association between MVPA and various measures of glycaemic variability (SD $r = -0.045$, $p = 0.771$; CV $r = 0.122$, $p = 0.426$). No differences were found between individuals completing over ($n = 24$, daily MVPA 42.74 ± 14.57 minutes) and under ($n = 21$, daily MVPA 8.78 ± 7.03 minutes, $p < 0.01$) 150 minutes of MVPA for age ($p = 0.833$), duration of diabetes ($p = 0.482$), HbA1c ($p = 0.708$) or BMI ($p = 0.147$). Additionally, there were no differences in any CGM parameters ($p > 0.05$).
Conclusion: This is the first observational study to compare objectively measured physical activity with glycaemic control under free-living conditions in T1D. We show that greater time spent doing MVPA is not associated with increased glycaemic variation, hypoglycaemia or time out of range. Moreover, there were no clinical or glycaemic differences between those individuals achieving over 150 minutes of physical activity compared to those below. Future research is needed to explore how accumulative PA bouts impact upon glycaemic variability and time in range.
Supported by: DRWF awarded to D.W
Disclosure: **G.S. Taylor:** Grants; Diabetes Research & Wellness Foundation award to DW.

910

Relationship between severe hypoglycaemia, impaired awareness of hypoglycaemia, psychological distress, quality of life and cognition in type 1 diabetes

E. Sepulveda[1], D. Carvalho[2], D. Seixas[3], S.A. Amiel[4], S.G. Vicente[1];
[1]Centre for Psychology at University of Porto, Faculty of Psychology and Educational Sciences, University of Porto, Porto, Portugal, [2]Department of Endocrinology, Diabetes and Metabolism, Centro Hospitalar São João, Porto, Portugal, [3]Institute for Biomedical Imaging and Life Sciences, University of Coimbra, Coimbra, Portugal, [4]Diabetes Research Group, King's College London, London, UK.

Background and aims: Impaired awareness of hypoglycaemia (IAH) in type 1 diabetes increases risk of severe hypoglycaemia (SH) six-fold. SH is associated with impaired cognitive function acutely, with reports of an association with long-term cognitive dysfunction. This study examined age, diabetes duration, psychological distress, quality of life (QoL), and cognition in adults with type 1 diabetes with intact hypoglycaemia awareness (HA) and IAH, according to the Gold score (GS) or Guy's and St Thomas' Minimally Modified Clarke Hypoglycaemia Survey (MMCHS), and also in those reporting presence or absence of ≥1 SH in the past 6 months; and sought predictors of moderate or severe cognitive impairment in global cognition and executive functions.
Materials and methods: We recruited 137 adults with type 1 diabetes. Hypoglycaemia awareness status was determined using the GS (one-item) and MMCHS (eight-item), with ≥4 = IAH. SH was evaluated by one question (question 3) in the MMCHS. Participants also completed the Montreal Cognitive Assessment (MoCA; global cognition: visuospatial abilities, executive functions, language, delayed recall, and orientation); the INECO Frontal Screening (IFS; executive functions: inhibition and set shifting, abstraction, and working memory); the Diabetes Health Profile (DHP; QoL: barriers to activity [BA], psychological distress [PD], and disinhibited eating [DE]); and the Hospital Anxiety and Depression Scale (HADS).
Results: Participants' mean age, diabetes duration and HbA1c were 38.1 years, 19.7 years, and 8.0%, respectively; 51.8% were women. Patients with IAH, according to the MMCHS, had a longer diabetes duration ($U = 947.0$, $p = 0.012$) and higher scores on HADS depression scale ($U = 910.5$, $p = 0.012$) than HA ones. Patients with SH in the past 6 months had higher scores on HADS total ($U = 1572.5$, $p = 0.022$) and DHP total ($U = 1498.5$, $p = 0.035$), higher depression on HADS ($U = 1594.5$, $p = 0.027$), and lower performance on z-score MoCA executive functions domain ($U = 1012.5$, $p = 0.032$) than patients with no SH. No diferences were found in age, QoL, and cognition between HA and IAH assessed by GS or MMCHS, or in age and diabetes duration between patients with and without SH. Being female (Exp (B) = 4.327, $p = 0.005$), having peripheral neuropathy (PN) (Exp (B) = 6.656, $p = 0.018$), and lower education (Exp (B) = 0.878, $p = 0.034$) were independent predictors of worse performance in IFS total.
Conclusion: While SH has sometimes controversially been associated with impaired cognitive function, the impact of IAH alone has not been studied. Our findings suggest that IAH was related with depression but not with cognitive impairment, while SH was related with both. The different relationships of IAH and SH with executive dysfunction suggest that SH may be a necessary event for cognitive dysfunction, although the direction of the link cannot be established from this study. In addition to SH, PN, female gender, and lower education may impact executive functioning.
Disclosure: **E. Sepulveda:** None.

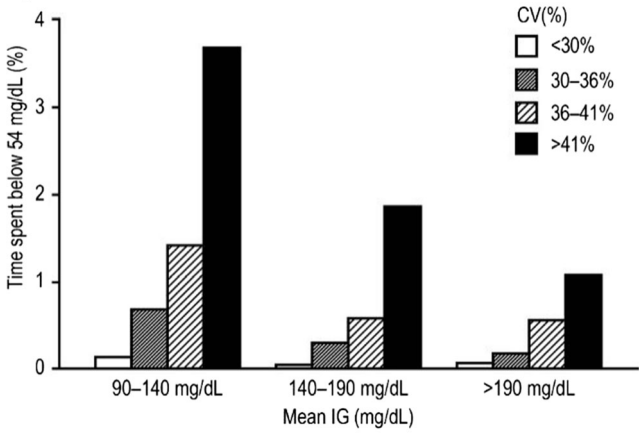
911

Reducing glycaemic variability decreases time in hypoglycaemia independent of mean glucose: data from real-world continuous glucose monitoring in type 1 diabetes patients

R. Bergenstal[1], E. Hachmann-Nielsen[2], A. Kaas[2], K. Kvist[2];
[1]International Diabetes Center, Minneapolis, USA, [2]Novo Nordisk A/S, Søborg, Denmark.

Background and aims: The increasing amount of continuous glucose monitoring (CGM) data reveals insights to better understand glucose control in diabetes and opens up for valuable indicator metrics like time in range, time in hypoglycaemia and time in hyperglycaemia. Previous studies have related lower HbA_{1c} with higher risk of hypoglycaemia, but less strict HbA_{1c} control does not protect against hypoglycaemia. CGM data can assist in answering the question: what is the role of glycaemic variability in diabetes management? The objective was to explore the association between glucose variability (coefficient of variation [CV%]), mean interstitial glucose (IG) and time spent in hypoglycaemia (TIHypo; % time with IG <54 mg/dL [3.0 mmol/L]). In addition, the link between CV and time spent in hyperglycaemia (TIHyper; % time with IG >250 mg/dL [13.9 mmol/L]) and time in range (TIR; % time with IG between 70 and 180 mg/dL [3.9 and 10.0 mmol/L]) was explored for different levels of mean IG.
Materials and methods: Patients volunteered to upload their data via Cornerstone4Care (C4C), a freely available digital patient management programme (app) for diabetes mellitus. The app supports diabetes self-management and captures self-reported patient data and real-world, real-time CGM data. A total of 112 type 1 diabetes (T1D) patients uploaded their CGM data via the C4C app for analysis. The CV was calculated based on non-overlapping 2-week intervals of CGM data. For each 2-week period, a CV index, mean IG, proportion of TIHypo, TIR and TIHyper was determined. The impact of glycaemic variability (CV) on TIHypo, TIR and TIHyper was analysed across a range of IG values (Figure). Definitions and data analysis was done in accordance with directions from the International Consensus on Use of Continuous Glucose Monitoring: a minimum of 14 consecutive days of data with approximately 70% of possible CGM readings over those 14 days. Stable glucose levels were defined as a CV <36%.
Results: A total of 369 non-overlapping 2-week periods with a high rate of available data (>70%) was available for analysis. There was a significant association between CV and TIHypo ($p = 0.0007$), linking higher glucose variability (CV) to more TIHypo. However, there was no significant association between CGM-derived mean IG and TIHypo ($p = 0.051$), indicating that, in these data, a lower mean IG does not significantly result in more TIHypo. When adjusting for the levels of CV, the association with mean IG becomes significant for TIHypo ($p = 0.0019$); likewise, CV significantly increased the TIHypo for all levels of mean IG (Figure, $p = 0.0006$). Both TIHyper and TIR showed significant associations to mean IG and CV in a multivariate setting ($p < 0.01$).
Conclusion: Hypoglycaemia can be a problem across all levels of CGM-derived mean IG. Independent of mean IG, there is a significant value of reducing variability to minimise time spent in hypoglycaemia.

Figure: The association between glycaemic variability (CV%) and TIHypo by CGM-derived mean IG



CGM, continuous glucose monitoring; CV, coefficient of variation; IG, interstitial glucose; TIHypo, time spent in hypoglycaemia.

Supported by: Novo Nordisk
Disclosure: **R. Bergenstal:** Employment/Consultancy; Novo Nordisk, Abbott Diabetes Care, Calibra, Eli Lilly, Hygieia, Roche, Sanofi, DexCom, Medtronic, United HealthCare, Onduo. Stock/Shareholding; Merck – family inheritance. Other; Volunteer for ADA and JDRF, Novo Nordisk, Abbott Diabetes Care, Boehringer Ingelheim, AstraZeneca, Calibra, Eli Lilly, Hygieia, Roche, Sanofi, Takeda, DexCom, Medtronic.

912

Common yet overlooked: non-severe hypoglycaemia and its risk indicators in type 2 diabetes (InHypo-DM Study)

N.H. Au, A. Ratzki-Leewing, B.L. Ryan, S. Mequanint, J.E. Black, S. Reichert, J.B. Brown, S. Harris;
Western University, London, Canada.

Background and aims: Non-severe hypoglycemia (NSH) is a common adverse event among people with type 2 diabetes mellitus (T2DM) using insulin and/or secretagogues. Its reoccurrence induces fear and other patient behaviors, which can lead to changes in glycemic therapies thereby impeding optimization of glycemic control. In addition, NSH can increase the risk of severe hypoglycemia (SH) and associated clinical sequelae. Much attention has focused on the importance of SH with less understanding of the risk indicators and impact of NSH. As NSH is self-treated and frequently under-reported, its clinical relevance has often been overlooked and its occurrence is too commonly not captured in electronic medical records/claims databases. Our current understanding of NSH has mainly come from clinical trials, which may not be reflective of the real-world incidence of NSH due to the stringent sampling frames used. Drawing on the strength of self-reported NSH, the population-based InHypo-DM study explored the association between relevant risk indicators and the rate of NSH among individuals with T2DM.
Materials and methods: Adults with diabetes (≥18 years of age) treated with insulin and/or secretagogues were recruited from a nationally-representative online panel in Canada. A validated questionnaire elicited self-reported frequencies of NSH, socio-demographic and clinical characteristics. Multivariable negative binomial regression, informed by univariable analyses ($p \leq 0.2$) and subsequent backward selection ($p < 0.05$), identified pertinent risk indicators of NSH frequency among complete cases.
Results: This analysis is based on 432 individuals with T2DM (male: 56.3%, mean age: 53.0 years (SD: 14.7), mean duration of diabetes: 11.7 years (SD: 7.8)). Over half (54.2%, 95% CI: 49.5% to 58.9%) of the respondents self-reported at least one NSH event in the past 30 days. The annualized incidence rate of NSH was 28.7 (95% CI: 26.9 to 30.5) events/person-year. Multivariable analysis suggested that being employed (versus other) and presence (versus absence) of comorbidities that interfere with hypoglycemia management increased the expected rate of NSH by a factor of 1.46 (95% CI: 1.01 to 2.10; $p = 0.0444$) and 2.08 (95% CI: 1.52 to 2.84; $p < 0.0001$), respectively. Lower annual household income brackets ($p < 0.0001$) and higher levels of HbA_{1c} ($p = 0.0067$) were independently associated with increased rates of NSH. Longer duration of diabetes ($p = 0.0005$) and younger age ($p < 0.0001$) were also identified as risk indicators of increased rates for NSH.
Conclusion: Our study identified relevant socio-demographic and clinical risk indicators for NSH in a real-world setting. Most notably, lower annual household income, being employed, and presence of comorbidities were associated with higher rates of NSH. Results suggest the influence of determinants beyond traditional clinical factors on hypoglycemia frequency, such as socio-economic and situational context. The InHypo-DM study presents compelling real-world evidence of the frequent burden imposed by NSH on adults with T2DM. These findings may identify patients at risk of higher rates of NSH, creating clinically relevant opportunities to improve glycemic management and initiate upstream prevention of NSH and SH in T2DM.

Supported by: Unrestricted grant from Sanofi Canada
Disclosure: **N.H. Au:** None.

913

Hypoglycaemia and risk of all-cause mortality in people with dementia and diabetes: a cohort study

K. Mattishent[1], K. Richardson[2], G. Savva[3], K. Dhatariya[4], C. Fox[1], Y. Loke[1];
[1]Norwich Medical School, Norwich, [2]School of Health Sciences, Norwich, [3]Quadram Institute Bioscience, Norwich, [4]Norfolk and Norwich University Hospital Foundation Trust, Norwich, UK.

Background and aims: A recent meta-analysis has found that hypoglycaemia may be associated with increased risk of mortality, cardiovascular events, falls and fractures. However, the included studies have not addressed serious consequences in specific patient groups at particularly high risk of hypoglycaemia, such as those with co-morbid dementia and diabetes. We aimed to determine the risk of all-cause mortality associated with hypoglycemia in older patients with diabetes and dementia.
Materials and methods: Retrospective cohort study of patients with diabetes (± dementia) in England aged >65 years based on the Clinical Practice Research Datalink with linkage to Hospital Episode Statistics and mortality data from Office of National Statistics (April 1997 to March 2016). We constructed three cohorts:Cohort 1: patients with dementia and diabetes and no hypoglycemic episodes;Cohort 2: patients with comorbid dementia and diabetes at first hypoglycemic episode;Cohort 3: patients with diabetes and first hypoglycemic episode (no dementia). The exposure/index date was defined as the first hypoglycemic episode captured on healthcare database after 1 April 1997. We evaluated mortality data for up to 5 years after index hypoglycemia, or end of available HES linkage. First, we compared whether the presence or absence of hypoglycemic episodes was associated with mortality in patients with dementia and diabetes (cohort 2 compared with cohort 1). Secondly, we compared whether hypoglycemic episodes have a different association with mortality in patients with dementia and diabetes compared to those with diabetes but no dementia (cohort 2 compared with cohort 3). We estimated Hazard Ratios (HR) and 95% confidence intervals (CI) for the association between hypoglycemia and subsequent mortality using Cox proportional hazard regression models with adjustment for age, sex, sociodemographics, co-morbidities and medications.
Results: We enrolled 19995 participants and found that hypoglycemia was associated with greater subsequent mortality in patients with diabetes and dementia (cohort 2). See Table 1 for number of deaths during follow-up and adjusted HR for association between hypoglycemic episodes and mortality.
Conclusion: Hypoglycemia has serious consequences in older people with diabetes and dementia. The consequences of hypoglycemia are worse in patients with comorbid dementia compared to those with diabetes alone. Prevention and reduction of hypoglycemia in older people with dementia and diabetes should be a top priority.

Table 1: Number of deaths and adjusted HR for association between hypoglycemic episodes and mortality

Cohort	Deaths during follow-up	Adjusted HR (95% CI)	
1 (n=6134)	3853 (63%)	Effect of hypoglycemia on mortality in patients with dementia (cohort 2 vs cohort 1)	Effect of dementia on mortality in patients with hypoglycemia (cohort 2 vs cohort 3)
2 (n= 1679)	1369 (82%)		
3 (n=12182)	6494 (53%)		
		1.66 (1.51-1.81)	1.67 (1.54-1.80)

Supported by: Alzheimer's Society Grant number: 324 (AS-CTF-16-001)
Disclosure: **K. Mattishent:** None.

914

Alexithymia, perfectionism, and attachment insecurities in type 1 diabetes patients with impaired awareness of hypoglycaemia: a pilot study

A. Naito[1,2], M. Nwokolo[2], E. Smith[1], N. De Zoysa[1], C. Garrett[3], P. Choudhary[1,2], S.A. Amiel[1,2];
[1]Diabetes Department, King's College Hospital NHS Foundation Trust, London, [2]Diabetes Research Group, Faculty of Life Sciences and Medicine, King's College London, London, [3]Diabetes and Psychiatry Research Group, Department of Psychological Medicine, King's College London, London, UK.

Background and aims: Up to a third of people with type 1 diabetes (T1D) have Impaired Awareness of Hypoglycaemia (IAH), increasing their risk of severe hypoglycaemia (SH) six-fold. Qualitative studies have identified cognitive barriers to behaviour change around hypoglycaemia avoidance and management in individuals with IAH that may diminish potential benefit from interventions to reduce SH such as structured education and technology. This study explores the hypothesis that IAH may be associated with specific personality traits of alexithymia (difficulty in identifying and expressing one's own emotions) and clinical perfectionism (striving for excessively high personal standards despite adverse consequences, combined with overly critical self-evaluation) and examines emotional dysregulation and attachment insecurities as possible factors distinguishing people with the condition.
Materials and methods: Five validated self-reported questionnaires, the Total Alexithymia Scale (TAS-20); the Frost Multidimensional Perfectionism Score (FMPS) exploring specific aspects of personality; the Reflective Functioning Questionnaire (RFQ); the Difficulties in Emotion Regulation Scale (DERS) and the Experiences in Close Relationships-Revised Scale (ECR-R) exploring attachment types; plus Attitudes to Awareness Questionnaire (A2A) exploring attitudes and beliefs about hypoglycaemia were distributed to 19 people with IAH [Gold score >4; age 40.7 ± 11.0 (mean ± SD) years, diabetes duration 26.5 ± 10.3 years, HbA1c 7.8 ± 0.8% (61.5 ± 8.8 mmol/mol)] and 15 with intact hypoglycaemia awareness (HA) [Gold score <3; age 39.5 ± 12.2 years, diabetes duration 26.7 ± 12.3 years, HbA1c 7.7 ± 1.0% (60.7 ± 11.3 mmol/mol), all $p > 0.05$ except Gold scores, $p < 0.001$] in a pilot study.
Results: Compared to those with HA, participants with IAH were more likely to have alexithymia on TAS-20 with a trend to significance (21% vs. 0%, χ^2 (1) = 3.58, $p = 0.059$) and had significantly greater variations in the Difficulty Identifying Feelings subscale ($p = 0.042$). There was a large variation in the Concerns over Mistakes (CM) subscale of the FMPS ($p = 0.014$). There were trends towards higher Impulse subscale score on the DERS: ($p = 0.126$) and the dismissing attachment type on ECR-R (29% vs. 7%, χ^2 (1) = 2.44, $p = 0.118$). Scores on the Prioritising Hyperglycaemia Avoidance subscale on the A2A correlated strongly with the Concerns over Mistakes scores in the FMPS ($r = 0.736$, $n = 26$, $p < 0.001$).
Conclusion: This pilot study demonstrates the feasibility of assessing aspects of personality, emotion regulation, and attachment type in people with T1D with IAH. Early evidence suggests that specific psychological traits are associated with higher risk of IAH and SH, which may characterise the psychological processes underlying this presentation. Alexithymia may be a good candidate as a predictor for development of IAH. Confirmation of these findings in a larger cohort may help clinicians in assessing which patients are at higher risk of IAH and help structure the psychological support offered in addition to structured education and technology in order to prevent SH and retain or regain hypoglycaemia awareness.
Supported by: NIHR CLAHRC - South London
Disclosure: **A. Naito:** None.

915

Risk factors for severe hypoglycaemia in community-based patients with type 2 diabetes: the Fremantle Diabetes Study Phase II

T.M.E. Davis, W.A. Davis;
Medical School, University of Western Australia, Fremantle, Australia.

Background and aims: Data from the Fremantle Diabetes Study Phase I (FDS1) collected between 1998 and 2006 showed that the risk factors for incident severe hypoglycaemia (that requiring ambulance/hospital attendance) in type 2 diabetes were duration of insulin treatment, renal impairment, peripheral neuropathy, education beyond primary level and past severe hypoglycaemia. Given subsequent changes in clinical management and outcome, the aim of this study was to perform a parallel analysis in FDS Phase II (FDS2) >10 years later to determine whether the predictors of severe hypoglycaemia had also changed.
Materials and methods: The FDS2 is community-based observational cohort study that enrolled representative patients from an urban population of 150,000 people between 2008 and 2011. Severe hypoglycaemia was ascertained to end-2013 from: i) public/private hospitalisations, ii) Emergency Department presentations, and iii) ambulance attendances. For i) and ii), relevant International Classification of Diseases codes were required. For i), episodes were identified from a validated data linkage system and the case-notes accessed where possible to validate coding and provide detailed concomitant data. Cox proportional hazards modeling with imputation of missing data was used to identify clinically plausible independent predictors of the first episode of severe hypoglycaemia during follow-up.
Results: The 1,551 FDS2 patients with type 2 diabetes were of mean age 65.7 years, 51.9% were male, and their median diabetes duration was 9.0 years. Sixty-three (4.1%) experienced 83 episodes of severe hypoglycaemia during 6,195 patient-years (mean 4.0 ± 1.2 years) of follow-up with an incidence of 1.34 (95% CI 1.07–1.66)/100 patient-years. In the Cox model, age, current smoking, sulfonylurea and/or insulin treatment, prior severe hypoglycaemia, renal dysfunction, retinopathy, and the plasma N-terminal pro-B-type natriuretic peptide concentration (ln(NT-proBNP)) were positive independent predictors of time to first episode, while Southern European ethnicity was protective (see Table for hazard ratios (95% CIs) and *P* values).
Conclusion: These data confirm that older age, insulin/secretagogues, progressively worsening renal function, and prior severe hypoglycaemia are potent risk factors for severe hypoglycaemia complicating type 2 diabetes. The association with smoking may reflect reduced insulin clearance. Microangiopathy is a recognised risk factor but the present data show that retinopathy is predictive independently of nephropathy. The relatively low severe hypoglycaemia risk in Southern Europeans parallels evidence of geographic variation in other observational studies that reflects differences in anthropometric, lifestyle and clinical management between populations. The link with NT-proBNP suggests that patients with heart failure, an increasingly recognised chronic complication, may require intensive glycaemic monitoring and use of therapies with a low risk of hypoglycaemia.

Age (increase of 1 year)	1.04 (1.01-1.07)	0.005
Southern European ethnicity	0.28 (0.09-0.91)	0.035
Current smoker	2.64 (1.31-5.35)	0.007
Diabetes pharmacotherapy: Sulfonylurea (±other glucose-lowering drug)	2.15 (1.23-3.77)	0.007
Insulin only	2.81 (1.15-6.85)	0.023
Insulin+other glucose-lowering drug	3.92 (2.13-7.20)	<0.001
History of severe hypoglycaemia	3.76 (1.96-7.21)	<0.001
Any retinopathy	1.78 (1.02-3.11)	0.044
Estimated glomerular filtration rate (CKD-EPI): 45-59 ml/min/1.73m^2	2.16 (1.07-4.34)	0.031
30-44 ml/min/1.73m^2	3.20 (1.50-6.80)	0.003
<30ml/min/1.73m^2	4.60 (1.76-12.0)	0.002
Ln(NT-proBNP (pg/mL)) [increase of 1=2.72-fold NT-proBNP increase]	1.26 (1.03-1.53)	0.021

Supported by: NHMRC, Fremantle Hospital Research Foundation, Spinnaker
Disclosure: **T.M.E. Davis:** None.

PS 081 Sweet mothers - big babies

916

Maternal and foetal complications in women with gestational and pre-gestational diabetes: a population study

G. Di Cianni[1], E. Gualdani[2], C. Lencioni[1], E. Lacaria[1], P. Francesconi[2], G. Seghieri[2];
[1]Diabetes and Metabolic Diseases Unit, Health Local Unit Nord-West Tuscany, Livorno and Lucca Hospital, [2]Epidemiology Unit, Agenzia Regionale Sanità, Florence, Italy.

Background and aims: Both gestational (GDM) and pre-gestational diabetes (PGDM) are associated with an increased risk of maternal and fetal complications. Less known is the situation in the real world where pregnant women are monitored for metabolic conditions as well as for main risk factors throughout pregnancy. By means of available data concerning pregnant women who have performed an OGTT in Tuscany, a region of central Italy, we conducted a study to evaluate the risk of macrosomia, as well as that of neonatal or delivery outcomes (such as Apgar index or emergency cesarean section), comparing women with GDM and PGDM with a population of women without diabetes.
Materials and methods: We studied all women aged >15 aa living in Tuscany who delivered in the years 2012–2016. Women were identified by means of their childbirth certificate (CeDAP) and performed an OGTT after the 16th week of gestation. The diagnosis of GDM was performed using a validated algorithm including as parameters: having received a first prescription of insulin during pregnancy later interrupted after delivery, having at least a prescription for a visit by a diabetologist, having received an educational program or having performed an OGTT after 6 months of delivery. PGDM was diagnosed from the regional database of people with diabetes prior to index pregnancy. Women with normal glucose tolerance (NGT), were compared with those with GDM and PGDM, as for the risk of macrosomia (neonatal weight >4 Kg), of elective or urgent Caesarean section and of neonatal suffering (Apgar index <7).
Results: On a total of 85075 deliveries, as compared to NGT women, the relative risk of macrosomia was twice as high in women with pre-gestational diabetes: IRR: 2.00 (95% CI: 1.54–2.58); $p < 0.0001$, but on the contrary it was significantly reduced in women with GDM: IRR: 0.89 (0.81–0.98); $p = 0.02$. The risk of caesarean section both in urgency and in election was increased in women with GDM: IRR: 1.11 (1.05–1.17); $p = 0.0002$ and IRR: 1.38 (1.31–1.45), respectively; $p = 0.0001$) and was also significantly higher in PGDM: 1.28 (1.05–1.57) and 2.02 (1.72–2.37) respectively; $p = 0.0001$. These results did not change after adjustement for neonatal sex and for gestational age. In GDM and PGDM there was a trend of non-significant increase in neonatal distress (Apgar Index <7): IRR:1.12 (0.92–1.39) and 1.59 (0.80–3.16), p = NS for both.
Conclusion: Women with GDM had a reduced risk of macrosomia, suggesting an effective prevention of this complication in our population.The risk of macrosomia was instead significantly increased in women with PGDM. The risk of caesarean section was increased in both GDM and PGDM, without any evidence of increased risk of neonatal distress at delivery.
Disclosure: **G. Di Cianni:** None.

917

Gestational diabetes (GDM <24 weeks) is associated with worse pregnancy outcomes despite early treatment, when compared with GDM diagnosed at 24–28 weeks gestation

M. Mustafa, A. Khattak, D. Bogdanet, L. Mcknenna, L.A. Carmody, B. Kirwan, G. Gaffney, P. O'Shea, F. Dunne;
Galway Diabetes Research Centre, National University of Ireland Galway, Galway, Ireland.

Background and aims: Gestational Diabetes Mellitus is associated with more adverse pregnancy outcomes compared to women with normal glucose tolerance in pregnancy. WHO recommends screening at 24–28 weeks gestation. However some women are screened earlier due to symptoms or because of prior GDM. Those screened and diagnosed earlier in pregnancy have a longer period of intervention which may have an impact on pregnancy outcome. Information on the outcomes of women with GDM diagnosed <24 weeks gestation is limited. we aimed to examine pregnancy outcomes of women with GDM diagnosed <24 weeks gestation compared to those diagnosed at 24–28 weeks in a large treated European cohort.
Materials and methods: A retrospective cohort study was carried out of 1471 pregnancies from women with GDM diagnosed using IADPSG criteria. Women were classified as early GDM diagnosed (<24 weeks) (n = 275), or standard GDM diagnosed (24–28 weeks) gestation (n = 1,196).
Results: Women with early GDM had higher BMI at diagnosis than women diagnosed between 24–28 weeks [34.522 ± 7.33 versus 31.88 ± 5.96 (P value <0.001, mean difference 95%CI (1.63, 3.64)]. Although there was no significant differences between the 2 groups in Antenatal OGTT, Post partum OGTT was significantly higher in early GDM group [32% vs. 15.6% (P value <0.001, mean difference 95%CI (0.09, 0.24)], fasting glucose (5.253 ± 0.726 vs. 4.994 ± 0.723 P value <0.001, 95%CI (0.13, 0.38), 2 hour Glucose 5.69 ± 1.90 vs. 5.30 1.69 P value 0.012, 95%CI (0.07, 0.70). Table 1 below shows pregnancy outcomes.
Conclusion: Women with early GDM are more likely to develop hypertensive disorders and have an operative delivery. Stillbirths, preterm delivery and requirement for NNU care are more common in offspring of mothers with early GDM. Although birth weights are similar, a greater number of babies from mothers with early GDM are born LGA. In view of the greater number of early GDM women displaying abnormal OGTT post-partum, this may reflect a more advanced state along the pathway to diabetes.

Maternal outcome	<24 week (n=279,18.9%)	24-28 week (n=1197,80.1%)	P-value	95% CI for difference
Total C-Section	47 %	41.5 %	0.08	(0.008, 0.13)
Pregnancy induced Hypertension	12.4%	5.3%	<0.01	(0.03, 0.12)
Preeclampsia	3.8%	0.9%	<0.05	(0.004, 0.05)
Post-Partum Hemorrhage	8.7%	2.4%	<0.05	(0.01, 0.084)
Neonatal outcome Stillbirth	1.4%	0.5 %	<0.01	(0.11, -0.04)
Preterm birth (< 37 weeks)	10.9%	6.6%	0.03	(0.004,0.08)
Gestational week of delivery	37.40 -/+ 6.15	39.03 -/+ 1.90	<0.01	(2.36, -0.89)
Neonatal unit care (NNU)	30.7%	22.1%	<0.05	(0.02, 0.15)
Neonatal Morbidities	10.5%	8.4%	0.29	(0.02, 0.06)
Mean birth weight	3.487-/+0.680	3.425-/+0.680	0.17	(0.27,0.15)
Large Gestational Age (LGA)	19.1%	13.4%	<0.05	(0.01, 0.1)
Small Gestational Age (SGA)	7%	6.3%	0.68	(0.02, 0.04)

Disclosure: **M. Mustafa:** None.

918

The effect of baseline maternal weight and weight gain on pregnancy outcome in women with untreated, mild gestational diabetes

Z. Szili-Janicsek[1], T. Tänczer[1], E. Szabó[2], B. Domján[1], V. Ferencz[1], Z. Kerényi[3], A. Péterfalvi[3], Á.G. Tabák[1,4];
[1]1st Department of Medicine, Semmelweis University, Budapest, Hungary, [2]Szent Imre Hospital, Budapest, Hungary, [3]Diabetes Outpatient Clinic Tóth Ilona Health Service, Budapest, Hungary, [4]Department of Epidemiology and Public Health, University College London, London, UK.

Background and aims: Both obesity and GDM are associated with an increased fetal and maternal risk during and after pregnancy. However, knowledge is limited on the combined effect of these risk factors on pregnancy outcomes. We aimed to clarify the extent by which adverse pregnancy outcomes of women with "mild GDM" are explained by maternal body weight. Mild GDM was diagnosed if glucose tolerance test was normal according to WHO-1999 but GDM was proved by WHO-2014 diagnostic criteria (fasting blood glucose between 5.1 and 6.9 mmol/l and 2-h post load blood glucose <7.8 mmol/l).
Materials and methods: Between 2002–2005 n = 5335 pregnant women participated in a population-based screening program using 75 g OGTT. After excluding pregestational diabetes, twin pregnancies, and GDM according to WHO-1999, data on n = 4362 pregnancies were analyzed. We assessed adverse fetal (large for gestational age, LGA ≥90 percentage, 1-minute Apgar ≤7), maternal (pregnancy induced hypertension, preeclampsia), and delivery (preterm delivery <37 weeks of gestation, Caesarean delivery) outcomes. Associations of these adverse outcomes with mild GDM were analyzed by logistic regression. Models then were further adjusted for maternal weight at delivery, weight gain during pregnancy and other known risk factors of adverse outcomes.
Results: Mean maternal age was 29.5 ± 0.7 (mean ± SD) years, body mass index (BMI) 22.6 ± 0.4 kg/m^2, fasting glucose 4.5 ± 0.4 mmol/l, 2-h glucose was 5.2 ± 1.7 mmol/l. Mild GDM was diagnosed in n = 510 women. Mild GDM was associated with an increased risk of LGA (OR: 1.57 95%CI: 1.25–1.98), pregnancy induced hypertension (OR: 2.27 95%CI: 1.50–3.45), preeclampsia (OR: 1.88 95%CI: 0.90–3.92), preterm delivery (OR: 1.70 95%CI: 1.04–2.78), and Caesarean section (OR: 1.3 95%CI: 1.02–1.65). No association between mild GDM and the 1-min Apgar score was found. Odds ratios almost halved after adjustment for maternal BMI at delivery, all (except for preterm delivery where the risk remained the same) became non-significant after further adjustment in multivariate models.
Conclusion: Our results suggest that mild GDM based solely on fasting glucose had little effect on pregnancy outcomes when maternal body weight and other known pregnancy risk factors are taken into account. Randomized controlled trials are required to determine whether lifestyle recommendations focusing on optimizing weight gain are sufficient to treat GDM cases diagnosed based solely on elevated fasting glucose.
Supported by: OTKA 68575/2007
Disclosure: **Z. Szili-Janicsek:** None.

919

Foetal abdominal overgrowth already affected at diagnosis of gestational diabetes (GDM) in elderly and obese women persists until delivery despite of treatment

Y. Kim[1], W. Kim[1], W. Park[1], J. Kim[1], S. Park[2];
[1]Kangnam CHA Medical Center, CHA University, Seoul, Republic of Korea, [2]Department of Epidemiology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, USA.

Background and aims: We previously observed that altered glucose metabolism already existed, and affected fetal growth at the time of GDM diagnosis in elderly and obese women. The aims of this study was to determine whether fetal abdominal overgrowth already affected

at the time of diagnosis of GDM in the elderly with/without obese women persist until delivery despite appropriate GDM treatment.

Materials and methods: Medical records of 1419 singleton pregnancy including 384 GDM and 1035 NGT (normal glucose tolerance) subjects were reviewed. GDM was diagnosed by 100-g OGTT after 50-g glucose screening tests according to ADA criteria. We collected serial HbA1C and fetal biometry data measured both at diagnosisof GDM and near term. Fetal ultrasonographic measurements of biparietal diameter(BPD), abdominal circumference(AC), and femur length(FL) were converted to each estimated gestational ages(GA-BPD, GA-AC, GA-FL) and estimated fetal weight (EFW) was calculated by Shinozukafomula. Ratio of GA-AC/GA-LMP(gestational age by LMP), GA-AC/GA-BPD, and GA-AC/GA-FL were calculated as indices for fetal abdominal obesity. GDM subjects were divided into 4 groups: group1 (age <35 and prepregnant BMI <25, $n = 147$), group 2 (<35 & ≥25, $n = 23$), group 3 (≥35 & <25, $n = 163$), and group 4 (≥35 & ≥25, $n = 51$). NGT subjects were compared with 4 subgroups of GDM subjects.

Results: 1) Frequency of insulin treatment in group2 and 4 GDM subjects were significantly higher than in group 1 and 3 GDM subjects (17.4% and 15.7% vs. 8.2% and 9.8%, $p < 0.05$). **2)** HbA1C of group 2 and 4 GDM subjects were significantly higher than group 1 and 3 GDM subjectsmeasured at diagnosis of GDM (5.6 ± 0.6 and5.6 ± 1.0% vs. 5.2 ± 0.3 and 5.2 ± 0.3%, $p < 0.0001$) and near term (5.9 ± 0.5 and 5.8 ± 0.7% vs. 5.5 ± 0.4 and 5.5 ± 0.4%, $p < 0.005$). **3)** GA-ACof group3 GDM were significantly higher than that of NGT at diagnosisof GDM (27.5 ± 1.4 vs. 27.1 ± 1.4, $p < 0.01$) and near term (38.7 ± 2.3 vs. 37.9 ± 2.4, $p < 0.0005$) but GA-BPD, GA-FL, and EFWof other subgroups were not different from NGT subjectsboth at diagnosisof GDM and near term.**4)** Only group3 and 4 GDM subjectsshowed fetal abdominal obesity with significantly higherratio of GA-AC/GA-LMP (1.05 ± 0.04 and1.05 ± 0.04 vs. 1.03 ± 0.04, $p < 0.001$), GA-AC/GA-BPD(1.03 ± 0.04 and 1.03 ± 0.04 vs. 1.01 ± 0.05, $p < 0.05$), and GA-AC/GA-FL (1.02 ± 0.05 and 1.03 ± 0.04 vs.1.01 ± 0.04, $p < 0.001$)compared with NGT subjects at diagnosis of GDM. **5)** Also near term, group 3 and 4 GDM subjects showed significantly higher ratio of GA-AC/GA-LMP (1.08 ± 0.05and1.09 ± 0.05 vs. 1.06 ± 0.05, $p < 0.0001$), GA-AC/GA-BPD(1.04 ± 0.05 and 1.06 ± 0.07 vs. 1.02 ± 0.06, $p < 0.0005$), and GA-AC/GA-FL (1.05 ± 0.05 and 1.05 ± 0.05 vs. 1.03 ± 0.05, $p < 0.005$) compared with NGT subjects. **6)** But fetal abdominal obesity indices of group 1 and 2 GDM subjects were not different from those of NGT subjects both at diagnosis of GDM and near term.

Conclusion: Fetal abdominal overgrowth already affected in the elderly with/without obeseGDM mothersat the time of GDM diagnosis at 24–28 weeks' gestation persisted until delivery despite appropriate GDM treatment. These findings suggest that active interventions before or early in pregnancy to normalize metabolic derangement and more strict glucose control during pregnancy would be necessary in the elderly with/without obese women.

Disclosure: **Y. Kim:** None.

920

What is the impact of weight gain less than that recommended by IOM on pregnancy outcome for women with GDM and BMI ≥30

D. Bogdanet[1], A. Khattak[1], M. Mustafa[1], L. Carmody[1], B. Kirwan[1], P. O'Shea[1], G. Gaffney[1], F. Dunne[2];
[1]Galway University Hospital, Galway, [2]National University of Ireland Galway, Galway, Ireland.

Background and aims: The Institute of Medicine (IOM) recommends gestational weight gain (GWG) of 5–9 kg in women with a body mass index of ≥30. Debate continues as to whether GWG less than that recommended is safe. Concern relates to whether this would result in an increase in small for gestational age (SGA) infants, or an increase in rates of prematurity.

Materials and methods: We examined pregnancy outcomes for mothers with GDM and a BMI ≥30 and their offspring ($n = 752$) of whom 473 were treated with insulin. Women were categorised into 3 groups (1) those with weight loss or weight gain 0–5 kg, (2) weight gain 5–9 kg (3) weight gain >9 kg, from the first antenatal visit to delivery. We examined pregnancy outcomes for groups 1 and 2 in women treated by diet only (GDM-D) and in those treated with insulin (GDM-I). Rates of maternal outcomes (pregnancy induced hypertension (PIH), pre-eclampsia (PET), antepartum (APH) and postpartum haemorrhage (PPH), week of delivery, caesarean section (CS) delivery) and neonatal outcomes (birth weight, large for gestational age (LGA), small for gestational age (SGA), macrosomia, prematurity <37 weeks, neonatal morbidities) were compared between groups.

Results: Maternal age BMI and week of GDM diagnosis were similar between groups. In the GDM-D group ($n = 120$) the baseline systolic (123 vs 117 mmHg; $p = 0.03$) and diastolic (73 vs 68 mmHg; $p = 0.01$) pressure was higher in group 1 vs 2. Women in Group 1 ($n = 91$) were more likely to deliver earlier (38.9 vs 39.8 weeks, $p < 0.01$), to develop PIH (15.4% v 0%; $p = 0.02$) or have a PPH (13.2% vs 0, $p = 0.03$) compared to GDM-D women in Group 2 ($n = 29$). Rates of prematurity were higher in group 1 vs 2 (14.3% vs 0%, $p = 0.03$). All other neonatal outcomes were similar with no excess of LGA, macrosomia, SGA, neonatal morbidities or need for NNU care. In the GDM-I group ($n = 192$), women in Group 1 ($n = 144$) had similar delivery time, rates of PIH, PET and CS as women in Group 2 ($n = 48$) but rates of PPH were higher (7.9% vs 0, $p = 0.05$). Rates of LGA, macrosomia, SGA, prematurity, neonatal morbidities and need for NNU care were similar between groups.

Conclusion: Weight gain less than IOM recommendations appears safe for infants of women with GDM. Reasons for an excess of maternal PPH or PIH are not apparent and require further investigation in other cohorts.

Disclosure: **D. Bogdanet:** None.

921

Heterogeneity in insulin sensitivity and insulin secretion in gestational diabetes relates to differences in pregnancy outcomes

L.R. Madsen[1,2], K. Gibbons[3], D. McIntyre[3,2];
[1]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark, [2]Danish Diabetes Academy, Odense, Denmark, [3]Mater Research Institute, South Brisbane, Australia.

Background and aims: Varying clinical phenotypes exist within the overarching "diagnosis" of Gestational Diabetes Mellitus (GDM), encompassing women with predominant defects in insulin sensitivity, insulin secretion or a combination of both. We aimed to determine if GDM phenotypes as defined by estimates of insulin sensitivity and secretion were independently associated with birthweight, LGA (large for gestational age), preterm delivery, caesarean delivery (CS), and a composite of GDM-related adverse pregnancy outcomes (LGA, neonatal hypoglycemia, or caesarean delivery), when adjusted for potential confounders.

Materials and methods: Using data from OGTTs at mean gestational week 28 in the Brisbane HAPO study cohort, we estimated insulin sensitivity (Matsuda index) and secretion (HOMA β) in 1245 women. In women with GDM (10.5%, when using IADPSG criteria), defects in insulin sensitivity and/or insulin secretion were defined as <25th percentile in non-GDM women. This approach yielded four subgroups named by the predominant defect; low insulin sensitivity (GDMsens), low insulin secretion (GDMsec), both defects (GDMmixed), or no detectable defects (ND). We created linear and logistic regression models adjusted for maternal age, maternal height, BMI, smoking, gravidity, parity, family history of diabetes, mean arterial BP, and HbA_{1c}. No women received GDM treatment during pregnancy.

Results: Relative to non-GDM women, women in the GDMsens group (52.7% of all GDM) had higher BMI (33.8 vs 28.6 kg/m^2, $p < 0.001$), higher mean arterial BP (87 [SD 7] vs 83 [SD 7] mmHg, $p < 0.001$), gave birth to heavier infants (birth weight z scores 0.67 [SD 1.12] vs 0.19 [SD 0.98], $p < 0.001$) with a higher odds of LGA (OR 2.34; 95% CI 1.33,

4.12; $p = 0.003$); had higher odds of preterm delivery (OR 2.62; 95% CI 1.14, 6.04; $p = 0.024$), and higher odds of delivering by CS (OR 1.89; 95% CI 1.15;3.10, $p = 0.012$). Relative to non-GDM women, women with GDMsec defects (17.6%) were older (33.6 [SD 4.5] vs 29.2 [SD 5.2] years, $p < 0.001$), but pregnancy outcomes were similar. Relative to non-GDM women, women in the GDMmixed (14.5%) group had higher BMI (32.8 [SD 6.5] vs 28.6 [SD 5.5] kg/m^2, $p = 0.003$) and showed similar trends in outcomes to the GDMsens group, though none achieved significance. The ND women (15.3%) did not differ from non-GDM women. When adjusting for confounders including BMI, only the GDMsens group were still at increased odds for adverse outcomes; preterm delivery (OR 2.56; 95% CI 1.02, 6.46; $p = 0.046$), and birth weight z score of +0.30 (95% CI 0.060, 0.55; $p = 0.015$) in comparison to non-GDM women. After adjusting for BMI, the odds of CS and LGA babies were no longer higher in the GDMsens group. We found no increased risk of the composite GDM-related adverse pregnancy outcome (LGA, neonatal hypoglycemia, or caesarean delivery) in any subgroup.

Conclusion: Different clinical phenotypes in GDM are associated with differing risks of LGA infants, preterm delivery, and caesarean delivery. Women with GDM, predominantly due to a defect in insulin sensitivity have higher risks of adverse outcomes; only partly explained by BMI and other confounders.

Supported by: DDA, APMøller, HNF, AF, DanishDiabetesAssociation, Oticon

Disclosure: **L.R. Madsen:** Grants; Danish Diabetes Academy funded by the Nordisk Foundation, AP Møller Foundation, Oticon Foundation, Augustinus Foundation, Danish Diabetes Association, Familien Hede Nielsens Fond.

922

Preconceptional care in diabetes: results from a single reference centre

A. González Lleó[1], B. Vega-Guedes[2], A. López-Alonso[2], M. Alberiche[1], A. Arencibia[2], O. Ramírez[2], L. Maya[2], A. Wägner[1,3];
[1]Endocrinology and Nutrition Department, Complejo Hospitalario Universitario Insular Materno-Infantil de Gran Canaria, Las Palmas de Gran Canaria, [2]Obstetrics and Gynaecology Department, Complejo Hospitalario Universitario Insular Materno-Infantil de Gran Canaria, Las Palmas de Gran Canaria, [3]Instituto Universitario de Investigaciones Biomédicas y Sanitarias (IUIBS), Las Palmas de Gran Canaria, Spain.

Background and aims: Preconceptional care (PC) is associated with improved pregnancy outcomes in previous studies in women with pregestational diabetes. The aim of this study was to assess the frequency of preconceptional planning in our population and compare the features and outcomes of women with and without PC.

Materials and methods: All women with pregestational diabetes who were followed at our centre during pregnancy and delivered between 1st January 2011 and 31st December 2017 were included in the analysis. PC was defined as attending our preconceptional clinic at least once before pregnancy, regardless of whether green light for pregnancy had been given or not. Comparisons were made between women with and without PC (Student's *t* and chi-squared, $p < 0.05$).

Results: Of the 401 women with pregestational diabetes (48% type 1) who delivered during the study period, 60 (15%) had attended PC (18.8% of those with type 1 vs 11.6% of those with type 2 diabetes, $p = 0.046$). There were no significant differences in the distribution according to Priscilla White classification of diabetes. Women with PC were more frequently nulliparous (87.8 vs 52.7%, $p < 0.0005$), but there were no differences in age or body mass index. PC was associated with lower HbA1c before pregnancy (6.3 ± 1.7 vs 7.8 ± 1.7%, $p < 0.0005$) and in the 1st trimestre (6.1 ± 0.7 vs 7.0 ± 1.3% $p < 0.0005$), although these differences were attenuated in the 2nd (5.9 ± 0.7 vs 6.0 ± 0.8% $p = 0.091$) and 3rd trimestres (6.1 ± 0.7 vs 6.2 ± 0.8%, $p > 0.1$). Women with PC had less often large for gestational age (LGA, $p >$ p90) babies (36.7% vs 51.5%, $p = 0.03$). There were no significant differences in the frequencies of caesarean section, shoulder dystocia, preterm delivery, intrauterine growth restriction, preeclampsia or admission to intensive care of the newborns.

Conclusion: The frequency of PC in our population with pregestational diabetes is low, especially among women with type 2 diabetes. PC is associated with lower pregestational and 1st trimestre HbA1c and with a lower frequency of LGA babies.

Disclosure: **A. González Lleó:** None.

923

When is HbA_{1c} during pregnancy correlated with macrosomia of the newborn in women with type 1 diabetes?

P. Kazakou[1], P. Antsaklis[2], P. Kontou[1], M. Mitropoulou[1], V. Sarantopoulou[1], G. Daskalakis[2], E. Anastasiou[1];
[1]Department of Endocrinology-Diabetes Center, General Hospital of Alexandra, Athens, [2]1st Department of Obstetrics and Gynecology, General Hospital of Alexandra, Athens, Greece.

Background and aims: It is well documented in the literature that poor glycemic control in pregnant women with type 1 diabetes is related with increased risk of adverse fetal and maternal outcomes, including macrosomia of the newborn. However, there are limited data regarding the time window during pregnancy that glycemic control can affect fetal growth and determine the development of macrosomia. The aim of this study was to investigate the correlation of A1C during pregnancy with macrosomia and birth weight of newborn in women with type 1 diabetes.

Materials and methods: This is a retrospective study which included a total of 184 pregnant women with type 1 diabetes followed in our department since 2010. Data regarding medical history, parameters of glycemic control, fetal ultrasounds and neonatal outcomes were recorded.

Results: Mean age was 30.3 ± 6.4 years and average duration of diabetes was 11.5 ± 8.7 years. 45.2% of the women were nulliparous and 54.8% multiparous. Mean gestational age at delivery was 37.0 ± 2.3 weeks and mean birthweight was 3163 ± 720 g. The rate of cesarean section was 87.1%. Of the newborns 46.7% were male and 53.3% were female. Mean daily insulin dose/weight kg was 0.63 U/kg (±0.27) during 1st trimester, 0.73 U/kg (±0.29) during 2nd trimester and 0.90 U/kg (±0.37) during 3^{d} trimester. The increase rate of daily insulin/kg dose throughout gestation was 42.4%. Mean A1C was 7% ± 1.38 for the 1st trimester, 5.6% ± 1.1 for the 2nd trimester, and 5.5% ± 0.8 for the 3^{d} trimester. For the fetuses with abdominal circumference (AC) >75th percentile during 3^{d} trimester, the levels of A1C of the 1st trimester were statistically higher (7.06% ± 1.77 vs 5.94% ± 0.86) ($p = 0.012$). The prognostic value of A1C of the 1st trimester remained statistically significant when the maternal weight gain was also taken into account. Linear regression analysis showed that A1C of the 1st trimester correlated with birthweight ($p = 0.011$) independently of the week of gestation, the fetal sex or maternal weight gain.

Conclusion: These findings suggest that macrosomia of the fetus/newborn, as documented by fetal ultrasound and birthweight, correlates with poor maternal glycemic control during the 1st trimester of gestation. The improvement of glycemic control subsequently doesn't seem to slow the accelerated fetal growth. This observation might be due to nonreversible pancreatic disorder of the fetus.

Disclosure: **P. Kazakou:** None.

PS 082 From pregnancy to breastfeeding

924

Sensitivity and specificity of the glucose challenge test for gestational diabetes using the 2013 World Health Organization criteria

K. Benhalima[1], P. Van Crombrugge[2], C. Moyson[1], J. Verhaeghe[1], S. Vandeginste[2], H. Verlaenen[2], C. Vercammen[3], T. Maes[3], E. Dufraimont[3], C. Deblock[4], Y. Jacquemyn[4], K. De Clippel[5], A. Loccufier[6], R. Devlieger[1], C. Mathieu[1];
[1]UZ Gasthuisberg, Leuven, [2]OLV ziekenhuis Aalst-Asse-Ninove, Aalst, [3]Imelda ziekenhuis, Bonheiden, [4]Antwerp University Hospital, Antwerp, [5]Kliniek St-Jan, Brussel, Belgium, [6]AZ St Jan, Brugge, Belgium.

Background and aims: A two-step screening strategy with a 50 g glucose challenge test (GCT) and the 2013 World Health Organization (WHO) criteria for gestational diabetes (GDM), might be a valuable alternative to the one-step approach with the 75 g oral glucose tolerance test (OGTT) with the advantage of limiting the number of OGTT's. The aim was to determine the sensitivity and specificity of the GCT in a universal two-step screening strategy for GDM using the 2013 WHO criteria.
Materials and methods: We performed a multi-centric prospective cohort study enrolling 2006 women before 14 weeks of pregnancy. Women without (pre)diabetes in early pregnancy, received both a GCT and 75 g OGTT between 24–28 weeks of pregnancy. Participants and health care providers were blinded for result of the GCT. The diagnosis of GDM was based on the 2013 WHO criteria. The performance of the GCT was assessed by a receiver-operating characteristic (ROC) curve and area under the curve (AUC). Sensitivity and specificity were analyzed across a wide range of GCT thresholds, with or without the addition of clinical risk factors. Women's preferences for the different screening strategies were also evaluated.
Results: Of all participants, 1811 (90.3%) received both a GCT and OGTT. Based on the OGTT, GDM prevalence was 12.5% (231). Using a two-step strategy with a GCT, GDM prevalence increased from 7.5% (136) to 9.1% (165) and 10.3% (187) when the threshold for consequent OGTT was lowered from 140 mg/dl to 130 mg/dl and 120 mg/dl. The ROC curve showed a AUC of 0.77 (95% CI 0.74–0.81) for the GCT. Sensitivity increased with decreasing GCT threshold [59.6% (95% CI 53.0–66.1), 72.4% (95% CI 66.1–78.1) and 82.0% (95% CI 76.4–86.8) for 140 mg/dl, 130 mg/dl and 120 mg/dl], at the expense of specificity [81.0% (95% CI 79.0–82.9), 70.2% (95% CI 67.9–72.4) and 56.0% (95% CI 53.5–58.4)]. Using a GCT threshold of 130 mg/dl, 65% of all OGTT's could be avoided compared to a one-step approach, with little added value of clinical risk factors. 20.6% (377) of women had complaints of the GCT and 43.4% (784) of the OGTT. More women preferred a two-step strategy or had no clear preference.
Conclusion: The GCT has a moderate diagnostic accuracy in a universal two-step screening strategy for GDM using the 2013 WHO criteria. A GCT threshold of 140 mg/dl had only a sensitivity of 59.6% and can therefore not be recommended in a two-step approach for GDM using the 2013 WHO criteria. To achieve sensitivity rates ≥70%, the threshold of the GCT would need to be reduced to at least 130 mg/dl.
Clinical Trial Registration Number: NCT02036619
Supported by: Belgian Lottery, Fund Academic studies UZ Leuven
Disclosure: **K. Benhalima:** Grants; National Lottery.

925

When timely gestational diabetes screening should occur in in vitro fertilisation pregnancies and how important is intensive glucose management?

P. Thomakos[1], O. Kepaptsoglou[1], A. Trouva[1], A. Sklavounos[2], E. Kapetanakis[3], A. Korantzis[4], D. Trouvas[2], P. Karantzis[5], C. Barreto[1], I. Taraoune[1], I. Sklavounos[5], C.S. Zoupas[1];
[1]Diabetes Center & Clinic, Hygeia General Hospital, Athens, [2]Mitera Maternity Hospital, Athens, [3]IVF center Chalandri, Athens, [4]IASO Maternity Hospital, Athens, [5]IVF department, Mitera Maternity Hospital, Athens, Greece.

Background and aims: In Vitro Fertilization (IVF) is a popular method of assisted reproduction. Gestational Diabetes Mellitus (GDM) is increased in IVF pregnancies. We aimed to investigate the effect of GDM in IVF pregnancies and its impact on maternal and fetal outcomes.
Materials and methods: Our study included a total of 408 singleton pregnancies [102 IVF with GDM (a) vs 102 spontaneous conception with GDM (b) vs 102 IVF without GDM (c) vs 102 normal pregnancies (d)]. The clinical characteristics of the study groups are (Mean ± SD): [age: 38.2 ± 4 vs 34.1 ± 3 vs 37.4 ± 4 vs 37.4 ± 5 years, $p < 0.001$ (a vs b) (b vs c) (b vs d); BMI: 25.8 ± 5 vs 23 ± 4 vs 24.8 ± 3 vs 22.7 ± 4 kg/m^2, $p <$ 0.001 (a vs b) (a vs d); HbA1c: 5.2 ± 0.5 vs 5.2 ± 0.7 vs 4.9 ± 0.3 vs 4.7 ± 0.3% , $p < 0.001$ (a vs c) (a vs d) (b vs c) (b vs d); Fasting Blood Glucose: 84.1 ± 8 vs 84.2 ± 7 vs 79.2 ± 8 vs 78.2 ± 5 mg/dl, $p < 0.001$ (a vs c) (a vs d) (b vs c) (b vs d), 1-h postprandial BG: 103.6 ± 11 vs 106.5 ± 10 mg/dl, p = NS (a vs b); week of diagnosis GDM: 21.8 ± 5.2 vs 23.8 ± 6.2, $p =$ 0.03 (a vs b); week of starting insulin: 22.8 ± 5 vs 24.8 ± 5, $p = 0.03$ (a vs b); insulin dose: 34.7 ± 10 vs 29.5 ± 16 iu/day, $p = 0.03$ (a vs b); smoking history: 30.5 vs 31.2 vs 31.3 vs 28.4%.
Results: The summary of obstetric and neonatal history between the 4 groups are: Maternal weight gain: 10.4 ± 4 vs 11.9 ± 3 vs 11.8 ± 3 vs 10.5 ± 2 kg, $p = 0.03$ (a vs b) (a vs c) (b vs d) (c vs d); week of delivery: 36.9 ± 2 vs 37.4 ± 1 vs 37.8 ± 1 vs 38.1, $p = 0.04$ (a vs b) $p < 0.001$ (a vs c) (a vs d) (b vs c) $p = 0.01$ (b vs d); neonatal birth weight: 2857 ± 517 vs 2891 ± 341 vs 3049 ± 379 vs 3117 ± 347 g, $p < 0.001$ (a vs c) (a vs d) (b vs c) (b vs d); women experienced hypoglycemia episodes: 24.5 vs 26.5%, $p =$ NS, pre-eclampsia rate: 4.9 vs 3.9 vs 2.4 vs 1.9%; Respiratory Distress Syndrome: 14.7 vs 12 vs 10.5 vs 5.8%, $p = 0.03$ (a vs d); Neonatal hypoglycemia: 17.6 vs 14.7%, p = NS, Neonatal Intensive Care Unit admittance: 14.7 vs 15.6 vs 10.5 vs 5.8% $p = 0.03$ (a vs d) $p = 0.02$ (b vs d), Caesarean Section: 86.3 vs 56.9 vs 74.4 vs 41.1%. There were 2 cases of perinatal mortality in each IVF groups. 1-hour Postprandial BG was associated with maternal-fetal complications ($r = 0.504$, $p < 0.001$). Maternal hypoglycemia did not affect fetal outcome. Age and BMI were not correlated with the week of GDM diagnosis.
Conclusion: GDM screening in IVF pregnancies should be much earlier than 24–28 weeks. Strict postprandial metabolic control with early insulin therapy limits adverse pregnancy outcomes, while maternal hypoglycemia does not seem to have an effect. Further studies are necessary to clarify the role of GDM in increased fetal complications, especially in IVF pregnancies.
Disclosure: **P. Thomakos:** None.

926

Screening for early gestational diabetes is not associated with a better pregnancy prognosis than no early screening: an observational study including 9975 women

E. Cosson[1], D. Sandre-Banon[1], F. Gary[1], I. Pharisien[2], J.-J. Portal[3], I. Banu[1], L. Bianchi[1], C. Cussac-Pillegand[1], S. Chiheb[1], E. Vicaut[3], P. Valensi[1], L. Carbillon[2];
[1]Department of Endocrinology Diabetology Nutrition, Bondy, [2]Department of Gynecology-Obstetrics, Bondy, [3]Clinical Research Unit, Fernand Vidal Hospital, Paris, France.

Background and aims: In addition to screening for gestational diabetes mellitus (GDM) after 24 gestational weeks (GW), the IADPSG proposed to screen in early pregnancy and to refer women with early GDM (eGDM) for immediate care. The usefulness of such a strategy is still unknown.
Materials and methods: We included women with a singleton pregnancy, without personal history of diabetes or bariatric surgery, who delivered

in our University hospital between 2012 and 2016. We compared the incidence of preeclampsia or large for gestational age infant or shoulder dystocia (predefined composite criterion) whether an early screening (before 22 WG) -leading to care for eGDM (FPG 92–125 mg/dL) or diabetes in pregnancy (DIP: FPG ≥126 mg/dL)- was performed (screened group) or not (unscreened group).

Results: Compared with women in the unscreened group ($n = 5190$, 53.0%), those in the screened group ($n = 4605$) had different ethnicities ($p < 0.0001$) and more risk factors for GDM including overweight (unscreened 45.9 *vs* screened 48.7%, $p < 0.01$), family history of diabetes (25.3 *vs* 27.9%, $p < 0.01$), personal history of GDM (4.3 *vs* 6.2%, $p < 0.01$) and macrosomic infant (2.7 *vs* 3.4%, $p < 0.05$). Early screening increased from 2012 to 2016 ($p < 0.0001$) and was associated with less maternal smoking during pregnancy (7.5 *vs* 6.2%, $p = 0.027$). Glycaemic status was different ($p < 0.001$) in the unscreened group (GDM 16.8% and DIP 1.2%) and the screened group (eGDM 8.5%, GDM 12.1% and DIP 0.9%), with a lower rate of insulin therapy (6.0% *vs* 8.9%, $p < 0.001$) and a higher gestational weight gain (11.4 ± 5.5 *vs* 11.1 ± 5.4 kg, $p = 0.013$) in the unscreened group. Composite criterion rate was similar in the unscreened and the screened groups (11.6 *vs* 12.0% respectively, odds ratio 1.040 (95% confidence interval 0.920–1.176) $p = 0.53$), also after adjusting for a propensity score considering factors associated with early screening (OR 1.034 (0.911–1.175), $p = 0.6036$) alone or in addition to predictors of pregnancy outcomes that were forced in the model (OR 1.057 (0.928–1.205), $p = 0.4022$). The incidence of other outcomes was similar in both groups. The results were similar considering only the 6656 women with risk factors for GDM (composite criterion 11.6 *vs* 12.0% respectively, OR 1.051 (0.915–1.207) $p = 0.4822$).

Conclusion: Our observational results show that a strategy including early measurement of FPG during pregnancy increases the number of GDM cases and care with more insulin therapy and lower gestational weight gain, but suggest that it may not improve pregnancy prognosis.

Supported by: Lilly France

Disclosure: **E. Cosson:** None.

927

Effect of probiotics on glucose and lipid metabolism in pregnant women: a systematic review and meta-analysis

M.M. Han[1], J.F. Sun[2], C.H. Wu[1], X.Y. Zhu[1], L. Li[1];
[1]Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, [2]Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, China.

Background and aims: Gut microbiome are altered in pregnancy. These alterations may influence the maternal glucose and lipid metabolism and contribute to gestational diabetes mellitus (GDM). Probiotics could be a method to alter the gut microbiome, however, little is known about their use in influencing the metabolic environment of pregnancy. The aim of this meta-analysis was to assess the effects of probiotics supplementation in pregnancy on relevant maternal metabolic control and rate of GDM.

Materials and methods: We searched PubMed, Web of Science and the Cochrane Library for reports published up to October 2017. We performed a meta-analysis of randomized controlled trials (RCTs) on the relationship between probiotics supplementation and glucose and lipid metabolism in pregnant women. After study selection, two authors performed quality assessment and data extraction independently, and mean differences (MD) or standard mean differences (SMD) or relative risk (RR) with 95% confidence intervals (CIs) were calculated with the random-effects model in this meta-analysis.

Results: From 648 citations, a total of 13 articles (10-RCTs) with 1139 participants met the inclusion criteria; it was at a low risk of bias. We found that there were no significant differences on quantitative insulin sensitivity check index (QUICKI) (MD 0.01, $p = 0.06$) or maternal weight change (MD −0.27 kg, $p = 0.13$). However, the meta-analysis showed that probiotics supplement significantly reduced the fasting blood glucose level (MD −0.11 mmol/L, $p = 0.0003$), serum insulin level (MD −2.06 μU/mL, $p < 0.00001$), homeostasis mode assessment of insulin resistance (HOMA-IR) (MD −0.38, $p < 0.00001$). And the Meta-analyses showed that a reduction in the rate of GDM (risk ratio (RR) 0.52, $p = 0.003$) when women are randomized to probiotics early in pregnancy. Additionally, there was statistically significant reduction in total cholesterol following probiotic supplementation (SMD −0.56, $p = 0.03$). However, there was no significant reduction in other lipid profiles following probiotic supplementation.

Conclusion: Probiotics supplementation is associated with improved glucose and lipid metabolism in pregnant women, and may contribute to reduce the risk of GDM. The use of probiotics supplementation is promising as a potential prevention and therapy to assist in the metabolic management of GDM.

Disclosure: **M.M. Han:** None.

928

The prevalence of microvascular complications during the third trimester in women with gestational diabetes

B.R. Shah, D.Z. Cherney, D.S. Feig, E. Herer, M.A. Hladunewich, A. Kiss, R.P. Kohly, L.L. Lipscombe;
University of Toronto, Toronto, Canada.

Background and aims: Chronic exposure to hyperglycemia is a key factor that promotes the development of microvascular complications of diabetes. However, women with gestational diabetes mellitus (GDM) with only short-term hyperglycemia may nonetheless be at risk for these complications. Our aim was to determine the prevalence of retinopathy and microalbuminuria in women in the third trimester of pregnancy with and without gestational diabetes.

Materials and methods: We recruited women between 32 and 40 weeks' gestational age. Women who developed hypertensive disorders of pregnancy including preeclampsia, and those with a positive postpartum oral glucose tolerance test, were retrospectively excluded. Blood pressure (BP), body-mass index, fundus photographs, urine albumin-creatinine ratio and A1c were obtained. Photographs were graded by an ophthalmologist, and those with ETDRS step ≥14 were considered to have retinopathy. Microalbuminuria was defined as an albumin-creatinine ratio ≥2.0 mg/mmol. The prevalence of retinopathy and microalbuminuria were determined among women with and without GDM, and bivariate analysis was performed to determine what baseline factors were associated with each outcome. Logistic regression was used to determine the independent predictors.

Results: We studied 213 women with GDM and 178 without. Their baseline characteristics are presented in the Table. Retinopathy was present in 10% of women with GDM and 11% without ($p = 0.7$), whereas microalbuminuria was found in 15% and 6%, respectively ($p = 0.007$). In additional bivariate analysis, systolic BP, diastolic BP, body-mass index, 1 hr glucose challenge test and A1c were all significantly associated with microalbuminuria. Age, gestational age, gravidity, parity and ethnicity were not. In multivariable modeling, after adjusting for A1c and diastolic BP, GDM was no longer significantly associated with microalbuminuria. Each 5 mmHg increase in diastolic BP increased the odds of microalbuminuria by 50%, while each 0.1% increase in HbA1c increased the odds by 11%. No baseline factors were associated with retinopathy in bivariate or multivariate analysis.

Conclusion: GDM increases the risk of microalbuminuria, but not retinopathy, after only a brief exposure to hyperglycemia. The risk for microalbuminuria was driven by differences in A1c and diastolic BP, potentially highlighting the importance of controlling these risk factors in patients with GDM.

Table. Baseline characteristics of study subjects

Characteristic	With GDM	Without GDM	p
Age (years)	34.6 ± 4.5	33.7 ± 3.5	0.03
Completed weeks of gestation	34.7 ± 1.8	36.6 ± 1.8	<0.0001
Gravidity	2.3 ± 1.4	2.2 ± 1.2	0.88
Parity	0.6 ± 0.8	0.7 ± 0.8	0.09
European ethnicity	33.7%	65.9%	<0.0001
Blood pressure			
Systolic	112 ± 11	107 ± 9	<0.0001
Diastolic	68 ± 9	66 ± 7	0.002
BMI (kg/m^2)	26.5 ± 5.5	23.6 ± 4.1	<0.0001
1 hr GCT result (mmol/L)	9.9 ± 1.5	6.3 ± 1.3	<0.0001
A1c (%)	5.4 ± 0.4	5.1 ± 0.3	<0.0001

Supported by: CIHR
Disclosure: **B.R. Shah:** None.

929

Gestational diabetes is associated with increased risk of non-alcoholic fatty liver disease: a population-based cohort study

A. Lavrentaki[1,2], A. Subramanian[3], N. Thomas[3], G. Valsamakis[2], K. Toulis[3,4], B. Daly[5], G. Mastorakos[2], A.A. Tahrani[1,6], K. Nirantharakumar[3];
[1]Institute of Metabolism and System Research, University of Birmingham, Birmingham, UK, [2]Endocrine Unit, Aretaieion University Hospital, Athens Medical School, Athens, Greece, [3]Institute of Applied Health Research, University of Birmingham, Birmingham, UK, [4]Department of Endocrinology, 424 General Military Hospital, Thessaloniki, Greece, [5]School of Nursing, University of Auckland, Auckland, New Zealand, [6]Centre for Endocrinology, Birmingham Health Partners, Birmingham, UK.

Background and aims: Gestational diabetes mellitus (GDM) is associated with adverse perinatal outcomes, and increased risk of postnatal type 2 diabetes and cardiovascular disease. However, whether GDM increases the risk of developing incident Non-alcoholic Fatty Liver Disease (NAFLD) is unclear and has not been well examined in previous studies. This is important considering the significant health burden of NAFLD and the opportunity to interfere in high risk population in order to reduce the risk of developing end-stage liver disease.
Materials and methods: We conducted a retrospective cohort study after extracting data from a large primary care database in the United Kingdom. The cohort consisted of 9,640 women with GDM diagnosis and 31,296 control women, matched for age, body mass index (BMI) and time of pregnancy. All study participants were free from NAFLD diagnosis at study entry.
Results: Mean (standard deviation) age of the whole cohort was 32.62 (SD:5.34) years and BMI 28.62 (SD:6.10) kg/m^2. There were 44 (0.46%) and 41 (0.13%) NAFLD incident diagnosis in the GDM and control population respectively over a median follow-up of 2.87 (IQR 1.16–5.81) years. Unadjusted incidence rate ratio (IRR) for NAFLD development was 3.28 (95% CI 2.14–5.02). After adjusting for age, Townsend (deprivation) quintile, smoking, BMI and Metformin usage; women with GDM remained at increased risk of developing NAFLD compared to women without GDM (IRR 2.95; 95%CI 1.91–4.55). Further adjustment for the diagnosis of polycystic ovarian syndrome, hypertension, hypothyroidism, and lipids lowering treatment did not change our findings (IRR 2.83; 95%CI 1.83–4.38).
Conclusion: Women diagnosed with GDM were at significantly increased risk of NAFLD development in their post-delivery life compared to women without GDM. Clinicians should have a low threshold to investigate women with history of GDM for the presence of NAFLD. Future studies need to examine whether lifestyle or pharmacological interventions could reduce the risk of developing NAFLD in women with history of GDM.

Supported by: National and Kapodistrian University of Athens
Disclosure: **A. Lavrentaki:** Grants; Scholarhip from National and Kapodistrian University of Athens.

930

Comparison of diagnostic value and cost-effectiveness in screening approaches for postpartum pre-diabetes and diabetes among women with history of gestational diabetes

L.-J. Li[1,2], I.M. Aris[3], Y. Chong[4], P. Gluckman[3], S. Ang[5,6], K. Tan[1,2];
[1]Division of O&G, KK Women's and Children's Hospital, Singapore, [2]O&g acp, Duke-NUS Medical School, Singapore, [3]Singapore Institute for Clinical Sciences, Agency for Science Technology and Research, Singapore, [4]Department of O&G, Yong Loo Lin School of Medicine, Singapore, [5]Division of Family Medicine, KK Women's and Children's Hospital, Singapore, [6]Duke-NUS Medical School, Singapore, Singapore.

Background and aims: Women with a history of gestational diabetes mellitus (GDM) are at high risk for developing pre-diabetes and type 2 diabetes (T2D) after delivery. However, current guidelines regarding the methods of postpartum routine screening on GDM mothers are inconsistent and not directive. We aimed to compare different screening methods [fasting HbA1c, fasting glucose level and 2-hour glucose level] for diagnosing pre-diabetes and T2D in women with GDM.
Materials and methods: Of participants from a Singapore birth cohort, 142 mothers attended both baseline (26–28 gestation weeks) and follow-up (5-year postpartum) visits. At baseline, we diagnosed GDM using a 75 g OGTT according to World Health Organization (WHO) guidelines. At follow-up, we assessed glucose tolerance using fasting HbA1c, fasting glucose level, and a 75 g oral glucose tolerance test (OGTT). We defined pre-diabetes when either of the following was present: a) fasting plasma glucose ranged between 6.1 mmol/Land 6.9 mmol/L; b) fasting plasma glucose <7.0 mmol/L and 2-h plasma glucose ranged between 7.9 and 11.0 mmol/L. We defined T2D if: a) fasting plasma glucose ≥7.0 mmol/L or 2-h plasma glucose ≥11.0 mmol/L, or b) has been diagnosed T2D during the 5 years' interval. We estimated area under the curve (AUC) to compare the diagnostic values, and conducted cost-effectiveness analysis for each of the three screening approaches.
Results: Of 142 mothers with GDM diagnosed at baseline, 57 (40.1%) developed pre-diabetes or T2D at the 5-year postpartum visit. AUC was 74.0, 73.5 and 76.5 for fasting HbA1c alone, fasting glucose alone, and the combination of fasting HBA1c and fasting glucose respectively. AUC predicted pre-diabetes and T2D perfectly (98.8%) if using 2-h glucose >7.7 mmol/L. Cost-effectiveness analysis showed that 2-h glucose level is a better postpartum screening tool for GDM mothers than fasting HbA1c and/or fasting glucose test.
Conclusion: Our study showed that either fasting HbA1c and fasting glucose level screening approach missed around a quarter of the pre-diabetes or T2D cases, among mothers with a history of GDM. Two-h glucose level and even OGTT may be the most optimal screening tool in terms of its accuracy in diagnostic value and economic cost-effectiveness.
Supported by: NMRC
Disclosure: **L. Li:** None.

931

Breastfeeding at night is rarely followed by hypoglycaemia in women with type 1 diabetes using carbohydrate counting and modern insulin therapy

L. Ringholm[1,2], A.B. Roskjær[1], S. Engberg[2], H.U. Andersen[2], A.L. Secher[1], P. Damm[1], E.R. Mathiesen[1];
[1]Center for Pregnant Women with Diabetes, Rigshospitalet, Copenhagen, [2]Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: Hypoglycaemia in association with breastfeeding is a feared condition in mothers with type 1 diabetes.

Thus, routine carbohydrate intake at each night-time breastfeeding is often recommended despite lack of evidence. We evaluated glucose levels during breastfeeding with focus on whether night-time breastfeeding induced hypoglycaemia in mothers with type 1 diabetes.

Materials and methods: Out of 43 consecutive mothers with type 1 diabetes, 33 (77%) were included prospectively one month after singleton delivery. Thereof 26 mothers (mean age 30.7 (SD ±5.8) years, 64% nulliparous, duration of diabetes 18.6 (10.3) years) were breastfeeding and 7 mothers (31.7 (5.6) years, 57% nulliparous, duration of diabetes 20.4 (6.2) years) were formula feeding. Thirty-two women with type 1 diabetes, who had not given birth or breastfed in the previous year, served as age and BMI matched controls. All 33 mothers were experienced in carbohydrate counting with 45% on insulin pump and 55% on multiple daily injections. Blinded continuous glucose monitoring (CGM) for six days was applied at one, two and six months postpartum in the breastfeeding mothers and they recorded breastfeedings and carbohydrate intake. CGM data were evaluated once in the control women and at one month postpartum in the formula feeding mothers.

Results: One month postpartum the percentage of night-time (11 pm to 7 am) spent with CGM <4.0 mmol/l in the breastfeeding mothers was comparable with the control women (median 4.6% (range 0–20.8) and 1.6% (0–39.1), $p = 0.68$). The figure for formula feeding mothers was 8.2% (0–14.8). Mean glucose level at night-time was lower in breastfeeding mothers than in control women (8.3 (1.7) and 9.7 (2.4) mmol/l, $p = 0.02$) and formula feeding mothers (9.4 (1.6) mmol/l). In the breastfeeding mothers the mean glucose levels at night were similar at one, two and six months postpartum (8.3 (1.7) mmol/l, 8.6 (2.5) mmol/l and 7.9 (1.6) mmol/l, $p = 0.55$). Symptomatic hypoglycaemia occurred 2–3 times per week in the breastfeeding mothers at one, two and six months postpartum and in the control women with no significant differences between the breastfeeding mothers and the control women. Severe hypoglycaemia was reported by one (3%) mother during the 6-month post-partum period and by one (3%) control woman in the previous year (0.74). In breastfeeding mothers the insulin dose at one month postpartum was 18% lower than before pregnancy ($p = 0.04$). At one, two and six months postpartum carbohydrate was not ingested at a total of 438 (93%) night-time breastfeedings, whereof 20 (4.6%) breastfeedings were followed by CGM <4.0 mmol/l within 3 hours.

Conclusion: The percentage of night-time spent with hypoglycaemia in the breastfeeding mothers with type 1 diabetes was at the level of that in the non-pregnant control women with type 1 diabetes. Among >400 documented night-time breastfeedings without carbohydrate intake, hypoglycaemia within 3 hours was rare. The recommendation of routine carbohydrate intake at night-time breastfeeding may be obsolete in mothers with type 1 diabetes on modern insulin therapy who count carbohydrate.

Clinical Trial Registration Number: NCT02898428

Disclosure: **L. Ringholm:** None.

PS 083 Diabetic pregnancy: experimental work

932

Diagnosis of gestational diabetes in fasting serum samples - biomarkers detected by GC-MS based metabolomics

B.A. Raczkowska[1], D. Rojo[2], B. Telejko[1], M. Paczkowska-Abdulsalam[3], J. Hryniewicka[1], A. Zielinska[1], M. Szelachowska[1], M. Gorska[1], M. Ciborowski[3], C. Barbas[2], A. Kretowski[1,3];
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland, [2]Centre of Metabolomics and Bioanalysis (CEMBIO), Universidad CEU San Pablo, Madrid, Spain, [3]Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland.

Background and aims: Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders in pregnancy which increases the risk of complications for mother and foetus, in particular the risk of developing diabetes type 2 later in life. Despite the fact that the criteria proposed by IADPSG were approved by WHO, the diagnosis of GDM varies considerably between individual countries and therefore it is still controversial, particularly with respect to the short and long term complications for mother and offspring. The aim of this study was to investigate the differences between fasting serum metabolites of GDM women with isolated impaired glucose tolerance (iIGT) or isolated impaired fasting glucose (iIFG) in comparison to normal glucose tolerance (NGT) pregnant women and to propose the potential biomarkers of GDM that can facilitate its diagnosis.

Materials and methods: Fasting serum samples were collected during 75 g oral glucose tolerance test (OGTT) between 24–28 weeks of gestation (gwk). Patients were diagnosed with GDM based on IADPSG criteria and divided into 2 subgroups: i) iIGT and ii) iIFG. Although there are no established criteria to identify prediabetes states i.e., iIGT and iIFG in pregnancy, for the aim of this research, we used these terms to clearly demonstrate the differences between GDM patients characterized by different glycemic status. Control group comprised of NGT pregnant individuals. Serum samples (discovery cohort, $n = 79$) were fingerprinted using gas-chromatography-mass spectrometry (GC-MS). Selected metabolites significantly discriminating iIGT from NGT group were quantified in a larger cohort (validation cohort, $n = 163$) by a targeted GC-MS. The statistical analysis was performed using the Student's t-test for normally distributed data and Mann-Whitney U test for the data without the normal distribution, followed by Benjamini-Hochberg post hoc correction. ROC curve analysis was performed to evaluate the clinical usefulness of proposed biomarkers.

Results: We identified a set of metabolites discriminating iIGT, iIFG and NGT groups. α-Hydroxybutyric acid (α-HB), β-hydroxybutyric acid (β-HB) and several fatty acids were found to be associated with iIGT. A combination of α-HB, β-HB and myristic acid was found highly specific and sensitive for diagnosis of GDM manifested by iIGT (AUC = 0.828).

Conclusion: Measurement of α-HB, β-HB and myristic acid in the fasting serum sample may allow for diagnosis of GDM manifested by iIGT without the need to perform OGTT.

Supported by: Leading National Research Centre in Bialystok (KNOW 2012-2017)

Disclosure: **B.A. Raczkowska:** None.

933

Effect of gestational diabetic blood soluble factors on beta cell function: in vitro physiological analysis combined with proteomics approach

H. Kenar[1], C.Y. Ozdogan[1], H. Keskin[1], E.E. Islek[2], E. Doger[3], A. Nalla[4], I. Tarkun[5], J.H. Nielsen[4], M. Kasap[2];

[1]Diabetes and Obesity Research Laboratory, Kocaeli University, Kocaeli, Turkey, [2]DEKART Proteomics Laboratory, Kocaeli University, Kocaeli, Turkey, [3]Department of Gynecology and Obstetrics, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey, [4]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [5]Department of Endocrinology and Metabolism, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey.

Background and aims: Pancreatic beta cell mass increases in pregnancy, but Gestational diabetes mellitus (GDM) may develop when adaptation of beta cells to compensate for the increased peripheral insulin resistance fails. The aim of this study was to investigate the effect of blood soluble factors from GDM and healthy glucose- tolerant (NGT) pregnants on beta cell function through *in vitro* physiological tests and proteomics approach.
Materials and methods: Blood plasma from 3rd-trimester GDM ($n = 8$) and NGT ($n = 10$) pregnant women was used in culture of INS-1E (rat insulinoma cell line) cells at 5% (v/v) concentration. After 24 h-culturing in RPMI 1640 supplemented with 5% (v/v) NGT/GDM blood plasma, glucose stimulated insulin secretion (GSIS) was performed under static conditions for 1 h in presence of 5% (v/v) NGT/GDM blood plasma and both secreted and intracellular insulin concentrations were determined by ELISA. Data were normalized to total DNA amount. Samples were studied in triplicate and data were statistically analyzed using Student's t-test; differences were considered significant when $p < 0.05$. Time dependent membrane depolarization and calcium influx into the cells upon glucose stimulation were determined in an automated spectrofluorometer. At the end of 1h - stimulation with 16.7 mM glucose, the cells were lysed and the proteins analyzed by 2D gel electrophoresis coupled with MALDI TOF-TOF MS/MS. Interactome analysis of the differentially regulated proteins was performed by using the STRING program.
Results: GDM blood plasma was associated with elevated insulin secretion ($p < 0.05$) from INS-1E upon exposure to 16.7 mM glucose, while caused no difference in intracellular insulin when compared with NGT group. Time dependent membrane depolarization of INS-1E and calcium influx into the cells occurred earlier and to a higher extent in the GDM group. Interactome analysis of the differentially regulated proteins in the GDM group revealed 4 glycolytic pathway proteins (Pkm, Aldoa, Eno 1, Gapdh), the mitochondrial glutamate dehydrogenase 1 (Glud1) and 2 proteins (HspA5 and HspA8) responsible for protein folding. In the NGT group, 7 of the proteins residing in the same interactome were related to energy metabolism - 3 Krebs cycle proteins (Atp5a1, Aco2, Cs) and 4 glycolytic pathway proteins (Gapdh, Pkm, Pgk1, TPI) - and still others were HspA8 and Hadh, an enzyme having essential role in mitochondrial beta-oxidation of short chain fatty acids.
Conclusion: These results demonstrated that glucose stimulated insulin secretion was elevated by GDM blood soluble factors and that under high glucose concentration the differentially regulated proteins in the beta cells mostly took role in the glycolytic pathway. On the contrary, NGT blood soluble factors caused differential regulation of both the glycolytic pathway and the Krebs cycle proteins.
Supported by: TUBITAK (Contract Number: 114S411)
Disclosure: **H. Kenar:** Grants; TUBITAK (Contract Number: 114S411).

934

The long-term maternal impact of impaired glucose tolerance during pregnancy: a role for placental kisspeptin signalling

L.I.F. Smith, T.G. Hill, S.J.S. Simpson, J.E. Bowe, P.M. Jones;
Department of Diabetes, School of Life Course Sciences, King's College London, London, UK.

Background and aims: During pregnancy increased maternal insulin resistance requires compensatory islet adaptation to maintain normoglycemia. Kisspeptin, released in high levels from the placenta, has previously been shown to potentiate glucose-induced insulin secretion through direct action on its receptor, GPR54, on the beta-cells. Beta-cell specific knockout of GPR54 (βGPR54ko) leads to impaired glucose tolerance in mice during pregnancy, but not in non-pregnant controls, whilst in pregnant women gestational diabetes (GDM) correlates with reduced plasma kisspeptin levels. GDM is also associated with an increased risk of T2DM in later life, though whether GDM is causative for T2DM development has not been fully determined. We monitored the glucose homeostasis of βGPR54ko mothers compared to age matched virgin controls to determine whether impaired glucose tolerance during pregnancy is causative for impaired glucose tolerance in later life.
Materials and methods: MIP-CreERT^{1Lphi}xGPR54*loxP* mice were administered tamoxifen (TMX) at 8 weeks to induce GPR54 knockout. Mice not administered TMX (Cre con) and TMX-injected mice lacking the Cre transgene (TMX con) were used as two separate control groups. Mice were maintained on either a standard chow diet (CD) or a high-fat high-sugar diet (HFHSD) from 12 weeks. At 18 weeks glucose tolerance was assessed through an intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT). Females were then either left as virgin controls or mated with males. IPGTT and IPITT were repeated at 4 weeks postpartum and every subsequent 4 weeks.
Results: Prior to mating, all female HFHSD groups had significantly worse glucose tolerance than their CD counterparts (CD vs HFHSD AUC: Cre con 1304 ± 115 vs 2903 ± 170, $P = 0.0002$; TMX con 1257 ± 50 vs 2196 ± 538, $P = 0.0567$; βGPR54ko 1390 ± 52 vs 2538 ± 383, $P = 0.0018$). However, there were no significant differences between control and knockout groups ($P = 0.2299$). At 4 weeks post-partum, CD mothers had similar glucose tolerance to age-matched virgins ($P = 0.8815$) suggesting a successful recovery of normal glucose homeostasis. However, βGPR54ko mothers on HFHSD had impaired glucose tolerance compared to virgins at 30 min after i.p. glucose (HFHSD βGPR54ko mothers vs βGPR54ko virgins 30 min glucose: 29.47 ± 2.02 mM vs 13.75 ± 0.65 mM, $P = 0.076$), a trend which is not observed in other groups (HFHSD Cre con mothers vs Cre con virgins 30 min glucose: 26.55 ± 4.75 mM vs 20.75 ± 5.65 mM, $P > 0.999$). In age-matched males, HFHSD caused an overall significant impairment of glucose tolerance ($P = 0.0002$), whilst there were no differences between control and knockout groups at 18 weeks ($P = 0.9728$), nor at any subsequent age.
Conclusion: Beta-cell specific GPR54 knockout does not impair glucose tolerance in males, nor in non-pregnant females on either a CD or HFHSD. This suggests that kisspeptin is likely to be a pregnancy-specific regulator of beta-cell function. On CD, females recover from the impaired glucose tolerance during pregnancy caused by GPR54 knockout and have similar long-term glucose homeostasis as virgin females. However, on HFHSD, females do not recover from the impaired pregnant glucose tolerance caused by GPR54 knockout. This suggests that the combined effects of HFHSD and a lack of islet kisspeptin signalling are causative for prolonged post-partum maternal glucose intolerance.
Supported by: BBSRC
Disclosure: **L.I.F. Smith:** Grants; BBSRC.

935

Association of gestational diabetes with low-grade inflammation and lymphocyte populations in subcutaneous and visceral adipose tissue

A. Cinkajzlova[1,2], K. Anderlova[3,4], P. Simjak[4], Z. Lacinova[1,2], J. Klouckova[1,2], H. Kratochvilova[1,2], H. Krejci[3,4], A. Parizek[4], M. Mraz[5,2], M. Krsek[3,6], M. Haluzik[1,2];
[1]Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, [2]Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University and General University Hospital, Prague, [3]3rd Department of Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, [4]Gynaecology and Obstetrics Department, First

Faculty of Medicine, Charles University and General University Hospital, Prague, [5]Department of Diabetes, Institute for Clinical and Experimental Medicine, Prague, [6]2nd Department of Internal Medicine, Third Faculty of Medicine, Charles University and University Hospital Královské Vinohrady, Prague, Czech Republic.

Background and aims: Low-grade inflammation accompanying obesity and metabolic syndrome is associated with changed immune cell composition and phenotype in adipose tissue. Nevertheless, little is known about subclinical inflammation in gestational diabetes mellitus (GDM). The aim of this study was to analyze lymphocyte populations in subcutaneous (SAT) and visceral adipose tissue (VAT) together with systemic levels and mRNA expression of selected cytokines in these depots and placenta in the context of GDM and normal pregnancy.
Materials and methods: Thirty-seven pregnant females - 21 with GDM (GDM group) and 16 without GDM (P group) - were included into the study. Blood samples were taken in 28^{th}–32^{th} and 36^{th}–38^{th} gestational week and 6 months after delivery and SAT, VAT and placental samples were obtained during delivery. Lymphocytes were assessed as % of CD45+ cells measured by flow cytometry.
Results: In both groups CD45+ lymphocytes were higher in VAT compared to SAT (19.2 ± 2.2 vs. $6.5 \pm 0.7\%$, $p < 0.001$ for GDM and 18.3 ± 3.4 vs.7.8 ± 1.6, $p = 0.008$ for *P*, respectively). In GDM group T helper (CD4+) cells were higher in SAT compared to VAT (37.6 ± 2.4 vs. $28.6 \pm 2.6\%$, $p = 0.019$), resulting in a higher ratio of CD4+/CD8+ cells. Similarly, the amount of B (CD19+) and NKT (CD16/56+CD3+) cells was higher in SAT relative to VAT in GDM subjects, but not in P group. NK (CD15/56+CD3-) cells were increased in VAT only in GDM group (17.9 ± 3.1 vs. $9.1 \pm 1.3\%$, $p = 0.015$). CRP levels did not differ between GDM and P group, while circulatory TNF-α levels were higher in GDM females throughout the study (7.92 ± 0.69 vs. 4.59 ± 0.61 pg/ml, $p = 0.001$). Placental mRNA expression of IL-10 and IL-8 was higher in GDM versus P group with no difference between adipose tissue depots.
Conclusion: GDM is associated with increased systemic TNF-α levels and changed lymphocyte content in subcutaneous and visceral adipose tissue. These changes could be partly responsible for increased risk of complications for both mother and child during and after pregnancy.
Supported by: AZV 15-27630A, RVO VFN64165 and MH CZ – DRO (IKEM, IN 000023001)
Disclosure: **A. Cinkajzlova:** None.

936

Polyphenols as preventatives for gestational diabetes

C. Nguyen-Ngo, S. Liong, R. Lim, M. Lappas;
University of Melbourne, Melbourne, Australia.

Background and aims: Low-grade maternal inflammation, oxidative stress and peripheral insulin resistance are key features of GDM, and can lead to multiple short- and long-term complications for both mother and offspring. Recent epidemiological data suggest diets high in fruits, vegetables and plant-based foods confer potent health benefits. Polyphenols are the active compounds of these foods, and they possess potent anti-inflammatory, anti-oxidant and anti-diabetic properties. However, the effects of polyphenols on inflammation, oxidative stress and insulin resistance associated with GDM has not yet been studied. Therefore, the aims of this study were to examine the effects of the polyphenols naringenin, nobiletin and curcumin on the expression of pro-inflammatory mediators, oxidative stress, and insulin resistance associated with GDM.
Materials and methods: An *in vitro* explant model was used to determine the effects of naringenin, nobiletin and curcumin on TNF-α-induced expression of pro-inflammatory mediators and anti-oxidants in human placenta and adipose tissue, and glucose uptake in skeletal muscle ($n = 6$ patients/treatment/tissue). For these studies, a repeated measures ANOVA was used, with Fisher's Least Significant Difference (LSD) post hoc testing to discriminate among the means. A mouse model of GDM was used to assess the effects of polyphenols on glucose metabolism and inflammation ($n = 6$–7 mice/treatment group). GDM mice were injected intraperitoneally with or without polyphenols from gestational day (gd) 10 to gd 18. An oral glucose tolerance test was performed to assess glucose tolerance, and placenta and adipose tissues collected to assess gene expression of pro-inflammatory markers. Unpaired Student's t-test was used to assess statistical significance between normally distributed data; otherwise, the nonparametric Mann-Whitney *U* (unpaired) test was used for comparisons between groups. For all data, statistical significance was assigned to *P* value <0.05.
Results: *In vitro*, naringenin, nobiletin and curcumin significantly reduced TNF-α-induced mRNA expression and protein secretion of pro-inflammatory cytokines and chemokines including IL-6, IL-8, growth-regulated oncogene (GRO)-α and monocyte chemotactic protein (MCP)-1 from human placenta and adipose tissue. Naringenin and curcumin significantly increased mRNA expression of the antioxidants thioredoxin reductase (TrxR), Glutathione reductase (GR) and catalase in placenta and adipose tissue. Naringenin, nobiletin and curcumin significantly attenuated the decrease in glucose uptake induced by TNF-α in skeletal muscle. *In vivo*, GDM mice treated with naringenin and nobiletin had significantly reduced fasting glucose levels. Additionally, when compared to control treated mice, naringenin and nobiletin significantly decreased mRNA expression of IL-1α, IL-1β, TNF-α, IL-6, GRO-α and or MCP-1 in placenta, visceral and subcutaneous adipose of GDM mice.
Conclusion: Naringenin, nobiletin and curcumin possess potent anti-inflammatory, anti-oxidant and anti-diabetic properties in both *in vitro* and *in vivo* models of GDM. These polyphenols may act as novel therapeutics for the prevention of GDM.
Supported by: CNN: Aus. Gov. RTP scholarship. ML: NHMRC #1047025, DA, RLCRF, AMRF, NBMRF.
Disclosure: **C. Nguyen-Ngo:** None.

937

Inflammation markers and insulin like growth factor binding protein 1 in women with gestational diabetes treated with metformin or insulin

M. Huhtala[1], K. Tertti[2], T. Rönnemaa[3];
[1]Department of Obstetrics and Gynecology, University of Turku, Turku, [2]Department of Obstetrics and Gynecology, Turku University Hospital, Turku, [3]Department of Medicine, University of Turku, Turku, Finland.

Background and aims: The prevalence of gestational diabetes (GDM) is increasing worldwide. Pregnancy as such influences inflammation markers. Modulation of inflammatory mediators such as high sensitivity C-reactive protein (hsCRP) and interleukine-6 (IL6) occurs in GDM. Insulin like growth factor binding protein 1 (IGFBP1) has been associated to obesity in pregnancy and GDM. Our aim was to characterise the inflammatory profile of GDM, its changes during pregnancy and the possible effects of metformin compared to insulin. Moreover, we examined the associations of birth weight with inflammation markers and IGFBP1.
Materials and methods: 217 pregnant women diagnosed with GDM were randomized to receive either metformin (n 110) or insulin (n 107). Fasting serum samples were collected at mean 30 gestational weeks (gw) and at 36 gw. In addition, baseline serum samples of 126 women with GDM achieving sufficient glycemic control with lifestyle modifications only were included in the study. Concentrations of hsCRP, IL6, matrix metalloproteinase-8 (MMP8), glycoprotein acetylation (GlycA), IGFBP1 and its entirely and partly phosphorylated isoforms, were measured using ELISA and nuclear magnetic resonance spectroscopy in the case of GlycA. Statistical analyses were done using Mann-Whitney *U*, t-test or Wilcoxon signed-rank test and Spearman's rank correlation.
Results: At baseline none of the variables differed significantly between diet and medically treated groups. In medically treated groups combined, hsCRP decreased ($p = 0.01$), whereas IL6 ($p = 0.002$) and GlycA ($p <$

0.001) increased from baseline to 36 gw. MMP8 was unchanged. All IGFBP1 isoforms increased ($p < 0.001$). GlycA (8.3% vs 5.6%, $p = 0.02$) and non-phosphorylated IGFBP1 (29% vs 18%, $p = 0.008$) increased more in the metformin than in the insulin group. At baseline BMI and fasting C-peptide correlated positively with hsCRP, IL6 and GlycA. On the contrary, IGFBP1s correlated inversely with BMI and C-peptide. Baseline, but not 36 gw, GlycA correlated positively and IGFBP1 inversely with birth weight, while MMP8 measured at 36 gw correlated inversely with birth weight. In regression analysis one SD change in MMP8 was associated to 72 g lower birth weight independent of BMI and smoking (95%CI: 8.1–150).

Conclusion: Serum concentrations of inflammation markers change significantly during the last trimester of pregnancy in GDM patients. The changes were parallel between medically treated groups. However, metformin associated with a higher increase in GlycA and a simultaneous decrease in hsCRP, thus not explicitly indicating either a pro- or anti-inflammatory environment. High IGFBP1, which has been associated to a healthier metabolic profile, rose more in metformin group. Most of the markers measured relate to BMI and C-peptide but respond differently to progression of pregnancy, thus together providing more detailed insight of inflammation in GDM. High MMP8, previously associated to cardiovascular risk and insulin resistance, did not correlate with BMI or fasting C-peptide in our data, unlike the other inflammation markers at baseline. Yet it was the only marker measured at 36 gw relating to birth weight. Our results suggest that compared to insulin, metformin does not have unfavourable effects on GDM pregnancy in terms of inflammation markers.

Clinical Trial Registration Number: NCT01240785

Supported by: TUH foundation, Government grant to TUH

Disclosure: **M. Huhtala:** Grants; Turku University Hospital Foundation.

938

Association of nuclear-mitochondrial epistasis with BMI in type 1 diabetic patients

A.H. Ludwig-Slomczynska[1], M.T. Seweryn[1], P. Kapusta[1], E. Pitera[1], K. Cyganek[2], U. Mantaj[3], L. Dobrucka[2], E. Wender-Ożegowska[3], M.T. Malecki[2], P. Wolkow[1];

[1]Center for Medical Genomics OMICRON, Jagiellonian University Medical College , Krakow, Poland, Krakow, [2]Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland, Krakow, [3]Poznan University of Medical Sciences, Department of Obstetrics and Women's Diseases, Poznan, Poland, Poznan, Poland.

Background and aims: Obesity results from an imbalance between energy intake and its expenditure. GWAS analyses have led to discovery of only about 100 variants influencing BMI, which explain only a small portion of genetic variability. Analysis of gene epistasis gives a chance to discover another part. Since it was shown that interaction and communication between nuclear and mitochondrial genome is indispensable for normal cell function we have looked for epistatic interactions between the two genomes to find their correlation with BMI.

Materials and methods: The analysis was performed on 366 T1DM female pregnant patients using Illumina Infinium OmniExpressExome-8 chip. Only genes which influence mitochondrial functioning were included in the analysis - variants of nuclear origin (MAF >5%) in 1158 genes and 42 mitochondrial variants (MAF >1%). Gene expression analysis was performed on GTex data. Association analysis between genetic variants and prepregnancy BMI was performed with the use of Linear Mixed Models as implemented in the package 'GENESIS' in R. Analysis of association between mRNA expression and BMI was performed with the use of linear models and standard significance tests in R.

Results: Among genes involved in epistasis between mitochondria and nucleus we have identified mitochondrial transcription factor TFB2M. It interacted with few mitochondrial variants localized to MT-RNR1 ($p = 0.0004$, MAF = 15%), MT-ND2 ($p = 0.07$, MAF = 5%) and MT-ND4 ($p = 0.01$, MAF = 1.1%). Analysis of the interaction between nuclear variant rs6701836 localized to TFB2M and rs3021088 localized to MT-ND2 mitochondrial gene has shown that the combination of the two led to BMI decrease ($p = 0.02411025$). Each of the variants on its own does not correlate with higher BMI (p(nuc) = 0.8566547, p(mito) = 0.1160552). Although rs6701836 is intronic it influences gene expression in thyroid ($p = 0.000037$). rs3021088 is a missense variant that leads to Alanine to Threonine substitution in the MT-ND2 gene which belongs to complex I of the electron transport chain. The analysis of the influence of genetic variant on gene expression has confirmed the trend explained above - each of the mRNAs on its own is associated with higher BMI (p-(mito) = 0.0244 and p(nuc) = 0.0269), however, their interaction leads to BMI decrease ($p = 0.0308$).

Conclusion: Our results show that nuclear-mitochondrial epistasis can influence BMI in T1DM patients. The correlation between transcription factor expression and existence of genetic variants will be subject of further analysis.

Supported by: UMO-2013/11/D/NZ5/03219

Disclosure: **A.H. Ludwig-Slomczynska:** None.

PS 084 Neuropathy: prevalence and clinical impact

939

Prevalence of and risk factors associated with distal symmetric diabetic polyneuropathy in the SDRNT1BIO cohort

A. Jeyam[1], A. Ochs[1], S.J. McGurnaghan[1], L.A.K. Blackbourn[1], P.M. McKeigue[2], H.M. Colhoun[1,3], on behalf of the SDRNT1BIO Investigators;
[1]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, [2]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, [3]Department of Public Health, NHS Fife, Kirkcaldy, UK.

Background and aims: Distal symmetric diabetic polyneuropathy (DSPN) is a major complication of diabetes, but its presence is difficult to assess before the pathology reaches an advanced stage with several clinical signs (e.g. foot sensation loss, ulcerations etc). We used the validated Michigan Screening Instrument Patient Questionnaire score threshold of ≥4 as a measure of DSPN to evaluate the contemporary prevalence of and cross sectional risk factor associations with DSPN in a large population representative cohort with type 1 diabetes (T1DM): the Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO).
Materials and methods: SDRNT1BIO is a large cohort ($N = 6127$) of adults with Type 1 diabetes recruited between 2010 and 2013 across primary and secondary care in Scotland. The characteristics of this cohort and their representativeness of the total adult T1DM population have been previously described in the literature. Patients completed the MSI at recruitment. Clinical risk factor data at time of recruitment was obtained by linkage to the comprehensive electronic health care record (SCI-Diabetes). The clinical factors examined for association with a MSI patient score of 4 or more were: HbA1c (mmol/mol), age at study date (years), duration of diabetes at study date (years), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), height (cm), weight (kg), eGFR (ml/min/1.73 m^2), total cholesterol (mmol/L) triglycerides level (mmol/L), smoking status, gender. These variables were entered simultaneously into the model and logistic regression was used for the analysis.
Results: Overall the prevalence of DSPN was 13.4% (based on the $N = 5486$ available MSI binary scores), ranging from 5.9% in those aged 16–25 ($N = 577$ available binary scores out of 646 patients in this age group) to 19.4% in those aged 75 and above ($N = 103$ available binary scores out of 126 patients). A significant independent positive association was found between duration of diabetes and the prevalence of DSPN: the odds of having diabetic neuropathy for individuals with a diabetes duration of 20 to 30 years was 1.55 (95% CI: 1.7–2.24) times higher than those with a diabetes duration lower than 5 years while the odds were 2.25 (95% CI: 1.55–3.27) higher for those with diabetes duration higher than 30 years. The odds of having diabetic neuropathy also increased significantly with age (OR = 1.06 per 5 years, 95%CI: 1.02–1.10), HbA1C levels (OR = 1.17 per 5 mmol/mol, 95%CI: 1.14–1.20), weight (OR = 1.06 per 5 kg, 95%CI: 1.03–1.09). Smoking increased the odds of presenting diabetic neuropathy by 1.55 (95%CI: 1.27–1.90) and an increase of one unit in triglyceride levels mmol/L was significantly associated with higher odds of diabetic neuropathy (1.25, 95%CI: 1.16–1.34). Height was inversely associated with DSPN (OR per 5 cm 0.91, 95%CI = 0.85–0.97), higher eGFR was also significantly associated with lower odds of diabetic neuropathy (OR: 0.99 per ml/min/1.73 m^2, 95%CI = 0.98–0.99).
Conclusion: These data indicate a substantial burden of DSPN still remains in those with T1DM. Factors other than glycaemic control are associated with DSPN and these factors are modifiable, including lipids and smoking.
Supported by: Diabetes UK and CSO
Disclosure: **A. Jeyam:** Grants; Diabetes UK, CSO.

940

Contemporary prevalence of diabetic neuropathy in type 1 diabetes: findings from the T1D Exchange

R. Pop-Busui[1], C. Boyle[2], V. Shah[3], G. Aleppo[4], J. McGill[5], R. Pratley[6], E. Toshchi[7], L. Ang[1], K. Mizokami-Stout[1];
[1]University of Michigan, Ann Arbor, [2]Jaeb Center for Health Research, Tampa, [3]Barbara Davis Center for Diabetes, Aurora, [4]Northwestern University, Chicago, [5]Washington University School of Medicine, St Louis, [6]Florida Hospital Diabetes Institute, Orlanda, [7]Joslin Diabetes Center, Boston, USA.

Background and aims: Diabetic peripheral neuropathy (DPN) is a major cause of disability, mortality and poor quality of life in patients with T1D, with prior reported prevalence rates of up to 35%. The contemporary prevalence of DPN in T1D patients was evaluated in T1D Exchange Registry centers throughout the United States.
Materials and methods: The Michigan Neuropathy Screening Instrument (MNSI), a validated 15-item self-administered questionnaire, was used to assess DPN in adults ≥18 years with ≥5 years of T1D duration. A score of ≥4 was used to define DPN. Diabetes-related characteristics and laboratory data were obtained through the most recent clinic update. Chi-square and t-tests were used to compare demographic and diabetes-related characteristics between those with and without DPN. Linear regression was used to determine the effect of DPN on HbA1c, adjusted for possible confounders.
Results: In preliminary analyses of 5,058 participants across 62 sites (mean age 39 ± 18 years, T1D duration 22 ± 14 years, 56% female, 88% non-Hispanic White, mean HbA1c 8.1 ± 1.6%), the prevalence of DPN was 10%. Those with DPN were older (52 ± 17 vs 37 ± 18 years), more likely to be female (61% vs 55%), had longer T1D duration (32 ± 16 vs 21 ± 13 years), lower annual household income (37% vs. 59% earning ≥$75K), and lower education level (55% vs. 69% with college degree) than those without DPN (all $p < 0.001$). They also had higher systolic blood pressure (126 ± 17 vs 123 ± 14 mmHg), triglycerides (117 ± 89 vs 95 ± 62 mg/dL), tobacco use (9% vs 4%) and prevalence of established CVD (26% vs 6%), despite higher use of CVD-modifying agents such as statins (64% vs 31%) and ACE-inhibitors/ARBs (45% vs 23%) (all $p < 0.001$). Participants with DPN had higher HbA1c (8.4 ± 1.7% vs 8.1 ± 1.6%), even after adjusting for multiple confounders ($p < 0.01$).
Conclusion: The prevalence of DPN in this national T1D cohort is lower than prior published reports, reflecting current clinical care practices, and highlighting other non-glycemic risk factors for DPN including CVD risk factors and socioeconomic status.
Supported by: Leona M. and Harry B. Helmsley Charitable Trust
Disclosure: **R. Pop-Busui:** None.

941

Sex differences in neuropathy and neuropathic pain in longstanding diabetes: results from the Canadian study of Longevity in type 1 diabetes

N. Cardinez[1], L.E. Lovblom[1], J.-W. Bai[1], A. Abraham[2], E. Lewis[1], J.A. Lovshin[1], A. Orszag[1], A. Weisman[1], H. Keenan[3], M. Brent[4], N. Paul[5], V. Bril[6], D. Cherney[7], B. Perkins[1];
[1]Division of Endocrinology and Metabolism, Department of Medicine, University of Toronto, Sinai Health System, Toronto, Canada, [2]Neuromuscular Diseases Unit of the Department of Neurology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, [3]Division of Endocrinology and Metabolism, Department of Medicine, Joslin Diabetes Center, Cambridge, USA, [4]Department of Ophthalmology and Vision Sciences, Department of Medicine, University of Toronto, Toronto, Canada, [5]Joint Department of Medical Imaging, Division of Cardiothoracic Radiology, University Health Network, Toronto, Canada, [6]Department of Medicine, University Health Network, University of Toronto, The Ellen and Martin Prosserman Centre for Neuromuscular Diseases, Krembil Neuroscience Centre, Division of

Neurology,, Toronto, Canada, [7]Division of Nephrology, Department of Medicine, University of Toronto, Toronto, Canada.

Background and aims: Neuropathy and neuropathic pain are common complications in T1D. We aimed to determine if sex-specific differences in the prevalence of neuropathic pain and neuropathy exist in patients with longstanding T1D.
Materials and methods: In Phase 1 of the study, 361 Canadians with ≥50 years of T1D completed questionnaires which included subjective assessment for neuropathy defined by Michigan Neuropathy Screening Instrument Questionnaire score ≥3, termed $NEUROPATHY_{MNSI-Q}$. In Phase 2 of the study, we studied a sub-cohort of 75 diabetes participants and 75 age- and sex-matched non-diabetic controls who completed objective neurological examinations which included assessment of abnormal nerve conduction studies (NCS) for neuropathy, termed $NEUROPATHY_{NCS}$.
Results: In the Phase 1 cohort, more females than males reported neuropathic pain [87(42%) vs 41(27%); $p = 0.003$)], but the presence of neuropathy ($NEUROPATHY_{MNSI-Q}$) did not differ by sex [87(42%) females vs. 66(43%) males, $p = 0.82$], and thus neuropathic pain was independent of the presence of neuropathy [adjusted OR for neuropathic pain in females compared to males, 2.7 (1.4–5.0; $p = 0.002$)]. In the Phase 2 participants, neuropathic pain was similar between the sexes (29% females vs 21% males, $p = 0.43$) while $NEUROPATHY_{NCS}$ was less prevalent among females (83% females vs 97% males, $p = 0.05$). Though not statistically significant, in a combined analysis of Phase 2 participants adjusted for $NEUROPATHY_{NCS}$, females had a tendency to a higher adjusted OR for neuropathic pain compared to males [OR 2.0 (95% CI 0.8–4.7), $p = 0.11$].
Conclusion: In conclusion, in patients with longstanding TID, neuropathic pain appears to be greater among females compared to males independent of the presence of neuropathy. Further research using larger datasets with objective neuropathy measures are required to further confirm and address these sex-specific differences.
Supported by: JDRF Canada, Canadian Clinical Trial Network, Diabetes Canada
Disclosure: **N. Cardinez:** Grants; Juvenile Diabetes Research Foundation operating grant 17-2013-312.

942

Clinical and genetic factors contributing to protection from neuropathy in extreme duration patients with type 1 diabetes

S. Azmi[1], M. Ferdousi[1], R. Donn[1], J. O'Sullivan[1], I. Petropoulos[2], G. Ponirakis[2], U. Alam[3], A. Marshall[1], A. Sankar[1], O. Asghar[1], A.J.M. Boulton[1], H. Soran[1], N. Efron[4], R.A. Malik[1,2];
[1]University of Manchester, Manchester, UK, [2]Weill Cornell Medical College Qatar, Doha, Qatar, [3]University of Liverpool, Liverpool, UK, [4]Queensland University of Technology, Queensland, Australia.

Background and aims: Individuals with Type 1 Diabetes Mellitus (T1DM) for more than 50 years (medallists) represent a unique cohort, to study factors, which protect individuals from developing complications. We aim to identify factors that may protect this cohort from complications, in particular neuropathy.
Materials and methods: Thirty-three medallists age (63.7 ± 1.4 years), duration of diabetes (58.5 ± 0.8 years) and HbA1c (65.9 ± 2.1 mmol/mmol) underwent detailed assessment of neuropathy and eight individuals with minimal/no evidence of neuropathy underwent exome sequencing.
Results: Medallists without neuropathy had a shorter duration of diabetes ($p = 0.006$), lower alcohol consumption ($p = 0.04$), lower cholesterol ($p = 0.04$) and LDL ($p = 0.02$), but no difference in smoking, BMI, HbA1c, HDL or triglycerides, compared to medallists with complications. They also had a significantly lower ACR ($p < 0.001$) and higher eGFR ($p = 0.001$), lower neuropathy symptom profile ($p = 0.002$), vibration perception threshold ($p = 0.02$), warm threshold ($p = 0.05$) and higher peroneal amplitude ($p = 0.005$) and velocity ($p = 0.03$), heart rate variability ($p = 0.001$), corneal nerve fibre density ($p = 0.001$), branch density ($p < 0.001$) and length ($p = 0.001$), compared to medallists with complications. Targeted enrichment and sequencing was performed on 200 ng of DNA extracted from peripheral blood and sequence data was mapped with BWA software with the hg19 human genome as a reference. Variants were called using GATK v2.4.7 software and filtered out if they were non-functional in dbSNP138 (unless seen in HGMD) and in our in-house variant database. Exome sequencing, replicated by Sanger sequencing failed to identify any unique variant in medallists without neuropathy.
Conclusion: Medallists with minimal complications have a lower cholesterol, LDL and alcohol consumption, but no evidence of any unique genetic variant contributing to this protection.
Supported by: JDRF
Disclosure: **S. Azmi:** None.

943

Looking for an early marker of diabetic neuropathy in type 1 diabetes

S. Frontoni[1], F. Picconi[1], M. Parravano[2], D. Schiano Lomoriello[2], L. Ziccardi[2], G. Mataluni[3], P. Pasqualetti[4], D. Ylli[5], L. Chioma[1], I. Malandrucco[1], G. Marfia[3];
[1]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, [2]IRCCS-G.B. Bietti Foundation, Rome, Italy, [3]Unit of Disimmune Neuropathies, University of Rome Tor Vergata, Rome, Italy, [4]AFaR Division, Fatebenefratelli Foundation for Health Research and Education, Rome, Italy, [5]Department of Clinical Semiotics and Imaging, University of Medicine Tirana, Tirana, Albania.

Background and aims: A painless, non-invasive, reproducible, cost-effective and clinically available tool is necessary for the early detection and diagnosis of diabetic neuropathy. Retinal neurodegeneration has been considered so far a predictive sign for the development of microvascular alterations in diabetic retinopathy (DR). However, few data are available on the association between neuroretinal damage and diabetic neuropathy. Our study, therefore, was aimed to investigate the possible role of retinal neurodegeneration, as an early marker of diabetic peripheral neuropathy (DPN).
Materials and methods: 15 type 1 diabetes mellitus (DM1) patients with no symptoms/signs of peripheral polyneuropathy, without DR or with very mild non-proliferative DR, and 14 healthy controls (C), matched for age and gender, were enrolled. All patients underwent corneal confocal microscopy, including the number of fibers, the number of fiber beadings, the degree of fiber branching, and the degree of fiber tortuosity of corneal sub-basal nerve plexus. Cardiovascular autonomic function was evaluated by cardiovascular autonomic reflex tests (CARTs). The following electrophysiological tests were also performed: standard nerve conduction studies (NCS), incremental motor unit number estimation (MUNE) from abductor hallucis (AH) and abductor digiti minimi (ADM) with assessment of AH and ADM average single motor unit potential (SMUP) size. Neuroretinal function was analyzed by multifocal electroretinogram measuring amplitude density (Amp) and implicit time (IT) of nasal (N)/ temporal (T)/superior (S)/ inferior (I) macular quadrants.
Results: Amp of all macular quadrants was significantly reduced in DM1 ($p < 0.001$) vs C. ADM MUNE and AH MUNE were significantly decreased in DM1 (p 0.039; $p < 0.0001$, respectively), and AH-SMUP significantly increased (p 0.002) vs. C. A positive correlation between Amp in N and I quadrant and AH MUNE (r 0.368, p 0.01; r 0.288, p 0.03, respectively) was observed in DM1 patients. A negative correlation between degree of corneal sub-basal nerve plexus fiber tortuosity and Amp in N, I, T quadrants (r −0.780, p 0.002; r −0.583, p 0.036; r −0.571, p 0.041 respectively) and between degree of fiber tortuosity and AH

MUNE (r −0.547, p 0.043) were observed in DM1. No abnormalities of NCS were found in any subject. A positive correlation between lying-to-standing (LTS) values and Amp S, I, T, N (r 0.826, p 0.002; r 0.749, p 0.008; r 0.797 p 0.003; r 0.694, p 0.018 respectively) were observed in DM1.

Conclusion: Neuroretinal dysfunction and motor unit loss are already present in DM1 patients without DPN. The association between neuroretinal changes and motor unit decline, corneal confocal microscopy parameters and autonomic function supports the hypothesis that neuroretina could represent a new "point of view" to detect the early overall neurogenic process in diabetes.

Disclosure: **S. Frontoni:** None.

944

Independent correlations between the presence of retinopathy and kidney disease in diabetes and measures of both metabolic control and neuropathy

D. Tesic[1], M. Mitrovic[1], J. Ljikar[2], D. Popovic[1], D. Tesic[3], M. Tomic[1], B. Vukovic[1];
[1]Clinic of Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Vojvodina, Novi Sad, [2]Clinic of Ophthalmology, Clinical Center of Vojvodina, Novi Sad, [3]Clinic of Cardiology, Institut for Cardiovascular Diseases, Sremska Kamenica, Serbia.

Background and aims: The aim of this study was to examine associations between measures of neuropathy, nephropathy and lower-extremity artery disease (LEAD) in people with diabetes complicated by nonproliferative retinopathy (NPDR), maculopathy and proliferative retinopathy (PDR).

Materials and methods: People were included who attended a specialist service for review between September 2012 and September 2017. Clinical examination of the eyes was performed through dilated pupils using a slit lamp. VPT was measured using a semiquantitative tuning fork C128 and ankle reflexes (AR) were recorded. Sudomotor function was determined as the time until total colour change using Neuropad®. LEAD was defined with ABPI >1.4 or <0.8 or Continous wave Doppler monophasic curve. In a fasting blood sample serum creatinine, lipids and lipoproteins were measured. Urinary protein excretion (mg/24 h) and the presence of coronary and cerebrovascular disease, neuropathy symptom score (NSS) of Veves et al (1994) were documented. Hypertension was defined as ≥140/90 mmHg or by antihypertensive therapy. All tests were undertaken as part of routine clinical care.

Results: Of 469 people, 46.1% were male and 68.4% had T2DM. 30 had PDR, 98 had maculopathy, 89 had NPDR and 252 had no evidence of retinopathy. Compared with people without retinopathy, those with retinopathy were older (58 ± 12.5 vs. 52.3 ± 15.1 years), with lower VPT (5.1 ± 2.8 vs. 6.6 ± 2), more often with missing ankle reflexes (2.9 ± 1.3 vs. 2.0 ± 1.6), higher prevalence of LEAD (18.4% vs. 7.9%) and arterial hypertension (52.9% vs. 36.5%), lower height (175.4 ± 8.5 vs. 177 ± 5.9 cm), higher waist circumference (96.3 ± 12.6 vs. 91 ± 13.6 cm) and longer diabetes duration (18.2 ± 8.7 vs. 12.2 ± 8.6 years); all $p < 0.01$. After multivariable logistic regression analysis (MVLR), the differences in VPT, AR and diabetes duration all persisted ($p < 0.01$). People with PDR compared with controls had worse VPT (3.8 ± 3.3 vs. 6.6 ± 2; $p < 0.001$). In a univariate model PDR was related to creatinine (OR 1.014 [95% CI: 1.005–1.023]), triglycerides (1.022 [1.02–1.46]), duration of insulin therapy (1.057 [1.015–1.101]); all $p < 0.01$. After MVLR the differences remained significant ($p < 0.01$) for creatinine and duration on insulin therapy. People with maculopathy had worse sudomotor neuropathy (10 ± 7.3 vs. 7 ± 5.7 min; $p < 0.001$). In a univariate model maculopathy was related to NSS (OR 2.19 [1.35–3.04]), Neuropad® time (1.07 [1.033–1.109]); T2DM (77.6 vs. 62.3%), HbA1c 1.083 [1.058–1.108]); all $p < 0.01$, and to fasting cholesterol (1.035 [1.05–1.71]; $p = 0.02$). After MVLG the significance remained: for NSS, Neuropad® time, HbA1c (all $p < 0.05$). Both PDR and maculopathy in univariate analyses was related to proteinuria (OR 1.000 [1.0–1.001]) and after MVLR with creatinine clearance (0.976 [0.98–0.99]) and highest life BMI (1.067 [1.029–1.106]); all $p = 0.000$.

Conclusion: Our data showed associations between the presence of different clinical measures and peripheral neuropathy with both retinopathy and kidney disease, complications associated with severe insulin deficient diabetes and severe insulin resistant diabetes. Diabetic neuropathy reinforce the need to strive to optimise metabolic control.

Disclosure: **D. Tesic:** None.

945

Assessment of diabetic peripheral neuropathy in patients with prediabetes, type 1 and type 2 diabetes using corneal confocal microscopy

M. Ferdousi[1], S. Azmi[1], A. Kalteniece[1], I.N. Petropoulos[2], G. Ponirakis[2], U. Alam[3], O. Asghar[1], A. Marshall[1], W. Jones[1], M. Jeziorska[1], H. Soran[1], N. Efron[4], A.J. Boulton[3], R.A. Malik[2];
[1]Institute of Human Development, Manchester, UK, [2]Weill Cornell Medicine-Qatar, Doha, Qatar, [3]Division of Endocrinology, Diabetes and Gastroenterology, Manchester, UK, [4]Faculty of Health, School - Optometry and Vision Science, Sydney, Australia.

Background and aims: Over the past decade corneal confocal microscopy (CCM) has been introduced as a surrogate end point for the assessment of diabetic neuropathy. We aimed to utilise CCM for the assessment of diabetic neuropathy and associated risk factors.

Materials and methods: 597 participants including 98 controls (age = 42.11 ± 15.06), 171 patients with T1DM (age = 49.02 ± 16.14, Duration: 27.69 ± 17.96), 271 with T2DM (age = 62.73 ± 9.56, Duration: 12.40 ± 13.5) and 57 with IGT (age: 59.55 ± 10.85, Duration: 6.96 ± 9.30) underwent the detailed assessment of neuropathy.

Results: Patients with T1DM had evidence of neuropathy with significant impairment of all neuropathy markers compared to controls. Corneal nerve fibre length (CNFL) was significantly associated with duration of diabetes ($r = -0.3$, $P < 0.0001$). Patients with T2DM also had neuropathy with all markers being significantly impaired ($P < 0.05$) apart from vibration perception threshold (VPT) and cold perception threshold (CPT) compared to the controls. In this cohort CNFL was significantly associated with HDL ($r = 0.2$, $P = 0.04$), Triglyceride ($r = -0.2$, $P = 0.004$), VPT ($r = -0.2$, $P < 0.0001$), CPT ($r = 0.24$, $P < 0.0001$). Patients with IGT had small fibre neuropathy with impaired corneal nerve fibre density (CNFD), branch density (CNBD), CNFL, WPT, CIP and HIP compared to controls. There was no association between CNFL, HbA1c, lipid profiles and other clinical measure of neuropathy in patients with IGT. The area under the curve for CNFD were 0.72 in T1DM, 0.55 in T2DM and 0.65 in patients with IGT.

Conclusion: CCM detects neuropathy in patients with IGT, T1DM and T2DM. CNFL was associated with other measurements of neuropathy only in patients with T2DM. CNFL was associated with duration of diabetes only in patients with T1DM and with lipid profile in T2DM highlighting the difference in aetiology between these 2 groups.

Parameters	Control (n=98)	T1D M(n=171)	T2DM (N=271)	IGT(N=57)
Corneal nerve fibre density (no./mm²)	31.6±0.883	22.49±0.61*$#	26.03±0.52*	28.27±1.05*
Corneal nerve branch density (no./mm²)	83.36±4.04	49.59±2.83*$#	62.79±2.38*	61.61±4.83*
Corneal nerve fibre length (mm/mm²)	25.33±0.78	18.23±0.54*$#	22.43±0.46*	22.82±0.93*
Intra epidermal nerve fibre density (no./mm²)	9.15±1.03	5.45±0.52*#	6.52±0.68*	7.82±0.77
NDS (0-10)	1.7±0.3	4.18±0.2*$#	3.1±0.17*	2.5±0.3
Vibration perception threshold (v)	11.09±1.26	18.11±0.78*$#	13.59±0.65	14.68±1.33
Cold perception threshold ©	26.96±0.71	23.53±0.45*$#	26.03±0.36	25.31±0.76
Warm perception threshold ©	38.15±0.53	41.26±0.34*	40.61±0.27*	39.95±0.57*
Cold induced pain ©	10.78±0.99	7.39±0.7*	5.86±0.68*	6.83±1.05*
Heat induced pain ©	45.14±0.38	46.88±0.27*	47.7±0.26*	46.95±0.41*
Sural nerve conduction Velocity	48.8±0.77	40.52±0.48*$#	45.98±0.4*#	50.21±0.82

All data presented as Mean±SE. * Significant difference compared to controls, $ significant difference compared to T2DM, # significant difference compared to IGT. All parameters have been adjusted for age using ANCOVA.

Supported by: JDRF, NIH

Disclosure: **M. Ferdousi:** None.

946

Physical and psychological determinants of fall risk in patients with diabetic neuropathy: a prospective investigation

S.J. Brown[1], L. Vileikyte[2], A.J.M. Boulton[2], N.D. Reeves[1];
[1]Manchester Metropolitan University, Manchester, [2]University of Manchester, Manchester, UK.

Background and aims: People with diabetic peripheral neuropathy (DPN) are more likely to fall and report diminished levels of physical activity (PA). However, determinants of DPN-related falls and PA are not well described. This prospective study examined the physical (DPN severity and unsteadiness) and psychological factors (fear of falling (FoF) and generalized distress) in their relationship to falling and PA levels.

Materials and methods: Twenty-two type 2 diabetes patients (18 males; age: 70 ± 9 years, Vibration Perception Threshold, VPT: 23 ± 11 V, Neuropathy disability score: 6 ± 3 score/10) wore hip mounted activity monitors for 4 weeks (adherence: 17 ± 6 days). Daily activity levels were separated into minutes of: sedentary, light, moderate and vigorous. Unsteadiness at baseline was measured objectively- by Berg balance test (48 ± 6 score/56) and subjectively - by patient self-report (2-item NeuroQoL; 3.7 ± 1 score/5). Diaries were used to self-record falls during the study (8/22 individuals fell at least once, median: 2 [range: 1–12] falls per faller). FoF at baseline was assessed with Falls Self-Efficacy-International Scale (FES-I; 29 ± 12 score/64); generalized distress-with Hospital Anxiety and Depression Scale (HADS; 18 ± 3 score/21). Associations between variables were assessed by Pearson's correlations.

Results: More severe DPN was associated similarly with self-reported unsteadiness ($r = 0.41$, $p = 0.03$) and with objective, Berg balance test ($r = 0.43$, $p < 0.02$). Berg and self-reported measures of unsteadiness were significantly correlated ($r = 0.49$, $p = 0.02$, Fig. 1), however, whilst self-reported unsteadiness was associated with greater FoF ($r = 0.64$, $p < 0.01$) and with fall incidence ($r = 0.68$, $p < 0.01$), objectively measured unsteadiness was associated with FoF only ($r = 0.68$, $p < 0.01$), and not reported fall incidence. Higher levels of FoF were strongly associated with increased fall incidence ($r = 0.81$, $p < 0.01$), while increased generalized distress was associated higher fall incidence ($r = 0.47$, $p = 0.04$). Higher levels of light activity were associated with more falls ($r = 0.73$, $p < 0.01$).

Conclusion: These findings suggest that subjective measures such as self-reported DPN-unsteadiness and fear of falling may be valuable indicators of fall risk and of at least similar value compared to simple laboratory measures of balance such as the Berg Balance test. This makes the case for incorporating psychological components in carefully designed multifactorial interventions. Moreover, as increments even in light activity levels are associated with more falls, potentially due to increased opportunities to fall, balance should be taken into consideration when designing interventions to improve physical activity.

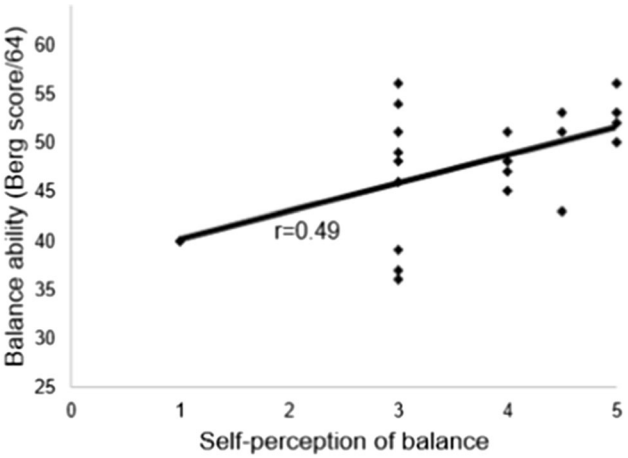


Figure 1: Relationship of between measured balance (Berg balance score) and self-perception of balance (Neuroqol)

Supported by: Manchester Metropolitan University - University of Manchester Joint Grant
Disclosure: **S.J. Brown:** None.

PS 085 Neuropathy: markers and remedies

947

A systemic inflammatory signature reflecting crosstalk between innate and adaptive immunity is associated with polyneuropathy: KORA F4/FF4 Study

C. Herder[1,2], J.M. Kannenberg[1,2], M. Carstensen-Kirberg[1,2], A. Strom[1,2], G.J. Bönhof[1], W. Rathmann[1,2], C. Huth[3,2], W. Koenig[4,5], M. Heier[3], J. Krumsiek[3,2], A. Peters[3,2], C. Meisinger[3,6], M. Roden[1,7], B. Thorand[3,2], D. Ziegler[1,7];
[1]German Diabetes Center, Duesseldorf, [2]German Center for Diabetes Research (DZD), Muenchen-Neuherberg, [3]Helmholtz Zentrum München, Neuherberg, [4]Technische Universität München, Munich, [5]German Center for Cardiovascular Research (DZHK), Munich, [6]Ludwig-Maximilians-Universität München am UNIKA-T Augsburg, Augsburg, [7]Medical Faculty, Heinrich Heine University Duesseldorf, Duesseldorf, Germany.

Background and aims: Data on the association between biomarkers of inflammation and distal sensorimotor polyneuropathy (DSPN) from large cohorts are scarce and limited to biomarkers of innate immunity. Therefore, we aimed to assess associations between biomarkers reflecting multiple aspects of immune activation and DSPN in a population-based cohort.
Materials and methods: This cross-sectional analysis included 1048 participants of the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study. Serum levels of biomarkers of inflammation were measured at baseline using proximity extension assay technology. The 92-biomarker panel covers a range of proteins including pro- and anti-inflammatory cytokines, chemokines, growth factors and factors involved in acute inflammatory and immune responses, angiogenesis, fibrosis and endothelial activation. Associations between biomarkers and the Michigan Neuropathy Screening Instrument (MNSI) score were estimated and followed-up by pathway analysis.
Results: Linear regression analysis showed that 27 out of 71 biomarkers of inflammation that passed quality control were positively associated with the MNSI score after adjustment for a range of anthropometric, metabolic, clinical and lifestyle factors ($P < 0.05$). Correction for multiple testing reduced the number of biomarkers that remained associated with the MNSI score to 20 (false-discovery rate/FDR <0.05). Ingenuity Pathway Analysis revealed an enrichment of the 27 biomarkers that were associated with incident DSPN in 22 canonical pathways (FDR <0.001). These results indicated that multiple cell types from innate and adaptive immunity may be involved in the development of DSPN and pointed towards hepatic fibrosis and autoimmunity as additional pathomechanisms. Potential upstream regulators for this set of differentially expressed biomarkers were the proinflammatory cytokines TNFalpha ($P = 2 \times 10^{-15}$) and IL-1beta ($P = 1 \times 10^{-14}$).
Conclusion: We identified multiple novel associations between biomarkers of inflammation and DSPN pointing to a complex cross-talk between innate and adaptive immunity in the pathogenesis of neuropathy.
Supported by: DZD, DDG
Disclosure: **C. Herder:** None.

948

Acute hyperoxia deteriorates nerve conduction velocity in type 1 diabetes: A possible effect on oxidative stress?

J.C. Laursen[1], C. Stevns Hansen[1], M. Bordino[2], E. Hein Zobel[1], S. Abitz Winther[1], P.-H. Groop[2], M. Frimodt-Møller[1], L. Bernardi[2], P. Rossing[1];
[1]Steno Diabetes Center Copenhagen, Copenhagen, Denmark, [2]Folkhälsen Research Center, Folkhälsen Institute of Genetics, Helsinki, Finland.

Background and aims: Cardiovascular autonomic dysfunction (CAD) and distal symmetric polyneuropathy (DSPN) are prevalent diabetic complications, associated with increased morbidity and mortality. In previous studies, acute hyperoxia and slow deep breathing (SDB) improved measures of CAD in patients with both type 1 diabetes (T1D) and type 2 diabetes (T2D). However, acute oxygen inhalation has been shown to deteriorate arterial function and increase blood pressure due to acute oxidative stress, while SDB improves both. Such effects have not been addressed in respect to DSPN. The aim of this study is to examine the effects of acute hyperoxia on measures of DSPN and blood pressure and whether these could be modified by albuminuria or existing autonomic dysfunction.
Materials and methods: Fifty-four patients with T1D (57% male) were enrolled in a cross-sectional study stratified by normoalbuminuria (n = 29) and presence of/or historical macroalbuminuria (n = 25). Mean age and diabetes duration were 59.8 (SD 9.5) and 37.5 (SD 14.4) years respectively. CAD was diagnosed when a minimum of two out of three standard cardiovascular autonomic reflex tests (CARTs) were abnormal. Patients were exposed to acute oxygen inhalation and SDB respectively, while obtaining continuous systolic blood pressure (SBP), diastolic blood pressure (DBP) and blood oxygen saturation (SAT). Patients were exposed to acute oxygen inhalation while obtaining measures of DSPN. DSPN was assessed by sural nerve conduction velocity (SNCV), action potential (SNAP) and electrochemical skin conduction (ESC). Effects were evaluated by linear regression analyses adjusted for baseline values of outcome variables.
Results: Acute oxygen inhalation was associated with an increase of 2.1% (95CI 1.7; 2.6), 3.9 mmHg (95%CI 1.3; 6.5) and 2.5% (95%CI 0.1; 5.0) in SAT, DBP and hands-ESC (μS) respectively. Conversely, acute oxygen inhalation was associated with a decrease of −1.7 ms (95%CI −3.22; −0.12) and −2.1% (−4.1;−0.01) in SNCV and feet-ESC (μS) respectively. SDB was associated with an increase of 1.2% (95%CI 1.0; 1.5) in SAT, compared to spontaneous breathing. SDB was associated with a decrease of −8.4 mmHg (95%CI −14.4; −2.4) and −3.6 mmHg (95%CI −5.8; −1.4) in SBP and DBP respectively. Interventions had no effect on SNAP. Oxygen inhalation induced a 6mmHg higher (95%CI 0.45; 11.55) response in DBP and a 4.8 ms higher (95%CI 1.4; 8.1) response in SNCV (ms) in patients with CAD compared with those without. Albuminuria or existing autonomic dysfunction did not modify any other associations.
Conclusion: Acute hyperoxia deteriorates blood pressure and nerve conduction velocity in T1D and SDB improves blood pressure. The effect of acute hyperoxia is stronger on blood pressure and diminished on nerve conduction velocity in patients with CAD. Similarly to the demonstrated effect of oxidative stress on blood pressure, we hypothesise that the acute worsening in SNCV, is a direct effect of oxidative stress (present in both hypoxia and hyperoxia) on nerve function. This novel finding indicates that peripheral neuropathy has a functional component that could be acutely modified by manipulation of oxygen. Further studies exploring the pathological pathways causing tissue hypoxia may improve the understanding and treatment of diabetic neuropathy.
Supported by: Novo Nordisk Foundation grant NNF14OC00
Disclosure: **J.C. Laursen:** None.

949

Associations between vitamin D and diabetic neuropathy in adolescents with type 1 diabetes

A. Heinesen[1,2], M.M.B. Christensen[1,2], J. Fleisher[3], M.E. Jørgensen[1,4], E.E. Hommel[5], C.S. Hansen[1];
[1]Department of clinical epidemiolgy, Steno Diabetes Center Copenhagen, Gentofte, [2]University of Copenhagen, Copenhagen, [3]Medical Research Laboratories, Clinical Institute of Medicine, Aarhus University, Aarhus, [4]University of Southern Denmark, Odense, [5]Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: Diabetic peripheral- and cardiovascular autonomic neuropathy (DPN, CAN) are severe and prevalent complications to diabetes, associated with increased morbidity and mortality. Manifest diabetic neuropathy is not reversible why early detection and prevention is essential e.g. in early diabetes. Vitamin D has been independently associated with diabetic neuropathy. Our aim is to investigate this association in a population of adolescent type 1 diabetes patients.
Materials and methods: 151 patients with type 1 diabetes (42% male, mean age 22 years (IQR 21; 23), mean diabetes duration 11.2 years (SD 5.1)) were screened for CAN and DPN. CAN was measured by 5 minute resting heart rate variability and three standard cardiovascular autonomic reflex tests (CARTs): lying to standing (30:15), deep breathing (E:I) and Valsalva Maneuver (VM). DPN was measured by light touch perception, pain perception, vibration perception threshold, electrochemical skin conductance, Sural nerve conductance velocity (SNCV) and Sural nerve action potentials. Models were adjusted for age, sex, diabetes duration, HbA1c, cholesterol, triglycerides, systolic BP, smoking, beta blockers and seasonal variation for vitamin D.
Results: Twenty patients (13.6%) had vitamin D deficiency (<25 nmol/l). The mean level of serum vitamin D was 62.6 nmol/l (IQR 35; 86), 32 patients (21.2%) used vitamin D supplements. Vitamin D was significantly associated with the VM (0.16% (95%CI: 0.019; 0.30) $p = 0.027$) and SNCV (2.9% (95%CI: 0.93; 4.8) $p = 0.004$), in a non-linear manner as an inverse U-shaped association for both outcomes (figure 1). No associations were established between vitamin D and other outcomes.
Conclusion: High and low serum vitamin D levels are associated with both CAN and DPN in young adults with type 1 diabetes. The associations were found despite a low prevalence of vitamin D deficiency. Our findings indicate that high and low levels of vitamin D may be a risk factor for diabetic neuropathy in young diabetes patients. Future studies could elucidate if vitamin D monitoring and management could have beneficial effects on diabetic neuropathy.

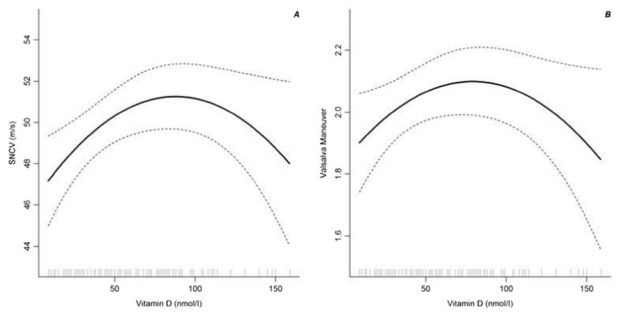


Figure 1
Complete lines show the predicted values of the SNCV (A) and VM (B) as a function of vitamin D. Dashed lines show 95% CI. Both models are estimates for a 22 year old, non-smoking, male with a diabetes duration of 11 years, a HbA1c at 69 mmol/mol, with serum cholesterol at 4.5 mmol/l, triglyceride at 1.3 mmol/l and a systolic BP of 125 who does not use beta blockers.

Supported by: The Augustinus Foundation, The Toyota Foundation
Disclosure: **A. Heinesen:** None.

950

Glycaemic variability and diabetic neuropathy in adolescents with type 1 diabetes

M.M.B. Christensen[1], E.E. Hommel[1], M.E. Jørgensen[1,2], J. Fleischer[3], C.S. Hansen[1];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]National Insitute of Public Health, University of Southern Denmark, Odense, [3]Clinical Institute of Medicine, Aarhus University, Aarhus, Denmark.

Background and aims: Glycemic control assessed by HbA1c is a risk factor for diabetic neuropathy. However averaged long-term glucose measurement does not necessarily reflect the daily glycemic variability (GV). Hypo- or hyperglycemic events may attribute to the pathogenesis of diabetic neuropathy. Early stages of diabetic peripheral neuropathy (DPN) and cardiovascular autonomic neuropathy (CAN) may be reversible, thus early detection and prevention is crucial. The aim of the study was to investigate the association between GV and DPN and CAN in a Danish population of adolescent patients with type 1 diabetes.
Materials and methods: Adolescent type 1 diabetes patients were enrolled in a single-center study. CAN was assessed by cardiovascular autonomic reflex tests (CARTs) and measures of 5 min. resting heart rate variability (HRV). CAN was diagnosed if two or three out of three tests were abnormal. HRV indices were analysed in time- and frequency-domain. Time-domain analyses included the root mean square of the sum of the squares of differences between consecutive R-R intervals (RMSSD) and standard deviation of normal-to-normal intervals (SDNN). Frequency-domain analyses included low-frequency power band (LF) (0.04–0.15 Hz), high-frequency power band (HF) (0.15–0.4 Hz) and the LH/HF-ratio. DPN was assessed by light touch perception, pain perception, vibration perception threshold (VPT), electrochemical skin conductance (ESC), sural nerve conduction velocity (SNCV) and sural nerve action potential (SNAP). Data on five-day continuous glucose monitoring was obtained. Coefficient of variation (CV), SD, mean amplitude of glucose excursions (MAGE), continuous overall net glycemic action (CONGA) and time spent in hypo- (<3.9 mmol/l), eu- (≥3.9; ≤10.0 mmol/l) and hyperglycemia (>10.0 mmol/l) were calculated. The associations between GV and neuropathy measures were assessed by logistic and linear regression analyses adjusting for age and gender (model 1) and subsequently BMI, exercise and HbA1c (model 2).
Results: The study comprised 133 adolescents (44% were males) with a mean age of 22 years (SD 1.6), a mean diabetes duration of 11 years (SD 5.2) and a median HbA_{1c} of 65.5 mmol/mol (IQR 57; 74). Mean CV and CONGA were 40% (SD 10) and 9.1 mmol/l (SD 2.2), respectively; median SD and MAGE were 3.9 mmol/l (IQR 3.2; 4.7) and 7.7 mmol/l (IQR 5.9; 9.9). Median percentage of time spent in hypo-, eu- and hyperglycemia was 4% (IQR 1.0; 10), 47.9% (SD 18.7) and 44.7% (SD 20.6), respectively. Higher CONGA was associated with increasing incidents of the composite measure of symmetric peripheral neuropathy, abnormal SNAP and SNCV and incidents of CAN, however significance was lost in model 2. Higher MAGE was associated with increasing SDNN (1.0(95%CI: 1.0; 1.0) $p = 0.037$), RMSSD (1.0(95%CI: 1.0; 1.0) $p = 0.041$) and HF (1.0(95%CI: 1.0; 1.0) $p = 0.0095$) in fully adjusted models. No other significant associations were found.
Conclusion: Modest associations between GV and measures of autonomic and peripheral neuropathy were found. Findings were confounded by relevant risk factors for diabetic neuropathy. This suggests that GV is not a risk factor for diabetic neuropathy in adolescents with type 1 diabetes. However long-term effects of GV excursions may still play a role in the pathogenic mechanisms leading to neuropathy in later life.
Supported by: The Augustinus Foundation, The Toyota Foundation
Disclosure: **M.M.B. Christensen:** None.

951

Relation of oxidative stress and glycaemic variability with in vivo corneal confocal microscopy parameters in type 1 diabetes

F. Picconi[1], R. Squitti[2], M. Siotto[3], D. Schiano Lomoriello[4], M. Parravano[4], P. Pasqualetti[5], D. Ylli[6], L. Chioma[1], I. Malandrucco[1], S. Frontoni[1];
[1]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, [2]Molecular marker laboratory, IRCCS Istituto centro San Giovanni di Dio-Fatebenefratelli, Brescia, Italy, [3]Don Carlo Gnocchi ONLUS Foundation, Milan, Italy, [4]IRCCS-G.B. Bietti Foundation, Rome, Italy, [5]AFaR Division, Fatebenefratelli Foundation for Health Research and Education, Rome, Italy, [6]Department of Clinical Semiotics and Imaging, University of Medicine Tirana, Tirana, Albania.

Background and aims: In vivo corneal confocal microscopy (CCM), measuring corneal sub-basal nerve plexus, is a validated screening tool, diagnosis method, and biomarker of diabetic sensorimotor polyneuropathy (DSP) in type 1 diabetes mellitus (DM1). Both copper and iron homeostasis and glucose variability have been implicated in the activation of oxidative stress pathways, and subsequent onset of diabetes and neurodegeneration. The aim of the present study is to evaluate the impact of metabolic and oxidative markers on corneal sub-basal nerve plexus parameters.
Materials and methods: 15 DM1 patients with no symptoms/signs of peripheral polyneuropathy and 15 healthy controls (C), matched for age and gender, were studied. All patients underwent CCM, including the number of fibers, the number and density of fiber beadings, the degree of fiber branching, and the degree of fiber tortuosity of corneal sub-basal nerve plexus. Oxidative stress was evaluated by the measurement of systemic variation of markers of iron and copper metabolism, such as serum iron, ceruloplasmin (Cp), transferrin (Tf), non-ceruloplasmin bound copper (nonCp-Cu), Cp specific activity (eCp/iCp) and ceruloplasmin to transferrin ratio (Cp/Tf). All DM1 persons underwent a 72-h continuous glucose monitoring (CGM), with the calculation of glycemic variability GV indexes
Results: Cp/Tf, nonCp-Cu and eCp/iCp were significantly increased and Tf decresed in DM1 group vs C group. A positive correlation between density of beadings of corneal sub-basal nerve plexus and Tf (r 0.760, p 0.003) and a negative with Continuous Overall Net Glycemic Action (CONGA) 1–2 −4 h indexes (r −0.755, p 0.005; r −0,790 p 0.002; r −0.818, p 0.001) were observed in DM1 group. No correlation was found between glycated hemoglobin and CCM parameters. After mutivariate analysis, only Cp/Tf ratio was an independent predictor of density of fiber beadings.
Conclusion: Oxidative stress, correlated to metal dysregulation and GV is associated with density of beadings, in DM1. Nerve beadings density is a parameter of metabolic activity of small nerve fibers, and represents the accumulation of mitochondria along the nerve. Mitochondria play a critical role in controlling nerve function and their morphological and functional anomalies are involved in development of diabetic neuropathy
Disclosure: **F. Picconi:** None.

952

A novel method for measuring vibration perception thresholds (VPTs) shows an improvement in VPTs in type 1 diabetic patients with improved metabolic control

E. Lindholm[1], T. Elgzyri[1], M. Löndahl[2], L.B. Dahlin[1];
[1]Lund University, Malmo, [2]Lund University, Lund, Sweden.

Background and aims: Whilst improvement of metabolic control has been shown to result in improvement in tests evaluating functional and structural measures of small diameter nerve fibres, no such improvement has been presented for large diameter (i.e. myelinated) nerve fibres detected by vibration perception tests or electrophysiology. A novel method for measurement of vibration perception thresholds (VPTs) was used to study if better metabolic control can improve VPTs in adults with type 1 diabetes.
Materials and methods: VPTs were investigated at six frequencies (4, 8, 16, 32, 64 and 125 Hz) using VibroSense Meter (VibroSense Dynamics AB) in the sole of the foot - at the first metatarsal head (MTH1) and at fifth metatarsal head (MTH5) - at two different occasions (mean follow up time 1.6 ± 0.3 years) in patients with type 1 diabetes (n = 122; 55 males, 67 females). VPTs at the right foot at baseline and at follow up were compared separately in the patients with lower HbA_{1c} (n = 70) and with the same or higher HbA_{1c} at follow up (n = 52). The significant p value after multiple comparisons (n = 24) was less than 0.002.
Results: In patients with lower HbA_{1c} at follow up, the VPTs improved at 64 Hz at the MTH1 and at 4 Hz, 64 Hz and 125 Hz at MTH5 (Table). No significant difference was seen in VPTs when the patients' HbA_{1c} was similar or higher at the follow up compared to the baseline.
Conclusion: Improving metabolic control measured by HbA_{1c} in patients with type 1 diabetes can improve VPTs, suggesting a reversible effect on nerve function by improved metabolic control. The use of the non-invasive method VibroSense Meter to assess early changes in nerve function can further motivate patients with diabetes to adhere to a strict treatment strategy.

Table. Difference in VPTs in patients according to changes in metabolic control when HbA_{1c} was lower at the follow up.

	baseline	follow up	P[a]
HbA_{1c}(mmol/mol)	65.8±11.5	56.7±11.0	**<0.00001**
MTH1 4 Hz (Db)	95.8[91.6-108.8]	95.1[90.1-102.2]	0.02
MTH1 64 Hz (Db)	128.5[117.2-138.4]	122.1[111.5-135.1]	**0.00006**
MTH1 125 Hz (Db)	136.0[121.3-147.3]	131.6[113.4-147.2]	0.011
MTH5 4 Hz (Db)	99.3[93.7-108.3]	94.5[89.3-101.9]	**0.00002**
MTH5 8 Hz (Db)	104.8[100.5-116.0]	103.2[94.9-111.1]	0.003
MTH5 16 Hz (Db)	112.2[105.9-122.9]	109.2[103.1-120.1]	0.003
MTH5 32 Hz (Db)	120.2[112.0-133.5]	117.7[111.3-125.9]	0.006
MTH5 64 Hz (Db)	127.7[118.9-139.2]	123.8[111.8-133.6]	**0.00006**
MTH5 125 Hz (Db)	133.0[119.6-143.5]	130.0[114.6-141.8]	**0.001**

[a]Significant after multiple comparisons (n=24) if p <0.002; Db=decibel. Numbers are mean ± standard deviation for the HbA_{1c} and median [25th-75th percentile] for the VPTs. For the VPTs at MTH1 8 Hz,16 Hz and 32 Hz p> 0.05 (data not shown).

Supported by: Swedish Governmental Agency for Innovation Systems
Disclosure: **E. Lindholm:** None.

953

Impact of normoglycaemia in reducing microvascular complications in patients with type 2 diabetes

M. Tavakoli[1], F. Ishibashi[2];
[1]University of Exeter Medical School, Exeter, UK, [2]Ishibashi Medical Centre, Hiroshima, Japan.

Background and aims: Hyperglycemia is associated with increased risk of microvascular complications in patients with type 2 diabetes (T2DM). The strict glycemic control is the only strategy for slowing DPN progression in T2DM. Thus, identifying potentially modifiable risk factors for neuropathy is crucial. The aim of present study was to investigate whether reduction of the level of HbA1c by tight glycemic control (GC) decreases the rate of microvascular complications and improves the neurological functions in patients with T2DM over 4 years period.
Materials and methods: Detailed clinical and neurological examinations including corneal confocal microscopy (CCM) were performed in 141 patients with type 2 diabetes and 60 age-matched control subjects at baseline and follow-up with GC for 4 years. Patients were stratified according to mean HbA1c level during follow-up into good (HbA1c <53.0 mmol/mol, mean; 47.5 mmol/mol), fair (53.0 mmol/mol ≤HbA1c <58.5 mmol/mol, mean; 55.6 mmol/mol), and poor (HbA1c ≥58.5 mmol/mol, mean; 68.9 mmol/mol) GC groups with similar HbA1c levels at baseline (84.5–88.2 mmol/mol).The patients assigned to the insulin-sensitizing or insulin providing strategy were treated with biguanides or pioglitazone, or with sulfonylureas or insulin, respectively. The HbA1c for patients was measured monthly during the follow-up period. And hence the mean HbA1c levels during follow-up are representative, and the linear regression provided changes in neuropathy outcomes by a reduction in mean HbA1c level per year of follow-up.
Results: At baseline, CCM revealed significant nerve damage in all patients compared to controls. The interval changes in most CNF parameters and neurophysiological functions were significantly related to mean HbA1c levels during follow-up. Interestingly the baseline HbA1c level did not impact on neurological functions at follow-up. Interval changes in neuropathy outcomes were associated with mean clinical factors during follow-up and hypoglycemic strategies. Good GC improved all nerve functions, including CNF branch density and bead, but not length and

main fibre density. Fair GC deteriorated some nerve functions. Poor GC compromised all neuropathy outcomes. Irrespective of GC levels retinopathy increased after follow-up period, while nephropathy decreased.After follow-up among the patient subgroups, despite strict glycemic control and improvement in mean HbA1c by around 30.6 mmol/mol, the cumulative incidence of neuropathy increased from 17.7% at baseline to 21.3% ($p = 0.383$) and retinopathy increased from 21.3% to 35.5% ($p < 0.001$), however the cumulative incidence of nephropathy reduced from 37.6% to 22.0% ($p < 0.001$) (Table 1). Neuropathy decreased insignificantly in subgroup-3 (those with the poorest glycemic control at baseline which suffered from severe neuropathy).

Conclusion: Despite strict GC, the retinopathy progressed in T2DM. The near normoglycemia improved most neuropathy outcomes. The HbA1c level close to 47.5 mmol/mol achieved mainly with insulin-sensitizing agents and lifestyle modification would be a safe glycemic goal for improving the outcome measures of DPN in patients with poorly controlled type 2 diabetes with mild to moderate neuropathy. The near-normoglycemia is effective for preventing the development of neuropathy and nephropathy, but not retinopathy.

Disclosure: **M. Tavakoli:** None.

954

Effects of methylcobalamin on diabetic peripheral neuropathy: a meta-analysis of randomised controlled trial

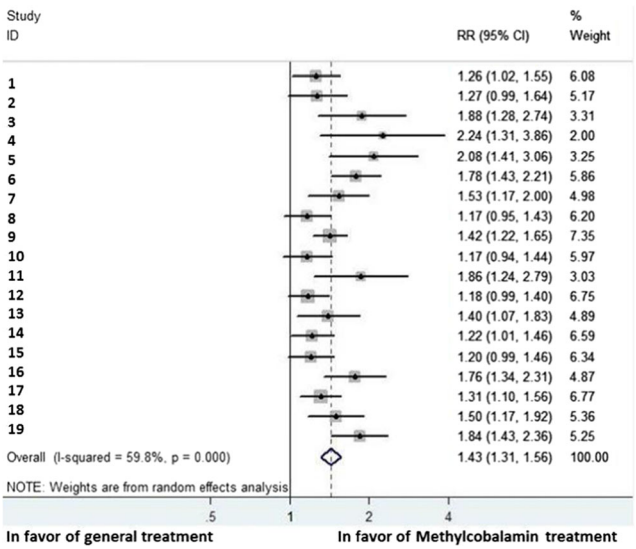
Q. Li[1], H. Wu[2];
[1]PLA Rocket Force General Hospital, Beijing, [2]Eisai China Inc., Shanghai, China.

Background and aims: Methylcobalamin has long been used for treatment of peripheral neuropathy. This study aimed to examine the effect of Methylcobalamin in the treatment of diabetic mellitus peripheral neuropathy (DPN) by performing a meta-analysis of all available relevant randomized controlled trials (RCTs).

Materials and methods: PubMed, Cochrane Library, Web of Science, Embase, and other databases were searched to retrieve articles published in English and Chinese up to February 28, 2018. RCTs evaluating effects of methylcobalamin on the treatment of DPN were included. Excluded from the analysis were studies without any type of control. Pooled relative risks (RRs) and 95% confidence intervals (CIs), and, standard mean difference (SMD) and 95% confidence intervals (CIs) were calculated with a random effect model to estimate the effect of methylcobalaminon on the treatment of DPN. Its effect on clinical signs and symptoms of DPN, conduction velocities of sensory and motor nerves were assessed where appropriate.

Results: 43 relevant RCTs with 3 619 patients with DPN were included in the meta-analyses. 1) In the pooled analysis with 19 RCTs wherein the control group receiving general treatment only, methylcobalamin showed significantly positive effects on the improvement of the signs and symptoms of DPN (RR: 1.43, 95%CI: 1.31–1.56, $P < 0.0001$). Compared to patients who were not treated with methycobalamin, patients treated with methylcobalamin showed greater increase of conduction velocities of Peroneal Nerve (sensory: SMD: 1.71, 95%CI: 1.28–2.15, $P < 0.0001$; motor: SMD: 1.09, 95%CI: 0.78–1.40, $P < 0.0001$) and Median Nerve (sensory: SMD: 1.01, 95%CI: 0.65–1.38, $P < 0.0001$; motor: SMD:1.13, 95%CI: 0.28–1.97, $P = 0.0090$). 2) In the pooled analysis with 24 RCTs wherein the control group receiving general treatment plus vitamin B agents, methylcobalamin showed significantly positive effects on the improvement of the signs and symptoms of DPN (RR: 1.87, 95%CI: 1.58–2.21, $P < 0.0001$). Compared to patients who were treated with vitamin B agents, patients treated with methylcobalamin showed greater increase of conduction velocities of Peroneal Nerve (sensory: SMD: 0.73, 95%CI: 0.42–1.04, $P < 0.0001$; motor: SMD: 0.64, 95%CI: 0.35–0.93, $P < 0.0001$) and Median Nerve (sensory: SMD: 0.73, 95%CI: 0.42–1.04, $P < 0.0001$; motor: SMD:0.75, 95%CI: 0.39–1.12, $P < 0.0001$).

Conclusion: Methylcobalamin appeared to be effective and better than vitamin B agents in treating DPN, and more rigorously designed, randomized, double-blinded, placebo controlled trials for DPN are needed.



Supported by: Eisai China Inc. Medical Support
Disclosure: **Q. Li:** None.

PS 086 Autonomic neuropathy

955

Effect of slow breathing and apnoea on arterial stiffness in type 2 diabetic and obese patients

P. Valensi[1], S. Chiheb[1], I. Banu[1], A. Rezki[1], E. Cosson[1], L. Bernardi[2], L. Bianchi[1];
[1]Jean Verdier Hospital, Bondy, France, [2]Folkhälsan Research Centre, Helsinki, Finland.

Background and aims: Several studies have shown that slow breathing (SLB) improves oxygen saturation (SaO_2) and the baro-chemoreflex interaction through the stimulation of parasympathetic nervous system. We recently reported that a short period of SLB can trigger the onset of apneas in patients with alterations of cardiorespiratory reflexes, particularly OSAS patients. The activation of sympathetic nervous system is a physiological response to apnea. The aim of this study was to evaluate the effects of SLB and apneas on arterial stiffness mediated by the autonomic nervous system.
Materials and methods: We included 66 patients (42 type 2 diabetic and 24 obese patients) who underwent the following protocol: spontaneous breathing (5 min), slow breathing at 6 cycles/min (5 min) and finally 10 minutes of spontaneous breathing (POST-SLB). Among them, 40 patients (26 diabetics, 53 ± 12 years, 14 men, BMI 36.6 ± 6.2 kg/m^2, HbA1c 6.9 ± 1.7%) developed apneas or hypopneas during the POST-SLB; 26 patients (16 diabetics, 53 ± 14 years, 11 men, BMI 33.8 ± 8.3 kg/m^2, HbA1c 7.3 ± 1.8%) did not develop respiratory abnormalities after SLB. We recorded heart rate (HR) and blood pressure (by Finapres®) continuously during the whole protocol. We calculated arterial stiffness (augmentation index, AIx and pulse wave velocity, PWV) with a software that reproduce the central aortic pressure waveform from the periphery (measured on finger) through a transfer function (validated from Sphygmocor ®).
Results: At baseline arterial stiffness was similar in both groups of patients: with apneas (APN+) and without apneas (APN-) (AIx 16.9 ± 9.2% *vs* 15.9 ± 7.4%, p = ns; PWV 8.3 ± 1.7 *vs* 7.9 ± 1.0 m/s, p = ns), as well as systolic (SBP 131 ± 22 *vs* 124 ± 19 mmHg, p = ns) and diastolic blood pressure (DBP 72 ± 15 *vs* 73 ± 12 mmHg). During SLB, all patients improved AIx (average −2.3% in APN+ and −2.0% in APN-, $p < 0.001$ *vs* baseline), PWV did not change (−0.2 m/s in APN+ and −0.1 m/s in APN-, p = ns *vs* baseline), SBP decreased (−8.8 mmHg in APN+ and −6.8 mmHg in APN-, $p < 0.001$ *vs* baseline) as well as DBP (−4.1 mmHg in APN+ and −3.2 mmHg in APN-, $p < 0.001$ *vs* baseline). During the POST-SLB, AIx increased in APN+ and became even higher than baseline values (+1.1%, $p < 0.05$) while in APN- AIx returned to baseline values (−0.1%, p = ns), PWV did not change in the two groups, SBP returned to baseline values in APN+ while it remained low in APN- (−6.7 mmHg, $p < 0.01$), DBP showed the same trend (in APN+, p = ns *vs* baseline; in APN- −2.6 mmHg, $p < 0.01$ *vs* baseline).
Conclusion: Slow breathing has shown the capacity to reduce blood pressure through the stimulation of parasympathetic nervous system and to influence the arterial stiffness favorably (AIx proner to acute changes than PWV). Conversely apneas induce sympathetic activation that increases blood pressure and worsens arterial stiffness. These important changes highlight the central role of the autonomic nervous system in the cardiovascular risk related to OSAS and suggest an acute benefit of slow breathing. The long-term outcomes of repeated sessions of SLB remain to be explored.
Disclosure: **P. Valensi:** None.

956

The impact of the interaction between obstructive sleep apnoea and cardiac autonomic neuropathy on eGFR decline in patients with type 2 diabetes: a longitudinal study

A. Tahrani[1], Q.-A. Altaf[2];
[1]Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, [2]Department of Diabetes and Endocrinology, University Hospital NHS Foundation Trust, Birmingham, UK.

Background and aims: Diabetes-related chronic kidney disease (CKD) is a leading cause of end-stage renal disease (ESRD). We have previously shown that both obstructive sleep apnoea (OSA) and cardiac autonomic neuropathy (CAN) are independently associated with renal function decline in patients with Type 2 diabetes (T2DM). We have also shown that OSA is associated with CAN in patients with T2DM. Hence, in this study we aimed to assess the impact of the interaction between OSA and CAN on renal function longitudinally.
Materials and methods: Patients with T2DM and no ESRD were recruited from a single tertiary diabetes centre in the UK 2009–2011. Renal function was assessed using MDRD-calculated estimated glomerular filtration rate (eGFR). Rapid eGFR decline was defined as 4% decline of eGFR/year. OSA was diagnosed if the apnoea hypopnea index (AHI) was ≥5 events/hour using overnight cardiorespiratory portable monitoring. CAN was assessed based on heart rate variability using ANSAR^{TM} technology. CAN was present if at least 2 of the standardized tests were abnormal. Patients were divided into 4 groups; Group 1: no OSA and no CAN (n = 45); Group 2: CAN with no OSA (n = 27); Group 3: OSA with no CAN (n = 67); and Group 4: OSA and CAN (n = 54).
Results: A total of 200 patients were included [mean age (57.4 (12) years, gender (male 57.5% (n = 115), diabetes duration 12.6 (7.7) years]. The mean follow-up duration was 2.5 (0.7) years. The OSA and CAN prevalence was 63% (n = 126) and 39.5% (n = 79) respectively. The mean baseline eGFR was lowest in patients with CAN and OSA (mean (SD); ml/min/1.73 m^2) [Group 1: 98.5 (22.2); Group 2: 87.7 (28.9); Group 3: 88.6 (25.9); Group 4: 76.7 (27); p = 0.001]. The eGFR decline (defined as % of baseline eGFR) was greater in patients with OSA and CAN [−1.9 (6.5) %; vs. −1.4 (11.7) %; vs. −3.2 (10.6) %; vs. −9.4 (13.2) % for group 1 to 4 respectively; p = 0.002). Rapid eGFR decline was more common in patients with OSA and CAN as compared with other groups [21.4% (n = 9) vs. 25% (n = 15) vs. 24% (n = 6) vs. 51% (n = 26) for groups 1 to 4 respectively, p = 0.005]. After adjusting for baseline eGFR, age, sex, ethnicity, diabetes duration, body mass index, mean arterial pressure, HbA1c, total cholesterol, triglycerides, insulin use, lipid lowering treatment, anti-hypertensive use, anti-platelets, oral anti diabetic agents, and smoking, having OSA and CAN predicted lower study-end eGFR (R^2 = 0.9; B = −7.2, p = 0.006), and greater drop in eGFR (R^2 = 0.13; B = −6.7, p = 0.01). After similar adjustments, OSA and CAN predicted rapid eGFR decline (OR = 3.38; 95% CI 1.08, 9.9; p = 0.04).
Conclusion: Detecting OSA and CAN in patients with T2DM identifies a high risk population for eGFR decline. Patients with T2DM and OSA and CAN are at increased risk of greater eGFR decline compared to those with either OSA only or CAN only or neither OSA nor CAN. Studies assessing the impact of OSA treatment on eGFR decline are ongoing.
Disclosure: **A. Tahrani:** None.

957

The effect of autonomic and sensory neuropathy on all-cause mortality: a retrospective cohort study

M.M. Svebis[1], V.J. Horváth[1], A.E. Körei[1], P. Fadgyas-Freyler[2], G. Korponai[2], T. Tänczer[1], B.A. Domján[1], P. Kempler[1], A.G. Tabák[1,3];
[1]1st Department of Internal Medicine, Semmelweis University, Budapest, Hungary, [2]Strategic Analysis Department, National Health Insurance Fund of Hungary, Budapest, Hungary, [3]Department of Epidemiology and Public Health, University College London, London, UK.

Background and aims: It is well-accepted that people with autonomic neuropathy have an increased risk of cardiovascular mortality. Much less is known in this respect about sensory neuropathy. Furthermore, there is a lack of information on the association between any neuropathy and all-

cause mortality. Thus our aim was to examine the effect of both autonomic and sensory neuropathy on all-cause mortality in a well-phenotyped cohort.

Materials and methods: Participants: patients living in the service area of the 1st Department of Medicine, Semmelweis University with detailed autonomic and sensory neuropathy assessments between 1997 and 2016 ($n = 1940$). Predictors: autonomic neuropathy (≥2 positive Ewing-tests), sensory neuropathy (≥1 abnormal result of one type of nerve fibre on both sides using Neurometer). Covariants: age, sex, type and duration of diabetes, comorbidities, medications, lifestyle factors. Outcome: all-cause mortality based on data from the National Health Insurance Fund of Hungary. Statistical analysis: Kaplan-Meier survival curves and Cox-regression models.

Results: Altogether $n = 1940$ patients had any neuropathy examinations. Full sensory assessment was available for $n = 1873$ cases (96.5%), autonomic neuropathy in $n = 1692$ cases (87.2%). Participants were 61.8 ± 12.0 years old at baseline, $n = 1311$ had diabetes (type 1 diabetes $n = 126$), 43.4% were male ($n = 813$). Autonomic neuropathy was found in $n = 492$ cases (29.0%), sensory neuropathy in $n = 673$ cases (35.9%), combination of both neuropathies in $n = 196$ cases (12.0%). During the 1–17-year follow-up, 788 participants died (40,6%). According to models adjusted for baseline age, sex, type and duration of diabetes, and comorbidities, patients with either autonomic or sensory neuropathy had an approximately 50% increased risk of mortality (hazard ratio [HR] 1.47, 95%CI: 1.25–1.74 and HR 1.55, 95%CI: 1.32–1.83, respectively). When both types of neuropathy were present together the risk more than doubled (HR 2.19, 95%CI: 1.74–2.77).

Conclusion: Our results confirm the association between autonomic neuropathy and all-cause mortality. Furthermore, they suggest that the presence of sensory neuropathy is at least as strongly related to all-cause mortality as autonomic neuropathy, and it is not only an important determinant of quality of life.

Clinical Trial Registration Number: SE TUKEB 36/2017

Disclosure: **M.M. Svebis:** None.

958

Effects of ivabradine, a selective I_{funny} channels blocker, on erectile function in streptozotocin-induced diabetic rats

S. Gur[1], D. Yilmaz-Oral[2], G. Koroglu[1], A. Onder[3], A. Gulpinar[3];
[1]Pharmacology, Ankara Universty Pharmacy Faculty, Ankara, [2]Pharmacology, Cukurova Universty Pharmacy Faculty, Adana, [3]Pharmacognosy, Ankara Universty Pharmacy Faculty, Ankara, Turkey.

Background and aims: High heart rate independent risk factor for the worsening of erectile dysfunction (ED), which is a major health issue in diabetic patients. The I_{funny} (I_f) or hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are involved in synaptic transmission and neuronal excitability under physiological conditions. Corlanor (ivabradine) is selective HCN-gated channel blocker in the sinoatrial node. This study is focused on the possible beneficial effect of intracavernosal injection of ivabradine, on ED in streptozotocin-induced diabetic rats.

Materials and methods: Adult Sprague-Dawley ($n = 20$) rats were equally divided into two groups: Control and diabetes, which was induced by single intraperitoneal injection of 45 mg/kg of streptozotocin. *In vivo* erectile responses were also repeated after intracavernosal injection of ivabradine (dose of 0.45 mg/kg) in anesthetized rats. Ivabradine relaxant responses were assessed in control rat corpus cavernosum (CC) strips after several inhibitors. The relaxant responses of CC strips were evaluated in the presence or absence of ivabradine (10 μM).

Results: Diabetic rats demonstrated significantly decreased ratio of intracavernosal pressure to mean arterial pressure (0.18 ± 0.02; $p < 0.001$) and total intracavernosal pressure (2058 ± 199 mmHg; $p < 0.01$) after were restored by intracavernosal administration of ivabradine (0.68 ± 0.05; 3691 ± 116 mmHg). Ivabradine causes a relaxant effect on rat CC tissues independent on the nitric oxide-cyclic guanosine monophosphate system, which may affect L-type Ca^{+2}, Ca^{+2}-activated K^+ and ATP-sensitive K^+ channels. No difference in the ivabradine-related relaxation response was observed between groups. In *in vitro* studies, the maximum nitrergic relaxation response to electrical field stimulation in diabetic CC (34.4 ± 2.8, $p < 0.001$) was enhanced after the presence of ivabradine (66.6 ± 3.4). Ivabradine increased acetylcholine (100 μM), sodium nitroprusside(10 nM) and sildenafil (1 μM)-induced relaxation in diabetic CC.

Conclusion: Our results firstly indicated that the beneficial effect of intracavernosal administration of ivabradine in the full recovery of erectile function and completely improved CC endothelial and neurogenic relaxation in diabetic rats. These data may support further clinical and preclinical studies using combinations of ivabradine with phosphodiesterase type 5 inhibitors for ED.

Disclosure: **S. Gur:** None.

959

Carotid baroreceptor magnetic activation increases heart rate variability in human, implications to treat type 2 diabetes

J. Gmitrov;
Krompachy Hospital, Agel SK inc., Krompachy, Slovakia.

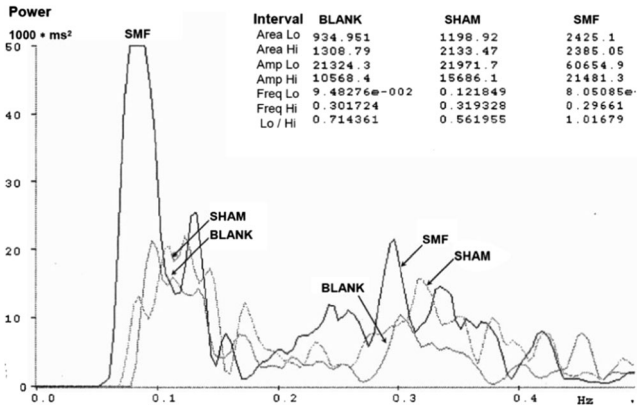
Background and aims: Increasing evidence suggests that positive feedback loop relationship exists between impaired autonomic function evidenced by reduced heart rate variability (HRV) and type 2 diabetes (T2DM). Lower HRV was found to increase risk of new onset T2DM which generates further decrease in HRV coupled with glucometabolic abnormalities, coronary heart disease and sudden cardiac death. This evokes emergent need to find a tool how to increase HRV, a marker of autonomic capacity to stabilize hemodynamic fluctuations and tissue perfusion finely adjusted to actual metabolic demands, severely impaired in T2DM. To test hypothesis that carotid baroreceptor (CB) magnetic stimulation has a modulatory effect on autonomic cardiovascular regulation, HRV was measured before and after CB exposure to static magnetic field (SMF).

Materials and methods: Spontaneous short-term HRV was measured in 16 supine healthy volunteers (mean age, 26 y) before and after CB local exposure to 120 mT intensity SMF generated by iron bar magnets, positioned with opposite poles over right and left carotid sinus area. For each patient three consecutive 300 cardiac intervals (5 min) were analyzed in blank control (BLANK), sham magnet (SHAM) and SMF exposure runs, using fast Fourier transform. HRV total power spectrum was divided into low (Lo) 0–150 mHz and high (Hi) 150–500 mHz frequency bands. The amplitude of the total power (ms^2), frequency-bounded area ($Hz \times ms^2$) and frequency corresponding to the peak of the power spectrum (mHz) were recorded. In addition, low and high frequency power spectrum ratio (Lo/Hi ratio) was calculated.

Results: In low frequency band, SMF induced increase of the power spectrum amplitude by 15.2%, area by 28.0% and Lo/Hi ratio by 20.6% accompanied by shift of the power spectrum peak to lower frequency region compared with SHAM magnet exposure: 102.1 ± 23.7 *vs.* 88.4 ± 14.7 mHz, $p < 0.05$. There were no significant changes of the power spectrum in high-frequency band. **Fig. 1. The frequency domain analysis of the HRV in healthy volunteer after CB magnetic activation.** SMF induced remarkable increase of the power spectrum area (Area) and amplitude (Amp) in low frequency band with maximal rise in 0.1-Hz frequency region which is strongly coupled with arterial baroreflex activation. The increase of the Lo/Hi ratio and the move of the power spectrum amplitude to lower frequency band suggests shift of the sympathovagal balance toward vagal predominance.

Conclusion: The important, novel finding demonstrated in this study is that SMF exerts stimulatory effect on CB in human reflected by increase in HRV. Each of SMF-enhanced HRV spectral components (Fig. 1) are depleted in T2DM even before clinical manifestation of the disease,

generating significant cardiovascular risk and a therapeutic opportunity to use CB stimulation techniques in T2DM where sympathetic overactivity, coupled with insulin resistance and endothelial nitric oxide deficit, triggers profound cardio-metabolic regulatory and structural damage, worsening cardiovascular outcomes substantially.



Disclosure: **J. Gmitrov:** None.

960

Saliva-derived extracellular vesicles carry distinct miRNAs in type 1 diabetic patients with altered cardiovascular tests

E. Favaro, T. Lopatina, C. Deregibus, C. Gai, M. Pomatto, S. Valerio, P. Passera, M. Porta, G. Camussi, M. Zanone;
University of Turin, Turin, Italy.

Background and aims: MicroRNAs (miRNAs), detected in body fluids, are becoming increasingly recognized as crucial regulators in gene expression both in physiological and pathologic processes, including diabetes and diabetic complications. Extracellular vesicles (EVs) released from healthy and diseased cells are potential biomarkers for ongoing pathological processes; they are recognised as an integral component of the cellular network, expressing surface receptors and carrying biologically active proteins, lipids, mRNAs, long non-coding RNAs and miRNAs, thus protected from plasma endogenous Rnases. miRNAs and miRNA gene polymorphisms have been recently described to be potentially associated with presence of, or susceptibility to, diabetic somatic and autonomic neuropathy. We aimed to characterize EVs and miRNA in the saliva obtained from type 1 diabetic patients and their correlation with neurological characteristics.
Materials and methods: Approximately 5–10 ml of saliva was obtained from 8 patients with type 1 diabetes of long duration (mean age 37.5 ± 2 , mean duration 29.4 ± 3, mean 1 year HbA1c 62.5 ± 7.6 mol/mol); neurological examination, autonomic CV tests and vibration perception threshold were assessed. EV were purified from saliva by charge-based precipitation. Briefly, the saliva samples were incubated with the protamine/polyethylene glycon precipitation solution (1 volume precipitation solution: 4 volume sample) overnight at 4°C. After centrifugation, the supernatant was discarded and the pellet was treated with different lysis buffer to study miRNA or protein expressions. miRNA expression levels were analysed using the Applied Biosystems TaqMan® Array Human MicroRNA A/B Cards (Applied Biosystems, Foster City, CA) to profile 754 mature miRNAs by qRT-PCR. The kit used gene-specific stem-loop reverse transcription primers and TaqMan probes to detect mature miRNA transcripts in a 2-step real-time reverse-transcription PCR assay. Western Blot analysis for neuronal markers was also performed.
Results: EVs were isolated and characterized by Nanosight instrument with a mean concentration of 5×10^8 particles/ml and with a mean size of 150 nm. Enolase and beta 3 tubulin, as as neuronal markers, were detected by Western blot analyses. In 2 patients with one altered CV test and in 1 patient with two altered CV tests, increased levels of miRNA146a, 146b, 29, 203 and 210 were detected.
Conclusion: EVs can be efficiently isolated from the saliva and may be exploited for the search of new biomarkers or pathogenetic mechanisms in diabetic complications. The miRNAs conveyed, with their involvement in inflammation and oxidative stress, suggest a role in diabetic autonomic dysfunction to be investigated.
Disclosure: **E. Favaro:** None.

961

The influence of clinically diagnosed neuropathy on respiratory muscle strength in type 2 diabetes

B.L.M. Van Eetvelde, D. Cambier, B. Celie, P. Calders;
Rehabilitation Sciences and Physiotherapy, University of Ghent, Ghent, Belgium.

Background and aims: Among all chronic disorders, Type 2 Diabetes Mellitus is the most common cause of peripheral and autonomic neuropathy (NP). The influence of NP on functional (dis)abilities is determined by the assessment of skeletal muscle strength and has been described extensively. This study investigated the influence of diabetes and diabetic neuropathy on respiratory muscle function.
Materials and methods: One hundred and ten diabetic patients and eighty controls, aged 60 years or more, were enrolled and allocated into 4 groups, depending on the presence or absence of neuropathy: patients with type 2 diabetes mellitus (T2DM) without NP (D-; $n = 28$), T2DM with NP (D+; $n = 82$), non-diabetic controls without NP (C-; $n = 35$), and non-diabetic controls with NP (C+; $n = 45$). Diabetes was diagnosed according to the Type 2 Diabetes ADA Diagnosis Criteria either by the specialist on the Department of Endocrinology or by the generalist of the participant. Neuropathy was examined based on clinical neurological examination, which consisted of Vibration Perception Threshold (VPT) and the Diabetic Neuropathy Symptom score (DNS). Respiratory muscle strength was registered by maximal static Inspiratory and Expiratory Pressure (PImax and PEmax), and Peak Expiratory Flow (PEF) measurements. Isometric handgrip strength and the Short Physical Performance Battery (SPPB) were used to capture peripheral skeletal muscle strength and physical performance. Univariate analysis of covariance (ANCOVA) was used with age, level of physical activity and BMI as covariates, to compare between groups and across the muscle strength conditions.
Results: Overall respiratory muscle strength (PI_{max}, PE_{max} and PEF; Table 1 - A) was higher in the controls without neuropathy (C-) compared to the other groups (C+, D- and D+). The presence of NP seemed to strongly affect the respiratory muscle strength (C+ versus C-) as well as the presence of T2DM (D- versus C-). The respiratory parameters in the diabetic patient group were less impacted when neuropathy was present (D+ versus D-). Significant differences were observed for SPPB total ($F = 4.588$; $p = 0.004$), Timed Chair Stands and gait (both subdomains of SPPB; respectively $F = 3.147$; $p = 0.027$ and $F = 7.505$; $p < 0.001$). For hand grip strength ($F = 2.396$; $p = 0.071$) and balance total (subdomain of SPPB, $F = 2.280$; $p = 0.083$) only a tendency was noticed between the four groups. SPPB total, and the subtests Timed Chair Stands and gait were affected by both presence of NP, T2DM and T2DM with NP in a similar way (Table 1 - B).

Conclusion: The presence of NP has an impact on respiratory muscle strength in T2DM patients as well as in non-diabetic controls. Therefore, it should be taken into consideration to integrate PI_{max}, PE_{max} and PEF measurements in the screening for respiratory muscle weakness as an indication for the presence of neuropathy.

Table 1: Univariate analysis of covariance (ANCOVA, corrected for age, BMI and physical activity) on respiratory muscle strength, and on peripheral muscle strength, balance and gait.

Panel A: Respiratory muscle strength

	F-value p-value	C-	C+	D-	D+
PI_{max} (cm H_2O)	F=3.509 p=0.017*	64,5 (28,83)	42,6 (28,56)	40,7 (25,22)	36,6 (23,71)*
PE_{max} (cm H_2O)	F=4.613 p=0.004*	100,6 (29,58)	69,3 (32,29)*	69,5 (29,97)	65,2 (31,20)*
PEF (l/min)	F=7.588 P<0.001*	471,2 (132,27)	357,9 (135,97)*	330,9 (152,07)*	314,5 (221,26)*

Panel B: Functional assessment (strength, balance and gait)

	F-value p-value	C-	C+	D-	D+
HGS_{max} (kg)	F=2.396 p=0.071	26,9 (12,36)	19,4 (9,56)	20,1 (10,15)	17,6 (9,50)
SPPB: total	F=4.588 p=0.004*	11 (4-12)	9 (2-12)	7 (1-12)*	6 (1-12)*
A. strength (TCS)	F=3.147 p=0.027*	3 (0-4)	1 (0-4)	1 (0-4)	0 (0-4)*
B. balance total	F=2.247 p=0.086	4 (3-4)	4 (0-4)	3,5 (0-4)	3 (0-4)
1. side-by-side	F=0.258 p=0.855	2 (2-2)	2 (0-2)	2 (0-2)	2 (0-2)
2. semitandem	F=0.650 p=0.584	2 (2-2)	2 (0-2)	2 (0-2)	2 (0-2)
3. tandem	F=2.558 p=0.058	2 (1-2)	2 (0-2)	1,5 (0-2)	1 (0-2)
C. gait	F=7.505 P<0.001*	4 (1-4)	4 (1-4)	2 (1-4)**	3 (1-4)**

C- = control group without NP. C+ = control group with NP. D- = T2DM without NP. D+ = T2DM with NP

PI_{max} = Maximum Inspiratory Pressure; PE_{max} = Maximum Expiratory Pressure; PEF = Peak Expiratory Flow
HGS = Hand Grip Strength; SPPB = Short Physical Performance Battery; TCS = Timed Chair Stands

All respiratory muscle parameters and HGS are expressed as mean (SD). All parameters of the SPPB are expressed as median (min-max).

* $p<0.05$ compared to C-
** $p<0.05$ compared to C+

Disclosure: **B.L.M. Van Eetvelde:** None.

PS 087 Foot ulcers: morbidity and mortality

962

Developing a foot ulcer risk algorithm: the reality of doing this in a real world primary care setting

G. Dunn[1], M. Lunt[2], M. Rutter[2], G. Moreno[3], M. Edmonds[4], E. Jude[5], A. Heald[2], A. Boulton[2];
[1]Department of Podiatry, East Cheshire Trust, Macclesfield, UK, [2]Academic Health Sciences Centre, University of Manchester, Manchester, UK, [3]Head of Medical Department, High Speciality Regional Hospital of Ixtapaluca, Mexico, Mexico, [4]Department of Diabetes, Kings College Hospital, London, London, UK, [5]Department of Diabetes, Tameside Hospital NHS Foundation Trust, Manchester, UK.

Background and aims: Foot ulceration is a major complication of type 1 and type 2 diabetes. The lifetime risk of foot ulceration in patients with diabetes is 25%. Foot ulceration is associated with significant morbidity and increased mortality. Our aim was to determine how data collected in the course of diabetes reviews of patients in UK primary care, can inform a risk algorithm to predict de novo foot ulcer presentation.
Materials and methods: We examined pseudo-anonymised electronic health records in a retrospective cohort of all men and women aged 16–89 years, attending 42 general practices (GPs) in Central and Eastern Cheshire, UK. The total population of the geographical area studied is 475,000 people. Data was available on 15,727 individuals without foot ulcers and 1,125 individuals with new foot ulcers over 12 year follow-up. Data search was performed through EMIS®. We examined all known risk factors (RFs) and added putative RFs in our model.
Results: People who developed foot ulcers were significantly older at baseline (mean age 77.9 ± (sd) 14.1 vs 73.8 ± 16.9) than those without, and had higher HbA1c% (mean 7.9 ± 1.9 vs 7.5 ± 1.7)/ HbA1C mmol/mol (62.8 ± 20.8/58.5 ± 18.5), creatinine (μmol/L) (99.9 ± 45.5 vs 93.0 ± 39.3) and social disadvantage a measured by Townsend Score (a higher score relates to greater social disadvantage) (−0.72 ± 2.84 vs −1.14 ± 2.70) ($p < 0.0001$ in all cases). Absence of monofilament was more common in cases (Left foot 21.5%; Right Foot 26.2% vs non-cases Left Foot 16.5%; Left Foot 18.8%) ($p < 0.0001$) as was absence of foot pulses ($p = 0.02$) (Table 1). There was no difference between cases and non-cases in smoking status, gender, history of stroke or foot deformity, although foot deformity was extremely rare (0.4% in cases, 0.6% in non-cases) (Table 1). Combining the 6 risk factors in a single logistic regression model gave modest predictive power, with an area under the ROC curve of 0.65. The absolute risk of ulceration in the bottom decile of risk was 1.8% and in the top decile 13.4% Thus the presence of all 6 risk factors gave a relative risk of 7.4 for development of a foot ulcer over time.
Conclusion: We have made progress in defining a usable algorithm for foot ulcer prediction. However more accurate determination of foot deformity and pedal circulation in the UK GP setting may improve the positive predictive value of the algorithm. It is clear that vascular dysfunction must be severe - much reduced in order to affect ulcer formation. However diminished sensation appears to be implicit in ulcer formation. Additional risk parameters will need to be identified to improve prediction to clinically useful levels to predict foot ulcers. We have made steps in the right direction.

Variable	Value	Non-cases N (%)	Cases N (%)	p-value for difference
Smoking Status	Never Smoked	3782 (38.55)	287 (39.81)	
	Not current	1257 (12.81)	87 (12.07)	
	Ex-smoker	3174 (32.35)	232 (32.18)	
	Current Smoker	1598 (16.29)	115 (15.95)	0.8917
History of Stroke	No	14676 (92.15)	1046 (92.81)	
	Yes	1250 (7.85)	81 (7.19)	0.4236
Gender	Female	7160 (45.51)	522 (46.32)	
	Male	8574 (54.49)	605 (53.68)	0.5974
Monofilament L Absent	No	13298 (83.50)	886 (78.62)	
	Yes	2628 (16.50)	241 (21.38)	0.0000
Monofilament R Absent	No	12936 (81.23)	832 (73.82)	
	Yes	2990 (18.77)	295 (26.18)	0.0000
Foot pulse L Absent	No	15297 (96.05)	1066 (94.59)	
	Yes	629 (3.95)	61 (5.41)	0.0160
Foot pulse R Absent	No	15296 (96.04)	1074 (95.30)	
	Yes	630 (3.96)	53 (4.70)	0.2165
Foot deformity (L)	No	15845 (99.49)	1125 (99.82)	
	Yes	81 (0.51)	2 (0.18)	0.1227
Foot deformity (L)	No	15846 (99.50)	1123 (99.65)	
	Yes	80 (0.50)	4 (0.35)	0.4946
Monofilament absent	0	12619 (79.24)	812 (72.05)	
on one or more feet	1	3307 (20.76)	315 (27.95)	0.0000
One or more foot	0	15162 (95.20)	1055 (93.61)	
pulses absent	1	764 (4.80)	72 (6.39)	0.0168
Deformity on one	0	15832 (99.41)	1123 (99.65)	
or more feet	1	94 (0.59)	4 (0.35)	0.3125
HbA1C above 9.5	No	11672 (89.17)	727 (83.28)	
	Yes	1418 (10.83)	146 (16.72)	0.0000
Creatinine above 150	No	9921 (96.12)	744 (93.23)	
	Yes	401 (3.88)	54 (6.77)	0.0001
Age above 55	No	2245 (14.27)	83 (7.36)	
	Yes	13489 (85.73)	1044 (92.64)	0.0000
Townsend score above 1	No	11935 (74.94)	798 (70.81)	
	Yes	3991 (25.06)	329 (29.19)	0.0020

Table 1: Differences between cases and non-cases for categorical variables

Disclosure: **G. Dunn:** None.

963

Uncensored incidence of diabetic foot ulcers to patients in remission

L. Lavery[1], J. Bloom[2], B. Petersen[2], G. Rothenberg[3];
[1]University of Texas Southwestern Medical Center; Department of Plastic Surgery, Dallas, [2]Podimetrics, Somerville, [3]University of Michigan School of Medicine, Ann Arbor, USA.

Background and aims: Diabetic foot ulcers (DFU) are known to be associated with increased morbidity, mortality, and resource utilization. Patients with history of DFU are among those at highest risk, with several prospective studies reporting annual incidence between 20% and 40% for those in remission. However, nearly all of these studies report DFU-free survival ("censored incidence") and right-censor outcomes by (1) disenrolling patients upon initial observation of DFU occurrence, and (2) characterizing multiple distinct DFU observed on the same date as a single occurrence. Reporting uncensored incidence, which includes all observed outcomes over the entire followup period, may more accurately reflect the true burden of DFU in high-risk populations. We hypothesized that the uncensored incidence is meaningfully larger than the censored incidence.
Materials and methods: A recent multi-center investigation recorded all DFU occurring in a cohort of 129 participants in remission from a DFU healing prior to enrollment. Participants were followed for 34 weeks or until withdrawing consent. We modeled DFU occurrences as a nonhomogeneous Poisson point process over time indexed from the participants becoming DFU-free prior to enrollment. From this, we estimated the instantaneous incidence through Savitzky-Golay smoothing of the counting process and numerical differentiation. We compared the time-dependent censored and uncensored incidence curves qualitatively, and assessed the aggregate difference qualitatively by testing censored and uncensored ratios of DFU/participant over the 34 week followup for significance.
Results: At least one DFU occurred to 37 participants, resulting in a censored ratio of 0.29 DFU/participant (37/129). The uncensored ratio was found to be 0.41 DFU/participant (53/129) given a total of 53 DFU during the study. The 16 additional DFU in the uncensored ratio occurred to eleven participants. Six of these participants presented with multiple DFU on the same date. Although the observed difference of 0.12 DFU/participant is not statistically-significant at the alpha = 0.05 level (p = 0.06), this study was not a priori powered to detect this difference. Both the censored and uncensored instantaneous incidence curves are unimodal with annualized peak rates of 0.42 DFU/participant/year and 0.71 DFU/participant/year during month four of remission. The censored and uncensored instantaneous incidences asymptote to the same baseline value of approximately 0.03 DFU/participant/year after month 18 of remission.
Conclusion: These data suggest a clinically-meaningful component of incidence that is underreported in the literature and potentially underappreciated by researchers and practitioners. Furthermore, it may be misleading to annualize DFU incidence by extrapolating the results from studies with followup shorter than one year because this approach may understate the true burden of DFU due to the strong temporal dependence of incidence during remission. Better characterizing DFU incidence for those in remission may enable improved allocation of resources, organization of care, and communication of prognosis, possibly resulting in reduced DFU-related morbidity, mortality, and resource utilization.
Clinical Trial Registration Number: NCT02647346
Disclosure: **L. Lavery:** None.

964

A risk prediction score for early-onset lower extremity arterial disease in Chinese type 2 diabetes

X. Zhang[1], X. Ran[2], Z. Xu[3], L. Ji[1,4];
[1]Endocrinology, Peking University International Hospital, Beijing, [2]Endocrinology, West China Hospital, Sichuan University, Chengdu, [3]Endocrinology, The 306th Hospital of PLA, Beijing, [4]Endocrinology and Metabolism, Peking University People's Hospital, Beijing, China.

Background and aims: Lower extremity arterial disease (LEAD) is highly prevalent in Chinese type 2 diabetes mellitus patients, but half of cases are underdiagnosed at incipient stage of onset because of diversified clinical presentations. Patients with early-onset LEAD are at increased risk of further cardiovascular comorbidities and mortality, but early initiation of secondary prevention is proved to improve prognosis. The present study was designed to develop a risk score for early-onset LEAD in diabetes patients, facilitating patient screening.
Materials and methods: 10,681 diabetes patients from the China DIA-LEAD Study, a multicenters, cross-sectional epidemiological investigation during June 2016 and January 2017, were selected as the training set to develop a weighted risk score associated with early-onset LEAD by multivariate regression. The risk score was confirmed by a validation dataset of 486 patients consecutively enrolled from a teaching hospital between Jul 2017 and Nov 2017. Receiver operator curves (ROC) with 95% CI were calculated to evaluate the discrimination capacity of the risk score. All patients were assessed for LEAD by ABI and/or lower limb ultrasonography according to 2013 Chinese guidelines on LEAD screening and management. The early-onset LEAD was defined as the ages at the date of first diagnosis <60 years.
Results: A total of 450 (4.2%) patients were diagnosed as early-onset LEAD in the training set. Of the candidate variables analyzed, factors (gender, body mass index, smoking, family history of premature cardiovascular disease, established coronary heart disease and skin test abnormality) correlated with early-onset LEAD in multivariate model resulted in weighted risk score of 0–20. The risk score discriminated patients with low risk (0–4, 0.8%), moderate risk (5–8, 3.0%), high risk (9–12, 7.4%) and very high risk (≥13, 12.2%) of early-onset LEAD, respectively. The prevalence of early-onset LEAD in different risk groups were 0.9%, 3.1%, 7.4% and 14.5% in the validation set. When the risk scores were analyzed by ROCs, a score of ≥13 was found to be optimal cut-off for

discriminating very high risk patients with the area under curve (aROC) of 0.82 (95%CI: 0.77–0.89), sensitivity of 0.79 (95%CI: 0.77–0.90) and specificity of 0.74 (95%CI: 0.69–0.88). Similar performance of aROC, sensitivity and specificity for different risk score cut-points (4, 8 and 12) were observed in the validation set.

Conclusion: The present study was the first attempt to develop an early-onset LEAD risk score system specific for type 2 diabetes mellitus patients based on a large population. The objective, weighted risk score for early-onset LEAD could reliably discriminate the occurrence of LEAD in patients younger than 60 years old, which may be helpful in a precise risk assessment, early diagnosis and treatment of LEAD. The continued refinement and multicenter validation of early-onset LEAD risk score with well-defined diagnostic procedure prospectively is required.

Disclosure: **X. Zhang:** None.

965

Incidence and clinical features of new onset diabetic foot ulcer post simultaneous pancreas-kidney transplantation

A. Sharma[1], S. Cohen[2], S. Thomas[3], T. Patel[4], J. Karalliedde[5], P. Vas[6];
[1]King's College London, London, [2]Renal unit, Guy's and St Thomas' NHS Trust, London, [3]Diabetes and Endocrinology, Guy's and St Thomas' NHS Trust, London, [4]Foot health team, Guy's and St Thomas' NHS Trust, London, [5]Cardiovascular Division, Faculty of Life Science & Medicine, King's College London, London, [6]Diabetes and Diabetes Foot Medicine, King's College Hospital, London, UK.

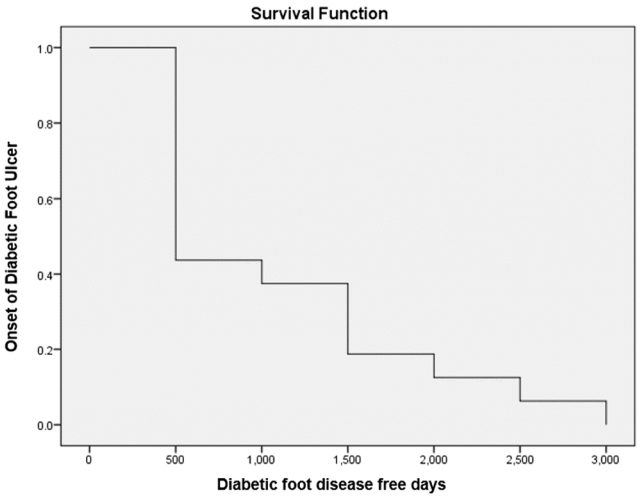
Background and aims: Patients with diabetes and renal dysfunction are at high risk of diabetic foot ulcers (DFU). Whether this risk is modified post simultaneous pancreas-kidney (SPK) transplantation is unknown with limited data on the incidence and predictors of new onset DFU in this population. We evaluated the incidence of new onset DFU post SPK in a single centre retrospective study. Patients who underwent SPK transplantation between 2004–2014 were evaluated.

Materials and methods: In total, 90 (51% male) patients were evaluated. Median (range) follow up was 6 (3 to 13) years. Median age was 49 (28 to 69) years and duration of diabetes was 32 (10 to 56) years. Electronic patient investigation records and podiatry medical notes were reviewed.

Results: Over the follow-up period, 16 (17%) patients developed a new DFU. Patients with a DFU were of similar age, duration of diabetes, and had similar pre-transplantation haemoglobin, HbA1c and estimated glomerular filtration rate as compared to those without a DFU. Patients who developed a DFU were more likely to have history of peripheral vascular disease (PVD) [37.5% vs. 4%, $p < 0.05$]. Of the cohort, 8 patients had a history of DFU pre-transplantation and all 8 developed a new onset DFU post SPK transplantation. Median (range) duration of healing was 7 (2–27) weeks. Site, Ischaemia, Neuropathy, Bacterial Infection, and Depth (SINBAD) classification score was <3 in 10 of the 16 DFU cases. Nearly 60% of DFU occurred within 500 days post-transplantation (Figure 1). Only 1 out of 16 needed a minor amputation. There was no significant difference in transplant failure between those with and without DFU (31% vs. 23.3%). When compared with UK national data - healing time, rates and severity of ulcer at presentation are significantly better in this cohort.

Conclusion: Nearly 1 in 6 patients developed a new DFU post SPK transplantation with greater than 50% of cases occurring within the first 500 days post-transplantation. A pre-transplantation history of PVD and DFU is associated with increased risk of new onset post-transplantation DFU. Our results highlight the need for greater awareness of regular foot evaluation post-transplantation and the burden and risks of DFU in this high-risk patient population.

Figure 1



Disclosure: **A. Sharma:** None.

966

Years of life lost due to diabetic foot complications during a 20-year follow-up

K. Ogurtsova[1], S. Morbach[2], G. Rümenapf[3], D. Ziegler[4], A. Icks[1,5];
[1]Institute for Health Services Research and Health Economics, German Diabetes Center, Düsseldorf, [2]Department of Diabetes and Angiology, Marienkrankenhaus, Soest, [3]Klinik für Gefäßchirurgie, Diakonissen-Stiftungs-Krankenhaus Speyer, Speyer, [4]Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, [5]Institute for Health Services Research and Health Economics, Faculty of Medicine, Heinrich-Heine-University, Düsseldorf, Germany.

Background and aims: Diabetic foot ulcers (DFUs) and consequent amputations, shorten patients' life. There is a lack of knowledge to which extent these conditions affect life expectancy (LE). The objection of this study is to estimate years of life lost (YLL) from DFUs and LE of patients with DFUs in a long-term follow-up study.

Materials and methods: 260 patients with new DFUs consecutively presenting to a single diabetes centre in Germany between June 1998 and December 1999 were included in this study and followed up until December 31st 2017 (mean age at baseline and death: 69.1 (SD: 10.8) 76.1 (SD 10.4) years, respectively), 59.2% male, 88.1% type 2 diabetes (T2DM). 13 (5.0%) patients had a first unilateral major amputation before study inclusion, 16 (10.0%) during the study period and 15 patients (5.8%) experienced bilateral major amputation. There are observations on 637 recurring ulcer episodes. The follow-ups were analysed by the Kaplan-Meier method to derive a cumulative hazard function. LE was estimated in the Cox-regression model with age, sex, smoking status (have ever smoked), type and duration of diabetes, as well as the presence of ischemic heart disease (IHD), peripheral arterial disease (PAD) and Charcot neuroosteoarthropathy at inclusion as covariates. Only death was considered as a final event; patients who dropped out due to other reasons were censored. YLL are calculated from the number of deaths multiplied by a standard LE at the age of death. The standard life tables were obtained from the Human Mortality Database (HMD) and matched with the sample by sex, age at deaths and calendar year when death occurred.

Results: 228 patients died and 19 were lost to follow-up. Of the 241 patients who were followed to death or are still under observation, 241, 233 and 229 have completed 18, 19 and 20 years of follow-up, respectively. At present 13 patients are alive and under observation. The Cox-

regression showed that the following baseline variables adjusted to other covariates are significantly associated with a probability of death: having any PAD (RR = 1.48, p value = 0.008), age (RR = 0.85 per year, p value <0.001). LE in a general German population over the follow-up period for men and women at age 65 was 80.2 and 84.0 years, accordingly. At the same time, the estimated LE for a counterpart at age 65 with DFUs and T2DM of 20 years' duration (a mean duration in the sample) ranges from 72.1 (CI = 70.76–77.62) years to 74.2 (CI = 73.60–77.62) years with all and without any complications included in the analysis for non-smokers while LE of smokers ranged from 70.5 (CI = 69.54 75.97) to 72.6 (CI = 71.89–74.82) years respectively, irrespective of sex. The YLL from DFUs and its complications was 10.0 (SD 6.3) years compared with the general population in Germany, 8.9 (SD 6.3) for female and 10.8 (SD 6.2) for male (t-test = −2.29, df = 212.82, p value = 0.023).

Conclusion: Our results confirm that diabetes in conjunction with DFUs and its related complications lie a great burden on patients by lessening their LE and long-term survival.

Disclosure: **K. Ogurtsova:** None.

967

Perioperative and long term mortality after lower limb amputation in patients with diabetes

M. López Valverde[1], J. Aragón Sánchez[2], V. Guerrero Cedeño[3], R. Tejedor Méndez[4], G. Víquez Molina[5];
[1]Endocrinology and Nutrition, Hospital San Pedro, Logroño, Spain, [2]General Surgery, Diabeti Foot Unit, Hospital La Paloma, Las Palmas de Gran Canaria, Spain, [3]Geriatry, Hospital San Jorge, Huesca, Spain, [4]General Medicine, Hospital San Jorge, Huesca, Spain, [5]Hospital San Juan de Dios, San José de Costa Rica, Costa Rica.

Background and aims: Long-term mortality of patients with diabetes who underwent a lower extremity amputation (LEA) has not been reported in Spain. The aim of the present research is to study in-hospital and long-term mortality in a retrospective cohort of patients with diabetes who underwent LEAs

Materials and methods: The retrospective cohort included all subjects who underwent LEA from January 1, 2005 to December 31, 2015 in San Jorge Hospital, Huesca, Spain. Perioperative mortality was defined as death within 30 days after the index procedure. Live status of every patient up to September 2017 and the date of the death were retrieved using the national death index from the civil registry of Huesca.

Results: The series included 203 patients who underwent LEA. One hundred sixteen patients (57.1%) underwent a minor amputation and 87 patients (42.9%) underwent a major amputation. Twenty-five patients (12.3%) died in the perioperative period. Significant risk factors of perioperative mortality were: undergoing an above-the-knee amputation (p = 0.04, OR 2.6, 95% CI 1.0–6.9), postoperative cardiac complications (p = 0.02, OR = 3.3, 95% CI 1.1–9.8), age >74 years old (p = 0.002OR 6.5, 95% CI1.9–22.1) and acute renal failure (p = 0.004, OR 17.8, 95% CI 2.5–124.1). Survival rates in patients who underwent a minor amputation at 1, 3 and 5 years were 90.6%, 72.8% and 55.5% compared with 70.8%, 41.3% and 34.4% in patients who underwent a major amputation. Log-rank test between the two groups was $\chi^2 = 12.7$ ($P < 0.01$). The Cox's proportional hazards model performed to analyse the association between the covariables and the mortality showed a significant hazard with: PAD (p = 0.04, Hazard ratio [HR] 1.6, 95% CI 1.0–2.7), major amputation (p = 0.01, HR 1.9, 95% CI 1.3–2.8), age >74 years (p < 0.01, HR 2.0, 95% CI 1.3–3.0) and previous amputation before the index amputation (p < 0.01, HR 4.1, 95% CI 2.4–6.9).

Conclusion: Long-term survival is worse in patients who underwent a major amputation with a five-years mortality of 65.6%. This mortality is worse than what has been reported for some types of common malignancies. Prevention and aggressive treatment of comorbidities should be implemented in order to reduce these poor outcomes.

Disclosure: **M. López Valverde:** None.

968

Presence, characterisation and clinical impact of anaemia in diabetic foot ulceration: a cross sectional study with longitudinal follow up of ulcer outcomes

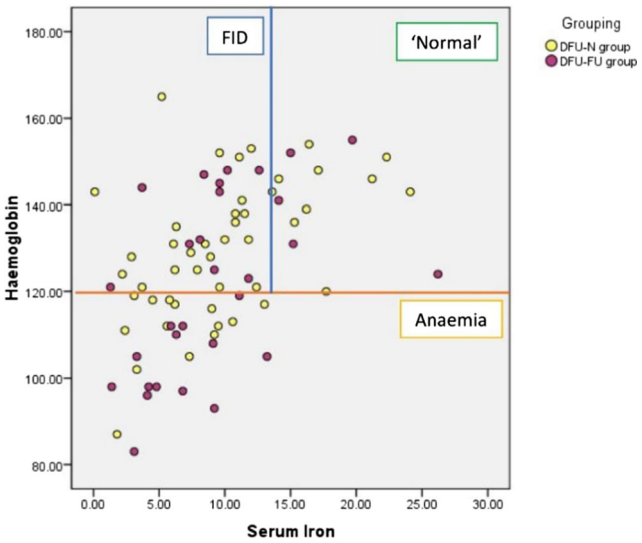
M. Anson[1], J. Karalliedde[1], I. Alejandro[2], M. Bates[2], C. Manu[2], M. Edmonds[2], I. Macdougall[2], P. Vas[2];
[1]King's College London, London, [2]King's College Hospital, London, UK.

Background and aims: Anaemia is a commonly understood to be associated with diabetic foot ulceration (DFU). However, the prevalence and the influence on DFU outcomes, is poorly understood and seldom researched. The aims of this study are to assess the prevalence of anaemia and functional iron deficiency (FID, low iron indices but normal haemoglobin) in patients attending a tertiary diabetic foot clinic, and to determine whether they are predictive of a poor DFU outcome.

Materials and methods: A cross sectional study with a prospective, observational intent was undertaken between November 2017 and February 2018. Patients were stratified into how they were assessed on visitation, into New (DFU-N, n = 48) or Follow up (DFU-FU, n = 31) groups. They were then subsequently classified into an anaemia, FID or normal subgroups. DFU was characterised using the SINBAD score. Those on iron/folate/vitamin B12 replacement and those receiving dialysis were excluded. The DFU-N cohort was followed up for a period of 6 weeks, and prognosis classified as favourable (healing or improvement in DFU size) or unfavourable (static, worsening DFU, amputation or death).

Results: There was no significant difference in age (66 ± 15 v 63 ± 12 years), gender (males 73% v 84%) HbA1C (8.3 ± 2.2 v 9.5 ± 2.9, p = 0.11) or DFU severity (p = 0.73) between the two groups. EGFR was lower in the DFU-FU group (72 ± 20 v 57 ± 21 ml/min, p = 0.007) and there was a trend for the DFU-FU to have a longer duration of diabetes (16 ± 11 v 22 ± 15 years, p = 0.053). Prevalence of anaemia and FID in the DFU-N group was 40% and 40% respectively; in the DFU-FU it was 55% and 32% respectively. For the whole cohort, haemoglobin value correlated with CRP (rho −0.282, p = 0.13), eGFR (0.282, p = 0.01) and serum albumin (0.393, p < 0.0001). Serum iron correlated with CRP (−0.591, p < 0.0001), White cell count (WCC) (−0.359, p = 0.001), albumin (0.233, p = 0.02). There was no correlation between serum iron and ferritin (0.31, p = 0.13), nor did it differ between the 2 groups (Z score −1.63, p = 0.1). None of the patients had low vitamin B12 or folate. In the follow up cohort, presence of anaemia and FID were both predictors of an unfavourable ulcer prognosis at 6 weeks with an unfavourable outcome in 82% of those with anaemia and 67% of FID compared to only 14% in those with normal haematinics (p < 0.05).

Conclusion: A very high prevalence of Anaemia and FID was noted in patients with DFU and to our surprise, there was little difference between DFU-N and DFU-FU groups. This was associated with poor DFU outcome, even at 6 weeks. Taken together, along with the association with CRP, WCC and albumin, our findings are suggestive that inflammation of any degree may initiate the pathway to anaemia development. Studies looking at larger, more diverse cohorts and settings with assessment of DFU outcomes over 12, 24 and 52-weeks are required to confirm our early findings.



Disclosure: **M. Anson:** None.

969

Non-neuronal control of proliferation and migration of keratinocytes on site of ulceration

E. Artemova[1], Z. Abdulvapova[1], E. Ivanov[2], A. Gorbacheva[1], S. Gavrilova[2], A. Tokmakova[1], G. Galstyan[1];
[1]Endocrinology Research Center, Moscow, [2]Lomonosov Moscow State University, Moscow, Russian Federation.

Background and aims: to assess proliferation and migration of keratinocytes at the nonhealing edges of neuropathic wounds, identify key enzymes for the synthesis of catecholamines in keratinocytes
Materials and methods: 25 patients (DM2 - 87.5%), with neuropathic ulcers (duration about 12 months) and 5 patients without diabetes with decubitus were enrolled. DF patients were underwent to standard treatment including debridement, atraumatic dressing, offloading with removable total contact cast, antibacterial therapy if it needs. Severity of peripheral neuropathy was assessed according to the NDS scales; was evaluated. Histological (hematoxylin and eosin) and immunohistochemical (Ki 67, α7nAChR, keratin K10, tyrosine hydroxylase) examination of wound edge were done during treatment (0, 10, 24 days).
Results: All patients have severe neuropathy according to NDSm (>8). The average size of DF ulcers before and on 10th day of treatment was of 5.56 cm^2 and 4.29 cm^2, respectively ($p < 0.004$). Neuropathic ulcers were characterized by hyperproliferative epidermis. Mitotically active keratinocytes reside throughout the suprabasal layers. Ki-67 expressed all layers of the epidermis, but a greater staining density was detected in the basal layer. The density of a7nAChR-positive cells increased from 0 to 24 days. Skin samples taken from patients on the 0th and 10th day of therapy were characterized by a low density for tyrosine hydroxylase, in contrast to samples taken on the 24th day. There was a low expression of K10 keratin differentiation markers before the beginning of therapy. There was formed a hyperproliferative epidermis, the cells of which lost the ability for terminal differentiation, the process of cornification was disturbed.
Conclusion: All layers of the epidermis of wound edge actively proliferated at conditionally separated stages of the wound process, which led to a pathological thickening of the epidermis, despite regular debridement. An increase in the expression of receptors for α7nAChR indicates a low migration potential of keratinocytes of the edge of neuropathic wounds. Due to the presence of severe neuropathy, the pathological pattern in the epidermis can be caused by a deficiency of nerve regulatory influences (the amount of mediator released by damaged nervous structures) and inadequate amounts of catecholamines synthesized in skin cells (skin cells expressed key enzymes of catecholamine synthesis), which led to the impaired migration and differentiation. The data obtained can serve as a basis for the development of local therapy for wound defects to enhance the migration of keratinocytes. It is necessary to search for signaling pathways that block excessive proliferation of epidermal cells that form a pathologically thickened epidermis.
Supported by: Russian Science Foundation № 16-15-13065
Disclosure: **E. Artemova:** Grants; The research was supported by Russian Science Foundation, project № 16-15-13065.

PS 088 Diabetic foot ulcers: How to prevent and how to treat

970

Arterial disease below the ankle in the diabetic foot: the final frontier

C.A. Manu, N.L. Petrova, P.J. Vas, K. Winkley, H. Rashid, M.E. Edmonds;
King's College Hospital, London, UK.

Background and aims: Recommendations from most guidelines suggests that peripheral arterial disease (PAD) is unlikely when Ankle Brachial Pressure Index (ABPI) is normal, that is between 0.9–1.3. The aim of this study was to evaluate the presence or absence of distal arterial disease below the ankle in limbs with normal ABPI between 0.9–1.3, as indicated by Toe Brachial Pressure Index (TBPI), Transcutaneous Oxygen ($TcPO_2$) and the associated clinical impact.
Materials and methods: The ABPI, TBPI and forefoot $TcPO_2$ were measured in both limbs of consecutive patients attending our outpatient clinic with diabetic foot ulceration. We used TBPI and forefoot $TcPO_2$ to diagnose the presence of arterial disease below the ankle, compared to measurements of ABPI per limb. We also assessed clinical outcome on a patient level, with regards to subsequent amputation and mortality.
Results: Measurements were taken in 154 patients, of which there were 121 limbs with a presumed absence of PAD as indicated by ABPI between 0.9–1.3. Within these limbs with normal ABPI range, 57% (69 limbs) had a low TBPI of <0.7, indicative of distal disease below the ankle (Group 1). The remaining 52 limbs (Group 2), had both ABPI and TBPI in the normal range. Absolute ankle pressures were similar in both groups, 159 ± 32 mmHg vs 159 ± 25 mmHg in Group 1 and Group 2 respectively, [$p = 0.478$]. However, the forefoot $TcPO_2$ was significantly lower in Group 1, 48 ± 15 mmHg vs 54 ± 12 mmHg in Group 2, [$p = 0.010$], as was their absolute toe pressure, 72 ± 21 mmHg vs 112 ± 19 mmHg respectively, [$p = 0.001$]. There were 43 patients in Group 1 and 21 patients in Group 2. More patients in Group 1 underwent minor amputation over the subsequent year; 26% vs 5%, [$p = 0.0455$]. Over the subsequent 18 months 2/43(5%) in Group 1 underwent a major amputation but none in Group 2. There was also a higher 2 year mortality in Group 1 patients, 14% vs 5% mortality in Group 2, but did not meet statistical significance, [$p = 0.267$].
Conclusion: A normal ABPI does not exclude PAD below the ankle in patients with diabetes. Over 50% of patients with normal ABPI between 0.9–1.3, have distal arterial disease in the foot which is associated with significant morbidity and mortality.
Supported by: King's College Hospital Charity
Disclosure: **C.A. Manu:** None.

971

Post-exercise transcutaneous tissue oxygen tension in the detection of latent peripheral arterial disease in patients with diabetic foot

V. Fejfarova[1], J. Matuška[2], P. Piťhová[3], M. Flekač[4], J. Venerová[5], K. Roztočil[6], V. Wosková[1], M. Dubský[1], R. Bém[1], A. Němcová[1], A. Jirkovská[1], V. Lánská[1];
[1]Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague, [2]MATMED, Vascular ambulance, Hodonín, [3]2nd Faculty of Medicine, Teaching Hospital Motol, Prague, [4]1st Faculty of Medicine, Charles University, Prague, [5]Military University, Prague, [6]Department of Transplant Surgery, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Background and aims: Assessment of arterial supply is one of the key goals in diabetic foot (DF) management. In addition to assessment of macrocirculation by ankle-brachial (ABI) or toe-brachial (TBI) indexes, the status of microcirculation is also controlled (usually by transcutaneous tissue oxygen tension, TcPO2). However, all diagnostic procedures have their own limitations in relationship to diabetes mellitus. The aim of our study was to refine TcPO2 measurement using astimulation test to enhance the detection ofa latent form of peripheral arterial disease (PAD) or arterial restenosis in previously diagnosed PAD.
Materials and methods: We initiated a multicentre trial with 26 patients with or without PAD (17/9 patients) who were treated in out patient foot clinics for DF (mean age 66.5 ± 12.8 years, diabetes duration 20.9 ± 10.1 years, HbA1c 60.4 ± 14.8 mmol/mol), underwent evaluation of macrocirculation (ankle/toe pressures, ABI, TBI, duplex ultrasound) and had baseline TcPO2 values between 30 and 50 mmHg. Each patient had TcPO2 measurement supplemented by a modified Ratschow stress physical activity test (lower limb elevation and rhythmic exercise for 2 minutes). TcPO2 values before, during (the lowest values) and after stress test and their changes (Δ = baseline TcPO2 minus the lowest TcPO2 during stress test) were evaluated in all study subjects with a total of 38 angiosomes. Patients and their limbs were divided into two groups based on duplex ultrasound findings in relevant evaluated angiosome - group A ($n = 13$) with triphasic flow, and group B ($n = 25$) with monophasic flow or proved arterial obliteration. These groups were compared in terms of all examined TcPO2 values and their possible correlations with macrocirculation findings.
Results: The stimulation test lead to a significant decrease in TcPO2 values in group A (42.8 ± 5.2 before vs. 34.4 ± 5.4 mmHg during test; $p < 0.001$), this decline was more expressed in group B (40.9 ± 5.9 vs. 25.8 ± 11.5; $p < 0.001$). Both study groups differed significantly in Δ TcPO2 (8.4 ± 2.4 in group A vs. 15.1 ± 9.3 mm Hg in group B; $p = 0.003$).These differences were most marked especially in those patients with a TcPO2 range of 30–40 mmHg (the lowest TcPO2 during the stimulation test 29.6 ± 3.6 mm Hg in group A vs. 16.7 ± 9.8 in group B; $p = 0.004$ and Δ TcPO2 7.8 ± 3.5 vs. 17.7 ± 9.5; $p = 0.02$). The lowest TcPO2 values during the stress test correlated positively with ABI ($p = 0.03$) and also with toe pressures ($p = 0.01$).
Conclusion: Stress physical activity test supplementing TcPO2 measurement used in daily podiatric practice could improve with a combination of ABI and TBI the detection of latent forms of PAD or restenosis in previously diagnosed PAD in patients with DF where the clinical signs of PAD are not always fully expressed.
Supported by: the project 00023001 (IKEM, Prague, Czech Republic)
Disclosure: **V. Fejfarova:** None.

972

Neurovascular response to pressure: a new potential predictive marker of diabetic foot ulcer

J. Vouillarmet[1], A. Josset-Lamaugarny[2], J. Saumet[2], P. Michon[1], P. Abraham[3], B. Fromy[4], D. Sigaudo-Roussel[2];
[1]Centre hospitalier Lyon sud, Pierre Benite, [2]UMR CNRS 5305, Lyon, [3]CHU d'Angers, Angers, [4]UMR CNRS 5305, Pierre Benite, France.

Background and aims: We previously described a specific cutaneous skin blood flow in response to an increase non-noxious progressive local pressure. This neurovascular reactivity permits an increase local blood flow that contribute to limit skin ischemia and by the way ulceration. This neurovascular response is impaired in patients with type 1 and type 2 diabetes but no data were available in patients with diabetic foot ulcer (DFU). We analysed, in this study, skin blood flow response of locally applied pressure in patients with DFU and in patients without DFU.
Materials and methods: Patients were recruited in a single diabetic centre. All patients had a complete record of diabetes history and foot problem. Neuropathy was assessed using the neuropathy sensitivity score (NSS), the neuropathy disability score (NDS) and by sensory tests. Basal blood flow, endothelium-dependent and endothelium-independent vasodilatations, maximal vasodilatation capacity by local heating to 44°C and skin blood flow in response to locally applied pressure were measured. All measurement were realised on the same tibia. Vasodilator responses were expressed as the maximal percent increase in cutaneous blood flow

from the baseline. Data were compared to ten healthy age-matched control subjects previously recruited (ID: NCT00160927).

Results: A total of 59 patients with type 2 diabetes were included; 29 without DFU and 30 with DFU. Patients were predominantly men (78%) with a mean age of 65 ± 11 years. Patients with DFU have a significant higher NDS score than patients without DFU (6.0 ± 0.5 vs 2.8 ± 0.6) but no difference between the two groups for NSS score (3.4 ± 0.5 vs 4.2 ± 0.5), and for cutaneous pressure perception threshold (CPPT) before (2.9 ± 0.1 vs 3.3 ± 0.2 g) and after local application of lidocaïne (4.0 ± 0.1 vs 4.2 ± 0.2 g). Basal Skin blood flow was significantly reduced in both diabetic groups compared to healthy subjects but with no difference in skin blood flow after local heating, Ach and SNP stimulation. By contrast, skin blood flow in response to pressure was significantly impaired in both diabetic groups compared to healthy subjects. The vasodilator capacity to pressure was significantly more altered in patients with DFU compared to those without DFU and lidocaine did not further decrease the vasodilation capacity to pressure in DFU group whereas it was reduced in patients without DFU.

Conclusion: This study revealed an incremental defect in skin blood flow response to pressure locally applied in patients with type 2 diabetes with a significantly more severe impairment in those with DFU. This effect occurs despite the absence of difference of the CPPT and of vascular reactivity to Ach and SNP suggesting a specific pathway.

Clinical Trial Registration Number: NTC01963559

Supported by: a grant from ARC2 Région Rhône-Alpes and CNRS

Disclosure: **J. Vouillarmet:** None.

973

Efficacy of long-term remote ischaemic conditioning on vascular and neuronal function in type 2 diabetes patients with peripheral arterial disease

C. Hansen[1], M.E. Jørgense[2,3], J. Fleischer[4], H. Bøtker[5], P. Rossing[1,6];
[1]Complications research, Steno Diabetes Center Copenhagen, Gentofte, [2]Clinical Epidemiology, Steno Diabetes Center Copenhagen, Gentofte, [3]National Institute of Public Health, Southern Denmark University, Copenhagen, [4]Clinical Institute of Medicine, Århus, [5]Complications research, Department of Cardiology, Århus, [6]Department of Clinical Medicine, Faculty of Health, Aarhus University AND University of Copenhagen, Aarhus, Copenhagen, Denmark.

Background and aims: Peripheral arterial disease (PAD) is a major challenge in the diabetes community, causing foot ulcers and amputations and often associated with neuropathy. When risk factor management fails to prevent PAD, only surgical intervention is offered to patients. New treatment options are therefore needed. Remote ischemic conditioning (RIC) has shown to provide cardioprotection in ischemic heart disease and improve vascular function. We aimed to investigate the efficacy of RIC on vascular and neuronal functions in individuals with type 2 diabetes and PAD.

Materials and methods: Individuals with type 2 diabetes and moderately reduced toe pressure (40 mmHg to 70 mmHg) were enrolled in a randomized placebo-controlled double blinded trial at a tertiary diabetes outpatient clinic. Patients were equally allocated to twelve weeks once-daily upper arm cuff-based treatment of either RIC treatment (4 cycles of 5 minute ischemia followed by 5 minute reperfusion) or similar treatment with a sham device with no ischemia. Primary outcome was transcutaneous tissue oxygen tension ($TcPO_2$) of the instep of the feet. Secondary outcomes were aortic pulse wave velocity, toe pressure and toe-brachial index and tertiary outcomes were markers of peripheral and autonomic nerve function: Sural nerve conductance velocity, Sural nerve action potentials, vibration perception threshold, electrochemical skin conductance and cardiovascular autonomic neuropathy indices.

Results: We enrolled 36 patients in the trial, 83% male. Participants had a mean (SD) age of 70.7 years (6.8), diabetes duration of 18.4 years (8.3), HbA_{1c} of 59.7 mmol/mol (11.2), toe pressure of 61.3 mmHg (15.1) and $TcPO_2$ average of both feet of 51.3 mmHg (10.7). Eighty percent had peripheral symmetrical neuropathy. Between group difference in change in $TcPO_2$ from baseline was −0.03 mmHg (95%CI −0.1; 0.04). *P* for group difference = 0.438. RIC did not elicit any change in secondary (Table 1) or tertiary outcome variables (not shown). Participants in the RIC group and placebo group applied treatment in 92.1% (SD 13.8) and 82.1% (SD 18.1) of possible days, respectively. Three participants experienced transient petechiae (micro bleeds) in the skin distal from the cuff. Treatment elicited no permanent adverse effects.

Conclusion: Repeated remote ischemic conditioning treatment may have no effect on tissue oxygenation, vascular or neuronal function in individuals with type 2 diabetes and moderate PAD despite excellent compliance to treatment during twelve weeks.

Table 1

	Randomization			Week 12		
	RIC	Placebo	p-value for group difference at baseline	RIC	Placebo	Difference in change from baseline, RIC vs. Placebo
Primary outcome						
Transcutaneous oxygen tension, mean feet (mmHg)	50.8 (9.9)	51.8 (11.8)	0.763	50.1 (10.0)	50.8 (9.0)	-0.03 (-0.1; 0.04) [0.438]
Secondary outcomes						
Pulse wave velocity (m/s)	2.62 (2.41;2.75)	2.54 (2.5;2.74)	0.487	2.67 (2.49;2.84)	2.56 (2.47;2.72)	0.07% (-0.08; 0.22) [0.385]
Toe pressure, mean feet (mmHg)	61.4 (15.7)	61.2 (15.0)	0.967	73.3 (14.8)	75.7 (15.3)	-0.04 (-0.2; 0.11) [0.603]
Toe brachial index, mean of both sides	-0.89 (-1.16;-0.74)	-1.02 (-1.12;-0.79)	0.684	-0.68 (-0.81;-0.62)	-0.71 (-0.83;-0.43)	-0.06% (-0.28; 0.17)[0.627]

Data are means (SD) or medians (IQR). Estimates of treatment effect are in % or absolute values (95% CI) [P values for group difference]. Models have been adjusted for baseline values of the given outcome.

Clinical Trial Registration Number: ClinicalTrials.gov No NCT02749942

Supported by: The project was funded by unrestricted research grant from The Augustinus F

Disclosure: **C. Hansen:** None.

974

The efficacy of sucrose-octasulphate dressing in neuro-ischaemic DFU considering factors influencing wound closure rate: a post-hoc analysis of the Explorer RCT

G. Rayman[1], M. Edmonds[2], R. Lobmann[3], J. Lázaro-Martínez[4], J. Martini[5], J.-M. Petit[6], A. Piaggesi[7];
[1]The Diabetes Centre, Ipswich, UK, [2]Diabetic Foot Clinic, King's College Hospital, London, London, UK, [3]Department of Endocrinology, Diabetology and Geriatrics, Klinikum Stuttgart – Bad Cannstatt Hospital, Stuttgart, Germany, [4]Diabetic Foot Unit, Complutense University of Madrid, Madrid, Spain, [5]Department of Endocrinology, Rangueil University Hospital, Toulouse, Guadeloupe, [6]Department of Endocrinology, Diabetology and Metabolism, François Mitterrand University Hospital, Dijon, France, [7]Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy.

Background and aims: According to most recent guidelines, no treatment added to optimal Standard of care (SOC) including efficient offloading has shown clear benefit in the management of diabetic foot ulcer (DFU). Efficacy of a sucrose octa-sulfate wound dressing (TLC-NOSF dressing) versus a neutral dressing (TLC) in addition to the same standard of care, in patients presenting with a neuro-ischaemic DFU was assessed in a European RCT.

Materials and methods: This double-blind RCT was conducted in 43 centres in patients presenting with a non-infected neuro-ischaemic DFU (grade IC/IIC, Texas Classification), and a surface area >1 cm^2. The primary outcome was the wound closure rate by week 20 in the ITT population (binary logistic analysis). Secondary outcomes included time to closure and adverse events occurrence (infection, notably).

Results: A total of 240 patients were randomised and received either the treatment dressing (n = 124) or the control dressing (n = 114). At Week 20, wound closure occurred in 34 patients (30%) in the control group and in 60 patients (48%) in the treatment group (adjusted odds ratio 2·60 [95% CI 1·43 to 4·73], p = 0·002). Post-hoc analysis were undertaken, considering parameters that may influence the tissue repair process (wound duration, wound area, wound location, vascular status, patients'

characteristics···), always showing favourable outcomes for the sucrose-octasulfate dressing, whatever the characteristics of the treated wound.
Conclusion: Sucrose octasulfate dressing and good standard of care is significantly more effective than neutral dressing, in the management of neuro-ischaemic DFUs, and specifically when treatment is initiated early in the wound evolution.
Clinical Trial Registration Number: NCT01717183
Disclosure: **G. Rayman:** None.

975

Real life experience of VACOped boots in the management of diabetic foot ulcers

W.T. Lim[1], H. Robinson[2], S. Rajbhandari[2];
[1]Manchester Medical School, Manchester, [2]Lancashire Teaching Hospital, Chorley, UK.

Background and aims: Offloading with Total contact cast (TCC) is the gold standard treatment for diabetic foot ulcers (DFU) and is used as standard off-loading method in our clinic. However its use can be limited due to underlying conditions such as infection or ischaemia or patients' reluctance. We use VACOped boot as a removable walker in such cases. The VACOped has an outer light weight shell and the inner lining. The inner lining is the cushion filled with thousands of styrofoam pearls that surrounds the foot. When air is extracted from the cushion with a pump, the body shaped cushion becomes hard as a cast just in a few seconds. This avoids pressure to the DFU and provides stability due to a perfectly shaped orthosis. This aim of this study was to analyse the results of VACOped boots in the healing of DFU in real life scenario.
Materials and methods: In this retrospective study we analysed records of all subjects supplied with VACOped boot from 2011 to 2017 for the treatment of DFU. The outcome of their ulcers was noted from clinical records. If patients stopped using VACOped for any reason their outcome was grouped with those stopped using it. If they changed from other off-loading device to VACOped it was analysed as the outcome of this device.
Results: VACOped were supplied to 42 patients (35 males) to treat 83 episodes of DFU during this period. The mean age was 56.7 (±11.2) years and duration of diabetes was 18.9 (±13.5) years. Of the 83 episodes of ulcers 41 (49.4%) healed in the median duration of 17 weeks with the use of VACOped. 13 (15.6%) are still continuing treatment and 29 (34.9%) stopped using it. The reason for stopping its use was the change to other device ($n = 12$), infection ($n = 3$), amputation ($n = 3$), lost to FU ($n = 7$) and other reason ($n = 4$). Out of 83 DFU episodes, 8 episodes were those who changed from TCC to VACOped. Similarly in 10 ulcer episodes, VACOped was changed to TCC.
Conclusion: The healing time with VACOped was longer than TCC but these included patients with underlying infection and ischaemia, in whom TCC is relatively contraindicated. Our data shows that VACOPed is preferred by patients, can be reused when patient has re-ulceration and is as effective as other removable cast walkers.
Disclosure: **W.T. Lim:** None.

976

Severe diabetic foot infection and osteomyelitis can be successfully treated with outpatient parenteral antimicrobial therapy

S. Rajbhandari[1], A. Muir[2], J. Purcell[3], J. Orton[4];
[1]Lancashire Teaching Hospital, Chorley, UK, [2]Microbiology Dept, Lancashire Teaching Hospital, Preston, [3]Microbiology Dept, Lancashire Teaching Hospital, Chorley, [4]Lancashire Teaching Hospital, Preston, UK.

Background and aims: Administration of intravenous antimicrobials to a patient with severe diabetic foot infection can be done by outpatient parenteral antimicrobial therapy (OPAT) either at home or at day wards without the need for an overnight stay in hospital. Our microbiology department started OPAT service in 2012 and accepts referrals from hospital inpatients to facilitate early discharges and also directly from outpatient clinics to avoid admissions. Diabetes foot team has been using OPAT for severe limb threatening diabetic foot infections since it started. The aim of this study was to assess the clinical outcome of OPAT services and calculate the savings made by avoiding hospital admissions.
Materials and methods: List of subjects accepted by OPAT service over a period last 5 years from diabetes foot team was obtained from database. 49 patients (8 males) with mean age of 60.4 (±12.4) years had 57 episodes of severe diabetic foot infections and their medical records were analysed retrospectively. All patients had appropriate offloading, revascularization and regular debridement as per clinical practice. Choice of parental antibiotics was guided by allergy history and culture results. Outcome of foot ulcer was obtained from clinical record and confirmed with photographic record.
Results: 32 episodes were stepped down from hospital admission and 25 were direct referral from diabetic foot clinic. The site of infection was MTP area in 17 cases, great toe in 13, other toes in 9, hind foot in 6, rocker bottom deformity in 5, dorsum of foot in 2 and residual infection of amputation site in 5 cases. Underlying osteomyelitis was present in 47 cases and remaining 10 cases had cellulites. Cetriaxone 2 gm IV daily was given in 38 cases and Teicoplanin 10 mg/kg IV daily in 19 cases along with metronidazole for anaerobic cover. The mean duration of OPAT was 20.7 (±17.9) days and it was followed by oral antibiotics as needed. Most patients had PICC sited for central venous access and were reviewed twice a week by infection specialist and weekly at Diabetic foot clinic. Complete healing of ulcer was obtained in 42 (84%) episodes. 6 (12%) ended with amputations and 2 (4%) died. 7 cases, all of which have underlying osteomyelitis are still undergoing treatment with parental or oral antibiotics. Regarding osteomyelitis, 82.9% healed with OPAT and oral antibiotics. One patient with CKD 4 needed hospital admission due to worsening of renal function as serious adverse event. Two had minor adverse event in the form of rash. There were no cases of septicaemia or c. diff infection.
Conclusion: Our findings show that severe diabetic foot infections including osteomyelitis can be successfully treated with OPAT. It is safe, well-liked by patients and in the last 5 years OPAT service spared 1138 hospital bed days from severe diabetic foot infection treatment which saved £273120 (Euro 310, 363).
Disclosure: **S. Rajbhandari:** None.

977

Bedside Blind Bone Biopsy (B4) for suspected diabetic foot osteitis: A reliable tool to manage medical treatment?

F. Féron, D.-C. Gauthier, M. Laloi-Michelin, A.-L. Munier, L. Salle-Teyssières, G. Pean-de-Ponfilly, E. Lecorche, F. Mougari, H. Jacquier, P.-O. Sellier, J.-D. Laredo, J.-P. Riveline, J.-F. Gautier, J.-P. Kevorkian;
Hôpital Lariboisière, Paris, France.

Background and aims: Performed by a diabetologist, B4 has been recently shown as an easy and safe procedure. To assess Exclusive Medical Treatment (EMT: offloading, wound care ± antibiotherapy) determined by B4 in suspected Diabetic Foot Ulcer (DFU) osteitis, we compared DFU outcome between proved (B4+) and ruled out osteitis (B4-) with microbial culture.
Materials and methods: A prospective observational study was conducted in our Diabetology Unit during a 21-month period (12.17.2015–09.04.2017). Among 291 patients admitted with DFU, we included 33 (11%) consecutive patients with Type 2 Diabetes Mellitus (T2DM) and clinical and/or radiological suspicion of osteitis. For each, B4 was decided by our DFU multidisciplinary board. B4 was performed ≥2 weeks after antibiotics discontinuation in case of prior exposure. A 6-week targeted antibiotherapy was indicated for proven osteitis by prolonged culture (B4+). In case of negative cultures (B4-), no antibiotherapy was

prescribed.The primary outcome was the complete DFU healing and no recurrence with EMT at 6 months.
Results: Patient characteristics and results are summarized in table 1. Mean follow-up after B4: 14 ± 6 months. Around 50% of suspected osteitis was B4-, which prevented from unneeded antibiotics without poorer healing outcomes.
Conclusion: B4 might be a reliable tool for managing EMT in suspected DFU osteitis.

	Total (n=33)	B4+ (n=16, 48,5%)	B4- (n=17, 51,5%)	p
Sex (% men)	76	81	71	0,69
Age (years, sd)	70±13	74±9	67±14,5	0,11
T2DM duration (years, sd)	18±11	19±8,5	17±12	0,27
HbA1C (%, sd)	8±1,8	7,3±1,4	8,5±2	0,08
IDSA Scale = 4 (n, %)	13 (39)	6 (37,5)	7 (41)	0,99
Severe PAD (n, %)	8 (24)	6 (38)	2 (12)	0,04
Complete healing EMT (n, %)	21 (63)	10 (63)	11 (64)	0,69
Mean duration between B4 and complete healing with EMT (days, sd)	102±75	122±78	84±72	0,66

Table 1: Patients characteristics. *p* levels from Chi-2 or Mann-Whitney U tests. IDSA= Infectious Diseases Society of America; PAD= Peripheral Arterial Disease

Disclosure: **F. Féron:** None.

978

Ten years outcome of diabetic foot ulcer with osteomyelitis in Egypt
H. Gawish, F. Kyrillos, A. Albehairy, M. Elsayed, M. Sherif, M. El-Nahas, O. State, M. Tarshoby;
Diabetes and Endocrinology Unit, Mansoura UNiversity, Egypt.

Background and aims: Osteomyelitis is an important contributing factor to impaired wound healing. However, the selection of the appropriate therapy and the effect of therapy on ulcer healing is still unclear. The study aims to identify the management outcome of patients with diabetic foot ulcer associated with osteomyelitis at Mansoura Diabetic Foot centre, Egypt and different variables affecting it
Materials and methods: A retrospective single center including all registered cases, who presented with diabetic foot ulcer complicated by osteomyelitis, treated conservatively, were recruited between July 2005 and December 2015 at our tertiary center.
Results: Of all 3544 Diabetic Foot Ulcer (DFU) presented to the clinic for this 10 years period; 331 were associated with osteomyelitis. Data of 161 ulcer were excluded; because they were either referred to orthopedic surgery or dropped their follow up. The mean age of patients was 57 ± 9.91 yrs, with male predominance of 61.8%. PAD was only present in 2.35% and severe infection in 10.6% of patients. Admission was done in 7.1% of the whole group. Sharp debridement was done for 96.5% patients while ultrasound debridement was done for 3.5% patients. Patients were classified into group 1 who showed complete healing of their ulcers during the study period and group 2 who did not show complete healing. The healing rate was 65.88% among the group and the time to heal (was 127.05 ± 82.87 days in the first group. None or delayed healing was present in 34.1% of patients. There was no significant difference between the 2 groups as regards the age, gender distribution, or other system comorbidity. Toe ulcers with osteomyelitis showed higher healing rate than ulcers over Metatarsal Heads ($P \leq 0.000$). Non-significant difference was present at the other sites of the foot ulceration. The commonest antibiotics used among the healed ulcer group were combination of quinolones and linezolid in 31.4%. Recurrence rate of ulceration was 8.9% at the same site of ulcer and new ulceration at other sites occurred in 19.6% of the healed group during the study period.
Conclusion: Around two thirds of patients presenting with osteomyelitis healed conservatively without undergoing surgical bone resection. Quinolones/linezolid combination therapy were the commonest antibiotic regimen used among the healers. Osteomyelitis affecting the metatarsal heads was the site significantly associated with delayed ulcer healing or the need for surgical intervention.
Disclosure: **H. Gawish:** None.

PS 089 The impact of retinopathy

979

Decreased occurrence of early diabetic retinopathy in lifestyle intervention group of the Finnish Diabetes Prevention Study
M. Uusitupa[1], A. Aro[2], A. Kauppinen[3], P. Summanen[4], G. Von Wendt[4], N. Kivinen[5], T. Selander[6], K. Kinnunen[5], J. Tuomilehto[7], S. Keinänen-Kiukaanniemi[8], J. Lindström[9], K. Kaarniranta[10];
[1]Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland, [2]Department of Ophthalmology, Karelia Central Hospital, Joensuu, Finland, [3]School of Pharmacy, University of Eastern Finland, Kuopio, Finland, [4]Department of Ophthalmology, University of Helsinki, Helsinki, Finland, [5]Department of Ophthalmology, Kuopio University Hospital, Kuopio, Finland, [6]Research Service Unit, Kuopio University Hospital, Kuopio, Finland, [7]Dasman Diabetes Institute, Dasman, Kuwait, [8]Center for Life Course Health Research, University of Oulu, Oulu, Finland, [9]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland, [10]Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland.

Background and aims: Diabetic retinopathy may occur at the time of diagnosis of Type 2 diabetes. The Finnish Diabetes Prevention Study was the first individually randomised controlled trial showing that Type 2 diabetes is preventable by changing lifestyles. The aim of the study was to find out whether participation in earlier intervention had an effect on the occurrence of retinopathy in study participants. We also examined risk factors (age, sex, fasting and 2-h glucose, HbA1c, blood pressure, serum lipids) for early retinal changes.
Materials and methods: The study included 522 individuals (mean 55, range 40–64 years) with impaired glucose tolerance who were randomised into intervention (weight loss, healthy diet and physical activity, $N = 265$) and control groups ($N = 257$). Intervention lasted for median of 4 years in 1993–2000, after which annual follow-up visits at study clinics were conducted. In years 2002–2006 (at least 5 years after stopping intervention), fundus photography was offered for all study participants in four of the five study clinics. Photographs were assessed by two experienced ophthalmologists (AA and KK), blinded for the group assignment. After exclusions of poor quality photographs the data of 214 individuals ($N = 114$ for intervention and $N = 100$ for control group) were included in the present study. Statistical comparisons were done by independent samples t-test or Chi-square with Fisher's exact test. Binary logistic regression model was used to examine multivariable adjusted association of risk factors with the risk of microaneurysms. P values <0.05 were set to indicate statistical significance.
Results: The occurrence of retinopathy (microaneurysms) was significantly higher in the control (37/100, 37%) than in the intervention group (27/114, 24%; $p = 0.029$). The only risk factor that predicted the occurrence of microaneurysms was serum triglycerides at baseline (mean ± SD 1.82 ± 0.82 vs. 1.52 ± 0.78, mmol/l, $p = 0.008$, with and without microaneurysms, respectively). In the model including age, sex, diabetes diagnosis before the retinal assessment, BMI and treatment group, the odds ratio for microaneurysms was markedly lower in intervention group (OR 0.53; 0.28–0.98, $p = 0.042$). Interestingly, adding serum triglycerides in the model weakened the association to non-significant level (OR 0.56, $p = 0.076$).
Conclusion: Lifestyle intervention in overweight and obese individuals with impaired glucose tolerance was associated with decreased occurrence of retinal microaneurysms. Elevated serum triglycerides significantly contributed to the development of early diabetic microangiopathy.
Clinical Trial Registration Number: NCT00518167
Disclosure: **M. Uusitupa:** None.

980

Frequent physical activity is associated with reduced incidence of severe retinopathy in type 1 diabetes: a prospective FinnDiane Study

H. Tikkanen-Dolenc[1], J. Wadén[1], C. Forsblom[1], V. Harjutsalo[1], L.M. Thorn[1], M. Saraheimo[1], N. Elonen[1], K. Hietala[2], P. Summanen[2], H.O. Tikkanen[3], P.-H. Groop[1];
[1]FinnDiane Study Group, Helsinki, [2]Helsinki University Central Hospital, Helsinki, [3]Clinic for Sports and Exercise Medicine, Helsinki, Finland.

Background and aims: Only a few studies have investigated the relationship between physical activity and diabetic retinopathy in type 1 diabetes. So far, no clear associations have been found. The aim was to study how baseline Leisure-Time Physical Activity (LTPA) and its components (intensity, single-session duration and frequency) are associated with the development of severe diabetic retinopathy during follow-up.
Materials and methods: This is a prospective observational study which is part of the Finnish Diabetic Nephropathy (FinnDiane) Study and the mean follow-up time was 10.7 ± 4.6 years. The study population consisted of 1612 patients with type 1 diabetes. Of the population, 44.7% were men and the duration of diabetes was 18.9 ± 11.7 years. LTPA was assessed at baseline using a validated self-report questionnaire. Severe retinopathy was defined as the initiation of laser treatment due to proliferative retinopathy or diabetic maculopathy or blindness (identified from the Care Register for Health Care).
Results: Of the patients 261 received laser treatment during follow-up. Higher frequency of LTPA was associated with lower incidence of diabetic retinopathy ($p \equiv 0.024$). The finding remained significant after adjustment for gender, duration, age at onset of diabetes, kidney function (eGFR), BMI, triglycerides and systolic blood pressure. When HbA_{1c} and smoking were added to the Cox regression model the association decreased to a non-significant level.
Conclusion: Frequent LTPA was associated with a lower incidence of severe diabetic retinopathy during follow-up. The total amount or the other components of LTPA (intensity or the duration of a single session) were not associated with retinopathy during follow-up.
Disclosure: **H. Tikkanen-Dolenc:** None.

981

Prevalence of diabetic retinopathy in China: a nationwide survey by AI-based automatic screening system

Z. Sun[1], J. Kuang[2], J. Liu[3], F. Wang[4], N. Wang[4], R. Wang[1], X. Liu[5], Z. Wang[6], Y. Luo[7];
[1]Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, School of medicine, Southeast University, Nanjing, [2]The 4th People's Hospital of Shenyang, Shenyang, [3]Gansu Provincial Hospital, Lanzhou, [4]Beijing Tongren Hospital, Beijing, [5]Beijing Shanggong Medical Technology Co., Ltd, Beijing, [6]Suzhou Metro Health Medical Technology Co., Ltd, Suzhou, [7]Merck Sereno Co., Ltd, Beijing, China.

Background and aims: Diabetic Retinopathy (DR) is one of the major Diabetic Microvascular Complications. With the growing number of DM patients in China, the prevalence of DR has also dramatically increased. Nationwide study showing prevalence of DR in China is limited and current DR prevalence data varies between 24.7–37.5% in T2DM patients. The present study aims to discover accurate DR prevalence nationwide in China. An AI-based automatic screening system, AutoEye, is used to help Chinese endocrinologists to screen and evaluate DR. Prevalence were therefore assessed in 133,905 DM patients.
Materials and methods: In the present project, China Diabetic Retinopathy Screening and Prophylaxis Project, from January 2017 to November 2017, 133,905 DM patients were screened for Diabetic Retinopathy. 65,695 patients were remotely diagnosed by ophthalmologist and 68,210 patients were AI-based automatic diagnosed. 154 hospitals from 24 provinces of China participate in this project. Project is led by Chinese society of microcirculation. All of the patients were screened by retinoscopy. Diagnosis of DR is evaluated by AutoEye system (an AI-based DR screening cloud platform).
Results: Among 133,905 screened DM patients, 50351 i.e. 37.60% of DM patients were found to have DR. Mean age of subjects were 54.83 yr and mean age of DR subjects were 56.20 yr. 51.15% of screened subjects were male, whereas 48.85% were female. 32.92% subjects were from Southern region and 67.08% were from Northern region of China. Per AutoEye, the prevalence of DR in China DM patients were 37.60%. However, there was no significant difference between the prevalence of Southern and Northern area; i.e. 37.15% vs 37.83%. The present study also discovered that prevalence of DR in males and females are comparable. Prevalence of DR and the percentage of severe DR was closely related to patients' fasting plasma glucose level (FPG). For patients whose FPG was over 7%, DR prevalence was close to 46%. Interestingly, DR prevalence was also related to patients' age. DR prevalence was significantly increasing for patients over 43 yr. Therefore, DR screening should be strongly recommended for DM patients, especially those over 43 yr.
Conclusion: DR prevalence in Chinese diabetic patient is as high as 37.60% based on the AI-based automatic screening system. Mid-aged & elderly and not well controlled blood glucose level are risk factors for DR. Early screening is strongly recommended to DM patients especially those over 43 yr.
Disclosure: **Z. Sun:** None.

982

Screening for retinopathy in Danish children with type 1 diabetes

C. Herskin[1], B. Olsen[1], M. Madsen[2], P. Kjaersgaard[3], S. Fredheim[1], A. Johansen[4], K. Kristensen[5], N.H. Birkebaek[5], J. Svensson[1], K.A. Pilgaard[6], J. Johannesen[1];
[1]Department of Paediatrics, Herlev University Hospital, Herlev, [2]Aalborg University Hospital, Aalborg, [3]Herning Hospital, Herning, [4]Copenhagen University Hospital Rigshospitalet, Copenhagen, [5]Aarhus University Hospital, Aarhus, [6]Nordsjællands Hospital, Hillerød, Denmark.

Background and aims: Children with type 1 diabetes (T1D) should be screened regularly for long-term complications including retinopathy, neuropathy and nephropathy to ensure early detection and appropriate treatment. According to Danish national guidelines, screening for retinopathy should take place at the age of 12, 15 and 18 years after at least 3 years of diabetes. The aim of the study is to investigate the prevalence of retinopathy in children with T1D and to investigate if screening at 12 years of age is indicated in Denmark.
Materials and methods: All Danish children with onset of T1D in the period from 2003–2013 were included in the study ($N = 2943$). Data on retinopathy screenings from fundus photography and annual HbA1c determinations performed from 2003–2017 was retrieved from the Danish national diabetes register, DanDiabKids. The prevalence of retinopathy was determined at age 12, 15 and 18 years. In children with retinopathy, subsequent screenings were studied to reveal if retinopathy was persistent or temporary.
Results: The cohort comprised 2943 children of which 81.2% were screened at least once. Retinopathy was demonstrated in 3.1% of children who underwent screening. Of those with retinopathy 73.0% had unilateral changes and 94.6% had minimal background retinopathy (grade 1). For children with retinopathy, mean HbA1c was 73.8 ± 18.3 mmol/mol at time of first occurrence of a pathological retinopathy status. At 12 years, 1277 children were screened and retinopathy was identified in 11 children (0.9%) with mean HbA1c of 66.7 ± 9.8 mmol/mol. None presented with more than grade 1 retinopathy. Ten out of eleven children were re-screened and no retinopathy could be demonstrated at re-screening. At 15 years, retinopathy was demonstrated in 37 (2.3%) out of 1640. Mean HbA1c of those with retinopathy was 68.9 ± 17.1 mmol/mol. Nineteen of these children underwent subsequent screenings and five (26.3%) had

persistent retinopathy. At 18 years, 26 (3.1%) of 848 had retinopathy changes. Mean HbA1c was 82.6 ± 19.5 mmol/mol. 33% had persistent retinopathy. For children older than 18 years, 2 (4.4%) out of 45 had retinopathy. Mean HbA1c was 80.5 ± 10.6 mmol/mol. For the whole group of children with retinopathy, 33 were screened again and 7 (21.1%) had persisting retinopathy.

Conclusion: The overall prevalence of retinopathy in Danish children with T1D was 3.1% for all ages together. For children screened at 12 years, the prevalence of retinopathy was 0.9% and all had minimal background retinopathy. The changes were found to be temporary in those who were re-screened. Based on these results, we find no absolute indication to screen Danish children with T1D at 12 years of age for retinopathy. We propose an individualized approach for retinopathy screening at the age of 12 and for the national retinopathy screening program to begin at the age of 15 years.

Disclosure: **C. Herskin:** None.

983

Diabetic retinopathy in children and young people with type 1 diabetes in Wales

R.L. Thomas, J.M. Rafferty, S.D. Luzio, D.R. Owens;
Diabetes Research Unit Cymru, Swansea University, Swansea, UK.

Background and aims: Screening programmes for diabetic retinopathy (DR) were introduced in the UK from 2003 onwards. Annual screening from the age of 12 years was recommended by the Royal College of Ophthalmologists. In Wales, there is a single national community-based screening programme (Diabetic Eye Screening Wales, DESW) which screens all those with diabetes registered with a GP in Wales. We report the DR screening results for those Children and Young People (CYP) with type 1 diabetes aged <18 years undergoing screening for DR in Wales.

Materials and methods: The DESW employs standardised quality assured image capture and grading protocols. Canon DGi cameras are used to capture 2x45^{o} digital images per eye following mydriasis. Grading is performed by trained graders using the DESW standard grading protocol. Referable DR (RDR) is the level at which further assessment by hospital-based ophthalmologists is required and includes pre-proliferative DR (PPDR), proliferative DR (PDR) and exudative maculopathy. Data (age, gender, duration of diabetes and retinopathy grade) of CYP screened (2,347) between 2009 and 2017 were extracted from the DESW database This was linked with data (HbA_{1c}, blood pressure, cholesterol level, creatinine and BMI) from primary care.

Results: A total of 2,347 CYP (1,222 Male) were screened with a total of 10,375 screening events. At first screening the mean (SD) age was 15.2 (2.2) years, duration of diabetes median (IQR) was 5.0 (2.4–8.7) years, HbA_{1c} 79.1 (22.5) mmol/mol, systolic blood pressure 119.1 (13.8) mmHg, diastolic blood pressure 70.3 (8.8) mmHg and BMI 23.5 (4.5) kg/m^2. Of the 2,347 invited for screening 504 (21.4%) did not attend. Those who attended 1,331 (72.1%) did not have evidence of DR and 285 (15.4%) had background DR. 3 (0.2%) had RDR and a similar number 3(0.2) required referral for other eye lesions. Additionally, of those 1,331 people without DR a total of 153 (11.5%) progressed to DR during the study, developing background DR firstly with 19 (1.4%) subsequently developing RDR and 22 (1.7%) requiring referral (DR and other lesions). Of those who had DR (438) during the study DR developed at ages 12, 13, 14, 15, 16 and 17 years (in 39 [8.9%], 32 [7.3%], 48 [11.0%], 74 [16.9%], 103 [23.5%] and 142 [32.4%] respectively). The final logistic regression model after removal of non-significant variables retained duration of diabetes, HbA_{1c} and diastolic blood pressure. The presence of DR was associated with an increased duration of diabetes (OR1.27 95% CI 1.22, 1.32), increased HbA_{1c} (OR 1.03 95%CI 1.02, 1.04) and diastolic blood pressure (OR 1.03 95%CI 1.01, 1.04) after adjustment.

Conclusion: Almost 10% of DR detected occurred in those aged up to 12 years but with the majority (67.6%) occurring in those under the age of 17 years. However, only 1.2% had developed RDR by the end of the study period requiring referral and an additional 0.3% for other eye lesions. The risk of developing DR increased with increasing duration of diabetes, glycaemic control and diastolic blood pressure. More effort should be made to ensure early screening with emphasis on the importance of screening as well as enabling CYP with type 1 diabetes to understand the risks of developing and promoting healthy lifestyles.

Disclosure: **R.L. Thomas:** None.

984

Incidence of diabetic retinopathy in newly diagnosed subjects with type 2 diabetes over 5 years: contribution of beta cell function

S. Roy Chowdhury[1], R.L. Thomas[2], G.J. Dunseath[2], S. Luzio[2], F.S. Wong[3], D.R. Owens[2];
[1]Diabetes and Endocrinology, Princess of Wales Hospital, Bridgend, [2]Diabetes Research Unit Cymru, Swansea University Medical School, Swansea, [3]Centre for Endocrine and Diabetes Science, Cardiff University School of Medicine, Cardiff, UK.

Background and aims: Identifying risk factors in development of Diabetic Retinopathy (DR) is essential to prevent visual impairment in type 2 diabetes (T2DM). Prolonged hyperglycaemia is acknowledged as the main risk factor, represented by HbA1c, fasting plasma glucose (FPG) and to a lesser extent 2 hour post-prandial plasma glucose (PPG). The relationship with indices of β cell function remains inadequately addressed. This study aims to examine incident DR over 5 years in persons with newly-diagnosed treatment naïve T2DM with no DR at diagnosis.

Materials and methods: 233 T2DM participants were screened for DR (digital photography) and had a standardised Meal Tolerance Test (MTT) at baseline and 1, 2 and 5 years post diagnosis involving serial plasma glucose and insulin levels over 4 hours. Fasting (M_0) and postprandial (M_1) β cell responsiveness were estimated (Calculating Pancreatic Response (CPR) Program) along with HOMA-B. We compared subjects with no DR (NDR) throughout the 5 years ($n = 179$) with those who developed DR ($n = 54$). Following comparison of means of the two groups, the putative risk factors were evaluated using logistic regression methods. Multivariate analyses were adjusted for age, gender, BMI, systolic blood pressure and total cholesterol using SPSS 20 ($p < 0.05$ taken as statistically significant). We also calculated the average exposure to all measured metabolic variables (Mean) as an indicator of diabetes control over the 5 year study period.

Results: Of 233 subjects, 145 male, 88 female, mean age (SD) 54(9) years, 76.8% (179) never developed DR with 23.2% (54) developing DR during 5 years post diagnosis. 22% developed background DR after 1 year, 28% after 2 years and 50% after 5 years. All with DR at Year 5, when compared to those without, had at diagnosis a higher HbA_{1c} ($p = 0.017$) and during MTT a higher FPG ($p = 0.031$) with a reduced basal ß cell secretory function, M_0 ($p = 0.025$) and HOMA-B ($p = 0.044$). In the postprandial state, those with DR at Year 5 had a higher PPG ($p = 0.009$) associated with a significantly lower estimated β cell responsiveness: M_1 ($p = 0.000$). The contribution of fasting, postprandial, overall glycaemia and β cell function at diagnosis and over 5 years to incident DR are represented in the attached table.

Conclusion: We established an independent association between $HbA_{1c,}$ FPG and PPG, both at diagnosis and over the 5 years period, with incident DR. β cell function, fasting (M_0 and HOMA-B) and postprandial (M_1) also at diagnosis and over 5 years were independently associated with DR development. Our data further emphasizes the contribution of overall glycaemiac exposure (HbA1c) and postprandial hyperglycaemia to the risk of developing DR within 5 years of diagnosis. A reduced ß cell function is a significant contributor to this risk of developing DR.

Table: Univariate and Multivariate logistic regression depicting variables independently associated with development of Diabetic Retinopathy by 5 years from diagnosis of T2DM.

		Univariate		Multivariate	
	Number	Crude OR (95% CI)	p	OR (95% CI) (fully adjusted **)	p
HbA1c (%)	233	4.27 (1.21, 15.13)	**0.024**	4.48 (1.26, 15.96)	**0.021**
Fasting Glucose (mmol/L)	230	2.77 (1.01, 7.59)	**0.047**	2.78 (1.02, 7.64)	**0.045**
Postprandial Glucose (mmol/L) (120 mins)	230	3.44 (1.34, 8.85)	**0.011**	3.44 (1.34, 8.86)	**0.011**
M_0 (*10^9 pmol/kg/min)	227	0.62 (0.41, 0.93)	**0.022**	0.59 (0.39, 0.91)	**0.015**
M_1 (*10^9 pmol/kg/min)	224	0.48 (0.33, 0.70)	**0.000**	0.46 (0.32, 0.68)	**0.000**
HOMA-B (%)	224	0.70 (0.50, 0.98)	**0.040**	0.60 (0.41, 0.87)	**0.007**
Mean HbA1c (%)	233	1.51 (1.14 – 2.01)	**0.004**	1.48 (1.12 – 1.97)	**0.008**
Mean Fasting Glucose (mmol/L)	230	1.15 (0.96 – 1.38)	**0.143**	1.13 (0.93 – 1.36)	**0.227**
Mean Postprandial Glucose (mmol/L) (120 mins)	230	1.15 (1.02 – 1.30)	**0.022**	1.15 (1.02 – 1.30)	**0.023**
Mean M_0 (*10-9 pmol/kg/min)	227	0.89 (0.78 – 1.02)	**0.100**	0.81 (0.68 – 0.96)	**0.014**
Mean M_1 (*10-9 pmol/kg/min)	224	0.93 (0.89 – 0.97)	**0.001**	0.93 (0.86 – 0.97)	**0.001**
Mean HOMA-B (%)	224	0.10 (0.98 – 1.01)	**0.505**	0.99 (0.97 – 1.01)	**0.207**

** adjusted for age, sex, BMI, SBP, TCh. SBP = Systolic Blood Pressure; TCh = Total Cholesterol, BMI = Body Mass Index FPG = Fasting Plasma Glucose; PPG = Post Prandial Glucose, Fasting (M_0) and Postprandial (M_1) β-cell responsiveness. Mean = Average of all metabolic variables (Yr 0, 1, 2 and 5) as an indicator of diabetes control over the 5 year study period.

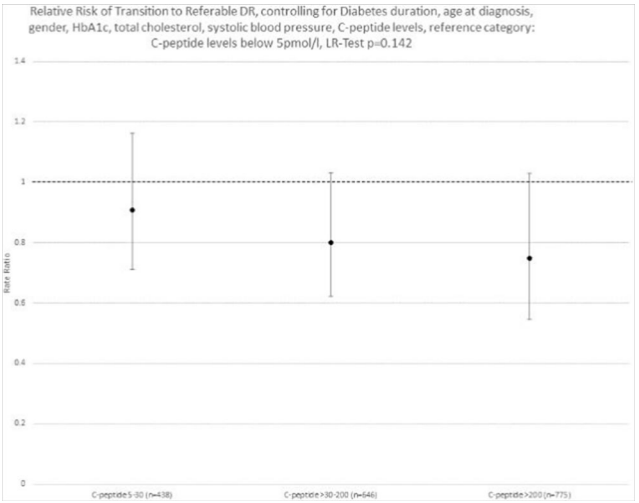
Disclosure: **S. Roy Chowdhury:** None.

985

The association between C-peptide levels and the transition to referable retinopathy in patients with type 1 diabetes

A. Ochs[1], S.J. McGurnaghan[1], L.A.K. Blackbourn[1], P.M. McKeigue[2], H.M. Colhoun[1], on behalf of the SDRNT1BIO Investigators;
[1]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, [2]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, UK.

Background and aims: Type 1 diabetes mellitus (T1DM) involves the autoimmune destruction of pancreatic β-cells. Research over the recent years using C-peptide levels, a well-established marker of endogenous insulin secretion levels, has challenged the dogma that T1DM is associated with complete destruction of pancreatic β-cells and established that a number of patients have detectable C-peptide levels. Previous research has shown associations between C-peptide levels, risk of hypoglycaemia and glucose management. The consequences of C-peptide persistence on diabetes complications are less well known. This study examines the association between C-peptide levels and the subsequent progression to referable diabetic retinopathy (DR), in a large cohort of T1DM patients.
Materials and methods: This study used a large population representative sample with T1DM, the Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO), in whom biosamples have been collected and who have been linked to electronic health care records and diabetic retinopathy screening data. C-peptide levels of individuals were measured on a single random measure at recruitment. The lower limit of detection of C-peptide was 3 pmol/l. Screening information was obtained from the routine screening data within the Scottish Diabetic Retinopathy Screening Programme, which has invited every diabetes patient in Scotland aged 12 and older for regular screening since 2007. Generalised linear regression techniques with complementary loglog link function for interval-censored data were used to assess the effect of (i) C-peptide persistence and (ii) C-peptide levels on transition to referable DR. Models were adjusted for previous DR grade, age at diagnosis, diabetes duration, HbA1c, total cholesterol and systolic blood pressure at recruitment. The effects of C-peptide was assessed by comparing a model excluding and including C-peptide using a Likelihood-Ratio (LR) Test.
Results: Out of the 6127 participants in SDRNT1BIO, 4595 patients attended at least two DR screenings and did not transit to a referable DR state before their recruitment. 4486 patients had a measure of C-peptide reported. The analysis was based on 18348 screenings for these patients. The absence of detectable levels of C-peptide was statistically significantly associated with increased risk of transition to referable DR (OR 1.191, LR $p < 0.05$). The graph suggests further that an increase tends to reduce the risk of transition to referable DR, however the relationship is not clear cut and a threshold of no further protection from C-peptide is not clearly apparent.
Conclusion: This study showed that C-peptide is significantly associated with transition to referable DR, a threshold of protective C-peptide levels needs to be detected.



Supported by: Diabetes UK and CSO
Disclosure: **A. Ochs:** Grants; Diabetes UK, CSO.

986

Neuroretinal versus vascular changes in patients with type 2 diabetes compared to healthy controls

S. Jung[1], A. Bosch[2], N. Kohler[2], C. Ott[2,3], D. Kannenkeril[2], T. Dienemann[2], J.M. Harazny[2,4], G. Michelson[5], R.E. Schmieder[2];
[1]Cardiology, Friedrich-Alexander-University, Erlangen-Nuremberg, Germany, [2]Nephrology and Hypertension, Friedrich-Alexander-University, Erlangen-Nuremberg, Germany, [3]Nephrology and Hypertension, Paracelsus Medical School, Nuremberg, Germany, [4]Pathophysiology, University of Warmia and Mazury, Olsztyn, Poland, [5]Ophthalmology, Friedrich-Alexander-University, Erlangen-Nuremberg, Germany.

Background and aims: Optical coherence tomography (OCT) has evolved into an accurate and reliable high-resolution imaging technique for the measurement of retinal layer thickness in a variety of metabolic and neurological disorders. The aim of the present study was to characterize neuroretinal alterations in patients with early type 2 diabetes mellitus (T2DM) determined by spectral-domain (SD-) OCT as opposed to early vascular changes evaluated by arteriovenous ratio (AVR).
Materials and methods: In a prospective, cross-sectional, observational single center study we included a total of 73 subjects - 35 patients with early T2DM and 38 healthy controls. Clinical characteristics of all participants were obtained and fasting plasma glucose, HbA1c and other biochemical parameters were measured. To determine possible neurodegenerative retinal changes related to T2DM, retinal layer thicknesses including circular scans of the retinal nerve fiber layer (RNFL) of both eyes were measured by SD-OCT. Additionally, AVR as a maker of retinal arteriolar narrowing was determined by fundus photography.
Results: The group of patients with T2DM consisted of 12 female (34%) and 23 male (66%) patients aged 61.6 ± 6.9 years with an average HbA1c of 6.8 ± 0.9%, fasting plasma glucose of 135.3 ± 36 mg/dl and an average T2DM duration of 6.87 ± 5.9 years. The group of healthy controls included 28 female (74%) and 10 male (26%) subjects aged 57.9 ± 10 years. Age-adjusted inner retinal layer (comprising RNFL, ganglion cell layer and inner plexiform layer thickness) was thicker in patients with T2DM than in the group of healthy controls (6.46 ± 0.42 vs. 6.27 ± 0.38 mm³,

$p = 0.032$) as was total retinal thickness (8.70 ± 0.43 vs. 8.51 ± 0.41 mm³, $p = 0.037$). Mean AVR was lower among patients with T2DM compared to controls (0.769 ± 0.10 vs. 0.827 ± 0.06, $p = 0.042$). After adjustment for age and gender, the above mentioned differences in inner retinal layer thickness ($p = 0.100$) and total retinal thickness ($p = 0.101$) disappeared, whereas the difference in AVR between the two groups stayed significant ($p = 0.009$). A subanalysis of the 22 male subjects with T2DM (compared to male healthy controls) revealed a higher thickness of the circular RNFL scans in the temporal right (78.19 ± 12.84 vs. 68.90 ± 9.53 μm, $p = 0.034$) and temporal superior left sectors (138.0 ± 9.94 vs. 123.50 ± 19.60 μm, $p = 0.048$). There were no significant differences in other retinal layers or total RNFL thickness among the two subgroups, whereas AVR was lower in the group of male T2DM patients (0.778 ± 0.06 vs. 0.840 ± 0.07, $p = 0.033$).

Conclusion: In our patients with T2DM (early stage of disease, well-controlled) we observed a lower AVR than in elderly healthy controls whereas no clear signal of neurodegeneration, visualized by retinal layer thickness measurement, was detected. Our findings indicate that vascular changes precede neuronal retinal changes in patients with early T2DM. Nevertheless, evaluation of RNFL thickness by means of SD-OCT may emerge as a new and attractive option to assess neuroretinal changes during disease progression.

Disclosure: **S. Jung:** None.

PS 090 Retinopathy: there is more than hits the eye

987

Functional analysis of miRNAs shuttled by extracellular vesicles from diabetic subjects reveals their role in diabetic retinopathy

A. Mazzeo, E. Beltramo, C. Gai, T. Lopatina, M. Trento, M. Porta; Dept. of Medical Sciences, University of Turin, Torino, Italy.

Background and aims: Extracellular vesicles (EVs) derived from mesenchymal stem cells cultured in diabetic-like conditions enter the pericytes, causing their detachment and migration, and stimulating angiogenesis. Diabetic patients have different EV patterns in comparison with healthy subjects. In particular, our data suggest a role for miR-150-5p, miR-21-3p and miR-30b-5p as putative biomarkers of the onset and development of diabetic retinopathy. The functional KEGG pathways of these 3 miRNAs showed that they are involved in pathways strictly correlated to the dysfunctions occurring in the early phases of retinopathy, such as adherents junctions, ECM-receptor interactions, TGF-β signaling. In this work, we aimed at further investigating the functional role of the 3 miRNAs on the homeostasis of retinal microvascular cells and characterizing EVs derived from diabetic subjects with/without retinopathy by mRNA content analysis.

Materials and methods: EVs were extracted from plasma of 7 type-1 diabetic subjects with severe retinopathy (DR, gender: 3F/4M, age 39.3 ± 5.9, disease duration 28.0 ± 12.8), age- and gender-matched with 7 healthy controls (CTR, gender: 3F/4M, age: 41.0 ± 10.6) and 7 diabetic subjects without retinopathy (noDR, gender: 3F/4M, age: 46.1 ± 11.7, disease duration: 27.3 ± 14.2). As we found miR-21-3p and miR-30b-5p increased, and miR-150-5p decreased in EV of DR patients, human retinal pericytes (HRP) and endothelial cells (HMEC) were transfected with mimics or inhibitors, as appropriate, of the 3 miRNAs, to evaluate their functional role in angiogenesis (vessel-like formation assay) and migration of retinal microvascular cells. Furthermore, EV expression of genes involved in angiogenesis was measured by *Human Angiogenesis RT² Profiler PCR Array* and confirmed by qRT-PCR and Western blotting (WB).

Results: After 48 hrs from transfection, modulation of miRNA expression increases migration in microvascular cells and vessel formation *in vitro*, confirming that the 3 miRNAs are involved in angiogenesis. mRNA analysis revealed different expression of 7 genes involved in angiogenesis in the 3 groups, while subsequent qRT-PCR and WB confirmed decreased expression of angiopoietin-1 (involved in vessel stabilization) and increased expression of the pro-angiogenic HIF-1α in DR vs CTR.

Conclusion: In conclusion, the analysis of EV mRNA content reveals differences between diabetic patients with microvascular complications, and healthy controls. miR-150-5p, miR-21-3p and miR-30b-5p, differentially expressed in EVs from DR patients and controls, seem to be related to diabetic retinopathy by inducing features of retinopathy in *in vitro* models of retinal microvasculature. These miRNAs might be taken into account as potential biomarkers of the onset/development of the disease and considered as specific targets for the prevention of this complication.

Clincial Trial Registration Number: CS/236

Supported by: EFSD/Lilly - MIUR

Disclosure: **A. Mazzeo:** Grants; EFSD/Lilly Fellowship 2016 - Italian Ministry of Education, Universities and Research.

988

The SRPK1 inhibitor SPHINX31 stabilises retinal permeability in models of diabetes

K.P. Arkill, N. Malhi, C.L. Allen, K. Horton, J. Batson, D.O. Bates; University of Nottingham, Nottingham, UK.

Background and aims: In diabetic retinopathy (DR) microvascular damage results from ischaemia driven production of pro-angiogenic vascular

endothelial growth factor (VEGF) inducing angiogenesis and increased permeability in the retina. Small molecular inhibitors of serine-rich protein kinase-1 (SRPK1) have been shown to inhibit choroidal neovascularisation in mice by decreasing pro-angiogenic and increasing anti-angiogenic VEGF isoforms. SRPK1 inhibitors such as SPHINX31 may therefore switch splicing in DR and prevent increased vascular permeability of both the junctions or the endothelial glycocalyx.

Materials and methods: Retinal pigment epithelial (RPE) cells were exposed to either normoglycaemia (NG, 7.5mM) or hyperglycaemia (HG, 37.5mM) under normoxic (Nx, 20% O_2) or hypoxic (Hx, 1% 0_2) conditions with and without SPHINX31 (1 μM and 3 μM). SRPK1 activity and monolayer permeability were assessed by immunofluorescence and Electric Cell-substrate Impedance Sensing (ECIS). Fluorescent fluorescein angiography (FFA) was performed in streptozotocin induced diabetic Norway Brown rats ($n = 12$, 250–300 g) on day 0 and 7, using the Micron IV retinal microscope (Phoenix Technology Group, US). Animals received twice daily topical eye drops of eye formulation control buffer ($n = 5$) or SPHINX31 (200 μg/ml, $n = 6$). The ratio of interstitial to vascular fluorescence was calculated and plotted against time to determine an estimate of permeability.

Results: The ratio of nuclear to cytoplasmic SRSF1 increased significantly ($p < 0.05$) in cells treated with HG from 5.03 (NG) to 6.12 (HG). Hx induced a further increase ($p < 0.05$) to 7.25. SPHINX31 blocked these increases at both 1 μM and 3 μM whilst having no effect on the normoglycaemic, normoxic cells. Expression of tight junctional protein ZO-1 was decreased in Hx ($p < 0.001$). Inhibition of SRPK1 increased impedance across a RPE monolayer in NG and not HG. Retinal permeability was shown to significantly increase ($p < 0.01$) on day 7 $(12.67 \pm 1.09) \times 10^{-4}$ cm^{s-1} compared to day 0 $(8.85 \pm 1.29) \times 10^{-4}$ cm^{s-1} in the eye formulation control group. Following a weekly regimen of twice daily topical eye drop treatment with SPHINX31 retinal permeability stabilised on day 7 $(7.92 \pm 1.65) \times 10^{-4}$ cm^{s-1} compared to day 0 $(8.15 \pm 2.33) \times 10^{-4}$ cm^{s-1} and the control group. Our preliminary immunofluorescence results on retinal and choroidal endothelial cells also agree with the literature that there is a loss of both endothelial glycocalyx and ZO-1 proteins.

Conclusion: SPHINX31 protected the retinal endothelial permeability barrier from diabetes-associated loss of integrity and reduced the progression. SPHINX31 may therefore be a potential alternative and more specific topical therapeutic for DR.

Supported by: NERC, MRC

Disclosure: **K.P. Arkill:** None.

989

Thiamine transporter-2 is involved in high glucose-induced damage and altered thiamine metabolism in cell models of diabetic retinopathy

E. Beltramo, A. Mazzeo, M. Porta;
Dept Medical Sciences, University of Turin, Torino, Italy.

Background and aims: High glucose-induced damage in microvascular cells *in vitro* and progression of retinopathy and nephropathy in diabetic animals are prevented by thiamine supplementation. Impaired thiamine availability facilitates metabolic damage, and renal loss of this vitamin is described in diabetic patients. Two SNPs located in the SLC19A3 gene encoding for the thiamine transporter-2 (ThTR-2) are associated with resistance to development of proliferative diabetic retinopathy and end-stage renal disease in type 1 diabetic subjects, but the mechanisms of these protective effects remain to be understood. We previously showed that diabetic-like conditions modulate ThTR-2 expression in human retinal cells. Our aim was to further investigate the involvement of the two thiamine transporters ThTR-1 and ThTR-2 and their transcription factor Sp1 in high glucose-induced damage and altered thiamine metabolism in cell models mimicking the diabetic retinopathy microenvironment.

Materials and methods: Human retinal pericytes (HRP), human microvascular endothelial cells (HMEC) and human Müller cells (MIO-M1) were cultured for 8 days in physiological glucose (NG), stable high glucose (HG) or intermittent high glucose (intHG). Cells were also cultivated in thiamine-deficient medium (noT) or high thiamine conditions (HT), to evaluate substrate influence. Transketolase (TK) activity and intracellular thiamine concentration were studied through metabolic assays, and cellular localization of the transporters by immunofluorescence staining (IF). To better mimic the retinal microenvironment and the complex intercellular exchanges, triple co-cultures were established. ThTR-1, ThTR-2 and Sp1 mRNA and protein expression were checked by RT-PCR and Western blotting.

Results: TK activity and intracellular thiamine were markedly decreased in HRP and HMEC cultured in noT, as expected, and increased in HRP cultured in HT. Increased intracellular thiamine and TK activity were found in MIO-M1 cultured in HG and intHG and, surprisingly, in HG and intHG without thiamine. IF staining evidenced cytoplasmic localization of ThTR-1 and Sp1 in all cell types. ThTR-2 showed a characteristic nuclear speckle distribution in HRP and MIO-M1, while in HMEC it was uniformly distributed in the cytoplasm. As regards co-culture models, ThTR-1 and Sp1 expression were unchanged in all cell types regardless of treatment, whereas ThTR-2 mRNA and protein expression were decreased in HRP, HMEC and MIO-M1 cultured in HG and intHG conditions, showing differences from data in single cultures.

Conclusion: Down-regulation of ThTR-2 in co-culture models mimicking the diabetic retinal microenvironment suggests its major role in thiamine transport in retinal cells and its involvement in high glucose-induced damage and impaired thiamine metabolism. As expected, altered thiamine supplementation influences TK activity. The increased intracellular thiamine and TK activity in Müller cells following deficient thiamine supplementation may be interpreted as an adaptive mechanism of these cells to counteract lack of substrate.

Supported by: EFSD/Boehringer Ingelheim - MIUR

Disclosure: **E. Beltramo:** Grants; EFSD/Boehringer Ingelheim, MIUR.

990

Effects of topical administration of SOCS1-derived peptide on retinal neuroinflammation and vascular leakage in experimental diabetes

C. Hernández[1], P. Bogdanov[1], C. Gómez-Guerrero[2], J. Sampedro[1], C. Solà-Adell[1], M. García-Ramirez[1], J. Egido[2], R. Simó[1];
[1]Vall d'Hebron Reserach Institute, UAB and CIBERDEM, Barcelona, [2]IIS Fundación Jiménez Díaz, UAM and CIBERDEM, Madrid, Spain.

Background and aims: Current treatments for diabetic retinopathy (DR) target late stages when vision has already been significantly affected. Accumulating evidence suggests that neuroinflammation plays a major role in the pathogenesis of DR, resulting in the disruption of the blood-retinal barrier, the main cause of diabetic macular edema. Suppressors of cytokine signaling (SOCS) are cytokine-inducible proteins that function as a negative feedback loop regulating cytokine responses. On this basis, the aim of the present study was to evaluate the effect of a SOCS1-derived peptide administered by eye drops (2 weeks) on retinal neuroinflammation and early microvascular abnormalities in db/db mouse model.

Materials and methods: SOCS1-derived peptide (10 mg/ml; 5 μl twice/daily) ($n = 10$) or vehicle (PBS; 5 μl twice/daily) ($n = 10$) eye drops were administered directly onto the superior corneal surface of each eye using a micropipette in 8 week-old db/db mice. Ten non-diabetic mice (db/+) matched by age served as the control group. The treatment (SOCS1-derived peptide or vehicle) was administered twice daily for 15 days. Retinal analyses were performed *in vivo* by electroretinography and *ex vivo* by using RT-PCR, Western blot and immunofluorescence measurements. Glutamate was quantified by HPLC. In addition, vascular leakage was examined by the Evans blue method.

Results: We found that SOCS1-derived peptide significantly reduced glial activation and neural apoptosis induced by diabetes, as well as retinal

levels of proinflammatory cytokines. Moreover, a significant improvement of electroretinogram parameters was observed, thus revealing a clear impact of the histological findings on global retinal function. Finally, SOCS1-derived peptide prevented the disruption of the blood-retinal barrier.
Conclusion: Our results suggest that topical administration of SOCS1-derived peptide is effective in preventing retinal neuroinflammation and early microvascular impairment. These findings could open up a new strategy for the treatment of early stages of DR.
Supported by: PI16/00541, SAF2015-63696-R, PI14/00386, PI17/01495 and DTS-2017/00203
Disclosure: **C. Hernández:** None.

991

δ opioid receptor agonism preserves the outer retina barrier in an experimental model of diabetic retinopathy: a novel therapeutic approach to treat diabetic macular edema

D.A. Duarte[1], R. Simó[2], M. García-Ramirez[2], J.B. Lopes de Faria[1], J.M. Lopes de Faria[1];
[1]Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, Sao Paulo, Brazil, [2]Vall d'Hebron Research Institute (VHIR) and CIBERDEM, Barcelona, Spain.

Background and aims: Macular edema is a common vision-threatening feature in patients with diabetic retinopathy (DR). Diabetic macular edema (DME) is frequently resistant to corticoid or anti-angiogenic agent treatments. Previously, we demonstrated that the agonism of the δ opioid receptor (DOR) by epicatechin (a potential scaffold for the opioid receptor ligands of the C2 and C3 positions of the epicatechin molecule) preserves the tight junction proteins in ARPE-19 cells under diabetic conditions. The aim of this study was to investigate whether the agonism of δ opioid receptor (DOR), preserves the outer blood-retinal barrier (BRB) in experimental model of diabetic retinopathy.
Materials and methods: C57BL mice were experimentally induced diabetes through streptozotocin and treated with (−)-epicatechin (used as a δ opioid receptor-agonist) in drinking water for 12 weeks ($n = 13$). To better demonstrate the possible role of the DOR in the observed effects of epicatechin, diabetic mice ($n = 13$) were underwent to intravitreous injection shRNA-DOR (10^8 TU/ml) and randomized to receive or not oral epicatechin in drinking water (aproximately 2.3 g/Kg/day). At the end, the animals were euthanized, the eyes enucleated and the retinas extracted. Human postmortem eyes were obtained from diabetic ($n = 7$) and non-diabetic donors ($n = 7$) matched by age. To be included in this study, an ophthalmoscopic examination documenting the absence of microvascular abnormalities associated with diabetic retinopathy or only mild non-proliferative diabetic retinopathy must have been performed within 2 years prior to the individual's death.
Results: The expression of DOR was demonstrated in retinal tissue in RPE and inner plexiform layers and did not change in presence of diabetes or with treatment with epicatechin. The treatment with DOR agonist prevented the upregulation of the early markers of DR (GFAP, VEGF) and the downregulation of PEDF, and tight junction proteins occludin, claudin-1 and ZO-1 ($p < 0.01$). The reduction of about 30% in DOR protein in diabetic mice submitted to intravitreous shRNA-DOR expression abolished partially the protective effects of epicatechin in DR markers and outer BRB tight junction proteins. The preservation of the RPE function observed in diabetic mice treated with oral epicatechin led to increase of the endogenous anti-angiogenic factor PEDF ($p < 0.0001$), counterbalancing the increase of VEGF levels ($p < 0.0001$). In human retina specimen, we did not found any significant difference between diabetic and non-diabetic human donors in protein content and mRNA levels of DOR in RPE or neuroretina samples. The immunohistochemistry images revealed that DOR is located in human retina mainly in RPE and inner retinal layers.
Conclusion: This set of experiments strongly indicates that (−)-epicatechin preserves the functions of the RPE and reduced the early markers of DR through the DOR agonism in diabetic retinal tissue. These novel findings designate DOR as a potential therapeutic tool to treat DR, especially diabetic macular edema.
Supported by: EFSD/Sanofi and FAPESP
Disclosure: **D.A. Duarte:** None.

992

Glucosamine is neuroprotective in diabetic retina

R. Eshwaran[1], M. Kolibabka[2], K. Kohl[2], H.-P. Hammes[2], T. Wieland[1], Y. Feng[1];
[1]Experimental Pharmacology, European Center of Angioscience, Medical Faculty Mannheim, Heidelberg University, Mannheim, [2]5th Medical Clinic, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

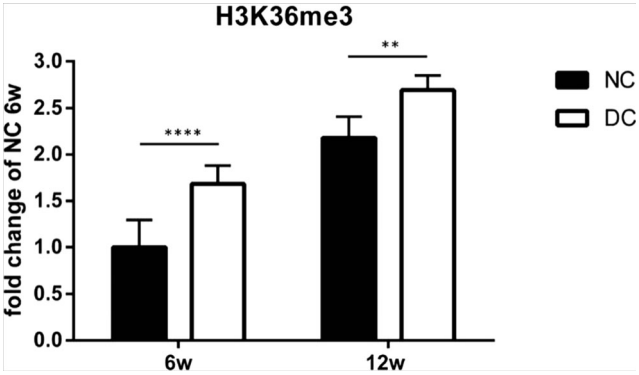
Background and aims: Diabetic retinopathy occurs in over 80% of patients suffering from diabetes for more than 20 years and is one of the leading causes of blindness in adults. Hyperglycemia hampers not only the vascular but also the neuronal function in diabetic retinopathy. Glucosamine is a hexose sugar that might be involved in glucose metabolism via the hexosamine pathway. Glucosamine exhibits anti-inflammatory and anti-oxidative effects and participates in the modulation of endothelial cell activation. It is widely used as an oral supplement in patients with osteoarthritis. The effect of glucosamine is still controversial. In this study, we compared the effects of glucosamine in the mouse retina under non-diabetic and diabetic conditions.
Materials and methods: Diabetes was induced by i.p. injection with streptozotocin in 8-week-old wild type and NDPK-B KO mice, which mimic the obvious vascular degeneration without neuronal degeneration. The mice were treated with glucosamine from 9 weeks of age on, and analyzed at 6 months. Optical Coherence Tomography (OCT) was performed to analyze retinal thickness and electroretinogram (ERG) was used to analyze the neuronal function. Retinal morphometry was assessed in retinal digest preparations.
Results: Glucosamine did not alter blood glucose and HbA1c levels, which were elevated in diabetic animals. A significant increase in body weight was seen in wild type non-diabetic animals treated with glucosamine compared with control wild type animals. In diabetic wild type and NDPK-B KO mice, treatment with glucosamine did not change the body weight. No change in food and water intake of the mice was observed with glucosamine treatment. Retinal thickness did not differ between any of the groups upon analysis with OCT. There was no alteration in the a-wave of the ERG after glucosamine treatment in wild type and NDPK-B KO retinas. However, glucosamine treatment was able to rescue the loss of the b-wave seen in diabetic wild type animals compared with non-diabetic controls. Glucosamine did not impact the b-wave in NDPK-B KO retinas. Pericyte loss and formation of acellular capillaries were not altered in non-diabetic and diabetic wild type retinas after treatment. In NDPK-B KO retinas, the formation of acellular capillaries was significantly increased, while pericyte numbers showed no change after glucosamine treatment. The ratio of endothelial cells to pericytes was increased in non-diabetic and diabetic retinas after glucosamine treatment in both wild type and NDPK-B KO retinas.
Conclusion: Our results suggest that glucosamine is neuro- but not vasoprotective in the diabetic retina.
Supported by: EFSD, DFG
Disclosure: **R. Eshwaran:** None.

993

H3K36me3 associated pericyte loss in early diabetic retinopathy

K. Kohl, M. Kolibabka, P. Friedrichs, H.-P. Hammes;
5th Medical Department, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany.

Background and aims: Pericyte loss is one of the earliest vascular signs of diabetic retinopathy. Capillaries are hereby destabilized leading to vasoregression during the course of the disease. Gene expression changes of microarray analyses of a streptozotocin-induced diabetic mouse model point towards a distinct state of H3K36 trimethylation in the retina as early as six weeks of diabetes. The epigenetic modification H3K36me3 regulates chromatin transcription and occurs on constituous as well as facultative heterochromatin. The exact mechanisms for pericyte dropout and incipient diabetic retinopathy are not yet fully understood and need further clarification. We investigated H3K36me3 as an early mechanism in relation to pericyte loss.
Materials and methods: Pericyte numbers were determined in retinal digests by quantitative morphometry. H3K36me3 was measured photometrically in an immune-based assay.
Results: After 12 weeks of STZ-Diabetes, pericyte count of diabetic animals was lower than in normoglycemic animals (NC: 1571 ± 69.80 vs. DC: 1978 ± 93.52; $p < 0.01$; [pericytes/mm^2]; $n = 6$). After six weeks of STZ-diabetes, levels of H3K36me3 were higher in diabetic than in normoglycemic mice persisting until week 12 (6 w: 1.69 vs. 1.00; $p < 0.0001$; 12 w: 2.69 vs. 2.18; $p < 0.05$; [fold change of NC 6 w]; $n = 6$). These findings suggest that H3K36me3 is changed early during experimental diabetic retinopathy, which could lead to more severe signs of the disease.
Conclusion: Further investigations will assess the pericyte status in this animal model after six weeks to establish a timely correlation between H3K36 trimethylation and pericyte loss in early diabetic retinopathy.



Supported by: DFG within the IRTG 1874 "DIAMICOM"
Disclosure: **K. Kohl:** Grants; DFG within the IRTG 1874 "DIAMICOM".

994

Exposure of embryonic retinal cultures to starvation unravels early neurovascular mechanisms responsible for later effects in diabetic retinopathy

R. Jain[1], T. Özgümüs[2], A. Fedotkina[2], A. Zhydenko[3], I. Artner[1], V. Lyssenko[1,2];
[1]Diabetes and Endocrinology, Department. of Clinical Sciences, Lund University Diabetes Center, Malmö, Sweden, [2]Department of Clinical Science, KG Jebsen Center for Diabetes Research, University of Bergen, Bergen, Norway, [3]Chernihiv Regional Hospital, Chernihiv, Ukraine.

Background and aims: Diabetes retinopathy (DR) is a progressive disease leading to severe vision impairment and blindness. It is characterized by elevated vascular endothelial growth factor (VEGF) levels, which then alters the vascular tone (non-proliferative DR) and increases angiogenesis (proliferative DR). Although anti-VEGF therapies have been very successful in stopping the progression of DR at the proliferative DR stage, there are no drugs available to stop the progression of DR in the non-proliferative stage. Search for novel treatment modalities has not been easy, as precise molecular mechanisms by which, metabolic risk factors accelerate retinal damage are not fully elucidated. In particular, the early changes in the neurovascular unit are not well described.
Materials and methods: To study early effects of energy deficits, we developed a model of starvation exposure in retinal cultures from E18.5 embryos. We used short-term (6 hours) exposure to starvation in the *in vitro* model from the primary rodent retina. The retinal cells were after that cultured under normal physiological conditions in order to investigate long-term changes. The effects were assessed on the mRNA levels of genes involved in growth and glucose metabolism. Starvation-induced changes in transcriptomics and morphology of neurons were studied.
Results: As a validation of the model, RT-QPCR analyses showed that, immediately after starvation (short-term effects), the mRNA expression of *Vegf* and *Vegfr2* ($p < 0.05$) (expressed on neurons) was significantly upregulated compared to non-starved control retinal cultures. When starved embryonic retinal cultures were cultured for 6 days under normal physiological conditions (long-term effects), *VegfA* expression remained elevated ($p < 0.01$), while *Vegfr2* was significantly reduced ($p < 0.01$). Short-term exposure to starvation resulted in upregulation of *Glut1*, *Hif1a*, *Ppargc1a* and *Ppargc1b* genes involved in glucose, hypoxia and mitochondrial metabolism ($p < 0.01$), while downregulation of *Txnip* as compared to control retinal cultures ($p < 0.01$). After exposure was removed, expression of *Hif1a*, *Ppargc1b* and *Txnip* returned to that observed in controls, but *Ppargc1a* was significantly downregulated ($p < 0.01$). We also measured the lengths of axons in the retinal cultures. Short-term exposure had significant inhibiting effect on the length of the longest neurite (by 11 nm), while long-term effects showed significantly shorter mean total axonic length of starved retinal neurons (by 3.7 nm) ($p < 0.01$).
Conclusion: Our results demonstrate that an *in vitro* model of embryonic retinal culture, exposed to starvation may unravel early mechanisms involved in the pathogenesis of diabetes retinopathy.
Supported by: SRC-VR, NNF, UiB and BRF
Disclosure: **R. Jain:** None.

PS 091 Biomarkers of nephropathy

995

Biomarkers associated with early stages of kidney disease in adolescents with type 1 diabetes

M.L. Marcovecchio[1], M. Colombo[2], R.N. Dalton[3], P.M. McKeigue[2], H.M. Colhoun[4], D.B. Dunger[1];
[1]Department of Paediatrics, University of Cambridge, Cambridge, [2]Usher Institute for Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, [3]Guy's and St Thomas' NHS Foundation Trust, London, [4]MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK.

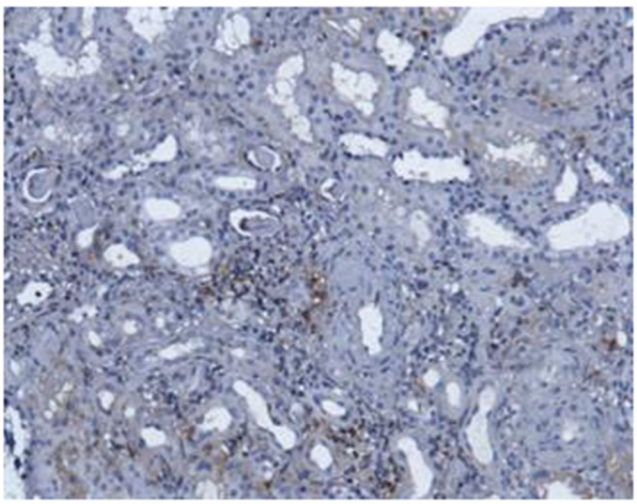
Background and aims: To identify biomarkers associated with glomerular filtration rate (GFR) and urinary albumin-creatinine ratio (ACR) in a cohort of young people with type 1 diabetes (T1D) recruited into the Adolescent Type 1 diabetes cardio-renal Intervention Trial (AdDIT).
Materials and methods: Biomarkers were measured in non-fasting blood samples collected at the baseline AdDIT visit from 553 adolescents (45.2% females) with a median [interquartile range] age of 13.9 [12.6, 15.2] years and diabetes duration of 5.4 [3.4, 8.2] years, using the Myriad RBM platform (25 biomarkers). Participants were followed for a median of 3.9 [3.1, 4.1] years with 6-monthly visits including collection of clinical data and blood and urine samples. Estimated GFR (eGFR) was calculated with a modified Schwartz formula. eGFR trajectories were calculated for each participant by a linear slope and two categories were defined: rapid decliners, including participants with eGFR slopes <−3 ml/min/1.73 m^2 per year; and rapid increasers for participants with eGFR slopes >5 ml/min/1.73 m^2 per year. ACR was calculated as the geometric mean of three early morning urine samples and microalbuminuria defined as an ACR >3.5 (males) or >4 (females) mg/mmol in at least 2 out of three samples. Associations between individual biomarkers and eGFR and ACR were assessed using regression models adjusted for age, sex, diabetes duration (and eGFR when modelling ACR).
Results: At baseline median ACR was 1.1 [0.7, 1.5] mg/mmol and 96.4% of the study participants were normoalbuminuric. Median eGFR was 133 [119, 151] ml/min/1.73 m^2 and eGFR slopes −4.0 [−7.1, −0.5] ml/min/1.73 m^2; 46.8% of the study participants had a baseline eGFR in the hyperfiltration range (>135 ml/min/1.73 m^2). After setting a Bonferroni-corrected significant threshold of 2.0×10^{-3}, three biomarkers: Trefoil factor 3, Cystatin C and Beta-2 microglobulin were independently associated with eGFR at baseline (B coefficient [95%CI]: −0.19 [−0.27, −0.12], $p = 7.0 \times 10^{-7}$; −0.18 [−0.26, −0.11], $p = 5.1 \times 10^{-6}$; −0.12 [−0.20, −0.05], $p = 1.6 \times 10^{-3}$, respectively). Osteopontin was significantly associated with eGFR slopes (−0.25 [−0.34, −0.16], $p = 4.5 \times 10^{-8}$) as well as with the rapid decliners category (OR: 1.80 [1.40, 2.37], $p = 9.3 \times 10^{-6}$), whereas Fibroblast Growth Factor 23 (FGF23) was significantly associated with the rapid increasers category (1.74 [1.29, 2.34], $p = 2.2 \times 10^{-4}$). No significant associations were found between any of the measured biomarkers and ACR at baseline or its changes during follow up.
Conclusion: In this young population with T1D and high rates of hyperfiltration, Trefoil factor 3, Cystatin C and Beta-2 microglobulin were associated with eGFR at baseline, whereas FGF3 was associated with eGFR decline over time and Osteopontin with eGFR increases. In contrast, in this cohort predominantly normoalbuminuric there were no significant associations with ACR, in contrast to previous results in adult cohorts with T1D.
Clinical Trial Registration Number: NCT01581476
Supported by: JDRF
Disclosure: **M.L. Marcovecchio:** None.

996

Urinary exosomal CCL21 mRNA as biomarker of diabetic nephropathy

Y. Feng, L. Lv, W. Wu, X. Zhong, B. Liu;
Zhong Da hospital, Southeast University, Nanjing, China.

Background and aims: Diabetic nephropathy (DN) is one of the common complications of diabetes characterized by variable histological changes and clinical course. Currently, renal biopsy and pathological assessment remain the standard approach in the diagnosis and prognosis of DN. Given the invasive procedures and unpredictable post-operative complications of biopsy, novel and noninvasive biomarkers are needed. We aimed to find noninvasive biomarkers reflecting the histological injury and progression of renal function in DN.
Materials and methods: A screening cohort of 4 biopsy-proven DN patients and 4 diabetic patients with normal renal function(DM) and a validation cohort of patients with 28 biopsy-proven DN patients and 24 DM patients were enrolled in our study. We isolated exosomes from urine samples at the time of renal biopsy. Urinary exosomes was identified by Western blotting (using Alix, CD63 and CD9 as exosomal markers). Kidney histological damage of DN patients was scored according to Tervaet standard. Urinary exosome profile of the packing inflammatory response related genes were assessed and its correlation with clinic and histological injury parameters were analyzed.
Results: Known exosome markers including Alix, CD63 and CD9 were identified by Western blotting. Profile of the packing inflammatory related mRNA revealed CCL-21 was remarkably upregulated in urinary exosomes of DN patients compared with DM patients ($p < 0.05$). Validation study confirmed the findings and found the correlation of CCL-21 with levels of proteinuria ($r = 0.590$, $p < 0.05$) and eGFR ($r = 0.591$, $p < 0.05$) in DN patients. Furthermore, CCL-21 was positively correlated with tubulointerstitial damage. DN patients with severe tubulointerstitial damge showed the highest expression of CCL-21 compared with DN patients with mild and moderate damage. Impressively, CCL-21 showed good performance in discriminating patients with different levels of tubulointerstitial damage.
Conclusion: In summary, urinary exosomal CCL-21 mRNA may be promising noninvasive biomarkers of diabetic nephropathy reflecting renal histological injury and renal function deterioration.



Clinical Trial Registration Number: 2017ZDSYLL107-Y01
Supported by: National Natural Scientific Foundation No.31671194
Disclosure: **Y. Feng:** None.

997

Differential expression of urinary exosomal microRNAs in type 2 diabetic kidney disease

J. Zang[1], D.A. Simpson[2], A.P. Maxwell[1], G.J. McKay[1];
[1]Centre for Public Health, Queen's University Belfast, Belfast, [2]Centre for Experiment Medicine, Queen's University Belfast, Belfast, UK.

Background and aims: Diabetic kidney disease (DKD) is a progressive microvascular complication that leads to a decline in renal function and is the most frequent cause of end stage renal disease. Currently, there is a need for improved biomarkers for the early detection of DKD. MicroRNAs (miRNAs) are short, non-coding regulatory RNA molecules that are commonly found in urinary exosomes and may reflect differential gene expression ongoing in the kidney during the disease process. We evaluated differential urinary exosomal miRNA expression in type 2 DKD (T2DKD) compared to control subjects with type 2 diabetes (T2D) and normal renal function.
Materials and methods: Exosomes were harvested from 1.1 ml of cell-free urine according to the protocol for miRCURY Exosome Urine Kit (Qiagen). RNAs were extracted using miRCURY RNA Isolation Kit (Qiagen) and cDNAs synthesised using Universal cDNA Synthesis Kit (Qiagen).Differential urinary exosomal miRNA profiles were evaluated in 14 participants with T2DKD and 15 age and gender matched individuals with T2D and normal renal function using miRCURY LNA miRNA Focus PCR Panel (Qiagen). The panels consisted of 87 miRNAs previously reported in human urinary exosomes. All miRNAs expressions were normalised using the reference genes selected by the NormFinder algorithm, using GenEx software for the analysis of quantitative PCR data. Statistical analyses were undertaken to compare the expression profiles between cases and controls using independent sample t tests and binary logistic regression to adjust for appropriate confounders (SPSS v.22).
Results: Quantitative expression data were normalised according to the NormFinder algorithm using the 3 most stably expressed miRNAs in the panels (miR-200b-3p, 30c-5p and 27b-3p). Urinary miR-21-5p, 23b-3p and let-7e-5p were significantly upregulated in T2DKD cases compared to T2D controls with good renal function ($P < 0.05$). Conversely, miR-30b-5p and 125b-5p expression were significantly lower in T2DKD cases compared to T2D controls with normal renal function ($P < 0.05$). In a binary logistic regression analysis adjusted for age, sex and mean arterial blood pressure, only miR-21-5p remained significantly associated with T2DKD (OR = 3.28, CI: 1.14–9.43; $P = 0.03$), while miR-30b-5p, 23b-3p, 125b-5p and let-7e-5p rose just above the established significance threshold ($P > 0.05$).
Conclusion: The results suggest miR-21-5p, 30b-5p, 125b-5p, 23b-3p and let-7e-5p are differentially expressed in individuals with T2DKD, although only miR-21-5p remained significant in the fully adjusted model. Further independent validation and replication are ongoing to confirm the role of these miRNAs in T2DKD aetiology, and to evaluate their sensitivity as potential biomarkers of T2DKD.
Supported by: CSC and NIKRF
Disclosure: **J. Zang:** None.

998

Serum mitochondrial inhibition activity is an independent risk factor for rapid decline of renal function: a Korean Genome Epidemiologic Study

H. Lee[1], S. Lee[1], J. Kim[1], H. Choi[2], Y.K. Pak[3];
[1]Internal Medicine, Eulji University Medical School, Seoul, [2]Internal Medicine, Kangwon National University Medical School, Chuncheon, [3]Physiology, KyungHee University Medical School, Seoul, Republic of Korea.

Background and aims: Serum contains persistent organic pollutants which act through aryl-hydrocarbon receptor (AhR) and inhibit mitochondrial function. Previously we reported serum mitochondrial inhibitor (MI) activity correlates with AhR bioactivity and insulin resistance. As insulin resistance is linked to the deterioration of renal function. We tested if AhR bioactivity or MI activity is associated with a decline of renal function.
Materials and methods: We measured serum AhR bioactivities and MI activities in 1,537 subjects participating Korean Genome Epidemiologic study. AhR bioactivity was measured with a novel cell-based assay using a cell line which is genetically modified to respond to AhR ligands. MI activity of serum was measured by intracellular ATP contents after treating cultured cells with serum. Excluding missing serum creatinine measurement at follow up, we analyzed 1456 subjects for their clinical parameters, specifically estimated glomerular filtration rate (eGFR), and tested if MI activity is associated with eGFR changes.
Results: Of 1456 participants, mean age was 60.3 years and 43.9% were men. Mean baseline eGFR was 80.7 ml/min/1.73 m^2. After 4 years' follow-up, mean eGFR change was −2.9 ml/min/1.73 m^2/year, and 229 (15.7%) people experienced rapid eGFR decline. In multivariate linear regression analysis, MI activity was positively associated with yearly eGFR change [beta 0.01, 95% confidence interval (CI) 0.00–0.02; $P = 0.008$]. In multivariate logistic regression analysis, the odds ratio of the first vs. forth quartile for rapid eGFR decline (eGFR decline more than 5 ml/min/1.73 m^2/year.) was 1.67 (95% CI 1.04–2.69, $P = 0.035$). Serum AhR bioactivity did not show any significant correlations, even though it correlated with MI activity.
Conclusion: Increased serum mitochondrial function inhibitor level was an independent risk factor for rapid eGFR decline, suggesting that increased MI activity is a novel risk factor for declining renal function. Further studies are needed to confirm and explore meanings of these results.
Supported by: HI14C2700 Korean Health Technology R&D Project (to YKP)
Disclosure: **H. Lee:** None.

999

Markers of collagen formation and degradation reflect renal function and predict adverse outcome in type 1 diabetes

S. Pilemann-Lyberg[1], T.W. Hansen[1], N. Tofte[1], S.A. Winther[1], S. Theilade[1], D.G.K. Rasmussen[2], S.H. Nielsen[2], M.A. Karsdal[2], F. Genovese[2], P. Rossing[1];
[1]Steno Diabetes Center Copenhagen, Soeborg, [2]Nordic Bioscience, Herlev, Denmark.

Background and aims: Type 1 diabetic (T1D) patients have higher risk of developing chronic kidney disease (CKD), cardiovascular events (CVE) and mortality than the general population. We hypothesize that PRO-C6, a product generated during collagen type VI formation, and C3M, collagen type III degradation fragments, may be associated with renal function and have prognostic value for adverse outcome in patients with T1D.
Materials and methods: PRO-C6 and C3M in serum (sPRO-C6; sC3M) and urine (uPRO-C6; uC3M) was measured by ELISAs in 663 patients with T1D and various degrees of albuminuria ranging for normal albuminuria (<30 mg/24 h) to macroalbuminuria (≥300 mg/24 h). In 2016, patients were traced through the Danish National Death Register and the Danish National Health Register, from which data for CVE and mortality are gathered. Estimated glomerular filtration rate (eGFR) was calculated based on data from outpatient visits. Endpoints: mortality from all causes, CVE (cardiovascular death, non-fatal myocardial infarction, non-fatal stroke and coronary or peripheral arterial interventions) and eGFR-decline of ≥30%. Median follow-up ranged from 5.1 to 6.2 years. Cross sectional associations were analyzed with linear regression, and the follow-up data with Cox regressions models. Adjustment included sex, age, LDL cholesterol, smoking, HbA_{1c}, systolic blood pressure, urinary albumin excretion rate (UAER) and baseline eGFR.
Results: Of the 663 participants, 384 (56%) were male; mean ± SD age was 55 ± 13 years and baseline eGFR 81 ± 26 ml/min/1.73 m^2. Median (interquartile range) baseline UAER was 17 (8–68) mg/24-h. Higher sPRO-C6, uPRO-C6 and sC3M were at baseline associated with lower eGFR and higher UAER in both the unadjusted and adjusted analysis ($p \leq 0.007$). Higher uC3M was associated with higher eGFR and lower UAER ($p < 0.001$), but the association with UAER was lost after

adjustment. During follow up higher sPRO-C6 was associated with higher risk of mortality ($n = 58$) in both the unadjusted and adjusted analysis (HR (95% CI): 2.33 (1.37–3.96), $p \leq 0.002$). Higher sC3M was only associated with mortality in the unadjusted analysis (HR (95% CI): 2.07 (1.26–3.43), $p = 0.004$). uPRO-C6 and uC3M was not associated with mortality ($p \geq 0.07$). Higher sPRO-C6 and sC3M was associated with higher risk of CVE ($n = 94$) and eGFR-decline ≥30% ($n = 93$) in the unadjusted analysis (HR (95% CI): ≥1.81 (1.44–2.28), $p \leq 0.003$), but the associations were lost after adjustment ($p \geq 0.19$). Higher uPRO-C6 was associated with higher risk of eGFR-decline ≥30% in the unadjusted analysis (HR (95% CI): 1.30 (1.09–1.56), $p = 0.0037$), after adjustment higher uPRO-C6 was associated with lower risk of eGFR-decline ≥30% (HR (95% CI): 0.80 (0.65–0.98), $p = 0.032$). Higher uC3M was associated with lower risk of eGFR-decline ≥30% in both unadjusted and adjusted analyses (HR (95% CI) ≤0.69 (0.49–0.97), $p \geq 0.035$).

Conclusion: In type 1 diabetic patients, higher levels of serum markers representing collagen type VI formation (PRO-C6) and collagen type III degradation (C3M) were independently associated with presence of diabetic kidney disease. Moreover, higher serum PRO-C6 was an independent predictor of mortality. U-PRO-C6 and uC3M was associated with lower risk of decline in GFR.

Disclosure: **S. Pilemann-Lyberg:** None.

1000

HDL-cholesterol but not triacylglycerol variability is associated with progression of diabetic nephropathy in type 1 diabetes

F.J. Jansson[1,2], C. Forsblom[1,2], E.H. Dahlström[1,2], L.M. Thorn[1,2], V. Harjutsalo[1,3], N. Elonen[1,2], N. Sandholm[1,2], P.-H. Groop[1,2], The FinnDiane Study Group;
[1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, [2]Abdominal Center Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, [3]The Chronic Disease Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland.

Background and aims: Lately, a growing interest in the association between diabetic nephropathy (DN) and intraindividual variability of clinical parameters has emerged, with the impact of glycaemic instability receiving special focus. Abnormal lipid levels are known to predict both development and progression of renal complications in type 1 diabetes. We aimed to assess whether higher intraindividual variability of HDL-cholesterol and triacylglycerol add to this risk.

Materials and methods: To this prospective observational study, adults with type 1 diabetes were recruited from the nationwide, multicentre Finnish Diabetic Nephropathy Study. Progression of DN was defined as change to a more advanced level of albuminuria or as initiation of renal replacement therapy. Serial HDL-cholesterol and triacylglycerol measurements until progression or most recent date of sustained renal status were covered. Variability was assessed using coefficient of variation (CV, standard deviation/mean) in individuals with ≥3 lipid measurements and verified renal status (normal albumin excretion rate/micro-/macroalbuminuria) at baseline. Thus, 3,015 individuals (median 9 measurements during 10.1 years of follow-up) and 2,820 individuals (median 8 measurements during 10.3 years of follow-up) were included in analyses concerning HDL-cholesterol and triacylglycerol variability, respectively. To avoid overfitting, an Akaike-based fast-backward variable elimination method was implemented to reduce the number of covariates in the final Cox proportional hazards model.

Results: Intraindividual variability of HDL-cholesterol was higher among individuals experiencing progression of DN (CV 17.5 ± 7.9%) compared to those with sustained renal status (15.1 ± 6.6%), $p < 0.001$. After adjusting for covariates in the final model (BMI, use of RAAS-inhibitor, baseline triacylglycerol, HbA_{1c}, eGFR, and history of smoking), the hazard ratio for progression was 1.16 (95%CI 1.02, 1.33) for 10 percentage point increase in HDL-cholesterol variability. An independent association persisted even after inclusion of HDL-cholesterol level. Similarly, individuals with sustained renal status showed less variation of triacylglycerol (CV 29.8 ± 14.5%) than individuals who progressed (33.0 ± 17.5%), $p < 0.001$. However, in multivariable analyses comprising triacylglycerol serial mean, no association between variability and the outcome was observed.

Conclusion: In type 1 diabetes, intraindividual variability of HDL-cholesterol is associated with progression of DN, even after adjusting for HDL-cholesterol levels. We also observed higher triacylglycerol variability among progressors, however, variability does not appear to add to the robust predictive value of single-measured triacylglycerol or triacylglycerol serial mean.

Supported by: Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation

Disclosure: **F.J. Jansson:** Grants; Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation. Honorarium; P-HG has received lecture honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi. Lecture/other fees; P-HG is an advisory board member of AbbVie, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi.

1001

Collagen type III degradation is associated with deterioration of kidney function in patients with type 2 diabetes with microalbuminuria

M. Frimodt-Moller[1], F. Genovese[2], T.W. Hansen[1], D.G.K. Rasmussen[2], S.H. Nielsen[2], H. Reinhard[1], B. von Scholten[1], F. Persson[1], P.K. Jacobsen[1], H.-H. Parving[3], M.A. Karsdal[2], P. Rossing[1,4];
[1]Steno Diabetes Center, Gentofte, [2]Nordic Bioscience, Herlev, [3]Department of Medical Endocrinology, Rigshospitalet, Copenhagen, [4]University of Copenhagen, Copenhagen, Denmark.

Background and aims: In diabetes one of the main features of the progression to diabetic kidney disease is a pathological deposition of extracellular matrix components triggering renal fibrosis. The main structural component of the fibrotic core is collagen. One of the most prominent collagens is type III (COL III), which is excessively synthesized and incorporated into the fibrotic extracellular matrix. Multiple studies in both humans and mice have suggested that MMP-9 activity is increased in diabetic kidney disease. We investigated whether a neo-epitope fragment of COL III generated by MMP-9 (C3M) was associated with deterioration of kidney function in a well-characterised type 2 diabetic population with microalbuminuria and without symptoms of coronary artery disease.

Materials and methods: The cohort included 200 participants, followed for 6.1 years. We measured C3M levels in serum (S-C3M) and urine (U-C3M) at baseline. To adjust for urine output, levels of U-C3M were normalized for urinary creatinine. The investigated endpoint was a decline in eGFR of more than 30% ($n = 42$). Cox proportional hazards regression analysis was performed for S-C3M and U-C3M both unadjusted and adjusted for traditional risk factors (sex, age, systolic blood pressure, LDL-cholesterol, smoking, HbA_{1c}, creatinine and urinary albumin excretion rate). To assess whether S-C3M or U-C3M improved risk prediction beyond traditional risk factors we calculated the relative integrated discrimination improvement (rIDI).

Results: The hazard ratio per doubling of S-C3M was 3.00 (95% CI 1.52–5.90, $p = 0.002$) in unadjusted analyses and 2.84 (95% CI 1.35–5.97, $p = 0.006$) after adjustment. Addition of S-C3M to a model containing traditional risk factors improved the rIDI by 19.8 percentage points ($p = 0.007$). U-C3M was not associated with declining eGFR.

Conclusion: S-C3M was independently associated with decline in renal function, and added significant improved discriminatory power to a model containing traditional risk factors.

Disclosure: **M. Frimodt-Moller:** None.

1002

C-type natriuretic peptide as a candidate marker of diabetic kidney disease

H. Lunt[1,2], T. Prickett[2], J. Warwick[1], H. Heenan[1], E. Espiner[2];
[1]Christchurch Hospital, Christchurch, [2]Medicine, University of Otago, Christchurch, New Zealand.

Background and aims: Our current clinical predictor of early diabetic kidney disease, urinary albumin, lacks sensitivity and specificity. Better markers of early kidney injury are therefore needed. One such candidate marker is C-type natriuretic peptide (CNP), as increased expression of CNP within the kidney is reportedly an early response to renal injury. CNP is a member of a family of structurally related peptides known for their roles in electrolyte homeostasis, fluid balance and chondrogenesis, which also have anti-inflammatory actions. CNP is synthesized as a precursor (proCNP) which is cleaved to release CNP and the bio-inactive amino-terminal peptide fragment (NTproCNP). We have recently identified an intact form of NTproCNP in urine collected from diabetic participants with impaired renal function which was largely absent from urine obtained from healthy participants without diabetes. This study aimed to evaluate the utility of a urine NTproCNP as a marker of early renal disease in participants with diabetes.
Materials and methods: Adult participants with established (>5-year duration) T1DM (Type 1 diabetes), or with T2DM (Type 2 diabetes) were recruited from diabetes outpatient clinics at Christchurch Hospital, New Zealand. Participants were selected to ensure that a broad range of eGFR and albumin creatinine ratio (ACR) values were present, in both T1DM and T2DM subgroups. Exclusions included prior bariatric surgery, life threatening co-morbidities and serum creatinine >250 μmol/L. Along with demographic and clinical data, blood was drawn (non-fasting) for HbA1c, serum creatinine and NTproCNP. A spot urine sample was obtained for ACR and intact NTproCNP creatinine ratio (NCR). A second urine sample was collected within three weeks to assess replication. Urine NTproCNP was analysed by a radioimmunoassay that specifically measures intact NTproCNP in urine.
Results: To date 59 T1DM (median age 54 years) and 80 T2DM (median age 64 years) participants have been recruited. The median HbA1c (mmol/mol) for T1DM participants of 63 (53–72, IQR), was lower than the median HbA1c of 70 (58–89) for T2DM participants ($p = 0.026$). Median ACR (mg/mmol) was also lower in T1DM participants when compared to T2DM participants; 1.1 (0.6–5.0) compared to 5.3 (1.1–50), $p = 0.009$. When considering results from all participants combined, the coefficients of variation for repeat spot urine measurements for NCR and ACR were 33% and 51% respectively. Median NCR was 0.22 (0.17–0.27) pmol/μmol in participants with impaired eGFR (<60 ml/min/1.73 m^2) compared to 0.13 (0.08–0.20) pmol/μmol in participants with normal, or near-normal, eGFR (>70 ml/min/1.73 m^2), $p = 0.008$. The significant inverse association of NCR with eGFR (Spearman's rho, $r = -0.36$) was stronger than that observed between ACR and eGFR; $r = -0.17$. When considering T1DM and T2DM participants separately, the significant inverse association of NCR with eGFR was observed in both these subgroups ($r = -0.34$ for both subgroups), whereas the relationship between ACR and eGFR was $r = -0.09$ and -0.15 respectively.
Conclusion: These preliminary results support the hypothesis that urine NTproCNP excretion reflects declining renal function and may be superior to urinary albumin excretion, as a marker of kidney injury in diabetes.
Supported by: NZSSD
Disclosure: **H. Lunt:** Grants; New Zealand Society for the Study of Diabetes and Diabetes Christchurch (New Zealand).

PS 092 Diabetic nephropathy: Predictions are hard to make

1003

Prediction models for the risk of developing nephropathy in people with type 2 diabetes. A systematic review

A.A. van der Heijden, M. Gort, P.J.M. Elders, G. Nijpels, J.W.J. Beulens;
VUMC University Medical Center, Amsterdam, Netherlands.

Background and aims: Early detection and treatment of nephropathy in people with type 2 diabetes (T2D) can be facilitated by prediction models. We aimed to identify all prediction models for nephropathy applicable to people with T2D and to assess their quality and accuracy.
Materials and methods: A systematic search was performed in PubMed and Embase in February 2017. A study was included when (I) the model was applicable to people with T2D, (II) any stage of nephropathy was the outcome, and (III) the follow-up of the development study was at least one year. Exclusion criteria were: (I) not written in English, (II) a study population with other serious physical conditions or a post-surgical population, (III) not an original model, or (IV) reporting a univariable model. Screening, full-text assessment, data-extraction (CHARMS-checklist) and risk of bias assessment (PROBAST-tool) was performed independently by two reviewers. Performance of the models was evaluated by discrimination (ability to identify those at risk), and calibration (ability to quantify the absolute risk).
Results: Of the 8,339 studies, 25 met the inclusion criteria. These studies accounted for 36 prediction models, of which 23 were based on a T2D population, and 13 on a general population and included T2D as a predictor. Only ten of the models were externally validated. The models showed poor to good discrimination during internal validation (range C-statistic: 0.59–0.97) and external validation (range C-statistic: 0.67–0.95). Calibration of the models ranged from poor to excellent in internal validation and from poor to good in external validation. Overall higher C-statistics were reported in models based on the general population. Risk of bias could be present in the models due to the lack of adjustment for model overfitting and optimism, and inappropriate handling of missing data. The models described by Schroeder et al and Elley et al showed good performance using routinely obtained patient information.
Conclusion: A high number of nephropathy prediction models are available. Less than one third of these models were externally validated, showing variable results, indicating the need for external validation of these models. The models by Schroeder et al and Elley et al seem promising considering their study quality, performance and simplicity.
Supported by: Dutch Diabetes Research Foundation
Disclosure: **A.A. van der Heijden:** None.

1004

Blood pressure response to RAAS inhibition predicts all-cause mortality for individuals with type 1 diabetes and albuminuria

R. Lithovius[1,2], S. Mutter[3], V.-P. Mäkinen[3], P.-H. Groop[1,2];
[1]Folkhälsan Institute of Genetics, Helsinki, Finland, [2]Abdominal Center Nephrology, Helsinki, Finland, [3]South Australian Health & Medical Research Institute, SAHMRI, Adelaide, Australia.

Background and aims: As type 1 diabetes usually manifests within the first two decades of life, the affected individuals are at high life-time risk to develop diabetic kidney disease that can substantially lower their quality of life and shorten their life span. No targeted cure exists for diabetic kidney disease, but clinical trials have shown the benefits of inhibiting the renin-aldosterone-angiotensin system (RAAS) to slow down or even halt disease progression. However, there might be patients whose RAAS treatment could be optimised to achieve better long-term outcomes. Therefore, accurate information on how patients respond to RAAS inhibition in real-world settings is highly valuable.

Materials and methods: The present pilot study is part of the Finnish nationwide FinnDiane Study. We included 287 patients with type 1 diabetes who had been prescribed a RAAS inhibitor for the first time (index date) between 1996 and 2013. Patients were then followed up to the first BP measurement after the index date (0.9 years ± 0.6 years). Inclusion criteria were as follows: index date after joining the FinnDiane study, no RAAS prescriptions ≥3 years before index date, at least one RAAS prescription for every six months after the index date, BP measured in the two years before and after the index date, and albuminuria status and HbA1C measured before and after the index date. Patients were classified into two groups: normal urinary AER (AER <20 μg/min or AER <30 mg/24 h) or albuminuria above those thresholds (end-stage renal disease excluded). A beneficial BP response was defined as a reduction in BP below 140/90 mmHg, otherwise the BP response was considered poor. In a Cox regression analysis, patients were further followed up (9.6 years ± 3.9 years) until death (19 events) or until the end of 2015.
Results: After the initiation of RAAS blockade, systolic BP was reduced by 6 mmHg and diastolic BP by 3 mmHg. Overall, 111 (39%) patients achieved a beneficial BP response. We compared three groups: patients with normal AER ($n = 201$), patients with albuminuria and a beneficial BP response ($n = 33$) and patients with albuminuria and poor response ($n = 53$). Only patients with albuminuria and poor BP response showed higher all-cause mortality (HR = 3.41 [95% CI 1.27, 9.18], $p = 0.02$) with respect to the normal AER group, whereas no excess mortality was observed in those with a beneficial BP response (HR = 2.11 [0.56, 7.97], $p = 0.27$). Albuminuria and a poor BP response was associated with a younger age of diabetes onset (11.8 vs. 17.0 and 17.9 years, $p = 0.02$) and a higher systolic BP (150 vs. 135 and 148 mmHg, $p < 0.01$) at index date compared to the beneficial BP response and the normal AER groups. There were no differences between the three groups in age, diastolic BP and HbA1C at index date, or in BMI, WHR and sex at the study enrolment.
Conclusion: The initial BP response in individuals with type 1 diabetes and albuminuria predicts all-cause mortality. Therefore, poor BP response is an important predictive biomarker for diabetic kidney disease. Further research on the causes for this phenomenon is warranted to find more effective means to protect the kidneys.
Disclosure: **R. Lithovius:** None.

1005

Sex differences in outcomes in treated diabetic kidney disease

B. Fernandez-Fernandez, M. Sanchez-Niño, A. Ortiz;
Nephrology and Hypertension department, IIS-Fundación Jiménez Díaz-Universidad Autónoma de Madrid, Madrid, Spain.

Background and aims: There is recent interest and controversy on the reasons behind the lower incidence of renal replacement therapy in women, despite a higher prevalence of chronic kidney disease (CKD). Being diabetic kidney disease (DKD) the most frequent cause of end-stage kidney failure, exploring the impact of sex on DKD progression under routine clinical practice conditions may contribute to solve the controversy. The aim was to analyze the progression of CKD and predictive factors in men and women with diabetes and CKD.
Materials and methods: Prospective cohort study of diabetic patients referred to an outpatient diabetic CKD clinic in a tertiary hospital within a healthcare system without limitations in access to nephrological care, and treated according to existing guidelines.
Results: 261 new patients were referred to the DKD clinic, two thirds of them men. Women had lower albuminuria (99.5 [15–403] vs 187 [50–592] mg/g, p 0.013) and were more frequently non-smokers (71/91, 78.0% vs 37/170, 21.9%, $p < 0.001$), but did not differ from men in other parameters such as age and baseline glomerular filtration rate (eGFR). Over a mean follow-up of 30 ± 10 months, eGFR changed by −1.2 [−4.6; 2.3] ml/min/1.73 m²/year in men and −0.8 [−4.1; 3.5] ml/min/1.73 m²/year in women. However, 48/261 (18%) patients experimented rapid progression, defined as loss of ≥5 ml/min/1.73 m²/year over the follow-up period. Women represented 15/48 (31%) of rapid progressors and 70/199 (35%) of non-progressors (ns). Albuminuria remained stable in men changing by 3.1 [−7.3; 20.3] %/year, but decreased in women, changing by −4.8 [−12.9; 7.4] %/year. The best multivariate model to predict rapid progression in men and women differed. UACR and Fractional excretion of phosphate were part of the best multivariable model predicting rapid progression in men (AUC 0.92, 95% CI 0.85 to 0.98), but were absent from the best model for women (AUC 0.90, 95% CI 0.8 to 1). The AUC for predicting rapid progression by UACR was 0.71 (95% CI 0.61 to 0.81) for men and 0.62 (95% CI 0.43 to 0.82, thus overlapping the 0.50 mark) for women, with cut-off points of 811 and 213 mg/g respectively. Around 20% of women rapid progressors had UACR >300 mg/g, vs 36% of men rapid progressors. Additionally, in women, the percentage of rapid progressors with UACR<30 mg/g was significantly higher than in men (33% vs 3%; $p = 0.0085$).
Conclusion: In women with DKD treated according to current guidelines, CKD progression is slower overall than in men and albuminuria decreases over time. However, the proportion of rapid progressors was similar in both sexes, although factors predicting rapid progression differed: albuminuria was a better predictor in men than in women. This information may be used to enrich clinical trials in rapid progressors and in women with high risk of progression.
Supported by: JR14/00028
Disclosure: **B. Fernandez-Fernandez:** None.

1006

Insulin resistance predicts incident hypertension and increasing systolic blood pressure in a Swedish cohort study

B. Daka[1], K. Ottarsdottir[1], L. Råstam[2], M.I. Hellgren[1], U. Lindblad[1];
[1]Department of Public Health and Community Medicine, Medicine, Göteborg, [2]Department of Clinical Science, Medicine, Malmö, Sweden.

Background and aims: To investigate whether insulin resistance predicts increase in systolic blood pressure and the development of hypertension in a representative cohort in Sweden.
Materials and methods: Between 2002–2005 a representative cohort based on census data was defined and 2816 ($M = 1400$) subjects (76% participation rate at baseline) were included in a survey. Data regarding lifestyle, socio-economy, stress and depression were collected using validated questionnaires. During 2012–2014 a follow-up visit was completed in 1327 ($M = 657$) (70% participation rate at visit 2) subjects consecutively recruited and similar protocols were used. Blood pressure was measured at baseline and follow up and hypertension was defined according to Joint National Comittee 7 (JNC 7) guidelines. An oral glucose tolerance test was completed at baseline and at follow-up in subjects without diabetes and diabetes was defined based on WHO recommendations 1999. Insulin resistance was defined based on homeostatic model assessment of insulin resistance (HOMA-Ir) both at baseline and at follow-up. Morning fasting blood samples were collected in all participants and stored in biobank. Multivariable logistic regressions were computed to investigate how HOMA-Ir at baseline predicts incident cases of hypertension during follow-up. Multivariate linear regressions were also computed to investigate how the change in HOMA-Ir influenced the change in systolic blood pressure during follow-up. Subjects with hypertension and or diabetes mellitus at baseline (206) and those with lack of information regarding HOMA-Ir (40) were excluded from all analysis while 1081 subjects participated in this study. All analyses were computed in IBM SPSS version 24. The interaction between sex and HOMA-Ir on incidence of hypertension and the increase of blood pressure was investigated and no significant interaction were found ($p = 0.661$). Thus, the analyses were computed for all subjects and were adjusted for sex.
Results: During the follow up of 9.6 years (SD 1.4) 156 new cases of hypertension were registered (15%), systolic blood pressure increased by 5.9 (SD 12) mmHg in the overall population and HOMA-IR increased by

12% in average. In age adjusted cross-sectional linear regression analyses HOMA-Ir was strongly associated with systolic blood pressure at baseline ($B = 1.2$ CI 0.7–1.6 $p < 0.001$) and at follow-up ($B = 1.5$ CI 0.9–2.0 $p < 0.001$). HOMA-Ir at baseline predicted the development of hypertension independent of age, sex, waist hip ratio, systolic blood pressure at baseline (OR = 1.27 CI 1.02–1.59). The change in HOMA-Ir was strongly associated with change in systolic blood pressure regardless of age, sex, systolic blood pressure, HOMA-Ir, waist hip ratio at baseline ($B = 0.8$ CI 0.3–1.3 $p = 0.002$).

Conclusion: Insulin resistance predicts the development of hypertension and the increase of blood pressure. Lifestyle intervention to improve insulin sensitivity may be effective to lower blood pressure and to prevent hypertension.

Supported by: Regionala FoU-medel Västra Götalandsregionen

Disclosure: **B. Daka:** None.

1007

Insulin resistance prognosticate kidney disease in individuals without diabetes

M.I. Hellgren, B. Daka, U. Lindblad;
Department of Public Health and Community Medicine/Primary Health Care, Institution of Medicin, Gothenburg, Sweden.

Background and aims: Renal disease is one of the major complications in patients with Diabetes Mellitus (DM). The aim of this study is to explore whether insulin resistance affects renal function even before the development of manifest DM in men and women.

Materials and methods: In the Vara Skövde Cohort 2816 randomly selected individuals (men 49.7%) were carefully examined with blood-pressure, body weight and height, waist-circumference and fasting venous samples, drawn in the morning for serum concentrations of insulin, cholesterol, HDL, LDL and triglycerides. All participants also completed an oral glucose tolerance test (OGTT). Questionnaires concerning social life and lifestyle were completed. After ten years (9.7 years SD 1.4), a total of 1327 of the original participants consequently recruited were re-examined following the same protocol as at base-line. EGFR was calculated using CKD-EPI formula. Renal status was defined according to KDIGO taking both eGFR and albuminuria into account. According to the KDIGO classification kidney impairment is divided into four different categories depending on risk for kidney failure; normal, mild/moderately increased risk, high risk and very high risk. In this study, kidney impairment was defined as at least moderately increased risk of chronic kidney disease (eGFR ≤60 ml/min/1.73 m^2 or albuminuria 30–300 mg/g 3–30 mg/mmol) and insulin resistance was defined based on homeostatic model assessment insulin resistance (HOMAir). Participants with manifest DM ($n = 58$), with missing data for creatinine or albumin excretion in urine ($n = 38$) or with impaired kidney function ($n = 44$) at baseline were excluded from the calculations in this study, leaving 1187 for the final analyses. Binary logistic regression was used to investigate the association of HOMAir at baseline and risk of CKD at follow-up.

Results: Mean age at baseline was 48.1 years (SD 11), mean eGFR 90 ml/min/1.73 m^2 (SD 13), mean body mass index 27 kgm^{-2} (SD 3) and mean systolic blood pressure 120/70 mm Hg (men 123/72 mmHg, women 118/68 mmHg). At follow-up 47 men and 37 women had developed moderately increased risk of CKD according to KDIGO. Insulin resistance predicted an increased risk for CKD in men when adjusted for age, eGFR, albumin/creatinine ratio, BMI and hypertension at baseline, OR 1.8 (CI 1.19–2.73; $P = 0.005$). Concerning women BMI seemed to be the decisive factor, OR 1.1 (CI 1.04–1.26; $P = 0.007$) and not insulin resistance OR 1.1 (CI 0.91–2.07; $P = 0.129$).

Conclusion: Insulin resistance in men and overweight in women are associated with an increased risk to develop kidney failure after 9.7 years and risk factors in individuals with prediabetes should thus be treated intensively.

Supported by: The Västra Götaland region, The scientific board of Sweden,

Disclosure: **M.I. Hellgren:** None.

1008

Renal resistive index predicts post-bariatric surgery renal outcome in severely obese non diabetic individuals

L. Giannini, M. Seghieri, E. Santini, F. Parolini, S. Taddei, R. Bruno, A. Solini;
University of Pisa, Pisa, Italy.

Background and aims: Bariatric intervention has shown positive effects on renal function in obese subjects, even though studies where glomerular filtration rate (GFR) and renal plasma flow (RPF) have been measured rather than estimated are scanty. Postoperatively, amelioration in subclinical atherosclerosis, arterial stiffness and endothelial function may also be observed; this positive systemic effect on vasculature might be beneficial at the renal level too, potentially improving GFR. Aims of this study were to demonstrate whether metabolic surgery is able 1) to improve GFR when measured by a gold-standard technique in morbidly obese non-diabetic individuals and 2) to identify clinical, renal and systemic vascular predictors of GFR improvement.

Materials and methods: Twenty-five severely obese subjects were studied before and one year after gastric bypass surgery. GFR and RPF were measured with iohexol clearance and hippurate infusion, respectively. By ultrasonographic technique, measurements of baseline (RI) and dynamic (DRIN) renal resistive indices, renal visceral fat and systemic vascular parameters were performed.

Results: Pre-intervention subjects (47 ± 13 years, 16 F, BMI: 44.8 ± 6.0 kg/m^2) had a measured GFR (mGFR) of 86.9 ± 15.2 ml/min/1.73 m^2 and an estimated GFR (eGFR, by CKD-EPI) of 100.8 ± 18.6 ml/min/1.73 m^2. At follow-up, BMI decreased by 31% and fasting plasma glucose from 5.42 ± 1.03 to 4.75 ± 0.47 mmol/L ($p = 0.041$). eGFR did not change significantly, while mGFR increased to 109.0 ± 18.2 ml/min/1.73 m^2, ($p < 0.001$), but only when expressed by BSA, and not by height. RPF did not vary (612 ± 170 *vs* 573 ± 181 ml/min, $p = 0.345$). RI decreased (0.62 ± 0.06 *vs* 0.59 ± 0.05, $p = 0.047$), while DRIN was unmodified. Peri and pararenal fat were also reduced (from 2.10 ± 0.65 to 1.27 ± 0.52 cm and from 1.77 ± 0.67 to 0.96 ± 0.41 cm, respectively; both $p < 0.01$). Systemic vascular data showed an improvement in endothelium-dependent function (flow-mediated dilation, FMD: 4.55 ± 2.56 to 6.90 ± 2.30%, $p < 0.01$), while brachial artery diameter and baseline and hyperemic shear rate were reduced; conversely, response to nitrates was unmodified. Systemic arterial stiffness improved, as aortic pulse wave velocity (PWV) was reduced (8.34 ± 1.13 to 7.40 ± 1.12 m/s, $p = 0.03$), even when covarying by mean BP ($p = 0.05$). Carotid IMT was significantly decreased, and all parameters of carotid elasticity were significantly improved. Searching for clinical predictors of mGFR improvement after bariatric surgery (ΔmGFR), younger age ($r = -0.734$, $p < 0.001$), lower baseline fasting glucose ($r = -0.599$, $p = 0.007$) and Hb1Ac ($r = -0.603$, $p = 0.017$) were significantly associated. Interestingly, ΔmGFR was not significantly related with either ΔBMI or basal BMI. Among vascular variables, a greater improvement in ΔmGFR was inversely correlated to smaller brachial artery ($r = -0.705$, $p = 0.005$), carotid diameter ($r = -0.573$, $p = 0.032$) and lower RI ($r = -0.562$, $p = 0.029$). Such significant correlations were maintained when GFR was indexed by height. None of the measures of adiposity at baseline was associated to ΔmGFR.

Conclusion: In obese subjects with fully preserved renal function, bariatric surgery induces an improvement of GFR, renal and systemic stiffness with no relevant effect on RPF. A better glucose control, younger age and lower renal intravascular resistance can predict such improved renal function, maximising such favourable effects in relatively young individuals with healthy arteries.

Supported by: Italian Society of Diabetes

Disclosure: **L. Giannini:** None.

1009

Higher pulse pressure predicts initiation of dialysis in Japanese patients with diabetes: analysis using a nationwide claim database

T. Osawa[1], K. Fujihara[1], M. Yamamoto[1], M. Harada[1], M. Ishizawa[1], H. Seida[2], N. Yamanaka[2], Y. Matsubayashi[1], S. Matsunaga[1], T. Yamada[1], H. Sone[1];
[1]Department of Internal Medicine, Niigata University Faculty of Medicine, Niigata, [2]Japan Medical Data Center Co., Ltd, Tokyo, Japan.

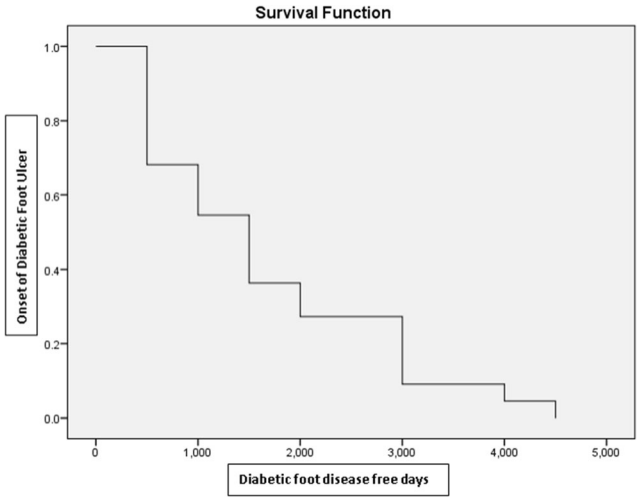
Background and aims: Since dialysis is known to predispose patients with diabetes to a lower quality of life as well as higher rates of cardiovascular events and mortality, identifying predictors for starting dialysis is of clinical relevance in diabetes management and care. However, only a few studies have investigated predictors for initiation of dialysis.
Materials and methods: To clarify this, data from a nation-wide claim database *involving* 18,935 *participants with diabetes during 2008–16 were analyzed.* We compared 2 groups (*with and without* dialysis) by a Kaplan-Meier curve and log-rank test. Multivariate Cox regression model *was used to identify variables related to* starting *dialysis*. Hazard ratios (HRs) were compared among 4 groups divided according to combinations of HbA1c (<8 or ≥8%), systolic blood pressure (SBP) (<140 or ≥140 mmHg) and pulse pressure (PP) (<60 or ≥60 mmHg) values.
Results: Baseline of the proportion of men, BMI, SBP, diastolic blood pressure (DBP), PP, HbA1c, and prevalence of coronary artery disease *were higher in the group who started dialysis than did not. The multivariate Cox model showed that PP was most significant and independent predictor of the initiation of* dialysis. *The* HRs for initiation of dialysis for each 1 SD elevation in SBP and 1 SD elevation in PP were 1.09 (95% CI 0.81–1.48) and 1.54 (1.14–2.08), respectively. Compared with HbA1c <8% and PP <60 mmHg, the HR for those with HbA1c ≥8% and PP ≥60 mmHg was 6.32 (3.42–11.7). On the other hand, compared with HbA1c <8% and SBP <140 mmHg, the HR for those with HbA1c ≥8% and SBP ≥140 mmHg was 4.26 (2.32–7.85).
Conclusion: These findings reveal that although SBP and PP were independent predictors for starting dialysis, PP was more potent. Especially, a high risk for starting dialysis was found for those with PP ≥60 mmHg along with HbA1c ≥8% which is useful for targeting high-risk patients.
Supported by: Japan Society for the Promotion of Science
Disclosure: **T. Osawa:** Grants; Japan Society for the Promotion of Science.

1010

Incidence, predictors, and clinical outcomes of new onset diabetic foot ulceration after renal transplantation

P.R.J. Vas[1], A. Sharma[2], S. Cohen[3], T. Patel[3], S. Thomas[3], J. Karalliedde[2];
[1]King's College Hospital, London, [2]King's College London, London, [3]Guy's and St Thomas' Hospital, London, UK.

Background and aims: Patients with diabetic kidney disease are at high risk of diabetic foot ulceration (DFU). Whether this risk is modified after renal transplant is unclear. Importantly, there is a paucity of information on the burden and the risk factors associated with new-onset DFU development after renal transplantation. We evaluated the incidence and predictors of new-onset DFU post renal transplantation in a single centre retrospective study. Patients who underwent renal transplantation for diabetic kidney disease between 2004–2016 were evaluated.
Materials and methods: In total 144 (66% male, 26% Type 1 DM) patients were evaluated. Median (range) of follow up was 6 (3 to 13) years. The median (range) age was 62 (28 to 80) years and duration of diabetes 23 (7–60) years. Electronic patient investigation records and podiatry medical notes were reviewed.
Results: Over the follow-up period, 22 (15%) patients developed a new DFU. Patients with a DFU were of similar age, body mass index, diabetes duration and had similar pre-transplantation haemoglobin, as compared to those without a DFU. Patents who developed a DFU were more likely to have Type 1 diabetes than type 2 diabetes (29% vs. 10%), history of peripheral vascular disease (PVD) [32% vs. 8%], had higher pre-transplantation HbA1c, mean ± standard deviation (7.5 ± 1.2% vs. 6.8 ± 1.4%) and serum creatinine (809 ± 243 μmol/l vs. 660 ± 202 μmol/l) $p < 0.05$ for all. Of the cohort, 8 patients had a history of DFU pre-transplantation and all 8 developed a new onset DFU post-transplantation. Median (range) duration of healing was 5 (1–26) weeks. Site, Ischemia, Neuropathy, Bacterial Infection, and Depth (SINBAD) classification score was <3 in 14 of the 22 DFU cases. Nearly 50% of all DFU occurred within the first 1000 days post-transplantation (Figure 1). Of the 22 cases, 6 needed a minor amputation; no major amputations were documented. Patients with DFU had more than a twofold increased risk of transplant failure as compared to those without DFU (50% vs 23.3% $p = 0.02$). Mortality was 27.3% in patients with DFU compared to 20.3% without DFU $p = 0.25$
Conclusion: Close to 1 in 7 patients post renal transplantation develop a new onset DFU. Type 1 diabetes, higher pre-transplantation HbA1c, serum creatinine, history of PVD and prior DFU are associated with increased risk of new-onset DFU post-transplantation. Importantly, in this group, DFU is associated with a nearly two-fold incidence of transplantation failure. When compared with UK national audit data healing time, rates and severity of ulcer at presentation are significantly better in our cohort. Nonetheless, our results indicate a high burden of DFU post-transplantation and emphasise the requirement for regular foot surveillance in this high-risk population.



Disclosure: **P.R.J. Vas:** None.

PS 093 Nephropathy: from markers to real life

1011

Plasma lipids are associated with diabetic kidney disease: a study of plasma lipidomics in type 1 diabetes

N. Tofte[1], T. Suvitaival[1], L. Ahonen[1], S. Theilade[1], S.A. Winther[1], M. Frimodt-Møller[1], T.S. Ahluwalia[1], P. Rossing[1,2];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]University of Copenhagen, Copenhagen, Denmark.

Background and aims: The pathophysiology of diabetic kidney disease (DKD) is incompletely understood. The aim of this study was to perform lipidomics analysis to, first, evaluate cross-sectional associations between plasma lipid levels and measures of DKD in type 1 diabetes (T1D) and, second, validate the strongest associations as predictors of the development of a combined kidney endpoint (CKE) in survival analysis.
Materials and methods: In total the study comprised 670 patients with T1D among which 312 had normoalbuminuria, 168 had microalbuminuria and 190 had macroalbuminuria. Mean ± SD baseline estimated GFR (eGFR) 82 ± 28 ml/min/1.73 m^2. Non-targeted lipidomics analyses were performed on plasma samples using ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry. Data were pre-processed with MZmine2. Lipids with a maximum of 20% missing values were included and imputed with k-nearest neighbour algorithm. Endpoints were traced through National Health and Death Registers and eGFR was obtained from electronic laboratory records. CKE was defined as the first event of ≥30% decline in eGFR, all-cause mortality or ESRD (chronic dialysis, kidney transplantation or GFR/eGFR <15 ml/min/1.73 m^2). Median follow-up was 5.2 years. Cross-sectional associations between single lipid species and low eGFR or categorical group (normo-, micro- and macroalbuminuria) were analysed by linear regression and analysis of covariance, respectively. Correction for multiple testing was done with the Benjamini-Hochberg method. In longitudinal data, adjusted HR for the development of CKE was calculated for the lipid species that had the strongest cross-sectional association to low eGFR and albuminuria, respectively, using the Cox proportional hazards model. All models were adjusted to age, gender, HbA1c, systolic BP, smoking, BMI, statin treatment, plasma triglycerides, total plasma cholesterol and, further, eGFR and/or urinary AER, where appropriate.
Results: A total of 121 lipids from 4 different lipid classes (phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), triacylglycerols (TGs), and sphingomyelins (SMs)) were qualified and included in the analyses. Cross-sectional data demonstrated both positive and negative associations between PCs and low eGFR ($p < 0.05$), whereas PCs were all decreased in macroalbuminuria vs. normoalbuminuria ($p < 0.05$). Several SMs and medium-chain-length TGs were positively associated to low baseline eGFR and decreased in macroalbuminuria vs. normoalbuminuria ($p < 0.05$). In the longitudinal validation of the top-ranking lipids from the cross-sectional analyses, the PC(O-36:2) and SM(d40:1), were associated with a decreased adjusted HR for the development of CKE ($n = 117$; HR: 0.73 (0.57–0.95); $p = 0.02$, and HR: 0.68 (0.52–0.88); $p = 0.004$, respectively).
Conclusion: Alterations in the plasma lipid levels were associated with decreased eGFR and macroalbuminuria in this T1D study cohort (after covariate adjustment). Further, the top-lipids from the cross-sectional analysis were validated for a longitudinal predictive association to the combined kidney endpoint. The results indicate broad changes in the lipidome in relation to the pathophysiology of DKD and suggest potentially protective lipid biomarkers.
Supported by: NNF14OC0013659
Disclosure: **N. Tofte:** Grants; Novo Nordisk Foundation grant NNF14OC0013659 "PROTON Personalising treatment of diabetic nephropathy".

1012

Apolipoprotein C3 in diabetic nephropathy in type 1 diabetes and its role in cardiovascular disease

L. Stechemesser[1], C. Forsblom[2,3], N. Tolonen[2,3], M.-R. Taskinen[4], R. Weitgasser[5], P.-H. Groop[2,3];
[1]Paracelsus Medical University - LKH, Salzburg, Austria, [2]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, Finland, [3]Abdominal Center Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [4]Heart and Lung Centre, University of Helsinki, Helsinki, Finland, [5]Department of Medicine, Diakonissen-Wehrle Hospital, Salzburg, Austria.

Background and aims: Apolipoprotein C3 (ApoC3) is a key regulator of triglyceride metabolism via its inhibitory effects on lipolysis and hepatic remnant uptake. Emerging evidence indicate that ApoC3 is an independent risk factor for cardiovascular events. The fact that glucose and insulin regulates ApoC3 expression raises the role of ApoC3 and cardiovascular risk in diabetes. We, therefore, investigated ApoC3 and its association with cardiovascular disease (CVD) in patients with and without diabetic nephropathy.
Materials and methods: This cross-sectional and prospective analysis was part of the prospective, ongoing Finnish Diabetic Nephropathy (FinnDiane) Study. Between 1994 and 2015 data were obtained from 3926 type 1 diabetes (T1D) patients at more than 80 hospitals or health centers across Finland. ApoC3 levels were explored by groups of albuminuria, CKD stages, presence of CVD as well as prediction of CVD and death. Survival curves were calculated by Cox regression analysis.
Results: At baseline, normo-, micro- and macroalbuminuria were present in 71.7%, 13.8% and 14.5% of the population ($n = 3926$, females 48%, age 37.8 ± 12.2 yrs, diabetes duration 23.2 ± 13 yrs, HbA1c 8.4 ± 1.5%). CKD stage 3–5 was diagnosed in 14.3%. Coronary heart disease or stroke (CVD) were present in 5.5% at baseline, while 16.3% developed CVD during 15-year follow-up. Compared to normoalbuminuria ApoC3 was elevated in the presence of micro- ($p = 0.013$) or macroalbumiuria ($p < 0.001$). Increasing ApoC3 levels were observed alongside progression of CKD stage ($p < 0.001$). Notably, higher baseline ApoC3 correlated with presence of CVD at baseline, development of CVD during follow-up and death. Differences in survival of those with the highest quartile of ApoC3 were independent of eGFR, triglycerides or HbA1c.
Conclusion: Baseline ApoC3 levels were elevated in T1D patients with micro- and macroalbuminuria, with impaired renal function, and with CVD. ApoC3 also predicted the development of CVD and death during follow-up.
Supported by: Folkhälsan Research Foundation
Disclosure: **L. Stechemesser:** None.

1013

Neutrophil elastase and myeloperoxidase mRNA levels are elevated in diabetic patients with overt nephropathy and are associated with albuminuria

S.K. Biswas[1], R.M. Rinta[1], F. Haider[1], M. Fariduddin[2], M.I. Arslan[1];
[1]Biochemistry and Molecular Biology, Bangabandhu S M Medical University, Dhaka, [2]Endocrinology, Bangabandhu S M Medical University, Dhaka, Bangladesh.

Background and aims: Neutrophil elastase (NE) and myeloperoxidase (MPO) enzymes are abundantly expressed by activated neutrophils and protect us from infection by killing pathogens. However, activation of these enzymes is known to induce oxidative stress, inflammation and tissue damage. Experimental study in mouse model of 5/6th renal ablation showed that the MPO contributes to the development and progression of chronic kidney disease. However, the contribution of NE and MPO in the pathogenesis and progression of diabetic nephropathy (DN) is not yet established. In the present study, NE and MPO mRNA levels, which are likely to be more sensitive than their circulating protein levels or

activities, were quantified in the peripheral blood leukocytes (PBL) in long-term diabetic patients. The aim of this study was to explore the association of DN with NE and MPO mRNA expressions in the PBL.

Materials and methods: A total of 70 diabetic patients with duration of diabetes >10 years were recruited and divided into 3 groups on the basis of urine albumin to creatinine ratio (ACR): normoalbuminuric or control (ACR <30 mg/g), microalbuminuric or incipient DN (ACR 30–299 mg/g) and macroalbuminuric or overt DN (ACR ≥300 mg/g). Diabetic patients with kidney failure and patients on dialysis were excluded. Total RNA from PBL was extracted and converted to cDNA. The mRNA levels of NE and MPO genes were quantified by real-time qPCR, analyzed by comparative Ct method and expressed as percentage of expression of reference gene beta-actin.

Results: The control ($n = 25$), incipient DN ($n = 27$) and overt DN ($n = 18$) groups were found similar in terms of age (mean ± SD, 53 ± 9 yrs), sex (m = 36, f = 34), body mass index (25.4 ± 3.6 kg/m^2), duration of diabetes (14 ± 2 yrs), fasting glucose (11.4 ± 4.2 mmol/L), lipid profile and leukocyte counts. Systolic BP and serum creatinine levels were found elevated in overt DN group compared to control group ($p < 0.05$). Urine albumin level [median (IQR)] was found 9 (5–16), 75 (43–168) and 1008 (402–1472) mg/L; and ACR was found 17 (7–20), 78 (55–201) and 829 (374–1790) mg/g for control, incipient DN and overt DN groups, respectively ($p < 0.001$). However, the NE mRNA level was found significantly elevated in overt DN group [1.40% (0.69–3.48)] compared to control [0.44 (0.12–1.10); $p = 0.015$] and incipient DN [0.43 (0.20–0.77); $p = 0.009$] groups. Similarly, the MPO mRNA level was also found significantly elevated in overt DN group [1.50 (0.55–2.58)] compared to control [0.27 (0.04–1.55); $p = 0.03$] and incipient DN [0.22 (0.04–1.08); $p = 0.008$] groups. However, neither NE nor MPO mRNA levels showed any significant difference between control and incipient DN groups. The NE mRNA level showed significant positive correlation with urine albumin level ($r = 0.39$, $p = 0.01$) and ACR ($r = 0.43$, $p = 0.007$). Similarly, MPO mRNA level showed significant correlation with urine albumin level ($r = 0.35$, $p = 0.03$) and ACR ($r = 0.42$, $p = 0.007$).

Conclusion: We showed here for the first time that the NE and MPO mRNA levels are elevated in the PBL in overt DN, but not in incipient DN, and are associated with albuminuria. This particular pattern of gene expression, in spite of cross-sectional nature of the study, suggests that increased expression of NE and MPO mRNA may be a consequence, rather than a cause, of DN and may contribute to the increased risk of cardiovascular disease in DN.

Supported by: HEQEP CP3073

Disclosure: **S.K. Biswas:** None.

1014

Sleep disordered breathing during rapid eye movement sleep and diabetic kidney disease in patients with type 2 diabetes

A. Nishimura[1], T. Kasai[2], S. Ikeda[1], T. Uchida[1], S. Kikuno[1], K. Nagasawa[1], M. Okubo[1], K. Narui[3], Y. Mori[1];
[1]Endocrinology and Metabolism, Toranomon Hospital, Tokyo, [2]Cardio-Respiratory Sleep Medicine, Department of Cardiology, Juntendo University School of Medicine, Tokyo, [3]Sleep Center, Toranomon Hospital, Tokyo, Japan.

Background and aims: Sleep disordered breathing (SDB) can induce hyperglycemia, hypertension and oxidative stress. SDB, therefore, may exacerbate diabetic kidney disease (DKD). Although several studies showed potential association between SDB and DKD, almost all studies used unattended portable monitors. Recently, a few studies showed that apnea hypopnea index during rapid eye movement sleep (REM-AHI), which can be measured only by polysomnography (PSG), is associated with glycated hemoglobin (HbA1c) and hypertension whereas non-REM-AHI is not. There are no studies, however, evaluating the association between REM/non-REM-AHI and DKD.

Materials and methods: We conducted a cross-sectional study in 303 patients with type 2 diabetes (T2D) followed in our diabetes clinic, who underwent a fully-attended PSG with no history of heart failure, active lung disease, urologic diseases or pretreated SDB. DKD was defined as the presence of albuminuria (≥3.4 mg/mmol creatinine) or declined estimated glomerular filtration rate (eGFR) (<60 mL/min/1.73 m^2). Logistic regression analysis was performed to clarify the effect of AHI or REM-AHI on the prevalence of DKD adjusted for age, sex, BMI, T2D duration, smoking, hypertension, HbA1c, non-HDL cholesterol, insulin use, ACE inhibitor/angiotensin receptor blockers use, statin use and sleep pills use. In REM-AHI analysis, patients with REM sleep <30 minutes during PSG were excluded.

Results: Characteristics of the patients are summarized in the table. Categorical AHI was independently associated with DKD ($p = 0.011$ for linear trend, OR and 95% CI for 15 ≤AHI <30, 1.541 (0.640–3.710); 30 ≤AHI, 3.078 (1.364–6.943) compared with AHI <15). Furthermore, quartile of REM-AHI was independently associated with DKD ($p = 0.034$ for linear trend, OR and 95% CI for Q2, 3.142 (1.099–8.980); Q3, 3.827 (1.263–11.601); Q4, 4.968 (1.596–15.460) compared with Q1) whereas quartile of non-REM-AHI was not ($p = 0.124$ for linear trend). Similar results were obtained when continuous AHI or REM/non-REM-AHI variables were included in the model. In addition, quartile of minimum oxygen saturation or percentage of time spent with SO2 <90 were independently associated with DKD ($p = 0.001$ and $p < 0.001$ for linear trend).

Conclusion: This is the first study to demonstrate independent association between REM-AHI and DKD in patients with T2D. REM-AHI could be a potential risk factor for DKD.

Table Characteristics of the patients

	Overall (n=303)	REM-AHI (n=182)	REM-AHI Q1	Q2	Q3	Q4	*P* value
Age, years	58.5 ± 11.9	57.8 ± 11.8	58.3 ± 12.3	60.5 ± 10.4	57.3 ± 12.5	55.3 ± 12.0	0.202
Male sex, %	85.8	86.8	86.4	89.4	88.9	82.6	0.590
T2D duration, years	5 (0-13)	5 (1-12)	6 (1-11)	8 (0-14)	5 (0-10)	4 (1-11)	0.829
DKD, %	44.9	39.0	20.9	42.9	46.3	47.4	0.014
Hypertension, %	68.6	64.3	47.7	66.0	64.4	78.3	0.005
BMI, kg/m²	27 (24-29)	26 (24-29)	24 (23-27)	25 (24-29)	27 (25-29)	28 (25-32)	< 0.001
HbA1c (NGSP), %	7.3 (6.8-8.4)	7.4 (6.8-8.5)	7.4 (6.7-8.9)	7.1 (6.7-7.7)	7.9 (7.2-9.0)	7.4 (7.0-8.5)	0.005
non-HDLC, mg/dL	145 (124-166)	141.3 ± 32.1	140.6 ± 33.8	140.0 ± 27.6	146.2 ± 33.9	138.7 ± 33.4	0.689
Insulin use, %	5.9	7.1	2.3	6.4	15.6	4.3	0.385
AHI, events/h	34.6 (20.4-55.1)	29.8 (18.0-45.4)	15.6 (8.0-29.8)	29.7 (19.0-39.9)	33.9 (21.8-48.8)	42.3 (27.5-64.6)	< 0.001
REM-AHI, events/h	39.7 (21.1-55.2)	35.4 (21.1-53.3)	12.8 (7.2-17.7)	28.6 (23.7-31.3)	44.1 (41.2-48.2)	62.1 (56.1-69.8)	< 0.001

Data are mean ± SD, median (interquartile range) or %. REM, rapid eye movement; AHI, apnea hypopnea index; T2D, type 2 diabetes; DKD, diabetic kidney disease. Analysis performed using the Cochran-Armitage trend test for categorical variables, the analysis of variance (ANOVA) for normally distributed variables and the Kruskal-Wallis test for skewed variables.

Disclosure: **A. Nishimura:** None.

1015

The pathological mechanism of anaemia in diabetic patients with chronic kidney disease

K. Harada[1], Y. Yamaguchi[2], Y. Akai[3];
[1]Department of Nephrology, Diabetes and Renal Replacement Therapy, Nishinara Central Hospital, Nara, [2]Department of Nephrology and Rheumatology, Rakuwakai-Otowa Hospital, Kyoto, [3]Department of Community-based Medicine, Nara Medical University, Kashihara, Japan.

Background and aims: Anemia is a common complication in chronic kidney disease (CKD), affecting over half of all patients. Diabetes, as the major causes of CKD worldwide, is therefore the most principal cause of renal anemia. CKD patients with diabetic nephropathy (DN) develop renal anemia earlier for their degree of renal impairment than those without DN. How the renal pathological alterations in DN affect renal anemia remains to be elucidated. We conducted this study to investigate association between anemia and renal pathological findings in diabetic patients with CKD.

Materials and methods: Eighty-six patients with type 2 diabetes mellitus (DM) and proteinuria and/or renal impairment were enrolled in

this study. Renal biopsy was performed for all the patients in order to obtain definite renal diagnosis.
Results: Renal biopsy revealed that 60 patients (69.8%) had diabetic lesions and were diagnosed as DN. Hemoglobin was lower in patients with DN than in those without DN (12.0 ± 2.11 g/dL vs 13.3 ± 2.52 g/dL, $p = 0.0084$), whereas there was no significant difference of transferrin saturation (TSAT) and serum ferritin levels between both groups. DN patients had severer tubulointerstitial ($p < 0.05$) and vascular ($p < 0.0001$) damages than non-DN, however, urinary excretion of albumin/protein and estimated glomerular filtration rate (eGFR) showed no statistically significant relationship to the histological diabetic changes. To evaluate the significance of clinical and pathological parameters in the progression of anemia using multiple regression analysis, hemoglobin was positively correlated with eGFR and TSAT (eGFR; correlation coefficient 0.238, $p = 0.0313$, TSAT; correlation coefficient 0.245, $p = 0.0120$, respectively) and was inversely correlated with the severity of interstitial fibrosis and tubular atrophy (IFTA) (correlation coefficient −0.278, $p = 0.0352$).
Conclusion: Severity of IFTA could be closely associated with the development of renal anemia in CKD patients with DN. Severer tubulointerstitial damage in DN than non-DN could explain earlier manifestation of renal anemia.
Disclosure: **K. Harada:** None.

1016

Elevated systemic iron levels aggravate diabetic nephropathy

S. Altamura[1], J. Schmidt[1], K. Schreckenberger[1], K. Müdder[1], V. Peters[2], A. Wagner[3], P. Nawroth[4], M. Muckenthaler[1];
[1]Department of Pediatric Haematology, Oncology and Immunology, University of Heidelberg, Heidelberg, [2]Centre for Paediatric and Adolescent Medicine, University of Heidelberg, Heidelberg, [3]Department of Cardiovascular Physiology, University of Heidelberg, Heidelberg, [4]Department of Internal Medicine I and Clinical Chemistry, University of Heidelberg, Heidelberg, Germany.

Background and aims: Iron and diabetes are strictly related. This is clinically evident in patients affected by hereditary hemochromatosis, in which the prevalence of diabetes is about 20%. On the other side, recent studies demonstrate that in diabetic patients plasma iron and/or ferritin levels could be prognostic factors for the development of the disease. The aim of this project is to understand the role of increased iron levels in the generation of diabetic late complications. For this purpose, we have generated and characterized a mouse model of combined hereditary hemochromatosis type 4 and type 2 diabetes mellitus.
Materials and methods: To assess how increased iron levels affect the pathogenesis of diabetes and its complications, Lepr(db/db) mice affected by T2DM have been mated with the FpnC326S mouse model of hereditary hemochromatosis type 4. The main diabetic and iron parameters have been evaluated. Tissue iron content and distribution were assessed in histology while alterations in the expression of iron-related genes were analyzed in qRT-PCR and western blot. Kidney carnosine levels and PAS staining were used as markers of diabetic nephropathy.
Results: Already at 15 weeks of age, both diabetic and diabetic/hemochromatotic mice present the major hallmarks of the early T2DM: obesity, hyperglycemia and hyperinsulinemia. Diabetic mice show elevated circulating iron content which are not translated into increased hepcidin production, confirming our previously reported "iron resistance" phenotype (Altamura, 2017). The iron resistance mechanism is present also in diabetic/hemochromatotic mice, which show a 1,5-fold increase in systemic iron content compared to hemochromatotic mice. At molecular level, the liver is correctly sensing the elevated circulating iron levels, as shown by the activation of the BMP/SMAD pathway, further demonstrating that the iron resistance mechanism is dominant over this pathway. Analysis of the liver also revealed a decreased hepatic iron content in both diabetic and diabetic/hemochromatotic animals, a phenotype that occurs despite the elevated systemic iron content and the increased TfR1 mRNA expression. Histological analysis of the kidney revealed iron accumulation in the proximal tubules and in the glomeruli of 40-week old diabetic/hemochromatotic mice. At the same age, iron accumulation correlates with an increase in PAS staining and with decreased carnosine levels.
Conclusion: This study shows that type 2 diabetes mellitus causes an "iron resistant" phenotype where increased systemic iron levels are not correctly translated into hepcidin production. This mechanism is persistent also in the presence of an hemochromatotic background, further increasing the amount of circulating iron. Iron accumulation in the kidney correlates with a decrease in carnosine levels, a protective factor for diabetic nephropathy and increased PAS staining, a marker of diabetic kidney dysfunction. Taken together, these results indicate that elevated systemic iron levels, which can be considered as a diabetic complication, could aggravate diabetic nephropathy.
Supported by: SFB1118
Disclosure: **S. Altamura:** None.

1017

Serum levels of angiopoietin-like protein 4 in relation to glomerular function and nephropathy severity in type 2 diabetic patients

Y. Feng, L. Yang, C. Yu, S. Yuan, Y. Wang, N. Zhang, D. Zhao;
Beijing Key Laboratory of Diabetes Prevention and Research, Endocrinology Center, Lu He hospital, Capital Medical University, Beijing, China.

Background and aims: Diabetic nephropathy (DN) is one of the major diabetic microvascular complications that leads to end-stage kidney disease. Urinary Angiopoietin-like 4 was reported increased in STD-induced rats and in diabetic patients with microalbuminuria or macroalbuminuria. Nevertheless, whether ANGPTL4 could relate to glomerular function and DN severity is not defined in type 2 diabetic mellitus (T2DM) patients.
Materials and methods: The study complied with the Helsinki Declaration for investigation of human subjects and was approved by the competent Institutional Review Boards of the Lu He hospital. From July 2015 until January 2017, 1321 T2DM patients were recruited in the Endocrinology Center at Lu He hospital. After exclusion of the subjects with missing data or outliers, totaled 1241 patients were analyzed. The stages of DN were categorized by KDOQI Clinical Practice Guidance Diabetes and CKD based on urinary albumin-to-creatinine ratio (UACR) and/or estimated glomerular filtration rate (eGFR). Serum levels of angiopoietin-like 3 (ANGPTL3), ANGPTL4, high-sensitive C-reactive protein (CRP), vascular adhesion molecule-1 (VCAM-1), transforming growth factor-β1 (TGF-β1), neutrophil gelatinase associated lipocalin (NGAL) and vascular endothelial growth factor (VEGF) were quantified by ELISA. Multivariable-adjusted logistic analysis was performed to estimate the association of each biomarker and DN stage. Multivariable-adjusted linear regression was performed to study the association of each biomarker and eGFR or UACR. The confounding factors included were age, sex, body mass index, waist-to-hip ratio, mean arterial blood pressure, Hemoglobin A1c (HbA1c), total cholesterol, smoking, diabetic and hypertension history, anti-diabetic drugs, anti-hypertensive drugs (in class) and statins. For database management and statistical analysis, we used the SAS system, version 9.4 (SAS Institute Inc., Cary, NC).
Results: In the 1241 T2DM patients (47.62% women), age averaged (SD) 57.7 (14) years, body mass index 26.2 (3.8) kg/m^2 and eGFR 96.8 (21.4) mL/min/1.73m^2. When categorized by DN stage, 693 (55.84%) had no DN, 462 (37.23%) were in stage 3, 85 (6.85%) were in stage 4, and 1 (0.08%) was in stage 5. All patients received anti-diabetic treatment, among which 704 (56.7%) took metformin and 680 (54.8%) took insulin injection. The multivariable-adjusted odds ratios expressing the risk of DN stage ≥3 ($n = 548$) *vs.* DN <3 ($n = 693$) was 2.41 (95% CI, 1.94–3.0, $p < 0.0001$) for ANGPTL4. Except ANGPTL4, DN severity did not associate with other biomarkers ($p > 0.08$). One-SD increase of

serum ANGPTL4 levels were negatively associated with eGFR by −9.2 (95% CI, −11.9 to −6.4) ml/min/1.73 m^2 ($p < 0.0001$). Likewise, 1-SD increase of serum ANGPTL4 levels or ANGPTL3 levels were positively associated with UACR by 1.6 (95% CI, 1.3 to 1.9 mg/mmol, $p < 0.0001$) or 1.3 (95% CI, 1.0 to1.6 mg/mmol, $p = 0.0497$), respectively. Except that, other biomarkers were neither associated with eGFR nor with UACR ($p > 0.08$).

Conclusion: Serum levels of ANGPTL4 are strongly associated with DN severity. Blockade of ANGPTL4 in kidney might postpone the onset and development of DN in T2DM patients.

Supported by: the National Science Funding in China (#81470566 and #81670765)

Disclosure: **Y. Feng:** None.

1018

Post transplantation diabetes in kidney transplant recipients: time of diagnosis, impact on graft function and infectious complications

D. Cieniawski, E. Ignacak, A. Prokop, P. Miarka, K. Krzanowska, M. Kuzniewski, W. Sulowicz;
Department of Nephrology Jagiellonian University, Krakow, Poland.

Background and aims: Post transplantation diabetes mellitus (PTDM) is one of the most common complication affecting patients after organ transplantation. Development of PTDM is mainly connected with immunosuppressive treatment and individual tendency. The diagnostic difficulties are associated with high doses of drugs in the early period after transplantation and transient hyperglycemia observed in first weeks. Regardless of this, disturbances of carbohydrate metabolism are associated with a worse prognosis for both recipients and organs and the greater frequency of complications The aim of the study was to assess the incidence of PTDM, time of its diagnosis and impact on graft function and frequency of infectious complications.

Materials and methods: In prospective study we enrolled 212 consecutive kidney transplant recipients from years 2013–2014. Follow-up period was 24 months. The main endpoint was confirmation of PTDM diagnosis. All disease history reports and patients laboratory results available in the electronic systems from the first two years after transplantation were analyzed. The diagnosis of PTDM was determined according to standard criteria - two measurement of fasting plasma glucose ≥7.0 mmol/l, 2 hours glucose in OGTT (oral glucose tolerance test) ≥11.1 mmol/l or casual glycemia ≥11.1 mmol/l with standard symptoms. The frequency of infections was obtained from the hospital system information and questionnaire from patients. As clinically significant infection necessary of hospital admission or antibiotic use was assumed.

Results: The full-time follow-up was finished by 195 patients. Before kidney transplantation (ktx) diabetes was present in 13 patients. From other 199, PTDM was diagnosed in 56 cases (28.14%). In 34 graft recipients PTDM developed in first month after transplantation (60.7%). Between 2 and 6 months after ktx diabetes was confirmed in 9 patients (16.1%) and after fisrt six month in another 13 (23.2%). Patients with PTDM had significantly worse kidney graft function after 24 months of observation (eGFR 51.5 vs. 61.4 ml/min/1.73 m^2; $p = 0.002$). The same dependence was confirmed when the whole group with DM was analyzed (eGFR 52.0 vs. 61.4 ml/min/1,73m^2; $p = 0.0005$). The frequency of respiratory and urinary tract infections in first two years after ktx was higher in patients with DM (respectively 48.3% vs. 37.6%; $p = 0.170$, 46.7% vs. 41.0%; $p = 0.473$) but differences were not significantly important. The average number of respiratory tract infection was higher in patients with DM (1.24 vs. 0.94; $p = 0.167$), but reversed situation was observed in case of urinary tract infections - higher average value was obserwed in patients without DM (2.64 vs. 2.25; $p = 0.442$).

Conclusion: The incidence of PTDM is still high and is associated with a worse graft function. The highest risk of PTDM development is observed in first month after ktx. Patients with PTDM are exposed to a higher frequency of infectious complications in first two years. Frequency of respiratory and urinary tract infections is very high in first two years after ktx regardless of DM occurrence.

Disclosure: **D. Cieniawski:** None.

1019

Ramadan fasting effects on renal function in type 2 diabetic patients

M. Abushady[1], M. Samy[1], M. bekhet[1], A. Abdullah[2];
[1]Endocrinology and Metabolism Department, Ain Shams University, Internal Medicine, Cairo, [2]Internal medicine Department, Specialized Damietta Hospital, Damietta, Egypt.

Background and aims: Ramadan fasting is a religious pillar carried out by Muslims all over the world. Concerns have been raised over how the practice of fasting from dawn to sunset affects kidney functions in diabetic Muslim patients as it represents a major shift in meal timing and content for practicing Muslims. It is hypothesized that patients with diabetic kidney may experience worsening of their kidney functions. We aimed to evaluate the effects of Ramadan fasting on kidney functions in type 2 diabetic patients.

Materials and methods: We recruited 90 patients with type 2 DM intending to fast Ramadan (2016); where the average fasting time was about 16 hours from 3 am to 7 pm. They were be divided into 30 patients with normal kidney functions and no albuminuria, 30 patients with albuminuria and normal kidney functions and 30 with albuminuria and renal impairment. Before Ramadan by two weeks and after Ramadan by two weeks, fasting blood glucose (FBG), 2hours plasma glucose (2hPG), hemoglobin A1c (HbA1c), fructosamine, serum creatinine, BUN, eGFR and albumin/ creatinine ratio were measured.

Results: On comparing the studied groups before and after fasting Ramadan, a significant reduction in HbA1c was found in all studied groups ($11.23 \pm 2.67\%$ vs $9.09 \pm 1.95\%$, $11.09 \pm 2.40\%$ vs $8.80 \pm 1.78\%$, $9.28 \pm 2.41\%$ vs $8.21 \pm 1.45\%$, $p < 0.001$, $p < 0.001$, $p = 0.04$ respectively). Regarding the kidney function parameters; there was no significant change in group I but there was a significant decline in these parameters in groups II and III; serum creatinine (1.33 ± 0.05 mg/dl vs 1.41 ± 0.23 mg/dl ,0.66 ± 0.11 mg/dL vs 0.93 ± 0.17 mg/dL , 0.70 ± 0.12 mg/dL vs 0.84 ± 0.16 mg/dL, $p = 0.101$, $p < 0.001$, $p < 0.001$ respectively), eGFR (63.07 ± 3.27 ml/min/1.73 m^2 vs 59.73 ± 13.25 ml/min/1.73 m^2, 114.00 ± 18.74 ml/min/1.73 m^2 vs 77.83 ± 16.48 ml/min/1.73 m^2, 111.70 ± 18.60 ml/min/1.73 m^2 vs 97.50 ± 21.19 ml/min/1.73 m^2, $p = 0.186$, $p < 0.001$, $p = 0.008$ respectively), urinary albumin/creatinine ratio (88.40 ± 64.86 mg/g vs 86.03 ± 86.52 mg/g, 71.43 ± 21.17 mg/g vs 112.33 ± 72.40 mg/g, 16.18 ± 7.99 mg/g vs 41.67 ± 22.0 mg/g, $p = 0.905$, $p = 0.004$, $p < 0.001$ respectively). Concerning hypoglycemia related events and need for dose reduction were significant in group I ($P < 0.001$) and ($p = 0.050$) respectively whereas group II and III showed non-significant difference in between as regards hypoglycemic events and dose modification need.

Conclusion: Ramadan fasting appears to a have significant effect on improvement of glycemic control in type 2 diabetic patients with no decline in kidney functions except in those patients with already existing albuminuria with or without abnormal kidney function.

Disclosure: **M. Abushady:** None.

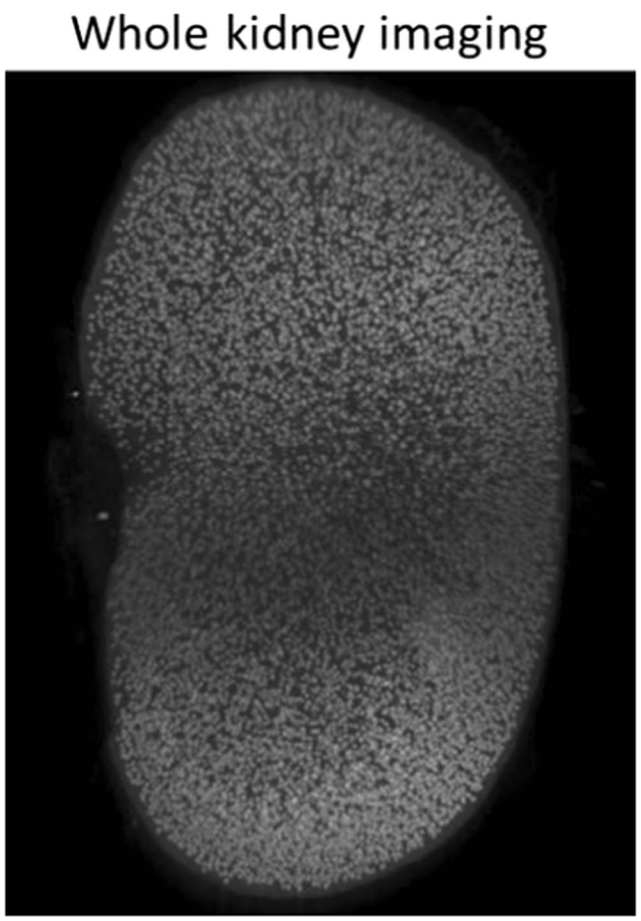
PS 094 Animal studies on nephropathy

1020

3D kidney imaging for assessment of glomerular number and size in a mouse model of diabetic nephropathy

J. Hecksher-Sørensen, K. Fabricius, T.X. Pedersen, T. Johansen, L.N. Fink, N. Vrang, J. Jelsing, T. Secher;
Gubra, Hørsholm, Denmark.

Background and aims: Diabetic nephropathy (DN) is a major long-term complication of diabetes characterized by hypertrophy and hyperfunction of the kidney. To facilitate a rapid and unbiased evaluation of drug efficacy on glomerular size and number in preclinical studies, we investigated the use of light sheet microscopy as a new high-end 3D methodology to study glomerular changes in whole kidneys. Diabetic db/db mice subjected to unilateral nephrectomy (UNx) was used as a model.
Materials and methods: Unilateral nephrectomy (UNx) was performed in diabetic db/db mice to accelerate the development of nephropathy. The surgery was performed in 18 weeks old male db/db mice and terminated 6 weeks later. Prior to the UNx operation, mice were randomly assigned into two groups (control or UNx; $n = 8$) based on blood glucose levels. To determine the effect of accelerated DN on glomerular morphology, mice were injected with Lectin_594 prior to termination and the intact kidneys were scanned using light sheet microscopy. Using 3D image analysis, the total number of glomeruli were quantified and segmented according to their individual size.
Results: Diabetes development remained similar in db/db control and db/db UNx mice. However, at termination, kidney weight was increased in the db/db UNx group indicative of renal insufficiency leading to kidney hypertrophy. In agreement with stereological observations on histologically processed tissue sections, 3D light sheet imaging confirmed an increase in glomerular size in the db/db UNx mouse kidneys compared to db/db control mice, while glomerular numbers as expected were maintained (app. 14.000 glomeruli per kidney; see figure 1). The imaged kidneys were subsequently analyzed for Wt1, Collagen IV and podocin expression using conventional immunohistochemistry. All antigens were readily detected confirming that whole organ imaging is compatible with antibody detection and further substantiated the db/db UNx mice as a useful model of DN.
Conclusion: We have successfully applied light sheet microscopy to assess renal and glomerular hypertrophy at the whole organ level in uni-nephrectomised db/db mice. The analysis of intact organs offers a new approach for evaluating changes in key glomerular markers of DN, while maintaining the ability to perform conventional immunohistochemistry on the same tissue. This detailed analysis of all glomeruli enables improved pharmacological testing of compounds targeting this disease.



Disclosure: **J. Hecksher-Sørensen:** None.

1021

Next generation of spontaneous diabetic model of FATZO mice with intact leptin signalling develop nephropathy after high fat and high sucrose induction

J. Gorski, G. Sun, G. Zhang, Y. Wang;
Crown Biosciences, San Diego, USA.

Background and aims: Progressive albuminuria and bi-phasic changes in glomular filtration rate (GFR) are hall marks of functional deterioration of the kidney in diabetic nephropathy. However, not all diabetic rodent models can capture the development of such changes in the kidney. FATZO, a new generation of diabetic mouse model with intact leptin signaling pathway shows all the metabolic dysfunctions that mimic human patients such as obesity, dyslipidemia, diabetes and NASH. Here, we sought to further characterize the model for the evaluation of its development in diabetic nephropathy.
Materials and methods: Male FATZO mice on 5008 diet or on western diet with addition of 10% fructose (WD+fru) from age of 11 weeks were subject to monthly urine collection for albumin and creatinine measurement for 28 weeks, after which animals were treated with Lisinopril (15 mg/kg) and pioglitazone (20 mg/kg) daily for 6 weeks ($n = 8$ for each group). Animals were also monitored for GFR using a subcutaneous monitoring device (Medibeacon, US).
Results: Animals on WD+fru were heavier than those on 5008 diet. Notably, WD+fru markedly elevated urine albumin levels compared to 5008 diet in FATZO mice which resulted in higher albumin/creatinine ratio and urine albumin excretion rate (UAE) from 2 weeks on diet, while

Lisinopril treatment for 7 weeks could reduce the levels respectively. In addition, GFR was slightly elevated after 6 weeks of diet induction, which could be normalized by either pioglitazone or Lisinopril treatment.
Conclusion: We have created a mouse model with progressive albuminuria and elevated GFR that can be reversed by angiotensin-converting-enzyme (ACE) inhibitor. The model with associated dysmetabolic phenotypes including obesity and dyslipidemia can be used as an ideal tool for studying anti-diabetic nephropathy treatment.
Disclosure: **J. Gorski:** None.

1022

A novel mechanism of prostaglandin E1 attenuating high glucose-induced apoptosis in renal tubular epithelial cells: a key role for the JNK/Bim pathway

Y. Zhang[1], Z. Zou[2], C. Guo[3], L. Liao[1], J. Dong[2], Z. Zhang[1];
[1]Department of Internal Medicine, Shandong Provincial Qianfoshan Hospital, Shandong University, Ji'nan, Shandong Province, [2]Department of Internal Medicine, Qilu Hospital of Shandong University, Ji'nan, Shandong Province, [3]First Clinical Medical College, Shandong University of Traditional Chinese Medicine, Ji'nan, Shandong Province, China.

Background and aims: Renal tubular damage is a critical process underlying diabetic kidney disease (DKD). Our previous study has suggested that prostaglandin E1 (PGE1) ameliorates renal tubule apoptosis in DKD rats. However, the precise mechanism remains unclear. We hypothesize that PGE1 alleviates tubular apoptosis of DKD by modulating primarily the c-Jun N-terminal kinase (JNK), a key regulator of cell apoptosis, while alleviating the gene expressions such as Bim, Bax and cleaved caspase-3 downstream related to intrinsic mitochondrial apoptosis.
Materials and methods: In the present study, we investigated the mechanism of PGE1 on the diminished apoptosis in the uninephrectomized streptozotocin (STZ)-induced diabetic rats. Renal proximal epithelial tubular cell line (HK-2) exposed to high glucose (HG) ambiance was also studied. Flow cytometry and TUNEL assays were used to detect apoptosis of renal tubular epithelial cells. Bim expression was detected by immunofluorescence. Immunohistochemical staining was introduced to detect the Bim, Bax and Bcl-2 expressions in renal tissue. The expressions of apoptosis related proteins Bim, Bax and cleaved caspase-3 were detected with western blot.
Results: Chronic exposure of the rodent tubular epithelial cells and HK-2 cells to high levels of glucose induced apoptosis ($p < 0.01$) and apoptosis related protein Bim, Bax and cleaved caspase-3 expressions ($p < 0.05$). Simultaneously, high glucose increased the phosphorylation level of JNK over time (1h, 6h, 12h, 24h, 48h), followed by the increased expression of Bim subsequently. PGE1 notably ameliorated renal tubular apoptosis in DKD rats and HK-2 cells in vitro, which was accompanied with the decreased expressions of Bim, Bax, and cleaved caspase-3 ($p < 0.05$). Treatment with anisomycin (AM), a JNK activator, resulted in an increased activation of phosphorylated JNK (p-JNK) in HK-2 cells ($p < 0.05$). Furthermore, PGE1 also reversed p-JNK expression in HK-2 cells when incubated with high glucose ($p < 0.05$) or combination of high glucose and AM ($p < 0.05$), followed by the decreased Bim protein expression.
Conclusion: Our results demonstrated that PGE1 ameliorated tubular apoptosis by modulation of mitochondrial apoptosis pathway via JNK-related Bim signaling.
Disclosure: **Y. Zhang:** None.

1023

Diabetic human carnosinase 1 transgenic BTBR$^{ob/ob}$ mice have reduced renal carnosine concentrations and display a higher degree of glomerular filtration barrier impairment

J. Qiu[1], F. Siegerist[2], A.-N. Rodriguez[1], D.O. Pastene[1], N. Endlich[2], B.K. Krämer[1], B.A. Yard[1];
[1]5th Medical Department, University Hospital Mannheim, Mannheim, [2]Department of Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany.

Background and aims: A trinucleotide repeat polymorphism in the *CNDP1* gene is associated with susceptibility to develop diabetic nephropathy (DN). Serum carnosinase 1, which is encoded by the *CNDP1* gene, degrades substrates that have been shown to exert beneficial effects in rodent models of DN, e.g. carnosine. Because of the complete lack of serum carnosinase in rodents, translation of these experimental findings to humans is difficult. We, therefore, have made human *CNDP1* transgenic mice in the BTBR$^{ob/ob}$ background to assess the role of renal carnosine in preventing glomerular damage.
Materials and methods: Chip-based gene expression profiling was performed using renal tissue obtained from diabetic wild-type and hCNDP1 transgenic BTBR$^{ob/ob}$ mice. Renal carnosine concentrations were assessed by HPLC and podocyte effacement by 3D structured illumination microscope.
Results: Renal tissue obtained from diabetic hCNDP1 displayed ~10 fold decreased levels of carnosine as compared to age-matched wild-type diabetic mice. Gene expression profiling revealed in the transgenic diabetic kidney upregulation of genes associated with kidney disease (9 of the 15 most upregulated genes) and a decreased expression of genes related to cell cycle and cell death (5 of the 15 most downregulated genes). KEGG and GO annotation analysis revealed cell adhesion and tight junction as most enriched amongst all up-regulated genes and phosphorylation/kinase activity amongst all downregulated genes. Transgenic mice displayed higher albuminuria and proteinuria levels. Using 3D structured illumination microscopy, a significantly higher slit diaphragm density indicating less podocyte foot process effacement was detected in transgenic diabetic kidneys.
Conclusion: Carnosinase 1 overexpression decreases renal tissue carnosine and aggravates glomerular filtration barrier impairment. This might be a consequence of a pathological slit membrane due to altered expression of cell adhesion and tight-junctional proteins. Further histological analysis to confirm the latter is warranted.
Supported by: German research foundation (GRK 1874)
Disclosure: **J. Qiu:** None.

1024

Apoptosis resistant modified endothelial progenitor cell (EPC) transplantation improves diabetic kidney disease (DKD)

N. Kundu, L. Asico, C. Domingues, P. Jose, S. Sen;
George Washington University, Washington, USA.

Background and aims: DKD is a major vascular complication of diabetes, which is associated with glomerulosclerosis and poor perfusion. Therefore, improving the renal perfusion may help to treat the sclerotic kidney disease. EPCs have been shown to improve tissue perfusion. Here, we investigated whether transplanting apoptosis resistant genetically modified mouse EPCs (that has p53 gene silenced, transiently, using Adenovirus ex-vivo), could improve angiogenesis and renal perfusion, in a hyperglycemic type 1 diabetes milieu. Mesenchymal stromal cells (MSC) transplantation has also been used in DKD however concern for epithelial to mesenchymal transformation following MSC transplantation remains. .
Materials and methods: We compared the efficacy of p53shEPC with MSC, Null-EPC and saline in STZ-induced type 1 diabetic C57BL/6J mouse model. 8 weeks after STZ delivery stable hyperglycemia, polyuria and proteinuria was confirmed followed by transplantation of 0.3 million EPC/ MSC bilaterally, under each kidney capsule. We compared our results with non-STZ normal C57Bl6J mouse. Urine was collected weekly for volume, protein and creatinine estimation. Blood creatine and renal blood flow was measured by laser Doppler, at sacrifice. qRT-PCR on

harvested kidneys were performed focusing on genes associated with angiogenesis
Results: Polyuria was reduced in p53shEPC transplanted animals with urine volume close to non DM animals and half the volume compared to saline and MSC group at week 4 and 6. Protenuria levels were the lowest in p53shEPC group. Plasma creatinine was lowest in p53shEPC group (3-fold lower) compared to Null EPC group Enhanced blood flow by laser doppler was also noted with delivery of p53sh-EPCs vs controls, such as null EPC (1.2 fold), saline (3 folds) at wk4&6. Markers for angiogenesis, such as eNOS mRNA (4.5 fold, $p = 0.002$) and VEGF-A mRNA (1.5 fold, $p = 0.03$) upregulated significantly post p53 silenced EPC transplantation compared to null EPC group. Capillary density staining is pending.
Conclusion: Transient silencing of p53 gene in EPCs help to improve proteinuria, plasma creatinine, diabetic polyuria and renal blood flow, most likely by helping to increase angiogenesis and perfusion and may have a prominent therapeutic role in DKD. MSC transplantation results were consistently less efficacious compared to p53silenced EPC transplantation.
Supported by: American Heart Association
Disclosure: **N. Kundu:** None.

1025

Modulation of TIMP3-ADAM17 dyad limits the progression of diabetic nephropathy
R. Menghini[1], V. Casagrande[1], S. Menini[2], G. Iuliani[1], L. De Angelis[1], M. Mavilio[1], G. Pugliese[2], M. Federici[1];
[1]University of Rome Tor Vergata, Rome, [2]Sapienza University, Rome, Italy.

Background and aims: The tissue inhibitor of metalloproteinase (TIMP)3 is the most highly expressed TIMP in the kidney and we and others have recently demonstrated that loss of TIMP3 contributes to the onset and progression of Diabetic Kidney Disease in mouse models of diabetes, at least in part through the regulation of A Disintegrin and Metalloproteinase (ADAM)17. Our aim was to provide evidences that in vivo manipulation of the TIMP3/ADAM17 system may limit the onset and the progression of diabetic kidney complications.
Materials and methods: We generated a mouse model with cell-targeted overexpression of TIMP3 in myeloid cells (MacT3), which results in kidney overexpression of TIMP3 consequently to macrophage accumulation induced by diabetes, and a podocyte-specific ADAM17 knockout mice (ΔPodA17). WT, MacT3 and ΔPodA17 mice ($n = 10$ for each group) were rendered diabetic through i.p. injections of STZ (50 mg/kg) for 5 consecutive days. After 12 weeks, 24h Albuminuria was determined by ELISA and kidney morphometry was analyzed by periodic acid-shift staining (PAS). RT-PCR and western blot analysis were performed on mRNA and protein extracted from kidney cortex.
Results: in both mouse models, morphometric analyses showed a reduction in renal lesions, as assessed by lower increase in mean Glomerular Area (mGA), mean Mesangial Area (mMA) and fractional Mesangial Area (fMA) ($p < 0.002$). Consistently, 24h urine Albuminuria was significantly reduced ($p < 0.05$). Moreover, preliminary analysis of mRNA and proteins derived from renal cortex of MacT3 mice indicated that expression of TIMP3 in macrophages led to an increased TIMP3 mRNA expression ($p = 0.02$), rescuing impaired inflammatory markers (F4/80, MCP1 and IL6 ($p < 0.05$)) observed in diabetic WT mice. Protein expression of podocytes markers Podocin an WT1 ($p = 0.01$) were significantly improved whereas fibrosis markers Collagen IV and TGF beta were reduced in MacT3 diabetic mice compared with diabetic control mice.
Conclusion: Our data indicate that in vivo manipulation in rodent models of TIMP3/ADAM17 system may rescue kidney defects induced by diabetes, representing a valid approach to characterize the pathogenesis of Diabetic Nephropathy and to develop new avenues to diagnose and treat this disorder.
Supported by: EFSD/Boehringer Ingelheim Research; JDRF
Disclosure: **R. Menghini:** None.

1026

Salidroside ameliorates diabetic albuminuria through inhibition of Bim-mediated mitochondrial apoptosis in renal tubular cells
C. Guo, L. Liao;
Shandong Provincial Qianfoshan Hospital, Jinan, China.

Background and aims: Accumulating studies indicate that the apoptosis of tubular cells is implicated in progression of the diabetic kidney disease (DKD). How to prevent or treat the injury remains unsolved. Here, we investigate whether salidroside, a natural phenolic antioxidant compound can protect tubular cells from high glucose-induced apoptosis.
Materials and methods: Salidroside, at a dose of 70 mg/kg body weight, was orally administrated to uninephrectomized streptozotocin-induced DKD rats for up to 8 weeks. Renal function related parameters, such as 24-h urine albumin and serum creatinine (SCr) of the rats were measured. TUNEL, immunohistochemistry and western blot examinations were conducted to evaluate the anti-apoptosis activity of salidroside in DKD rats. Subsequently, human renal proximal tubule cells line (HK-2 cells) were used to further verify the activity and explore the mechanisms in vitro. Finally, the underlying pathways involved in the nephroprotective effect of salidroside were predicted by a network pharmacology approach.
Results: The vivo studies in DKD rats showed that the amounts of cells underwent apoptosis were significantly more in tubule regions than that in glomerular regions. Salidroside treatment dramatically decreased the apoptosis rate of tubular cells, inhibited the expression of Bax, cleaved caspase-3 protein, and reversed the increased serum creatinine and albuminuria in the DKD rats. In vitro studies further demonstrated that high glucose reduced cell viability and increased apoptosis by elevating Bim protein expression, which is a proapoptotic inducer of mitochondrial apoptosis. Silencing of Bim protein reduced the expression of Bax and cleaved caspase-3 protein. Salidroside significantly downregulated Bim expression and ROS production. The enrichment analysis of network pharmacology verified the above results that the nephroprotective effect of salidroside was associated with various pathways involved in anti-apoptosis signing pathwayand antioxidant processes.
Conclusion: We conclude that salidroside could significantly relieve DKD. The possible mechanisms might be the decrease in apoptosis of tubular cells via Bim and oxidative stress inhibition.
Supported by: National Natural Science Foundation of China
Disclosure: **C. Guo:** None.

1027

Metformin is nephroprotective in a progressive renal disease model
C.M. Borges[1], C.K. Fujihara[2], V.F. Ávila[2], D.A. Duarte[1], J.M. Lopes de Faria[1], J.B. Lopes de Faria[1];
[1]Renal Pathophysiology Laboratory, Investigation on Diabetes Complications, State University of Campinas, Campinas, [2]Faculty of Medicine, University of São Paulo, São Paulo, Brazil.

Background and aims: Chronic kidney disease (CKD) is one of the most common metabolic diseases, worldwide. Currently, the treatment of CKD is suboptimal since a large number of individuals with CKD progress to end-stage renal disease, thus requiring dialysis and/or kidney transplantation. It has been demonstrated that metformin exerts pleitropic efffects beyond its actions as glucose-lowering agent in the treatment of diabetes mellitus. The use of rats with 5/6th renal ablation (Nx) is a well-established progressive renal disease model. Previously, it has been demonstrated that metformin prevented kidney fibrosis in the Nx model. However, treatment with metformin was initiated before the development of CKD in the nephrectomised rats. The present study investigated whether metformin is nephroprotective when treatment is initiated after the rats had developed higher levels of albuminuria and higher blood pressure level.
Materials and methods: Adult male Munich-Wistar rats underwent Nx. Thirty days after the nephrectomy, the rats with a systolic blood pressure

(SBP) above 170 mmHg and albuminuria levels >40 mg/24 h were randomised into four groups: Sham group; Nx, no treatment group; Nx+M group (receiving metformin 300 mg/Kg/day - in drinking water), and Nx+L group (receiving losartan 50 mg/Kg/day - in drinking water). Twenty-four-hour urine was collected in individual metabolic cages to assess albuminuria levels using enzyme-linked immunosorbent assay (ELISA); systolic blood pressure (SBP) was estimated using a tail-cuff method. The treatments were maintained for 120 days. At the end of the study, the rats were euthanised and the kidneys were harvested and processed for histological analysis.

Results: At baseline, the 24 h urinary albumin excretion rate (UAE) and SBP were significantly higher in the Nx group than in the Sham group. After 60 days of treatment, UAE decreased from 105 ± 37 mg/24 h to 39 ± 32 mg/24 h ($p = 0.0001$) and SBP decreased from 226 ± 21 mmHg to 167 ± 17 mmHg ($p = 0.0001$) in the Nx+L group in comparison to the untreated Nx group ($n = 10$). After 120 days, UAE was 162 ± 82 mg/24 h in the Nx group; it decreased to 54 ± 38 mg/24 h in the Nx+L group ($p = 0.0001$) and to 110 ± 41 mg/24 h in the Nx+M group ($p = 0.0125$). The effect of metformin on albuminuria was independent of any change in the blood pressure and glycaemia levels. Interstitial fibrosis was significantly higher in the Nx group than in the Sham group, and it was decreased in both the Nx+M and Nx+L groups after 120 days of treatment.

Conclusion: Metformin was able to prevent a further increase in albuminuria levels and to reduce kidney interstitial fibrosis in rats with established CKD, elevated blood pressure and albuminuria. Thus, the nephroprotection confered by metformin was independent of blood pressure and glycaemic controls.

Supported by: FAPESP/CNPq

Disclosure: **C.M. Borges:** None.

PS 095 Diabetic nephropathy: on the bench

1028

Approaches for the generation of human kidney organoids for modelling nephropathies

K. Uusi-Rauva[1,2], A. Pirttiniemi[1,2], S. Lehtonen[3], V. Fellman[1,4], P.-H. Groop[1,2], M. Lehto[1,2], FinnDiane Study Group;
[1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, Finland, [2]Abdominal Center Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [3]Department of Pathology, University of Helsinki, Helsinki, Finland, [4]Department of Clinical Sciences, Pediatrics, Lund University, Lund, Sweden.

Background and aims: Induced pluripotent stem cell (iPSC)-derived kidney cells enable dissection of diabetic nephropathy, or other kidney diseases, in patient-derived cells. Several approaches for the differentiation of kidney cells from iPSCs have been published, including the generation of kidney organoids. However, cell line characteristics and culture conditions may affect the end result of the differentiation. In addition, current organoid protocols cannot be used directly for high-throughput applications required for extensive functional analyses or subsequent drug screenings. Therefore, the reproducibility of one of the previously published protocols for the generation of iPSC-derived kidney organoids was tested using different cell lines and initial cell confluences. In addition, four different modified approaches with different culture systems, multiwell plate products, and amounts of cells were tested to set up more efficient and consistent organoid protocol in order to better address the needs of high-throughput analyses.

Materials and methods: This study was based on pre-established iPSC lines derived from healthy volunteers and a patient with GRACILE syndrome (OMIM:603358). The survival and the amount of intrinsic structures positive for well-established markers for kidney cells of differentiating organoids were analysed by light and immunofluorescence microscopy.

Results: Kidney organoids were managed to be generated from three out of five iPSC lines tested. However, the amount and appearance of intrinsic structures were variable between separate cell lines but also from experiment to experiment. Initial cell confluence had different effects on the outcome between separate cell lines. In general, the amount and appearance of nephrin-positive glomeruli seemed to be cell line-dependent, although in case of GRACILE kidney organoids, this phenomenon may be a sign of potential disease-associated phenotype. All tested modified approaches also allowed formation of structures positive for kidney cell markers but the total outcome of them all was not comparable to the organoids generated using the original protocol, and separate cell lines appeared to respond differently to different approaches. Nevertheless, some of the tested approaches were found to be highly promising, and may enable rather easy processing of a plateful of 24–96 kidney organoids simultaneously.

Conclusion: These results confirm that initial culture conditions may have substantial effects on the kidney differentiation, and thus, each cell line should be tested using different culture systems and cell confluences, and potential phenotypes of affected organoids need to be controlled with appropriate isogenic controls. In the future, kidney organoids generated from iPSCs of patients with nephropathy, their healthy relatives, and isogenic control cell lines using the here established, more efficient differentiation approach(es) can and will be utilised for the functional analyses of diabetic and other nephropathies.

Supported by: Folkhälsan Research, Wilhelm and Else Stockmann & Novo Nordisk foundations

Disclosure: **K. Uusi-Rauva:** Employment/Consultancy; Per-Henrik Groop is an advisory board member of AbbVie, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi. Honorarium; Per-Henrik Groop has received lecture honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi.

1029

Modelling the metabolic reprogramming of diabetic nephropathy using hESC-derived 3D kidney organoids

C. Hurtado del Pozo, P. Prado Peralta, B. Coppe, E. Garreta, N. Montserrat;
IBEC, Barcelona, Spain.

Background and aims: Diabetic Nephropathy (DN) is the leading cause of end-stage renal disease (ESRD). To date, treatment of DN is mainly based on drugs acting on glycaemic and blood pressure control, as there is no validated therapy able to stop the progression towards renal failure. One of the main impediments for developing new therapies for DN has been the lack of a good preclinical models which can recapitulate important functional, structural, and molecular features of advanced human diabetic kidney disease. Due to the increasing evidences that links kidney fibrosis and metabolic reprograming in DN, we hypothesize that early DN progression is promoted by the metabolic alterations occurring in diabetic patients. To test our hypothesis, we have first developed a DN model taking advantage of human Embryonic Stem Cell (hESC) derived kidney organoids. In our hands, this innovative system recapitulates the in vivo signature of DN including phenotypic and genetic alterations.
Materials and methods: **Kidney organoid differentiation:** Differentiation protocol was developed in our lab (Garreta et al, *under review*). **Isolation of tubular epithelial cells (TECs) from organoids:** TECs were isolated by flow cytometry from kidney organoids after incubation with fluorescein-conjugated Lotus Tetragonolobus Lectin (LTL) for five hours and further dissociation with Accumax. LTL^+ cells were cultured in REGM media. **Purification of Total RNA and Quantitative RT-PCR:** Isolation of total RNA was performed using Tri-Reagent®. 1 ug of RNA was used to synthesize cDNA. cDNAs (25 ng/well) were used to quantify gene expression by Quantitative RT-PCR (qPCR). All absolute data were first normalized to *RPLP0*. **Collagen measurement:** collagen quantification was performed using Collagen Assay Sircol™ **Trichome Masson Staining**: Trichrome Stain (Masson) Kit was used to stain paraffin sections of kidney organoids to ascertain fibrotic-like deposition.
Results: Under hypoxia and high glucose environment (diabetogenic condition) kidney organoids showed an increase in the mRNA levels of core pro-fibrotic genes (*COL1A1*, *COL3A4*, *COL4A1*, *ACTA2*) and collagen synthesis by colorimetric assay (Collagen Assay SircolTM) To check if kidney organoids can recapitulate the metabolic reprograming, previously reported in the diabetic kidney, we compared TECs from diabetic or control patients with TECs sorted from kidney organoids exposed to control and diabetogenic cell culture conditions. We observed that both cellular systems exhorted a similar metabolic profile assessed by Seahorse analysis and qPCR. Next, we inhibited fatty acid oxidation (FAO-main source of energy for TECs) exposing kidney organoids to etomoxir and further analysed by qPCR and Trichome mason staining the extent of a fibrotic-like response. The results showed that FAO inhibition results in a metabolic reprogramming (from FAO to glycolytic) together with an increase in the levels of expression of core pro-fibrotic genes.
Conclusion: The information gained here opens the door for the development of a DN model in kidney organoids. As a first proof of concept we interrogated for the effect of FAO inhibition in this system and showed that TECs metabolic reprogramming resulted in a fibrotic-like response at both transcriptomic/phenotypic level. Our model is currently being explored for the screening of epigenetic modifiers targeting FAO components serving as an unprecented tool for drug discovery in DN.
Supported by: This project has received founding form the European Research Council (ERC)
Disclosure: **C. Hurtado del Pozo:** None.

1030

Activated TGF-β1 reduces GPx-4 protein abundance in murine podocytes

C. Hangel[1], R.H. Henning[2], A.H. Wagner[1], M. Hecker[1];
[1]Division of Cardiovascular Physiology, Institute of Physiology and Pathophysiology, Heidelberg, Germany, [2]Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Background and aims: Glutathione peroxidases (GPx) protect cells from oxidative damage by catalyzing the reduction of both lipid and hydrogen peroxides. Oxidative stress has been reported to be a causative factor in the progression and complications of renal diseases. We previously showed that GPx-1 and GPx-4 expression was significantly reduced in kidney glomerular podocytes of people with type 2 diabetes (T2D). These individuals have also higher serum leptin levels, inducing the release of growth factors like, e.g. transforming growth factor β-1 (TGF-β1) from glomerular endothelial cells. As endothelial cells and podocytes are in close proximity in the glomerulus, we investigated *in vitro* the paracrine signaling mechanism causing a reduction of GPx-4 protein abundance in podocytes.
Materials and methods: Conditionally immortalized murine microvascular endothelial cells (CI-muMECs) were cultured until reaching 100% confluence and exposed to different concentrations of leptin (10–1000 ng/ml leptin) for 24 hours. Medium supernatants were analyzed for TGF-β1 concentration measured by enzyme linked immunosorbant assay (ELISA). Quantitative real-time PCR was used to detect and quantify TGF-β1 mRNA in CI-muMECs. The podocyte cell line E11 was incubated with different concentrations of TGF-β1 (1–50 ng/ml) as well as different concentrations of leptin (5–200 ng/ml) and harvested after 5 days of treatment for GPx-4 protein analysis using standard Western blot techniques.
Results: No significant difference in TGF-β1 mRNA levels between non-stimulated and stimulated CI-muMECs could be detected. In contrast, a significant ($p < 0.05$) 1.6 fold increase of activated TGF-β1 (30 ng/ml) in the medium supernatant of the endothelial cells could be detected after stimulation with 100 ng/ml leptin. Subsequently, using this concentration of TGF-β1 in co-culture experiments with podocytes resulted in a significant ($p < 0.05$) 50% decrease in the GPx-4 protein content of these cells after 5 days incubation time.
Conclusion: These results show that elevated levels of leptin in obese people with T2D may have an indirect effect on kidney podocytes via leptin binding to glomerular endothelial cells and increased release of activated TGF-β1. This paracrine TGF-β1 signaling might cause a reduction of GPx-4 protein abundance in podocytes leading to ferroptosis, a non-apoptotic iron-dependent form of regulated cell death. Ferroptosis is characterized by the execution of GPx-4 and a subsequent accumulation of lipid peroxides. This might explain a fundamental mechanism causing kidney damage in people with diabetes.
Supported by: GRK 1874 SP10
Disclosure: **C. Hangel:** None.

1031

DPP-4 release in human podocytes

E. Benetti[1], V. Bordano[1], F. Barutta[2], G. Gruden[2], C. Fedele[1], A. Rosa[1], T. Klein[3], G. Miglio[1];
[1]Scienza e Tecnologia del Farmaco, University of Turin, Turin, Italy, [2]Department of Medical Sciences, University of Turin, Turin, Italy, [3]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

Background and aims: Dipeptidyl-peptidase (DPP)-4 is the pharmacological target of gliptins, drugs approved for the treatment of Type-2 diabetes mellitus. It is a membrane-bound protein expressed by different cell types, including podocytes. However, a soluble DPP-4 form is also found in serum, thus indicating the existence of at least two forms of this

enzyme, possibly playing a distinct pathophysiological role. To date, however, the mechanisms involved in determining the relative proportion of the known DPP-4 forms have not be fully elucidated. By employing human immortalized podocytes as a cellular model, the effect of relevant stimuli for this cell type on the release of DPP-4 was studied

Materials and methods: Human immortalized podocytes were exposed (48 h) to angiotensin II (1 μM), transforming growth factor (TGF)-β (10 ng/ml), phorbol 12-myristate 13-acetate (PMA; 50 nM) or forskolin (10 μM). The level of the soluble DPP-4 was evaluated in cell-conditioned media by ELISA. Moreover, DPP-4 enzymatic activity was determined both in cell extracts and conditioned media by measuring the cleavage of the substrate H-Ala-Pro-7-amido-4-trifluoromethylcoumarin. Statistical significance was assessed by the Student's t test and a $P < 0.05$ was adopted as threshold.

Results: DPP-4 was highly expressed in human immortalized podocytes. In comparison to the cell-conditioned media, a ~100-fold higher enzymatic activity was determined in cell extracts. Soluble DPP-4 level was significantly increased in the media of cells treated with angiotensin II ($P < 0.05$ *vs*control), but not with TGF-β. DPP-4 release was also dramatically increased by PMA ($P < 0.005$ *vs* control), but not by forskolin.

Conclusion: In human podocytes DPP-4 release is promoted by angiotensin II. This effect could be mediated by the activation of phospholipase C isoenzymes. Overall, modulation of DPP-4 release could contribute to the effects exerted by specific stimuli affecting podocyte biology. In addition, a better characterization of the DPP-4 biology could add new piece of evidence to the gliptin pharmacology.

Supported by: Boehringer Ingelheim International GmbH

Disclosure: **E. Benetti:** Grants; Boehringer Ingelheim International GmbH.

1032

(-)-Epicatechin and the colonic metabolite 3,4-dihydroxyphenylacetic acid protect against high glucose-induced stress via SIRT1 in renal tubular cells

D. Álvarez-Cilleros[1], M.-Á. Martín[1,2], L. Goya[1], S. Ramos[1];
[1]Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), Madrid, [2]CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM), Madrid, Spain.

Background and aims: Persistent oxidative stress plays a main role in the development and progression of the diabetic nephropathy, which is accompanied by increased production of free radicals and/or impaired antioxidant defences. Flavonoids have been found to possess beneficial effects on health and have drawn attention because of their safety. Oligomeric and polymeric flavanols are metabolised by the intestinal microbiota into various phenolic acids of low molecular weight, which are well absorbed in the colon. (-)-Epicatechin (EC), a main flavanol in cocoa, and its colonic metabolites, such as 3,4-dihydroxyphenylacetic acid (DHPAA), 2,3-dihydroxybenzoic acid (DHBA) and 3-hydroxyphenylpropionic acid (HPPA), could display some antidiabetic effects, but the mechanisms for their preventive activities related to oxidative stress in the kidney remain largely unknown. In the present work, the effects of EC and the mentioned microbial metabolites on the redox status are studied in renal proximal tubular NRK-52E cells treated with high glucose.

Materials and methods: NRK-52E cells treated for 2 h with realistic concentrations of EC, DHPAA, DHBA or HPPA (10 μM) were further exposed to 30 mM glucose for 22 h. In the experiments with the inhibitors, cells were pre-incubated with 10 μM DPI or 10 μM EX-527 for 1 h prior to EC or DHPAA incubation for 2 h followed by the glucose challenge. Generation of reactive oxygen species (ROS) and glutathione levels (GSH) were evaluated by fluorimetric methods. Activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) were assayed by spectrophometric methods. Total levels of proteins related to oxidative stress such as ERK, JNK, p38, SIRT-1 and NOX-4 were analysed by Western blot.

Results: Pre-treatment of cells with EC or DHPAA reverted the enhanced generation of ROS induced by glucose (30 mM), but not when they were treated with DHBA or HPPA. EC and DHPAA pre-treatment also prevented the inactivation of GPx, GR, CAT and SOD induced by glucose, showing a glutathione content similar to those of control cells. Furthermore, glucose induced a diminution of SIRT-1 levels, which was avoided by EC and DHPAA. Pre-treatment of cells with EC and DHPAA prevented the increase in phosphorylated MAPKs and total NOX-4 levels provoked by 30 mM glucose. Inhibition of NOX-4 and SIRT-1 in EC- and DHPAA-pre-treated cells showed the involvement of both proteins in EC- and DHPAA-mediated protection.

Conclusion: EC and the flavanol colonic metabolite DHPAA at concentrations that are not toxic to tubular renal cells and are reachable through the diet, possess a renoprotective effect. EC and DHPAA contributed to the cellular redox balance by preventing excessive ROS generation, and activation of stress related key proteins (MAPKs and NOX-4), as well as by averting the diminution of antioxidant defences and SIRT-1 levels induced by high glucose. Further efforts are needed to define the precise role of EC and its colonic metabolites on the protection of renal function, but it could be suggested that a diet rich in EC and/or cocoa may be a potential chemopreventive tool useful for the management of diabetes.

Supported by: AGL2015-67087, MINECO/FEDER, UE

Disclosure: **D. Álvarez-Cilleros:** None.

1033

Endothelial FGFR1 is essential for the anti-fibrotic and anti-EndMT effects of N-acetyl-seryl-aspartyl-lysyl-proline in diabetic mice

K. Kanasaki, J. Li, K. Nitta, D. Koya;
Kanazawa Medical University, Kahoku, Ishikawa, Japan.

Background and aims: Endothelial-to-mesenchymal transition (EndMT) and epithelial-to-mesenchymal transition (EMT) have been shown to contribute in organ fibrogenesis, and we have reported that the anti-EndMT effect of N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) was associated with restoring expression of diabetes-suppressed fibroblast growth factor receptor (FGFR)1. We recently reported that the deficiency in endothelial FGFR1 resulted in EndMT and AcSDKP could not suppress EndMT in FGFR1 deficient endothelial cells. Here we investigated whether endothelial FGFR1 is critical for anti-EndMT and anti-fibrotic action of AcSDKP in diabetic kidney.

Materials and methods: In vitro analysis was performed by using human dermal microvascular endothelial cells (HMVECs) and HK-2 tubular cells. In vivo, endothelial specific FGFR1 deficient mice (FGFR^{endo}: *FGFR1*$^{fl/fl}$; *VE-cad Cre(+)*) were generated and streptozotocin was utilized for the induction of diabetes.

Results: The conditioned-media from EndMT cells (FGFR1 deficient endothelial cells) stimulated transforming growth factor-β-dependent EMT in HK2 cells. In vivo, the kidney fibrosis was induced in diabetic both control mice (*FGFR1*$^{fl/fl}$; *VE-cad Cre(-)*) and FGFR1^{endo}; the fibrosis is significantly severe in diabetic FGFR1^{endo}. The intervention with AcSDKP significantly ameliorated the kidney fibrosis in diabetic control mice, but only partially suppressed in diabetic FGFR1^{endo}. In addition, AcSDKP had no effect on EndMT but suppressed EMT in kidney of diabetic FGFR1^{endo}.

Conclusion: These data demonstrated that the endothelial FGFR1 is functional target of AcSDKP and the key molecule for combating diabetes-associated kidney fibrosis in diabetes by suppressing EndMT.

Disclosure: **K. Kanasaki:** Honorarium; Japan Boehringer Ingelheim, Sanofi, Japan Eli Lilly, Tanabe Mitsubishi.

1034

The role of TNF-a-Induced Protein 2 in diabetic nephropathy

F. Barutta, S. Bellini, B. Corbetta, G. Gruden;
University of Turin, Turin, Italy.

Background and aims: Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins. The cytosolic protein TNF-α-Induced Protein 2 (TNFAIP2) is induced by TNF-α, an inflammatory cytokine implicated in the pathogenesis of DN. Moreover, TNFAIP2 expression is affected by retinoic acid, which is a key regulator of podocyte phenotype. This raises the possibility that TNFAIP2 may play a role in DN; however, there is no information on TNFAIP2 in diabetic or other kidney diseases. Our aim was, thus to assess if TNFAIP2 expression is altered in both human and experimental DN and to investigate the potential role of TNFAIP2, by studying both functional and structural abnormalities of DN in diabetic mice knockout for TNFAIP2.
Materials and methods: *Human kidney biopsies*: TNFAIP2 protein expression was studied in kidney biopsies from type 2 diabetic patients with overt DN ($n = 15$) and control non-diabetic subjects ($n = 6$) by immunohistochemistry/double immunofluorescence. *Experimental diabetes*: TNFAIP2 expression was measured in both renal cortex sections and isolated glomeruli obtained from both diabetic (DM $n = 15$) and control (ND $n = 15$) animals after 14 weeks of diabetes by RT-PCR, immunohistochemistry, immunoblotting. *In vitro experiments*: podocytes were exposed to high glucose, mechanical stretch, TNF-α, or retinoic acid for various time periods and TNFAIP2 expression analysed by either RT-PCR or immunoblotting. Control cells were studied in parallel. *In vivo study*: Both wild type ($TNFAIP2^{+/+}$) and TNFAIP2 knockout ($TNFAIP2^{-/-}$) mice were made diabetic by intraperitoneal injection of streptozotocin. Control mice were injected with citrate buffer alone. Fourteen weeks after the induction of diabetes, mice were individually placed in metabolic cages for urine collections and blood samples taken for blood glucose and glycated haemoglobin measurements. Albumin excretion rate and creatinine clearance were measured by enzyme-linked immunosorbent assay and HPLC, respectively. Expression of podocin, synaptopodin, and fibronectin was assessed by either immunofluorescence or RT-PCR.
Results: Glomerular TNFAIP2 expression was greater in patients with DN than healthy subjects and showed a predominant podocyte distribution. Similarly, glomerular TNFAIP2 expression was upregulated in diabetic mice as compared to controls. Cultured podocytes constitutively express TNFAIP2 at both mRNA and protein level and TNFAIP2 expression was increased by high glucose, TNF-α, and retinoid acid. Diabetes was associated with reduced body weight and elevations in both plasma glucose and glycated haemoglobin levels, but no differences were seen between $TNFAIP2^{+/+}$ and $TNFAIP2^{-/-}$ mice. Albumin/creatinine ratio was significantly ($p < 0.05$) increased in the wild type diabetic animals (DM: 68.2 ± 14.0) as compared to the controls (ND: 36.7 ± 7.6) and further enhanced by TNFAIP2 deletion [(ND $TNFAIP2^{-/-}$:28.8 ± 7.9; DM $TNFAIP2^{-/-}$:144.1 ± 36.5; $p < 0.05$ DM $TNFAIP2^{+/+}$ vs DM $TNFAIP2^{-/-}$]. Moreover, diabetes-induced both podocin and synaptopodin down-regulation was further exacerbated in diabetic mice lacking TNFAIP2. Histological assessment by PAS staining showed that the degree of mesangial expansion was greater in diabetic mice $TNFAIP2^{-/-}$ than in wild type mice. Consistently, diabetes-induced fibronectin overexpression was significantly increased by TNFAIP2 deletion.
Conclusion: These results suggest a protective role of TNFAIP2 in DN.
Supported by: JDRF
Disclosure: **F. Barutta:** None.

1035

Mesenchymal stem cells prevent progression of diabetic nephropathy by improving mitochondrial function in tubular epithelial cells

K.-U. Lee, E. Koh, J. Jang, C. Woo, S. Lee;
Asan Medical Center, Seoul, Republic of Korea.

Background and aims: Mitochondrial dysfunction is considered a major pathogenic factor in diabetic nephropathy. Renal proximal tubular epithelial cells (TECs), which are abundant in mitochondria, play an important role in its progression. Administration of mesenchymal stem cells (MSCs) was shown to attenuate overt, as well as early, diabetic nephropathy in rodents, but the mechanism of this prevention is largely unknown.
Materials and methods: We examined the preventative effects of human umbilical cord blood-derived MSCs on proximal tubular injury in streptozotocin-induced diabetes, and whether MSCs ameliorate mitochondrial dysfunction. We also investigated the molecular mechanism by which MSCs can improve mitochondrial function in TECs.
Results: MSC administration to diabetic mice reversed albuminuria and prevented both inflammation and proximal tubular injury in the kidneys. The expression of pro-inflammatory M1 macrophage markers, and arginase 1 (*Arg1*), a representative M2 macrophage marker, increased and decreased, respectively, in diabetic kidneys. Treatment with MSCs notably prevented these changes. Correspondingly, co-culture of macrophages with MSCs significantly decreased M1 macrophage markers, with a concomitant increase of *Arg1* expression. Inflammatory cytokines produced by macrophages decreased mitochondrial biogenesis in adipocytes. Similarly, conditioned media from lipopolysaccharide-activated macrophages decreased the expression of peroxisomal proliferator-activated receptor gamma coactivator 1α (*Pgc-1α*) and impaired mitochondrial function in cultured TECs. These effects were reversed by co-culture of macrophages with MSCs. In addition, *Arg1*-overexpression in macrophages reversed the *Pgc-1α* suppression observed in TECs.
Conclusion: Treatment with MSCs prevents progression of diabetic nephropathy by reversing mitochondrial dysfunction in TECs via induction of *Arg1* in macrophages.
Supported by: NRF and KHIDI
Disclosure: **K. Lee:** None.

PS 096 Of drugs and kidneys

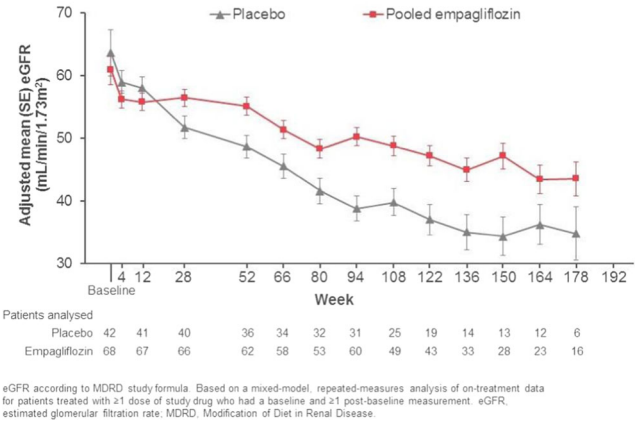
1036

Empagliflozin and progression of chronic kidney disease in type 2 diabetes complicated by nephrotic-range proteinuria: insights from the EMPA-REG OUTCOME trial

P. Ruggenenti[1], S. Inzucchi[2], B. Zinman[3], S. Hantel[4], A. Koitka-Weber[4,5], M. von Eynatten[6], C. Wanner[7];
[1]Istituto di Ricerche Farmacologiche Mario Negri, Clinical Research Center for Rare Diseases "Aldo e Cele Daccò" (IRCCS), Bergamo, Italy, [2]Section of Endocrinology, Yale University School of Medicine, New Haven, USA, [3]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital and Division of Endocrinology, University of Toronto, Toronto, Canada, [4]Boehringer Ingelheim International GmbH, Biberach, Germany, [5]Dept of Diabetes, Central Clinical School, Monash University, Melbourne, Australia, [6]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [7]Dept of Medicine, Division of Nephrology, Würzburg University Clinic, Würzburg, Germany.

Background and aims: In patients with diabetic kidney disease, nephrotic-range proteinuria is a major risk factor for accelerated glomerular filtration rate (GFR) loss, cardiovascular disease and all-cause mortality. In this *post-hoc* analysis of the EMPA-REG OUTCOME trial, we evaluated the effects of the sodium glucose co-transporter 2 inhibitor empagliflozin (EMPA) in patients with type 2 diabetes, established cardiovascular disease and nephrotic-range proteinuria at study inclusion.
Materials and methods: In the EMPA-REG OUTCOME trial, patients were randomised to receive EMPA 10 or 25 mg/day, or placebo (PBO), in addition to standard of care. Median observation time was 3.1 years. According to Kidney Disease: Improving Global Outcomes (KDIGO) criteria, nephrotic-range proteinuria was defined as urine albumin:creatinine ratio (UACR) ≥2200 mg/g. A mixed-model repeated measures analysis was used to evaluate changes in estimated GFR (eGFR) over time. Treatment differences in the average rate of annual loss of eGFR were assessed for pooled EMPA vs PBO using a random coefficient model. A Cox proportional hazards model was used to investigate the risk of all-cause hospitalisation as ascertained by investigator 'serious adverse event' reporting.
Results: We identified 112 patients with nephrotic-range proteinuria (PBO, $n = 42$; pooled EMPA, $n = 70$). At baseline, mean [SD] eGFR (PBO, 63.6 [23.5]; EMPA, 60.3 [19.5] mL/min/1.73 m²) and median UACR [interquartile range] (PBO, 3676 [2713–4865]; EMPA, 3532 [2701–4879] mg/g creatinine) were balanced between groups. After an acute fall in eGFR during the first 4 weeks in both groups, the PBO group experienced a steeper decline in eGFR than the EMPA group (Figure). Between week 4 to last value on treatment the annual loss of eGFR was 10.7 mL/min/1.73 m² with PBO and 4.5 mL/min/1.73 m² with EMPA; thus, yearly eGFR loss was 6.1 mL/min/1.73 m² slower with EMPA than PBO ($p = 0.0098$). Moreover, EMPA significantly reduced the risk of all-cause hospitalisation by 47% versus PBO (hazard ratio 0.53 [0.30–0.93]; $p = 0.0263$).
Conclusion: EMPA could be a new treatment option to slow GFR decline and reduce all-cause hospitalisations in patients with type 2 diabetes and cardiovascular disease at high risk for rapid loss of renal function due to nephrotic-range proteinuria.

Figure. eGFR over time in patients with nephrotic-range proteinuria at baseline



eGFR according to MDRD study formula. Based on a mixed-model, repeated-measures analysis of on-treatment data for patients treated with ≥1 dose of study drug who had a baseline and ≥1 post-baseline measurement. eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease.

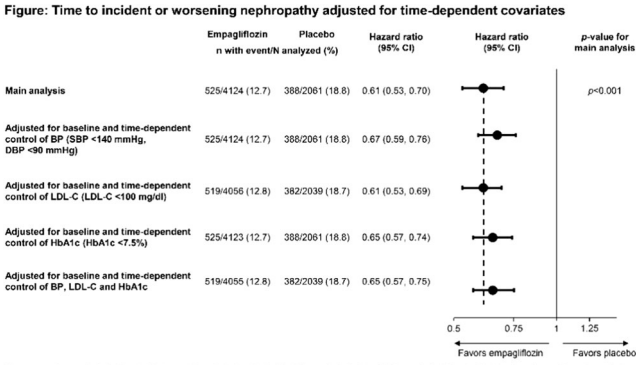
Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **P. Ruggenenti:** Non-financial support; Boehringer Ingelheim.

1037

Empagliflozin improves kidney outcomes irrespective of control of blood pressure, low-density lipoprotein cholesterol and HbA_{1c}

C. Wanner[1], M.E. Cooper[2], S.E. Inzucchi[3], B. Zinman[4], U. Hehnke[5], M. von Eynatten[5], A. Koitka-Weber[2,6];
[1]Dept of Medicine, Division of Nephrology, Würzburg University Clinic, Würzburg, Germany, [2]Dept of Diabetes, Central Clinical School, Monash University, Melbourne, Australia, [3]Section of Endocrinology, Yale University School of Medicine, New Haven, USA, [4]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital and Division of Endocrinology, University of Toronto, Toronto, Canada, [5]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [6]Boehringer Ingelheim International GmbH, Biberach, Germany.

Background and aims: The EMPA-REG OUTCOME trial demonstrated the renoprotective role of the sodium-glucose co-transporter 2 inhibitor empagliflozin (EMPA) by reducing the risk of incident or worsening nephropathy by 39% vs placebo (PBO) in patients with type 2 diabetes and established cardiovascular (CV) disease. We investigated the effects of controlling the CV risk factors of blood pressure (BP), low-density lipoprotein cholesterol (LDL-C) and HbA1c on treatment differences in kidney outcomes.
Materials and methods: In EMPA-REG OUTCOME, patients were randomised 1:1:1 to EMPA 10 mg, EMPA 25 mg, or PBO. Risk of incident or worsening nephropathy was assessed in the pooled EMPA group vs PBO adjusting for control of BP, LDL-C and HbA1c at baseline and during the study as time-dependent covariates. Control of the various parameters was defined as systolic BP <140 mmHg and diastolic BP <90 mmHg, LDL-C <100 mg/dL, and HbA1c <7.5%.
Results: Adjusting for control of BP, LDL-C or HbA1c individually, HRs for time to incident or worsening nephropathy with EMPA vs PBO ranged from 0.61 to 0.67 (Figure). Adjusting for control of all 3 parameters, the HR (95% CI) was 0.65 (0.57, 0.75) (Figure).
Conclusion: EMPA reduced the risk of incident or worsening nephropathy to the same extent when analyses were adjusted for control of BP, LDL-C and HbA1c over time. These data suggest that risk reductions in kidney outcomes were preserved irrespective of control of conventional CV risk factors.



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **C. Wanner:** Grants; Boehringer Ingelheim. Non-financial support; Boehringer Ingelheim.

1038

Luseogliflozin inhibits HIF-1alpha expression in renal proximal tubular epithelial cells

Y. Takiyama, R. Bessho, T. Ota;
Department of Medicine, Asahikawa Medical University, Asahikawa, Japan.

Background and aims: Recent clinical trials have demonstrated the renoprotective effects of SGLT2 inhibitors in diabetic nephropathy. However, the mechanisms of SGLT2 inhibitors on prevention of diabetic nephropathy have not been fully elucidated. Hypoxia-induced tubulointerstitial fibrosis is considered to be a common pathway for various progressive kidney diseases including diabetic nephropathy. Hypoxia inducible factor (HIF)-1α plays an important role in these pathological processes. In the present study, we assessed the effects of luseogliflozin, an SGLT2 inhibitor, on HIF-1α expression in both cultured human renal proximal tubular epithelial cells (HRPTECs) and the kidneys of diabetic *db/db* mice.
Materials and methods: We examined the expression of HIF-1α protein by Western blot, HIF-1 targeted genes by qRT-PCR and cellular hypoxia using a hypoxia-sensitive dye pimonidazole in HRPTECs. Next, eight-week-old male diabetic *db/db* mice were treated with 15 mg/kg luseogliflozin for 8 weeks. Body weight, glucose tolerance, blood pressure, and urinary albumin excretion were measured. The renal histology, immunohistochemistry for HIF-1α and megalin, hypoxia-probe pimonidazole accumulation and confocal microscopic analysis of albumin endocytosis were evaluated at the end of the study.
Results: Hypoxia (1% O_2 for 24h) markedly increased HIF-1α protein in HRPTEC, whereas luseogliflozin inhibited the expression of hypoxia-induced HIF-1α protein. In addition, luseogliflozin inhibited mRNA expression for HIF-1-targeted genes, PAI-1 and GLUT-1 under hypoxia.The inhibitors of mitochondrial respiratory complex I, rotenone or complex III, antimycin A equally suppressed hypoxia-induced HIF-1α expression. Luseogliflozin decreased staining of pimonidazole in HRPTECs under hypoxic conditions. Eight weeks after administration, luseogliflozin lowered plasma glucose levels and decreased glomerular mesangial matrix expansion as well as glomerular and interstitial fibronectin accumulation in the kidney of *db/db* mice. However, luseogliflozin failed to attenuate urinary albumin excretion. Interestingly, luseogliflozin decreased megalin expression in *db/db* mice and led to decrease Texas Red-albumin uptake, suggesting that luseogliflozin induces albuminuria in *db/db* mice through the inhibition of megalin expression, but not renal injury. Electron microscopic study revealed that luseogliflozin decreased the length of mitochondria in the renal proximal tubules of the S1 segment. Furthermore, luseogliflozin attenuated diabetes-induced HIF-1α accumulation in the kidney of *db/db* mice. These results suggest that luseogliflozin inhibits hypoxia-induced HIF-1α accumulation at least partly by suppressing mitochondrial respiration. However, luseogliflozin augmented pimonidazole staining of the S3 segment of the proximal tubules cells which expressed SGLT1, in the outer stripe of the kidney of the *db/db* mice. Because pimonidazole and HIF have different kinetics and different hypoxia thresholds, these signals showed only partial overlap at the cellular level in *db/db* mice.
Conclusion: These results suggest that luseogliflozin inhibits hypoxia-induced HIF-1α expression and dynamically changes the oxygen metabolism in the renal proximal tubular cells. The SGLT2 inhibitors may protect diabetic kidney from hypoxia-induced renal fibrosis by attenuating the expression of HIF-1α and profibrotic molecules.
Supported by: Taisho Pharmaceutical Co., Ltd.
Disclosure: **Y. Takiyama:** Other; Taisho Pharmaceutical Co., Ltd.

1039

Relation between urinary glucose reabsorption and kidney function in diabetic patients

O. Matar[1], L. Potier[1,2], M. Hallot-Feron[1], F. Fumeron[2], R. Roussel[1,2], G. Velho[2], M. Marre[1,2];
[1]Diabétologie, Hôpital Bichat, APHP, Paris, [2]INSERM U 1138, Paris, France.

Background and aims: Glycosuria induced by SGLT2-inhibitors protects against end stage kidney disease, but no association has been established between urinary glucose reabsorption and kidney function in diabetic patients. We examined the relationship between urinary glucose reabsorption and kidney involvement in patients with diabetes.
Materials and methods: The diabetic patients attending our tertiary referral centre were studied consecutively from September 2017 to January 2018 for their urinary glucose reabsorption according to their kidney functions: eGFR (CKD-EPI equation) >90, 90–60, <60–30, <30 mL/min/1.73 m^2, and urinary albumin excretion (normo-, micro-, and macroalbuminuria), taking into account their prevailing plasma glucose (<6, 6–11, and >11 mmol/L). Urinary glucose reabsorption was calculated as 1- Fractional Excretion of Glucose (FEGlu), where FEGLu = (Urinary glucose concentration (UGlu) × urine volume)/GFR, and GFR = Creatinine Clearance. Thus, FEGLu = (UGlu × Plasma Creatinine)/(Plasma Glucose × Urinary Creatinine concentration).
Results: 650 diabetic patients, without SGLT2 inhibitors, were studied (aged 60 ± 14 years; 41% women; 10% type 1, 76% type 2, 14% other diabetes types; BMI 29.1 ± 6.3 kg/m^2; HbA1c 9.1 ± 2.4%; median diabetes duration 14 (interquartiles (IQ) 6–22) years ; systolic/diastolic blood pressure 131(19)/73(13) mmHg), 43% with eGFR >90, 33% 90–60, 17% <60–30, 7% <30 mL/min/1.73 m^2, and 48% with normo-, 41% micro-, and 11% macroalbuminuria. The median urinary glucose reabsorption was 0.9977 (IQ 0.9665–0.9992), and differed in the patients with plasma glucose <6 mmol/L: 0.9990 (IQ 0.9978–0.9993), with 6–11 mmol/L: 0.9991(IQ 0.9963–0.9994), and with >11 mmol/L 0.9636(IQ 0.8593–0.9947), (ANOVA $p < 0.0001$). Plasma glucose accounted for only 37% of inter-individual variance of urinary glucose reabsorption. The latter varied with eGFR: >90 (0.9927), 90–60 (0.9987), <60–30 (0.9987) to <30 mL/min/1.73 m^2 (0.9920), multi-adjusted ANOVA $p = 0.0046$, and increased from normo- to macroalbuminuria ($p = 0.0105$). Urinary glucose reabsorption was significantly affected by the interactions between increasing plasma glucose levels and eGFR stages ($p < 0.0001$), and with normo-, micro-, macroalbuminuria stages ($p < 0.0001$). In patients with plasma glucose >11 mmol/L, urinary glucose reabsorption increased from 0.9458(IQ 0.8125–0.9931) for eGFR >90 mL/min/1.73 m^2 to 0.9728(IQ 0.9013–0.9976) for 90–60 mL/min/1.73 m^2 to 0.9912(IQ 0.9276–0.9982) for <60–30 mL/min/1.73 m^2 and to 0.9946(0.9764–0.9980) for <30 mL/min/1.73 m^2 ($p = 0.0087$); it was

0.9606(IQ 0.8308–0.9975) in patients with normo-, 0.9656(IQ 0.9413–0.9973) in those with micro-, and 0.9876(IQ 0.9420–0.9957) in those with macroalbuminuria ($p = 0.01$).

Conclusion: There is a strong association between elevated capacities of urinary glucose reabsorptions and severity of kidney disease in patients with diabetes. Although cross-sectional, these results support the view that mechanisms involved in urinary glucose reabsorption contribute to risk for kidney disease in diabetes.

Disclosure: **O. Matar:** None.

1040

Effects of sodium-glucose cotransporter 2 inhibitors on renal outcomes in patients with type 2 diabetes: a systematic review and meta-analysis

J. Bae[1], E. Park[2], S. Kim[2], K. Kim[1], J. An[1], H. Kim[1], J. Kim[1], S. Kim[1], S. Hahn[2], N. Kim[1];

[1]Korea University College of Medicine, Seoul, [2]Seoul National University College of Medicine, Seoul, Republic of Korea.

Background and aims: To investigate the effects of sodium-glucose cotransporter 2 (SGLT2) inhibitors on renal outcomes in patients with type 2 diabetes mellitus (T2D)

Materials and methods: We searched Medline, Embase, and the Cochrane Central Registration of Controlled Trials to identify randomized controlled trials (RCTs) of SGLT2 inhibitors up to Sep 18, 2017. RCTs comparing SGLT2 inhibitors with placebo or other oral antidiabetic drugs and reporting at least one kidney-related variable including changes in urine albumin-to-creatinine ratio and estimated glomerular filtration rate (eGFR), and development of microalbuminuria, macroalbuminuria, or end-stage renal disease in patients with T2D were selected. We performed a meta-analysis using fixed-effects model and random-effects model to calculate mean differences and relative risk for renal outcomes.

Results: A total of 45 eligible RCTs involving 45,654 patients with T2D were included in the analysis. Compared with placebo or other oral antidiabetic drugs, SGLT2 inhibitors significantly decreased urine albumin-to-creatinine ratio (mean difference −7.84 mg/g, 95% confidence interval [CI] −12.84 to −2.84, $P = 0.0021$). In addition, SGLT2 inhibitors were associated with the lower risk of developing microalbuminuria (relative risk [RR] 0.73, 95% CI 0.54 to 1.00, $P = 0.0505$) and macroalbuminuria (RR 0.68, 95% CI 0.60 to 0.77, $P < 0.0001$) compared with controls. The overall change in eGFR was comparable between SGLT2 inhibitors and controls (mean difference −0.08 mL/min/1.73 m^2, 95% CI –0.66 to 0.49, $P = 0.7833$). However, in subgroup analyses according to study duration and baseline eGFR, SGLT2 inhibitors decreased eGFR in T2D patients with the study duration of <26 weeks and baseline eGFR of <45 mL/min/1.73 m^2 compared with controls. The risk of developing ESRD tended to be reduced in SGLT2 inhibitors compared with controls (RR 0.78, 95% CI 0.43 to 1.41, $P = 0.4114$).

Conclusion: SGLT2 inhibitors were associated with the lower risk of development or progression of albuminuria, although the overall changes in renal function were comparable to placebo or other oral antidiabetic drugs in patients with T2D.

Disclosure: **J. Bae:** None.

1041

Empagliflozin and linagliptin alleviate podocyte injury and activate glomerular autophagy in a model of type 2 diabetes

A.I. Korbut[1], V.V. Klimontov[1], Y.S. Taskayeva[1], N.P. Bgatova[1], E.L. Zavjalov[2];

[1]Research Institute of Clinical and Experimental Lymphology – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, [2]Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russian Federation.

Background and aims: Podocyte injury is believed to be a cornerstone in pathogenesis of diabetic kidney disease. Recent data indicate emerging role of autophagy downregulation in diabetic podocytopathy. Accordingly, diminished autophagy could be the therapeutic target in diabetes. Inhibitors of sodium-glucose cotransporter-2 (SGLT2) and dipeptidyl peptidase-4 (DPP4) are considered as promising therapeutic agents in diabetic nephropathy, but little is known about the effects of these agents on podocytes. Thus, we aim of our study to assess the effects of SGLT2 inhibitor empagliflozine, DPP4 inhibitor linagliptin and their combination on podocyte injury and autophagy in a model of type 2 diabetes.

Materials and methods: Eight-week-old male *db/db* mice (BKS.Cg-Dock7^{m}+/+Lepr^{db}/J) were treated with empagliflozine (10 mg/kg), linagliptin (10 mg/kg), combination of these agents, or placebo for 8 weeks. Non-diabetic heterozygous *db*/+ mice were acted as control. The concentrations of insulin, glucagon, leptin and resistin in blood plasma were determined by Multiplex analysis, and body composition was assessed by MRI at week 0 and 8 of experiment. Renal structural changes were analyzed quantitatively from the light and electron microscopic images. To estimate autophagy, beclin-1 staining in glomeruli was assessed by immunohistochemistry, and volume density of autophagosomes, lysosomes, and autolysosomes in podocytes were estimated.

Results: Diabetic *db/db* mice became obese and hyperglycemic before the start of experiment and demonstrated elevated levels of leptin and insulin and increased fat percentage at week 0 and week 8 (all $p < 0.00001$). Podocytopathy in these mice was manifested by the reduction in mean number of foot processes and increase in their width. In all treated groups the number of podocyte foot processes increased significantly ($p = 0.003$ for linagliptin, $p = 0.03$ for empagliflozin and combination) and the width of podocyte foot processes decreased ($p = 0.003$ for empagliflozin and linagliptin, $p = 0.001$ for combination). Vehicle-treated diabetic mice had weak staining for beclin-1 in glomeruli (for volumetric density $p = 0.002$ vs. *db*/+ mice) and reduced autophagosome volume density in podocytes ($p = 0.04$). The volumetric density of beclin-1-positive area correlated with volume density of autophagosomes and lysosomes (both $r = 0.43$, $p = 0.04$) and width of foot processes ($r = -0.64$, $p = 0.0008$). Under the treatment, glomerular staining for beclin-1 was increased ($p = 0.03$ for empagliflozin, $p = 0.008$ for linagliptin, $p = 0.003$ for combination). Empagliflozin and linagliptin, either alone or in combination, increased volume density of autophagosomes ($p = 0.04$ for empagliflozin and combination, $p = 0.008$ for linagliptin) and autolysosomes ($p = 0.03$ for empagliflozin and combination, $p = 0.05$ for linagliptin) in podocytes.

Conclusion: The data from the current study demonstrate that both empagliflozin and linagliptin ameliorate podocyte injury and enhances autophagy in a model of type 2 diabetic nephropathy. The data provide further explanation for the mechanism of nephroprotective effect of SGLT2 and DPP4 inhibitors in diabetes.

Supported by: RFMEFI61914X0005, RFMEFI62114X0010

Disclosure: **A.I. Korbut:** None.

1042

Effect of vildagliptin added to insulin on glycaemic control in haemodialysis patients with type 2 diabetes: a randomised multicentre prospective study

A. Smagala[1], M. Munch[2], L. Meyer[3], A. Klein[1], B. Guercy[4], L. Frimat[4], S. Borot[5], D. Ducloux[5], D. Fleury[6], O. Verier[7], F. Alenabi[8], F. Chantrel[9], L. Kessler[3];

[1]CH Colmar, Colmar, [2]CH Strasbourg, Strasbourg, [3]CHRU de Strasbourg, Strasbourg, [4]CHRU de Nancy, Nancy, [5]CHRU de Besançon, Besançon, [6]CH Valenciennes, Valenciennes, [7]CHRU de Valenciennes, Valenciennes CEDEX, [8]CH de Mulhouse, Mulhouse, [9]CHRU de Mulhouse, Mulhouse, France.

Background and aims: Type 2 diabetic patients (T2D) undergoing chronic dialysis occurs for almost half of the patients starting dialysis in

European Western countries. Hemodialysis T2D patients are usually treated with insulin. Standard schema for glycemic management doesn't exist in this population with high cardiovascular risk mainly due to high risk of hypoglycemia and the limitations of HbA1C. Vildagliptin can be used in severe renal impairment and protects against hypoglycemia. The aim of the study was to evaluate the efficacy and safety of vildagliptin (50 mg/day) added to insulin, in a population of hemodialysis T2D patients.

Materials and methods: Sixty-five hemodialysis T2D patients treated with multiple daily insulin injections were randomized Insulin + Vildagliptin (Group 1: G1, $n = 32$) or Insulin regimen (Group 2: G2, $n = 33$) in a multicenter, prospective, open-label, 12 weeks-study. Glycemic control was assessed through 48h-Continuous Glucose Monitoring (*CGM- IPro2*, *Medtronic*) performed at baseline, repeated at 3-month follow-up with probe analysis. The primary end-point was the variation of mean interstitial glucose at CGM, after 3-month treatment. Secondary criteria were: CGM-Time in Range (70–180 mg/dl), time spent in hyperglycemia (>180 mg/dl) or hypoglycemia (<70 mg/dl), glycemic variability, HbA1c, glycated albumin, daily insulin requirements, tolerance, cardiovascular outcomes. Statistical analysis was performed in intention to treat.

Results: At baseline both Groups (G1 vs G2) were comparable for age (69.7 ± 7.4 vs 71.3 ± 9.6 years), duration of diabetes (23 ± 3.4 vs 23 ± 13 years), HbA1c (7.3 ± 1.1 vs 7.3 ± 1.1%) glycated albumin (611.3 ± 322 vs 666 ± 339 μmol/l, *normal range 100–285*), daily insulin needs (51.3 ± 25.4 vs 47.7 ± 29 IU/d), BMI (34.5 ± 6.4 vs 32.1 ± 6 kg/m²), severe hypoglycemia (4 vs 3 per 12 month prior to inclusion), mean glucose at CGM (163.9 ± 41.9 vs 167.4 ± 33.8 mg/dl). After 12 weeks, mean glucose of CGM decreased respectively from 163.9 ± 41.9 to 147.6 ± 32.7 mg/dl ($p = 0.2703$) in G1 and from 167.4 ± 44.8 to 160.0 ± 42.9 mg/dl ($p = 0.415$) in G2. At 12 weeks in G1 *vs* G2, time in range was higher (77.2 vs 69.5%, $p < 0.05$), time spent in hyperglycemia was lower (21.2 vs 39%, $p < 0.05$), and time spent in hypoglycemia was comparable (1.5 vs 1.4%, $p = 0.53$). Daily insulin needs (46.1 ± 23.4 vs 48.7 ± 31.7 IU/d, ns) and HbA1c (6.9 ± 0.9 vs 7.2 ± 1.1%, ns) were no different, whereas glycated albumin was lower (520.4 ± 159 vs 658.9 ± 386, $p < 0.001$). Coefficient of variation, AUC <70 mg/dl and >180 mg/dl were no different between the two groups. Two severe hypoglycemia occurred in each group, no cardiovascular outcome occurred, and digestive symptoms were comparable without acute pancreatitis

Conclusion: Adding vildagliptin to insulin in hemodialysis T2D patients doesn't modify mean glucose at CGM, but increases time in range, reduces time spent in hyperglycemia, without increasing time in hypoglycemia, and reduces glycated albumin

Clinical Trial Registration Number: 5704

Supported by: VILDDIAL PRI

Disclosure: **A. Smagala:** None.

1043

Safety of liraglutide vs placebo in patients with type 2 diabetes and chronic kidney disease in the LEADER trial

J. Mann[1], V. Fonseca[2], O. Mosenzon[3], I. Raz[3], H. Frimer-Larsen[4], B. von Scholten[4], T. Idorn[4], N. Poulter[5], The LEADER Trial Steering Committee and Investigators;

[1]KfH Kidney Center, Munich, Germany, [2]Tulane University Health Sciences Center, School of Medicine, New Orleans, USA, [3]Diabetes Unit, Hadassah Hebrew University Hospital, Jerusalem, Israel, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Imperial College London, London, UK.

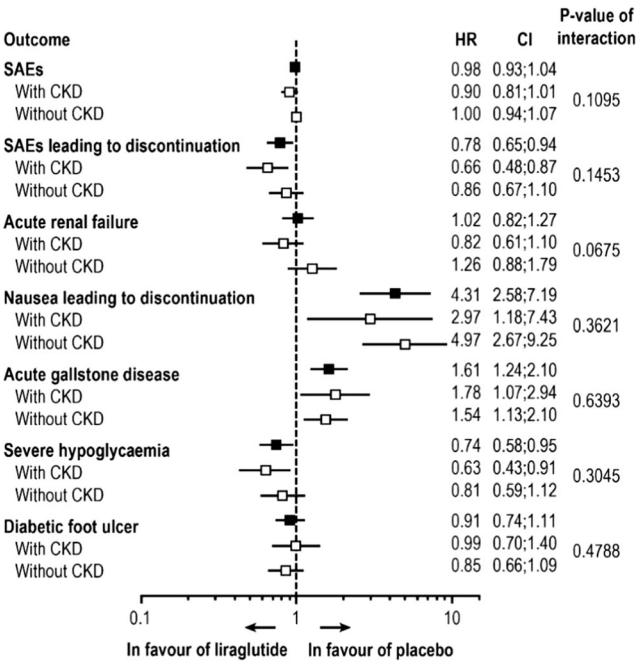
Background and aims: We assessed the safety of liraglutide vs placebo (PBO) in patients with chronic kidney disease (CKD) in the LEADER trial.

Materials and methods: LEADER was a randomised, double-blind, multicentre, placebo-controlled, cardiovascular (CV) outcome trial assessing CV and long-term safety of liraglutide up to 1.8 mg/day vs PBO plus standard of care for 3.5–5 years in 9340 patients with type 2 diabetes (T2D) at high risk for CV disease. We stratified participants according to baseline estimated GFR (eGFR) <60 (with CKD) or ≥60 ml/min/1.73 m^2 (without CKD) and analysed: serious adverse events (SAEs), SAEs leading to discontinuation, acute renal failure, nausea leading to discontinuation, acute gallstone disease, severe hypoglycaemia and foot ulcers.

Results: Mean eGFR in patients with ($n = 2158$) or without CKD ($n = 7158$) was 45.7 ± 10.9 and 90.8 ± 21.6 mL/min/1.73 m^2, respectively. There was no increased risk of SAEs or SAEs leading to discontinuation with liraglutide vs PBO in those with and without CKD (Figure); and no conclusive risk of acute renal failure in those with CKD (HR 0.82, CI 0.61;1.10) or without CKD (HR 1.26, CI 0.88;1.79) with liraglutide vs PBO. There was no difference in the risk of nausea leading to discontinuation or acute gallstone disease in patients with and without CKD. Severe hypoglycaemia risk was significantly reduced with liraglutide by 37% (with CKD: HR 0.63, CI 0.43;0.91) and non-significantly reduced by 19% (without CKD: HR 0.81, CI 0.59;1.12). Frequency of hypoglycaemia increased with decreasing eGFR. Diabetic foot ulcer risk was not increased with liraglutide in those with and without CKD (Figure).

Conclusion: In LEADER, liraglutide was as well tolerated in patients with CKD as in those without CKD.

Figure: Risk of selected adverse events with liraglutide vs placebo according at CKD at baseline



Estimated glomerular filtration rate was based on the Modification of Diet in Renal Disease formula.
CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio; SAE, serious adverse event.

Clinical Trial Registration Number: NCT01179048

Supported by: Novo Nordisk A/S

Disclosure: **J. Mann:** Other; Funding: Novo Nordisk A/S.

1044

Exenatide LAR improves in hypertensive type-2 diabetic patients plasma levels of cytokines involved in hypertension: an 8-month prospective intervention study

A. Rizvi[1], G. Li Volti[2], D. Nikolic[3], R. Chianetta[3], G. Castellino[3], A. Patti[3], R. Giglio[3], R. Citarrella[3], F. Provenzano[4], V. Provenzano[4], G. Montalto[3], M. Rizzo[3];

[1]Endocrinology, Diabetes and Metabolism, University of South Carolina, Columbia, USA, [2]University of Catania, Catania, Italy, [3]DIBIMIS, University of Palermo, Italy, Palermo, Italy, [4]Internal Medicine,

Partinico Hospital, Partinico, Italy.

Background and aims: Hypertension is often associated to type 2 diabetes (T2DM) and this combination increases the overall cardiovascular (CV) risk of such patients. Interleukin-2 (IL-2) and plasminogen activator inhibitor-1 (PAI-1) seem to be associated with incident of hypertension, while monocyte chemotactic protein 1 (MCP-1) is known to be a powerful mediator of inflammatory responses in hypertensive subjects. We here assessed the effects of exenatide once-weekly (long-acting release, LAR) on cardio-metabolic control, including the assessment of the 3 aforementioned cytokines in T2DM subjects with and without hypertension.
Materials and methods: Sixty T2DM subjects (41 men and 19 women; age: 60 ± 10 yrs), naïve to incretin-based therapies, were treated with exenatide LAR as add-on to metformin for 8 months. Exclusion criteria included a previous major CV event, moderate and severe renal and liver function. The cohort of subjects was subdivided in those with hypertension ($n = 18$) and without hypertension ($n = 42$). The three cytokines were measured by multiplex analysis using Luminex Magpix®. Endothelial function was assessed by flow mediated dilation (FMD) of the brachial artery, while cIMT was assessed by B-mode real-time ultrasound. Paired t-test and ANOVA were performed.
Results: Although cardio-metabolic control was similarly achieved in both groups of patients after exenatide LAR therapy (Table), plasma levels of the investigated cytokines reduced only in the subgroups of subjects with hypertension as following: IL-2 (from 8.7 ± 4.7 to 6.2 ± 2.0 pg/ml, $p = 0.0029$), MCP-1 (from 19.6 ± 7.0 to 15.9 ± 7.2 pg/ml, $p = 0.0087$), PAI-1 (from 1.7 ± 1.5 to 1.2 ± 1.4 ng/ml, $p = 0.0369$).
Conclusion: Exenatide LAR treatment was associated with similar cardio-metabolic control in T2DM patients with vs. without hypertension. However, improvements in cytokines associated with inflammation at the endothelial level and with the development of hypertension were only seen in subjects with hypertension. Whether this finding would translate into an effective CV prevention in this category of patients remains to be established by future studies.

Clinical Trial Registration Number: NCT02380521

	With hypertension (n=18)			Without hypertension (n=42)			
	before exenatide LAR therapy	after exenatide LAR therapy	p*	before exenatide LAR therapy	after exenatide LAR therapy	p*	p** (between groups)
BMI	34±10	32±6.0	**0.0658**	30±5.0	29±5.0	*0.1648*	*0.4837*
Waist circumference (cm)	111±13	108±17	**0.0177**	104±13	102±17	*0.1373*	*0.8570*
Glycemia (mmol/l)	9.0±3.0	7.0±2.0	**0.0003**	8.0±3.0	7.0±2.0	**0.0240**	*0.6396*
HbA1c (%)	8.0±0.0	7.0±1.0	**<0.0001**	8.0±0.0	7.0±1.0	**<0.0001**	*0.6448*
Total cholesterol (mmol/l)	4.0±1.0	4.0±1.0	**0.0163**	5.0±1.0	4.0±1.0	**0.0289**	*0.1656*
HDL-cholesterol (mmol/l)	1.0±0.0	1.0±0.0	*0.2417*	1.0±0.0	1.0±0.0	*0.2427*	*0.0525*
LDL-cholesterol (mmol/l)	2.0±1.0	2.0±1.0	**0.0028**	3.0±1.0	2.0±1.0	**0.0151**	*0.2188*
Triglycerides (mmol/l)	1.0±1.0	1.0±1.0	*0.4158*	2.0±1.0	1.0±1.0	*0.0936*	*0.2434*
Endothelial Fuction (%)	6.0±1.0	7.0±2.0	**<0.0001**	6.0±1.0	7.0±2.0	**0.0015**	*0.6928*
cIMT	1.0±0.1	0.9±0.1	**<0.0001**	1.0±0.1	0.8±0.2	**<0.0001**	*0.1347*

Supported by: AstraZeneca
Disclosure: **A. Rizvi:** None.

PS 097 Unexpected comorbidities

1045

Pulmonary function in prediabetes: dysfunction appears before the development of type 2 diabetes

A. Lecube[1], E. Sánchez[1], C. López-Cano[1], M. Sánchez[1], A. Gaeta[1], F. Purroy[1], R. Pamplona[1], M. Ortega[1], T. Vidal[1], À. Betriu[1], X. Gómez[1], M. Hernández[1], J. Olsina[1], C. Hernández[2], R. Simó[2];
[1]Arnau de Vilanova, IRBLleida, UdL, Lleida, [2]Vall d'Hebron, VHIR, UAB, Barcelona, Spain.

Background and aims: Patients with type 2 diabetes have been considered a susceptible group for pulmonary dysfunction. However, little is known regarding about the potential pulmonary dysfunction in prediabetes.
Materials and methods: We assessed pulmonary function in 4,464 non-diabetic subjects, aged between 45 and 70 years, without vascular disease and chronic pulmonary obstructive disease from the ongoing cross-sectional study ILERVAS. A normal forced expiratory volume in the first second (FEV1) was defined as a value equal or higher than 80% of that predicted, and a "non-obstructive ventilatory defect" by a forced vital capacity (FVC) <80% of the predicted value with a FEV1/FVC ratio ≥70% (Global initiative for chronic Obstructive Lung Disease). Prediabetes was defined by glycosylated haemoglobin (HbA1c) between 5.7 and 6.4% (American Diabetes Association criteria).
Results: The study population was composed of 52.1% women, age of 57 [53;63] years old, and a BMI of 28.6 [25.8;31.8] kg/m^2. The prevalence of prediabetes was 31.2%. Subjects with prediabetes, compared to the population with a normal glucose metabolism, had lower FVC (93 [82;105] vs. 96 [84;106] % of predicted, $p < 0.001$) and maximum FEV1 (94 [82;107] vs. 96 [84;108] %, $p = 0.011$), as well as a higher percentage of subjects with non-obstructive ventilatory defect (16.5% vs. 13.6%, $p = 0.015$) and FEV1 <80% (20.3% vs. 17.2%, $p = 0.017$). In addition, in the prediabetes group, HbA1c was negatively correlated with both pulmonary parameters (FVC: $r = -0.113$, $p < 0.001$; FEV1: $r = -0.079$, $p = 0.003$). The multinomial logistic regression model showed that there was a significant association between HbA1c with both non-obstructive ventilatory defect [OR = 1.42 (1.10 to 1.83), $p = 0.008$] and FEV1 <80% [OR = 1.50 (1.19 to 1.90), $p = 0.001$].
Conclusion: The negative effect of type 2 diabetes on lung function is already initiated in prediabetes, and it is related with metabolic control.
Clinical Trial Registration Number: ClinTrials.gov Identifier: NCT03228459
Supported by: ISCIII (PI12/803, PI15/260) and FEDER.
Disclosure: **A. Lecube:** None.

1046

Diabetes duration, BMI, and HbA_{1c} have greater effects on Pulmonary Function (PF) than inhaled Technosphere Insulin (TI)

D.M. Kendall[1], J. Brain[2], J. Buse[3], D. Klein[4], Y. Ma[5], M. Grant[4], F. Pompilio[1], K. Smith[1];
[1]MannKind Corporation, Westlake Village, [2]Department of Environmental Health, Harvard University, Boston, [3]Medicine, University of North Carolina, Chapel Hill, [4]MannKind Corporation, Danbury, [5]Maxwell Consulting, Jericho, USA.

Background and aims: Rapidly absorbed inhaled insulin may lead to improved patient compliance and outcomes. Small, rapidly developing, non-progressive and reversible decreases in PF are seen with TI. How do these changes compare with PF changes caused by diabetes per se? Factors that contribute to PF decline were characterized and differences between diabetic subjects treated with TI versus usual care (UC) compared.
Materials and methods: The effects of diabetes duration (DUR), body mass index (BMI), and HbA1c on baseline PF and changes in PF (ΔPF) over 24 months' treatment were compared in TI versus UC patients. Two-

year PF follow up study with T1D ($N = 446$), T2D ($N = 1108$), and healthy controls ($N = 145$) were analyzed. ANCOVA identified the factors contributing to baseline PF. Mixed-model with repeated-measures analysis was performed to assess treatment effects on change in PF from baseline to end of study.
Results: Baseline analyses: In T1D, DUR was negatively correlated with FEV1 (−13 ml/y, $p = 0.003$) and FVC (−16 ml/y, $p = 0.006$). BMI and HbA1c were not significant factors. In individuals with T2D, both baseline BMI and HbA1c, but not DUR, were correlated with PF. FEV1 was negatively correlated with both BMI (-25 ml per kg/m^2, $p < 0.001$) and HbA1c (−47 ml per %HbA1c, $p < 0.001$). HbA1c at baseline was also associated with lower FVC (−97 ml/%, $p < 0.001$) and DLco (−0.35 ml/min/mmHg/%, $p = 0.004$). **24 month treatment with either TI or UC:** In T1D, baseline BMI was correlated with changes in both FEV1 (−6 ml per kg/m^2, $p = 0.019$) and FVC (−10 ml per kg/m^2, $p = 0.003$). In T2D subjects, BMI was associated with an additional 4 ml decrease in FVC per kg/m^2 ($p = 0.008$) while HbA1c affected FEV1 (−12 ml/%, $p < 0.001$), FVC (−14 ml/%), $p = 0.001$) and DLco (−119 ml/min/mmHg/%, $p = 0.0175$). The effects of baseline characteristics on PF were the same across treatment groups (TI or UC). For comparison, treatment with TI has been shown to be associated with a modest, non-progressive and reversible reduction of approximately 40 ml in FEV1.
Conclusion: The duration of diabetes, BMI, and HbA1c impact pulmonary function. Importantly, elevated BMI and HbA1c are associated with reductions in PF that exceed the observed and reversible changes seen with inhaled insulin treatment. Demographic factors impacted baseline findings equally across treatment groups. Diabetics experience above normal declines in PF as they age compared to healthy controls. TI does not produce an accelerated decline in PF after a small drop which disappears after TI cessation.
Clinical Trial Registration Number: NCT00308737
Disclosure: **D.M. Kendall:** None.

1047

Prevalence of and risk factors for gustatory sweating amongst people with type 2 diabetes

P.L. Kristensen[1], C. Dam[1], B. Thorsteinsson[1], L. Tarnow[2];
[1]Department of Endocrinology and Nephrology, Nordsjællands University Hospital, Hillerød, [2]Steno Diabetes Center Sjælland, Region Sjælland, Denmark.

Background and aims: Gustatory Sweating (GS) is known as a complication to diabetes mellitus (DM) and is characterised by profuse sweating during or immediately after ingestion of food. Most reports on gustatory sweating have been case reports suggesting it to be a rare late diabetic complication. The aim of this study was to determine the prevalence of gustatory sweating in an unselected cohort of patients with type 2 diabetes. To generate hypothesis on the pathophysiology of GS, associations between GS and classic complications of DM were explored.
Materials and methods: In a cross-sectional study all people with type 2 DM in the DM outpatient clinic at Nordsjællands Hospital, Denmark, received a questionnaire by mail with eight questions regarding GS. Answers were paired with medical data from the electronic patient records. Prevalence of GS was primary endpoint. Association between GS and complications to DM were secondary endpoints. Univariate and multivariate logistic regression analyses were performed including the following variables: Sex, age, HbA1c, albuminuria, retinopathy and neuropathy. Variables associated with or nearly associated with GS in the univariate analyses (defined by $p < 0.15$) were included in the multivariate analysis.
Results: Out of 991 persons receiving the questionnaire, 510 people answered, four were excluded, leaving a response rate at 51%. 22% of the patients sweat in relation to ingestion of food, and 13% (95% CI: 10–16%) also in relation to non-spicy foods. In the multivariate logistic regression analysis, we found that decreasing age ($p = 0.007$) was associated with increasing probability of GS. Also presence of severe peripheral neuropathy (threshold of biothesiometry >50 V) was associated with GS ($p = 0.01$).
Conclusion: A prevalence of gustatory sweating of 13% was found in a large unselected cohort of people with type 2 DM. Gustatory sweating was associated with lower age and severe peripheral neuropathy.
Clinical Trial Registration Number: NOH-2016-029
Disclosure: **P.L. Kristensen:** None.

1048

Hearing loss as a complication of type 2 diabetes: preliminary findings from the population-based Hoorn study

M. Stam[1], F. Rutters[2], E. Urry[3], S.E. Kramer[1], P.M. Elders[4], J.M. Beulens[2,5], G. Nijpels[4];
[1]Otolaryngology-Head and Neck Surgery, section Ear & Hearing, VU University Medical Center and Amsterdam Public Health research institute, Amsterdam, Netherlands, [2]Epidemiology and Biostatistics, VU University Medical Center and Amsterdam Public Health research institute, Amsterdam, Netherlands, [3]Science & Technology, Sonova AG, Staefa, Switzerland, [4]General Practice and Elderly Care, VU University Medical Center and Amsterdam Public Health research institute, Amsterdam, Netherlands, [5]Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, Netherlands.

Background and aims: It is generally accepted that type 2 diabetes induces microvascular and neuropathic changes that may lead to complications of the auditory pathway from the cochlea to the cortex. However, results from epidemiological studies, which assess the association between diabetes and hearing impairment, is mixed and requires clarification. The aim of the current explorative study was to investigate the prevalence of hearing loss in a population-based diabetes cohort in the Netherlands and to assess the associations between metabolic parameters and self-reported hearing problems.
Materials and methods: Data for the present cross-sectional data analyses ($n = 1223$, aged 54–85 years) were derived from The Hoorn Study cohort. The Hoorn Study investigates the prevalence and risk factors of impaired glucose metabolism and type 2 diabetes in the Dutch city of Hoorn. Standardized procedures were conducted to measure glucose metabolism (fasting plasma glucose, 75-g oral glucose tolerance test (OGTT) and haemoglobin A_{1c} (HbA_{1c})); anthropometrics (height, waist and hip circumference); blood plasma lipid levels (triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)); blood pressure; and smoking behaviour. Hearing status was determined by self-report: "Do you have severe hearing problems?" (yes/no). Preliminary data analyses included descriptive statistics, independent t-tests, chi-square tests and multiple logistic regression.
Results: The prevalence of self-reported hearing problems was 8% in the study sample ($n = 93$). Of these participants, 47 (50.5%) could be classified as having normal glucose metabolism, 35 (37.6%) as having impaired glucose metabolism, and 11 (11.8%) as having diabetes according to the WHO 2011 and American Diabetes Association 2012 criteria. The prevalence of metabolic syndrome, impaired glucose tolerance and/or diabetes was comparable between adults with and without self-reported hearing problems ($p > 0.05$). However, 2-hour glucose levels after OGTT and waist circumference were significantly higher in participants with severe hearing problems, compared to those without: 6.6 mmol/litre vs. 6.0 mmol/litre ($p = 0.02$); 95.5 cm vs. 92.3 cm ($p < 0.01$), respectively. Results from the regression analyses showed that 2-hour glucose levels after OGTT and waist circumference were significantly associated with severe hearing problems: OR: 1.11, 95% CI: 1.02–1.21; $p = 0.022$; OR: 1.03, 95% CI: 1.01–1.05; $p = 0.006$).
Conclusion: These preliminary results indicate that adults with severe hearing problems may have poorer metabolic profiles than their normally-hearing peers.
Supported by: Sonova AG project grant
Disclosure: **M. Stam:** Grants; Project grant from Sonova AG, Switzerland.

1049

Association between hearing and renal function in young adult type 1 diabetic patients

M. Dąbrowski[1], G. Mielnik-Niedzielska[2], A. Nowakowski[3];
[1]Faculty of Medicine, University of Rzeszów, Rzeszów, [2]II Faculty of Medicine, Medical University of Lublin, Lublin, [3]Professor emeritus, Madical University of Lublin, Lublin, Poland.

Background and aims: Retinopathy and nephropathy are well-known complications of type 1 diabetes. Also impaired function of auditory organ is observed in this population. The aim of this study was to evaluate associations between hearing and renal function in a group of young adult patients with type 1 diabetes mellitus.
Materials and methods: The study group consisted of 31 patients (9 women) with type 1 diabetes, aged below 45 (mean 29.5 ± 7.0 years), with a disease duration less than 10 years (mean 4.6 ± 2.6 years) and without overt hearing impairment. In all subjects blood pressure, weight and height were measured and BMI was calculated, blood samples for laboratory tests (lipid profile, creatinine and HbA_{1c} level) were obtained and urinary albumin excretion (UAE) in the first morning urine sample was assessed. Estimated glomerular filtration rate (eGFR) was calculated according to CKD-EPI formula. Then in all patients pure-tone audiometry, transient evoked otoacoustic emissions (TEOAE) and auditory brainstem responses (ABR) were evaluated. Also eye fundus was examined within the 3 months window from audiological evaluations.
Results: In three patients eGFR was between 75.0 and 90.0 ml/min/1.73 m^2, the rest had normal eGFR, in one case UAE was at microalbuminuria range and in three patients early background retinopathy was found. In pure-tone audiometry mild hearing impairment was revealed in seven patients. These subjects had significantly lower eGFR compared to the patients with normal hearing, 108.8 vs. 121.7 ml/min/1.73 m^2, $p = 0.047$. Significant positive linear correlation was found between hearing threshold and creatinine level at frequencies 6 kHz (coefficient factor $r = 0.404$, $p = 0.001$) 8 kHz ($r = 0.372$, $p = 0.003$) and 12 kHz ($r = 0.440$, $p < 0.001$) and negative between hearing threshold and eGFR at frequencies 4 kHz ($r = -0.259$, $p = 0.042$), 6 kHz ($r = -0.411$, $p < 0.001$), 8 kHz ($r = -0.373$, $p = 0.003$) and 12 kHz ($r = -0.431$, $p < 0.001$). No association between hearing threshold and UAE was revealed. In TEOAE evaluation patients with absence of TEOAE in at least one ear (amplitude <6.0 db) had significantly higher creatinine level (0.93 vs. 0.70 mg/dl) and lower eGFR (103.1 vs. 123.3 ml/min/1.73 m^2), $p < 0.001$ in both cases. For UAE such association was not found, however, it tended to correlate negatively with TEOAE amplitude, but it did not reach significance level ($r = -0.230$, $p = 0.077$). In ABR significant negative linear correlation was found between creatinine level and latency of wave III ($r = -0.290$, $p = 0.033$) and interval I-III ($r = -0.271$, $p = 0.048$), while positive correlation with interval III-V ($r = 0.269$, $p = 0.049$). Positive correlation of eGFR with wave III latency ($r = 0.406$, $p = 0.002$) and interval I-III ($r = 0.324$, $p = 0.017$) was revealed. Also positive linear correlation between UAE and latencies of wave III ($r = 0.415$, $p = 0.002$), wave V ($r = 0.299$, $p = 0.020$) and interval I-III ($r = 0.328$, $p = 0.018$) were found.
Conclusion: This study indicate possible relationship between hearing and renal function in young adult type 1 diabetic patients, seen also at the subclinical level. Pathways directly linking hearing and renal function are unknown. Our results indicate that creatinine/eGFR and microalbuminuria are linked with hearing function through different mechanisms. Obviously, larger studies using also other markers are necessary to further analyze these relationships.
Disclosure: **M. Dąbrowski:** None.

1050

Social jetlag and sleep quality are independently associated with poor glycaemic control in type 1 diabetes

A. Rusu[1], C. Bala[1,2], A. Cerghizan[2], D. Ciobanu[1], G. Roman[1,2];
[1]Diabetes and Nutrition Diseases, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, [2]Diabetes Centre, Emergency Clinical County Hospital Cluj, Cluj-Napoca, Romania.

Background and aims: Social jetlag (SJL) is a small recurrent circadian rhythm disruption and the most frequent form of circadian rhythm misalignment. It results from the mismatch between imposed social rhythms and internal circadian clock. Although it is accepted that circadian rhythm is involved in the regulation of metabolic processes, only recently circadian misalignment has been regarded as a potential contributor to glucose dysregulation outside of shift work settings. The main objective of this cross-sectional study was to investigate in real-life settings the effect of SJL on glycaemic control, as assessed by HbA1c, in persons with type 1 diabetes.
Materials and methods: 115 consecutive adults with type 1 diabetes presenting for an appointment in the outpatient clinic of the Diabetes Centre, Cluj-Napoca, Romania between May 2017 and January 2018 were enrolled and analysed. Data on bedtime, sleep-onset latency, and wake-up time on weekdays and weekends during the previous month were collected and used to calculate SJL, mid-sleep time during free days (MSF, an indicator of chronotype), sleep duration and sleep debt. Sleep quality over the previous month was assessed by Pittsburgh Sleep Quality Index (PSQI). A PSQI score >5 was considered as an indicator of poor sleep quality. Based on the presence of SJL, study participants were divided in 2 groups: with SJL ≥1 h and with SJL <1 h. Linear univariate and multivariate regression analyses were used to assess the association of HbA1c with SJL ≥1 h and other sleep-related parameters. Parameters associated with HbA1c in regression analysis were tested for interaction with regards to HbA1c values in addition to main effects of individual variables.
Results: Based on SJL duration, 53 participants had a SJL duration ≥1 h and 62 had a SJL duration <1 h. Compared to persons with SJL <1 h, those with SJL ≥1 h were younger, had a shorter diabetes duration, lower daily insulin doses and a lower frequency of diabetes-related retinopathy and peripheral neuropathy ($p < 0.05$ for all). Persons with SJL ≥1 h had statistically significant higher HbA1c values than those with SJL <1 h (8.7% vs. 8.0%, $p = 0.029$), independent of age, gender, diabetes duration, daily insulin dose and BMI. In multivariate regression analysis employing sleep and chronotype parameters (SJL, sleep duration, sleep debt, poor sleep quality, MSF) as predictors, only SJL ≥1 h and poor sleep quality were significant predictors of HbA1c values, explaining 22.7% ($p = 0.041$) and 23.5% ($p = 0.019$), respectively, of the HbA1c variation. Associations remained statistically significant after adjustment for age, gender, diabetes duration, daily insulin dose and BMI ($\beta = 0.233$ [$p = 0.042$] for SJL ≥1 h; $\beta = 0.235$ [$p = 0.027$] for poor sleep quality). In the analysis of interaction, SJL ≥1 h by poor sleep quality interaction terms were not statistically significant associated with HbA1c neither in the unadjusted nor in the adjusted model (p values for interaction 0.994 in unadjusted model and 0.990 in adjusted model).
Conclusion: In persons with type 1 diabetes SJL is associated with a poor glycaemic control and this association is independent of age, gender, diabetes duration, total daily insulin dose, BMI and other sleep and circadian rhythm measurements. We also showed that SJL acts independently of sleep quality in exerting a deleterious effect on glycaemic control.
Disclosure: **A. Rusu:** None.

1051

Type 2 diabetes is complicated by sleep apnoea syndrome: focussing in comorbidities

S. Kawasaki[1], H. Misawa[1], R. Kaneda[1], T. Shizuku[1], Y. Tamura[1], T. Kondo[1], Y. Kondo[2], Y. Terauchi[3];
[1]Shonan Fujisawa Tokushukai Hospital, Fujisawa, [2]Odawara Municipal Hospital, Odawara, [3]Yokohama City University Graduate School of Medicine, Yokohama, Japan.

Background and aims: Sleep apnea syndrome (SAS) is a disorder characterized by repeated episodes of apnea and hypopnea during nocturnal

sleep. Risk factors for SAS include obesity, male gender, and advanced age. Diabetic patients often have various diseases such as vascular diseases and other comorbidities. SAS in such patients may lead to acute deterioration due to nocturnal hypoxia, highlighting the importance of evaluation and appropriate treatment of SAS in routine clinical practice. The present study examined risk factors for SAS in patients with type 2 diabetes (T2DM), with special reference to microangiopathy and stable comorbidities.

Materials and methods: Among the 1,367 ambulant T2DM patients who were being treated at our institution, 483 (313 men and 170 women) who agreed to undergo a portable sleep polygraph test were evaluated to examine the prevalence of SAS and the relationships between SAS and microangiopathy or other comorbidities.

Results: Patient characteristics were as follows: age, 67.0 ± 11.9 (mean ± standard deviation) years; estimated diabetes duration, 16.4 ± 10.3 years; body mass index (BMI), 25.2 ± 4.4 kg/m^2; fasting blood sugar (FBS), 142 ± 44 mg/dL; HbA1c, 7.1 ± 1.2%; fasting blood C-peptide immunoreactivity (CPR), 3.1 ± 2.1 ng/mL; homeostatic model assessment of insulin resistance (HOMA-IR), 6.11 ± 10.8; creatinine (Cr), 1.06 ± 1.14 mg/dL; apnea-hypopnea index (AHI), 15.4 ± 14.3 (16.8 ± 14.5 in men and 12.9 ± 13.8 in women); prevalence of SAS (AHI ≥15), 40.6% (127/313) in men and 29.4% (50/170) in women. The incidences of microangiopathy were as follows: retinopathy (147/483, 30.6%) and nephropathy (233/483, 48.5%). The incidences of stable comorbidities were as follows: intracranial lesions (106/483, 22.0%), respiratory diseases (48/483, 9.9%), cardiovascular diseases (192/483, 39.8%), cancer (78/483, 16.1%), thyroid diseases (36/483, 7.7%), and mental illnesses (27/483, 5.8%). Analysis of the relationships of SAS with microangiopathy and other comorbidities showed that intracranial lesions, cardiovascular diseases, and cancer were more common among patients with SAS than among those without SAS (AHI <15) ($p < 0.05$ each), while no such association was found for microangiopathy. Multivariate analysis adjusting for known risk factors for SAS (BMI, male gender, and age) identified BMI (odds ratio, 1.25; 95% confidence interval, 1.18–1.33; $p < 0.0001$), male gender (2.11; 1.36–3.34; $p < 0.001$), and intracranial lesions (1.74; 1.07–2.83; $p < 0.05$) as independent risk factors for SAS.

Conclusion: The intracranial lesions identified were stable and diverse, ranging from infarction and hemorrhage to benign brain tumor and encephalitis. The association of intracranial lesions with hypoxia may be related to the respiratory centers, although the underlying mechanism is unknown. T2DM patients often present with various diseases such as angiopathy, infection, and cancer, and occasionally experience hyperglycemia and hypoglycemia. Therefore, it is considered that T2DM patients with obesity, male gender, and stable intracranial lesions should be carefully monitored for nocturnal hypoxia, particularly during emergency hospitalization. The present results also suggest the importance of evaluation and appropriate treatment of SAS in routine clinical practice.

Disclosure: **S. Kawasaki:** None.

1052

Autoimmune hypothyroidism increases risk of microangiopathic complications in adult patients with type 1 diabetes

A. Rogowicz-Frontczak[1], B. Falkowski[2], A. Grzelka[1], A. Uruska[1], A. Araszkiewicz[1], D. Zozulinska-Ziolkiewicz[1];
[1]Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poznan, [2]Poznan University of Medical Sciences, Poznan, Poland.

Background and aims: Type 1 diabetes (T1DM) often coexists with other autoimmune diseases, most commonly with hypothyroidism. To date, the influence of co-occurrence of autoimmune hypothyroidism (AHT) in the course of chronic microangiopathic complications of autoimmune diabetes has not been established. Aim of the study was to assess the relationship between (AHT) and the occurrence of chronic type 1 diabetes complications.

Materials and methods: The study population comprised 342 European Caucasian participants with T1DM including 165 (49.7%) men and 167 (50.3%) women. Median participants` age was 32 (interquartile range [IQR]: 26–41) years and T1DM duration was 14 (IQR: 9–21) years. Exclusion criteria were: age <18 years, diabetes duration <5 years, hyperthyroidism, iodotherapy or thyroidectomy in the medical history, eGFR below 30 mL min^{-1}·1.73 m^{-2}, ALT or AST three times above reference interval, diabetic ketoacidosis or ketonuria at the time of enrollment to the study, neoplasms, anemia, taking drugs affecting glucose metabolism, anti-thyroid drugs and drugs affecting thyroid function except L-thyroxine. T1DM was diagnosed based on American Diabetes Association criteria and at least one out of three examined autoantibodies (against islet cells [ICA], glutamic acid decarboxylase [GAD], insulinoma-associated tyrosine phosphatase [IA-2]) had to be positive. AHT was recognized in state overt or subclinical hypothyroidism and the presence of anti-thyroid autoantibodies: ATPO (anti-thyroperoxidase) and/or ATg (anti-thyroglobulin) and ultrasonography (hypoechogeneity, parenchymal heterogeneity, lymph nodes).

Results: In the study group, 161 (48.5%) participants were diagnosed with at least one microangiopathic complication (retinopathy - 41.6%, diabetic kidney disease - 13.9%, autonomic neuropathy - 14.3% and peripheral neuropathy-20.8%). At the time of enrollment, 16.3% participants were diagnosed with AHT or had AHT in medical history. Among patients with AHT 82.7% were treated with L-thyroxine and 67.4% of them were treated effectively what means TSH concentration within reference interval. Patients with AHT were characterized by higher prevalence of microangiopathy (64.8% vs. 45.3% respectively; $p = 0.009$) in all and retinopathy (55.6% vs 38.9%; $p = 0.02$) as compared with participants without AHT. The group with microangiopathy differed significantly with classic risk factors of chronic diabetes complications such as age, T1DM duration, SBP, DBP, HbA_{1c}, TG, eGFR and hypertension prevalence. AHT turned out to be an independent predictor of microangiopathy in multivariate logistic regression analysis (odds ratio, 2.35; 95% confidence interval [CI], 1.14–4.84; $p = 0.02$] after an adjustment for other potential explanatory factors such as age, BMI, DBP, HbA_{1c} and TG.

Conclusion: Co-occurrence of autoimmune hypothyroidism with type 1 diabetes is associated with a higher incidence of microvascular complications.

Disclosure: **A. Rogowicz-Frontczak:** None.

PS 098 Factors affecting cardiovascular outcome

1053

Heart rate variability indices in patients with micro- and macrovascular complications of type 2 diabetes: a cross sectional study

P.S. Wadhokar[1], L. Phadke[2], S. Bhat[1];
[1]Medicine, Smt. Kashibai Navale Medical College, Pune, [2]Physiology, Smt. Kashibai Navale Medical College, Pune, India.

Background and aims: Meta-analyses of various studies have shown that in comparison to healthy subjects, reduced heart rate variability a marker of cardiac autonomic activity is strongly associated with hypertension, diabetes & myocardial infarction.There is paucity of data, suggesting the degree of association between heart rate variability indices (HRV) in type II diabetes with and without micro & macro vascular complications. It will be interesting to explore whether there is a progressive reduction in HRV with complications of type II diabetes. Results of study may pave the way for utility of HRV indices in risk stratification of complications in type II diabetes. The aim of present study was to explore the Heart Rate Variability and degree of association of HRV indices in complicated and uncomplicated type II Diabetes mellitus.
Materials and methods: 60 consenting type II diabetes patients between 30 to 70 years of both genders, enrolled during a visit at Kashibai Navale Medical College Pune India were included in the study which was approved by Institutional Ethics Committee. Exclusion Criteria: Factors affecting HRV like Previous myocardial infarction, congenital heart disease, Uncontrolled cardiac arrhythmias, Parkinsonism, Cerebrovascular accident, Thyroid disorders, Hypertrophic cardiomyopathy, Drugs (Tricyclic antidepressants, β & Calcium channel Blockers, ACE inhibitors). All patients were subjected to exercise stress test with multistage Bruce protocol, those with positive stress test were subjected to Coronary Angiography for confirmation of CHD. All patients were also tested for- 1) Presence of diabetic nephropathy (KDOQI guidelines), 2) Diabetic retinopathy (ETDRS guidelines). 3) Diabetic Peripheral Neuropathy (Toronto Expert Panel on Diabetic Neuropathy). A high sampled ECG (1 KHz) -Chronovisor HRV DX system & HRV analysis software suit version 1.1.487 Promorphosis Pvt. Ltd.Pune) in resting supine position for 15 minutes was recorded for HRV analysis. Patients were grouped as Group I - Type II DM without complications & Group II- Type II DM with Micro/Macrovascular complications.
Results: Group comparison was done using Mann-Whitney test and association was tested by χ^2 test using Epi Info.7. P value of <0.05 was considered as significant.In comparison to group I there was significant reduction in all HRV indices (SDNN, LF and HF (ms2)) in group II. HRV indices have shown a strong association with type II diabetes with complication (Odds ratio for group with complications) and with longer duration of diabetes.
Conclusion: Results have highlighted that in type II diabetes there is significant & progressive reduction in HRV, and association of HRV indices with complications were stronger compared to uncomplicated type II diabetes. Progressive reduction of HRV indices & stronger association of indices with complications of diabetes may be useful for screening of complications in type II diabetes.

Parameter	Type II DM With Complications			Type II DM Without Complications		P value
Avg. Heart Rate	90.1 ± 14			76.7 ± 10		0.0001**
HbA1C	8.4 ± 1.5			7.5 ± 1.6		0.02
HF(ms2)	60.1 ± 93			137.3 ± 127		0.01
LF(ms2)	65 ± 116			199.1 ± 221		0.005
SDNN	18.6 ± 10			32.6 ± 12		0.0001**
	Odds Ratio			X^2		P value
HF(ms2)/Complication	7.6			12.1		0.0005**
LF(ms2)/Complication	8.5			8.4		0.003*
SDNN /Complication	6.2			7.6		0.005*
Type II DM with complications stratified for duration of Diabetes						
Duration of DM	Less than 5 Years			More than 5 years		
	Odds Ratio	X^2	P value	Odds Ratio	X^2	P value
HF(ms2)	7.5	3.8	0.04	10	4.7	0.02*
LF(ms2)	7.9	6.9	0.008	6.5	4.1	0.002*
SDNN	4.3	2.8	0.09	10	4.5	0.01*

Disclosure: **P.S. Wadhokar:** None.

1054

Heart rate variability in type 2 diabetes: a systematic review and meta-analysis

T. Benichou[1], B. Pereira[1], M. Mermillod[2], D. Pfabigan[3], I. Tauveron[1], S. Maqdasy[1], F. Dutheil[1];
[1]CHU Clermont-Ferrand, Clermont-Ferrand, France, [2]CHU Grenoble, Grenoble, France, [3]Peking University, Beijing, China.

Background and aims: Cardiac autonomic neuropathy in type 2 diabetes mellitus (T2DM) patients is frequent and associated with high cardiovascular mortality. Heart rate variability (HRV) is the gold standard to measure cardiac autonomic neuropathy. We aimed to conduct a systematic review and meta-analysis to evaluate the impact of T2DM on HRV parameters.
Materials and methods: The PubMed, Cochrane Library, Embase and Science Direct databases were searched on 1st October 2017 using the keywords "diabetes" AND ("heart rate variability" OR "HRV"). Included articles had to report HRV parameters in T2DM patients and healthy controls measured during 24 hours with a Holter-electrocardiogram. Measurements of HRV retieved were: RR-intervals (or Normal to Normal intervals - NN), standard deviation of RR intervals (SDNN), percentage of adjacent NN intervals differing by more than 50 milliseconds (pNN50), square root of the mean squared difference of successive RR intervals (RMSSD), total power, Low Frequency (LF), High Frequency (HF) and LF/HF ratio, as per Task Force recommendations.
Results: We included twenty-five case-control studies with 2932 patients: 1356 with T2DM and 1576 healthy controls. We noted strong evidence that T2DM patients had significantly lower RR intervals (effect size −0.61 [95% CI −1.21, −0.01]; $p = 0.01$; $I^2 = 91.6\%$), lower SDNN (−0.65 [−0.83, −0.47]; $p < 0.001$; $I^2 = 65.1\%$), lower RMSSD (−0.92 [−1.37, −0.47]; $p < 0.001$; $I^2 = 94.0\%$), lower pNN50 (−0.46 [−0.84, −0.09]; $p = 0 < 0.001$; $I^2 = 85.5\%$), lower total power (−1.52 [−2.13, −0.91]; $p < 0.001$; $I^2 = 93.5\%$), lower LF (−1.08 [−1.46, −0.69]; $p < 0.001$; $I^2 = 91.3\%$), and lower HF (−0.79 [−1.09, −0.50]; $p < 0.001$; $I^2 = 85.6\%$). LF/HF did not differ between groups (0.02 [−0.38, 0.43]; $p = 0.914$; $I^2 = 90.1\%$). Higher levels of HbA1c were associated with shorter RR intervals. Blood glucose levels were associated with both an increase in LF and HF, and with an increase in RMSSD and SDNN. Time from diagnosis of T2DM was linked with a lower level of total power and an increase in RMSSD and SDNN.
Conclusion: T2DM is associated with an overall decrease in the HRV of T2DM patients. We demonstrated that both sympathetic and parasympathetic activity were decreased, which can be explained by the metabolic deleterious effects of blood glucose levels on HRV, leading to cardiac autonomic neuropathy. Some of these parameters are modifyied by independent variables.
Disclosure: **T. Benichou:** None.

1055

Determinants of aspirin loss of efficacy in type 2 diabetic patients

E. Paven[1], J. Dillinger[1], C. Bal dit Sollier[2], J. Riveline[3], J. Launay[4], J. Gautier[3], L. Drouet[2], P. Henry[1];
[1]Cardiology, AP-HP Hôpital Lariboisière, Paris, [2]Institut des Vaisseaux et du Sang, Paris, [3]Diabetology, AP-HP Hôpital Lariboisière, Paris, [4]INSERM UMRS-942, Paris, France.

Background and aims: Aspirin is clinically less effective for preventing atherosclerotic events in type-2 diabetic patients (T2DP). Aspirin loss of efficacy in this population is mainly related to an accelerated platelet turnover with persistent platelet reactivity or increased level of thromboxane 24h after last aspirin intake. The aim of this study was to investigate the main mechanisms associated with aspirin loss of efficacy in this population.
Materials and methods: This prospective study included consecutive stable T2DP coming for yearly check-up between March and July 2016. Aspirin loss of efficacy was defined as a persistent high platelet reactivity (HPR) using light transmission aggregometry with arachidonic acid (0.5 mg/ml - threshold >20% residual aggregation) and confirmed with serum thromboxane B2 measurement (TXB2). Assessment of aspirin efficacy was performed 24 h after last aspirin intake in patients treated for at least 8 days. An extensive study of diabetes status, insulin resistance and inflammation was performed. Data are presented as mean ± SD or median [25–75%] - Student T test, Mann-Whitney test and Spearman's rank were used for comparisons.
Results: 116 patients (mean age 65 ± 9 years old, 69% men) were included. Mean duration of diabetes was 15 y. [11–24] and 50% were treated with insulin for 9 [4–14] y. Most of the population presented associated cardiovascular risks factors (dyslipidemia: 85% - hypertension: 77% - current smoking: 16%). 43% had history of coronary artery disease or stroke. Mean BMI was 28 kg/m^2 [24–32] with an android obesity (waist circumference: 103 ± 13 cm). Using light transmission aggregometry, HPR was found in 27 (23%) patients. There was no significant difference in mean age, sex ratio and cardiovascular risk factors, in patients with or without HPR. Median duration of diabetes and insulin treatment were significantly longer in patients with HPR ($p = 0.01$ for both). Median fasting glucose was significantly higher in patients with HPR ($p = 0.02$) but HbA1c was not significantly different. HPR was strongly related to all markers of insulin resistance especially waist circumference ($p = 0.007$), HOMA-IR ($p = 0.002$), QUICKI index ($p = 0.002$), leptin ($p = 0.01$) and mean dose of weight adjusted dose of long-lasting insulin in patients treated with insulin ($p = 0.006$). Surprisingly, there was no relationship with thrombopoietin ($p = 0.38$) and inflammatory markers: IL6 ($p = 0.86$), IL10 ($p = 0.68$), IDO activity ($p = 0.98$), TNFalpha ($p = 0.31$), usCRP ($p = 0.08$). TXB2 concentration was also strongly correlated with all insulin resistance parameters, especially with HOMA-IR ($r = -0.42$, $p = 0.002$), QUICKI index ($r = -0.44$, $p = 0.0006$) and long lasting insulin dose/kg ($r = -0.43$, $p = 0.0007$) but not to inflammatory parameters.
Conclusion: Aspirin loss of efficacy is frequent in T2DP. It appears to be strongly related to insulin resistance but not to inflammation. This result could help to select a population who could benefit to alternative antiplatelet treatment.
Disclosure: **E. Paven:** None.

1056

Risk stratification for thrombosis in type 2 diabetic patients

S. Daniels[1], D. Moreno-Martinez[1], H. Soran[2], S. Adam[2,3], J. Thachil[4], N. Dempsey-Hibbert[1], M.Y. Alexander[1], S. Jones[1];
[1]Department of Biomedicine, Manchester Metropolitan University, Manchester, [2]University Department of Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, [3]Faculty of Medical and Human Sciences, University of Manchester, Manchester, [4]Department of Haematology, Manchester Royal Infirmary, Manchester, UK.

Background and aims: The leading cause of mortality in type 2 diabetes mellitus (T2DM) patients is cardiovascular disease, with patients exhibiting platelet hyperreactivity and prothrombotic propensity. The mechanisms that underpin these effects however are unclear. The aim of this study was to determine which of the biochemical features associated with T2DM are responsible for increased platelet reactivity.
Materials and methods: Blood was collected from fasted healthy controls and T2DM patients. Full blood counts and biochemical profiles were analysed and platelet aggregation and flow cytometry performed to assess platelet reactivity.
Results: Platelets from healthy donors supplemented with glucose (5.0–30.0 mmol/l) for 1 hour at room temperature ($n = 10$), exhibited a significant elevation in surface expression of activated GPIIb/IIIa ($p = 0.032$), and a significant increase in mean platelet volume (MPV) (one-way ANOVA, $p < 0.001$), which was not observed with the osmotic control mannitol. Platelet aggregation in response to ADP or collagen however was not increased by acute hyperglycaemia in platelet rich plasma (PRP) or whole blood and there was no significant increase in alpha-granule secretion or GPIIb/IIIa activation following ADP stimulation. Furthermore, in T2DM patients ($n = 25$), there was no correlation with platelet reactivity and fasting glucose or HbA1c, but HbA1c did positively correlate with MPV (Pearson, $p = 0.013$). The strongest association with platelet reactivity in T2DM was with LDL-C. A significant positive correlation was observed between LDL-C and ADP-activated platelet aggregation (Spearman, $p = 0.002$, $n = 26$). Moreover, patients with LDL-C levels above 2.0 mmol/l ($n = 13$) had significantly higher platelet aggregation than patients with LDL-C below 2.0 mmol/l ($n = 13$; Mann-Whitney U test, $p = 0.027$).
Conclusion: The data demonstrates that hyperglycaemia primes rested platelets and increases platelet size, but does not alter platelet reactivity as previously reported. The most significant risk factor for platelet hyperreactivity is elevated LDL-C. This highlights the potential for LDL-C to be used as a clinical biomarker to identify diabetic patients who would benefit most from antithrombotic therapy, and suggests that lipid lowering medication may indirectly provide antithrombotic benefit.
Clinical Trial Registration Number: 11/NW/0731
Supported by: Centre for Biomedicine, MMU
Disclosure: **S. Daniels:** None.

1057

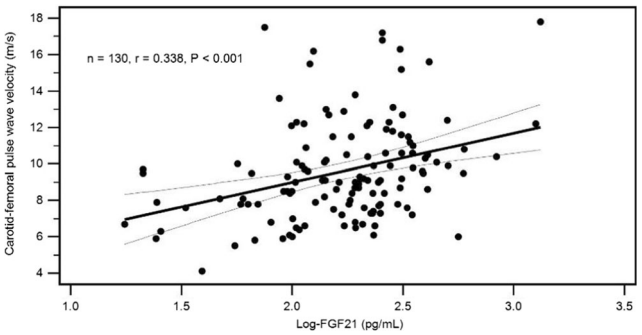
Serum fibroblast growth factor 21 levels are positively associated with aortic arterial stiffness in patients with type 2 diabetes

B.-G. Hsu[1], Y.-C. Chen[2], D.-A. Wu[3];
[1]Tzu Chi General Hospital, Hualien, [2]Department of Psychiatry, Dalin Tzu Chi General Hospital, Chiayin, [3]Division of Metabolism and Endocrinology, Tzu Chi General Hospital, Hualien, Taiwan.

Background and aims: Elevated serum fibroblast growth factor 21 (FGF-21) levels are associated with atherosclerosis and obesity. Aortic arterial stiffness assessed by carotid-femoral pulse wave velocity (cfPWV) provides a high predictive value for the future cardiovascular disease and mortality. This study evaluated the correlation between serum FGF-21 levels and aortic stiffness in patients with type 2 diabetes mellitus (T2DM).
Materials and methods: Fasting blood samples were collected from 130 patients with T2DM. cfPWV value was measured by a validated tonometry system and cfPWV >10 m/s were used to define the high aortic arterial stiffness group according to the ESH-ESC 2013 guideline. Serum FGF-21 concentrations were determined using a commercially available enzyme immunoassay kit.
Results: In total, 45 T2DM patients (34.6%) had high aortic arterial stiffness, and showed older age ($p = 0.015$) and higher systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), body fat mass ($p = 0.019$), triglycerides ($p = 0.016$), fasting glucose ($p = 0.043$), glycated hemoglobin ($p = 0.034$), creatinine ($p = 0.006$), urine albumin-

to-creatinine ratio ($p = 0.004$), serum FGF-21 ($p < 0.001$) levels, and lower estimated glomerular filtration rate ($p = 0.001$) than those in a low aortic arterial stiffness group. After adjusting for factors significantly associated with aortic arterial stiffness by multivariate logistic regression analysis, serum FGF-21 level (odds ratio: 1.006, 95% confidence interval: 1.002–1.010, $p = 0.002$) was the independent predictor of aortic arterial stiffness in patients with T2DM. Multivariate forward stepwise linear regression analysis also showed that logarithmically transformed FGF-21 level (log-FGF-21, $\beta = 0.369$, $p < 0.001$) was positively associated with cfPWV values in T2DM patients.

Conclusion: Serum FGF-21 level is positively correlated with cfPWV values and is the independent predictor of aortic arterial stiffness in patients with T2DM.



Clinical Trial Registration Number: IRB103-136-B
Disclosure: **B. Hsu:** None.

1058

Association between H-ficolin and mortality in type 1 diabetes

J.A. Oestergaard[1], C. Forsblom[2], S. Thiel[3], L. Thorn[2], F. Jansson[2], T.K. Hansen[4], P.-H. Groop[2,5], FinnDiane Study Group;
[1]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark, [2]Folkhälsan Inst of Genetics, Folkhälsan Res Center; Abd Center Nephr; Res Prog Unit, Diab & Obesity, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Biomedicine, Aarhus University, Aarhus, Denmark, [4]Steno Diabetes Center Aarhus, Aarhus, Denmark, [5]Department of Diabetes, Monash University, Melbourne, Australia.

Background and aims: Circulating levels of complement factor H-ficolin has been associated with incidence of microalbuminuria in diabetes, but it remains unknown if H-ficolin is associated with mortality. In this study, we aimed to test the hypothesis that high H-ficolin concentration is associated with mortality. In addition, we investigated the external validity of the previous finding on microalbuminuria.
Materials and methods: We studied 2439 type 1 diabetes patients from the Finnish Diabetic Nephropathy (FinnDiane) study. The study included adult patients with type 1 diabetes diagnosed before the age of 40 who had insulin treatment initiated within 1 year of diagnosis, and where data on renal status and mortality was available. 2146 patients were included in analysis of renal outcomes omitting patients with end-stage renal disease at baseline (ESRD, $n = 232$) or missing follow-up data ($n = 61$). Data on death was received from Statistics Finland until December 31 2015. Patients were grouped by the quartiles of baseline H-ficolin concentration in accordance with a previous the study. Akaike information criterion (AIC) was used to choose best adjusted Cox regression model.
Results: The patients were followed through a median of 16.6 years (IQR 15.2–17.3). 1428 patients had normal albumin excretion at baseline, 318 had microalbuminuria, 461 had macroalbuminuria and 232 patients had ESRD. All-cause mortality rate was higher among patients with H-ficolin concentration within the highest quartile (Q4) as compared with the combined group of the lower three quartiles (Q1–3), HR 1.48 (CI95% 1.17–1.88) in unadjusted Cox analysis. In adjusted analysis, HR for all-cause mortality was 1.45 (1.07–1.97) comparing Q4 to Q1–3 including the following covariates; age at baseline, age at diabetes onset, BMI, systolic blood pressure, estimated GFR, 24-hour albumin excretion rate, HbA1c and smoking status. Data on 24-hour albumin excretion rate was missing for 450 patients. However, omitting this variable from the analysis of mortality did not alter the estimate significantly. Median follow-up on renal data was 8.5 years (IQR 5.5–12.7). HR for progression from normoalbuminuria to microalbuminuria was 2.26 (CI 95% 1.62–3.18) comparing Q4 with Q1–3 in unadjusted Cox regression analysis. In an adjusted model, including 24-hour albumin excretion rate and HbA1c as covariates, HR decreased to 1.40 (CI95% 0.93–2.11) and lost statistical significance. H-ficolin was not associated with progression from microalbuminuria to macroalbuminuria or with progression from macroalbuminuria to ESRD.
Conclusion: High concentration of H-ficolin was associated with all-cause mortality in the present cohort of type 1 diabetes patients. H-ficolin was also associated with progression to microalbuminuria, but this was not statistically significant in adjusted analysis.
Supported by: Folkhälsan Res Foundation, Stockmann Foundation, Danish Diabetes Association
Disclosure: **J.A. Oestergaard:** Grants; Danish Diabetes Association, Danish Diabetes Academy.

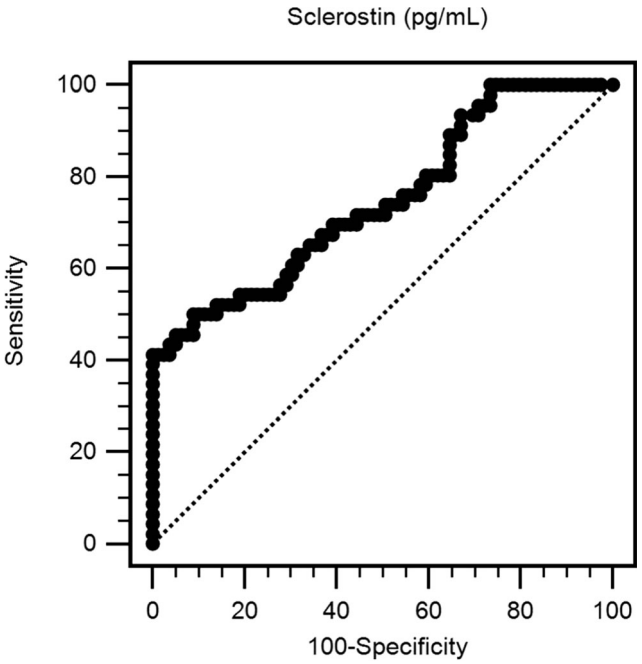
1059

Positive correlates of sclerostin and association with aortic arterial stiffness in patients with type 2 diabetes

Y.-C. Chen[1], D.-A. Wu[2], B.-G. Hsu[3];
[1]Department of Psychiatry, Dalin Tzu Chi General Hospital, Chiayin, [2]Division of Metabolism and Endocrinology, Tzu Chi General Hospital, Hualien, [3]Division of Nephrology, Tzu Chi General Hospital, Hualien, Taiwan.

Background and aims: Sclerostin or dickkopf-1 (DKK1) is a canonical Wnt/β-catenin signaling pathway inhibitor and Wnt/β-catenin signaling pathway is thought to be implicated in the development of arterial stiffness. Carotid-femoral pulse wave velocity (cfPWV) is novel method to assess for aortic arterial stiffness. It is interesting to investigate whether sclerostin or DKK1 level is correlated with aortic arterial stiffness in patients with type 2 diabetes mellitus (DM).
Materials and methods: Fasting blood samples were collected from 125 patients with T2DM for biochemical data, sclerostin, DKK1 levels. cfPWV value was measured by a validated tonometry system and cfPWV >10 m/s were used to define the high aortic arterial stiffness group according to the ESH-ESC 2013 guideline. Serum sclerostin and DKK1 concentrations were determined using a commercially available enzyme-linked immunosorbent assays.
Results: Forty-six type 2 DM patients (36.8%) were defined as the high aortic arterial stiffness. Patients in the high aortic arterial stiffness had older age ($P = 0.001$) and higher systolic blood pressure ($P < 0.001$), diastolic blood pressure ($P = 0.029$), blood urea nitrogen ($P = 0.029$), creatinine ($P < 0.001$), urine albumin-to-creatinine ratio ($P < 0.001$), serum sclerostin ($P < 0.001$) levels, and lower high-density lipoprotein cholesterol (HDL-C, $P = 0.016$), and estimated glomerular filtration rate ($P < 0.001$) than those in a low aortic arterial stiffness group. After adjusting for factors significantly associated with aortic arterial stiffness by multivariate logistic regression analysis, serum sclerostin level (odds ratio (OR): 1.005, 95% confidence interval (CI): 1.002–1.007, $P = 0.002$) and HDL-C (OR: 0.949, 95% CI: 0.902–1.000, $P = 0.048$) were the independent predictors of aortic arterial stiffness in patients with type 2 DM. Multivariate forward stepwise linear regression analysis also showed that serum sclerostin level ($\beta = 0.374$, adjusted R2 change: 0.221, $P < 0.001$) was positively associated with cfPWV values in type 2 DM patients. The area under the receiver-operating characteristic

(ROC) curve predicting central arterial stiffness by sclerostin level in DM patients was 0.748 (95% CI: 0.661–0.820, $P < 0.001$).
Conclusion: Serum sclerostin level, but not DKK1, is positively correlated with cfPWV values and is the independent predictor of aortic arterial stiffness in patients with type 2 DM.



Clinical Trial Registration Number: IRB103-136-B
Disclosure: **Y. Chen:** None.

1060

HbA_{1c} coefficient of variation is an independent risk factor for chronic complications in type 2 diabetes in the FIELD study

E.S. Scott[1,2], A.S. Januszewski[1,3], R. O'Connell[1], S. Colagiuri[4], G.R. Fulcher[2,5], A. Keech[1], A.J. Jenkins[1,3], on behalf of the FIELD study investigators;
[1]NHMRC Clinical Trials Centre, Sydney, [2]Department of Endocrinology and Diabetes, Royal North Shore Hospital, Sydney, [3]Department of Medicine, St Vincent's, University of Melbourne, Melbourne, [4]The Boden Institute, University of Sydney, Sydney, [5]Northern Clinical School, University of Sydney, Sydney, Australia.

Background and aims: Glycaemic variability (GV) is implicated in the pathogenesis of chronic diabetes complications. GV can be measured long- and short-term by fluctuations in HbA1c or fasting plasma glucose respectively. Study aims were to assess whether GV is associated with the development of micro- and macrovascular complications in the Fenofibrate Intervention and Event Lowering In Diabetes (FIELD) study, and to determine what baseline factors predict GV.
Materials and methods: The FIELD study randomised 9795 individuals to fenofibrate 200 mg daily or placebo and followed the development of vascular complications for a median of 5-years. GV was calculated as coefficient of variation (CV) and standard deviation (SD) of HbA1c and fasting plasma glucose (measured at baseline, annually and at study-end). We assessed whether GV was a predictor of microvascular and/or macrovascular complications from study year-2 onward using logistic and Cox proportional hazards regressions with adjustment for significant covariates. The primary composite endpoint was the development of microvascular disease (defined as presence of either retinopathy, albuminuria, neuropathy and/or microvascular amputations), and subsidiary composite endpoints: macrovascular disease (cardiovascular mortality, myocardial infarction, stroke, coronary and carotid revascularization), total mortality and individual vascular complications.
Results: A logistic regression model with adjustment for age, gender, glycaemic control, baseline complications and treatment allocation demonstrated that HbA1c CV was an independent risk factor for the development of microvascular complications (OR 1.13 95% CI (1.04–1.22) $P = 0.002$). All other GV parameters had similar effects. Although HbA1c CV was significantly associated with the macrovascular outcome in univariate Cox proportional hazards regression ($P = 0.002$), significance was lost in multivariate analysis. Multiple logistic regression demonstrated that HbA1c CV (quartiles Q1 = reference) was significantly associated with the development of nephropathy at 2-years (Q2: OR 1.24 (95% CI 0.97–1.59), Q3: OR 1.43 (1.12–1.82), Q4: OR 1.77 (1.38–2.25) $P < 0.001$). HbA1c CV (quartiles) was significantly associated in Cox proportional hazards multiple regression with the development of stroke (Q2: OR 1.48 (95% CI 0.93–2.35), Q3: OR 1.52 (0.96–2.41), Q4: 1.99 (1.27–3.14) $P = 0.02$) and total mortality (Q2: OR 0.97 (95%CI 0.73–1.28), Q3: 1.24 (0.95–1.62), Q4: OR 1.66 (1.29–2.14) ($P < 0.001$)). In subjects randomised to fenofibrate HbA1c CV increased compared to placebo across the study (HbA1c CV 8.98 (5.13) vs 8.58 (5.06) ($P < 0.001$, (Diff 0.39, 95% CI 0.19–0.60).
Conclusion: In Type 2 diabetes HbA1c CV is an independent risk factor for microvascular complications, stroke and total mortality. Despite higher HbA1c CV in individuals randomised to fenofibrate, fenofibrate therapy significantly reduced total cardiovascular events and all microvascular outcomes in the FIELD study.
Disclosure: **E.S. Scott:** None.

PS 099 Don't forget!

1061

Diabetes and cognitive function: longitudinal study of adult health (ELSA-Brasil)

M. Teixeira[1], V. Passos[1], S. Barreto[1], M. Schmidt[2], B. Duncan[2], A. Beleigoli[1], M. Fonseca[3], P. Vidigal[1], L. Araujo[4], M. Diniz[1];
[1]Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, [2]Faculty of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, [3]Public Health School, Fundação Oswaldo Cruz, Rio de Janeiro, [4]Public Health School, Universidade Federal do Ceara, Fortaleza, Brazil.

Background and aims: Some studies show worse performance in cognition tests for diabetes mellitus (DM) patients. Our aim is to assess the association between cognitive performance and DM. Detection of early alterations in cognitive performance among individuals with DM could be important for future potential interventions

Materials and methods: Cross-section analysis, baseline (2008–2010) of a large multicentric cohort of a developing country that has been experiencing a socio-demographic and nutritional transition in the last three decades: Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Cognitive domains was assessed by word-list learning, word-list delayed recall, word recognition tests, semantic and phonemic verbal fluency tests, and trail making test B. Multiple linear regression and generalized multiple regression with algorithmic link and gamma distribution were used to estimate the association between DM and cognitive performance. The participants were located in three subgroups: no DM, new-onset (NDM) and previous DM(PDM). Analyses were adjusted for age, sex, education, comorbidity, habits and lipids. In the final model were included results with $p < 0.05$

Results: There were 14,480 participants in this study, 45.7% male, mean 52.0 ± 9.1 years-old, 52.8% with University degree, 76.7% sedentary, 57.1% never smoked, 63.1% with BMI >25.0 kg/m^2. PDM was associated with the poorest performance in memory tests (early memory, delayed memory and words recognition) (β −0.67 [−0.98; −0.36] R2 0.21) when compared to no or to NDM, with a dose-response gradient. Results show a better cognitive performance in women and worse performance in older individuals, with lower schooling, high alcohol consumption, smoking and with higher ratio total cholesterol/ HDL-c. The results of the models between verbal phonemic fluency and new-onset and PDM shows similar performance in both groups (β −0.48 [−0.72; −0.24] R2 0.18) and (β −0.49 [−0.72; −0.26] R2 0.18), respectively after adjustments. Final model demonstrate a lower cognitive performance in the older individuals, with low schooling, hypertensive, smoking and with low physical activity level

Conclusion: There was a significant association between diabetes and cognitive performance in this population

Table 1 - Multivariate regression final models with Memory test and Phonemic verbal fluency test and diabetes at ELSA-Brasil (2008-2010)

Variable	Memory test (learning, recall and word recognition test)			Phonemic verbal fluency		
	Model 1 β[CI95%] R²-0.21	Model 2 β[CI95%] R²-0.20	Model 3 β[CI95%] R²-0.21	Model 1 β[CI95%] R²-0.17	Model 2 β[CI95%] R²-0.18	Model 3 β[CI95%] R²-0.18
Diabetes						
No	1	1	1	1	1	1
New-onset	-0.12[-0.41,0.18]	-0.04[-0.37,0.28]	-0.07[-0.37,0.23]	-0.54[-0.77,-0.30]***	-0.49[-0.73,-0.25]***	-0.48 [-0.72,-0.24]***
Previous	-0.71[-1.02,0.41]***	-0.71[-1.0,-0.38]***	-0.67[-0.98,-0.36]***	-0.50[-0.73,-0.27]***	-0.47[-0.70,-0.24]***	-0.49[-0.72,-0.26]***
Age	-0.13[-0.14,0.12]***	-0.12[-0.13,-0.11]***	-0.12[-0.13,-0.11]***	-0.05[-0.06,-0.04]***	-0.04[-0.05,-0.36]***	-0.05[-0.06,-0.04]***
Female Sex	2.08[1.91,2.26]***	2.20[2.01,2.39]***	2.03[1.84,2.22]***	-	-	-
Education(years)						
<8	1	1	1	1	1	1
8-10	2.19[1.68,2.70]***	2.27[1.64,2.67]***	2.16[1.65,2.67]***	1.57[1.20,1.96]***	1.51[1.12,1.90]***	1.57[1.19,1.94]***
11-14	4.18[3.76,4.60]***	4.17[3.69,4.54]***	4.12[3.70,4.55]***	3.53[3.22,3.84]***	3.54[3.23,3.85]***	3.54[3.23,3.85]***
>14	7.10[6.69,7.51]***	7.14[6.56,7.39]***	6.95[6.55,7.38]***	5.79[5.49,6.09]***	5.79[5.48,6.10]***	5.78[5.48,6.09]***
Smoking						
No			1			1
Former			-0.35[-0.55,-014]**			0.43[0.28,0.59]***
Current			-0.81[-1.08,-0.54]***			0.16[-0.04,0.37]
Alcohol (g/week)						
1-175			1		-	-
176-350			0.66[0.30,1.03]***			
>350	-		0.43[-0.13,0.99]			
Total Col-HDL-C	-	-	-0.14[-0.23,-0.06]**	-	-	-
Hypertension	-	-	-		-0.31[-0.47,-0.16]***	-0.30[-0.45,0.15]***
Physical activity	-	-	-	-	-	
Low						1
Moderate						0.27[0.08,0.46]**
High						0.13[-0.13,0.40]

β[CI95%]- β coefficient[95% confidence intervals] ; R²- Adjusted R²; * p value<0.05 **p value <0.01 ***p value<0.001

Disclosure: **M. Teixeira:** None.

1062

The diabetes and dementia (DIADEM) project: assessing inpatient admissions and trends in management and hospitalisation of patients with diabetes and dementia

A. Ali, S. Dhuna, A. Puttanna, P. De;
Sandwell and West Birmingham NHS Trust, Birmingham, UK.

Background and aims: Continued advances in medicine have contributed to an ageing population and incidence of type 2 diabetes is also on the rise. As of 2015 there are 46.8 million people with dementia in the world with this rising to over 131.5 million by 2050. The worldwide prevalence of diabetes in 2015 was 387 million and due to rise to 592 million by 2035. Consequently, the combined co-morbidity of diabetes and dementia is on the increase. The DIADEM project was devised to target this specific population of patients, assessing current trends in hospital admissions with a view to reducing the health and economic burden The aim was to evaluate the current trends and outcomes of hospital admissions in patients with dementia and diabetes including availability of recent Hba1c and medication regimens for complexity and overtreatment.

Materials and methods: Patients admitted over the previous year with a coded diagnosis of dementia and type 2 diabetes were analysed for information relating to medication, admission reasons as well as in hospital glycaemic control and Hba1c prior to admission.

Results: 350 patients were assessed, median age 84 years (IQR 79–88). Admission reasons included respiratory [75 (21.4%)], urinary [68 (19.4%)] , neurological [47 (13.4%)] , cardiac [41 (11.7%)] , trauma [40 (11.4%)]. Medication history was obtained for 256 (73.1%) patients with 94 (26.9%) on diet alone, 70 (20%) treated with sulphonylurea (SU), 46 (13.1%) were treated with oral and insulin therapies and 58 (16.6%) treated with insulin alone. 311 (88.9%) patients had a recent Hba1c with 39 (11.1%) never having a recorded Hba1c. Median 137 (IQR 52–285.5) days between Hba1c reading and date of admission. Median Hba1c for the cohort was 51(IQR 44–64) mmol/mol, with 163 (46.6%) having Hba1c <53 mmol/mol and 135 (38.6%) Hba1c <48 mmol/mol. 13 (8%) had episodes of hypoglycaemia (BG <3 mmol/l) during their admission. 11 (6.8%) of these patients had Hba1c <48 mmol/mol. Only three had their diabetic medication adjusted on discharge with the rest continuing on their admission medication. Stratified by medication history for insulin, combined insulin, sulphonylurea therapies and both SU/insulin therapies, 25 (7.4%), 11 (3.1%), 11 (3.1%) and 2 people (0.6%) had Hba1c <53 mmol/mol

Conclusion: This is the first project specifically focussing on this subgroup of patients. A large number of patients are over-treated. Clinicians must consider de-intensification, favouring less complex regimes with fewer side effects and reduced risk of hypoglycaemia to reduce health and economic burden for these patients.

Disclosure: **A. Ali:** None.

1063

Impacts of metabolic health and obesity status on the development of dementia: a population-based cohort study

M. Lee[1], J.-Y. Lee[1], K. Han[2], J. Bae[1], B. Lee[1,3], E. Kang[1,3], B.-S. Cha[1,3], Y.-H. Lee[1,3];
[1]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, [2]Department of Biostatistics, The Catholic University, Seoul, [3]Institute of Endocrine Research, Yonsei University College of Medicine, Seoul, Republic of Korea.

Background and aims: The risk for dementia among subjects who are metabolically healthy obese (MHO), remains uninvestigated. We aimed to evaluate the association between late-life metabolic health and obesity status and risk of incident dementia.

Materials and methods: Using the National Health Insurance System of South Korea, study population comprised 5,669,488 adults aged ≥60

years who underwent the health examinations from 2009 to 2012 without history of dementia at baseline. Subjects who met not more than one criterion using the Adult Treatment Panel-III were determined as metabolically healthy and obesity was defined as a body mass index 25 kg/m^2. Subjects were classified into four groups by metabolic profiles and obesity status, and were followed for incident overall dementia, Alzheimer's disease (AD), and vascular dementia (VaD).

Results: During a median follow up of 65 months (interquartile range [IQR] 51–74 months), dementia was developed in 363,932 subjects (6.4%). The MHO group had the lowest risk for overall dementia (hazard ratio [HR] 0.87, 95% confidence interval [CI] 0.86–0.88), whereas metabolically unhealthy non-obese (MUNO) subjects were at the highest risk (HR 1.20, 95% CI 1.19–1.21) compared to the metabolically healthy non-obese (MHNO) group. Metabolically unhealthy profiles raised the risk (up to 41% in VaD), and obesity reduced the risk (up to 15% in AD).

Conclusion: The MHO phenotype in late life was associated with decreased risk for overall dementia. Further studies in other populations are warranted to better understand current results and to predict individuals who have the most increased risk for developing dementia.

Disclosure: **M. Lee:** None.

1064

Associations between brain grey matter volumes and adipose tissue metabolism in healthy adults

J. Raiko[1], J. Tuulari[1], T. Saari[1], R. Parkkola[2], N. Savisto[1], P. Nuutila[3], K. Virtanen[1];
[1]Turku PET Centre, University of Turku, Turku, [2]Department of Radiology, Turku University Hospital, Turku, [3]Department of Endocrinology, Turku University Hospital, Turku, Finland.

Background and aims: Grey matter (GM) volume in different brain loci has been shown to vary in obesity and diabetes, but the mechanisms conveying the changes remain unresolved. Few studies have examined the associations between adipose tissue metabolic activity and brain GM volumes. We examined fatty acid metabolism in different adipose tissue depots and their associations with GM volumes in brain loci in healthy and overweight adults.

Materials and methods: 36 subjects (M/F: 12/24, mean age 39.7 ± 9.4 years and BMI 27.5 ± 5.6 kg/m^2) were imaged with positron emission tomography using fatty acid analog [^{18}F]FTHA for brown (BAT) and white (WAT) fat depots and with [^{15}O]H2O for brown fat only during cold exposure. T1-weighted MRI at 1.5T was performed to all subjects (brain and whole body scans). VBM was used to measure brain GM volumes and ROI definitions to measure intra-abdominal fat volume. 2-hour hyperinsulinemic euglycemic clamp was performed to measure whole-body insulin sensitivity (M-value).

Results: BAT NEFA uptake in cold correlated directly with GM volumes in anterior cerebellum ($r = 0.411$, $P = 0.014$), occipital lobe ($r = 0.398$, $P = 0.018$) and temporal lobe ($r = 0.408$, $P = 0.015$), while BAT perfusion in cold was linked with GM volume in anterior ($r = 0.369$, $P = 0.04$) and posterior cerebellum ($r = 0.367$, $P = 0.04$) and midbrain ($r = 0.427$, $P = 0.017$). BAT NEFA uptake associated directly with GM volume in anterior cerebellum and occipital lobe (P in both ≤0.04) when adjusted for age, gender and intra-abdominal fat volume. When intra-abdominal fat volume was replaced with M-value as a regressor of no interest, associations with anterior cerebellum, limbic lobe and temporal lobe GM volumes remained significant (P always ≤0.04).

Conclusion: BAT fatty acid metabolism associates with GM volume in anterior cerebellum and occipital lobe independently of visceral obesity and with GM volume in anterior cerebellum, limbic lobe and temporal lobe independently of whole-body insulin sensitivity. Adulthood BAT activity might be a crucial resilience biomarker that attenuates the metabolic effects of obesity and related metabolic changes.

Supported by: SWFCF, Paulo Found., UTU Found., Finn. Med.Found.

Disclosure: **J. Raiko:** None.

1065

Localised brain volume differences and cognitive status in subjects with type 2 diabetes

I.D. Wilkinson[1], L. Hunt[1], K. Teh[1], S. Tesfaye[2], D. Selvarajah[1];
[1]Academic Radiology & Metabolism, University of Sheffield, Sheffield, [2]Diabetes and Endocrinology, Sheffield Teaching Hospitals, Sheffield, UK.

Background and aims: Mild Cognitive Impairment (MCI) is thought to be a chronic sequelae of Type-2 Diabetes Mellitus (T2DM). Cerebral atrophy is known to be associated with cognitive decline, particularly in the various types of dementia. Prodronal dementia has also been associated with changes in brain parenchymal structure. The aim of this study was to identify and compare regional differences in brain volume in T2DM patients with and without MCI.

Materials and methods: Seventy-six age and gender matched subjects [30, T2DM+normal cognition (T2DM); 17, T2DM+MCI (T2DM/MCI) and 29 non-diabetic healthy volunteers (HV)] were recruited. All subjects underwent clinical and questionnaire (Addenbrooke's Cognitive Assessment [ACE-R]) assessments and high-resolution, 3D T1-weighted Magnetic Resonance Imaging at 3T. Cerebral volumes were analysed using voxel based morphometry (VBM, FSL, Oxford).

Results: Demographic data indicated that all three groups were age-matched (mean age 69.3–71.5 years, ANOVA, $p = 0.164$). Group mean T2DM/MCI ACE-R score (mean ± SD; 83 ± 4) was significantly lower compared to those of other groups (HV = 96 ± 2, T2DM = 94 ± 3; ANOVA, $p < 0.001$). The T2DM/MCI group had significantly lower regional grey matter volumes compared to HV in the left ($p < 0.0005$) and right hippocampi ($p < 0.05$), left putamen ($p < 0.05$), caudate ($p < 0.05$) and amygdala ($p < 0.05$).

Conclusion: The current study demonstrates significantly lower cortical brain volumes in areas associated with cognition (including short-term memory-retrieval) in patients who have T2DM and mild cognitive impairment. Detailed changes in neuroanatomical make-up may help elucidate diabetes-related pathological mechanisms that lead to a change in cognition associated with T2DM.

Supported by: G-WF & Wellcome Trust

Disclosure: **I.D. Wilkinson:** None.

1066

Impaired olfactory function is associated with insulin resistance in adults with type 1 diabetes

A. Duda-Sobczak[1], B. Falkowski[1], A. Araszkiewicz[1], M. Chudzinski[1], M. Urbas[2], L. Borucki[2], D. Zozulinska-Ziolkiewicz[1];
[1]Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poznan, [2]Department of Otorhinolaryngology, Raszeja City Hospital, Poznan, Poland.

Background and aims: Diabetes mellitus contributes to the central nervous system degeneration and impaired olfaction is a clinical manifestation of central diabetic neuropathy. Hyperglycemia and oxidative stress play a role in the development of olfactory dysfunction in diabetic subjects, however other diabetes-related factors might also be important. The aim of the study was to assess olfactory function and insulin sensitivity in adults with type 1 diabetes (T1DM).

Materials and methods: We included 113 participants with T1DM (60 men), median age 36 (IQR 29–43), disease duration 20 (IQR 13–26) years, HbA1c 8.0 (IQR 7.2–8.9)%. The patients underwent ENT examination with nasal endoscopy to exclude other factors disabling sense of smell. Olfactory function was assessed with "Sniffin" Sticks. For the assessment of odor identification 12 pens with different odors were used and patient should select 1 of 4 presented items which best described each odor for every pen (score 0–12, normosmia: score 10–12). We assessed the metabolic control of diabetes. To assess insulin sensitivity we used indirect markers: body mass index (BMI), waist-to-hip ratio (WHR),

visceral fat index, VFI (bioimpedance analysis), triglyceride to high density lipoprotein cholesterol (TG/HDL) ratio and specific calculators (estimated glucose disposal rate, eGDR; visceral adiposity index, VAI).
Results: Hyposmia was found in 56 (49.6%) participants, median odor identification score 10 (IQR 9–11). We showed lower insulin sensitivity in hyposmia compared to normosmia group: BMI [25.5 (21.9–29.5) vs. 23.3 (21.8–26.2) kg/m^2; $P = 0.047$], WHR [0.91 (0.83–0.96) vs. 0.83 (0.76–0.89); $P < 0.0001$], VF index [5 (3–9) vs. 3 (2–5); $P = 0.005$], TG/HDL ratio [0.70 (0.45–1.09) vs. 0.51 (0.34–0.74); $P = 0.007$], eGDR [8.0 (4.9–9.3) vs. 8.9 (6.3–10.0) mg/kg/min; $P = 0.01$], VAI [2.2 (1.6–3.5) vs. 1.6 (1.2–2.8); $P = 0.01$]. Odor identification score correlated with WHR (Rs = –0.40; $P < 0.0001$), TG (Rs = –0.24; $P = 0.01$), VF index (Rs = –0.22; $P = 0.03$), TG/HDL ratio (Rs = –0.25; $P = 0.008$), VAI (Rs = –0.24; $P = 0.01$) and eGDR (Rs = 0.23; $P = 0.01$). In multivariate linear regression analysis higher WHR was independently associated with impaired olfactory function ($\beta = -0.38$; $P = 0.002$) after adjustment for age, sex, TG, autonomic and peripheral neuropathy ($R^2 = 0.25$; $P < 0.0001$). The receiver-operating characteristic (ROC) analysis indicated a WHR cut-off of 0.92 [AUC: 0.737; 95%CI: 0.647–0.828, $P < 0.0001$] best predicting olfactory dysfunction (sensitivity 0.50, specificity 0.86).
Conclusion: Higher degree of olfactory dysfunction is observed in patients with type 1 diabetes and indirect markers of insulin resistance
Supported by: Poznan University of Medical Sciences
Disclosure: **A. Duda-Sobczak:** None.

1067

Linagliptin (LINA) restores the dopaminergic impairment induced by experimental diabetes in striatum and counteracts the detrimental effects of aging

G. Lietzau[1], E. Candeias[1], G. Magni[2], J. Kehr[3,4], T. Yoshitake[3], J. Skogsberg[5], T. Nyström[1], T. Klein[6], M.P. Abbracchio[2], S. Ceruti[2], V. Darsalia[1], C. Patrone[1];
[1]Dept. of Clinical Science & Education, Södersjukhuset, Internal Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Dept. of Pharmacological & Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy, [3]Dept. of Physiology & Pharmacology, Karolinska Institutet, Stockholm, Sweden, [4]Pronexus Analytical AB, Bromma, Sweden, [5]Boehringer Ingelheim AB, Stockholm, Sweden, [6]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

Background and aims: Recent data suggest that type 2 diabetes (T2D) may be implicated in the pathogenesis of motor system disorders, including Parkinson's disease (PD) but the underlying mechanisms are mostly unknown. We hypothesize that T2D impairs the dopaminergic system during aging in combination with structural changes in the basal ganglia, and that LINA, a dipeptidyl peptidase-4 (DPP-4) inhibitor used for the treatment of T2D, may reverse these effects. Specifically, we aimed to determine whether: 1) T2D impairs the basal and stimulated extracellular levels of dopamine (DA) in the mouse striatum, 2) T2D induces changes in neuronal and glial cellular populations, 3) LINA treatment counteracts these effects.
Materials and methods: Adult and non-diabetic/T2D middle-aged C57BL7/6 mice were used. T2D was induced by 12-month-intake of high-fat diet. LINA was administered in the chow (daily intake ~5 mg/kg/bw) for the last 3 months. To evaluate the effect of T2D on the dopaminergic system, microdialysis and HPLC were performed. To assess potential structural changes in substantia nigra and striatum, neurons (TH$^+$, DARPP-32$^+$, parvalbumin$^+$) and glia (Iba-1$^+$, GFAP$^+$, Olig2$^+$, GSTp$^+$) were quantified by immunohistochemistry.
Results: Versus control mice, middle-aged T2D mice showed a decrease in extracellular DA levels of 82% (3.38 ± 0.92 vs. 18.81 ± 6.89 fmol/10 µl, $p = 0.035$) and a blunted response to amphetamine challenge in the striatum. However, DA intracellular levels were unchanged in the diabetic vs. non-diabetic mice ($p = 0.23$). Chronic LINA treatment resulted in a strong trend towards an increase in extracellular DA (75% vs. control; $p = 0.13$) and a restoration of striatal DA signaling in T2D mice. On the structural level, T2D had no effect on neuronal and glial markers. However, we identified an age-induced decrease in the density of the parvalbumin$^+$ interneurons (70.78 ± 6.3 vs. 52.48 ± 2.5 cells/mm^2, $p = 0.035$), age-induced increase in both neuroinflammation (174.1 ± 12.65 vs. 274.3 ± 12.79 Iba-1$^+$ cell volume/µm^3, $p < 0.0001$) and gliosis (78.25 ± 6.25 vs. 166.6 ± 12.52 GFAP$^+$ cells/mm^2, $p < 0.0001$), as well as a decrease in the density of proliferating (16.41 ± 1.45 vs. 8.23 ± 1.35% of PCNA/Olig2$^+$ cells, $p = 0.0046$) and mature oligodendrocytes (1.35 ± 0.097 vs. 0.72 ± 0.137 GSTp$^+$ cells/µm^2, $p = 0.012$). LINA treatment alleviated all these age-related effects.
Conclusion: This study provides new knowledge on how T2D impairs the dopaminergic system during aging. These effects could represent one of the pathogenic mechanisms predisposing T2D patients to develop motor dysfunction disorders such as PD. We also show that LINA alleviated these changes. Finally, we identified T2D-independent aging effects in the striatum that could be normalized by LINA treatment. Overall, our results suggest a potential role for DPP-4 inhibitors against the detrimental effects of T2D and aging on the motor system.
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **G. Lietzau:** Non-financial support; Boehringer Ingelheim.

1068

Increased glycosuria reduces the risk of hyperuricaemia in subjects with newly diagnosed diabetes: a cross-sectional study

L. Fan[1,2], J. Chen[1], Z.L. Sun[3];
[1]Medical School, Southeast University, Nanjing, [2]China Eastern Airlines, Nanjing, [3]Department of Endocrinology, Affiliated Zhongda Hospital of Southeast University, Nanjing, China.

Background and aims: Accumulating evidences have demonstrated that sodium glucose cotransporter 2 inhibitors lowers the serum uric acid level (SUA) by increased glycosuria. However, the association between SUA and urine glucose excretion (UGE) has not been fully investigated. In this study, we evaluated the relationship between SUA and UGE in subjects with newly diagnosed diabetes (NDD).
Materials and methods: We performed a cross-sectional study. Inclusion criteria were the following: aged 18–65 years, with no previous history of diabetes, consent to participate in the study. All participants were given an oral glucose tolerance test. Urine samples were collected within 2 h of oral glucose loading for the measurement of glucose. Blood glucose, lipid profile, and uric acid were assessed. Multiple linear regression analysis and multivariate logistic regression analysis were performed to determine the association of SUA with UGE.
Results: 445 people with NDD were included in the final analysis. The prevalence of hyperuricemia in subjects with NDD was 12.8%. The low SUA group exhibited significantly higher age, plasma glucose, and UGE compared with high SUA group ($p < 0.001$), while triglycerides and BMI significantly lower than high SUA group. Multiple linear regression analysis with SUA as a dependent variable, log-transformed UGE were negatively associated with SUA when adjusted for age, sex, and BMI ($\beta = -26.4$, SE: 5.7, 95% CI: −37.6 to −15.3, $p < 0.001$). Multivariable logistic regression model showed that log-transformed UGE was significantly associated with a decreased odds ratio of hyperuricemia (Table 1). In addition, no significant correlation was found between SUA and blood glucose (Table 1).
Conclusion: In this cross-sectional study, increased UGE was negatively related to SUA in subjects with NDD. These findings suggest that subjects with high UGE have decreased risk of hyperuricemia.

Table 1 Multivariate logistic regression analysis of the factors influencing the prevalence of hyperuricemia.

Category	β	SE of β	OR	95% CI	p value
Age (years)	-0.042	0.017	0.96	0.93-0.99	0.013
FPG (mmol /L)	-0.100	0.126	0.91	0.71-1.16	0.427
2h-PG (mmol /L)	-0.054	0.073	0.95	0.82-1.09	0.565
BMI (kg/m^2)	0.104	0.044	1.11	1.02-1.21	0.018
Gender *	-1.477	0.389	0.23	0.11-0.47	< 0.001
lg (UGE)	-0.596	0.253	0.55	0.34-0.90	0.018
Cholesterol (mmol /L)	0.158	0.264	1.17	0.70-1.97	0.550
Triglycerides (mmol /L)	0.137	0.074	1.15	0.99 -1.33	0.065
HDL-C (mmol /L)	-0.186	0.669	0.83	0.22-3.08	0.781
LDL-C (mmol /L)	0.205	0.289	1.23	0.70-2.17	0.478

* 1=male, 2= female; OR, odds ratio, CI, confidence interval; FPG, fasting plasma glucose; 2h-PG, 2h- plasma glucose; lg (UGE), log-transformed urine glucose excretion; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index.

Supported by: 2016YFC1305700
Disclosure: **L. Fan:** None.

PS 100 Brain functionalities

1069

Nrf2 mediated protection against hypoglycaemia induced cognitive deficits in type 1 diabetes

A.D. McNeilly, J. Gallagher, A. Dinkova-Kostova, R.J. McCrimmon; University of Dundee, Dundee, UK.

Background and aims: Hypoglycaemia in Type 1 diabetes (T1D) is associated with an increase in oxidative stress. We have previously demonstrated that recurrent intermittent hypoglycaemia (RH) in a rodent model of T1D is associated with impaired cognitive function and activation of the Nrf2 antioxidant system. This study sought to investigate whether pre-treatment with a potent Nrf2 inducer would ameliorate these cognitive deficits.
Materials and methods: A chronic stable model of insulin-treated T1D was achieved using streptozotocin (125 mg/kg i.p.) and insulin implants (Linbit). Diabetic (male C57bl6 mice $n = 8$–10/group) mice were randomly allocated to one of 3 groups: (i)T1D, (ii) T1D+RH, (iii) T1D+RH+SFN and subjected to repeated episodes of insulin-induced hypoglycaemia (3 episodes per week for 4 weeks). Sulforaphane (SFN; 50 mg/kg i.p.) or Vehicle (1% DMSO/PBS) was administered 24 hr prior to each hypoglycaemic episode. Cognition was subsequently assessed by novel object recognition (NOR) and spontaneous alternation tasks.
Results: Pre-treatment with SFN had no impact upon body weight or fasting blood glucose glucose (both p = ns). In contrast HbA1c levels were significantly lower in SFN treated animals ($p < 0.01$). Furthermore, SFN improved cognitive performance in the 24 hr NOR task ($p < 0.01$) and the spontaneous alternation task ($p < 0.01$) when compared to those receiving vehicle.
Conclusion: Treatment with SFN significantly improves RH induced cognitive impairments in a rodent model of T1D. These improvements were associated with a significant improvement in HbA1c levels. Therefore, activation of the Nrf2 antioxidant pathway offers a novel therapeutic target for the treatments of cognitive impairments associated with RH in T1D.
Supported by: DUK
Disclosure: **A.D. McNeilly:** None.

1070

Decreased O-GlcNAcylation to tau phosphorylation at Thr212 site ratio is associated with mild cognitive impairment in type 2 diabetic patients

R. Huang, S. Tian, R. Cai, D. Guo, H. Lin, J. Wang, K. An, S. Wang; Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China.

Background and aims: Aberrant O-GlcNAc modification has been associated with insulin resistance and type 2 diabetes mellitus (T2DM), as well as the pathogenesis of neurodegenerative diseases. We aimed to investigate the association between global O-GlcNAcylation, tau phosphorylation levels and mild cognitive impairment (MCI) in the whole blood of T2DM patients.
Materials and methods: Sociodemographic, clinical characteristics and cognitive performances of the enrolled T2DM subjects were extensively assessed. Global O-GlcNAcylation and tau phosphorylation levels in the whole blood were also determined using Western blot.
Results: According to Montreal Cognitive Assessment score, 24 T2DM with MCI patients and 24 with normal cognition subjects were enrolled in this study. In addition to elevated fasting blood glucose, glycated hemoglobin A1c (HbA1c), decreased fasting C-peptide and uric acid, T2DM with MCI subjects displayed a decreased O-GlcNAcylation level, while increased tau phosphorylation levels than cognitively normal controls (all $p < 0.05$). In order to reflect the combined effect, relative ratios of O-GlcNAcylation to tau phosphorylation levels showed that O-GlcNAc/

Tau-5, O-GlcNAc/p-S396, O-GlcNAc/p-S404, O-GlcNAc/p-T212 and O-GlcNAc/p-T231 were all significantly decreased in MCI subjects (all $p < 0.05$). Multivariable logistic regression analysis revealed that higher HbA1c was an independent risk factor, while increased O-GlcNAc/p-T212 was an independent protective factor for MCI in T2DM patients (OR = 2.452, 95%CI 1.061–5.668, $p = 0.036$; OR = 0.028, 95%CI 0.002–0.388, $p = 0.008$, respectively). With regard to each cognitive domain, O-GlcNAc/p-T212 was positively correlated with the scores of Auditory Verbal Learning Test-delayed recall ($r = 0.377$, $p = 0.010$), which represented delayed learning and memory function.

Conclusion: Our study suggests that decreased O-GlcNAcylation to tau phosphorylation at Thr212 site ratio in the whole blood is associated with MCI, especially with delayed memory dysfunction in T2DM subjects.

Clinical Trial Registration Number: ChiCTR-OCC-15006060
Supported by: NSFC (No.81570732, SW)

Disclosure: **R. Huang:** None.

1071

The implications of rs1887922 polymorphism of insulin degrading enzyme gene in the cognitive impairments for patients with type 2 diabetes

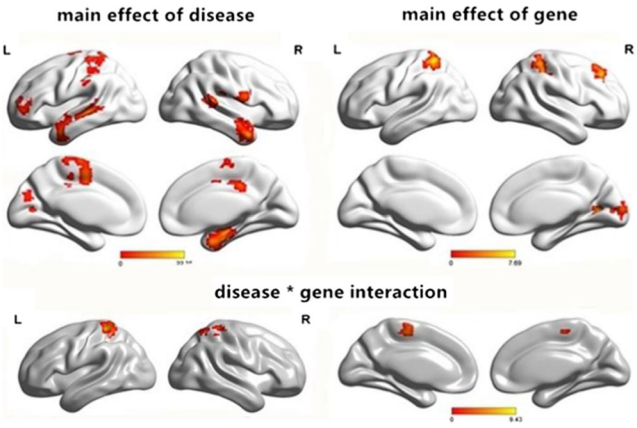
J. Huang;
Southeast University, Nanjing, China.

Background and aims: In recent years, efforts have been made to explore the mechanisms regarding how type 2 diabetes (T2D) increases the risk of Alzheimer's disease (AD), which will help to reveal the pathomechanisms of AD and find ways to relieve cognitive impairments in both diseases. Gene association analyses make great contribution in this flied, and the insulin degrading enzyme (IDE) gene represents a very promising gene that influences cognitive performance because of its biological function and location near late-onset Alzheimer's disease (AD) linkage peaks, i.e., the chromosome 10q. Previous molecular investigations have suggested the associations with IDE rs1887922 polymorphism and AD-related pathology, however little in known regarding its in vivo neurobiology. The present study aimed to reveal the associations between IDE rs1887922 and brain default mode network in T2D patients via genetic-imaging approach.

Materials and methods: A total of 160 patients with T2D were enrolled in the present study. 80 of the participants had intact cognitive performance, and the remaining 80 subjects were mild cognitive impairment subjects. All the participants went through cognitive evaluations, structural and functional MRI scans, and genotyping of IDE rs1887922 polymorphism. To explore the influence of rs1887922 on brain functional network, a mixed analysis of covariance (ANCOVA) with disease status (2 levels: MCI and HC) and IDE rs1887922 (2 levels: C and T) as fixed factors was performed. Finally, the behavior significances of the brain regions with gene × disease interactions were tested, and the partial correlations were applied.

Results: The main effects of disease were detected within bilateral temporal cortex, left parietal lobule and left medial prefrontal gyrus. For the IDE polymorphism, main effects were detected within right posterior cingulate, bilateral inferior parietal lobule and right medial frontal gyrus. Regarding the interaction of disease and gene, regions within bilateral superior parietal lobules were reported (corrected $P < 0.05$, determined by Monte Carlo simulation). The partial correlation analyses implied the cognitive significance of regions with interactive effects, as the functional activity within left superior parietal lobule were significantly related to the performance of episodic memory (scores of Rey-Osterrieth Complex Figure Test with 20-min delayed recall, rho = 0.67, $P = 0.002$) and general cognition (scores of MMSE, rho = 0.47, $P = 0.017$) for MCI subjects.

Conclusion: The genetic-imaging approach expanded our understanding for the mechanisms underlying the implications of IDE genetic polymorphism in T2D-related injuries of brain network, and improving the dysfunction of IDE may be a promising approach to relieving neurological and psychiatric disorders for patients with T2D.



Disclosure: **J. Huang:** None.

1072

Elevated biomarkers of oxidative stress and endothelial dysfunction are associated with reduced cognitive performance in type 2 diabetes in the CAROLINA® trial

G. Biessels[1], O. Groeneveld[1], E. van den Berg[2], S. Schnaidt[3], K. Hermansson[4], B. Zinman[5], M. Espeland[6], O. Johansen[7];
[1]Department of Neurology, University Medical Center Utrecht, Utrecht, Netherlands, [2]Department of Neurology, Erasmus MC - University Medical Center, Rotterdam, Netherlands, [3]Biostatistics and Data Sciences, Boehringer Ingelheim, Ingelheim, Germany, [4]Clinical operations, Boehringer Ingelheim, Stockholm, Sweden, [5]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada, [6]Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, USA, [7]Therapeutic area cardiometabolism, Boehringer Ingelheim, Asker, Norway.

Background and aims: Type 2 diabetes (T2D) is associated with cognitive dysfunction and an increased risk of dementia, but underlying mechanisms are largely unclear. T2D is also associated with increased inflammation and oxidative stress, as well as endothelial dysfunction. We explored relationships between circulating biomarkers of inflammation, oxidation, and endothelial function, and cognitive functioning at baseline in patients with T2D at elevated cardiovascular (CV) risk participating in the CAROLINA trial.

Materials and methods: CAROLINA is a CV outcome trial assessing impact of linagliptin or glimepiride. Prior to first study drug treatment, a subset of patients without a history of dementia were recruited to participate in the cognition substudy. Circulating biomarkers of inflammation (CRP, IL-6, TNF-α), oxidation (8-iso-Prostaglandin $F_{2\alpha}$ [8-iso $PGF_{2\alpha}$]), and endothelial dysfunction (Asymmetric dimethylarginine [ADMA], Endothelin-1) were analysed at baseline. All patients underwent the Cambridge Neuropsychological Test Automated Battery, assessing psychomotor speed, mental flexibility, memory, working memory, and attention. The relation between biomarkers and cognitive functioning was evaluated with linear regression analysis adjusted for age, gender, and education.

Results: Mean ± SD age of the 37 patients (92% males, history of CHD/cerebrovascular disease 27.0%/21.6%) with T2D was 67 ± 9 years and years of formal education was 11.4 ± 4.4. Median (interquartile range) HbA1c was 6.9% (6.6%, 7.3%), diabetes duration was 8.3 (3.4, 11.1) years and MMSE was 29 (27, 30). 8-iso $PGF_{2\alpha}$ was significantly associated with worse performance on mental flexibility and attention (standardized regression coefficients −0.47 and −0.34, respectively) (table).

ADMA was associated with significant worse performance on psychomotor speed and attention (standardized regression coefficients −0.39 and −0.34, respectively) (table). No significant associations between markers of inflammation and cognitive functioning were observed. Additional adjustments for HbA1c, vascular risk factors, macrovascular disease, and use of anti-inflammatory drugs did not attenuate the results.

Conclusion: Elevated circulating biomarkers of oxidative stress and endothelial dysfunction are associated with reduced psychomotor speed, mental flexibility, and attention in T2D.

Relation between circulating biomarkers of inflammation, oxidation, and endothelial function and cognitive functioning

	Median (IQR)	Standardized regression coefficient (95% confidence interval)[1, 2]				
		Psychomotor Speed	Mental flexibility	Memory	Working memory	Attention
Inflammation						
CRP	1.0 mg/L (0.7, 3.1)	-0.05 (-0.40, 0.30) p=0.77	-0.05 (-0.42, 0.32) p=0.79	0.13 (-0.18, 0.44) p=0.39	-0.02 (-0.39, 0.34) p=0.90	-0.19 (-0.54, 0.15) p=0.26
IL-6	3.0 pg/mL (2.4, 5.4)	-0.06 (-0.42, 0.30) p=0.73	-0.02 (-0.41, 0.36) p=0.90	-0.14 (-0.46, 0.17) p=0.36	-0.03 (-0.41, 0.35) p=0.86	-0.23 (-0.58, 0.13) p=0.20
TNF-α	2.82 pg/mL (2.63, 3.18)	-0.13 (-0.52, 0.26) p=0.50	-0.05 (-0.5, 0.4) p=0.83	0.001 (-0.41, 0.41) p=0.99	0.11 (-0.30, 0.51) p=0.60	-0.11 (-0.52, 0.3) p=0.59
Oxidation						
8-iso $PGF_{2\alpha}$	23.4 pg/mL (18.2, 27)	-0.26 (-0.59, 0.06) p=0.11	**-0.47 (-0.78, -0.15) p=0.005**	-0.21 (-0.50, 0.08) p=0.15	-0.01 (-0.37, 0.34) p=0.95	**-0.34 (-0.66, -0.03) p=0.03**
Endothelial dysfunction						
ADMA	0.51 µmol/L (0.48, 0.54)	**-0.39 (-0.71, -0.07) p=0.02**	-0.15 (-0.52, 0.22) p=0.41	-0.09 (-0.40, 0.23) p=0.58	-0.23 (-0.59, 0.13) p=0.19	**-0.34 (-0.68, -0.006 p=0.046**
Endothelin-1	1.7 fmol/mL (1.07, 4.51)	0.10 (-0.25, 0.44) p=0.58	-0.17 (-0.54, 0.19) p=0.34	-0.13 (-0.43, 0.18) p=0.40	0.07 (-0.30, 0.43) p=0.71	0.04 (-0.31, 0.39) p=0.83

[1]Adjusted for age, gender and formal years of education, [2] Type 3-p value. Abbreviations: CRP, C-reactive protein; IL-6, interleukin 6; TNF-alpha, tumor necrosis factor-alpha; 8-iso $PGF_{2\alpha}$, 8-iso prostaglandin F2 alpha; ADMA, asymmetric dimethyl arginine

Clinical Trial Registration Number: NCT01243424
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **G. Biessels:** Grants; Boehringer Ingelheim.

1073

Cerebral blood flow and cognitive function in type 2 diabetes

L. Hunt[1], D. Selvarajah[1], K. Teh[1], S. Tesfaye[2], I.D. Wilkinson[1];
[1]Academic Radiology & Metabolism, University of Sheffield, Sheffield, [2]Diabetes and Endocrinology, Sheffield Teaching Hospitals, Sheffield, UK.

Background and aims: There is approximately a 2-fold increase in the risk of developing mild cognitive impairment (MCI) in patients who have Type 2 Diabetes Mellitus (T2DM). Brain perfusion Single Photon Emission Computed Tomography is often used in the clinical work-up of patients with suspected cognitive decline. However, in the context of T2DM, Cerebral Blood Flow (CBF) status in relation to MCI has not been fully investigated. This study sought to assess regional CBF in matched T2DM patients with and without MCI using a non-invasive Magnetic Resonance Imaging (MRI) technique.

Materials and methods: Seventy-four age and gender matched subjects [28, T2DM+normal cognition (T2DM); 17, T2DM+MCI (T2DM/MCI) and 29, healthy volunteers (HV)] were recruited. All subjects underwent clinical and questionnaire (Addenbrooke's Cognitive Assessment [ACE-R]) assessments along with Arterial Spin Labelling (ASL) Magnetic Resonance Imaging (MRI) to assess parenchymal perfusion within the brain. Imaging was performed at 3T. The ASL data was modelled to yield quantitative arterial CBF maps in neuroanatomical regions involved with various cognitive and memory functions.

Results: Mean T2DM/MCI ACE-R score (mean ± SD; 83 ± 4) was significantly lower in the MCI group compared to the other groups (HV = 96 ± 2, T2DM = 94 ± 3; ANOVA $p < 0.001$). There was a significantly lower mean CBF in T2DM/MCI compared to T2DM and HV in the medial temporal lobes (CBF 76.8 ml/100 g/min, ANOVA $p < 0.05$), insula (CBF 67.5 ml/100 g/min ANOVA $p < 0.005$), and frontal lobes (CBF 71.8 ml/100 g/min, ANOVA $p < 0.005$). Significant correlations were observed between ACE-R score and regional CBF measurements in the medial temporal lobes, ($p < 0.05$, $r = 0.25$) thalamus ($p < 0.05$, $r = 0.23$) and the insula ($p < 0.05$, $r = 0.29$).

Conclusion: This study demonstrates significantly lower CBF in T2DM MCI subjects in neuroanatomical regions associated with cognitive processing and memory functions. This may be essential in helping our understanding of the pathological mechanisms that occur behind the increased risk of developing cognitive impairment associated with T2DM.
Supported by: G-WF & Wellcome Trust
Disclosure: **L. Hunt:** None.

1074

The association between cognitive functioning and cerebral perfusion in patients with type 2 diabetes

B. Mankovsky[1,2], N. Zherdova[1,2], S. Makeev[3], I. Dalhuisen[4,5], E. van den Berg[4], J. de Bresser[5,6], G.-J. Biessels[4];
[1]Department of Diabetology, National Medical Academy for Postgraduate Education, Kyiv, Ukraine, [2]Center for Innovative Medical Technologies of the National Academy of Sciences of Ukraine, Kiev, Ukraine, [3]The State Institution Romodanov Neurosurgery Institute National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine, [4]Department of Neurology, University Medical Center Utrecht, Utrecht, Netherlands, [5]Department of Radiology, University Medical Center Utrecht, Utrecht, Netherlands, [6]Department of Radiology, Leiden University Medical Center, Leiden, Netherlands.

Background and aims: Type 2 diabetes mellitus (T2DM) is associated with cognitive impairment, an increased risk of dementia and occurrence of cerebral small vessel disease. Impaired cerebral perfusion is one of the features of cerebral small vessel disease. We therefore investigated the association between cognitive functioning and cerebral perfusion in patients with T2DM.

Materials and methods: We examined 95 patients with T2DM (32 males, mean age 62.2 ± 5.5 years, diabetes duration 9.7 ± 6.6 years). Cognitive functioning was assessed using a standard neuropsychological test battery covering the domains memory, executive functioning, and processing speed. Cerebral perfusion was assessed using SPECT scans and quantified by comparison to a database of healthy individuals (expressed as a standard deviation). Linear regression analyses adjusted for age and gender were performed to study the association of cognitive functioning and global and regional (frontal, occipital, parietal, temporal, cerebellum, caudate nucleus, putamen, and thalamus; both left and right) cerebral perfusion.

Results: There were no statistically significant associations between cognitive functioning and global cerebral perfusion (memory B (95%-CI): −0.106 (−0.349↔0.136), $p = 0.386$; executive functioning: 0.001 (−0.216↔0.218), $p = 0.996$; processing speed: −0.189 (−0.405↔−0.028), $p = 0.087$). A worse memory domain score was associated with reduced perfusion in the thalamus (left: −0.278 (−0.242↔−0.053), $p = 0.003$; right: −0.283 (−0.237↔−0.054), $p = 0.002$). A worse processing speed domain score was associated with reduced perfusion in the left frontal lobe (−0.289 (−0.388↔−0.088), $p = 0.002$).

Conclusion: We found a strong association between reduced perfusion in the thalamus and memory impairment in patients with T2DM. This reduced perfusion might be one of the underlying functional correlates of cognitive impairment in patients with T2DM.
Supported by: EFSD Program New Horizons
Disclosure: **B. Mankovsky:** None.

1075

Influence of glycaemic variability on the results of neuropsychological testing in patients with type 1 diabetes

M. Rotkank, I. Samoilova, M. Matveeva, N. Zhukova, I. Tolmachev;
Siberian State Medical University, Tomsk, Russian Federation.

Background and aims: One of the complications of type 1 diabetes mellitus (DM type 1) is diabetic encephalopathy, the main symptoms of which are cognitive impairment. The variability of glycemia in DM type 1 plays an important role in the pathogenesis of vascular complications of DM. The aim was to determine the relationship between the results of performing neuropsychological testing and the measures of glycemic variability in patients with DM type 1.
Materials and methods: 58 patients with DM type 1 at the age of 29 (25–32) years and 20 people without DM, comparable by sex and age, were examined. All participants underwent neuropsychological testing with the Montreal Cognitive Evaluation Scale (MoCa-test), the evaluation of carbohydrate metabolism and the calculation of the measures of glycemic variability with the EasyGV calculator: standard deviation (SD), continuous overlapping net glycemic action (CONGA), lability index (LI), J-index, low blood glucose index (LBGI), high blood glucose index (HBGI), mean of daily differences (MODD), mean amplitude of glycemic excursions (MAGE), average daily risk range (ADRR). The statistical processing of the results was carried out in the IBM SPSS Statistics 20.0.0 program (significant differences were considered when $p < 0.05$).
Results: Assessment of the state of carbohydrate metabolism showed that the average level of fasting glycemia in patients with DM type 1 was 8.6(7.3–9.6)mmol/L, the average level of HbA1c was 8.4(7.5–8.9)%. According to the results of the MoCa-test, a statistically significant decrease in the score was observed in patients with DM type 1 in comparison with the control group ($p < 0.001$). Analysis of individual tasks of the MoCa-test revealed significant decrease in scores for tasks attention ($p < 0.001$), clock-drawing ($p = 0.002$) and delayed recall ($p < 0.001$). The measures of glycemic variability were calculated: SD 6.25 (3.1–7.7) mmol/L, CONGA 4.65 (3.3–7.3) mmol/L, LI 4.25 (3.3–5.1) (mmol/L)^2/h, J-index 54,15 (22.4–73.6), LBGI 3.85(2.6–5.2), HBGI 7.75 (5.6–12.5), MODD, 3.85 (2.9–5.6) mmol/L, MAGE 7.6 (4.6–8.9) mmol/L, ADRR 45.95 (28.9–57.8). Evaluation of the relationship between the results of neuropsychological testing and the measures of glycemic variability revealed a negative correlation with the measures LI ($p = 0.008$), MODD ($p = 0.005$) and ADRR ($p = 0.032$).
Conclusion: The present study showed an inverse relationship between the results of neuropsychological testing and glycemic variability. Thus, a decrease of the variability of glycemia can be used in cognitive rehabilitation in patients with DM type 1.
Disclosure: **M. Rotkank:** None.

1076

Novel therapeutic potential of RAAS blockers in diabetes comorbid depression

L. Lenart[1], D.B. Balogh[1,2], J. Hodrea[1], A. Barczi[1,2], A. Molnar[1,2], A. Hosszu[3], A. Vannay[4], L. Wagner[3], A.J. Szabo[4,2], A. Denes[5], A. Fekete[1,2]; [1]MTA-SE „Lendület" Diabetes Research Group, Budapest, [2]1st Department of Pediatrics, Semmelweis University, Budapest, [3]Department of Transplantation and Surgery, Semmelweis University, Budapest, [4]MTA-SE Pediatrics and Nephrology Research Group, Budapest, [5]MTA Institute of Experimental Medicine, Budapest, Hungary.

Background and aims: Comorbid depression is commonly occurring in diabetes mellitus (DM), which worsens quality of life and increases mortality. It has recently indicated that all components of renin-angiotensin-aldosterone system (RAAS) are produced also within the central nervous system and overactivated in DM. RAAS can modulate the level of brain-derived neurotrophic factor (BDNF), which is important in the neurobiology of depression and its antidepressant action. However, the interaction between the BDNF system and RAAS in DM comorbid depression has not yet been investigated. Therefore, our aim was to investigate the effect of RAAS blockers in the development of DM comorbid depression.
Materials and methods: After 5 weeks of streptozotocin-induced DM, adult, male Wistar rats ($n = 8$/group) were treated *po.* for 2 weeks with non-pressure dose of enalapril (40 mg/bwkg/day), losartan (20 mg/bwkg/day), spironolactone (50 mg/bwkg/day) or eplerenone (50 mg/bwkg/day). Untreated diabetic and healthy rats served as controls. Blood pressurewas measured and depressive-like behaviour was evaluated. Localization of BDNF was determined. The protein and mRNA levels of pro and mature BDNF, their receptors (TrkB and p75Ntr) and the potential downstream signaling molecules (p75Ntr signaling: pJNK, *Bax* and TrkB signaling: pERK, pCREB, *Bcl2*) were measured in the hippocampus.
Results: Depressive-like behaviour was observed in DM, which was improved by all RAAS blockers. BDNF is mainly produced by astroglia in hippocampus. Pro and mature BDNF, TrkB (receptor of mature BDNF), pERK, pCREB and anti-apoptotic *Bcl2* levels were decreased in DM, but were elevated by RAAS blockers. Neither DM nor RAAS blockers did not influenced the levels of p75NTR (receptor of pro BDNF), pJNK and pro-apoptotic *Bax*. Neither diabetes nor RAAS inhibitors influenced the blood pressure indicating that all these effects of RAAS blockers were independent of their antihypertensive effect.
Conclusion: Our results support that RAAS inhibition mitigates DM-associated depression. Here we identified that BDNF - TrkB - CREB signaling pathway is crucial in the development of DM-associated depression or the antidepressant effect of RAAS blockers. These findings exploring a new therapeutic horizon for RAAS inhibitors in treatment of depression in DM and signaling could be a basis of future drug development treating DM-induced complications.
Supported by: LP008/2017, OTKA-K112629-K116928-FK124491-NN-11460,VKE-2017-00006,EEMOFAKT
Disclosure: **L. Lenart:** None.

PS 101 Understanding vascular complications

1077

Glycation gap variation in human diabetes is associated with fructosamine-3-kinaseSNP rs3848403 but does not explain linked difference in enzyme activity

S.J. Dunmore[1], F. Naseem[1], A. Watkins[2], J.E. Brown[3], A.U. Nayak[4], A. Nevill[1], B.M. Singh[1,5];
[1]Diabetes Research Group, University of Wolverhampton, Wolverhampton, [2]Division of Child Health, Obstetrics and Gynaecology, University of Nottingham, Nottingham, [3]Aston Research Centre for Healthy Ageing, Aston University, Birmingham, [4]Department of Endocrinology & Diabetes, University Hospitals of North Midlands NHS Trust, Stoke on Trent, [5]Wolverhampton Diabetes Centre, New Cross Hospital, Wolverhampton, UK.

Background and aims: The glycation gap (GGap) is a consistent discrepancy between HbA1c and prevailing glucose concentrations (estimated HbA1c based on fructosamine (fHbA1c)) which we have shown to be associated with risk of diabetic complications and recently demonstrated to be potentially caused by erythrocyte activity of the deglycating enzyme fructosamine-3-kinase (FN3K). We hypothesised that the 3-fold difference in FN3K activity between dichotomised highly positive GGap (higher than expected HbA1c compared with fHbA1c, exhibiting a higher incidence of complications) and negative GGap (HbA1c<fHbA1c) might be explained by differences in genotype of known FN3K SNPs.
Materials and methods: We evaluated FN3K SNPs rs1056534, rs1046896, and rs3848403 using real-time PCR of genomic DNA extracted from whole blood of patients with diabetes dichotomised for GGap status ($n = 130$, 73 -ve, 57 +ve GGap, matched demographics). Ethical approval from UK National Research Ethics Service Committee.
Results: The FN3K SNPs rs1056534 and rs1046896 did not differ between the -ve GGap and +ve GGap groups. The SNP rs3848403 had 3 allele combinations C/T (85%), T/T (12%) and C/C (3%), the distribution of which was significantly between GGap groups ($X^2 = 6.901$, $p = 0.032$). These were reallocated into heterozygous (C/T) and homozygous (T/T or C/C) and this distribution again differed significantly between groups ($X^2 = 5.458$, 6.901, $p = 0.019$). In binary logistic regression ($X^2 = 54.469$, $p < 0.001$) GGap status was associated with BMI ($p < 0.001$) and the dichotomised allelic status of C/T vs T/T or C/C ($p = 0.044$) but not age, type of diabetes or the rs3848403 SNP triple status independently of the dichotomised status. The Odds Ratio (95% CI) of -ve GGap being associated with allelic heterogeneity or +ve GGap with homogeneity was 3.7 (1.0 to 13.1).
Conclusion: We have shown for the first time that a FN3K SNP, rs3848403, is associated with glycation gap status, a SNP previously associated with variations in HbA1c only. There is a 35–40% misalignment with GGap status in groups that really divergent for this characteristic. We did not find that the FN3K activity /concentration appeared to be dictated by the SNP although there is a very strong link between GGap and FN3K. We are currently undertaking transcriptomic studies of these dichotomised GGap samples in order to further understand possible contributors to FN3K activity variation such as splice variation.
Supported by: Rotha Abraham Trust
Disclosure: **S.J. Dunmore:** None.

1078

Impact of hypoglycaemia on the platelet activity and fibrinolysis in patients with type 1 diabetes

I.R. Jarek-Martynowa[1], K. Sarkisova[2], E. Koksharova[1], E. Mishina[1], M. Shestakova[1], L. Nikankina[1], L. Chirkova[1], M. Shamkhalova[1];
[1]Endocrinology Research Centre, Moscow, [2]I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.

Background and aims: Hypoglycemia can be a risk factor for adverse cardiovascular and cerebrovascular events. However, changes in platelets and coagulation hemostasis during hypoglycemia have not been extensively studied. During hypoglycemia, a wide spectrum of physiologic responses are activated that could have potential vascular biological effects. To date, the role played by catecholamines, the sympathetic nervous system, and neuroendocrine hormones on activating adhesion molecules and influencing fibrinolytic balance is incompletely understood. The aim of this study was to assess the impact of insulin-induced hypoglycemia on the platelet activity, and fibrinolysis in patients with type 1 diabetes.
Materials and methods: We studied 15 patients with type 1 diabetes (9 male and 6 female, age 24.4 ± 5.6, A1C 9.07 ± 2.3%) without microvascular complications during hyperinsulinemic (1 mU/kg/min) hypoglycemic clamp protocol. Induced platelet aggregation in whole blood using thrombin receptor activating peptide 6 (tRaP-6), collagen, arachidonic acid, adenosine-diphosphate, ristomycin was measured during hypoglycemia (plasma glucose(pg) 2.3 ± 0.1 mmol/l), euglycaemia (pg 4.4 ± 0.4 mmol/l), hyperglycemia (pg ≥12 mmol/l) and recovery phase by multiple electrode platelet aggregometry (Multiplate). Plasminogen activator inhibitor (PAI-1), tissue plasminogen activator (tPA) was determined by ELISA. Statistical analysis was performed with SPSS 22.0 for Windows, $p < 0.05$.
Results: Platelets aggregation induced collagen ($p = 0.001$), thrombin ($p = 0.003$), adenosine- diphosphate ($p = 0.016$), arachidonic acid ($p = 0.05$) was significantly increased during 20-min of hypoglycemia compared with euglycemia. Plasma PAI-1 activity were significantly different during hypoglycemia as compared with euglycemia ($p = 0.001$) and as compared with recovery phase ($p = 0.018$). Plasma concentrations of tPA did not alter during either hypoglycemic clamp in individuals with type 1 diabetes.
Conclusion: The present study confirmed that platelet activation is promoted by hypoglycemia and that hypoglycemia decreases systemic fibrinolytic balance by increasing PAI-1 activity while maintaining tPA values. Thus, at least two separate mechanisms for increasing thrombosis are activated by hypoglycemia in individuals with type 1 diabetes.
Disclosure: **I.R. Jarek-Martynowa:** None.

1079

Elastic and adhesive properties of erythrocyte and platelet membranes in patients with type 2 diabetes

V. Shyshko[1], G. Melnikova[2], T. Mokhort[3], E. Konstantinova[2], T. Tolstaya[2], A. Petrovskaya[2];
[1]City Endocrinology Dispensary, Minsk, [2]A.V. Luikov Heat and Mass Transfer Institute of the National Academy of Science of Belarus, Minsk, [3]Belarusian State Medical University, Minsk, Belarus.

Background and aims: In this study we propose the use of the atomic force microscopy (AFM) method to analyze the changes in the elastic properties of erythrocyte and platelet membranes in patients with type 2 diabetes mellitus (T2D) at the nanolevel. Aim**:** To evaluate the elastic and adhesive properties of erythrocyte and platelet membranes in patients with T2D.
Materials and methods: Patients with T2D were included. The mean age was 55 (50, 59) years, mean HbA1c level was (9.1 (8.30, 10.4)%.). Men and women were age-matched, the duration of T2D was 5 to 10 years. Blood was taken at the time of hospitalization in the department of endocrinology. Criteria for exclusion: presence of acute vascular events (MI, stroke) in anamnesis, reconstructive interventions on large vessels. Erythrocytes for studies were isolated from stabilized venous blood of patients with ethylenediamine triacetate potassium K3EDTA (Aldrich), fixed with 0.5% glutaraldehyde solution and applied to mica substrates at a temperature of 20 ± 5°C. Venous blood was stabilized with 3.8% r-rum sodium citrate to isolate platelets.The elastic modulus (E, MPa) and the adhesion strength (F, nN) were estimated by the AFM method of the Johnson-Kendell-Roberts model using an atomic force microscope using standard silicon probes NSC 11, rigidity 3 N/m ("MikroMacsh"), radius

the curvature is 50 nm. The strength of adhesion was calculated based on the measured values of the tip detachment from the sample surface.
Results: The results of the study of erythrocytes have shown that two ranges of values can be distinguished-less than and above 100 MPa. For the strength of adhesion, two ranges of values were identified - less than and more than 15 nN. For erythrocyte membranes, E values less than 100 MPa and F above 15 nN prevail, while the mean values of these parameters for men and women do not differ within the experimental error. Similar to the characteristics of red blood cells for platelet membranes, there are no differences in E for men and women, the tendency of the predominance of higher adhesion strengths persists. Platelets F in women (16.8 (12.6, 26.4) is significantly higher than in men (12.0 (8.6; 12.8) ($p = 0.022$).A negative correlation was established between platelet F (15.9 (10.4;24.3) and the age of the patients ($R = -0.30$, $p < 0.05$). It has been established that an increase in erythrocytes F correlates with platelet F ($R = 0.64$, $p < 0.05$) in patients with T2D, in particular in women ($R = 0.67$, $p < 0.05$), but not in men. F erythrocytes at a level of HbA1c of less than 6.5% is significantly higher (24.35 (19.9; 25.5) than in HbA1c more than 6.5% (15.40 (11.8, 20.8) ($p < 0.05$) (more than 60%).
Conclusion: 1The method of atomic force microscopy makes it possible to study the properties of erythrocyte and platelet membranes in patients with T2D that will later allow the use of results in the development of drugs for the treatment of microangiopathies. In patients with T2D, E values of erythrocytes prevail values less than 100 MPa and F above 15 nN. An increase in age in patients with T2D is accompanied by a decrease in F erythrocytes. As the level of HbA1 increases, there is a decrease in F erythrocytes.
Disclosure: **V. Shyshko:** None.

1080

Diabetes augments atherosclerotic inflammation in diabetic LDLr-/- mice

A. Midtgaard-Thomsen[1,2], L. Thoren[1], T.K. Hansen[3], G. Rakipovski[1], C.K. Mogensen[1];
[1]Global Research, Måløv, [2]Medicinsk Endokrinologisk Afdeling, Aarhus, [3]Steno Diabetes Center Aarhus, Aarhus, Denmark.

Background and aims: Mice are the most frequently used preclinical species for atherosclerotic studies. However, mice never develop unstable plaque; hence the translation of these mouse models for human atherosclerotic lesion and increased diabetic cardiovascular risk is unclear. The aim of this study was to characterize the cellular and molecular composition of the atherosclerotic aorta and atherosclerotic plaque in a diabetic mouse model.
Materials and methods: LDLr-/- mice (JAX, USA, Stock 2207; 6–8 weeks of age) were divided into three groups ($n = 23$/group) having two groups on a western type diet (WD) and one group on standard low caloric chow diet. After 8 weeks of diet intervention one of the WD fed groups were rendered diabetic by low dose streptozotocin injections (I.P., 60 mg/kg × 3). Mice were kept on their respective diets for another 9 weeks before termination. HbA1c, body weight and blood glucose were monitored throughout the study period. At termination, aortas were dissected free and images were taken for *en face* analysis. Immune cell composition of murine aortas was analyzed by flow cytometry and gene expression in plaque and plaque-free areas of the atherosclerotic aortas was analyzed.
Results: It was demonstrated that diabetes causes a significant increase ($p < 0.05$) in percentage of monocytes and neutrophils ($VD^-Cd45^+Cd11b^+Ly6c^+$ as % of $VD^-CD45^+CD11b^+$) in murine aortas even though diabetes caused no difference to the plaque area measured by *en face*. Furthermore, diabetes suppresses M2-like macrophage appearance (CD206 MFI on $VD^-CD45^+Cd11b+$). Gene expression analyses showed diabetes increases IL-6 expression in plaque-free area of aorta ($p < 0.05$) whereas IL-6 expression in plaque areas was not altered by diabetes.
Conclusion: Recruitment and infiltration of monocytes and neutrophils and increased levels of IL-6 can drive the plaque progression in mice, which has also been correlated to increased risk of cardiovascular events in humans. This may potentially play an important role for the increased cardiovascular risk observed in humans with diabetes. Looking at cellular and molecular composition of murine aortas for plaque areas and plaque free-areas can aid in the understanding of plaque development in mice and hopefully be able to better understand the development in humans.
Supported by: IF DK and NN
Disclosure: **A. Midtgaard-Thomsen:** Employment/Consultancy; Novo Nordisk A/S. Grants; Innovation Fund Denmark.

1081

Serum calcification propensity is associated with all-cause mortality in type 1 diabetes

D.J. Mulder[1], P.R. van Dijk[2,1], H. Hop[1], A. Pasch[3], F. Waanders[4], H. van Goor[5], H.J.G. Bilo[1,2];
[1]Internal Medicine, University of Groningen, Groningen, Netherlands, [2]Diabetes Centre, Isala, Zwolle, Netherlands, [3]Department of Biomedical Research, University of Bern, Bern, Switzerland, [4]Internal Medicine, Isala, Zwolle, Netherlands, [5]Department of Pathology and Medical Biology, Division of Pathology, University of Groningen, Groningen, Netherlands.

Background and aims: Type 1 diabetes mellitus (T1DM) is accompanied by increased incidence of atherosclerosis and mortality. A novel in vitro blood test provides an overall measure of calcification propensity by monitoring the maturation time (T_{50}) of calciprotein particles in serum. Accelerated T_{50} indicates a diminished ability of serum to resist calcification. Moreover, T_{50} levels strongly and independently predict all-cause mortality in various patient populations. Aim of our study was investigate the longitudinal association of T_{50} with micro- and macrovascular complications and all-cause mortality in T1DM.
Materials and methods: As part of a prospective cohort study, 283 T1DM patients were examined annually. Clinical and biochemical data from measures from 2002 until last available follow-up were analysed.
Results: T_{50} levels were measured among 216 patients (57% male) with a mean age of 45 (11) years, diabetes duration of 23 [16, 30] years, BMI of 26 (4) kg/m^2, systolic blood pressure of 130 (18) mmHg and HbA1c of 60 [51, 68] mmol/mol. Mean T_{50} level among all patients was 339 (59) minutes. There were no significant correlations between T_{50} levels and baseline characteristics. Patients in the upper T_{50} tertile (369 to 466 seconds) had a significant longer diabetes duration as compared to patients in the middle (317 to 368 seconds) and lowest (129 to 316 seconds): 25 [19, 33] vs. 21 [16, 30] and 20 [14, 29] years. During the 15 [6, 16] year follow-up period, there were 93 new micro- and 44 macrovascular events and 25 patients died. There were no differences between T_{50} tertiles for both micro- and macrovascular complications. Patients in the lowest tertile had the best overall survival (15.9 years (95% 15.5, 16.4)) as compared to both the middle (14.6 years (95%CI 13.8, 15.5), $p = 0.003$) and the highest tertile (15.0 (95%CI 14.2, 15.8), $p = 0.004$).
Conclusion: This is the first study to investigate the calcification propensity T_{50} score in T1DM. Higher T_{50} values were associated with all-cause mortality in this T1DM population. Although these results need confirmation in larger populations, it may lead to novel diagnostic or therapeutic approaches.
Supported by: Isala Wetenschaps en Innovatiefonds
Disclosure: **D.J. Mulder:** None.

1082

Glucagon-like peptide-1 receptor agonist, liraglutide, alters immune populations during regression of atherosclerosis

R. Bruen[1], S. Curley[2], S. Kajani[2], M.E. O'Reilly[2], F.C. McGillicuddy[2], O. Belton[1];
[1]Diabetes Complications Research Centre, School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin,

Dublin, [2]Diabetes Complications Research Centre, School of Medicine, Conway Institute, University College Dublin, Dublin, Ireland.

Background and aims: Atherosclerosis development is governed by biologically active macrophages and dendritic cells (DCs). We recently showed that the glucagon-like peptide-1 receptor (GLP-1R) agonist, liraglutide (Lir), alters these immune cell subsets. We hypothesised that Lir can alter immune populations halting progression and inducing regression of pre-established atherosclerosis in the apolipoprotein E deficient ($ApoE^{-/-}$) atherosclerotic mouse model.
Materials and methods: Wild-type C57BL/6 bone marrow-derived macrophages (BMDMs) were treated with Lir to examine phenotypic shifts. Alongside this, a high-fat (60%) high-cholesterol (1%) diet (HFHCD) was fed to $ApoE^{-/-}$ mice for 8–12 weeks to induce atherosclerotic disease. Mice received 300 μg/kg Lir daily during weeks 3–8 to investigate disease progression and weeks 8–12 to examine regression of established disease. *En face* analysis was employed to quantify atherosclerotic lesions in aortae from $ApoE^{-/-}$ mice. Human atherosclerotic plaques and serum from patients pre- and post-Lir treatment were also investigated. Gene expression analysis, ELISA and flow cytometry were used to interrogate inflammatory mediators.
Results: Lir halted early disease progression in $ApoE^{-/-}$ mice (HFHCD 2.03 ± 0.18% vs HFHCD+Lir 1.36 ± 0.28%, $*p < 0.05$). This result mirrored significant decreases in inflammatory M1 (TNF-α, HFHCD 2.50 ± 0.38 vs HFHCD+Lir 0.15 ± 0.01, $**p < 0.01$) and increases in anti-inflammatory M2 gene expression (IL-10 HFHCD 0.84 ± 0.07 vs HFHCD+Lir 2.46 ± 0.32, $**p < 0.01$) in BMDMs. Pro-inflammatory monocytes were also markedly reduced (HFHCD 59.27 ± 2.97 vs HFHCD+Lir 16.79 ± 4.47, $**p < 0.01$). Pro-resolving M2 BMDMs were significantly elevated (HFHCD 37.45 ± 2.49% vs HFHCD+Lir 73.36 ± 3.84%, $*p < 0.05$). Importantly, Lir induced regression of pre-established atherosclerotic lesions (HFHCD 6.81 ± 0.33% vs HFHCD+Lir 4.02 ± 0.38%, $***p < 0.001$), decreased M1 (HFHCD 18.07 ± 3.32% vs HFHCD+Lir 5.67 ± 1.71%, $**p < 0.01$) and increased M2 populations (HFHCD 71.32 ± 13.04% vs HFHCD+Lir 94.24 ± 1.71%, $**p < 0.01$). DCs from lymph nodes of Lir-treated mice were also elevated (HFHCD $5.63e^5 \pm 1.05e^5$ vs HFHCD+Lir $1.37e^7 \pm 1.14e^7$ ns, $p = 0.0952$), during regression. Serum samples from patients pre- and post-Lir treatment resulted in significant reductions of pro-atherogenic sCD163 (Pre-Lir 3.18 ± 0.30 vs Post-Lir 2.69 ± 0.17, $***p < 0.001$) and atherosclerotic plaques showed reduced TNF-α secretion (PBS 135.10 ± 67.48 vs Lir 61.32 ± 37.55, ns $p = 0.0927$).
Conclusion: Lir is an atheroprotective agent both altering early disease progression, late stage disease regression and human atherosclerotic disease via modulating immune cells towards pro-resolving mediators.
Supported by: EFSD Clinical Diabetes Research Programme
Disclosure: **R. Bruen:** None.

1083

The macrophage activation marker sCD163 during prolonged fasting

N. Rittig[1], A. Bak[1], N. Jessen[2], J. Møller[3], H. Grønbæk[4];
[1]Department of Internal Medicine and Endocrinology (MEA) and Medical Research Laboratory, Aarhus University Hospital, Aarhus C, [2]Research Laboratory for Biochemical Pathology, Institute for Clinical Medicine,, Aarhus C, [3]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus C, [4]Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus C, Denmark.

Background and aims: Prolonged fasting causes insulin resistance and accelerated lipolysis. These metabolic features are characteristic for conditions such as obesity, diabetes mellitus type 2 (DM2) and non-alcoholic fatty liver disease (NAFLD). Plasma concentration of the macrophage activation marker sCD163 is known to be elevated in subjects suffering from obesity, DM2- and NAFLD compared with healthy lean subjects. We have earlier shown how LPS exposure caused elevated sCD163 concentrations and was positive correlated with insulin resistance as well as accelerated lipolysis in both healthy and diabetic patients. For these reasons we hypothesized that sCD163 concentrations would increase during prolonged fasting.
Materials and methods: We investigated nine healthy lean (mean BMI = 21 kg/m^2) and nine obese (mean BMI = 36 kg/m^2) male subjects in a randomized crossover trial with two interventions: I) following an overnight fast (control) and II) following a 72 h fast period. Both interventions were followed by similar metabolic measurements including ^{3}H-glucose and ^{3}H-palmitate tracer infusion to investigate glucose and lipid metabolism, indirect calorimetry to estimate lipid oxidation rate, and a hyperinsulinemic euglycimic clamp to quantify insulin sensitivity. Two-way repeated measure ANOVA (RM-ANOVA) and a linear regression analysis were used in the statistical analyses.
Results: We found a significant 17% reduction in sCD163 concentrations when comparing 72 h fasting with control conditions (RM-ANOVA main effect, $p = 0.002$) with no difference between lean and obese subjects (RM-ANOVA interaction, $p = 0.36$). We did not find any linear correlations between the reduction in sCD163 concentration and the changes in glucose infusion rate ($p = 0.90$), endogenous glucose production ($p = 0.91$), palmitate rate of appearance ($p = 0.15$), free fatty acid concentrations (0.56) and lipid oxidation rate ($p = 0.87$).
Conclusion: To our surprise we found a reduction in sCD163 concentrations during prolonged fasting but no association with changes in insulin resistance. These results are novel and may suggest involvement of distinct mechanisms in the physiologic and pathophysiologic development of insulin resistance and accelerated lipolysis.
Clinical Trial Registration Number: M-2010-0182
Disclosure: **N. Rittig:** None.

1084

Anti-atherosclerotic activity of trigonal GLP-1R/GIPR/GCGR agonists in ApoE KO mice

T. Hübschle[1], S. Meister[1], D. Hein[1], S. Schmidt[1], M. Medem[1], D. Albrecht[1], A. Schröder[1], D. Ströbel[2], B. Alka[2], T. Tran[2], S. Pfeiffer-Marek[2], M. Bossart[2];
[1]Sanofi-Aventis Deutschland GmbH, R&D Diabetes TA, Frankfurt am Main, [2]Sanofi-Aventis Deutschland GmbH, R&D, Frankfurt am Main, Germany.

Background and aims: Evaluate the potential anti-atherosclerotic effect of trigonal GLP-1R/GIPR/GCGR agonists in the ApoE knockout (KO) mouse model, an animal model that is characterized by atherosclerotic plaque development with a morphology resembling human atherosclerosis.
Materials and methods: Male ApoE KO mice were implanted with osmotic minipumps (ALZET™) filled with either vehicle (sterile acetate buffer, pH 4.5), the trigonal GLP-1R/GIPR/GCGR agonists SAR1 or SAR2 (150 μg/kg/day) or with liraglutide (600 μg/kg/day) for four months. Vehicle treated mice of the background strain (C57Bl/6J) were used as healthy controls. Body weight and food intake was monitored throughout the study on a weekly basis.
Results: In contrast to wild type C57Bl/6J mice, ApoE KO mice receiving vehicle developed early atherosclerotic lesions on the total inner surface of the aorta as measured with oil red O staining in absolute and relative plaque area using quantitative and automated image analysis. Treatment with the trigonal GLP-1R/GIPR/GCGR agonists SAR1 or SAR2 agonists at the dose of 150 μg/kg/day led to a significant reduction of atherosclerotic plaques by 63% and 73%, respectively, relative to vehicle treated controls. A 4-fold higher dose of the GLP-1R monoagonist liraglutide reduced aortic plaque burden in ApoE KO mice by 37%. However, the efficacy seen with the trigonal GLP-1R/GIPR/GCGR agonists was superior to the benefit induced by liraglutide. Compared to vehicle treatment, both trigonal GLP-1R/GIPR/GCGR agonists robustly

lowered the blood lipids total cholesterol, LDL-cholesterol and triglycerides. Further, their efficacy on blood lipids was superior to the beneficial effect elicited by the GLP-1 analog liraglutide.
Conclusion: Trigonal GLP-1R/GIPR/GCGR agonists SAR1 and SAR2 exhibited strong anti-atherosclerotic activity in ApoE KO mice. The benefit was superior to the effects elicited by the GLP-1R monoagonist liraglutide.
Disclosure: **T. Hübschle:** Employment/Consultancy; Thomas Hübschle and the other co-authors are employees of Sanofi-Aventis Deutschland GmbH.

PS 102 The pump and the barrier

1085

Carbohydrate metabolism disorders in heart failure patients: a complex relationship

B. Kurmanbekova, G. Osmankulova;
Chronic heart failure, NCCIM, Bishkek, Kyrgyzstan.

Background and aims: Simultaneous flow of diabetes mellitus (DM) and chronic heart failure (CHF) determine the adverse prognosis of patients, creating difficulties in their management for both cardiologists and endocrinologists. Identifying the latent flow of carbohydrate metabolism disorders (CMD) in CHF patients in a timely manner, as well as influence on the key pathogenetic links in their development, we can achieve a significant reduction in the social and economic burden. Study purpose: to evaluate the prevalence of latent CMD in association with neurohormonal profile indicators in patients with CHF
Materials and methods: 174 hospitalized patients with CHF II-IV (NYHA) ischemic etiology without CMD in anamnesis, receiving basic for CHF and CHD therapy. Such parameters as: height, weight, BMI, systolic and diastolic blood pressure, FINDRISC scale points, fasting and postprandial glucose levels, HbA1C, lipid profile, eGFR, daily proteinuria, Nt-proBNP, aldosterone and insulin concentrations with assessment of HOMA-IR and standart oral glucose tolerance test were conducted.
Results: Among 174 patients with CHF II-IV (NYHA) using OGTT, in 50.6% patients was revealed latent CMD: 59 (33.9%) patients had impaired glucose tolerance (IGT), in 29 (16.7%) - DM was newly diagnosed, 86 (49.4%) patients were without CMD. The largest number of FINDRISC points was observed in group with newly diagnosed DM compared with patients with IGT and without CMD. Insulin concentrations were increased in each study groups with CHF and CHD, but was significantly higher in patients with CMD compared with patients without CMD. IR was identified in all patients with CHF and CHD, what is more, its level increased with the growth of CMD explicity and was highest in DM group compared with IGT and without CMD groups. Elevated levels of Nt-proBNP had a linear relationship with CHF severity, but did not differ in groups. Aldosterone levels increased in all patients with CHF, regardless of the CMD presence, but was significantly higher in DM group. Correlation analysis determined strong interrelation between IR and: BMI, FINDRISC points, fasting and postprandial glucose levels, HbA1C, insulin concentrations, but not with ejection fraction, NYHA class and CHF duration.
Conclusion: The presence and progression of IR in association with neurohormonal profile in patients with CHF ischemic etiology emphasizes the importance of early detection of latent CMD. It will improve quality of life, prognosis and their survival, and will also reduce the financial costs of health care in all countries
Clinical Trial Registration Number: 0007095
Disclosure: **B. Kurmanbekova:** None.

1086

Chronic hyperglycaemia in GLUT4-overexpressing H9C2-cells reveals diabetic heart failure mechanisms

B. Stratmann, Y. Mattern, D. Tschoepe;
Diabeteszentrum, Herz- und Diabeteszentrum NRW, Ruhr Universität Bochum, Bad Oeynhausen, Germany.

Background and aims: Chronic hyperglycaemia is an established cardiovascular risk factor. The mode of action of glucose or glucotoxic metabolites on the cellular entity and metabolic system remains elusive. Glucotoxic induced enhanced GLUT4 surface presentation may account as a possible mechanism to the development of diabetic heart failure. We established a GLUT4 overexpressing H9C2-cell line to analyse effects of hyperglycemia on GLUT4 trafficking and cellular metabolism.

Materials and methods: A stably GLUT4-*c-myc* overexpressing H9C2-cell line was generated for analyzing GLUT4 presentation, glucose influx as well as the effects on metabolic key components. Analysis of functional effects was achieved by means of flow cytometry, automated cell counting procedures, mRNA and protein analysis by RT-QPCR and Western blotting and glyoxalase enzyme activity assay. Chronic hyperglycaemia was induced by adjusting medium glucose content from 5 to 30 mM glucose.
Results: Under chronic hyperglycaemia a 6.7fold increase of GLUT4 was detected onto the cell surface by flow cytometry, resulting in a more than 3fold glucose influx (measured by NBDG-uptake for 60 min) and pronounced lactate production (1.3fold increase compared to 5mM). Increased GLUT4 presentation was not caused by pronounced protein expression due to hyperglycaemia. Cell proliferation decreased under hyperglycaemic conditions as well as cell viability (measured as Trypan Blue-Uptake for necrotic cells) indicating hyperglycaemia-induced apoptosis. This could be verified by PI/Annexin staining (1.5fold increase compared to 5mM) and cleavedPARP/PARP analysis using Western Blotting (2.2fold increase in cleavedPARP per PARP compared to 5 mM glucose). Increasing levels of Ddit3 point to methylglyoxal-induced apoptosis. Under hyperglycaemic conditions the addition of 10 μM methylglyoxal to the culture medium further increased GLUT4 presence on the cell surface indicating a possible role of glycotoxic metabolites in this scenario. Concomitantly, the glyoxalase 1 activity was found to be diminished under hyperglycaemic conditions. Chronic hyperglycaemia (30 mM for more than 3 months) induced a diabetic heart failure phenotype with significantly reduced levels of IRS1 (−31% RNA expression vs. 5 mM, $p < 0.01$), CPT1 (−17% protein expression vs. 5 mM, $p < 0.05$), AMP-kinase as an indicator of energy starvation (−22% protein expression vs. 5 mM, $p < 0.001$) and extraordinary high levels of Na-K-ATPase levels (+125% protein expression vs. 5 mM, $p < 0.05$).
Conclusion: The above described cell culture model is feasible for detection of metabolic alterations in cardiomyocytes following chronic hyperglycaemia and point to a contribution of reactive glucose metabolites to the scenario of diabetic heart failure. Substantial effects of long term hyperglycaemia are attributable to the generation of reactive glucose metabolites like methylglyoxal that accumulate due to increasing amounts of intracellular glucose and reduced activity of the detoxifying system Glyoxalase 1.
Disclosure: **B. Stratmann:** None.

1087

Exendin-4 alleviates diabetic cardiomyopathy in mice by regulating Sirt1/PGC1α

M. Guan, Y. Cai, S. Fang, L. Xu, Y. Zhang, L. Wang;
Dept. Endocri & Metab, Nanfang Hospital, Southern Medical University, Guangzhou, China.

Background and aims: To investigate the protective effect of Exendin-4 on diabetic cardiomyopathy in mice and the potential mechanism involved.
Materials and methods: Type 2 diabetic mice were induced by streptozotocin (STZ) combined with high-fat diet (HFD). C57BL/6J mice were randomly divided into 3 groups: control group (Con), diabetic control group (DM-Con) and diabetic group with Exendin-4 treatment (DM+Ex-4). Exendin-4 was administered intraperitoneally (i.p., 1nmol/kg, qd) to the DM+Ex-4 mice for 8 weeks. Physiological indicators, such as blood glucose and weight, were recorded. RT-PCR was used to examined the transcription levels of genes related to myocardial hypertrophy and fibrosis, and mitochondrial function related genes, including PGC1α, NRF and CytoC. The expressions of oxidative stress markers and Sirt1/PGC1 proteins were measured by Western blot. Myocardial structural changes were detected by HE staining.
Results: Compared with the Con group, the DM-Con group showed significantly increased blood glucose and blood lipids ($P < 0.001$), which were improved by Exendin-4 treatment. The expressions of ANP, BNP, TGFβ1, CytoC1 and NOX1 in diabetic myocardial tissue were significantly increased ($P < 0.05$), while Sirt1, PGC1α, NRF and SOD1 were markedly decreased ($P < 0.05$). Interestingly, the Exendin-4 treatment resulted in obviously reduced expressions of ANP, BNP, TGFβ1, CytoC1 and NOX1 compared with the DM-Con group ($P < 0.05$), and increased expressions of Sirt1, PGC1α, NRF and SOD1 ($P < 0.05$).
Conclusion: Exendin-4 prevents myocardial injury in diabetic mice by improving mitochondrial function and inhibiting oxidative stress through the Sirt1/PGC1α signaling pathway.
Supported by: NNSFC: 81628004, 31400992 and 81470047
Disclosure: **M. Guan:** None.

1088

Myocardial flow reserve assessed by Cardiac ^{82}Rb PET/CT is associated with albumin excretion in patients with type 1 diabetes

E. Hein Zobel[1], S. Abitz Winther[1], P. Hasbak[2], B. von Scholten[1], L. Holmvang[3], A. Kjær[2], P. Rossing[1], T. Hansen[1];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]Department of Clinical Physiology, Nuclear Medicine & PET and Cluster for Molecular Imaging, Rigshospitalet, Copenhagen, [3]Department of Cardiology, Rigshospitalet, Copenhagen, Denmark.

Background and aims: To evaluate myocardial flow reserve (MFR) and coronary artery calcium (CAC) in persons with type 1 diabetes with or without albuminuria and in non-diabetic controls; in addition we evaluated the association of MFR and CAC with retinopathy, another microvascular complication. MFR reflects the function of large epicardial arteries and myocardial microcirculation. CAC represents structural aspects of atherosclerosis.
Materials and methods: Cross-sectional study in type 1 diabetes, stratified by normoalbuminuria ($n = 30$) and presence of or historical macroalbuminuria ($n = 30$), and in non-diabetic controls ($n = 30$). MFR (pharmacological stress flow/rest flow) was evaluated by cardiac ^{82}Rb positron emission tomography/computed tomography. CAC content was quantified using the Agatston score.
Results: MFR was similar in patients with normomalbuminuria (NORMO) and controls (3.1 ± 0.8 *vs* 3.0 ± 0.79; $p = 0.74$). Patients with macroalbuminuria (MACRO) had lower (impaired) MFR compared to NORMO (2.1 ± 0.9 *vs* 3.1 ± 0.8; $p < 0.0001$). The CAC score (median[IQR]) was higher in NORMO compared to controls (72[22–247] *vs* 0[0–81], $p = 0.018$), and comparable between MACRO and NORMO. MFR was comparable in patients with diabetes and simplex or no retinopathy ($n = 24$ and $n = 12$, 2.8 ± 0.84 vs 3.3 ± 0.77, $p = 0.11$), but lower in proliferative ($n = 24$) compared to simplex retinopathy (2.1 ± 0.97 vs 2.8 ± 0.84, $p = 0.02$). The CAC score was comparable between groups of retinopathy. In multivariate linear regression lower MFR was associated with higher urinary albumin creatinine rate, higher age, lower eGFR and smoking ($R^2 = 0.39$). Higher CAC was associated with lower MFR, higher age and 24-hour systolic blood pressure ($R^2 = 0.34$).
Conclusion: Myocardial microvascular function was comparable in non-diabetic controls and patients with type 1 diabetes and normoalbuminuria; but impaired in the presence of microvascular complications (macroalbuminuria and proliferative retinopathy). Coronary calcification was elevated in diabetes, however not explained by albuminuria.
Supported by: NNF14OC0013659
Disclosure: **E. Hein Zobel:** None.

1089

Stereological quantification of key pathological features in a uninephrectomised db/db mouse model of diabetic nephropathy

T.T. Johansen, T.X. Pedersen, N. Vrang, J. Jelsing, T. Secher, L.N. Fink;
Gubra ApS, Hørsholm, Denmark.

Background and aims: Diabetic nephropathy (DN) is a long-term complication of diabetes characterized by increasing albuminuria and reduced

kidney function. Approximately one third of patients with type 2 diabetes develop DN with DN being the predominant cause of end-stage renal disease. To aid further understanding of disease mechanisms and development of new treatment options, translatable and stable rodent models that recapitulate features of human DN are essential.

Materials and methods: Uni-nephrectomy (UNx) was performed in female db/db mice to accelerate diabetes-induced renal pathology. UNx or sham surgery was performed in db/db or db/+ mice (7–8 weeks of age, $n = 8$–16 mice per group). The study was terminated 11 or 16 weeks after surgery. Plasma samples were obtained for assessment of blood glucose, HbA1c and blood urea nitrogen (BUN), and urine samples were collected for albumin-to-creatinine ratio (ACR) analysis. Estimates of renal hypertrophy were determined by stereology and renal fibrosis by image analysis of picrosirius red staining.

Results: UNx in db/db mice lead to a slight increase in fed blood glucose compared to db/db sham (16 weeks; 27.6 ± 1.6 vs. 22.3 ± 2.1 mmol/l; $p < 0.05$) but did not alter body weight or HbA1c (16 weeks; 8.8 ± 0.6 vs. 8.1 ± 0.5%). Total kidney and glomerulus volume were markedly higher in db/db UNx compared to both db/db sham and db/+ sham (16 weeks; 124.4 ± 6.0 vs. 85.9 ± 3.3 and 65.6 ± 2.9 kidney volume mm^3; $p < 0.001$; 2.66 ± 0.15 vs. 1.94 ± 0.12 and 1.46 ± 0.07 glomerulus volume mm^3; $p < 0.001$ db/db UNx vs. db/+ sham; $p < 0.01$, db/db UNx vs. db/db sham). Kidney fibrosis was increased in both db/db UNx and db/db sham vs. db/+ sham control (16 weeks; 12.7 ± 1.3 and 10.7 ± 1.8 vs. 5.7 ± 0.8 total collagen mg; $p < 0.05$). BUN was transiently increased in db/db UNx compared to both db/db sham and db/+ sham at 4 weeks post-surgery, but not at 11 or 16 weeks. Urine ACR increased progressively over time and was significantly increased in db/db UNx and sham at week 5, 10 and 15 compared to db/+ sham (15 weeks; 2263.1 ± 505.2 and 1748.4 ± 445.2 vs. 43.9 ± 6.9 ACR μg/mg; $p < 0.001$ db/db UNx vs. db/+ sham; $p < 0.01$ db/db sham vs. db/+ sham).

Conclusion: Diabetes and unilateral nephrectomy were combined to exacerbate renal pathology. Renal hypertrophy and increased glomerular volume in the db/db UNx model indicate renal compensation potentially leading to glomerular alterations and increased urinary ACR. Further characterization of renal features reflecting human DN is ongoing to refine the db/db UNx model and provide improved pre-clinical testing possibilities for drugs targeting DN.

Disclosure: **T.T. Johansen:** None.

1090

A novel surgery-induced rat model of diabetic nephropathy displaying kidney hypertrophy, albuminuria and pronounced tubular fibrosis

M.V. Østergaard, T. Secher, T. Johansen, P.G.J. Pedersen, N.E. Zois, T.X. Pedersen, J. Jelsing, N. Vrang, K. Fosgerau, L.N. Fink;
Gubra aps, Hørsholm, Denmark.

Background and aims: Diabetic nephropathy (DN) is a long-term complication of diabetes characterized by albuminuria and loss of kidney function, which affects up to one third of all diabetes patients. For more than a decade no new therapies targeting DN have been introduced, partly due to the lack of preclinical animal models reflecting key features of human DN. Here, we aimed to establish a novel preclinical model of DN in pancreatectomized uni-nephrectomized rats displaying progressive albuminuria and histopathological features of late stage DN.

Materials and methods: Diabetes was surgically induced in male Sprague-Dawley rats by 90% pancreatectomy (Px) resulting in stable blood glucose levels of >20 mmol/L. To accelerate the progression of DN, unilateral nephrectomy (UNx) was performed simultaneously to Px in another rat cohort. At 11 and 21 weeks after Px and 11 weeks after Px-UNx, urinary creatinine, albuminuria and NGAL, and plasma creatinine and cystatin C were quantified. Kidneys were processed for histology and stereology as well as next generation sequencing.

Results: Kidney hypertrophy was observed in Px rats 11 and 21 weeks post-surgery (0.53 ± 0.01 and 0.57 ± 0.01% of BW, respectively, vs 0.35 ± 0.02%, sham control, $p < 0.01$) and was further increased in Px-UNx rats 11 weeks post-surgery (Px-UNx 0.94 ± 0.04% of BW, $p < 0.001$ vs Px 11 wks and Px 21 wks). Renal fibrosis was significantly induced in Px vs Ctrl rats as determined by quantitative histology of picro-sirius red staining of total kidney collagen (127 ± 21 Px 11 wks and 227 ± 46 Px 21 wks vs 39 ± 11 mg Ctrl, $p < 0.05$ and $p < 0.001$, respectively). Renal fibrosis was further augmented in Px-UNx rats 11 weeks post-surgery (334 ± 81 mg, $p < 0.001$ vs Px 11 wks and $p < 0.01$ vs Px 21 wks). Urinary albumin excretion was significantly increased in Px-UNx rats vs Ctrl (albumin-to-creatinine ratio; 11,504 ± 5,366 vs 233+112 μg/mg, $p < 0.05$), while urinary NGAL excretion was increased by 21 weeks after Px and 11 weeks after Px-UNx vs Ctrl (NGAL-to-creatinine ratio; 3.2 ± 0.6 and 12.1 ± 3.5 vs 0.3 ± 0.023 μg/mg, both $p < 0.001$). Plasma creatinine and cystatin C were reduced 21 weeks post-surgery in Px vs Ctrl rats (creatinine; 13.3 ± 0.4 vs 17.9 ± 1.4 μmol/L, $p < 0.01$ and cystatin C; 1511 ± 87 vs 2411 ± 286 ng/mL, $p < 0.01$). Both plasma creatinine and cystatin C levels were significantly increased in Px-UNx rats 11 weeks vs Px 21 weeks post-surgery (creatinine; 20.9 ± 1.2 μmol/L, $p < 0.001$ vs Px 21 wks and cystatin C; 2463 ± 29 ng/mL, $p < 0.01$ vs Px 21 wks) and returned to levels similar to Ctrl rats. Finally, gene expression of proteins encoding podocyte markers nephrin, podocin, and WT-1 were significantly reduced in Px-UNx vs Ctrl rats, while gene expression of kidney injury markers NGAL and KIM-1 were significantly increased.

Conclusion: The novel Px-UNx model of DN develops extensive renal hypertrophy and fibrosis 11 weeks post-surgery; a nephropathy phenotype that is more progressed than in the Px model after 21 weeks. Furthermore, the Px-UNx model displays increased urinary albumin and NGAL excretion as wells as increased plasma creatinine and cystatin C. Thus, Px in combination with UNx in rats represents a novel, strong alternative to streptozotocin-induced diabetes and genetic models for pre-clinical drug development targeting DN.

Disclosure: **M.V. Østergaard:** None.

1091

Prediabetes and diabetes are associated with wider retinal arterioles and venules: the Maastricht study

W. Li[1], M. Schram[1], T. Berendschot[2], J. Schouten[2], A. Kroon[1], C. van der Kallen[1], R. Henry[1], A. Koster[3], P. Dagnelie[4], N. Schaper[1], F. Huang[5], B. ter Haar Romeny[5], C. Stehouwer[1], A. Houben[1];
[1]Department of Internal Medicine & School for Cardiovascular Diseases (CARIM), Maastricht University Medical Center+, Maastricht, [2]Department of Ophthalmology, Maastricht University Medical Center+, Maastricht, [3]Department of Social Medicine, Maastricht University, Maastricht, [4]Department of Epidemiology, Maastricht University, Maastricht, [5]Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands.

Background and aims: Retinal vascular calibers are biomarkers of cardio-metabolic risk. Previously, it has been shown in population-based cohort studies that wider retinal arterioles are associated with diabetes and impaired fasting glucose. However, the association of wider retinal venules and diabetes was found in non-caucasian ethnicities only. The aim of the present study was to investigate the association of glucose metabolism status with retinal arteriolar and venular diameters in a predominantly Caucasian population.

Materials and methods: In a population-based cohort study with oversampling of T2DM ($n = 2339$, 50.1% men, aged 59.7 ±8.2 years; 98.8% Caucasian), we determined retinal microvascular diameters [MU] (Rhino software) and glucose metabolism status (OGTT; normal glucose metabolism (NGM) [$n = 1363$], prediabetes [$n = 366$], or T2DM [$n = 610$]). Differences were assessed with multivariable regression analyses adjusted for age, sex, waist circumference, smoking, systolic-BP, lipid profile, the use of lipid-modifying and/or blood-pressure-lowering

medication, eGFR, albuminuria, and prior CVD. Individuals with retinopathy were excluded.

Results: Adjusted analyses showed that both central retinal arteriolar equivalent(CRAE) and central retinal venular equivalent(CRVE) were wider in prediabetes (CRAE: $B = 0.93$, 95% CI −1.43 to 3.30; CRVE: $B = 2.94$, 95% CI −0.78 to 6.66), and in T2DM (CRAE: $B = 3.47$, 95% CI 1.06 to 5.88; CRVE: $B = 3.88$, 95% CI 0.08 to 7.68) versus normal glucose metabolism (CRAE: P for trend = 0.006; CRVE: P for trend = 0.035). In addition, HbA1c was associated with wider CRAE after full adjustment ($B = 1.10$ [0.004 to 2.20], $P = 0.049$), but was not associated with CRVE.

Conclusion: Prediabetes and diabetes are independently associated with both wider retinal arterioles and venules, in a predominantly Caucasian population. These findings support the concept that microvascular dysfunction is an early phenomenon in disturbed glucose metabolism.

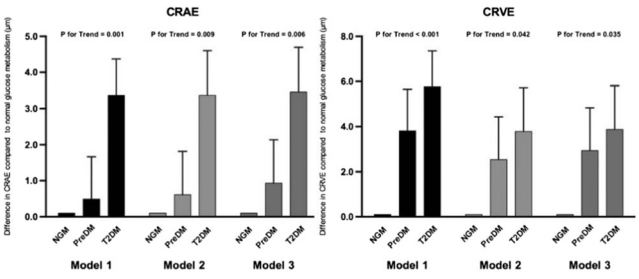


Figure 1. Multivariable adjusted differences in retinal microvascular diameters between individuals with prediabetes and T2DM compared with NGM. Bars represent the mean difference with standard error with NGM. NGM is the reference and is set to zero Model 1: adjusted for age and sex. Model 2: additionally adjusted for waist circumference, smoking, systolic-BP, lipid profile, the use of lipid-modifying and/or blood-pressure-lowering medication. Model 3: additionally adjusted for eGFR, albuminuria, and prior CVD.

Supported by: OP-ZUID
Disclosure: **W. Li:** None.

1092

Microvascular reactivity and nutrients profile in patients with type 2 diabetes and metabolically healthy volunteers with overweight or obesity

J. Veleba[1], L. Belinova[1], P. Janovska[2], H. Kahleova[1], H. Malinska[1], K. Velebova[1], E. Stolarikova[1], J. Kopecky, Jr.[1], K. Bardova[2], O. Kuda[2], J. Hansikova[2], V. Melenovsky[1], J. Kopecky, Sr.[2], T. Pelikanova[1];
[1]Institute for Clinicial and Experimental Medicine, Prague, [2]Academy of Sciences of The Czech Republic, Prague, Czech Republic.

Background and aims: Important features of type 2 diabetes (T2D) include endothelial dysfunction, insulin resistance and disruption of nutrients metabolism. The aim of our work is to investigate microvascular reactivity in relation to acute hyperinsulinemia, nutritional stimulation and adipose tissue composition by patients with T2D and overweight or obese subjects with normal glucose metabolism.

Materials and methods: We enrolled 20 patients with type 2 diabetes (T2D) on metformin treatment and 20 volunteers (C) with overweight or obesity and with normal glucose metabolism. All subjects underwent the hyperinsulinemic euglycemic clamp combined with indirect calorimetry, meal test using standard breakfast, biopsy of subcutaneous adipose tissue and assesment of plasma acylcarnitines (AcylCN) and aminoacids profiles. Parameters of the endothelial function - Augmentation Index (AI) reflecting arterial stiffness and Reactive Hyperemia Index (RHI) reflecting microvascular reactivity were measured by finger pulse plethysmography before and during the clamp and before and after the meal test.

Results: AI decreased in response to meal test in C (12.43 ± 1.86 vs. 4.02 ± 2.11; $p < 0.001$) as well as in DM2 (15.37 ± 2.37 vs. 4.13 ± 2.61; $p < 0.001$), FRHI did not change during the meal test neither in C (0.39 ± 0.08 vs 0.33 ± 0.09; ns.) nor in DM2 (0.14 ± 0.07 vs 0.1 ± 0.007; ns), but we found significant difference between the groups ($p < 0.001$). AI decreased in response to hyperinsulinemia in C (10.41 ± 1.13 vs. 6.4 ± 1.17; $p < 0.001$), whereas did not change in DM2 (8.2 ± 2.41 vs. 6.51 ± 2.11; ns.). FRHI tended to decrease in response to hyperinsulinemia (C: 0.39 ± 0.06 vs. 0.34 ± 0.06; ns. DM2: 0.14 ± 0.07 vs. 0.09 ± 0.07; ns.) and we found significant difference between the groups ($p < 0.01$). We proved higher level of C2-AcylCN and C4-AcylCN and lower level of C16-AcylCN in DM2. Glucogenic and branched aminoacids concentrations (alanine, proline, valine, leucine and isoleucine) were higher in DM 2 than in C. Concentrations of other amino acids did not differ significantly. We found a higher capillaries - adipocytes ratio in histological examination of adipose tissue in C (0.46 ± 0.11 vs. 0.40 ± 0.05; $p < 0.05$). Correlation between AcylCN concentrations and endothelial function parameters was clinically and statistically insignificant. We found a negative relationship between RHI and proline concentration ($r = -0.31$; $p < 0.01$), positive correlation between AI and phenylalanine concentration ($r = 0.34$; $p < 0.01$) and positive correlation between RHI and capillaries - adipocytes ratio ($r = 0.35$, $p < 0.01$).

Conclusion: Arterial stiffness decreased in DM 2 and C during postprandial state, whereas in response to hyperinsulinemia decreased only in C. Microvascular reactivity was not influenced by interventions, but was always higher in C. We observed higher concentrations of short chain AcylCN and glucogenic and branched aminoacids and lower vascularization of subcutaneous adipose tissue resulting in reduced vascular reactivity in DM 2.

Clinical Trial Registration Number: NCT01946347
Supported by: AZV15-27431A
Disclosure: **J. Veleba:** None.

1093

Dermal microvessel density in adults with type 1 diabetes is dependent on metabolic control

A. Adamska, A. Araszkiewicz, S. Pilacinski, A. Gandecka, A. Grzelka, K. Kowalska, A. Malinska, M. Nowicki, D. Zozulinska-Ziolkiewicz;
Poznan University of Medical Sciences, Poznan, Poland.

Background and aims: In patients with diabetes, functional changes in microcirculation and subclinical vascular pathology precede clinical manifestation of microangiopathic complications. Objective of the study was to evaluate the association between established vascular risk factors and density, maturity and reactivity of dermal blood vessels in adults with type 1 diebetes.

Materials and methods: We included 148 adult patients (87 men, 61 women) with type 1 diabetes, median (IQR) age 40.5 (30.5–49) years, diabetes duration 21 (17–29.5) years. Data on the medical history and treatment of diabetes were collected using a questionnaire. The participants underwent physical examination with anthropometric measurements. We determined parameters of metabolic control of diabetes and assessed accumulation of advanced glycation and products (AGEs) using AGE-Reader device. Skin biopsy was performed 10 cm above the lateral malleolus using 3 mm biopsy punch. In the immunohistochemical (IHC) analyses anti-CD133, anty-CD34, anti-CD31, and anti-vWF autoantibodies were used. All histology sections from a single patient were processed in the same IHC experiment. Microvessel density (MVD) was calculated using "hot spots technique". Microvascular function was examined by single-point laser-Doppler flowmetry (LDF). Data were analyzed using Statistica version 10.

Results: Median MVD, calculated for both papillary and reticular dermis, defined by CD31 antigen expression was 38 (19–56) per 1 mm^2. The median CD34+ blood vessel density was 121 (100–155) per 1 mm^2, CD133+ 79 (63–92) per 1 mm^2, and vWF 50 (40–69) per 1 mm^2. An average CD34/CD31 index in dermal biopsies was 2.78, vWF/CD31 ratio was 1.32 and CD133/CD31 ratio was 1.75. In multivariante regression analysis the CD34/CD31 index was positively associated with serum triglyceride concentration (Beta: 0.26, $p = 0.012$) and negatively associated with serum HDL cholesterol concentration (Beta: −0.22, $p = 0.027$), both independently from age, sex, diabetes duration, BMI, HbA1c value,

presence of hypertension, and eGFR. In LDF the area under thle blood flow/time curve (AUC) correlated positively with CD31+ MVD ($r = 0.21, p = 0.011$) and negatively with CD34+ MVD ($r = -0.20, p = 0.017$).
Conclusion: Atherogenic dyslipidemia is associated with increased formation of new blood vessels, characterized by high expression of CD34 and low reactivity in laser Doppler flowmetry. Conversely, chronic hyperglycemia and excessive formation of advanced glycation end products may result in decreased vascularity.
Supported by: Poznan University of Medical Sciences and Diabetes Poland
Disclosure: **A. Adamska:** Grants; Poznan University of Medical Sciences, scientific grant of Diabetes Poland.

PS 103 Diabetes from childhood to adults

1094

Gut microbiota in children with type 1 diabetes differs in composition and functionality in comparison with MODY 2 and healthy controls
L. Sánchez-Alcoholado[1], I. Leiva-Gea[2], B. Martín-Tejedor[2], D. Castellano-Castillo[1], I. Moreno-Indias[1], A. Urda-Cardona[2], F.J. Tinahones[1], J.C. Fernández-García[1], M.I. Queipo-Ortuño[1];
[1]Clinical Management Unit of Endocrinology and Nutrition, Virgen de la Victoria University Hospital, Laboratory of Biomedical Research Institute of Malaga (IBIMA), University of Malaga, CIBERobn, Málaga, [2]Clinical Management Unit of Pediatrics, Materno-Infantil Hospital, Málaga, Spain.

Background and aims: Type 1 diabetes mellitus (T1DM), an organ-specific autoimmune disease, is associated with compositional differences in gut microbiota. Maturity Onset Diabetes of the Young 2 (MODY2) is a monogenic cause of diabetes. We compared gut microbiota profile and functional capacity between healthy controls and children with T1DM and MODY2, and evaluated the relationship between intestinal microbiota, intestinal permeability and glycemic levels.
Materials and methods: Case-control study in 15 children with T1DM, 15 children with MODY2 and 13 healthy children. Metabolic control and potential factors modifying gut microbiota were controlled. Microbiome composition was determined by 16S rRNA pyrosequencing and bioinformatics analysis by QIIME software.
Results: We found a significant decrease in the microbiota diversity in T1DM with respect to healthy controls. A significant increase in the relative abundance of *Bacteroides*, *Ruminococcus*, *Veillonella*, *Blautia* and *Streptococcus* genera, and a decrease in the relative abundance of *Bifidobacterium*, *Roseburia*, *Faecalibacterium* and *Lachnospira* was found in T1DM. MODY2 showed a significant increase in *Prevotella* abundance and a significant decrease in *Ruminococcus* and *Bacteroides*. Moreover, significant correlations between the serum levels of IL-1B and gut microbiota composition were found in T1DM patients. Gut permeability (determined by serum zonulin levels) was significantly increased in MODY2 and T1DM. PICRUSt analysis found that T1DM was increased in genes related to lipid and amino acid metabolism, ABC transport, lipopolysaccharide biosynthesis, arachidonic acid metabolism, antigen processing and presentation and chemokine signaling pathways compared to MODY2 and healthy controls.
Conclusion: Gut microbiota in T1DM differs at taxonomic level respect to MODY2 and healthy controls, but also at functional level, involving different metabolic pathways. Moreover, T1DM was associated with a low-grade inflammatory gut microbita profile. The specific gut microbiota profile found in T1DM could represent an environmental risk factor associated with the autoimmunity process and, through gut microbiota modulation, might constitute a potential target for the prevention of T1DM.
Supported by: ISCIII, Fondos FEDER
Disclosure: **L. Sánchez-Alcoholado:** None.

1095

Metabolomic profiling of exhaled breath in relation to glycaemic variability in paediatric patients with type 1 diabetes: a prospective cross-sectional study
S.C. Schmidt[1], P. Trefz[2], J.K. Schubert[2], W. Miekisch[2], D.-C. Fischer[1];
[1]Paediatrics, University Medicine Rostock, Rostock, [2]Anaesthesiology and Intensive Care Medicine, University Medicine Rostock, Rostock, Germany.

Background and aims: Type 1 diabetes mellitus (T1DM) is the most common chronic metabolic disease in paediatric patients, for whom non-invasive and thus painless metabolic monitoring is particularly attractive. A prominent example is breath analysis, although previous studies on

exhaled concentrations of volatile organic compounds (VOCs) in diabetic breath yielded contradictory results. It is not yet known how the chemical composition of exhaled breath changes in the course of metabolic adaptation to endogenous insulin deficiency, and more specifically in relation to glycaemic variability.

Materials and methods: Blinded continuous glucose monitoring (CGM) and breath analysis with proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS) were combined in a prospective cross-sectional study design. Data was recorded every 5 minutes for 9 consecutive hours including 2 standardised meals. Glycaemic variability was assessed as SD and CV of interstitial glucose concentration as well as time in target range (3.9–10.0 mmol/L), and mean alveolar VOC concentrations were calculated as time-normalised AUCs. Between-group differences were tested for significance with either unpaired two-sample Student's t-test or Mann-Whitney *U* test, as appropriate, and bivariate rank correlation coefficients were calculated according to Spearman.

Results: Out of 360 quasi-molecular ions detected in exhaled breath of 45 children and adolescents, mean alveolar concentrations of methylamine (median [parts per billion by volume (ppbV)] 12.2 (range [ppbV] 5.4–36.9) vs 7.3 (3.4–18.3)), acetone (255.0 (212.6–295.2) vs 223.3 (183.5–261.7)), isopropanol (1015.6 (597.6–2740.0) vs 600.7 (319.6–1053.3)) and pentanal (6.3 (3.1–14.1) vs 3.5 (1.9–5.8)) were significantly elevated (all $p < 0.001$) in paediatric T1DM patients on either multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) compared to healthy controls matched for age (12–16 years) and sex. No significant correlations were found between mean alveolar VOC concentrations, mean interstitial glucose concentration and measures of glycaemic variability.

Conclusion: Distinct VOC profiles in exhaled breath mirror metabolic alterations occurring with T1DM, although not linearly related to well-established markers of glycaemic control, i.e. blood glucose concentration and glycaemic variability. Regardless, breath analysis might be a powerful tool for non-invasive real-time metabolic monitoring, especially in paediatric patients.

Supported by: DFG TR 1381/2-1, LGF M-V, FORUN 889004

Disclosure: **S.C. Schmidt:** Grants; DFG TR 1381/2-1, LGF M-V, FORUN 889004.

1096

Predictors of loss to follow up in youth with type 2 diabetes: comparing the US Pediatric Diabetes Consortium and European Pediatric Diabetes Prospective cohorts

S. Wiegand[1], G.J. Klingensmith[2], S. Lanzinger[3], W.V. Tamborlane[4], T. Reinehr[5], P. Cheng[6], M. Bauer[7], R.L. Gal[6], R.W. Holl[3], C. Kollman[6];
[1]Pediatric Endocrinology and Diabetology, Charité Universitätsmedizin Berlin, Berlin, Germany, [2]Department of Pediatrics, Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, USA, [3]Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany, [4]Pediatric Endocrinology, Yale University, New Haven, USA, [5]Department of Pediatric Endocrinology, Diabetes, and Nutrition Medicine, University of Witten/Herdecke, Datteln, Germany, [6]Jaeb Center for Health Research, Tampa, USA, [7]Department of Pediatric and Adolescent Medicine, University of Linz, Linz, Austria.

Background and aims: Youth with T2D have been shown to have greater social disadvantage than youth with type 1 diabetes or the general population. Clinicians also report that T2D youth are not seen in follow up consistently, thus creating difficulty in diabetes management and timely identification and treatment of complications and co-morbidities. This study was designed to determine predictors for loss to follow up (LFU) and assess differences in predictors between continents.

Materials and methods: Youth with T2D enrolled from Jan 2012 to Dec 2015, age 10 - ≤16.5 at the last visit, were identified in both the Pediatric Diabetes Consortium (PDC) and Central European Pediatric Diabetes Prospective Follow Up (DPV) Registry. T2D was determined by no beta cell autoantibodies detected in the presence of diabetes, using ADA/ISPAD guidelines. Characteristics at enrollment were aggregated to identify those associated with loss to follow up. In PDC, attempts to contact patients were made >3 times using several means before LFU was determined. In DPV, patients/families were contacted by phone if they missed follow up appointments.

Results: In all, 677 were eligible for inclusion, 463 in DPV and 214 in PDC. Both cohorts reported a high rate of LFU prior to 18 years of age (76% in DPV and 54% in PDC). In multivariable analysis, age predicted LFU in both cohorts with those <13 less likely to be LFU than those ≥15 years of age ($p < 0.001$). Youth ≥15 years had a 91% LFU in the DPV and 87% LFU in the PDC. Diabetes treatment was also a risk in both cohorts, $p = 0.03$ in PDC; $p = 0.02$ in DPV, patients treated with "lifestyle modification only" had highest risk for LFU. Of concern, 48% in PDC and 64% in DPV of those on insulin also were LFU. In the PDC but not in the DPV, those residing a greater distance from the clinical site (≥80 km) were at significantly higher risk of LFU than those within 8 km (82% vs 36%). Gender, minority status, diabetes duration, and HbA1c did not predict LFU in either cohort.

Conclusion: Loss to follow up is a serious problem in both T2D Registries. Identifying ways to help vulnerable T2D youth maintain consistent medical care is a major health issue in adolescent T2D and most likely contributes to poor outcomes.

Supported by: PDC: unrestricted grants NovoNordisk, Boehringer Ingelheim, Takeda. DPV: Ge

Disclosure: **S. Wiegand:** None.

1097

The effects of type 1 diabetes on hand and foot posture and mobility of young patients

P. Francia[1], L. Capirchio[2], U. Santosuosso[1], M. Sorelli[3], B. Piccini[2], A. Vittori[1], G. Iannone[4], M. Gulisano[1], S. Toni[2];
[1]School of Human Health Sciences, Florence, [2]Diabetes Unit Meyer Children's Hospital, Florence, [3]Dept. of Information Engineering, University of Florence, Florence, [4]National Association of Movement Sciences (ANIMO), Florence, Italy.

Background and aims: It is known that limited joints mobility can affect the quality of movement and could also adversely affect the body development of young subjects with Type 1 Diabetes Mellitus (T1DM). In particular, it is well known how diabetes mellitus can reduce hand and ankle joints range of motion and modify their posture. The aim of this study was to investigate using a new method how diabetes affects hand and foot posture in young T1DM patients.

Materials and methods: We enrolled 20 young T1DM patients: (M/F:11/9), mean age 13.8 ± 3.8 yrs, BMI: 19.5 ± 4.7 kg/m^2, diabetes duration 4.6 ± 3.6 yrs, mean HbA1c 8.2 ± 1.2% and 46 young subjects practicing soccer and dance: (M/F:30/16), mean age 12.6 ± 2.1 yrs, BMI: 18.9 ± 2.6 kg/m^2. In these subjects, we evaluated hand posture (analysis of frontal plane image of Prayer sign test). In particular, the inclination of the fifth metacarpal and phalanges bones were evaluated in addition to the angles at the metacarpophalangeal and interphalangeal joints. Ankle joint mobility and posture were evaluated (inclinometer and sagittal plane image of the ankle and lower limb) with the foot and leg in the same position used for the evaluation of joint mobility (patients lying). Moreover, trunk flexibility (sit & reach test), muscle strength (hand grip) and lifestyle (IPAQ-C, IPAQ-A) were evaluated. The individual sporting history was investigated by a specific questionnaire.

Results: The analysis of hand images showed the presence in diabetic patients of a higher extension of the fifth metacarpophalangeal joint (patients group: 34.7 ± 11.0°; control group: 18.6 ± 8.5°) and higher flexion of the proximal interphalangeal joint (patients group: 11.0 ± 5.1°; control group: 0.4 ± 9.8°). In comparison to controls, the patient group showed a higher inclination of the fifth metacarpal joint (42.4 ± 11.2° vs 34.4 ± 8.4°; $p < 0.005$) and a lower inclination of the proximal phalanx (4.9 ±

6.0° vs 15.7 ± 6.1°; $p < 0.001$). Moreover, the tests performed showed a significantly higher ankle joint mobility in young dancers (155.8 ± 10.3°) compared to patients and soccer group (126.8 ± 15.5°; $p < 0.001$) and patients group (127.3 ± 33.7°; $p < 0.001$). No significant correlations were found between the parameters investigated.

Conclusion: Young patients with T1DM can show abnormal posture of the hand. Regarding the analysis of the hand images the results of this pilot study indicate that the metacarpophalangeal joint and the proximal interphalangeal joint take a different posture in patients with T1DM. The prayer sign test could hinder the recognition of the presence of an abnormal distal interphalangeal joint flexion. All this indicates that diabetes could affect hand posture and mobility from the first years of disease onset.

Disclosure: **P. Francia:** None.

1098

Epidemiology of acute diabetes complications (coma) according to the Federal Diabetes register of the Russian Federation (2013–2016)

A.Y. Mayorov[1], O.K. Vikulova[1], A.V. Zheleznyakova[1], M.A. Isakov[1,2], O.G. Melnikova[1], I.V. Kononenko[1], M.V. Shestakova[1], I.I. Dedov[1]; [1]Diabetes Institution, Endocrinology Research Centre, Moscow, [2]Aston consulting group, Moscow, Russian Federation.

Background and aims: Despite the improvement in the quality of diabetes care in the Russian Federation (RF), the increased availability of modern hypoglycemic drugs and insulins, coma remain one of the causes of death in patients with diabetes. Aim of the study was to assess dynamic of epidemiological characteristic of acute complications in adult patients with type 1 diabetes (T1D) and type 2 diabetes (T2D) in 2013–2016.

Materials and methods: The database of the Federal Diabetes register of 81 regions included in the online register system. The data exported from online register, powered by Microsoft Dynamics CRM platform. The analysed number of patients with T1D/T2D: 0.23/3.48 mln in 2013, 0.23/3.65 mln in 2014, 0.24/3.75 mln in 2015, 0.24/3.81 mln in 2016. Statistics performed by SPSS, 24.0.0.1. The indicators of coma for 2013–2016 were estimated for 10,000 adult patients with diabetes (>18 years).

Results: In 2016, the prevalence of coma in the RF averaged 225.9 with T1D and 11.6/10,000 adults with T2D. Totally in 2016, 165 new cases of coma for both types of diabetes were registered, an average of 0.4/10,000 adults. At the same time, interregional differences in the prevalence of coma were from 0 to 4.2/10,000 adults. The frequency of new cases of coma in the dynamics of 2013–2016 had a tendency to decrease: from 0.9 to 0.4/10,000 adults. Depending on the type of diabetes, the frequency of new cases of coma was also reduced: with T1D - from 5.7 to 3.4, with T2D - from 0.6 to 0.2/10,000 adults. When assessing dynamics by type of coma, it was found that the frequency of ketoacidotic coma decreased with both types of diabetes: with T1D - from 3.6 to 1.6, with T2D - from 0.2 to 0.1/10,000 adults; frequency hypoglycemic coma: with T2D - without the dynamics of 0.1/10,000 adults, while with T1D there was an increase in the frequency of 0.9 to 1.5/10,000 adults. When evaluating the structure of coma in a dynamic, redistribution was evident in their forms. So in 2013, the most frequent was with T1D ketoacidotic coma - 79.9%, hypoglycemic coma - 17.2%. In 2016, the structure changed: the proportion of hypoglycemic coma increased to 40.7%, and ketoacidotic coma decreased to 56.6%. With T2D, the difference between the ratio of ketoacidotic and hypoglycemic coma in 2013 and 2016 expressed in a lesser degree than with T1D, but also a tendency to increase the proportion of hypoglycemic coma: in 2013 ketoacidotic - 51.7%, hypoglycemic - 37.5%, and in 2016 - 48.3% and 46.1%, respectively. The mean duration of diabetes at the time of coma development increased with T1D from 3.8 to 9.1 years, with T2D from 3.5 to 7.0 years.

Conclusion: It is established that the dynamics of the frequency of development of coma in 2013–2016 in adult patients with diabetes in the RF has a stable tendency to decrease: 1.5 times with T1D and more than 2.5 times with T2D. It can be assumed that this is due to the improvement in the quality of diabetes care and glycemic control in general, as well as the use of modern medicines. Attention is required to draw to the high frequency of coma in T1D, the development of coma with a longer duration of diabetes, an increase in the proportion of patients with hypoglycemic coma. Significant interregional differences in the frequency of coma registration require additional analysis.

Supported by: the state assignment of the Ministry of Health of RF

Disclosure: **A.Y. Mayorov:** Grants; The work was supported by the state assignment of the Ministry of Health of RF.

1099

Rehabilitation for children and adolescents with diabetes

R. Schiel[1], R. Stachow[2], T. Hermann[3], I. Satzke[4], T. Büttner[5], S. Koch[6], K. Enderlein[7], R. Bambauer[8], A. Steveling[9], E. Bollow[10], R. Holl[10], DPVWiss Initiative; [1]MEDIGREIF-Inselklinik Heringsdorf GmbH, Seebad Heringsdorf, [2]Fachklinik Sylt, Westerland, [3]Fachklinik Prinzregent Luitpold, Scheidegg, [4]MEDIAN Kinderklinik „Am Nicolausholz, Bad Kösen, [5]Deutsche Rentenversicherung Rheinland-Pfalz, Edelsteinklinik, Fachklinik für Kinder- und Jugendrehabilitation, Bruchweiler, [6]Fachklinik Gaißach der Deutschen Rentenversicherung Bayern Süd, Gaißach, [7]MEDIGREIF Parkklinik, Greifswald, [8]Formerly Institute for Blood Purification, Homburg, [9]Ernst-Moritz-Arndt-University, Internal Medicine A, Greifswald, [10]Institut für Epidemiologie und medizinische Biometrie, ZIMBT, University of Ulm, Ulm, Germany.

Background and aims: Medical rehabilitation plays an important role in the treatment of children/adolescents with diabetes. It was the aim of the survey to analyze trends in the number of patients admitted to rehabilitation, the quality of diabetes care, the incidence of acute complications, risk factors for cardiovascular co-morbidities and the familial status over a period of 13 years.

Materials and methods: Currently 7 hospitals offer in-patient rehabilitation for children/adolescents with diabetes in Germany. Six hospitals participated in the survey. All children/adolescents (n = 7163) who participated in an in-patient rehabilitation 01/01/2004–31/12/2016 were included. Clinical/familial data were assessed: age, sex, family situation, type/duration of diabetes, insulin dosage, self-monitoring, acute complications, height, body weight, blood pressure and laboratory parameters.

Results: During the study period the patients took part in 10,987 in-patient rehabilitation procedures. The yearly number of patients participating in rehabilitation remained stable. There was no change in the quality of diabetes control (HbA1c: $p = 0.30$, fasting blood glucose: $p = 0.80$). The incidence of severe hypoglycaemia decreased ($p < 0.001$). The incidence of ketacidosis remained stable ($p = 0.18$). The frequency of blood glucose self-monitoring increased ($p < 0.001$). The same was true for patients treated with CSII ($p < 0.001$), whereas the numbers of patients treated with CT or ICT decreased (both $p < 0.001$). There was no change in patients' total insulin dose ($p = 0.01$). Regarding the family status the survey revealed the following trends: During the study period there was a decrease in the number of patients living with both parents ($p < 0.001$), whereas the percentage of children and adolescents living with the mother or father alone increased ($p < 0.001$). Moreover the number of patients living in native German families decreased ($p < 0.001$). The percentage of children and adolescents with diabetes living in mixed cultural families or having a background of immigration increased ($p < 0.001$).

Conclusion: There is a change in medical rehabilitation: The number is stable, the proportion of patients using CSII increased, the number of patients living with single parents and the percentage of patients from culturally mixed families increased also.

Disclosure: **R. Schiel:** None.

1100

Acute effects of the combination of acarbose and gastric distension, with a water preload, on the postprandial blood pressure response to oral sucrose

H. Pham, L. Trahair, L. Phillips, C.K. Rayner, M. Horowitz, K.L. Jones;
Adelaide Medical School, University of Adelaide, Adelaide, Australia.

Background and aims: Postprandial hypotension (PPH), a fall in systolic blood pressure (BP) of >20 mmHg within 2 hours of a meal, predisposing to syncope and falls, occurs in ~15% of healthy people >65 years and ~35% of patients with type 2 diabetes (T2DM). There currently is no satisfactory treatment. The underlying mechanisms are heterogeneous, including the rate of small intestinal nutrient delivery and absorption, release of gastrointestinal hormones and changes in splanchnic blood flow and autonomic nerve function. We have shown that the α-glucosidase inhibitor, acarbose, used widely in the management of T2DM, attenuates the magnitude of the fall in BP, in healthy older subjects and patients with PPH. Nutrient or non-nutrient gastric distension also attenuates the fall in postprandial BP but the effects may be more transient. We aimed to determine whether gastric distension and acarbose have additive effects to attenuate the fall in BP induced by oral sucrose in healthy older subjects.
Materials and methods: 10 healthy older subjects (2M, 8F; mean age: 74 ± 1.4 yr; BMI: 26.2 ± 1.1 kg/m^2) were studied on 4 separate occasions in a randomised, crossover design. After an overnight fast, subjects received either (i) a drink of 100 g sucrose dissolved in 300 ml of water (control treatment: C), (ii) a 300 ml water 'preload' 15 minutes before a drink of 100 g sucrose in 300 ml of water (distension treatment: D), (iii) a drink of 100 g sucrose with 100 mg acarbose in 300 ml of water (acarbose treatment: A) or (iv) a 300 ml water 'preload' 15 minutes before a drink of 100 g sucrose with 100 mg acarbose dissolved in 300 ml of water (acarbose and distension treatment: AD). BP was measured with an automated device at baseline (before intervention) and at 3-min intervals for 120 min after the sucrose drink. The maximum fall in systolic BP was calculated as the primary endpoint. Data are mean values ± SEM.
Results: The studies were well tolerated. There were no differences in baseline (t = 0 min) systolic BP among the 4 treatments (C: 133 ± 4 mmHg vs. D: 128 ± 3 mmHg vs. A: 125 ± 4 mmHg vs. AD: 130 ± 4 mmHg; P = 0.19). Between t = 3 and 120 min, there was a treatment effect (P = 0.016) for acarbose, so that the maximum fall in systolic BP from baseline was less during treatments with acarbose (A: −10.9 ± 2.7 mmHg and AD: −10.9 ± 2.2 mmHg) compared with control (C: −17.6 ± 3.1 mmHg). There was no difference between the acarbose treatments with or without gastric distension (P = 0.55) and no effect of gastric distension alone (D: −20.8 ± 3.2 mmHg, P = 0.64) (*Figure*).
Conclusion: In healthy older subjects, an acute dose of acarbose (100 mg) attenuates the fall in systolic BP after an oral sucrose load. This effect was not potentiated by concurrent gastric distension. These observations support the use of acarbose, but not gastric distension, in the management of PPH in diabetes.

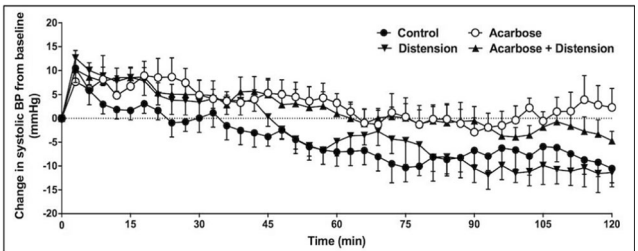


Figure: Acute effects of acarbose (100mg) with and without a 300ml water preload (distension), distension alone and control on the change in systolic BP from baseline after a 100g sucrose drink in healthy older subjects (n=10).

Clinical Trial Registration Number: ACTRN12618000152224
Supported by: RAH project grant
Disclosure: **H. Pham:** None.

1101

Intraocular lens implantation for cataract in type 2 diabetes: The Fremantle Diabetes Study Phase II

J.J. Drinkwater, W.A. Davis, D.G. Bruce, T.M.E. Davis;
School of Medicine, The University of Western Australia, Fremantle, Australia.

Background and aims: Cataracts are a leading cause of visual impairment. Although associated with type 2 diabetes (T2D), there are few contemporary data allowing quantification of the contribution of T2D to cataracts severe enough to warrant intraocular lens (IOL) implantation. This study aimed to compare the incidence of IOL implantation in community-based people with T2D and matched individuals without diabetes.
Materials and methods: The Fremantle Diabetes Study Phase II (FDS2) recruited a representative cohort of 1,499 people with T2D from a postcode defined community between 2008 and 2011. These participants were age-, sex- and postcode-matched to four de-identified people without diabetes randomly selected from the Australian electoral roll who were resident in the same catchment area. Hospitalisation data from the West Australian Data Linkage System were used to determine the cataract-associated IOL status of individuals in both groups during follow-up to end-December 2016. Those with prevalent IOL at baseline were excluded. Age-specific incident rates (IRs) and incident rate ratios (IRRs) for IOL implantation were calculated. A Cox regression model using age as time-scale and diabetes status as the sole independent variable was used to determine the effect of T2D on incident IOL implantation. This was then adjusted for age at baseline, sex, and Charlson's comorbidity index (CCI) excluding diabetes-specific components.
Results: The total eligible sample (n = 6316) comprised 1183 FDS2 and 5133 matched participants without diabetes (mean ± SD age 63.3 ± 11.0 years at entry, 52.6% male). The median [IQR] diabetes duration of those with T2D was 8.7 [2.0–14.0] years. During 6.1 [5.6–7.7] years of follow up, 311 FDS2 (26.3%) and 963 matched participants (18.8%) were hospitalised for first IOL implantation, representing IRs of 44.8 (95% CI 39.9–50.0) and 30.3 (28.4–32.2) per 1000 person-years, respectively. The crude IRR for FDS2 versus matched non-diabetic participants was 1.48 (1.30–1.68). Age-specific IRs, IRRs and incident rate differences are shown in Table 1. The IRRs decreased with increasing age from seven times higher in the 45–54 year age group to 31% higher in 75–84 year olds. The T2D participants had significantly higher IRs in all age-groups, apart from those aged >85 years. T2D increased the risk of IOL by 63% (HR 1.63 (1.43–1.84)) in an unadjusted Cox regression model and by 60% after adjustment (1.60 (1.40–1.81)). Male sex and younger age were associated with a significantly lower risk of IOL (HR 0.84 (0.75–0.94) and 0.81 (0.80–0.82) per year, respectively). There was no significant association between CCI and IOL implantation.
Conclusion: These data show that T2D adds substantially to the high rate of progression of cataracts to IOL implantation in a geographical area with a climate associated with increased ultraviolet light exposure. Younger people with T2D are at particularly high risk and should be encouraged to adopt multi-faceted cataract prevention strategies.

Table 1: Incident rates and incident rate ratios of IOL

Age Group	45-54	55-64	65-74	75-84	85+	45+
IR of IOL in T2D group*	9.19	17.37	53.65	103.74	147.91	45.82
IR in matched cohort*	1.28	8.22	33.93	79.06	84.74	31.39
IRR (Exact 95% CI)	**7.17 (2.07-27.86)**	**2.11 (1.39-3.16)**	**1.58 (1.28-1.94)**	**1.31 (1.06-1.61)**	1.75 (0.88-3.23)	**1.46 (1.28-1.66)**
IRD (Approx. 95%CI)	**7.91 (1.44-14.37)**	**9.16 (3.33-14.98)**	**19.72 (9.81-29.63)**	**24.69 (4.67-44.71)**	63.17 (-20.19 -146.54)	**14.43 (8.92-19.93)**

Abbreviations: IR- incidence rate, IRR-incidence rate ratio, IRD-incidence rate difference, IOL-intraocular lens, T2D- type 2 diabetes
*per 1000 person-years

Supported by: Warren Jones UWA Postgraduate Scholarship, AGRTP Scholarship, NHMRC grants
Disclosure: **J.J. Drinkwater:** Grants; National Health and Medical Research Council Project Grants 51.

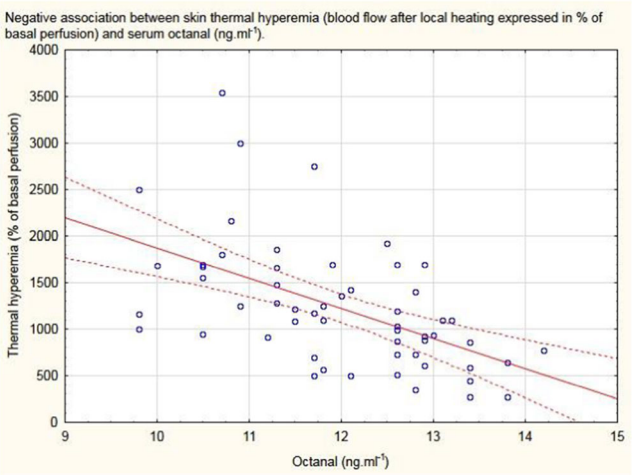
PS 104 Complications and treatment

1102

Lipid peroxidation is associated with impaired vascular function but not with glucose control or variability in type 1 diabetes

M. Prázný[1], J. Škrha jr.[1], T. Pelcl[1], J. Šoupal[1], P. Kačer[2], J. Škrha[1];
[1]3rd Department of Internal Medicine, Charles University in Prague, Prague, [2]BIOCEV, Prague, Czech Republic.

Background and aims: Oxidative stress plays an important role in the development of diabetes complications. It was hypothesized that high glucose variability, a typical clinical feature of Type 1 diabetes (T1DM), may additionally - beyond permanent hyperglycemia - contribute to the generation of oxidative stress. Although it may be useful to identify diabetic patients at high risk for vascular complications using biomarkers and/or functional tests of vasculature, no easy and reliable tests exist so far. To contribute to the development of such test(s), we evaluated the associations between skin microvascular reactivity (MVR), glucose variability (GV) and a novel group of serum biomarkers of oxidative stress - reactive aldehydes formed by lipid peroxidation.
Materials and methods: We included 56 T1DM patients younger than 50 years (mean age 32 ± 8 yrs, HbA_{1C} 62 ± 12 mM/M or 7.8 ± 1.5% DCCT, DM duration 14 ± 6 yrs). Reactive aldehydes with C-chain length from 6 to 12 (hexanal to dodecanal) and malondialdehyde (MDA) were measured by mass spectrometry. Masked continuous glucose monitoring collected glucose data for 12 days to evaluate mean blood glucose and parameters of GV (SD, CV and CONGA). Skin MVR was measured by laser Doppler fluxmetry on the forearm during post-occlusive reactive hyperemia (PORH) and thermal hyperemia (TH). Percent change in flow was calculated from baseline to peak value during stimulations.
Results: PORH was negatively associated with octanal and MDA ($r = -0.48$, $p = 0.0003$ and $r = -0.33$, $p = 0.017$, respectively). Time to maximal perfusion during PORH was negatively associated with nonanal, decanal, undecanal and dodecanal ($r = -0.31$, $p = 0.027$; $r = -0.31$, $p = 0.025$; $r = -0.47$, $p = 0.0005$ and $r = -0.29$, $p = 0.037$, respectively). TH was negatively associated with octanal, nonanal, decanal and MDA ($r = -0.55$, $p < 0.0001$; $r = -0.31$, $p = 0.021$; $r = -0.27$, $p = 0.048$ and $r = -0.43$, $p = 0.001$, respectively). No associations were found between glucose control, GV and reactive aldehydes and similarly, MVR was not associated with glucose parameters as well.
Conclusion: In our cross-sectional observational study, higher levels of reactive aldehydes originating in lipid peroxidation were associated with impaired skin MVR in T1DM. Parameters of glucose control and GV were not associated with lipid peroxidation or MVR in our study. We therefore suggest that other than simple glycemic mechanisms may be probably more important in the process of reactive aldehydes generation in T1DM. As our study was not designed to show causality between lipid peroxidation and vascular dysfunction, further research should evaluate the role of reactive aldehydes in the development and/or prediction of diabetic vascular complications.



Supported by: Q25/LF1/2, AZV 15-26705A
Disclosure: **M. Prázný:** Grants; Q25/LF1/2; 15-26705A.

1103

The p300 modulates eNOS expression in endothelial cells exposed to high glucose in vitro

G. Formoso, P. Di Tomo, P. Lanuti, M.P.A. Baldassarre, C. Pipino, S. Miscia, A. Pandolfi, A. Consoli;
University G. D'Annunzio, Chieti Scalo, Italy.

Background and aims: Vascular functional homeostasis is mainly controlled by endothelial Nitric Oxide Synthase (eNOS) expression and activity. In diabetes, the molecular mechanisms modulating eNOS cellular levels are not fully elucidated. Sirtuin 1 (SIRT1) is a deacetylase enzyme (HDAC) able to modulate histones and transcription factors acetylation in response to the cellular metabolic state. We previous demonstrated that in Human Umbilical Vein Endothelial Cells (HUVECs), 25mM glucose (HG) compared to 5mM (NG) increased cellular levels of both eNOS and SIRT1. Since cellular adaptation mechanisms are the result of a constant balance between the activity of acetylase (HAT) and deacetylase (HDAC) enzymes, aim of the present work was to investigate whether p300, a transcriptional co-activator with HAT activity required in key cellular processes, such as those regulated by Forkhead box protein O1 (FoxO1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), could be modulated by HG.
Materials and methods: HUVECs were obtained from umbilical cord by a standard procedure and were grown in the presence of NG (5mM) or HG (25mM) concentration for 48 hours. Cellular levels of eNOS and SIRT1 were assessed by Western Blotting as well as by Cytometric Analysis. FoxO1 and NF-kB nuclear localization was evaluated by Imaging Flow Cytometric Analysis (AMNIS). Data are expressed as fold increase over the NG condition (± Standard Deviation). Statistical analysis were performed using One-Way ANOVA or Student t-test. Value of $p < 0.05$ were considered statistically significant.
Results: In HG conditions, a significant increase in total and nuclear p300 protein levels ($p < 0.05$) was observed. This was associated with an enhanced total, nuclear and acetylated FoxO1 protein levels as well as with an augmented nuclear translocation of NF-kB p65 ($p < 0.05$). All of these modifications could contribute to increasing eNOS protein levels. Pretreatment with 20 μM Anacardic acid, a specific p300 inhibitor, was able to block ($p < 0.05$) the HG induced increase in total, nuclear and the acetylated FoxO1 protein levels, NF-kB p65 nuclear translocation, as well as the eNOS expression. This suggests that the eNOS transcription is regulated by HAT enzymes, such as p300.
Conclusion: In our cellular model, HG induced an increase in p300 (an HAT) which was necessary for the concomitant modulation of FoxO1 and

NF-kB levels and cellular distribution as well as for the enhanced eNOS protein levels. Thus, the elevated SIRT1 (a HDAC) levels observed after HG exposure could be interpreted as the result of a cellular adaptation in response to the increased p300 HAT activity. Our data extend the available information regarding the mechanisms potentially implicated in the hyperglycemia-induced modulation of the eNOS expression and activity, which in turn is responsible for the altered vascular Nitric Oxide bioavailability in diabetes.

Disclosure: **G. Formoso:** None.

1104

Circulating succinate concentrations are associated with arterial stiffness in type 1 diabetes

J.-M. González-Clemente[1,2], G. Llauradó[3,2], V. Ceperuelo-Mallafré[4,2], A. Cano[1], L. Albert[1], N. Keiran-Fernández[4,2], I. Mazarico[1], S. Fernández-Veledo[4,2], J. Vendrell[4,2];
[1]Department of Endocrinology and Nutrition, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, [2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid, [3]Department of Endocrinology and Nutrition, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, [4]Endocrinology and Nutrition Section, Hospital Universitari Joan XXIII, Institut d'Investigació Sanitària Pere Virgili (IISPV), Universitat Rovira i Virgili, Tarragona, Spain.

Background and aims: Cardiovascular disease (CVD) is the main cause of death in patients with type 1 diabetes mellitus (T1DM). Succinate, a citric acid cycle intermediate, is considered a marker of hypoxia and ischemia. The succinate/SUCNR1 axis has been linked to pathophysiological mechanisms involved in the development and progression of chronic diabetic complications, including CVD. The aim of our study was to evaluate the circulating succinate concentrations and to assess its relationship with arterial stiffness (AS) in T1DM patients.

Materials and methods: Eighty-four patients with T1DM (35–65 years old) and without established CVD were consecutively evaluated for: 1) clinical and anthropometric characteristics (including classical cardiovascular risk factors), 2) microvascular complications, 3) AS, measured by aortic pulse-wave velocity (aPWV) assessed by applanation tonometry (gold standard) and 4) circulating succinate concentrations using a fluorimetric assay. T1DM patients were compared with healthy controls (n = 30), patents with obesity (n = 40) and persons with type 2 diabetes mellitus (T2DM) (n = 20).

Results: Patients with T1DM [age 50.1 ± 9.3 years, 50% men, 36.9% active smokers, T1DM duration 19.0 (15.9–27.5), BMI 26.0 ± 4.2 kg/m^2 and HbA_{1c} 7.9 (7.1–8.7)%] had an increase in circulating succinate concentrations as compare to healthy subjects (85.5 (68.7–108.4) vs. 22.0 (11.5–39.2); p < 0.001), but these concentrations were not different from those in patients with obesity (85.5 (68.7–108.4) vs. 81.0 (65.0–96.5); p = 0.809) or with T2DM (85.5 (68.7–108.4) vs. 118.5 (89.5–135.0); p = 0.131). These results did not change after adjusting for age, gender and BMI. In T1DM, circulating succinate concentrations were positively associated with BMI (r = 0.288, p = 0.009) and aPWV (r = 0.280, p = 0.010). aPWV was one of the main independent variables associated with circulating succinate concentrations after adjusting for classical cardiovascular risk factors (β = 0.267; p = 0.014).

Conclusion: Patients with type 1 diabetes mellitus and without established cardiovascular disease have an increase in circulating succinate concentrations as compared to healthy controls. This increase is similar to that found in persons with obesity or with type 2 diabetes mellitus. Additionally, this increase is positively correlated with arterial stiffness, which supports a potential role of succinate in the development of cardiovascular disease in type 1 diabetes mellitus.

Supported by: PI15/00567 (National R+D+I and ISCIII/GEB-ERDF)

Disclosure: **J. González-Clemente:** None.

1105

Oral glycine treatment attenuated AGE-RAGE-ROS axis by restoring glyoxylase system in the aorta of diabetic rats

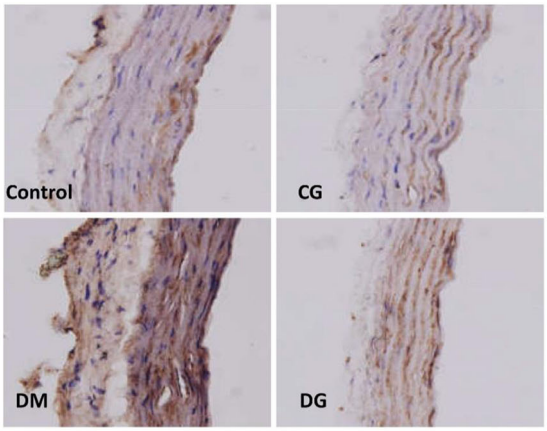
Z. Wang, J. Zhang, L. Chen, J. Li, H. Zhang, X. Guo;
Peking University First Hospital, Beijing, China.

Background and aims: The accumulation of advanced glycation endproducts (AGEs) can activate the receptor of AGEs (RAGE) and induce oxidative stress, thus underlying macrovascular complications in diabetes mellitus. Extensive studies have found that glyoxalase-1 (Glo-1) and its cofactor glutathione (GSH) can degrade a major precursor of AGEs, thus protecting against AGEs formation. Glycine is a crucial precursor of glutathione synthesis and its protective effects against oxidative stress have been reported. In this study, we aimed to investigate the effects of glycine on the AGE-RAGE-ROS axis in the aorta of diabetic rats and the possible underlying mechanisms.

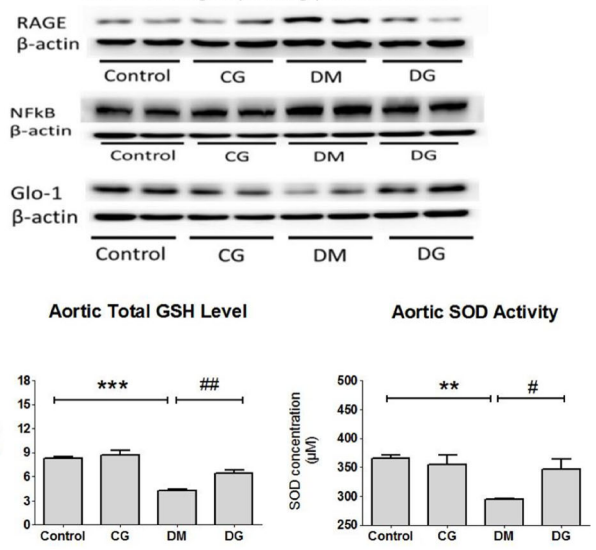
Materials and methods: The STZ-induced diabetic rats were treated with or without glycine (1% in drinking water) for 12 weeks. GSH, 3-nitrotyrosine, MDA and SOD were measured in the aorta homogenates. The AGEs in the aorta were measured by ELISA and immunohistology. The expressions of aortic RAGE, NF-κB and Glo-1 were analyzed by western blot and immunohistology.

Results: Compared with the control group, the expressions of AGEs (p < 0.05), RAGE (p < 0.01) and NF-κB (p < 0.001) exacerbated in diabetic rats, but were attenuated after glycine treatment (p < 0.05, p < 0.01, p < 0.01 respectively). The levels of aortic 3-nitrotyrosine and MDA increased in the diabetic rats (p < 0.05), but were down-regulated in the glycine-treated group (p < 0.05). The SOD activity in the aorta decreased in the diabetic group (p < 0.01), but was elevated after glycine treatment (p < 0.05). The expression and activity of Glo-1 (p < 0.01) and the levels of GSH (p < 0.001) decreased in the diabetic group, but were restored significantly after glycine treatment (p < 0.05, p < 0.01 respectively).

Conclusion: Our results showed that oral glycine treatment might work by promoting GSH synthesis and enhancing the activity and expression of Glo-1 to suppress both the formation of AGEs and the activation of AGEs-RAGE-ROS axis, thus protecting against the macrovascular complications in diabetic rats.



Immunostaining of aortic AGEs. Control: healthy rats receiving water. CG: control rats with glycine treatment. DM: diabetic group with water; DG: diabetic group with glycine treatment.



*p<0.05, **p<0.01,***p<0.001 *vs.* control group; #p<0.05, #p<0.01, ###p<0.001 *vs.* DM group.

Disclosure: **Z. Wang:** None.

1106

Glucose-dependent insulinotropic polypeptide suppresses arterial remodelling in mice: role of a calcium-mediated signalling pathway in vascular endothelial cells

Y. Mori, H. Kushima, M. Koshibu, T. Saito, M. Hiromura, K. Kohashi, M. Terasaki, T. Hirano;
Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine, Tokyo, Japan.

Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) exhibits direct cardiovascular actions in addition to its well-known insulinotropic effect. However, the role of GIP in peripheral artery disease remains unclear. Here we evaluated effects of GIP against peripheral arterial remodelling in mouse models.
Materials and methods: Nine-week-old male wild-type (C57BL/6) and GIP receptor knockout (GIPR-KO) mice were randomly assigned to treatment with either vehicle or GIP (50 mmol kg^{-1} day^{-1}) on Day 1, and subsequently subjected to left femoral artery wire injury to induce arterial remodelling on Day 3. Mice were killed by an overdose of anaesthesia and the injured arteries were collected to assess morphometric changes on Day 29, or endothelial regeneration on Days 4, 8, 13, and 17. Human umbilical vein endothelial cells (HUVECs) were used for western blotting and measurement of intracellular cAMP production, cytosolic calcium levels, and nitric oxide (NO) production.
Results: No difference was detected in physiological and biochemical parameters between the treatment groups. In the assessment of morphometric changes, the genetic deletion of GIPR led to exaggerated neointimal hyperplasia after arterial injury (1.3-fold). In contrast, GIP (1-42) treatment suppressed neointimal hyperplasia compared with vehicle treatment by 50%. We further assessed the effects of GIP on endothelial regeneration. Endothelial cells determined as being CD31-positive were almost completely absent from the lumen 1 days after wire insertion. Subsequently, endothelial cells gradually covered the lumen (5 and 10 days), mainly starting from the uninjured area, with endothelial regeneration being completed at 14 days after the injury. In comparison with vehicle treatment, GIP (1-42) treatment significantly increased the endothelial cell-covered area of the lumen assessed at 5 days after the injury. (1.5-fold). In HUVECs, GIPR protein levels were significantly higher than in human aortic smooth muscle cells, and GIP (1-42) increased cytosolic calcium levels without affecting intracellular cAMP levels. GIP also dose-dependently increased NO production, which was completely abrogated by inhibiting AMP-activated protein kinase (AMPK). Furthermore, GIP increased phosphorylation of AMPK along with endothelial NOS. GIP-induced AMPK phosphorylation was abolished by inhibiting phospholipase C or calcium-calmodulin-dependent protein kinase kinase, but not adenylate cyclase or liver kinase B1, suggesting the existence of a calcium-mediated GIPR signalling pathway. In high-glucose-culturing conditions (25 mmol/l), GIPR protein levels of HUVECs were significantly decreased compared with those in normal-glucose-culturing conditions (5.5 mmol/l). However, GIP still significantly increased AMPK phosphorylation under high-glucose conditions, even though this effect was slightly albeit not significantly (two-way ANOVA: interaction, $p = 0.25$) blunted compared with that observed normal glucose conditions (55% vs 35%).
Conclusion: We demonstrated that the activation of GIPR exhibits protection against peripheral arterial remodelling in mice, and the involvement of a novel calcium-mediated GIPR signalling pathway in vascular endothelial cells.
Supported by: Scholarship donation from MSD, Novatis, and Sanofi
Disclosure: **Y. Mori:** None.

1107

The cardiovascular benefits associated with liraglutide in the LEADER trial are sustained when analysing both first and recurrent MACE

S. Verma[1], S.C. Bain[2], T. Idorn[3], S. Rasmussen[3], D.D. Ørsted[3], M.A. Nauck[4], LEADER Publication Committee on behalf of the LEADER Trial Investigators;
[1]Division of Cardiac Surgery, Li Ka Shing Knowledge Institute of St. Michael's Hospital, University of Toronto, Toronto, Canada, [2]Swansea University Medical School, Swansea, UK, [3]Novo Nordisk A/S, Søborg, Denmark, [4]Diabetes Center Bochum-Hattingen, St Josef Hospital (Ruhr-Universität Bochum), Bochum, Germany.

Background and aims: In patients with type 2 diabetes and high risk for cardiovascular (CV) events, the LEADER CV outcomes trial (N = 9340) showed risk of a first major adverse CV event (MACE) was reduced with liraglutide vs placebo when added to standard of care. Here we further examined liraglutide treatment effects on both first and recurrent ("total observed") CV events, including: 1) a composite MACE endpoint: CV death, non-fatal stroke or non-fatal myocardial infarction; 2) expanded MACE (also included coronary revascularisation and hospitalisation for heart failure or unstable angina); and 3) individual CV endpoints.

Materials and methods: A post hoc analysis utilising an extension of Cox regression modelling of time to event data, with additional sensitivity analyses.
Results: In total, 1302 first and 303 recurrent MACEs occurred: liraglutide, 735 events; placebo, 870 events. Risk for total observed MACEs was reduced by 14% with liraglutide vs placebo (HR = 0.86, 95% CI 0.78–0.95). Corresponding analyses for all other CV endpoints suggested risk reductions with liraglutide, with the exception of hospitalisation for unstable angina (Table). Sensitivity analyses using other regression models confirmed the results (Table).
Conclusion: The reduction in risk of first event with liraglutide in LEADER was sustained in this post hoc analysis; this is of clinical relevance to individuals who are at risk of or who have experienced a MACE, and confirms the robustness of the data.

	First and recurrent ("total observed") CV endpoints				First CV endpoints only
Outcome	**No. of events** (lira vs placebo)	**HR*** (lira/placebo) **[95% CI]**	**Sensitivity analysis 1 (Prentice-Williams-Peterson model)† HR** (lira/placebo) **[95% CI]**	**Sensitivity analysis 2 (Wei-Lin-Weissfeld model) HR** (lira/placebo) **[95% CI]**	**Cox regression model HR** (lira/placebo) **[95% CI]**
MACE	735 vs 870	0.86 [0.78; 0.95]	0.87 [0.78; 0.95]	0.85 [0.77; 0.94]	0.87 [0.78; 0.97]
Expanded MACE	1721 vs 1958	0.92 [0.86; 0.99]	0.91 [0.86; 0.98]	0.90 [0.84; 0.96]	0.88 [0.81; 0.96]
CV death	219 vs 278	NA	NA	NA	0.78 [0.66; 0.93
Non-fatal stroke	174 vs 199	0.87 [0.71; 1.07]	0.88 [0.72; 1.08]	0.89 [0.72; 1.09]	0.89 [0.72; 1.11]
Non-fatal MI	342 vs 393	0.88 [0.76; 1.02]	0.90 [0.77; 1.04]	0.87 [0.75; 1.01]	0.88 [0.75; 1.03]
Coronary revascularisation	503 vs 559	0.93 [0.82; 1.05]	0.92 [0.81; 1.03]	0.92 [0.81; 1.04]	0.91 [0.80; 1.04]
Hospitalisation for heart failure	342 vs 389	0.94 [0.81; 1.10]	0.96 [0.83; 1.11]	0.93 [0.80; 1.08]	0.87 [0.73; 1.05]
Hospitalisation for unstable angina	141 vs 140	1.01 [0.80; 1.28]	1.01 [0.80; 1.27]	1.00 [0.79; 1.26]	0.98 [0.76; 1.26]

*Andersen-Gill intensity model, adjusted for previous events as a continuous time-dependent covariate. †Based on total time to event. CI, confidence interval; CV, cardiovascular; lira, liraglutide; MACE, major adverse cardiovascular event; MI, myocardial infarction; NA, not applicable.

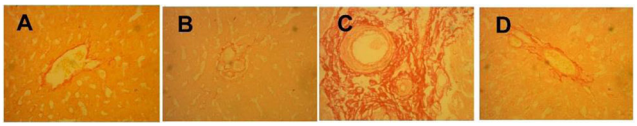
Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **S. Verma:** Non-financial support; Abstract funding: Novo Nordisk A/S.

1108

Raw yogurt supplementation prevents the dyslipidaemia, glucose intolerance and oxidative stress induced liver dysfunction in high fat diet fed obese rats

S.A. Nishad, J.F. Tisha, S. Lasker, F. Parvez, M. Zamila, M.M. Rahman, M.C. Shill, H.M. Reza, M.A. Alam;
Pharmaceutical Science, North South University, Dhaka, Bangladesh.

Background and aims: The main objective of this experiment was to determine and expose the responses of Yogurt supplementation on oxidative stress, diabetes, inflammation and fibrosis in High-Carbohydrate and high-fat (HCHF) diet induced obese rats.
Materials and methods: Twenty eight Wistar male rats of 175–195 g were divided into four groups: Control, Control + Yogurt, HCHF and HCHF + Yogurt. After 8 weeks of treatment with Yogurt (5% of powdered chow diet w/w) all animals were weighed and sacrificed.Blood and tissue samples were collected to evaluate biochemical assay as well as histological staining of liver tissue sections were also done.
Results: Yogurt supplementation showed improved blood glucose tolerance in HCHF + Yogurt group compared to the HCHF group. We have seen that liver enzymes ALP, ALT and AST were increased in HCHF group which is significantly normalized in HCHF + Yogurt group rats. Moreover, Yogurt supplementation also exhibited a significant reduction of the oxidative stress markers such as MDA, NO, and APOP level.Yogurt supplementation also prevented inflammatory cells infiltration, collagen deposition and fibrosis in HCHF diet fed rats.
Conclusion: The findings of this study suggest that Yogurt supplementation prevents metabolic syndrome as well as nonalcoholic fatty liver disease in rats.



Disclosure: **S.A. Nishad:** None.

1109

Trends of infections in adults with and without diabetes, U.S. 2000–2014

J. Harding[1], E. Gregg[1], M. Pavkov[1], L. Perreault[2];
[1]Centers for Disease Control and Prevention, Atlanta, [2]University of Colorado Denver School of Medicine, Aurora, USA.

Background and aims: People with diabetes (DM) are at an increased risk for infections compared with people without DM. As cardiovascular disease (CVD) and mortality complications of DM continue to decline, it is unknown if non-CVD complications, such as infections, are also decreasing. We estimated infection-related hospitalizations during 2000–2014 in adults aged ≥18 years with and without DM in the general U.S. population
Materials and methods: Infection-related hospitalisation rates were calculated using the National Inpatient Sample for the number of discharges (ICD-9 CM primary diagnosis code: 001-139, 480-486, 041.12, 682, 785.4, 040.0, 590.0; 060-066; 080-088; 042, 997.31, 136.9; 998.5) and the National Health Interview Survey for population estimates, by diabetes status. Joinpoint regression was used to assess trends over time.
Results: Among men with DM, age-standardised hospitalization rates per 1,000 persons, increased from 21.7 (95%CI: 20.4–23.0) in 2000 to 28.0 (27.1–28.9) in 2014, and from 9.2 (9.0–9.4) to 10.3 (10.1–10.4) in men without DM. Among women with DM, rates increased from 24.9 (23.5–26.2) in 2000 to 32.5 (31.5–33.4) in 2014, and from 8.5 (8.7) to 10.1 (10.0–10.3) in women without DM. All trends were significant $p < 0.05$, Figure.
Conclusion: Overall, rates of infection-related hospitalisations have increased since 2000. Adults with DM have greater excess risk of infection-related hospitalisations than those without DM, and this excess risk has increased over time.

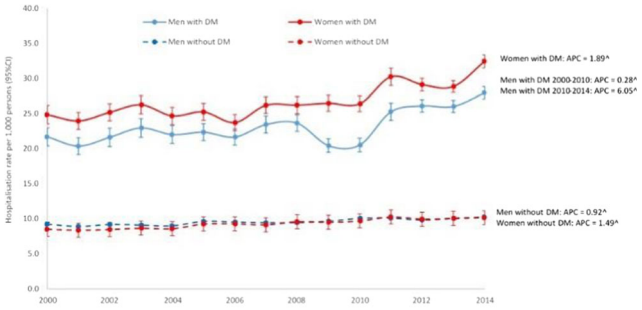


Figure Age-standardized rates of infection-related hospitalisations, per 1,000 persons, in men and women with and without diabetes between 2000 and 2014.
APC = Annual Percent Change. ^Indicates that the APC is significantly different from zero at alpha = 0.05

Disclosure: **J. Harding:** None.

PS 105 Bones and muscles

1110

The longitudinal association between type 2 diabetes and fractures in a large Dutch cohort of older women

P.J.M. Elders[1], T. Merlijn[1], A.A. van der Heijden[1], G. Nijpels[1], C. Netelenbos[2], K. Swart[1];
[1]General Practice and Elderly Care, VU University Medical Centre, Amsterdam, [2]Internal Medicine, Endocrine Section, VU University Medical Centre, Amsterdam, Netherlands.

Background and aims: Type 2 diabetes mellitus (T2DM) has been associated with an increased fracture risk. Glycemic control might be of influence. We examined the association of T2DM and fractures during a 3-yr follow up period in a large primary care sample of older women.
Materials and methods: Data were used from a randomized pragmatic trial among 25,613 women aged 65–90 years in a primary care setting. All participants with ≥1 established risk factors for fractures ($n = 11,331$) and a subsample of the participants without risk factors was followed up ($n = 5020$), including all remaining participants with T2DM. Outcomes were assessed by patient questionnaires after 18 and 36 months and fractures were verified in medical records. Self-reported baseline T2DM was verified with GP medical records, from which baseline HbA1c concentrations were derived as well. Baseline vertebral fractures assessment and BMD measurements were done in a subsample of participants with ≥1 risk factors for fractures ($n = 4310$). The main outcome was fractures over the 3-yr follow up period. Secondary outcomes were hip fractures, falling, baseline vertebral fractures, BMD of the hip and the lumbar spine. Depending on the outcome, Cox, logistic, or linear regression models were used to examine the associations.
Results: T2DM was verified in 1,578 out of 11,331 (13.9%) participants with ≥1 risk factors for fractures with a mean HbA1c of 51.7 mmol/mol. In the selective subsample of participants without risk factors for fractures 1,052 out of 5020 (21.0%) participants had T2DM, with a mean HbA1c of 49.3 mmol/mol. Oral anti-diabetes drugs was used in 78.5% and insulin in 15.4% of the participants with T2DM. Among the participants with ≥1 risk factors for fractures, 163 out of 1,578 (10.3%) participants with T2DM sustained a fracture versus 1,101 out of 9,537 (11.5%) participants without T2DM. T2DM was not associated with incident fractures of any type (adjusted HR = 1.00, 95% CI = 0.84 to 1.19). A significantly higher BMD of the hip and the lumbar spine was observed in the women who underwent a BMD measurement (Table). Among the women without risk factors for fractures, 70 out of 1,052 (6.7%) participants with T2DM sustained a fracture versus 380 out of 3968 (9.6%) participants without T2DM. T2DM was associated with a reduced fracture risk (adjusted HR = 0.68, 95%CI = 0.52 to 0.88) (Table). HbA1c among women with T2DM (analysed in quartile categories) was not associated with any of the outcomes.
Conclusion: T2DM was not associated with fractures in older women who already were identified with an increased fracture risk based on other factors. Moreover, women without fracture risk factors and T2DM had a reduced fracture risk. We postulate that this reduced risk is mediated through a higher BMD.

Table. Results of regression analyses of the association between T2DM and fracture related outcomes among women with and without established clinical risk factors for fractures.

	Prospective fractures HR (95%CI)	Prospective hip fractures HR (95%CI)	Prospective falling OR (95%CI)	Baseline vertebral fractures OR (95%CI)	Baseline BMD hip B (95%CI)	Baseline BMD lumbar spine B (95%CI)
Sample with ≥1 clinical risk factors for fractures (N=11331)						
Model 1	0.90 (0.77 to 1.06)	1.20 (0.88 to 1.65)	1.26 (1.03 to 1.55)	0.76 (0.57 to 1.02)	0.07 (0.06 to 0.08)	0.08 (0.06 to 0.09)
Model 2	1.00 (0.85 to 1.19)	1.30 (0.94 to 1.80)	1.03 (0.83 to 1.27)	0.79 (0.59 to 1.07)	0.04 (0.03 to 0.05)	0.03 (0.02 to 0.05)
Sample without clinical risk factors for fractures (N=5020)						
Model 1	0.68 (0.53 to 0.88)	0.68 (0.53 to 0.88)	0.97 (0.63 to 1.51)	-	-	-
Model 3	0.68 (0.52 to 0.88)	0.66 (0.32 to 1.36)	0.86 (0.55 to 1.35)	-	-	-

95%CI= 95% confidence interval; B= regression coefficient; BMD= bone mineral density; HR= hazard ratio; OR= odds ratio

Model 1: unadjusted

Model 2: adjusted for age, body mass index, a fracture history after age 50 years, a hip fracture in the family, rheumatoid arthritis, early menopause, malabsorption syndrome, chronic liver disease, and immobility.

Model 3: adjusted for age and body mass index (the other factors are all not applicable)

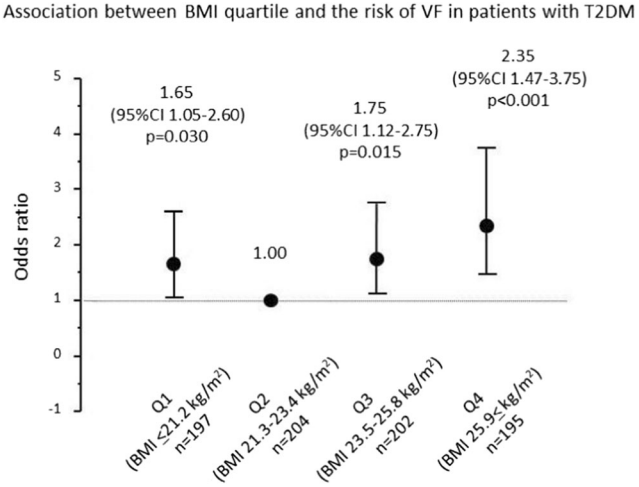
Clinical Trial Registration Number: NTR2430
Supported by: Stichting Achmea Gezondheidszorg
Disclosure: **P.J.M. Elders:** None.

1111

Association of body mass index with the risk of vertebral fractures in patients with type 2 diabetes

I. Kanazawa, M. Notsu, K.-I. Tanaka, T. Sugimoto;
Shimane University Faculty of Medicine, Izumo, Japan.

Background and aims: Several studies suggest that obesity may be a risk factor for fracture although the relationship between body mass index (BMI) and fracture risk is unknown in type 2 diabetes (T2DM). We thus aimed to examine the association between BMI and the prevalence of vertebral fracture (VF) in Japanese patients with T2DM.
Materials and methods: In this cross-sectional study, 798 subjects (500 men and 298 women) with T2DM were enrolled. Bone mineral density (BMD) of femoral neck (FN) was measured by the dual-energy X-ray absorptiometry. VF was defined by semi-quantitative method using lateral X-ray films of the thoracic and lumbar spine. The association of BMI quartiles (Q1; ≤21.2 kg/m^2, Q2; 21.3–23.4 kg/m^2, Q3: 23.5–25.8 kg/m^2, Q4; ≤25.9 kg/m^2) with the presence of VF was examined.
Results: According to BMI increase, urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX), a bone resorption marker, was significantly decreased, and FN-BMD, FN-T score, and FN-Z score were significantly increased. Subjects in Q2 quartile had less prevalence of VF (29.4%) compared to others in Q1 (43.1%), Q3 (28.6%), and Q4 (41.5%). Multiple logistic regression analyses adjusted for age, gender, duration of diabetes, HbA1c, estimated glomerular filtration rate, and serum albumin showed that Q1, Q3, and Q4 were significantly associated with an increased VF risk compared to Q2 as a reference [Q1; odds ratio (OR) = 1.91, 95% confidence interval (CI) 1.24–2.95, $p = 0.004$, Q3; OR = 1.65, 95%CI 1.07–2.55, $p = 0.023$, and Q4; OR = 2.18, 95%CI 1.39–3.41, $p < 0.001$]. Moreover, these associations remained significant after additional adjustment for femoral neck T score and uNTX (Figure). When the associations were examined separately in men and women, same tendencies were observed as the results of total subjects although some associations became insignificance because the number of the subjects was reduced.
Conclusion: This is the first study to show that both overweight and underweight were associated with the BMD-independent risk of VF in patients with T2DM. Therefore, body weight modification should be considered to protect diabetes-related bone fragility.



Multiple logistic regression analysis adjusted for age, gender, duration of diabetes, HbA1c, eGFR, albumin, FN-T score, and uNTX was performed with the presence of VF as a dependent variable and BMI quartile as an independent variable.

Disclosure: **I. Kanazawa:** None.

1112

Diabetes is associated with elevated risks of osteoarthritis, osteoporosis and rheumatoid arthritis

S. Molsted[1], A.-S.D. Bjørkman[1], M.B. Andersen[1], O. Ekholm[2];
[1]Department of Clinical Research, Nordsjaellands University Hospital, Hillerød, [2]National Institute of Public Health, University of Southern Denmark, København, Denmark.

Background and aims: Previous studies have reported elevated prevalence of musculoskeletal pain in patients with type 2 diabetes compared to age and gender matched general populations or non-diabetes populations. Musculoskeletal pain may be barriers to exercise training, which is important in the diabetes treatment. The question remains as to whether the pains are the results of an elevated prevalence of arthritis. The aim of this study was to investigate the association between diabetes (DM) and osteoarthritis (OA), osteoporosis (OP) and rheumatoid arthritis (RA).
Materials and methods: All data were self-reported and provided by the Danish National Health Survey 2013. The exposure variable was DM and the outcome variables included arthritis, back pain, shoulder/neck pain, and physical activity. Multiple logistic regression analyses adjusted for age, gender and BMI were performed.
Results: In total 109,218 individuals (≥40 years old) were included. Diabetes was reported by 9238 (8.5%), aged 65.6 ± 11.0 (mean ± SD) years, males 55.6%, and the BMI was 28.9 ± 5.5 kg/m^2. In those without DM ($n = 99{,}980$) the mean age was 59.2 ± 11.8 years, males 46.7%, and the BMI was 25.8 ± 4.4 kg/m^2. In individuals with DM vs those without DM, OA was reported by 43.5% vs 29.4%, $p < 0.0001$, OP by 6.4% vs 4.8%, $p < 0.0001$, and RA by 15.1% vs 7.6%, $p < 0.0001$, respectively. Back pain was reported by 60.6% vs 51.4%, $p < 0.0001$, and shoulder/neck pain was reported by 56.0% vs 51.5%, $p < 0.0001$, in individuals with and without DM, respectively. Diabetes was associated with OA (OR 1.33 (95% CI 1.25–1.41)), OP (1.29 (1.13–1.46)), and RA (1.71 (1.57–1.85)). Diabetes was associated with back pain 1.27 (1.21–1.34) and shoulder/neck pain 1.29 (1.22–1.36). In a sub analysis of those with DM, being physically active ($n = 6220$ (71.6%)) was inversely associated with back pain 0.65 (0.57–0.73) and shoulder/neck pain 0.76 (0.68–0.86).
Conclusion: Diabetes was associated with significantly elevated odds of having arthritis and musculoskeletal pain. The most frequent arthritis in individuals with DM was OA. The most pronounced association was found between DM and RA. The association between DM and RA in

this study may not be a link between the autoimmune diseases type 1 diabetes and RA as the majority of the individuals with DM may have had type 2 diabetes as result of the exclusion of individuals with an age below 40 years. The link between diabetes and RA may be a result of the chronic inflammation that is present in the two diseases. Another hypothesis of the association between DM and RA could be linked to medication. Whilst steroids are used in the treatment of RA, steroids also increases the risk of the development of type 2 diabetes. Furthermore, pains from RA may also increase the risk of physical inactivity, which is a type 2 diabetes risk factor. The reported pains may have negative impacts on the level of physical activity in individuals with diabetes. Health care professionals should remember to inform patients with DM, that musculoskeletal pain and arthritis not are contra-indications to exercise training. Thus, as exercise training is a recognized element in the treatment of DM and arthritis, it may have positive effects on glycemic control and musculoskeletal pain at the same time.
Disclosure: **S. Molsted:** None.

1113

Age-influenced higher cellular RAGE sensitivity associated with enhanced apoptosis and impaired osteogenic differentiation in type 2 diabetes

M. Phimphilai[1], P. Pothachareon[2], P. Kongtawelert[2];
[1]Internal Medicine, Chiang Mai University, Chiang Mai, [2]Biochemistry, Chiang Mai University, Chiang Mai, Thailand.

Background and aims: Preclinical studies have demonstrated impaired osteoblast differentiation in type 2 diabetes (T2DM), which is related to skeletal accumulation of advanced glycation end products (AGEs). Our previous study also showed impaired osteogenic differentiation in peripheral blood-derived mononuclear cell (PBMC) taken from patients with T2DM, which might be related to higher cellular receptor of AGEs (RAGE) sensitivity and cellular apoptosis. One of the potential mechanisms of that higher RAGE sensitivity is RAGE-dependent NF-κB activation that perpetuates *AGER* (RAGE) expression in a feed-forward manner, entailing NF-κB amplification and cellular apoptosis. This study aimed to further elucidate whether higher cellular RAGE sensitivity enhanced cellular apoptosis and retarded osteogenic differentiation, as well as to elucidate whether higher cellular RAGE sensitivity occurred in NF-κB-dependent manner.
Materials and methods: This cross-sectional study included 40 patients with T2DM and 30 age-matched non-diabetic controls (NDM). Venous blood was collected to measure serum pentosidine and isolate PBMC.
Results: Ninety percent of PBMC-isolated from NDM expressed osteoblast-specific genes including *RUNX2/PPAR*, *ALPL*, *COL1A1* and *BGLAP* (NDM-D) while only 40% of PBMC-derived from T2DM expressed those genes (DM-D). That 40% of cells in DM-D expressed *RUNX2/PPAR*, *ALPL*, *COL1A1* and *BGLAP* higher than that of PBMC-derived from DM with poor osteogenic differentiation (DM-ND) by 3.8, 7.3, 5.9 and 4.3 folds, respectively. Multivariate analysis demonstrated that being diabetes increased the risk for osteogenic differentiation impairment by 13.5 folds (OR 13.5; 95% CI 3.21–77.91; $p < 0.001$). By using age- and pentosidine-matched NDM-D as a reference group, *AGER* and *BAX/BCL2* expression in DM-ND were 6.6 and 5 folds higher than reference while the expression of those genes in DM-D were similar to those of reference, suggesting the existing of higher cellular RAGE sensitivity and apoptosis only in DM-ND. In contrast to the expression of *AGER* and *BAX/BCL2*, the expression of *IκB-α*, *IL1-β* and *TNF-α* was similar in both DM-D and DM-ND comparing to reference, suggesting that *NFκB*-associated genes were not overexpressed in DM-ND. Interestingly, *AGER* expression positively correlated with age ($r = 0.470$, $p = 0.003$). Multivariate analysis demonstrated that age, *AGER* and *BAX/BCL2* expression were factors determining osteogenic differentiation potential of the PBMC-derived from DM.
Conclusion: This study demonstrated *AGER* and *BAX/BCL2* overexpression only in PBMC-isolated from diabetes with poor osteogenic differentiation. Therefore, this study not only demonstrated the existing of higher cellular RAGE sensitivity but also strengthened the linkage between defect in osteogenic differentiation and either higher cellular RAGE sensitivity or apoptosis. That higher RAGE sensitivity increased with age and was conceivably occurred in NFκB-independent manner. Being diabetes was an independent risk factor of osteogenic differentiation impairment while age, higher cellular RAGE sensitivity and apoptosis were factors influencing osteogenic differentiation in type 2 diabetes.
Clinical Trial Registration Number: NCT02286128
Supported by: Merck
Disclosure: **M. Phimphilai:** Grants; Merck.

1114

Musculoskeletal impairment in patients with type 2 diabetes and arthritis is associated with beta cell dysfunction

O.P. Zaharia[1,2], D. Pesta[1,2], P. Bobrov[3,2], Y. Kupriyanova[1,2], K. Bódis[1,2], Y. Karusheva[1,2], J.-H. Hwang[1,2], V. Burkart[1,2], K. Müssig[1,4], J. Szendroedi[1,4], M. Roden[1,4];
[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, [2]German Center for Diabetes Research (DZD), München-Neuherberg, [3]Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, [4]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany.

Background and aims: Abnormal insulin secretion may affect the integrity of musculoskeletal structures in patients with type 2 diabetes (T2D). We hypothesized that in patients with T2D and arthritis (T2+A) musculoskeletal impairment is inversely associated with beta-cell function. To this end, we compared patients T2+A with T2D patients without arthritis (T2-A) and healthy humans (control, CTRL).
Materials and methods: Analyses included participants of the German Diabetes Study: T2+A: $n = 12$, age 62 ± 9 years, BMI 34.8 ± 7.2 kg/m^2; T2-A: $n = 22$, 59 ± 13 years, 32.8 ± 6.5 kg/m^2 and CTRL: $n = 18$, 42 ± 16 years, 25.3 ± 4.1 kg/m^2. Arthritis-related symptoms were assessed using validated questionnaires (Western Ontario and McMaster Universities Osteoarthritis Index). Knee extension force (KEF) and grip strength were measured by dynamometry and range of motion (ROM) by goniometry. Participants underwent glucagon-stimulation and intravenous glucose tolerance tests (IVGTT) to assess beta-cell function and hyperinsulinemic-euglycemic clamp tests for whole body insulin sensitivity (WBIS). Statistical analyses were adjusted for age, sex and BMI.
Results: Glycemic control was similar in T2+A and T2-A (HbA1c; 53 ± 6 vs. 51 ± 11 mmol/mol), but impaired compared to CTRL (33 ± 3 mmol/mol, both $p < 0.05$). WBIS was comparable in T2+A and T2-A, but approximately 2.5-fold lower than in CTRL (both $p < 0.05$). Across all groups, WBIS inversely correlated with scores for joint pain, stiffness and functional limitations ($r = -0.62$, $p < 0.05$). Beta-cell function, as assessed from C-peptide rise following glucagon stimulation (ΔC-peptide), was comparable in T2+A and T2-A (2.8 ± 1.3 vs. 3.3 ± 1.4 ng/ml) and lower than in CTRL (3.5 ± 1.6 ng/ml, both $p < 0.05$). ROM in T2+A patients was 8% lower compared to T2-A and 19% lower compared to CTRL (both $p < 0.05$). In T2+A patients, ROM was negatively correlated with total C-peptide secretion in IVGTT ($r = -0.64$, $p < 0.05$). KEF in T2+A patients was 18.7 ± 11.4 kg, 2.5-fold lower than in CTRL (46.9 ± 11.7 kg, $p < 0.05$) and was inversely correlated with ΔC-peptide ($r = -0.99$, $p < 0.05$). Grip strength and KEF were highly correlated in all groups ($p < 0.0001$). Consistently, in T2+A patients, grip strength also negatively correlated with parameters of beta-cell function (ΔC-peptide, $r = -0.62$, $p < 0.05$).

Conclusion: Patients with type 2 diabetes and arthritis exhibit lower muscle strength and range of motion, both of which associate with impaired beta-cell function.
Clinical Trial Registration Number: NCT01055093
Supported by: BMBF, DZD
Disclosure: **O.P. Zaharia:** None.

1115

Diabetes can accelerate sarcopenia in the diaphragm

M. Yamamoto[1], M. Takemoto[2], A. Matsuzaki[3], H. Masuyama[4], M. Koshizaka[1], Y. Maezawa[1], K. Yokote[1];
[1]Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, [2]School of Medicine, International University of Health and Welfare, Department of Diabetes, Metabolism and Endocrinology, Chiba, [3]International University of Health and Welfare, Ichikawa Hospital, Department of Urology, Chiba, [4]International University of Health and Welfare, Ichikawa Hospital, Department of Respiratory Internal Medicine, Chiba, Japan.

Background and aims: The diaphragm is a skeletal muscle that is involved not only in respiration but also in swallowing and lymphatic functions and maintaining posture. Diaphragm dysfunction may lead to a decrease in the activities of daily living, especially in the elderly, and this lower physical activity may in turn negatively affect blood glucose metabolism. Only a few studies have described diaphragm thickness in patients with diabetes. Diaphragm thickness is usually evaluated using ultrasonography (US). However, because evaluation of diaphragm thickness using US is not routinely performed, it is difficult to assess it in a retrospective manner. Therefore, in this study, we developed a new method to evaluate diaphragm thickness in patients with diabetes using computed tomography (CT) and evaluated the correlation between US and CT findings. Then, we evaluated the relationship among diaphragm thickness, age, and blood glucose metabolism.
Materials and methods: We prospectively evaluated the diaphragm thickness of 50 patients with diabetes using both US and CT. The relationship between the US and CT findings were evaluated using Spearman's rank correlation coefficient. Diaphragm thickness using US was evaluated between the 9th and 10th intercostal space in the neutral spine position, and the results were expressed as a sum of left and right measurements. The diaphragm thickness using CT was evaluated in the zone of apposition on contact with the thoracic cavity between the most frontal and dorsal positions, and the results were expressed as a sum of left and right measurements.
Results: The mean diaphragm thickness was 3.35 ± 0.64 mm and 3.73 ± 1.00 mm using US and CT, respectively. The diaphragm thicknesses evaluated using both US and CT were significantly correlated with each other ($r = 0.3809$, $P = 0.0063$). Then, we studied the data of 106 patients with tuberculosis who had undergone CT and had a high morbidity of diabetes. The diaphragm thickness was positively correlated with body weight ($r = 0.2911$, $P = 0.0088$) and negatively with age and HbA1c ($r = -0.4493$, $P < 0.0001$; $r = -0.5507$, $P < 0.0001$, respectively). Moreover, the diaphragm thickness was significantly lesser in patients with diabetes than in those without diabetes.
Conclusion: A thin diaphragm has been found to be related to poor prognosis among patients with acute pneumonia in intensive care units. Our results indicate that diabetes can accelerate sarcopenia of the diaphragm. Because the diaphragm has multiple functions, sarcopenia of the diaphragm in patients with diabetes might affect prognosis.
Disclosure: **M. Yamamoto:** None.

1116

Sarcopenia is associated with decreased insulin secretion and increased arterial stiffness in Japanese elderly patients with type 2 diabetes

S. Moyama[1,2], Y. Hamamoto[1,2], H. Kuwata[1,3], T. Kurose[2], D. Yabe[2], Y. Seino[2,3];
[1]Center for Metabolism and Clinical Nutrition, Kansai Electric Power Hospital, Osaka, [2]Center for Diabetes Research, Kansai Electric Power Medical Research Institute, Osaka, [3]Center for Diabetes, Endocrinology and Metabolism, Kansai Electric Power Hospital, Osaka, Japan.

Background and aims: It is known that factors such as inflammatory stress and insulin resistance in addition to the aging relate to the loss of skeletal muscle mass and arterial stiffness, but yet the underlying mechanisms are still unknown. Asian patients with type 2 diabetes mellitus (T2DM) are characterized by non-obese, decreased insulin secretion and less-insulin resistance compared to Caucasian, and the pathophysiology of sarcopenia and atherosclerotic disease in Asian T2DM remain unclear. The aim of this study is to examine the relationship between sarcopenia and insulin secretion/sensitivity and arterial stiffness in Japanese elderly patients with T2DM.
Materials and methods: This was a cross-sectional study. The subjects aged 65 years or older hospitalized for diabetes education during the period of 2016 and 2017 ($n = 108$; male = 54.6%, mean age 72.4 ± 5.3 years). The exclusion criteria were: (i) using insulin or steroid; (ii) having overt chronic diabetic complications; (iii) uncontrolled cardiovascular, pulmonary or peripheral artery disease. The grip strength (GS) and body composition (SMI; skeletal muscle index, PBF; percent body fat) were measured using the standard handgrip dynamometer and the bioelectricity impedance measuring device (InBody S10). Based on the diagnostic criteria of the Asian Working Group for Sarcopenia, subjects were divided into 3 groups; sarcopenia group (loss of SMI and GS), pre-sarcopenia group (loss of SMI or GS) and non-sarcopenia group (normal SMI and GS). We evaluated the arterial stiffness by brachial-ankle pulse wave velocity (baPWV) adjusted by age, gender and systolic blood pressure based on the previous report. We examined the relationship between sarcopenia/adjusted baPWV and age, body mass index, SMI, PBF, glycemic control (HbA1c), insulin secretion [the homeostasis model assessment of beta-cell function (HOMA-β), 24h-urinary C-peptide immunoreactivity (CPR), C-peptide index (CPI)] and insulin resistance [the insulin resistance index of HOMA (HOMA-IR)].
Results: The prevalence of sarcopenia (Group S), pre-sarcopenia (Group PS) and the rest (Group NS) were 17.6%, 16.7% and 62.0%, respectively. HbA1c, HOMA-IR and HOMA-β were not significantly different among 3 groups. However, 24h-urinary CPR and CPI in Group S were significant lower than those in Group NS. Furthermore, adjusted baPWV was higher in Group S compared to Group NS. The logistic regression analysis revealed the association of sarcopenia with age and adjusted baPWV.
Conclusion: Our data suggest that sarcopenia was associated with decreased insulin secretion and increased arterial stiffness in Japanese elderly patients with T2DM, suggesting its underlying mechanisms in this population.

Table. Comparisons of insulin resistance, insulin sensitivity and arterial stiffness

	Group S (n=19)	Group PS (n=18)	Group NS (n=56)
HOMA-IR	1.9 ± 1.0	2.7 ± 2.4	2.6 ± 1.5
HOMA-β	27.7 ± 16.5	39.2 ± 29.4	40.9 ± 35.1
24h-urinary CPR (μg)	56.9 ± 29.5**	83.8 ± 72.4	106.2 ± 44.6
C-peptide index	1.1 ± 0.5*	1.6 ± 0.9	1.5 ± 0.7
adjusted baPWV	14.6 ± 14.9*	9.0 ± 17.7	5.5 ± 12.4
Odds ratio (95% confidence interval) for the risk of sarcopenia from the logistic regression analysis			
Age	1.15 (1.03-1.27)		
HbA1c	1.35 (0.87-2.08)		
adjusted baPWV	1.06 (1.02-1.10)		

Values are expressed as mean ± standard deviation, *P < 0.05, **P < 0.01 (vs. Group NS)

Disclosure: **S. Moyama:** None.

1117

Type 2 diabetes, body mass index and cancer mortality: a population-based matched cohort study

N.N. Alam[1], A.K. Wright[2,3], M.K. Rutter[2,4], D. Ashcroft[3], M. Sperrin[5,6], A.G. Renehan[1];
[1]Division of Cancer Sciences, University of Manchester, Manchester, [2]Endocrinology and Gastroenterology, University of Manchester, Manchester, [3]Centre for Pharmacoepidemiology and Drug Safety, University of Manchester, Manchester, [4]Manchester Diabetes Centre, Manchester University NHS Foundation Trust, Manchester, [5]Farr Institute of Health Informatics Research, University of Manchester, Manchester, [6]Centre for Health Informatics, University of Manchester, Manchester, UK.

Background and aims: Compared to people without diabetes, those with type 2 diabetes (T2D) have higher cancer mortality - which is the second commonest cause of death in these individuals. Obesity may explain the link between T2D and cancer mortality. Here, we test the hypotheses that: (i) most cancer deaths are from obesity-related cancers (ORCs); and (ii) among individuals with T2D, there is a positive association between BMI (at diagnosis of T2D) and cancer mortality.
Materials and methods: Using the UK Clinical Practice Research Datalink, an incident cohort of 176,886 patients with T2D was derived and was matched to 852,946 controls without diabetes; all people had linked records for hospitalisation and mortality (1998 to 2015). The primary outcome was cancer morality, sub-divided into 13 ORCs or non-ORCs defined by the International Agency for Research on Cancer (2016). The secondary outcome was all-cause mortality. We derived gender-specific hazard ratios (HRs) and 95% confidence intervals (CIs), using Cox models, to determine: (i) the impact of T2D on risks for ORCs, non-ORCs and mortality relative to controls; and (ii) the associations of BMI with cancer mortality among individuals with T2D.
Results: Compared with the control population, T2D was associated with higher risk of total cancer mortality in men (HR: 1.22, 95% CI: 1.18–1.26) and women (1.31, 1.26–1.37), and a higher risk of death from ORCs in men (1.84, 1.72–1.96) and women (1.47, 1.39–1.56). T2D was associated with increased risk of death from non-ORCs in men (1.06, 1.02–1.11) and women (1.18, 1.12–1.25). Among 145,769 individuals with BMI recorded before T2D diagnosis, BMI was *negatively* related to all-cause mortality (obesity paradox), and we observe an inverse relationship with total cancer mortality. There was no association between BMI and mortality from ORCs combined, though there were strong positive associations for mortality from certain ORCs, such as endometrial cancer (e.g. obese II [BMI 35.0–39.9] versus normal, HR: 4.40, 1.51–12.84).
Conclusion: The findings support the notion that obesity-related mechanisms contribute to the higher cancer mortality in T2D compared to the general population. However, the elevated risk for non-ORC deaths suggests that other pathways are involved. In people with T2D, BMI predicts cancer mortality for only some ORC sub-types; a finding that requires further investigation.
Supported by: MCRC
Disclosure: **N.N. Alam:** None.

PS 106 From metabolism to vascular function

1118

Increased methylglyoxal protein modification in hyperglycaemia induces an inflammatory phenotype in endothelial cells by activation of the unfold protein response

M. Xue, Z. Irshad, A. Ashour, P.J. Thornalley, N. Rabbani;
Clinical Sciences Research Laboratories, University Hospital, Translational Medicine Section, Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, UK.

Background and aims: Endothelial cells in hyperglycemia develop an inflammatory phenotype characterized by increased inflammatory signalling, expression of adhesion molecules, secretion of inflammatory cytokines and processes supporting atherosclerosis. They accumulate methylglyoxal (MG) and MG-derived advanced glycation endproduct (AGE)-modified proteins in hyperglycemia - suppression of which prevents development of the inflammatory phenotype. Our aim is to identify cell signalling involved in promotion of the inflammatory phenotype by increased cellular MG protein glycation.
Materials and methods: Human aortal endothelial cells (HAECs) were incubated in primary culture with 5 mM (model normoglycemia) or 20 mM glucose (model hyperglycemia) glucose for 6 days. Activities of glyoxalase 1 (Glo1), MG reductase and MG dehydrogenase was assessed by spectrophotometric assay. For Glo1 knockdown studies, HAECs were transfected with GLO1 siRNA or non-targeting control siRNA. Cellular MG and cell protein content of major MG-derived AGE, MG-H1, was determined by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Markers of unfolded protein response (UPR) activation in the cytosol and endoplasmic reticulum (ER), heat shock protein 70 (HSP70) and glucose regulated protein-78 (GRP78), were assayed by Western blotting. Interleukin-8 (IL-8) was assayed by ELISA.
Results: When HAECs were incubated in high glucose concentration, the cellular content of MG was increased 2-fold, compared to low glucose concentration control (2.27 ± 0.21 versus 1.29 ± 0.03 pmol/10^6 cells, $n = 3$; $P < 0.01$). There were concomitant increases in cell protein MG-H1 content and flux of MG-H1 free adduct excretion into the culture medium. Glo1 activity of HAECs was 1862 ± 178 mU/mg protein and decreased 21% in high glucose concentration cultures. MG reductase and MG dehydrogenase activities were undetectable. High glucose concentration increased cellular HSP70 and GRP78 by 37% and 51%, respectively ($P < 0.01$), indicating activation of the UPR in cytosol and ER. Silencing of Glo1 had a similar effect in low glucose concentration controls and potentiated increase of HSP70 and GRP78 in high glucose concentration cultures, indicating that the UPR is activated by increased MG-modified proteins. UPR activation is linked to increased histone-lysine N-methyltransferase SET7 expression and inflammatory signalling, prevented by Glo1 overexpression in high glucose concentration cultures. A marker of this is secretion of IL-8 which was increased in high glucose cultures (28.2 ± 1.2 versus 16.5 ± 2.6 ng/ml, +71%, $P < 0.01$). Increased inflammatory response in high glucose concentration cultures was corrected by treatment with inducer of Glo1 expression, *trans*-resveratrol-hesperetin (tRES-HESP) combination.
Conclusion: Increased MG and MG-derived AGE formation in endothelial cells promotes an inflammatory phenotype through activation of the UPR. The UPR provides surveillance of proteome quality; the challenge of increased MG glycation proteome damage triggers inflammatory signalling. This provides a link between cellular AGE accumulation and vascular inflammation, implicated in vascular complications of diabetes for which tRES-HESP may provide a new approach to therapy.
Disclosure: **M. Xue:** None.

1119

Higher plasma methylglyoxal levels are associated with incident cardiovascular disease and mortality in individuals with type 2 diabetes

N.M.J. Hanssen[1], J. Westerink[2], J.L.J. Scheijen[1], Y. Van der Graaf[2], C.D.A. Stehouwer[1], C.G. Schalkwijk[1], SMART study group;
[1]Maastricht University, Maastricht, [2]University Medical Center Utrecht, Utrecht, Netherlands.

Background and aims: The dicarbonyl compounds methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG) are byproducts of glycolysis and highly reactive. Of these, MGO in particular has been identified as a potential key player in diabetic cardiovascular disease (CVD). Therefore, several compounds that inhibit formation of dicarbonyl compounds are under active investigation. Whether plasma dicarbonyl levels are associated with CVD in type 2 diabetes is unknown.
Materials and methods: We included 1003 individuals (mean age 59.1 ± 10.5 years, 69.3% male, 61.6% prior CVD) with type 2 diabetes from the Second Manifestations of ARTerial disease cohort (SMART). We measured plasma MGO, GO and 3-DG levels at baseline with mass spectrometry. Median follow-up of CVD events was 8.6 years. Data were analyzed with Cox regression, adjusting for sex, age, smoking, systolic blood pressure, total cholesterol, HbA1c, BMI, prior CVD and use of lipid-modifying, and blood pressure- and glucose-lowering medication. Missing data were replaced using a multiple imputation method. Hazard ratios (HR) are expressed per standard deviation Ln-transformed dicarbonyl.
Results: 287 individuals suffered from at least one CVD event ($n = 194$ fatal events, $n = 146$ myocardial infarctions, $n = 72$ strokes); 346 individuals died and 60 individuals underwent an amputation. Higher MGO levels were associated with total (HR: 1.26; 95%CI: 1.11–1.42) and fatal CVD (1.49; 1.30–1.71), and with all-cause mortality (1.25; 1.11–1.40), myocardial infarction (1.22; 1.02–1.45) and amputations (1.36; 1.05–1.76). MGO levels were not apparently associated with stroke (1.03; 0.79–1.35). Higher GO levels were significantly associated with fatal CVD (1.17; 1.00–1.37), but not with other outcomes. 3-DG was not significantly associated with any of the outcomes.
Conclusion: Plasma MGO and GO levels are associated with cardiovascular mortality in individuals with type 2 diabetes. Influencing dicaronyl levels may therefore be a target to reduce CVD in type 2 diabetes.
Suported by: Dutch Heart Foundation, Dutch Diabetes Research Foundation, EFSD
Disclosure: **N.M.J. Hanssen:** None.

1120

Carbamylated HDL and all-cause mortality in type 2 diabetes

K. Tan, S. Shiu, J. Lam, A. Lee, Y. Wong;
Medicine, University of Hong Kong, Hong Kong, Hong Kong.

Background and aims: Carbamylation, a process of post-translational modification of proteins, causes alterations in the structural and functional properties of proteins and contributes to the progression of chronic diseases like renal and cardiovascular disorders. Although carbamylation is previously considered only quantitatively important in uremic conditions, recent studies have shown that carbamylation of proteins can take place even in the absence of uremia by an alternative mechanism mediated by myeloperoxidase. We have previously shown that lipoproteins are subjected to carbamylation in diabetes and carbamylated HDL (cHDL) is dysfunctional. We aim to evaluate whether cHDL is associated with all-cause mortality in patients with type 2 diabetes.
Materials and methods: Plasma cHDL concentration was measured in the baseline samples of 990 type 2 diabetic patients followed up in a teaching hospital specialist diabetes clinic by an in-house sandwich ELISA using polyclonal rabbit anti-human cHDL antibody. All-cause mortality was ascertained from hospital electronic medical records.
Results: During a mean follow-up of 14 years, 102 subjects died from all causes. Baseline plasma cHDL levels were significantly higher in subjects with a fatal outcome (45.7 ± 19.6 ug/ml versus 35.6 ± 16.3, $p < 0.01$) whereas there were no significant differences in HDL-cholesterol levels (1.17 ± 0.30 mmol/l versus 1.22 ± 0.33 respectively). Plasma cHDL was a significant independent predictor of all-cause mortality even after adjustment for age, gender, body mass index, duration of diabetes, smoking, systolic blood pressure, HbA1c, LDL-cholesterol, cardiovascular disease and lipid lowering therapy at baseline ($p < 0.001$, odds ratio 1.027, 95% CI 1.016–1.038).
Conclusion: Elevated plasma cHDL was independently associated with all-cause mortality in patients with type 2 diabetes, and cHDL may play a pathological role and contributes to the adverse outcome.
Disclosure: **K. Tan:** None.

1121

Modulation of diagnostic strategy and treatment resulting from coronary artery calcium score assessment in type 2 diabetic patients

S. Charriere[1,2], C. Marsot[1,3], L. Balaire[1,3], M. Moret[1], S. Villar-Fimbel[1], A. Lecus[1], A. Villard[1], P. Douek[4,3], P. Moulin[1,2];
[1]Endocrinology department, cardiovascular hospital, Hospices Civils de Lyon, Lyon, [2]Lyon 1 university, INSERM U1060, Lyon, [3]Lyon 1 university, Lyon, [4]Radiology department, cardiovascular hospital, Hospices Civils de Lyon, Lyon, France.

Background and aims: New ESC-EASD guidelines propose coronary artery calcium score (CAC) to assess cardiovascular (CV) risk and prefilter functional explorations of silent coronary insufficiency in patients with type 2 diabetes (T2D). The aim of our study was to study the influence of the CAC score assessment on subsequent CV explorations and prescription of CV prevention therapies in a cohort of T2D patients in clinical practice.
Materials and methods: A retrospective monocentric study, including 461 T2D patients aged 45–85 years old in primary prevention, who consecutively had a CAC assessment between in jan-2015 and dec-2016, was conducted in our center. Demographic data, diabetes duration, microvascular complications, all treatments (including oral antidiabetic agents, hypolipidemic drugs, anti-hypertensive drugs, anti-aggregants) and subsequent CV explorations were systematically recorded from the electronic files (exhaustively was above 98%).
Results: Patients were 62.3 ± 8.9 (mean ± SD) years-old, with a 13.6 ± 9.1 years of diabetes duration. They had 2.1 ± 0.8 additional CV risk factors. CAC distribution is CAC 0-9 44%, CAC 1-299 35%, CAC ≥300 21%. In the group CAC ≥300, patients were older, with a longer diabetes duration, had more hypertension and microvascular complications, and were more frequently treated by insulin, statins, aspirin than in the two other groups ($p < 0.05$). In multivariate analysis, the CAC score was significantly associated with age, sex, diabetes duration, HTA and diabetic nephropathy ($p < 0.05$). In the group CAC ≥300, 75% of patients underwent a functional CV test, versus 21.6% in the group CAC 10-299 and 1.4% in the group CAC 0-9 ($p < 0.001$). In the group CAC ≥300, only 24% of patients had a significant myocardial ischemia and only 10% of positive coronary angiography. Whereas the prescription of renin-angiotensin system blocking drugs was not significantly influenced by the CAC score ($p = 0.169$), statins and anti-aggregants prescriptions were markly increased after the CAC from 66% to 92% ($p < 0.001$) for statins and from 38.5 to 99% for anti-aggregants ($p < 0.001$).
Conclusion: In clinical practice, the CAC score assessment can contribute to pre-select patients with an increased probability of for silent coronary insufficiency. However, the screening efficiency remains low in our cohort. Nevertheless, the CAC assessment appears very useful to guide clinicians for selectively intensifying CV prevention treatment such as statin or aspirin. The efficiency of such strategy based on CAC assessment remains to be tested in a randomized clinical trial.
Disclosure: **S. Charriere:** None.

1122

Change in circulating levels of endothelial progenitor cells in young adults with type 1 diabetes: a 2-year follow-up from the observational METRO study

M. Maiorino, G. Bellastella, M. Caputo, P. Cirillo, V. Pernice, M. Longo, K. Esposito;
Department of Medical, Surgical, Neurological, Metabolic, Sciences and Aging, University of Campania "Luigi Vanvitelli", Naples, Italy.

Background and aims: Diabetes mellitus is characterized by a defective mechanisms of vascular repair caused by an impaired regenerative capacity of endothelial progenitor cells (EPCs). EPCs reduction may represent one of the mechanisms linking the elevated vascular risk to diabetes mellitus. The aim of this study was to evaluate the change in circulating levels of EPCs in a population of young type 1 diabetic patients over a period of 2 years. To this purpose we used data of the Management and technology for Transition (METRO) study, a longitudinal observational study of type 1 diabetic patients in transition from the pediatric clinic to the adult diabetes care center.
Materials and methods: The study population included 204 type 1 diabetic patients aged 18–30 years which completed a 2-year follow-up: 84 were treated with continuous subcutaneous insulin infusion (CSII) and the remaining 120 were treated with multiple daily injections of insulin (MDI). Circulating levels of seven EPCs phenotypes were determined by flow cytometry. All clinical and biochemical measurements, including the indexes of glycemic control and glucose variability, lipid profile, and blood pressure, were collected at baseline and after 2 years.
Results: At baseline, both CSII and MDI groups were well matched for demographic and clinical characteristics. Similarly, EPCs cell counts did not differ between the two groups. There was a significant reduction of mean amplitude of glucose excursion (MAGE) (mean difference within group −1.1 ± 2.1 mmol/L, $P < 0.001$), and blood glucose standard deviation (BGSD) (mean difference within group −0.3 ± 1.1 mmol/L, $P < 0.001$) in the CSII group but not in MDI group. All EPCs phenotypes, except CD34+CD133+ cells, increased in CSII group, with significant differences as compared to MDI group regarding CD34+ [mean difference between groups 21, 95%CI (5 to 37)], CD34+KDR+ [13, 95%CI (5 to 20)], and CD34+KDR+CD133+ [6, 95%CI (2 to 10)] cell count. At univariate analysis, change in MAGE and SD negatively correlated with change in EPCs levels in CSII group, but not in MDI group. There was no association between changes in all clinical variables evaluated and EPCs in both groups. Multivariable regression analysis adjusted for age, smoking, BMI, and weight identified the reduction of MAGE as an independent predictor for increasing levels of both circulating CD34+ ($P = 0.020$) and CD34+KDR+ ($P = 0.004$) cell count ($P = 0.022$).
Conclusion: Over a 2 year follow-up, young type 1 diabetic patients treated with insulin pump showed an increase in circulating EPCs levels, which correlated with the improvement in glucose variability.
Disclosure: **M. Maiorino:** None.

1123

Pulse wave velocity is an independent risk factor for cardiovascular events, mortality and progression in diabetic kidney disease in patients with type 1 diabetes

T.W. Hansen[1], M. Frimodt-Møller[1], S. Theilade[1], S.A. Winther[1], N. Tofte[1], T.S. Ahluwalia[1], P. Rossing[1,2];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]University of Copenhagen, Copenhagen, Denmark.

Background and aims: The prognostic significance of carotid-femoral pulse wave velocity (cfPWV) - the gold standard measure of arterial stiffness - remains to be determined in patients with type 1 diabetes (T1D). We investigated the predictive value of cfPWV for development of cardiovascular events (CVE), mortality and decline in renal function in T1D.
Materials and methods: Prospective study including 652 patients with T1D and various degrees of albuminuria, ranging from normoalbuminuria (<30 mg/24 h), microalbuminuria (30–299 mg/24) to macroalbuminuria (≥300 mg/24 h). cfPWV was measured at baseline using the SphygmoCor device. Endpoints were traced through National Registers until 31st December 2016 and comprised: a composite CVE (cardiovascular death, non-fatal myocardial infarction, non-fatal stroke and coronary or peripheral arterial interventions); mortality; progression from normo- to micro/macroalbuminuria or from micro-to macroalbuminuria; and decline in estimated glomerular filtration rate (eGFR) ≥30%. Median follow-up ranged between 5.2–6.2 years. Slope estimates of eGFR and urinary albumin excretion rate (UAER) were calculated for a median of 5.5 years. Adjustment included sex, age, mean arterial pressure, LDL cholesterol, smoking, HbA_{1c}, UACR and eGFR at baseline. Hazard ratios (HR) were calculated per 1 standard derivation (SD) increase in cfPWV.
Results: Of the 652 participants, 363 (56%) were male; mean ± SD age was 54 ± 13 years, cfPWV 10.5 ± 3.38 m/s and eGFR 81 ± 26 ml/min/1.73 m^2. Median numbers of eGFR and UACR measures during follow-up were 6.0 and 17.0, respectively. Mean ± SD yearly decline in eGFR was 0.9 ± 2.5 ml/min/year and the median (interquartile range) of yearly change in UACR was −3.5 (−13.0–8.7)%. Higher cfPWV was associated with an increased risk of all endpoints in unadjusted analyses ($p \leq 0.0005$). After adjustment, higher cfPWV remained significantly associated with all endpoints: composite CVE ($n = 81$; HR: 1.31; $p = 0.045$); all-cause mortality ($n = 48$; HR: 1.39; $p = 0.033$); progression from normo- to micro/macroalbuminuria or from micro-to macroalbuminuria ($n = 95$; HR: 1.31; $p = 0.036$); and decline in eGFR ≥30% ($n = 90$; HR: 1.39; $p = 0.015$). Higher cfPWV was associated with a steeper decline in eGFR and a steeper increase in UACR in both unadjusted ($p \leq 0.002$) and adjusted ($p \leq 0.009$) analyses.
Conclusion: In patients with T1D, higher arterial stiffness was consistently associated with at higher risk of CVE, mortality and progression of diabetic kidney disease independent of other risk factors. Measurement of cfPWV may have a promising role in risk stratification in T1D.
Clinical Trial Registration Number: 2009-056
Disclosure: **T.W. Hansen:** None.

1124

The visceral adiposity index predicts cardiovascular events both in cardiovascular disease patients with and in those without diabetes

C.H. Saely[1,2], A. Vonbank[1,2], C. Heinzle[2], D. Zanolin[2], B. Larcher[1,2], A. Mader[1,2], A. Leiherer[2,3], A. Muendlein[2,3], H. Drexel[2,4];
[1]Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [2]Vorarlberg Institute for Vascular Investigation and Treatment, Feldkirch, Austria, [3]Private University in the Principality of Liechtenstein, Triesen, Liechtenstein, [4]Division of Angiology, Swiss Cardiovascular Center, University Hospital Berne, Berne, Switzerland.

Background and aims: The visceral adiposity index (VAI) is a validated tool for the evaluation of visceral adiposity, using waist circumference, serum triglycerides, age and gender to diagnose this metabolic abnormality. It has recently been associated with cardiovascular risk in primary care patients. No data are available on the association of the VAI with mortality in patients with cardiovascular disease (CVD).
Materials and methods: We therefore prospectively recorded the incidence of cardiovascular events over a mean follow-up period of 7.9 ± 3.1 years in a large cohort of 1858 consecutive patients with established cardiovascular disease (1599 patients with angiographically proven coronary artery disease and 259 patients with sonographically proven peripheral artery disease). The VAI was calculated according to the Amato formula; type 2 diabetes (T2DM) was defined according to the ADA Definition.
Results: At baseline, the VAI was significantly higher in CVD patients with T2DM than in those who did not have diabetes (347 ± 331 vs. 228 ± 200; $p < 0.001$). Prospectively, 585 vascular events occurred; the event rate was significantly higher in patients with T2DM than in those who did

not have diabetes (46.8% vs. 31.3%; $p < 0.001$). After multivariate adjustment, the VAI significantly predicted cardiovascular events in CVD patients with T2DM (standardized adjusted hazard ratio (HR) 1.24 [1.09–1.42]; $p = 0.007$) as well as in those who did not have T2DM (HR 1.18 [1.06–1.31]; $p = 0.014$).

Conclusion: We conclude that the VAI predicts cardiovascular events both in CVD patients with and in those without diabetes.

Disclosure: **C.H. Saely:** None.

1125

Effects of variability in blood pressure, glucose and cholesterol concentrations, and body mass index on mortality and cardiovascular outcomes in the general population

S.-H. Lee[1], M. Kim[1], K. Han[1], Y.-M. Park[2], H.-S. Kwon[1], K.-H. Yoon[1]; [1]The Catholic University of Korea, Seoul, Republic of Korea, [2]National Institutes of Health, Research Triangle Park, USA.

Background and aims: Variability in metabolic parameters, such as fasting blood glucose (FBG) and cholesterol concentrations, blood pressure (BP), and body weight can affect health outcomes. We investigated whether variability in these metabolic parameters has additive effects on the risk of cardiovascular outcomes in the general population.

Materials and methods: Using nationally representative data from the Korean National Health Insurance System, 6,748,773 people who were free of diabetes mellitus, hypertension, and dyslipidemia, and who underwent three or more health examinations during 2005–2012 were followed to the end of 2015. Variability in FBG and total cholesterol (TC) concentrations, systolic BP, and body mass index (BMI) was measured using the coefficient of variation (CV), standard deviation (SD), and variability independent of the mean (VIM). High variability was defined as the highest quartile of variability. Participants were classified numerically according to the number of high variability parameters; e.g., a score of 4 indicated high variability in all four metabolic parameters.

Results: There were 54,785 deaths (0.8%), 22,498 cases of stroke (0.3%), and 21,452 myocardial infarctions (MIs) (0.3%) during a median follow-up of 5.5 years. The risk of all-cause mortality, MI, and stroke increased significantly with the number of high-variability metabolic parameters. In the multivariable adjusted model comparing a score of 0 versus 4, the hazard ratios (95% confidence intervals) were 2.19 (2.03–2.36) for all-cause mortality, 1.56 (1.36–1.79) for stroke, and 1.47 (1.26–1.72) for MI. These relationships were independent of the baseline FBG, systolic BP, TC, and BMI values. Similar results were obtained when modeling the variability using the SD and VIM.

Conclusion: High variability of glucose and lipid levels, BP, and BMI was an independent predictor of cardiovascular events. There was a dose-response relationship between the number of high-variability parameters and cardiovascular outcomes.

Supported by: National Research Foundation of Korea

Disclosure: **S. Lee:** None.

PS 107 Big vessels

1126

Vascular effects of raivaroxaban compared to aspirin in type 2 diabetic patients with high cardiovascular risk

F. Pistrosch[1,2], H. Mehling[3], C. Axthelm[4], F. Schaper[5], E. Henkel[2], I. Weigmann[2], K. Kreutzfeld[2], U. von der Osten[6], C. Köhler[2], A.L. Birkenfeld[1,2], M. Hanefeld[2];
[1]Medizinische Klinik 3, Universitätsklinik Carl Gustav Carus, Dresden, [2]GWT TU Dresden GmbH, Dresden, [3]Studienzentrum Charite Berlin, Berlin, [4]Cardiologicum Pirna, Pirna, [5]Diabetesschwerpunktpraxis, Dresden, [6]Bayer Pharma GmbH, Leverkusen, Germany.

Background and aims: Endothelial dysfunction is most likely involved in both initiation and propagation of arteriosclerosis. Recent studies with the direct factor Xa inhibitor Rivaroxaban (RIV) in combination with low dose Aspirin (ASS) demonstrated a greater reduction of major cardiovascular events compared to ASS alone in patients with proven cardiovascular disease. Therefore we asked the question whether treatment with RIV could influence endothelial function in a high risk population for development of arteriosclerosis.

Materials and methods: We conducted a multi-centre prospective randomized open label study in type 2 diabetic patients with high cardiovascular risk and subclinical inflammation to compare the effects of RIV 5 mg b.i.d. vs. ASS 100 mg q.d. on forearm blood flow during reactive hyperemia (measured by forearm occlusion plethysmography), skin blood flow during reactive hyperemia (measured by laser Doppler fluxmetry) and arterial stiffness (measured by pulse wave velocity).

Results: 164 patients (mean age 64.2 ± 7.4 yr, mean diabetes duration 10.9 ± 5.2 yr) were eligible for analysis of the primary objective - the change of forearm blood flow during reactive hyperemia (20 weeks of treatment - baseline measurement) between ASS ($n = 81$) and RIV ($n = 83$) treatment. Baseline post-ischemic forearm blood flow was not different between treatment groups. As shown in figure 1, there was a significant improvement of post-ischemic forearm blood flow after RIV ($p = 0.016$; ANOVA for repeated measures) compared to ASS. Laser Doppler fluxmetry revealed a comparable finding: skin blood flow during reactive hyperemia increased by 11.7 ± 66.9 units after 20 weeks of RIV treatment compared to −8.4 ± 54.1 units after ASS ($p = 0.043$). Pulse wave velocity did not differ between RIV and ASS after 20 weeks of treatment (9.3 ± 1.3 m/s vs. 9.4 ±1.2 m/s). There were more bleeding events with RIV treatment (8 patients) compared to ASS (2 patients).

Conclusion: Treatment with direct factor Xa inhibitor RIV resulted in a significant improvement of forearm blood flow and skin blood flow during reactive hyperemia - a marker of endothelial function. Our findings suggest that besides anticoagulatory potency RIV has pleiotrophic effects on vascular function. However treatment with RIV was associated with an increased risk of bleeding.

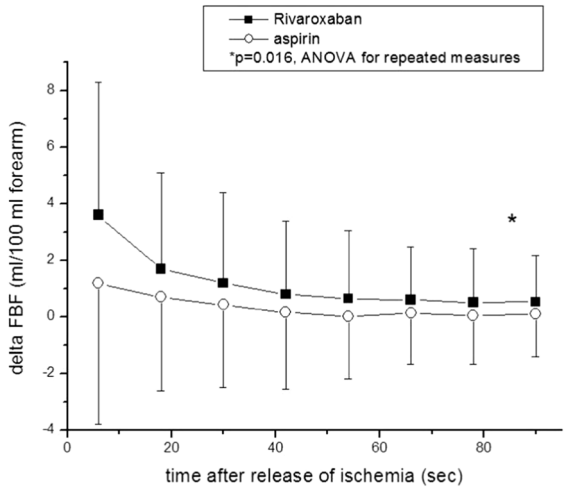


Figure 1: Post-ischemic forearm blood flow: difference between 20weeks of treatment and baseline measurement.

Clinical Trial Registration Number: NCT02164578
Supported by: Bayer provided a grant for conducting the study
Disclosure: **F. Pistrosch:** Grants; Bayer provided a grant for conducting the study.

1127

Second-hand-smoking and cardiovascular risk of women with type 2 diabetes

M. Gourine[1], K. Bentadj[2], S. Mostefa-Kara[2], A. Cherrak[3], S. Halimi[4], M. Belhadj[5];
[1]Internal Medicine, Medical School of Oran/University Hospital of Oran, Oran, Algeria, [2]Cardiovascular private hospital Kara, Oran, Algeria, [3]Internal Medicine, Medical School/University Hospital of Oran, Oran, Algeria, [4]University Joseph Fourier, Medical school, Grenoble, France, [5]Internal Medicine, Medical School, Oran, Algeria.

Background and aims: The increased relative risk of cardiovascular disease for women by diabetic status is not fully explained with the own presence of cardiovascular risk factors. As observed with active smoking, second hand smoking is linked to numerous adverse health effects, such as CHD. Nonetheless, it is not systematically looked for and is not included in the cardiovascular risk scores. A better understanding of the role of second hand smoking of women exposed to smoke is needed. So, our study aimed to investigate whether second hand smoking is linked with cardiovascular disease in type 2 diabetes women.
Materials and methods: We conducted a multicentre prospective case control study between January 2014 and June 2015 in outpatient clinic. Women with confirmed type 2 diabetes, aged 30 years or older, not pregnant and without history of neoplasia were included. Cases were defined as those who had a new confirmed diagnosis of cardiovascular disease. Cardiovascular disease was considered in the presence of acute coronary syndrome, silent myocardial infarction displayed at ECG, angina confirmed by a non-invasive test, stroke (ischemic, haemorrhagic or transitory), or peripheric vascular disease, diagnosed for less than 3 months. Controls were free from any cardiovascular disease. All the patients gave their fully oral informed consent for participating. We used the software SPSS.20 for all the statistical analysis.
Results: 714 patients met the inclusion criteria. Active smoking was not widespread among both groups (2 patients in the case group and 4 patients for controls). Finally, analyses were restricted to the 708 non-smokers women: 235 cases and 473 controls. Cases and controls shared similar characteristics on education, occupation and marital status. Patients with a cardiovascular disease were slightly older, had a longer duration of diabetes and a worse glycaemic control than controls. Hypertension, hypercholesterolemia, sedentary lifestyle, family history of CHD, microvascular complications were significantly more frequent in the case group when compared with the control group. Our study also disclosed that 16.9% of women were exposed to second-hand smoking. It was more often encountered in the case group (32.8%) than in the control group. After conditional logistic regression, second hand smoking was significantly associated with cardiovascular disease **(crude OR 4.87; CI95%: 3.22–7.38 ; $p < 10^{-3}$),** even after multiple adjustment **(adjusted OR 4.77; CI95%: 2.84–8.02).** *(Table 1)*
Conclusion: Second-hand smoking is frequent among algerian women with type 2 diabetes. It leads to a greater risk of cardiovascular disease and should be considered for individual risk evaluation in type 2 diabetic women.

Second hand smoking	OR	CI 95%	p-value
Unadjusted	4.87	3.22 – 7.38	$< 10^{-3}$
Adjusted on age	5.30	3.42 – 7.98	$< 10^{-3}$
Adjusted on age and cardiovascular risk factors	4.32	2,68 – 6.97	$< 10^{-3}$
Additional adjustment on HbA1c and duration of diabetes	4.32	2.55 – 6.80	$< 10^{-3}$
Additional adjustment on albuminuria	4.77	2.84 -8.02	$< 10^{-3}$

Cardiovascular risk factors : age, hypertension, sendentary lifestyle, family history of CHD, dyslipidemia, BMI.

Table 1 : association of second hand smoking and risk of cardiovascular disease by conditionnal logistic regression model

Disclosure: **M. Gourine:** None.

1128

Genome-wide association study on coronary artery disease in individuals with type 1 diabetes

N. Sandholm[1,2], E. Valo[1,2], E.H. Dahlström[1,2], C. Forsblom[1,2], V. Harjutsalo[1,3], P.-H. Groop[1,2];
[1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, [2]Abdominal Center Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, [3]The Chronic Disease Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland.

Background and aims: Cardiovascular deaths are the most common cause of premature mortality in type 1 diabetes (T1D), and the risk of cardiovascular disease (CVD) and mortality rates increase steeply in parallel with diabetic kidney disease. While there is evidence that genetic factors for CVD in the general population also affect individuals with diabetes, there are also genetic loci affecting CVD and CVD mortality particularly in individuals with diabetes. This study aims to assess the role of known genetic factors, and to identify additional genetic factors affecting the risk of coronary artery disease (CAD) in individuals with T1D.
Materials and methods: The study comprised 4824 Finnish individuals with T1D from the Finnish Diabetic Nephropathy (FinnDiane) study and the National Institute of Health and Welfare T1D studies with genome-wide genotyping data, and data on CAD available. CAD events included myocardial infarction, coronary bypass surgery or coronary balloon angioplasty. Controls were limited to those without hard CAD events, and with age ≥35 years and diabetes duration ≥15 years. Genotyping was performed with Illumina HumanCoreExome chips at the University of Virginia. After quality control, additional genotypes were imputed with Minimac3 using the 1000 Genomes Phase 3 reference panel. The genome-wide association study (GWAS) on CAD was analysed with score test using RVTESTS software, adjusted for sex, age, kinship matrix and genotyping batch. Data included 8,746,446 variants with minor allele frequency (MAF) ≥0.01 and imputation quality (R^2) ≥0.6.
Results: Data included 936 cases with CAD. Mean age at the time of first CAD event was 52.4 years (SD 10.2 years, range 20–90 years), and

duration of T1D was 38.1 years (SD 10.4, range 4–64 years). A total of 44% of CAD cases were women, a much higher proportion than in the general population. GWAS revealed a locus with genome-wide significant association (p value $<5 \times 10^{-8}$) near the defensin beta 127 (*DEFB127*) gene, with a MAF of 0.02 and an OR of 3.49 [95% CI 2.24–5.44]. Variants in 26 loci reached a suggestive p value $<10^{-5}$, including rs1537372 in the established 9p21 locus for CVD in the general population (OR = 1.30 [95% CI 1.17–1.45], $p = 1.54 \times 10^{-6}$); association in 9p21 reached genome-wide significance when the model was adjusted for the year of diabetes diagnosis. Among the previously identified loci, also rs2681472 in *ATP2B1* was nominally associated with CAD (p = 0.036), even though not withstanding correction for multiple testing.

Conclusion: We identified a novel susceptibility locus near *DEFB127* for CAD in individuals with T1D, along with 25 other suggestive loci that require further validation.

Supported by: Folkhälsan Res. Foundation, W&E Stockmann, JDRF, Academy of Finland, EFSD

Disclosure: **N. Sandholm:** Employment/Consultancy; P-HG is an advisory board member of AbbVie, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi. Grants; Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation, JDRF, Academy of Finland, EFSD. Honorarium; P-HG has received lecture honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi.

1129

Type 1 diabetes: defining the best cut-off points of arterial stiffness for predicting cardiovascular risk according to the Steno Type 1 Risk Engine

G. Llaurado[1,2], A. Cano[3], L. Albert[3], I. Mazarico[3], M. González-Sastre[4], J. Vendrell[5,2], J.-M. González-Clemente[3,2];
[1]Department of Endocrinology and Nutrition, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, [2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid, [3]Department of Endocrinology and Nutrition, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, [4]Ophthalmology Department, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, [5]Endocrinology and Nutrition Section, Hospital Universitari Joan XXIII, Institut d'Investigació Sanitària Pere Virgili (IISPV), Universitat Rovira i Virgili, Tarragona, Spain.

Background and aims: Cardiovascular disease (CVD) is the main cause of death in type 1 diabetes mellitus (T1DM). Recently, a specific score risk has been developed for predicting CVD: the Steno Type 1 Risk Engine. However, it includes 11 variables, which makes its clinical use somewhat cumbersome. The aim of our study was to assess the relationship between the Steno Type 1 Risk Engine and preclinical atherosclerosis measured as arterial stiffness (AS) in order to identify potential cut-off points of interest in clinical practice.

Materials and methods: One-hundred and seventy-nine patients with T1DM (18–65 years old) without established CVD were consecutively evaluated for: 1) clinical and anthropometric data (including classical cardiovascular risk factors), 2) microvascular complications and 3) AS measured by aortic pulse-wave velocity (aPWV) assessed by applanation tonometry (gold standard). The Steno Type 1 Risk Engine was used to estimate 10-year cardiovascular risk and patients were divided in 3 groups: low (<10%; $n = 105$); moderate (10–20%; $n = 53$) and high risk (≥20%; $n = 21$).

Results: 179 patients were included (50.8% men, age: 41.2 ± 13.1 yrs, duration of diabetes 16 (12–23) yrs). As compared to the low and moderate-risk groups, patients in the high-risk group were older (32.5 ± 8.3, 50.8 ± 6.0 and 60.7 ± 6.6 yrs; p for trend <0.001), had higher prevalence of hypertension (14.3%, 37.7% and 66.7%; p for trend <0.001) and dyslipidaemia (36.4%, 77.8% and 89.5%; p for trend <0.001). They also had higher BMI (24.3 ± 3.2, 26.6 ± 3.8 and 27.8 ± 4.4 kg/m^2; p for trend <0.001), higher insulin resistance (eGDR: 9.2 ± 1.8, 7.0 ± 2.1 and 5.5 ± 1.8 mg kg^{-1} min^{-1}; p for trend <0.001), worse glycaemic control (HbA1c: 7.6%, 8.0% and 8.5%; p for trend <0.001) and higher prevalence of microvascular complications (27.2%, 43.4% and 81.0%; p for trend <0.001). aPWV increased in parallel with estimated cardiovascular risk (6.4 ± 1.0, 8.4 ± 1.3 and 10.3 ± 2.6 m/s; $p < 0.001$; $r = 0.777$; $p < 0.001$). The C-statistic of aPWV was 0.914 (95% CI: 0.873–0.950) for predicting moderate-high risk and 0.879 (95% CI: 0.809–0.948) for high risk according to the Steno Type 1 Risk Engine. The best cut-off points of aPWV were 7.3 m/s (Sensitivity (Se): 86% and Specificity (Sp): 83%) and 8.7 m/s (Se: 76% and Sp: 86%) for moderate-high and high-risk, respectively.

Conclusion: Arterial stiffness was highly correlated with the scores obtained from Steno Type 1 Risk Engine. We have identified two cut-off points of arterial stiffness that can clearly discriminate moderate-high and high-risk T1DM patients, which could be of great interest for clinical practice.

Supported by: PI15/00567 (National R+D+I and ISCIII/GEB-ERDF)

Disclosure: **G. Llaurado:** None.

1130

A novel targeted approach for the reduction of vascular events in diabetes

M. Almutairi[1], C. Tiede[2], K. Kearney[1], N. Pechlivani[1], F. Hawkins[1], N. Oxley[1], D. Tomlinson[2], R. Ajjan[1];
[1]Division of Cardiovascular & Diabetes Research, Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), University of Leeds, Leeds, [2]School of Molecular and Cellular Biology, Astbury Building, University of Leeds, Leeds, UK.

Background and aims: Cardiovascular disease remains the main cause of mortality in patients with diabetes and an enhanced thrombotic environment is one of the mechanisms implicated. The increased incorporation of antifibrinolytic proteins into diabetes clots is contributes for hypofibrinolysis, a key abnormality precipitating vascular thrombosis in this condition. Our aim was to modulate incorporation of the potent antifibrinolytic protein, plasmin inhibitor (PI), into diabetes clots using a novel technology that involves small conformational proteins termed Affimers.

Materials and methods: A large library of random Affimers ($n = 3 \times 10^{10}$) was screened for PI binding using phage display. After multiple panning rounds, high affinity PI-binding Affimers were isolated and tested for modulation of fibrin clot lysis using *in vitro* and *ex vivo* techniques. Validated turbidimetric assays were employed to assess fibrinolysis, applying purified proteins, plasma and whole blood systems. Laser scanning confocal and electron microscopy were used to ensure integrity of the fibrin clots.

Results: A total of 167 high affinity PI-binding Affimers were isolated. Of these, 22 had distinct sequences and were subsequently subcloned and expressed in large quantities in *E. coli*. Using purified proteins, one Affimer (termed A68) consistently inhibited PI-induced prolongation of clot lysis at concentrations as low as 0.5 μg/ml. When tested in individual plasma samples form 12 patients with type 1 diabetes, the addition of A68 resulted in reduced clot lysis time from 549 ± 33 to 408 ± 23 seconds (26% reduction, $p < 0.01$). Similar results were obtained when A68 was tested using plasma from 12 patients with type 2 diabetes. Moreover, when tested in whole blood, A68 reduced clot lysis time from 2224 ± 113 seconds in control samples to 1436 ± 68 seconds after the addition of A68 (35% reduction in lysis time, $p < 0.01$). A68 had no significant effect on fibrin clot ultrastructure as assessed by confocal.

Conclusion: We describe, for the first time, the use of Affimer technology to modulate the hypofibrinolytic environment in diabetes. This targeted

approach has the potential to ameliorate the thrombotic milieu in diabetes without increasing the risk of bleeding. Future *in vivo* studies are warranted to assess the role of Affimers in preventing vascular thrombosis.
Supported by: General Directorate of Medical Services
Disclosure: **M. Almutairi:** None.

1131

Negative carotid artery remodelling in early type 2 diabetes and increased carotid plaque vulnerability in obesity as assessed by magnetic resonance imaging

E. Laugesen[1], P. Høyem[1], S. Thrysøe[1], E. Hansen[1], A. Mikkelsen[1,2], B. Kerwin[3], P.L. Poulsen[1], T.K. Hansen[1], W.Y. Kim[1];
[1]Aarhus University Hospital, Aarhus, Denmark, [2]Department of Procurement and Clinical Engineering, Aarhus, Denmark, [3]University of Washington, Seattle, USA.

Background and aims: Ischemic stroke from carotid plaque embolism remains a major cause of morbidity in type 2 diabetes (T2DM) patients. However, the effect of early T2DM and obesity on carotid remodeling and plaque burden remains elusive. We assessed carotid remodeling and plaque composition by carotid magnetic resonance imaging (MRI) in short duration T2DM patients compared to a sex- and age-matched control group.
Materials and methods: 100 T2DM patients (duration <5 years) and 100 sex- and age-matched controls underwent bilateral carotid artery MRI in a 1.5 T MRI scanner. Plaque burden was quantified by normalized wall index, maximum wall thickness, maximum wall area, and minimum lumen size. Plaque morphology was quantified by calcified plaque volume, necrotic core volume, and loose matrix volume.
Results: MRI data were available for 149 and 177 carotid arteries from T2DM patients and controls, respectively. Adjusted for age and sex, T2DM was associated with increased plaque burden indicated by a higher normalized wall index (ratio 1.03 (95% CI 1.002; 1.06), $p = 0.03$), and negative remodeling indicated by a lower minimum lumen area (ratio 0.81 (0.74; 0.89), $p < 0.001$), and lower maximum wall area (ratio 0.94 (0.88; 1.00), $p = 0.048$) compared to controls. In both T2DM and controls, BMI ≥30 kg/m^2 was associated with an 80% increase in total calcified plaque volume, and a 44% increase in necrotic core volume compared to BMI <25 kg/m^2.
Conclusion: Short duration T2DM was associated with increased carotid plaque burden and negative remodeling. Obesity was associated with increased carotid artery necrotic core volume and calcification independently of diabetes status.
Clinical Trial Registration Number: NCT00674271
Supported by: The Novo Nordic Foundation, The A.P. Møller Foundation and others
Disclosure: **E. Laugesen:** None.

1132

Risk of peripheral artery disease according to diabetic nephropathy and severe diabetic retinopathy in patients with type 1 diabetes

M. Kallio[1,2], V. Harjutsalo[2,3], C. Forsblom[2,4], P.-H. Groop[2,4], FinnDiane Study Group;
[1]Abdominal Center, Vascular Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, [2]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, [3]Chronic Disease Prevention Unit, National Institute for Health and Welfare, Helsinki, [4]Abdominal Center, Nephrology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.

Background and aims: Peripheral artery disease (PAD) is associated with increased premature mortality. Critical limb ischemia (CLI) is a severe form of PAD. Without revascularization the condition leads to lower limb amputation in 50% of patients. Most patients undergo revascularization and/or lower limb amputation. The aim of this study was to assess the impact of diabetic nephropathy (DN) and severe diabetic retinopathy (SDR) on the risk of CLI in patients with type I diabetes.
Materials and methods: The study comprised 4694 patients with type 1 diabetes who participated in the Finnish Diabetic Nephropathy Study (FinnDiane) before the year 2010. As ankle-brachial index (ABI) measurement alone or claudication are unreliable methods to detect PAD, especially in patients with diabetes who often have neuropathy attenuating the symptoms, we utilized clinical events of CLI as a sign of PAD. Therefore, PAD criteria were formed combining clinical data on ABI and toe pressure measurements, pulse palpation and radiological imaging data. Data on amputations and revascularizations were first identified from the standardized questionnaires at baseline as well as from the Finnish Care Register for Health Care. Thereafter, medical records were thoroughly reviewed to ascertain each event. DN status was categorized as having normo-, micro- or macroalbuminuria or end stage renal disease (ESRD). SDR was defined as a history of laser photocoagulation. Risk of PAD was evaluated using multivariable logistic regression analysis in a cross-sectional manner.
Results: There were 104 PAD events at the baseline visit with median duration of diabetes of 20.8 (IQR 11.3–30.5) years. The risk of PAD increased with each stage of DN. After adjustment for sex, age at onset of diabetes, duration of diabetes, HbA1c, HDL cholesterol, systolic blood pressure and history of smoking the risk in patients with ESRD was 14.9-fold (95% CI 6.4–34.6) compared with patients with normoalbuminuria. The odds ratio (OR) for patients with micro- and macroalbuminuria was 1.2 (0.4–3.8) and 3.2 (1.3–7.7), respectively. The risk of PAD was markedly increased with also in patients with SDR, OR = 3.0 (1.2–7.8).
Conclusion: We showed that in patients with type 1 diabetes DN is strongly associated with a higher risk of PAD. Also SDR independently from DN increased the risk of PAD. Early detection and treatment, as well as specific interventions, targeting changes both in the kidney and the retina could possibly lead to a decrease in the risk of PAD in patients type 1 diabetes.
Supported by: Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation, Acad
Disclosure: **M. Kallio:** Honorarium; P-HG has received lecture honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi. Other; P-HG is an advisory board member of AbbVie, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Novartis, Novo Nordisk, and Sanofi.

1133

Long-term role of peripheral angioplasty in diabetes patients with peripheral arterial disease

Z. Yan, Y. Li, L. Chang, Z. Zhao, H. He, H. Zhang, Z. Zhu;
Department of Hypertension and Endocrinology, Daping Hospital, Third Military Medical University, Center for Hypertension and Metabolic Diseases, Chongqing, China.

Background and aims: This study is aimed to evaluate the influence of peripheral angioplasty (PTA) on the long-term prognosis of diabetic patients with peripheral arterial diseases.
Materials and methods: A total of 312 diabetes patients with peripheral arterial diseases were given PTA treatment (lower extremity arterial balloon dilatation and/or stent implantation) from March 2009 to September 2017 in Daping hospital, Chongqing. 221 were followed up until September 2017. Their ankle-brachial index (ABI), blood pressure, blood glucose, glycosylated hemoglobin, blood lipids, serum creatinine, and high-sensitivity C-reactive protein (hsCRP) were measured in the 1, 3, 6 months, and every year after PTA treatment.
Results: The mean follow-up was 3.53 ± 1.12 years. The average ABI was 0.64 ± 0.21 before PTA treatment, and it was significantly increased on the 1, 3, 6 months and 1 year after PTA treatment (0.86 ± 0.19, 0.89 ± 0.14, 0.82 ± 0.20, 0.79 ± 0.22; $P < 0.05$ or 0.01). Among them, 15 (6.8%) patients received major amputations and PTA treatment were given in the

contralateral limb of 21 (9.5%) patients. Restenosis occurred in 36 (16.3%) patients and 14 (6.3%) of them suffered restenosis in 1 year after PTA treatment, higher than other time points ($P = 0.0127$). Logistic regression analysis showed that hs-CRP was an independent risk factor for restenosis (OR = 1.890, 95%CI: 1.011~3.902, $P = 0.031$).

Conclusion: PTA treatment can effectively improve blood supply of lower limb in diabetic patients with peripheral arterial disease. Lower extremity arterial restenosis most probably occurred in 1 year after PTA treatment. High hs-CRP level can predict the higher risk of lower extremity arterial restenosis.

Supported by: National Natural Science Foundation of China 81570761

Disclosure: **Z. Yan:** None.

PS 108 Adverse cardiovascular disease events

1134

Hospitalisations for major adverse cardiovascular events in patients with diabetes from 1989 to 2015 in the north of Portugal

C. Neves[1], J. Neves[2], F. Lopes[3], S. Castro Oliveira[2], M. Pereira[2], A. Oliveira[2], D. Carvalho[1];

[1]Department of Endocrinology, Diabetes and Metabolism, Centro Hospitalar São João; Institute for Research and Innovation in Health Sciences; Faculty of Medicine University of Porto, Porto, [2]Department of Endocrinology, Diabetes and Metabolism, Centro Hospitalar São João; Faculty of Medicine University of Porto, Porto, [3]Department of Codification, Centro Hospitalar São João, Porto, Portugal.

Background and aims: Diabetes is an important risk factor for major adverse cardiovascular events (MACE). Although the increased risk for MACE is well known, the variation over time of hospitalizations for MACE in patients with diabetes remains incompletely characterized. Our aim was to evaluate the proportion of hospitalizations due to MACE in a central hospital in the North of Portugal, comparing the periods between 1989–1998, 1999–2008 and 2009 and 2015.

Materials and methods: We evaluated retrospectively the hospitalizations due to MACE including stroke or transient ischemic attack (TIA), acute coronary syndrome (ACS) and heart failure from the Hospital Coding Centre. We have studied the distribution by age, causes and duration of admissions. Statistical analysis was performed with Student's t-test and chi-squared test. A two-tailed p value <0.05 was considered significant.

Results: A total of 1033842 hospitalizations occurred during the study period. We observed a significant decrease of the proportion of MACE hospitalizations over the three periods of evaluation, both in non-diabetic patients (23.5% in 1989–1998, 13.5% in 1999–2008 and 9.7% in 2009–2015, $p < 0.001$) and in patients with diabetes (29.9% in 1989–1998, 18.9% in 1999–2008 and 13.4% in 2009–2015, $p < 0.001$). The proportion of MACE admissions among all admissions remained significantly higher in patients with diabetes over the three periods (29.9% vs 23.5% in 1989–1998, 18.9% vs 13.5% in 1999–2008 and 13.4% vs 9.7% in 2009–2015, $p < 0.001$ for each period). Patients with diabetes were older that patients without diabetes in all periods (1989–1998: 67.3 ± 6.1 vs 65.3 ± 9.7 years, 1999–2008: 69.6 ± 8.5 vs 67.2 ± 1.2 years, 2009–2015: 71.6 ± 10.8 vs 69.4 ± 15.3 years; $p < 0.001$ for each period). There was a significant decrease in time of MACE hospitalization over the three periods, although it remained significantly higher among patients with diabetes over the period (1989–1998: 11.8 ± 9.2 vs 11.0 ± 9.1; 1999–2008: 10.2 ± 4.9 vs 9.3 ± 6.0; 2009–2015: 9.9 ± 9.6 vs 8.8 ± 8.5 days; $p < 0.001$ for each period). Patients with diabetes presented a higher proportion of stroke or AIT (1989–1998: 12.1% vs 8.1%; 1999–2008: 5.2% vs 4.0%; 2009–2015: 3.9% vs 3.5%; $p < 0.001$ for each period), acute coronary syndrome (1989–1998: 10.8% vs 9.5%; 1999–2008: 9.1% vs 6.6%; 2009–2015: 5.3% vs 3.8%, $p < 0.001$ for each period) and heart failure (1989–1998: 8.2% vs 6.1%; 1999–2008: 5.0% vs 3.2%; 2009–2015: 4.2% vs 2.4%, $p < 0.001$ for each period) compared with patients without diabetes in all three periods.

Conclusion: We observed a significant decrease of MACE hospitalization among patients with diabetes from 1989 until 2015 in a central hospital in the North of Portugal. However, MACE admissions remain higher in patients with diabetes comparing with patients without diabetes. The higher incidence of MACE in patients with diabetes highlights the importance of improving the prevention and treatment of cardiovascular disease in this population.

Disclosure: **C. Neves:** None.

1135

The maximum glucose peak during an oral glucose tolerance test is associated with greater arterial stiffness: the Maastricht Study

Y.D. Foreman[1,2], M.C.G. Brouwers[1,2], S.J.P. Eussen[2,3], M.M.J. van Greevenbroek[1,2], R.M.A. Henry[1,2], C.J.H. van der Kallen[1,2], K.D. Reesink[2,4], M.T. Schram[1,2], N.C. Schaper[2,5], C.D.A. Stehouwer[1,2];

[1]Dept. of Internal Medicine, Maastricht University Medical Centre +, Maastricht, [2]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, [3]Dept. of Epidemiology, Maastricht University, Maastricht, [4]Dept. of Biomedical Engineering, Maastricht University, Maastricht, [5]School for Public Health and Primary Care (CAPHRI), Maastricht University, Maastricht, Netherlands.

Background and aims: The 75-gram OGTT has traditionally been used for the formal diagnosis of (pre)diabetes. Recently, the maximum glucose peak during an OGTT has become a topic of interest, as it was found to be associated with an adverse cardiovascular risk profile. In the present study, we aimed to investigate the association between the maximum glucose peak during an OGTT and arterial stiffness, independent of glucose metabolism status (GMS), i.e. normal glucose metabolism, prediabetes and type 2 diabetes (T2D).
Materials and methods: Cross-sectional data from The Maastricht Study, an observational population-based cohort study enriched with individuals with T2D, was used. All participants with a complete 7-point OGTT were included for analysis ($N = 2{,}804$, aged 59.8 ± 8.2 years, 51.8% men). Maximum OGTT Glucose peak Increase from Baseline (MOGIB; mmol/l) was calculated as the highest glucose value during the OGTT minus the baseline glucose value. Carotid femoral pulse wave velocity (cf-PWV) and carotid distensibility coefficient (carDC) were used as measures of arterial stiffness. The associations of MOGIB with cf-PWV and carDC were investigated via multiple linear regression with stepwise adjustment for age, sex, GMS (or alternatively for HbA_{1c}), BMI, smoking status, mean arterial pressure, physical activity, lipid profile, use of lipid-modifying and antihypertensive medication, prior cardiovascular disease and retinopathy, estimated GFR, and urinary albumin excretion.
Results: In age- and sex-adjusted analyses, MOGIB was statistically significantly associated with cf-PWV (regression coefficient (β) 0.106 [95% confidence interval: 0.077; 0.134] m/s, $p < 0.001$), and carDC (−0.185 [−0.257; −0.114] 10^{-3}/kPa, $p < 0.001$). These associations remained statistically significant after additional adjustment for GMS or HbA_{1c}. The association between MOGIB and cf-PWV attenuated after full adjustment: β was 0.036 [−0.006; 0.078] m/s ($p = 0.091$) in the full model that included GMS, and 0.070 [0.034; 0.107] m/s ($p < 0.001$) in the full model that included HbA_{1c}. The association between MOGIB and cf-PWV was stronger with increasing age (p for interaction with age = 0.025). The association between MOGIB and carDC, however, was not statistically significant after full adjustment. The β was −0.082 [−0.185; 0.022] 10^{-3}/kPa ($p = 0.123$) and −0.026 [−0.117; 0.065] 10^{-3}/kPa ($p = 0.575$) in the full models that included GMS and HbA_{1c}, respectively.
Conclusion: These data show that the maximum glucose peak during an OGTT is independently associated with greater aortic stiffness (cf-PWV), which is known to be an independent risk factor for cardiovascular disease, but not with carDC. Further studies are needed to elucidate how these findings translate to glucose fluctuations in daily practice.
Supported by: ERDF, PoL, SDW, PSID, CVC, CARIM, CAPHRI, NUTRIM, SA, HFL, JC, NNF, SAN, MD
Disclosure: **Y.D. Foreman:** None.

1136

Quantitave myocardial blush evaluation correlates with infarct size and systolic left ventricle function in patients with type 2 diabetes and stemi

J. Gumprecht[1], K. Nabrdalik[1], H. Kwiendacz[1], E. Radzik[2], K. Pigoń[2], M. Basiak[2], E. Nowalany-Kozielska[2], A. Tomasik[2];
[1]Department of Internal Disease, Diabetology and Nephrology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Zabrze, [2]Second Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Zabrze, Poland.

Background and aims: HORIZONS AMI and CADILLAC studies provide conflicting results regarding validity of the visual assessment of myocardial blush grade (MBG) and TIMI myocardial perfusion grade (TMPG) in diabetic STEMI patients. Cardiac magnetic resonance studies, performed 48 hours up to 7 days post myocardial infarction episode, indicate clearly that diabetic patients present higher volume of oedema and microvascular obstruction. Since patients with type 2 diabetes (T2DM) are at high risk of worse prognosis following STEMI it is important to find the methods identifying patients who are at risk of having a larger infarct size and reduce LVEF early enough. The aim of the study was to evaluate the association between myocardial perfusion and infarct size as well as left ventricular function among STEMI patients with T2DM who were treated with primary percutaneous intervention (pPCI).
Materials and methods: A total of 104 consecutive STEMI patients with T2DM treated with pPCI were enrolled into this observational study. Myocardial perfusion was reassessed with the Quantitative Myocardial Blush Evaluator (QuBE). For infarct size assessment, we utilized peak activity of creatine kinase and troponin T concentration area under the curve (AUC) measured on hospital admission, at 12, 24, and 72 hours following STEMI. Echocardiographic evaluation of left ventricle systolic function was performed on the day of hospital discharge. Forward stepwise linear regression modeling has been used for assessment of relation between angiographic data and enzymatic infarct size and left ventricle function.
Results: Patients with T2DM and a QuBE score below the median value (9.0 arb. units) had significantly inferior procedural outcome than those with a QuBE score equal to or above the median value: epicardial flow in infarct-related artery was significantly slower (higher number of corrected TIMI frame count cTFC; $p = 0.004$) with a significantly higher peak CK-MB value ($p = 0.027$), troponin T AUC ($p = 0.01$) and worse EF ($p = 0.039$).
Conclusion: Diminished myocardial perfusion is associated with significantly larger infarct size and lower left ventricle systolic function among patients with T2DM. QuBE seems to be operator independent reliable predictor of infarct size and reduced left ventricle function in this group of patients.

Table 1. Demographic and clinical characteristics of patients divided according to median QuBE value of < 9 or ≥ 9.

	QuBE value below median	QuBE value equal or above median	Significance
Age, years	67.98±9.34	65.35±9.52	NS
Gender, M/F	28/24	26/26	NS
Time pain to balloon, min (±SD)	424.08±437.39	335.836±232.36	NS
cTFC, fps (±SD)	39.87±28.57	26.04±17.96	0.004
Peak CK-MB, IU/L (±SD)	264.94±325.4	151.23±167.30	0.027
Troponine T, AUC, ng/L*days, (±SD)	10332.14±4507.42	8289.18±3299.45	0.010
LVEF, %, (±SD)	40.84±10.48	45.35±11.31	0.039
BMI, kg/m2 (±SD)	29.16±4.46	28.82±3.27	NS
eGFR	76.33±23.84	88.49±25.39	0.013
Hypertension, n (%)	47	44	NS
Previous MI, n (%)	14	8	NS

Disclosure: **J. Gumprecht:** None.

1137

Differences in external validity of SGLT2-I cardiovascular outcome trials in general type 2 diabetes populations: a large observational study in European countries

K.I. Birkeland[1], J. Bodegard[2], A. Norhammar[3], J.G. Kuiper[4], W. Beekman-Hendriks[5], M. Thuresson[6], A. Kooy[7];
[1]Oslo University Hospital, Oslo, Nydalen, Norway, [2]AstraZeneca Nordic-Baltic, Södertälje, Sweden, [3]Karolinska Institutet, Stockholm,

Sweden, [4]PHARMO Institute for Drug Outcomes Research, Utrecht, Netherlands, [5]AstraZeneca, Den Haag, Netherlands, [6]Statisticon AB, Uppsala, Sweden, [7]University Medical Center and Bethesda Diabetes Research Center, Groningen, Netherlands.

Background and aims: Strict enrolment criteria for clinical trial patients assessing cardiovascular (CV) safety and efficacy of antihyperglycaemic drugs in type 2 diabetes (T2D) may not be representative for the general T2D population resulting in low external validity of the results. Recent cardiovascular outcome trials (CVOTs) have shown paradigm shifting results in the benefit of sodium-glucose cotransporter-2 inhibitors (SGLT-2is), and further studies will report soon (Table). Study criteria vary substantially between the CVOTs, and may affect the representativeness of a study population for the general T2D population. The aim of this analysis was to evaluate this representativeness in four SGLT-2i CVOTs collected from the general T2D populations in European countries.
Materials and methods: T2D patients in 2015 were included from the Netherlands (the PHARMO Database Network) and mandatory full-population registries in Norway and Sweden. Given the available data in each country, key inclusion and exclusion criteria were only defined by diagnoses, procedures, and drug treatment to facilitate comparability between countries. External validity was defined by dividing number of patients filling the CVOT's key enrolment criteria by the total enrolled T2D-population for the respective country.
Results: In total, a T2D population of 564,351 patients was identified in the Netherlands ($n = 36,213$), Norway, ($n = 149,782$), and Sweden ($n = 378,356$). All three populations showed a CV disease baseline prevalence of 25–35% and high proportions using cardiovascular (CV) preventive drugs. The CVOT patients were slightly younger compared to the general T2D population and had higher CV prevalence at baseline of 41%, 66% and 99% for DECLARE, CANVAS and EMPA-REG respectively. DECLARE had a more than 1.7-fold higher external validity compared to CANVAS and more than 3-fold compared to EMPA-REG or VERTIS. Variation in the representativeness between countries may be partly explained by the differences in age, CV disease prevalence and CV preventive drugs in the T2D populations, all being important to the key enrolment criteria.
Conclusion: In large T2D populations from European countries, consistent patterns of external validity for all studies were found despite some differences in patient characteristics. DECLARE had the highest external validity, followed by CANVAS, EMPA-REG, and VERTIS, indicating that DECLARE examines patients most representative of the general T2D patient in the studied countries.

	The Netherlands	Norway	Sweden
Baseline general T2D population			
Number of patients, n	36,213	149,782	378,356
Age, n (SD)	68.6 (11.5)	64.1 (13.4)	67.5 (12.3)
Female, n (%)	16,571 (45.8)	64,207 (42.9)	158,030 (41.8)
CV disease, n (%)	12,594 (34.8)	37,547 (25.1)	118,852 (31.4)
Antihypertensives, n (%)	26,750 (73.9)	105,220 (70.2)	298,238 (78.8)
Statins, n (%)	24,912 (68.8)	87,784 (58.6)	243,862 (64.5)
CVOT patient representativeness			
DECLARE (dapagliflozin)	26,317 (72.7%)	87 583 (58.5%)	245 864 (65.0%)
CANVAS (canagliflozin)	14 145 (39.1%) 1.9	50 239 (33.5%) 1.7	133 647 (35.3%) 1.8
EMPA-REG (empagliflozin)	4 608 (12.7%) 5.7	28 712 (19.2%) 3.0	79 487 (21.0%)
VERTIS (ertugliflozin)	2 637 (7.3%) 10.0	21 918 (14.6%)	67 295 (17.8%)

Supported by: AstraZeneca
Disclosure: **K.I. Birkeland:** Grants; AstraZeneca.

1138

The REMOVAL trial: metformin reduces progression of mean carotid intima-media thickness (cIMT) in never smokers with type 1 diabetes

J.R. Petrie[1], N. Chaturvedi[2], I. Ford[1], M.C.G. Brouwers[3], N. Greenlaw[1], I. Hramiak[4], A.D. Hughes[2], A.J. Jenkins[5], B.E.K. Klein[6], R. Klein[6], P. Rossing[7], C.D.A. Stehouwer[3], N. Sattar[1], H.M. Colhoun[8], for the REMOVAL Study Group;
[1]University of Glasgow, Glasgow, UK, [2]University College London, London, UK, [3]University of Maastricht, Maastricht, Netherlands, [4]St Joseph's Health Care, London, Canada, [5]University of Sydney, Sydney, Australia, [6]University of Wisconsin, Madison, USA, [7]Steno Diabetes Center, Gentofte, Denmark, [8]University of Edinburgh, Edinburgh, UK.

Background and aims: In REMOVAL, metformin reduced the rate of progression of the tertiary carotid outcome (averaged maximal cIMT) over three years in middle-aged adults with longstanding type 1 diabetes but did not significantly reduce progression of the primary outcome (averaged mean cIMT). We now report subgroup analyses for the primary outcome (the only subgroup analyses that were pre-specified).
Materials and methods: 3-way interaction terms with treatment and time were calculated for the following baseline variables: age, duration of diabetes, baseline HbA1c, BMI, LDL cholesterol, systolic BP (all by above or below median), baseline cIMT (by tertiles), sex (male or female), smoking (ever or never), insulin pump user (yes or no) and history of cardiovascular disease (present or absent).
Results: The 3-way interaction term was significant for only one of the 11 pre-specified subgroup analyses: smoking status ($p = 0{\cdot}0373$). Of 428 randomised participants, [(mean ± SD) age 55 ± 8·5 years, HbA1c 8·0 ± 0·82%, BMI 28·4 ± 4·3 kg/m², 59% male, duration of diabetes 34 ± 10·8 years, 12% with a history of cardiovascular disease, 82% on statin therapy], 227 (53%) were never smokers, 144 (34%) were ex-smokers and 57 (13%) were current smokers. Mean ± SD duration of smoking was 22 ± 13·2 years. The primary outcome mean cIMT was reduced by metformin in the 227 never smokers (−0·012 mm per year, 95% CI −0·021 to −0·002; $p = 0{\cdot}0137$) but not in the 201 ever smokers (0·003 mm per year, 95% CI −0·008 to 0·014; p=0·5767).
Conclusion: While subgroup analyses should be treated with caution, these data suggest that metformin may reduce progression of mean carotid IMT in type 1 diabetes in the absence of the powerful cardiovascular risk factor of smoking. This provides further support for a wider role of metformin in cardiovascular risk management.
Clinical Trial Registration Number: NCT01483560
Supported by: JDRF
Disclosure: **J.R. Petrie:** Non-financial support; Merck KGaA, Itamar Medical.

1139

Effects of liraglutide on cardiovascular events in patients with type 2 diabetes and polyvascular disease: results of the LEADER trial

B. Zinman[1], S. Verma[2], D.L. Bhatt[3], S.C. Bain[4], J. Mann[5], M.A. Nauck[6], R.E. Pratley[7], M.M. Michelsen[8], T. Monk Fries[8], S. Rasmussen[8], L.A. Leiter[2], LEADER Publication Committee on behalf of the LEADER Trial Investigators;
[1]Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Canada, [2]Li Ka Shing Knowledge Institute of St. Michael's Hospital, University of Toronto, Toronto, Canada, [3]Brigham and Women's Hospital Heart and Vascular Center and Harvard Medical School, Boston, USA, [4]Institute of Life Science, Swansea University, Swansea, UK, [5]Friedrich Alexander University of Erlangen, Erlangen, Germany, [6]Diabetes Center Bochum-Hattingen, St. Josef-Hospital (Ruhr-University Bochum), Bochum, Germany, [7]Florida Hospital Translational Research Institute, Orlando, USA, [8]Novo Nordisk A/S, Søborg, Denmark.

Background and aims: Polyvascular disease can predict cardiovascular (CV) events. In LEADER, liraglutide significantly reduced major adverse CV events (MACE) vs placebo. In a post hoc analysis, we assessed CV outcomes by history of single or polyvascular disease at baseline.

Materials and methods: In LEADER, 9340 patients with type 2 diabetes (T2D) and high CV risk were randomised 1:1 to liraglutide vs placebo, both as add on to standard of care (median follow-up = 3.8 years). The primary outcome (MACE) was a composite of CV death, nonfatal myocardial infarction, or nonfatal stroke. The secondary outcome (expanded MACE) also included hospitalisation for unstable angina, coronary revascularisation, or hospitalisation for heart failure. Cox regression was used to compare CV outcomes in patient risk groups stratified by number of atherosclerotic vascular territories (coronary, cerebrovascular and/or peripheral artery disease). Polyvascular disease was defined as ≥2 and single vascular disease as 1 atherosclerotic vascular territory.
Results: In LEADER, 6775 patients (72.5%) had documented atherosclerotic CV disease (ASCVD). Of these, 1536 patients (23%) had polyvascular and 5239 (77%) had single vascular disease. Patients with polyvascular disease had a higher risk of CV outcomes than those with single vascular disease (MACE: HR 1.52, 95% CI 1.33–1.73; expanded MACE: HR 1.45, 95% CI 1.31–1.62, CV death: HR 1.41, 95% CI 1.13–1.75). Liraglutide reduced MACE consistently in patients with polyvascular (HR 0.82, 95% CI 0.66–1.02) and single vascular disease (HR 0.82, 95% CI 0.71–0.95) vs placebo. In patients without ASCVD at baseline, the HR for liraglutide vs placebo for MACE was 1.08 (95% CI 0.84–1.38). Results were similar for expanded MACE and CV death (Table). No significant interactions were found across risk groups for CV outcomes (Table), with the exception of expanded MACE (p interaction = 0.03).
Conclusion: In patients with T2D, polyvascular disease was associated with greater risk of CV outcomes vs single vascular disease. Liraglutide appeared to reduce consistently CV outcomes in patients with single and polyvascular disease vs placebo. A trend towards a neutral response was observed in patients without ASCVD.

Table: Cardiovascular outcomes in patients treated with liraglutide vs placebo by number of vascular territories involved at baseline

Outcome	Vascular disease	n with event/N analysed (%)		HR [95% CI]	Treatment by subgroup interaction
		Liraglutide	Placebo		
MACE	Polyvascular	142/757 (18.8)	173/779 (22.2)	0.82 [0.66–1.02]	*p*=0.15
	Single	338/2646 (12.8)	398/2593 (15.3)	0.82 [0.71–0.95]	
	No ASCVD	128/1265 (10.1)	123/1300 (9.5)	1.08 [0.84–1.38]	
Expanded MACE	Polyvascular	220/757 (29.1)	255/779 (32.7)	0.86 [0.71–1.03]	*p*=0.03
	Single	541/2646 (20.4)	633/2593 (24.4)	0.82 [0.73–0.92]	
	No ASCVD	187/1265 (14.8)	174/1300 (13.4)	1.12 [0.91–1.38]	
CV death	Polyvascular	54/757 (7.1)	60/779 (7.7)	0.92 [0.63–1.32]	*p*=0.16
	Single	114/2646 (4.3)	165/2593 (6.4)	0.67 [0.53–0.85]	
	No ASCVD	51/1265 (4.0)	53/1300 (4.1)	0.99 [0.67–1.45]	

Interaction *p* value is for test of homogeneity of treatment group difference among all 3 subgroups with no adjustment for multiple tests. No ASCVD = no documented evidence of atherosclerotic disease in any of 3 vascular territories at baseline.

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **B. Zinman:** Other; Funding: Novo Nordisk A/S.

1140

Effect of 12 weeks continuous positive airway pressure on day and night arterial stiffness in patients with type 2 diabetes and obstructive sleep apnoea, a randomised trial

C. Krogager[1], A. Banghøj[2], P.L. Poulsen[3], M.G. Kirkegaard[4], L. Tarnow[2], K.W. Hansen[5], E. Laugesen[3];
[1]Clinical medicine, Aarhus University, Aarhus, [2]Cardiology, nephrology and endocrinology, Nordsjællands Hospital, Hillerød, [3]Endocrinology and internal medicine, Aarhus University Hospital, Aarhus, [4]Sleep Disorders Clinic, Elective Surgery Centre, Regional Hospital Silkeborg, Silkeborg, [5]Diagnostic centre, Regional Hospital Silkeborg, Silkeborg, Denmark.

Background and aims: Type 2 diabetes and obstructive sleep apnea (OSA) are associated with high cardio vascular risk. Both are characterized by increased arterial stiffness, which is a marker of cardiovascular risk capable of predicting cardio vascular events independent of traditional risk factors. Previous studies have reported a decrease in arterial stiffness when OSA is treated with continuous positive airway pressure (CPAP). Few trials are randomised and no studies are evaluating the effects in patients with diabetes. The aim of this randomised study is to evaluate the effect of CPAP treatment on arterial stiffness in patients with T2DM and OSA.
Materials and methods: We included 72 patients with type 2 diabetes, newly diagnosed OSA and apnea hyponea index (AHI) ≥15 events/hour. The patients were recruited from outpatient clinics at three Danish hospitals. Patients were randomised to 12 weeks CPAP treatment or to a control group. Arterial stiffness was evaluated by measurement of pulse wave velocity (PWV) at baseline, 4 weeks and after 12 weeks using the SphygmoCor device (office PWV). Ambulatory blood pressure and arterial stiffness were evaluated using the Mobil-O-Graph device (day and night PWV).
Results: At baseline AHI was 35 events/hour (±15.4), office PWV was 10.7 m/s (±2.6), nighttime PWV was 8.9 m/s (±1.1) and nighttime systolic blood pressure (BP) was 125 mmHg (±12.7). Apart form BMI, 36 vs. 33 kg/m^2, there were no difference at baseline between the two groups. In the CPAP group, the average median nightly CPAP usage was 5.41 hours. Primary outcome analysis was performed using a repeated measures analysis of variance. After 12 weeks of treatment, AHI in the CPAP group was 0.8 events/hour (±0.52). Nighttime PWV was 9.1 (±1.1) vs. 9.0 (±1.3) m/s in the control group and nighttime systolic BP was 125.2 (±13.7) vs. 126 (±12.9) mmHg in the control group, No difference in office PWV was seen, −0.62 m/s 95%CI (−1.45 to 0.20), $p = 0.14$ vs. −0.22 m/s 95%CI (−0.95 to 0.51), $p = 0.56$ in the control group. There was no difference in night time PWV after 12 weeks of CPAP treatment −0.036 m/s 95%CI (−0.17 to 0.10) $p = 0.61$. Change in nighttime BP was also non significant, −1.35 mmHg 95%CI (−5.54 to 2.85) vs. 2.54 mmHg 95% (−1.64 to 6.72) $p = 0.23$ in the control group. Furthermore, no difference in daytime BP or PWV was found.
Conclusion: In conclusion, among patients with type 2 diabetes and OSA, CPAP treatment for 12 weeks did not alter arterial stiffness or blood pressure when compared to a control group receiving no treatment.
Clinical Trial Registration Number: NCT02482584
Disclosure: **C. Krogager:** None.

1141

Association between allopurinol and cardiovascular events and all-cause mortality in diabetes: a population-based cohort study

A. Weisman, G.A. Tomlinson, L. Lipscombe, B.A. Perkins, G.A. Hawker;
University of Toronto, Toronto, Canada.

Background and aims: Higher uric acid (UA) is associated with cardiovascular events and mortality. Allopurinol, a UA-lowering therapy, may reduce risk of these outcomes. Despite the high prevalence of elevated UA in diabetes, the association between allopurinol and cardiovascular events and mortality in diabetes is unclear.
Materials and methods: A population-based cohort was constructed using administrative data in Ontario, Canada. Subjects with diabetes entered on receipt of a new prescription for allopurinol after age 66 (April 1/2002–March 31/2012) and were followed until a composite of all-cause mortality, stroke, myocardial infarction or revascularization. A Cox proportional hazards model was used for each sex, with time-varying allopurinol exposure modeled as yes/no, dose categories and cumulative dose.
Results: Over a median [IQR] follow-up time of 4.7[1.8–7.8] years, the composite outcome occurred in 16,262/23,103 males and 10,566/15,313 females. Allopurinol exposure was associated with a reduction in the composite outcome in a dose-response manner but there was no cumulative dose effect (Table 1).

Conclusion: Any allopurinol exposure and higher allopurinol doses were associated with reduced cardiovascular events and mortality in a large diabetes cohort. Potential mechanisms include an acute reduction of oxidative stress and endothelial dysfunction.

Table 1: Hazard ratios by sex

	Males (n=23,103)		Females (n=15,313)	
Allopurinol Exposure	**Unadjusted HR**	**Adjusted HR**	**Unadjusted HR**	**Adjusted HR**
Exposed time v. unexposed time	0.82 (0.79, 0.84)	0.77 (0.75, 0.80)	0.88 (0.85, 0.92)	0.81 (0.78, 0.84)
Dose categories				
0mg	-	-	-	-
>0 and ≤100mg	1.03 (0.99, 1.08)	0.84 (0.80, 0.88)	1.06 (1.01, 1.12)	0.86 (0.81, 0.90)
>100 and ≤200mg	0.82 (0.78, 0.85)	0.75 (0.72, 0.78)	0.83 (0.79, 0.87)	0.76 (0.72, 0.80)
>200mg	0.71 (0.68, 0.74)	0.75 (0.72, 0.78)	0.78 (0.73, 0.82)	0.81 (0.77, 0.86)
Cumulative Dose (per 100g increase)	0.99 (0.98, 1.00)	1.00 (0.99, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.00)

Disclosure: **A. Weisman:** None.

PS 109 Dyslipidaemia and diabetes

1142

Hypercholesterolaemia impairs GLP-1 action on platelets: effect of a lipid-lowering treatment with simvastatin

C. Barale[1], C. Frascaroli[2], K. Bonomo[2], F. Cavalot[2], I. Russo[1];
[1]Clinical and Biological Sciences, University of Turin, Orbassano, [2]San Luigi Gonzaga Hospital, Orbassano, Italy.

Background and aims: Glucagon-Like Peptide-1 (GLP-1) exerts a role in glucose homeostasis and cardiovascular system. GLP-1 in platelets increases the inibitory effects of the nitric oxide (NO)/PKG/VASP pathway, and reduces oxidative stress. Aim of the study was to verify GLP-1 effects on platelet function in hypercholesterolemia and the role of a lipid-lowering therapy with statin.
Materials and methods: In platelet samples from 45 hypercholesterolemics (M/F:26/19; age:50 ± 2 years, total cholesterol (TC):273 ± 7 mg/dl, c-HDL:62 ± 3 mg/dl, triglycerides:173 ± 16 mg/dl, c-LDL:183 ± 6 mg/dl) and 20 controls (M/F:12/8; age:51 ± 2 years) we evaluated GLP-1(7-36), or Liraglutide (100 nmol/l) influence on: i) antiaggregating effects of the NO-donor sodium nitroprusside (SNP) (Born's method); ii) the SNP-induced pVASP-ser239 levels; iii) the oxygen species (ROS) production (DCF-DA). These evaluations were repeated in hypercholesterolemics after a 3-month treatment with Simvastatin 40 mg/die ($n = 18$) or only diet therapy ($n = 22$).
Results: In hypercholesterolemics, if compared with controls, the effects of GLP-1(7-36) and Liraglutide on platelet responses were significantly lower. In particular, in the presence of GLP-1(7-36): i) the percent increase of the anti-aggregating effects of SNP was 8 ± 4 vs 23 ± 5 ($p < 0.03$) with ADP, 9 ± 3 vs 32 ± 7 ($p < 0.0001$) with collagen and 8 ± 3 vs 30 ± 8 ($p < 0.002$) with arachidonic acid (AA); ii) the percent increase of the SNP-induced pVASP levels was 5 ± 4 vs 33 ± 6 ($p < 0.0001$); iii) the percent reduction of ROS synthesis was 12 ± 5 vs 32 ± 9 ($p < 0.04$). In the presence of Liraglutide: i) the percent increase of the anti-aggregating effects of SNP was 11 ± 3 vs 34 ± 6 ($p < 0.0001$) with ADP, 17 ± 3 vs 35 ± 6 ($p < 0.004$) with collagen, 19 ± 2 vs 33 ± 5 ($p < 0.003$) with AA; ii) the percent increase of the SNP-induced pVASP was 5 ± 4 vs 40 ± 6 ($p < 0.0001$); iii) the percent reduction of ROS synthesis was 16 ± 7 vs 42 ± 9 ($p < 0.04$). In hypercholesterolemics, Simvastatin treatment induced a significant decrease of TC (from 288 ± 12 to 206 ± 9 mg/dl, $p < 0.0001$) and c-LDL (from 198 ± 10 to 120 ± 9 mg/dl, $p < 0.0001$), and in platelets: i) improved the sensitivity to the inhibitory NO/PKG/VASP pathway because the percent antiaggregating effects of NO rose from 51 ± 5 to 67 ± 3 ($p < 0.01$) with ADP, from 35 ± 4 to 55 ± 7 ($p < 0.02$) with collagen, from 41 ± 2 to 49 ± 3 ($p < 0.03$) with AA and the fold increase on basal values of SNP-induced pVASP rose from 8 ± 2 to 15 ± 2 ($p < 0.02$). However, Simvastatin treatment failed to modify platelet sensitivity to GLP-1 effects. For instance, in the presence of Liraglutide: i) the percent increase of the antiaggregating effects of SNP passed from 7 ± 3 to 9 ± 2 (ns) with ADP, from 8 ± 3 to 10 ± 3 (ns) with collagen, from 9 ± 1 to 11 ± 3 (ns) with AA, ii) the percent increase of pVASP, on values with SNP alone, passed from 6 ± 3 to 8 ± 2 (ns), iii) the percent reduction of the AA-induced ROS levels passed from 16 ± 5 to 20 ± 5 (ns). In hypercholesterolemics on diet therapy alone no significant difference was observed for lipid or platelet parameters.
Conclusion: Hypercholesterolemia is a condition of resistance to GLP-1 effects on platelets. The treatment with Simvastatin improved lipid profile and platelet sensitivity to NO but does not revert the impaired platelet responses to the *in vitro* GLP-1 effects.
Supported by: a SISA grant to CB
Disclosure: **C. Barale:** None.

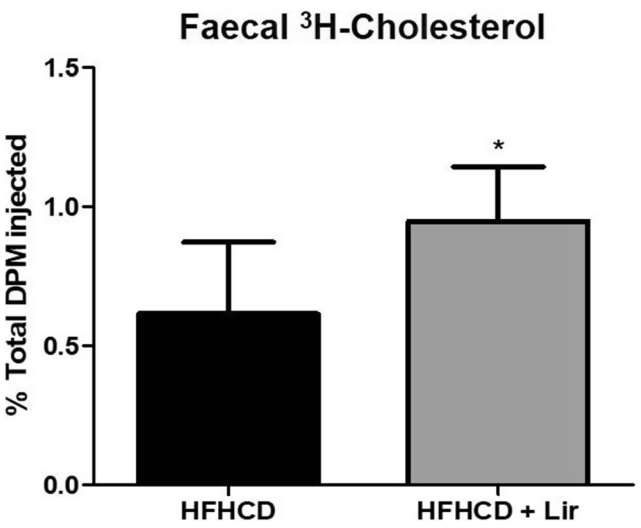
1143

Investigating the role of glucagon-like peptide 1 on reverse cholesterol transport in a state of early atherosclerosis

S. Curley[1], R. Bruen[2], S. Kajani[1], M. O'Reilly[3], E. Dillon[4], O. Belton[2], F. McGillicuddy[1];

[1]School of Medicine, University College Dublin, Dublin, [2]School of Biomolecular and Biomedical Sciences, University College Dublin, Dublin, [3]School of Public Health, University College Dublin, Dublin, [4]Conway Institute Proteomics Core, University College Dublin, Dublin, Ireland.

Background and aims: Glucagon-like peptide 1 (GLP-1) is a hormone secreted in the gut in response to food intake that promotes satiety as well as having anti-inflammatory effects. Clinical trials have indicated potential cardiovascular benefits of liraglutide, a GLP-1 analogue, in patients with diabetes. Diabetes is a major risk factor for development of atherosclerosis, a cardiovascular disease characterised by invasion of lipid-laden macrophages into the artery wall, inducing a chronic inflammatory state. Indeed, an important athero-protective pathway, reverse cholesterol transport (RCT), is attenuated by inflammation. This study hypothesized that liraglutide may exert cardioprotective effects via modulation of the RCT pathway *in vivo*.
Materials and methods: Apolipoprotein E knockout mice were fed a high-fat, high-cholesterol diet (HFHCD) for two weeks to induce atherosclerosis. Once-daily s.c. injections of either 300 μg/kg liraglutide ($n = 16$) or PBS ($n = 11$) were administered for a further six weeks, during which time the diets were maintained. Low-fat diet-fed (LFD) mice acted as a control. As an *in vivo* method to track cholesterol movement along the RCT pathway, ^{3}H-cholesterol loaded J774 macrophages were injected into the i.p. cavity and ^{3}H-cholesterol levels in the plasma, liver, bile and faeces analysed. Size-exclusion chromatography was performed on plasma samples to isolate the different lipoproteins.
Results: Consistent with its weight loss effect, liraglutide treatment decreased epididymal and subcutaneous adipose weight compared to HFHCD mice. Liraglutide had no significant effect on either plasma or liver ^{3}H-cholesterol levels however it did significantly increase cholesterol clearance to faeces ($p < 0.05$) compared to HFHCD (Figure 1) - Mann Whitney test. Cholesterol mass on isolated VLDL fractions was significantly increased with HFHCD feeding compared to LFD ($p < 0.001$), an effect that was almost completely abrogated by liraglutide treatment ($p < 0.01$) - two-way ANOVA. Mass spectrometry was performed on isolated HDL fractions, revealing significant changes in the HDL proteome, in particular the complement system, with liraglutide treatment ($p < 0.05$) - two sample t test.
Conclusion: We conclude that liraglutide plays a role in promoting cholesterol clearance in a state of early atherosclerosis. Results also indicate that liraglutide treatment affects lipoprotein particles - integral components of RCT - with cholesterol and protein composition being altered in VLDL and HDL respectively. As most patients present with an advanced state of the disease rather than a developing state, we are currently investigating the effect of liraglutide treatment in pre-established atherosclerosis.



Supported by: EFSD
Disclosure: **S. Curley:** Grants; EFSD.

1144

Role of HDL and apolipoprotein A1 in the modulation of glucagon levels

G.C. Mannino, A. Fuoco, E. Mancuso, C. Di Fatta, R. Spiga, C. Averta, F. Andreozzi, G. Sesti;
Department of Medical and Surgical Sciences, University of Catanzaro Magna Graecia, Catanzaro, Italy.

Background and aims: Individual's lipid pattern and lipoprotein levels are recognized as important predictors of the development of type 2 diabetes mellitus, and major contributors to the increased risk of cardiovascular disease. Several evidences suggest that HDL and Apolipoprotein A-1 (ApoA1) positively modulate β-cell function. However, their effects on α-cellular function are unknown. The aims of our study were to investigate the existence of a causal role of HDL/ApoA1 in the modulation of glucagon levels and to assess the molecular mechanisms underlying this association in an experimental model of pancreatic α-cells.
Materials and methods: We analyzed a cohort of 130 well-characterized Italian non-diabetic subjects enrolled in the CATAnzaro Metabolic Risk factors (CATAMERI) study, who underwent an oral glucose tolerance test. To assess HDL and ApoA-1 direct effects on glucagon secretion we treated 10 weeks old CD1 mice with HDL or ApoA1 (10 mg/kg; i.p.) for 3 consecutive days. In addition, pancreatic α-TC1 clone 6 cells were treated with HDL (32 μM) or ApoA-1 (20 μM) for 24 h and pre-exposed to AKT inhibitor VIII (210 nM) for 2 h. Specific siRNAs were employed to downregulate ABCA1 and ABCG1, which are the main mediators of ApoA-1 and HDL in pancreatic β-cells. Expression levels of pre-proglucagon were determined by RT-PCR, glucagon concentration was measured by ELISA assay, and the phosphorylation status of the PI3K/Akt/FoxO1 signaling pathway was estimated via Western blot.
Results: We observed a significant inverse correlation between circulating glucagon and HDL cholesterol levels ($r = -0.299$, $p < 0.003$), after adjusting for age, sex, BMI, smoking and dyslipidemic status. Mice exposed to HDL or ApoA1 showed a ~30% reduction in circulating glucagon levels following a hypoglycemic stimulus compared to controls ($p = 0.0001$). αTC1 cells pre-incubated with HDL or ApoA-1 respectively showed 40% and 47% reduction of glucagon expression and secretion after exposure to low glucose levels (2 mM, $p < 0.02$). Exposure of αTC1 cells to HDL and ApoA1 stimulated both Akt and FoxO1 phosphorylation; in this state, FoxO1 is excluded from the nucleus and unable to start preproglucagon transcription. The use of Akt inhibitor VIII blocked the

effects of HDL and ApoA1 on glucagon expression and secretion and restored αTC1 cell response to low glucose levels. The reduction of ABCA1 and ABCG1 expression by selective siRNA showed that the inhibitory effect of ApoA1 was specifically mediated by ABCA1, while HDL requires the expression of both receptors to achieve maximum effect.

Conclusion: These data suggest a new role of HDL and ApoA-1 on glucose homeostasis mediated by their effect on α-cellular function.

Disclosure: **G.C. Mannino:** None.

1145

Association of FADS1 genetic variation with free fatty acid levels and type 2 diabetes-related traits

H. Lokvancic[1], S. Mandal[2], M. Adilovic[1], M. Sterner[3], G. Gremsperger[3], E. Ahqvist[3], Z. Velija Asimi[2], B. Prnjavorac[4], A. Causevic[2], L. Groop[3], S. Semiz[1];
[1]International University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [2]University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [3]Lund University Diabetes Centre, Malmoe, Sweden, [4]General hospital Tesanj, Tesanj, Bosnia and Herzegovina.

Background and aims: Fatty acid desaturase (FADS) catalyzes the biosynthesis of highly unsaturated fatty acids (FA) from precursor essential fatty acids. Common variants of *FADS1* gene are associated with cardiometabolic traits, such as Type 2 diabetes (T2D), dyslipidemia, and coronary artery disease (CAD). Here we examined the association of rs174550 (T>C) polymorphism with T2D risk and its related traits, as well as with FA levels in a population from Bosnia and Herzegovina (BH), which has high T2D prevalence of 12.3%.

Materials and methods: Our study included 390 T2D patients and 252 unrelated nondiabetic control subjects. Biochemical parameters, including fasting glucose (FG), fasting insulin (FI), HOMA-B, HOMA-IR, TG, total cholesterol (TC), and lipoprotein levels, were measured in all participants. A subgroup of 96 T2D patients (NT-T2D) that were not treated with oral antidiabetics, lipid-lowering, and other drugs were also studied to dissect the potential drug effects on these phenotypic measures. Detection and quantification of 17 different free fatty acids (FFAs) was done by employing gas chromatography/mass spectrometry. Genotyping analysis was performed by Mass Array Sequenom iPlex platform in cooperation with Lund University Diabetes Centre, Malmo, Sweden.

Results: Importantly, here we report for the first time association of *FADS1* rs174550 variant with lower levels of C14:0 ($B = –0.109$ 95% CI –0.200; –0.019, $p_{add} = 0.019$) and C18:0 FFAs ($B = 0.066$ 95% CI 0.002; 0.129, $p_{add} = 0.042$), while positive association was observed for C18:1 FFA upon adjustment for age and gender ($B = 0.104$ 95% CI 0.013; 0.196, $p_{add} = 0.026$). Furthermore, our results demonstrated that upon adjustment for age and gender, rs174550 variant was also associated with waist circumference ($B = 0.042$ 95% CI 0.002; 0.082, $p_{add} = 0.038$) in control subjects and with BMI ($B = 2.907$ 95% CI 0.548; 5.265, $p_{add} = 0.016$) in NT-T2D patients. We showed that this *FADS1* variant was also associated with HOMA-B ($B = 18.10$ 95% CI 2.254; 33.94, $p_{rec} = 0.026$), FI ($B = 0.419$ 95% CI 0.006; 0.832, $p_{rec} = 0.047$), FG ($B = 0.052$ 95% CI 0.008; 0.097, $p_{add} = 0.022$), TC ($B = 0.505$ 95% CI 0.065; 0.945, $p_{add} = 0.025$) and HDL-cholesterol levels ($B = 0.120$ 95% CI 0.014;0.226, $p_{dom} = 0.027$) in control subjects upon adjustment for age, gender, and/or BMI. In T2D patients it was associated with FG levels ($B = −0.085$ 95% CI −0.162; −0.008, $p_{dom} = 0.031$), diastolic blood pressure ($B = 0.029$ 95% CI 0.003;0.055, $p_{add} = 0.028$), and C-reactive protein (CRP) levels ($B = −0.588$ 95% CI −1.026; −0.149, $p_{add} = 0.009$).

Conclusion: Importantly, here we report for the first time association of *FADS1* variant with levels of selected long-chain FAs that might modulate CAD risk in T2D patients. Interestingly, our previous studies suggested that these FFAs, such as C14:0 and C18:1, were also associated with progression and optimal control of T2D. In line with this, here we also showed the association of rs174550 with waist circumference, BMI, pancreatic B-cell function, fasting insulin and glucose levels in this sample of BH population. This *FADS1* variant was also associated with dyslipidemia, hypertension, and levels of CRP inflammatory marker, which is line with its suggested role in CAD development.

Supported by: Council of Ministers BH/MCA BH and FMES BH awarded to S.S.

Disclosure: **H. Lokvancic:** None.

1146

PEARL, a non-interventional study on real-world use of alirocumab in German clinical practice: results in patients with and without diabetes

K.G. Parhofer[1], B. von Stritzky[2], N. Pietschmann[2], W. Paar[2];
[1]Medical Department IV - Grosshadern, University of Munich, Munich, [2]Medical Department, Sanofi-Aventis Deutschland GmbH, Berlin, Germany.

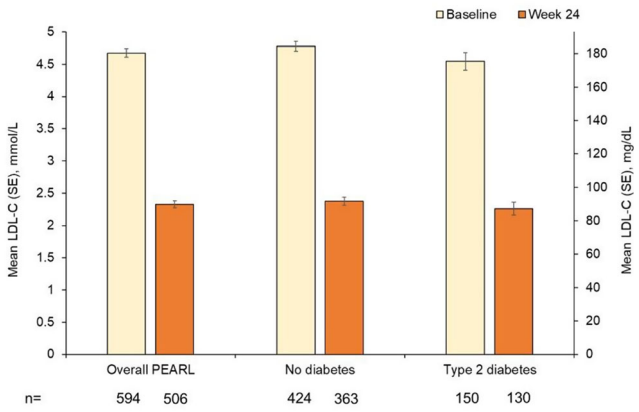
Background and aims: The updated 2017 ESC/EAS Task Force guidance recommends that PCSK9 inhibitors should be considered for patients with atherosclerotic cardiovascular (CV) disease who are not adequately treated with maximally tolerated statins. PEARL assessed efficacy and safety of the PCSK9 inhibitor alirocumab (ALI) in patients with hypercholesterolaemia in a real-world setting. Here, we present data from the overall PEARL population and those with no diabetes mellitus (DM) or type 2 DM (T2DM).

Materials and methods: PEARL was an open, prospective, multicentre, non-interventional study conducted in Germany. Enrolled patients ($n = 619$) should have LDL-C >1.81 or 2.59 mmol/L (70 or 100 mg/dL; depending on CV risk) despite maximally tolerated non-ALI lipid-lowering therapies (LLTs), and subsequently received ≥1 dose of ALI 75 or 150 mg every 2 weeks (Q2W) prior to enrolment. All patients received ALI; dose was adjusted based on physicians' clinical judgment throughout (duration: 24 weeks). The primary efficacy endpoint was LDL-C reduction from baseline (LDL-C prior to ALI therapy) to Week (W)24.

Results: In total, 27.6% of patients had DM, of whom 5.9% had type 1 DM (not further discussed) and 91.1% T2DM. Overall, 45.3% were statin intolerant (unable to tolerate ≥2 statins) and 27.6% were partially statin intolerant (unable to tolerate sufficient statin dose to reach LDL-C <1.81 or 2.59 mmol/L, depending on CV risk). Before the start of ALI therapy, 23.5% of patients were on statin only, 47.9% were on LLT (ezetimibe, fibrates and/or bile acid sequestrants) combined with statin, 10.1% were on LLT combination therapy without statin therapy and 1.8% were on other LLTs (no information available: 16.7%). A similar distribution was seen in patients with T2DM. Overall, initial ALI dose was 75 mg Q2W in 72.9% of patients and 150 mg Q2W in 24.5%, comparable with patients with no DM (72.8% and 24.9%) and with T2DM (73.4% and 24.0%). LDL-C levels at baseline and W24 are shown in Figure 1. Least-squares mean percent change from baseline to W24 in LDL-C was −48.6% for all patients, −49.0% for those with no DM and −47.4% for those with T2DM. During the study, 20.4% of all patients received a dose increase from 75 mg to 150 mg Q2W and 4.0% had a dose decrease from 150 mg to 75 mg Q2W. Corresponding percentages were 17.8% and 1.9% for patients with no DM and 28.1% and 0.7% for those with T2DM. In patients with DM, mean (SD) HbA1c level was 6.9 (1.2)% at baseline and 6.7 (1.0)% at W24. Overall, adverse events were reported in 10.3% of patients, with myalgia (7.3%) the most common; 13.4% of patients discontinued therapy.

Conclusion: PEARL showed that, in a real-world setting, ALI reduced LDL-C levels in patients with high CV risk, including those with no DM and with T2DM. ALI efficacy and safety were consistent with those observed in the ODYSSEY Phase 3 programme.

Figure 1. Mean LDL-C at baseline and W24 for the overall PEARL study population, patients with no DM and patients with T2DM† (ITT analysis)



†Patients with type 1 DM were not further analysed due to the low number of patients included in this group (n=10).
DM, diabetes mellitus; ITT, intention-to-treat; LDL-C, low-density lipoprotein cholesterol; SE, standard error; T2DM, type 2 diabetes mellitus; W, week.

Clinical Trial Registration Number: Nicht-interventionelle Studie number: 320
Supported by: Sanofi-Aventis Deutschland GmbH
Disclosure: **K.G. Parhofer:** Grants; Genzyme, Merck Sharp & Dohme, Novartis, Sanofi. Honorarium; Aegerion, Amgen, Fresenius, Genzyme, Kaneka, Kowa, Merck Sharp & Dohme, Novartis, Regeneron Pharmaceuticals, Inc., Roche, Sanofi.

1147

Frequency of high blood pressure and dyslipidaemia in type 1 and type 2 diabetes: results from the International Diabetes Management Practices Study (IDMPS)

H. Ilkova[1], G. Kaddaha[2], J.J. Gagliardino[3], P. Aschner[4], F. Lavalle[5], A. Ramachandran[6], J.C. Mbanya[7], M. Shestakova[8], J.-M. Chantelot[9], J.C.N. Chan[10];
[1]Istanbul University, Cerrahpasa Medical Faculty, Department of Internal Medicine, Division of Endocrinology, Metabolism and Diabetes, Istanbul, Turkey, [2]Government of Dubai, Dubai Health Authority, Dubai, United Arab Emirates, [3]CENEXA, Center of Experimental and Applied Endocrinology (La Plata National University – La Plata National Scientific and Technical Research Council), La Plata, Argentina, [4]Javeriana University School of Medicine and San Ignacio University Hospital, Bogotá, Colombia, [5]Facultad de Medicina de la Universidad Autónoma de Nuevo León, Monterrey, Mexico, [6]India Diabetes Research Foundation, Dr. A. Ramachandran's Diabetes Hospitals, Chennai, India, [7]Biotechnology Center, Doctoral School of Life Sciences, Health and Environment, and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, [8]Endocrinology Research Center, Moscow, Russian Federation, [9]Sanofi, Paris, France, [10]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, Hong Kong SAR, China.

Background and aims: Appropriate disease management in people with diabetes requires combined control of blood glucose levels and associated cardiovascular risk factors such as blood pressure and serum lipid profiles. We investigated the proportions of people with high blood pressure (HBP) and abnormal lipid profiles associated with their diabetes in the developing world.
Materials and methods: The IDMPS is a global observational survey on the management and patterns of care of people with T1D and T2D in the developing world. The proportions of people with HBP (>130/80 mmHg) and abnormal lipid profiles (LDL-C ≥2.59 mmol/l) associated with their diabetes were determined for participants enrolled from 24 countries across Africa, the Middle East, South Asia and Eurasia between 2016 and 2017.
Results: In people with T1D ($N = 2000$), 20% and 30% had HBP and abnormal lipid profiles, respectively (**Table**). All were prescribed corrective therapy; however, target levels were only achieved by 50% of the population. In people with T2D ($N = 6283$), the frequency of both HBP and abnormal lipid profiles increased over time; control of these complications was worse than in T1D. In total, 27.1% of people with T1D attained 2/3 targets (glycaemic, BP or lipid control) compared with 16.0%–26.5% of people with T2D. However, only 5.1% of people with T1D and T2D achieved the triple target of HbA_{1c} <7%, BP <130/80 mmHg, and LDL-C <2.59 mmol/l overall.
Conclusion: Many people, particularly those with T2D, have concomitant HBP and lipid abnormalities that are poorly controlled; these increase the burden of disease-induced complications and increase the costs of care.

Table. Blood pressure and lipid metabolism in people with T1D and T2D

	T1D N=2000	T2D life style N=50	T2D OGLD N=3637	T2D OGLD + insulin N=1936	T2D insulin alone N=660
Age (mean), years	34.0	54.7	56.4	57.8	59.9
Female sex	51.2	50.0	49.7	56.5	53.6
Disease duration (mean), years	13.1	4.4	7.6	12.8	13.5
Hypertension (HBP ≥130/80 mmHg)	19.9	48.0	61.3	74.3	71.4
HBP treated and BP <130/80 mmHg	48.6	14.9	19.6	16.8	15.8
Abnormal lipid profile	28.9	50.0	65.3	75.9	68.8
LDL-C <2.59 mmol/l	50.7	25.9	47.4	50.5	43.8
HDL-C ≥1.03 mmol/l	75.8	81.5	61.3	57.6	65.1
TG <1.69 mmol/l	85.9	74.1	72.0	71.3	78.1
Target attainment*					
2 of 3 targets attained	27.1	16.0	26.5	16.5	16.0
All targets attained	8.1	0	6.2	1.7	2.9

Data are % unless otherwise specified.
*BP control defined as BP ≥130/80 mmHg; lipid control defined as LDL-C <2.6 mmol/l; HbA_{1c} control defined as <7 %/individually defined.
HBP, high blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OGLD, oral glucose-lowering drug; TG, triglycerides

Supported by: Sanofi
Disclosure: **H. Ilkova:** Employment/Consultancy; Sanofi.

1148

Effects of a polyphenol-rich diet on postprandial lipoprotein composition

G. Della Pepa, C. Vetrani, M. Vitale, L. Bozzetto, G. Costabile, P. Cipriano, A. Mangione, L. Patti, G. Annuzzi, G. Riccardi, A.A. Rivellese; Federico II University, Naples, Italy.

Background and aims: Postprandial dyslipidemia is an independent cardiovascular risk factor that could be influenced by dietary habits. A polyphenol-rich diet has been shown to reduce postprandial triglyceride plasma levels in high cardiometabolic risk individuals. It is not known if dietary polyphenols could also influence lipids composition of plasma lipoproteins. Therefore, our aim was to evaluate the effects of a polyphenol-rich diet on postprandial lipoprotein composition in high cardiometabolic risk individuals.
Materials and methods: Seventy-eight individuals, 35–70 year-old, with high waist circumference and one more component of metabolic syndrome, were randomly assigned to follow a high-polyphenol (HighP) or low-polyphenol (LowP) diet. The two experimental diets were isocaloric, similar for macronutrient composition and fibre and vitamin content. At baseline and after 8-week intervention, the participants consumed a high-fat test meal with a similar composition as the assigned diet. At fasting and 2, 4, and 6 hours after the test meal, blood samples were collected for measuring cholesterol (Chol) and triglyceride (Tg) content in chylomicrons, VLDL1, VLDL2, LDL (separated by density-gradient ultracentrifugation) and HDL (phosphotungstic acid/magnesium chloride precipitation method). Apolipoprotein B-48 (Apo B-48) was measured (ELISA) in VLDL1 at fasting and at 4 hours and 6 hours after the test meal.

Results: Dietary adherence was optimal with no significant changes in body weight. VLDL1 postprandial areas under the curve (AUCs) were significantly lower after HighP than LowP diet for Chol (1.48 ± 0.98 vs. 1.91 ± 1.13 mmol/L·6 h, $p = 0.014$, final values corrected for baseline) and Tg (4.70 ± 2.70 vs. 6.02 ± 3.07 mmol/L·6 h, $p = 0.005$), with no significant changes in Chol/Tg ratio and apo B-48 concentration. LDL Tg AUCs were higher after HighP than LowP diet (1.15 ± 0.33 vs. 1.02 ± 0.35 mmol/L·6 h, $p < 0.001$), with a lower Chol/Tg ratio (14.6 ± 4.0 vs. 16.0 ± 3.8, $p = 0.007$). HDL Tg AUCs were lower after HighP than LowP diet (1.20 ± 0.41 vs. 1.34 ± 0.37 mmol/L·6 h, $p = 0.013$). Chol AUCs tended to be lower ($p = 0.07$) with no significant change in Chol/Tg ratio.
Conclusion: A diet naturally rich in polyphenols reduces lipid content in VLDL1 and modifies the composition of LDL that are richer in triglycerides and likely larger, and HDL that are instead poor in triglycerides. These modifications may configure a less atherogenic postprandial lipoprotein profile.
Clinical Trial Registration Number: NCT01154478
Supported by: European Community's Seventh Framework Programme FP7 and MIUR
Disclosure: **G. Della Pepa:** None.

1149

The burden of dyslipidaemia and association with metabolic parameters in young adults with type 1 diabetes

R. Zaidi[1], M. Belbehri[2], C. Lucas[2], S. Bajaj[2], J. Brake[1], F. Cook[1], P.J. Weston[1], A. Scroxton[1], K. Hassanin[2];
[1]Diabetes and Endocrine Department, Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, [2]School of Medicine, University of Liverpool, Liverpool, UK.

Background and aims: Dyslipidemia is a strong risk factor for cardiovascular disease in type 1 diabetes (T1D). The relationship of dyslipidemia with glycaemic control has been studied comprehensively in older adults with T1D, with limited data in young adults (18–25 years old). Recently, there has been a global rise of weight gain and obesity in this age group, including those with T1D, with rising prevalence of dyslipidemia. The aims of this study were: 1) to measure the prevalence of dyslipidemia and b) identify its relationship to glycaemic control and other metabolic markers in young adults with T1D at a tertiary hospital in the UK.
Materials and methods: We conducted a cross-sectional study of patients attending the Young Adult Diabetes Clinic over 12 months (2016–2017), using data from the online medical records and laboratory system. The eligibility criteria were: 1) patients aged 18–25 years old, 2) with T1D and 3) measured lipid profile/s in the preceding 12 months. Dyslipidemia was defined as total cholesterol/HDL-C ratio (TC/HDL-C) ≥3.5. Patients were then separated into two study groups based on the value of TC/HDL-C ratio - 'Normal lipid group' (TC/HDL-C <3.5) and 'dyslipidemia group' (TC/HDL-C ≥3.5). Baseline characteristics and metabolic features compared between the groups included a) gender difference, b) duration of T1D, c) percentage CHO counting d) HbA1c, e) BMI, f) total cholesterol (TC), g) triglycerides (TG), h) HDL-C, i) LDL-C, j) thyroid stimulating hormone (TSH), k) estimated glomerular filtration rate (eGFR) and l) liver function tests - alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gama glutamyltransferase (GGT).
Results: In total, there were 108 patients, including 34 in the dyslipidemia group (*v.* 74 in the normal lipid group). The prevalence of dyslipidemia, therefore, was 31.5%. In the dyslipidemia group, the male: female ratio was 1:1.6 (*v.* 1.2:1), with a mean duration of T1D of 10.51 years (*v.* 9.29 years, $p = 0.33$). 53% of patients were CHO counting (*v.* 44%, $p = 0.40$). The median BMI was 26.20 (*v.* 23.17, $p = 0.01$) with median HbA1c of 78.50 mmol/mol or 9.3% (*v.* 71.51 mmol/mol or 8.7%, $p = 0.02$). Median TC (4.92 *v.* 3.83 mmol/L, $p < 0.0005$), TG (1.8 *v.* 0.8 mmol/L, $p < 0.0005$) and LDL-C (2.93 *v.*1.86 mmol/L, $p < 0.0005$) levels were significantly higher, whilst HDL-C (1.15 *v.* 1.56 mmol/L, $p < 0.0005$) levels were significantly lower in the dyslipidemia group. Median ALT (14.0 *v.* 13.59 U/L, $p = 0.90$), ALP (83.38 *v.* 89.50 U/L, $p = 0.65$), TSH (1.93 *v.* 1.73 mU/L, $p = 0.09$) and eGFR (90 mL/min/1.73 m^2 in both groups) levels were similar in the two groups, but GGT levels (17.59 *v.* 12.0 U/L, $p < 0.0005$) were higher in the dyslipidemia group, albeit within normal range. None of the patients in either group were on lipid lowering therapy.
Conclusion: The pattern of dyslipidemia seen in this cohort of young adults with T1D is similar to that in the older population with other types of diabetes. The high prevalence of dyslipidemia in this population, particularly in females, who were overweight, with longer duration of T1D and more sub-optimal glycaemic control, is concerning for high risk of cardiovascular morbidity in the future. Whilst there is a lack of strong association of dyslipidemia with other metabolic parameters in this cohort, long-term follow up of these is required.
Disclosure: **R. Zaidi:** None.

PS 110 Treating cardiovascular disease in diabetes

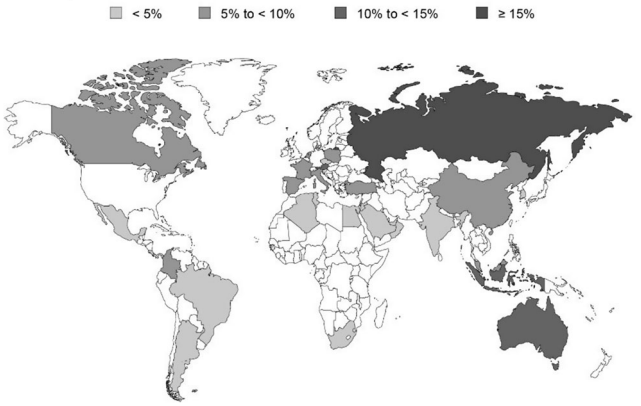
1150

Vascular events in patients with type 2 diabetes in the year following initiation of second-line therapy: the DISCOVER study

F. Surmont[1], H. Chen[2], J. Cid-Ruzafa[3], P. Fenici[4], M.B. Gomes[5], K. Khunti[6], S. Pocock[7], W. Rathmann[8], M.V. Shestakova[9], I. Shimomura[10], F. Tang[11], H. Watada[12], L. Ji[13], N. Hammar[14], M. Kosiborod[11];
[1]AstraZeneca, Luton, UK, [2]AstraZeneca, Gaithersburg, USA, [3]Evidera, Barcelona, Spain, [4]AstraZeneca, Cambridge, UK, [5]Rio de Janeiro State University, Rio de Janeiro, Brazil, [6]University of Leicester, Leicester, UK, [7]London School of Hygiene and Tropical Medicine, London, UK, [8]German Diabetes Center, Duesseldorf, Germany, [9]Endocrinology Research Center, Diabetes Institute, Moscow, Russian Federation, [10]Osaka University, Osaka, Japan, [11]Saint Luke's Mid America Heart Institute, Kansas City, USA, [12]Juntendo University, Tokyo, Japan, [13]Peking University People's Hospital, Beijing, China, [14]AstraZeneca Gothenburg, Mölndal, Sweden.

Background and aims: Vascular complications are the main cause of death and disability in people with type 2 diabetes (T2D). We assessed the occurrence of complications during the first year of follow-up of DISCOVER, a global, observational study of patients with T2D initiating second-line glucose-lowering therapy.
Materials and methods: Patients with data available at 6 or 12 months were included. Microvascular complications comprised new diagnoses or procedures related to retinopathy, neuropathy, nephropathy or erectile dysfunction. Macrovascular complications comprised new diagnoses or procedures related to coronary or peripheral artery disease or heart failure.
Results: In 11 430 patients from 34 countries, new microvascular and macrovascular complications were reported in 6.6% and 4.7% of patients, respectively, with substantial variations across countries. Proportions of patients with new macrovascular complications are shown in the figure. The proportion of patients with new microvascular complications was higher in those with vs without microvascular disease at baseline (9.8% vs 5.9%, $p < 0.001$). The same was observed for new macrovascular complications in patients with vs without macrovascular disease at baseline (16.9% vs 2.9%, $p < 0.001$).
Conclusion: Rates of new vascular complications over 1 year were strikingly high in presumably low-risk patients with short T2D duration, highlighting opportunities for early aggressive risk-factor modification.

Figure. Proportion of patients for whom new macrovascular complications were documented in the first year of follow-up.



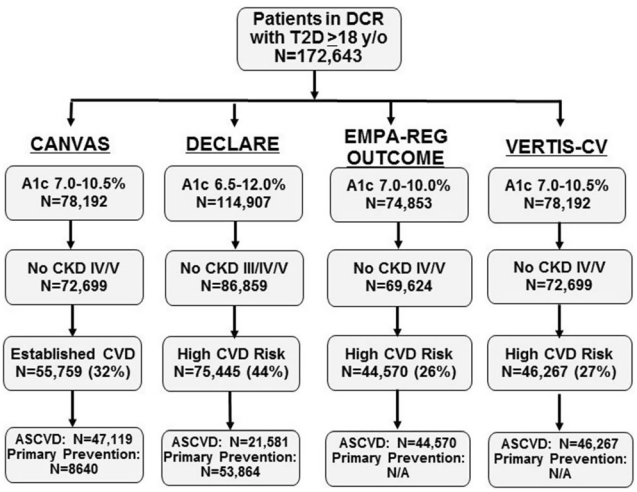
Clinical Trial Registration Number: NCT02322762
Supported by: AstraZeneca
Disclosure: **F. Surmont:** Employment/Consultancy; AstraZeneca.

1151

Eligibility varies across the 4 sodium-glucose cotransporter-2 inhibitor cardiovascular outcome trials in adults from the Diabetes Collaborative Registry

E.T. Wittbrodt[1], D. Chamberlain[1], S.V. Arnold[2], F. Tang[2], M. Kosiborod[2];
[1]Medical Affairs, AstraZeneca, Wilmington, [2]St. Luke's Mid America Heart Institute, Kansas City, USA.

Background and aims: The US FDA requires cardiovascular (CV) risk assessment for type 2 diabetes (T2D) medications. Due to differences in eligibility criteria among CV outcome trials (CVOTs), the generalizability of trial populations to US adults with T2D is unknown. We used the Diabetes Collaborative Registry (DCR) to assess the percentages of adults with T2D who would have met eligibility criteria for the pivotal CVOTs of the 4 US marketed sodium-glucose cotransporter-2 inhibitors (SGLT-2i).
Materials and methods: This was a retrospective cross-sectional study using data from the DCR, a US outpatient diabetes registry that comprises 374 sites and 5114 providers. For this analysis, we excluded adults with T1D, prediabetes, diet-controlled T2D, and missing A1C measurements. Major inclusion and exclusion criteria of the CVOTs, including A1C, CKD, and CV history, were used to determine the % eligible for each, all, or none of the CVOTs. The 10-year ASCVD risk for the primary prevention cohorts was calculated with ACC ASCVD risk estimator.
Results: Among 172,643 adults with T2D, mean age was 68 years, 43% were women, mean A1C was 7.8%, 64% had ASCVD, and 10-year risk was 29% in those without ASCVD. Proportions of potentially eligible adults for CVOTs were CANVAS 32%, DECLARE 44%, EMPA-REG OUTCOME 26%, and VERTIS-CV 27% (Figure); 20% were eligible for all 4 trials, and 48% for none of the trials. Patients who were ineligible for any of the trials were slightly younger (mean age 67 years), more likely women (47%), had less ASCVD (56%), and a lower 10-year risk of ASCVD among those without established disease (25%).
Conclusion: Large variability exists in the proportion of T2D adults potentially eligible for each of the SGLT-2i CVOTs. While patients who were ineligible for the CVOTs were lower risk than those eligible, the burden of ASCVD and underlying risk of these patients remains high. Eligibility for any of the CVOTs was greater than anticipated due to the relatively high CV risk profile of patients in the DCR compared to the general US population with T2D. The defined study populations in CVOTs must be carefully considered when comparing with patients seen in routine clinical practice.



Supported by: DCR is funded by AstraZeneca and Boehringer Ingelheim
Disclosure: **E.T. Wittbrodt:** Employment/Consultancy; AstraZeneca.

1152

Rates of major adverse cardiovascular events and mortality with basal insulin by liraglutide use: a DEVOTE sub-analysis

M.F. Ranthe[1], K. Brown-Frandsen[1], S.S. Emerson[2], S.P. Marso[3], D.K. McGuire[4], T.R. Pieber[5], N.R. Poulter[6], B. Zinman[7], R. Grøn[1], M. Lange[1], A.C. Moses[8], P. Örsy[1], R.E. Pratley[9], on behalf of the DEVOTE Study Group;
[1]Novo Nordisk A/S, Søborg, Denmark, [2]University of Washington, Seattle, USA, [3]HCA Midwest, Kansas City, USA, [4]University of Texas Southwestern Medical Center, Dallas, USA, [5]Medical University of Graz, Graz, Austria, [6]Imperial College London, London, UK, [7]Mount Sinai Hospital, University of Toronto, Toronto, Canada, [8]Novo Nordisk Inc., Plainsboro, USA, [9]Florida Hospital, Orlando, USA.

Background and aims: Cardiovascular (CV) safety profiles for insulin degludec (degludec) and insulin glargine 100 units/mL (glargine U100) were established by the DEVOTE and ORIGIN trials. In the LEADER trial, the glucagon-like peptide-1 analogue liraglutide significantly reduced the risk of major adverse cardiovascular events (MACE; CV death, non-fatal myocardial infarction or non-fatal stroke) and mortality vs. placebo in patients with type 2 diabetes (T2D) and high CV risk. This *post hoc* analysis of DEVOTE compared associations between concomitant liraglutide vs. no liraglutide use and the risk of MACE and all-cause mortality in patients with T2D and high CV risk, independent of the basal insulin assigned.

Materials and methods: In DEVOTE, patients with T2D and high CV risk ($n = 7637$) were randomised 1:1 to degludec or glargine U100. HRs for MACE and all-cause mortality were calculated using a Cox regression model adjusted for treatment and time-varying liraglutide use at any time in the trial, without interaction testing. Sensitivity analyses adjusted for baseline covariates included age, sex, smoking, T2D duration, CV risk, insulin therapy, race, BMI, HbA_{1c}, LDL, HDL and liver/kidney function.

Results: At baseline, 436 (5.7%) patients were on liraglutide: 187 (2.4%) started and 210 (2.7%) stopped liraglutide thereafter. Mean liraglutide exposure from randomisation was 731 days. Liraglutide use was associated with significantly lower HRs for MACE and all-cause mortality vs. no liraglutide use (Table). Multiple sensitivity analyses confirmed these results. There was no significant difference in the rate of severe hypoglycaemia with liraglutide use vs. no liraglutide use (HR: 0.79 $[0.51; 1.24]_{95\%\ CI}$). A similar result was obtained following adjustment for additional baseline covariates (HR: 0.89 $[0.57; 1.40]_{95\%\ CI}$). Mean total insulin doses were comparable for patients who received liraglutide at any time (0.9 ± 0.7 units/kg) and those who never received liraglutide (1.0 ± 0.8 units/kg).

Conclusion: In this *post hoc* analysis of DEVOTE, liraglutide use was associated with a lower MACE and all-cause mortality rate in basal insulin users providing additional support to the results from the LEADER trial.

Table: MACE and all-cause mortality by liraglutide use in DEVOTE

	Degludec/glargine U100 with concomitant liraglutide use		Degludec/glargine U100 with no concomitant liraglutide use		Liraglutide use vs no liraglutide use (HR [95% CI])	Two-sided *p*-value
	Number of events	Events per 100 patient-years of observation	Number of events	Events per 100 patient-years of observation		
MACE	25	2.91	656	4.74	0.62 [0.41; 0.92]	0.02
CV death	8	0.91	270	1.90	0.47 [0.23; 0.96]	0.04
Non-fatal MI	15	1.73	298	2.13	0.82 [0.49; 1.37]	0.45
Non-fatal stroke	5	0.57	145	1.03	0.56 [0.23; 1.37]	0.20
All-cause mortality	13	1.48	410	2.88	0.50 [0.29; 0.88]	0.02

HRs presented are for the time to the first confirmed event (in days).
CV, cardiovascular; glargine U100, insulin glargine 100 units/mL; MACE, major adverse cardiovascular events (CV death, non-fatal MI or non-fatal stroke); MI, myocardial infarction.

Clinical Trial Registration Number: NCT01959529
Supported by: Novo Nordisk
Disclosure: **M.F. Ranthe:** Employment/Consultancy; Novo Nordisk. Stock/Shareholding; Novo Nordisk.

1153

Liraglutide effects in insulin-treated patients in LEADER

C. Tack[1], S. Jacob[2], C. Desouza[3], S.C. Bain[4], M.A. Nauck[5], J. Petrie[6], N.R. Poulter[7], R.E. Pratley[8], H.B.K. Stegmann[9], H. Bosch-Traberg[9], E. Startseva[9], B. Zinman[10], LEADER Publication Committee on behalf of the LEADER Trial Investigators;
[1]Radboud University Medical Center, Nijmegen, Netherlands, [2]Praxis für Prävention und Therapie, Kardio Metabolisches Institut, Villingen-Schwenningen, Germany, [3]Veterans Affairs Medical Center, Omaha, USA, [4]Swansea University Medical School, Swansea, UK, [5]Diabetes Center Bochum-Hattingen, St Josef Hospital (Ruhr-Universität Bochum), Bochum, Germany, [6]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK, [7]International Centre for Circulatory Health, Imperial College, London, UK, [8]Florida Hospital Translational Research Institute, Orlando, USA, [9]Novo Nordisk A/S, Søborg, Denmark, [10]Lunenfeld–Tanenbaum Research Institute, Mt. Sinai Hospital, University of Toronto, Toronto, Canada.

Background and aims: Combining glucagon-like peptide 1 (GLP-1) analogues and insulin has complementary benefits, but long-term data are sparse. In the LEADER cardiovascular (CV) outcomes trial, rates of major CV events and hypoglycaemia were lower when liraglutide vs placebo was added to standard of care. A substantial number of patients in this trial were treated with insulin, providing detailed information on the combination insulin + GLP-1 for a median follow-up of 3.8 years.

Materials and methods: This post hoc subgroup analysis assessed metabolic parameters (HbA_{1c}, weight, systolic BP and LDL cholesterol), severe hypoglycaemia and CV outcomes by baseline insulin use: no insulin vs basal-only vs other. The LEADER trial included 9340 patients; at baseline, 5171 (55%) patients were not on insulin treatment, 3159 (34%) were on basal-only insulin and 1010 (11%) were treated with other insulin regimens (9.7% premix). Insulin use at baseline was balanced overall between randomised treatment groups, but fewer patients randomised to liraglutide (29%) vs placebo (43%) initiated in-trial insulin.

Results: In the basal-only subgroup, liraglutide reduced HbA_{1c} vs placebo (estimated treatment difference [ETD] −0.48%, 95% CI −0.57; −0.38), with a significant reduction in severe hypoglycaemia rate (estimated rate ratio 0.42, 95% CI 0.26; 0.68). Liraglutide also reduced weight (ETD −2.5 kg, 95% CI −3.0; −2.1) and there were trends for reductions in systolic BP (ETD −1.1 mmHg, 95% CI −2.4; 0.1) and LDL cholesterol (ETD −1.3 mg/dL, 95% CI −3.6; 1.0). The CV risk reduction observed with liraglutide in the full trial population was similar in the basal-only subgroup (estimated HR 0.84, 95% CI 0.70; 1.00). Results were similar in the no-insulin subgroup. There was heterogeneity in the results for the smaller, other insulin subgroup with reductions in HbA_{1c} and weight, but no significant differences in other endpoints.

Conclusion: In basal insulin-treated patients with T2D and high CV risk, treatment with liraglutide improved glycaemic control, reduced body weight and halved the severe hypoglycaemia risk, with a similar CV risk reduction to patients not on insulin.

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk
Disclosure: **C. Tack:** Other; Support by Novo Nordisk.

1154

Arrhythmias and heart rate increase in the LEADER trial and relation to risk of cardiovascular events

M. Husain[1], S.C. Bain[2], J.F.E. Mann[3], M.A. Nauck[4], N. Poulter[5], F.M.M. Baeres[6], B. Goldman[6], A.B. Thomsen[6], S. Marso[7], LEADER Publication Committee on behalf of the LEADER Trial Investigators; [1]Ted Rogers Centre for Heart Research, Toronto General Hospital Research Institute, Toronto, Canada, [2]Swansea University Medical School, Swansea, UK, [3]Friedrich Alexander University of Erlangen, Erlangen, Germany, [4]Diabetes Center Bochum-Hattingen, St Josef Hospital (Ruhr-Universität Bochum), Bochum, Germany, [5]Imperial College London, London, UK, [6]Novo Nordisk A/S, Søborg, Denmark, [7]HCA Midwest Health Heart & Vascular Institute, Kansas City, USA.

Background and aims: Epidemiological data suggest that a higher resting heart rate is associated with a higher risk of cardiovascular (CV) events and death. Glucagon-like peptide-1 receptor agonists can increase heart rate. In the LEADER trial, liraglutide significantly reduced the risk of major adverse CV events ([MACE]; CV death, non-fatal myocardial infarction and non-fatal stroke) by 13% vs placebo (PBO) in people with type 2 diabetes (T2D) and high CV risk.

Materials and methods: In a post hoc analysis from LEADER, we evaluated the frequency of arrhythmias and, for patients with heart rate increases <10 or ≥10 bpm at 6 months, the risk of CV events. In LEADER, 9340 patients with T2D and high CV risk were randomised 1:1 to add liraglutide or PBO to standard of care, and followed for 3.5–5 years. Serious adverse events and non-serious medical events of special interest related to heart rate were systematically collected and reviewed. Cox regression analysis was used to evaluate the risk of CV events in patients with heart rate increases <10 bpm or ≥10 bpm at 6 months.

Results: Mean heart rate increased by 3 bpm for liraglutide vs PBO. The overall frequency of cardiac arrhythmias was 4.9% in both arms, based on adverse event reporting. The types and rates of arrhythmias reported were generally similar in both arms, with low numbers for most arrhythmias and numerically fewer events of ventricular tachycardia and cardiac arrest in the PBO and liraglutide arms, respectively (Table). In total, 3002 (64.3%) patients receiving liraglutide and 3683 (78.8%) receiving PBO had a heart rate increase from baseline <10 bpm at 6 months. Among these patients, liraglutide significantly decreased the risk of MACE (HR [95% CI]: 0.84 [0.73–0.96], $p = 0.01$) and non-significantly decreased the risk of heart failure hospitalisation (0.81 [0.65–1.02]; $p = 0.07$) vs PBO. A total of 1435 (30.7%) patients receiving liraglutide and 750 (16.1%) receiving PBO had a heart rate increase from baseline ≥10 bpm at 6 months. In this subgroup, liraglutide non-significantly decreased the risk of MACE (HR [95% CI]: 0.92 [0.72–1.17], $p = 0.51$) and heart failure hospitalisation (0.94 [0.63–1.43], $p = 0.78$) vs PBO.

Conclusion: The increased mean heart rate observed with liraglutide was not accompanied by an overall higher frequency of arrhythmias vs PBO. Liraglutide decreased the risk of CV events vs PBO in both subgroups regardless of heart rate increase <10 or ≥10 bpm.

Table: Arrhythmia adverse events (AEs) with frequency ≥0.2%

	Liraglutide N=4668				Placebo N=4672			
	N	%	E	R	N	%	E	R
Cardiac arrhythmias*	228	4.9	293	16.4	229	4.9	279	15.7
Atrial fibrillation	91	1.9	115	6.5	99	2.1	116	6.5
Ventricular tachycardia	18	0.4	29	1.6	8	0.2	11	0.6
Atrial flutter	17	0.4	20	1.1	17	0.4	19	1.1
Cardiac arrest	20	0.4	20	1.1	31	0.7	31	1.7
Cardio-respiratory arrest	15	0.3	15	0.8	13	0.3	15	0.8
Bradycardia	11	0.2	11	0.6	11	0.2	11	0.6
Arrhythmia	10	0.2	11	0.6	6	0.1	6	0.3
Atrioventricular block complete	10	0.2	10	0.6	9	0.2	9	0.5

Full analysis set. *Serious AEs or non-serious medical events of special interest related to cardiac arrhythmia identified by reviewing events reported within group 'cardiac arrhythmias'. E, No. of events; N, No. of patients; R, rate of events/1000 patient-years of observation; %, proportion of patients.

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **M. Husain:** Other; Support: Novo Nordisk A/S.

1155

Effect of liraglutide on cardiovascular outcomes in patients with or without prior heart failure history in LEADER

H.A. Saevereid[1], M. Husain[2], S.C. Bain[3], J.F.E. Mann[4], M.A. Nauck[5], N. Poulter[6], B. Goldman[1], A.B. Thomsen[1], S. Marso[7], LEADER Publication Committee on behalf of the LEADER Trial Investigators; [1]Novo Nordisk A/S, Søborg, Denmark, [2]Ted Rogers Centre for Heart Research, Toronto General Hospital Research Institute, Toronto, Canada, [3]Institute of Life Science, Swansea University, Swansea, UK, [4]Friedrich Alexander University of Erlangen, Erlangen, Germany, [5]Diabetes Center Bochum-Hattingen, St. Josef Hospital (Ruhr-University Bochum), Bochum, Germany, [6]Imperial College London, London, UK, [7]HCA Midwest Health Heart & Vascular Institute, Kansas City, USA.

Background and aims: Some type 2 diabetes (T2D) therapies are associated with an increased risk of heart failure (HF). In the LEADER trial, liraglutide significantly reduced the risk of major adverse cardiovascular (CV) events (MACE) by 13% vs placebo (PBO) when added to standard of care in people with T2D and high CV risk. Here, we report post hoc analyses conducted to assess the risk of CV events, including HF hospitalisation, in LEADER participants with or without a history of New York Heart Association (NYHA) class I-III HF.

Materials and methods: In LEADER, 9340 patients with T2D and high CV risk were randomised 1:1 to add liraglutide or PBO to standard of care, and followed for 3.5–5 years. Chronic NYHA IV HF was an exclusion criterion.

Results: At baseline, 18% of patients in both treatment arms had a history of HF (NYHA I-III); 14% had a history of NYHA II-III HF. Overall, fewer patients were hospitalised for HF with liraglutide vs PBO during the trial (HR [95% CI]: 0.87 [0.73–1.05], $p = 0.14$; Table). There was no interaction between treatment and history of NYHA I-III HF for the risk of the primary CV endpoint, an expanded CV endpoint or HF hospitalisation (Table).

Conclusion: No increased risk of MACE or hospitalisation due to HF was observed in patients either with or without a history of HF in the LEADER trial. The point estimates were in favour of liraglutide for MACE and expanded MACE, in patients both with and without a history of HF.

Table: Risk of CV events with liraglutide or placebo by NYHA I–III HF status at baseline

	Number of patients with an event (%)		HR (CI)	*p*-value
	Liraglutide	Placebo		
Primary composite MACE: first occurrence of CV death, non-fatal myocardial infarction, or non-fatal stroke				
Overall	608/4668 (13.0)	694/4672 (14.9)	0.87 (0.78–0.97)	0.01
Baseline NYHA I–III HF status				
Without	466/3833 (12.2)	524/3840 (13.6)	0.88 (0.78–1.00)	0.53
With	142/835 (17.0)	170/832 (20.4)	0.81 (0.65–1.02)	
Expanded composite MACE: CV death, non-fatal myocardial infarction, non-fatal stroke, coronary revascularisation, or hospitalisation for unstable angina pectoris or HF				
Overall	948/4668 (20.3)	1062/4672 (22.7)	0.88 (0.81–0.96)	0.005
Baseline NYHA I–III HF status				
Without	704/3833 (18.4)	786/3840 (20.5)	0.89 (0.80–0.98)	0.72
With	244/835 (29.2)	276/832 (33.2)	0.86 (0.72–1.02)	
Hospitalisation for HF				
Overall	218/4668 (4.7)	248/4672 (5.3)	0.87 (0.73–1.05)	0.14
Baseline NYHA I–III HF status				
Without	110/3833 (2.9)	140/3840 (3.6)	0.78 (0.61–1.00)	0.22
With	108/835 (12.9)	108/832 (13.0)	0.98 (0.75–1.28)	

HRs and *p*-values estimated using Cox proportional hazards model with treatment as a factor. For subgroups with or without NYHA I–III HF at baseline, *p*-values are for interaction between treatment and subgroup.

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **H.A. Saevereid:** Other; Funding: Novo Nordisk A/S.

1156

Alogliptin and pioglitazone prevent palmitate-induced apoptosis and autophagy in human cardiac progenitor cells from control but not from type 2 diabetic subjects

R. D'Oria[1], M. Incalza[1], C. Caccioppoli[1], A. Leonardini[1], R. Schipani[1], A. Cignarelli[1], A. Natalicchio[1], S. Perrini[1], V. Margari[2], D. Paparella[2,3], L. Laviola[1], F. Giorgino[1];
[1]Department of Emergency and Organ Transplantation, University of Bari, Bari, [2]Cardiac Surgery Santa Maria Hospital, Bari, [3]Cardiac Surgery, University of Bari, Bari, Italy.

Background and aims: Physiological tissue turnover in the heart requires proper activation and viability of multipotent cardiac progenitor cells (CPCs). A defective CPC compartment, in terms of CPC number and pro-angiogenic capacity, contributes to diabetes- and hyperglycemia-related heart failure in humans. GLP-1-based therapies have been shown to promote myocardial survival and improve endothelial dysfunction. On the other hand, pioglitazone has demonstrated pleiotropic anti-oxidant and anti-atherogenic effects. Thus, we investigated the effects of alogliptin and pioglitazone, alone or in combination, on the viability of human CPCs challenged with the saturated fatty acid palmitate.
Materials and methods: Human CPCs were obtained from control subjects and type 2 diabetic patients (T2DM) undergoing cardiac surgery for coronary artery bypass grafting and/or valve surgery. Human CPCs were exposed to 0.25 mM palmitate for 16 h after pre-treatment with 10 μM alogliptin and/or 10 μM pioglitazone for 1 h. Apoptosis was assessed by ELISA assay. Autophagy was evidenced by immunoblotting of LC3-II. Akt and Erk phosphorylation was studied by immunoblotting.
Results: Exposure of human CPCs isolated from control subjects to alogliptin and/or pioglitazone for 16 h resulted in Akt, but not Erk, activation ($p < 0.05$). By contrast, neither alogliptin nor pioglitazone, used alone or in combination, induced Akt or Erk phosphorylation in human CPCs from T2DM patients. Exposure to palmitate resulted in increased apoptosis and autophagy in CPC from both control subjects and T2DM patients ($p < 0.05$). Pretreatment with alogliptin, alone or in combination with pioglitazone before exposure to palmitate, reduced palmitate-induced apoptosis and autophagy in human CPCs isolated from control subjects ($p < 0.05$) but not in CPCs from T2DM patients.
Conclusion: Palmitate induces apoptosis and autophagy in human CPCs from control subjects and T2DM patients. Alogliptin and pioglitazone and their combination prevent the palmitate-induced abnormalities of human CPCs isolated from control subjects, likely through enhancement of pro-survival signaling pathways. CPCs obtained from T2DM patients appear to be resistant to the protective effects of alogliptin and/or pioglitazone. Hence, diabetes may affect the viability of the CPC compartment by impairing the activation of protective signaling pathways.
Supported by: Takeda
Disclosure: **R. D'Oria:** None.

1157

Platelet reactivity and clinical outcome on prasugrel and ticagrelor in type 2 diabetic patients with acute myocardial infarction: real world single centre experiences

M. Samos[1], T. Bolek[1], R. Simonova[2], I. Skornova[2], F. Kovar[1], P. Galajda[1], P. Kubisz[2], J. Stasko[2], M. Mokan[1];
[1]Department of Internal Medicine I, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, [2]Department of Hematology and Blood Transfusion, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia.

Background and aims: Type 2 diabetes (T2D) is associated with high on-treatment platelet reactivity (HTPR) on clopidogrel, which might be overcome with prasugrel. Now, ticagrelor has been shown to be similarly effective as prasugrel in unselected population of acute coronary syndrome patients. The aim of this pilot study was to compare the on-treatment platelet reactivity and clinical outcome in prasugrel-treated and ticagrelor-treated T2D patients undergoing percutaneous coronary intervention (PCI) for acute myocardial infarction (AMI).
Materials and methods: A single centre, preliminary prospective study with observational design enrolling 23 T2D patients (11 prasugrel-treated, 12 ticagrelor treated) undergoing PCI was performed. On-treatment response was tested with vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) flow cytometry analysis. Samples were taken prior coronary angiography (sample 1) and on the next day after this procedure (sample 2). Composite primary major cardiac events endpoint (cardiovascular death, AMI, need for repeated urgent myocardial revascularization and stent thrombosis) was recorded in a 6-month period of clinical follow-up.
Results: The time interval form ADPRB loading dosing to blood sampling did not differ significantly in prasugrel-treated and ticagrelor-treated T2D patients. Similarly, no significant differences in VASP-P were found between prasugrel-treated and ticagrelor-treated T2D patients (sample1: 52.7 ± 25.5 versus 50.5 ± 21.1, $p = 0.62$; sample2: 26.3 ± 10.3 versus 29.9 ± 12.7, $p = 0.45$). In addition, there were no significant differences in cardiovascular mortality and in the incidence of primary composite endpoint comparing ticagrelor- and prasugrel-treated patients.
Conclusion: Ticagrelor therapy reaches similar on-treatment platelet reactivity. In addition, no significant differences were found in clinical outcome between ticagrelor- and prasugrel-treated T2D patients with AMI.
Supported by: APVV (Slovak Research and Development Agency) 16-0020
Disclosure: **M. Samos:** None.

PS 111 Metabolism, inflammation in metabolic liver disease

1158

Pyruvate dehydrogenase kinase 4 in the liver mediate hepatic gluconeogenesis by modulation of cAMP-PKA-CREB signal pathway

J.-B. Seo[1], B.-Y. Park[2], J.-H. Jeon[1], Y.-K. Choi[1], N.-Y. Kim[1], M.-J. Kim[1], J.-G. Kim[1], K.-G. Park[1], E.-H. Kim[3], I.-K. Lee[1];
[1]Department of Internal Medicine, School of medicine, Kyungpook National University, Daegu, [2]Department of Biomedical Science, Graduate School, Kyungpook National University, Daegu, [3]Department of Internal Medicine, Daegu Fatima Hospital, Daegu, Republic of Korea.

Background and aims: Hepatic gluconeogenesis is facilitated by glucagon, and it is mediated by cyclic AMP (cAMP)-protein kinase A (PKA)-cAMP response element binding (CREB) signaling pathway. In diabetic condition, it is well known that pyruvate dehydrogenase kinase 4 (PDK4) expression is increased in the muscle and liver. However, there are few studies on relationship between PDK4 and gluconeogenic signaling pathway in the liver.
Materials and methods: Primary mouse hepatocytes were used for quantitative real-time PCR, western blot and glucose production assay. Metabolic flux analysis was performed to determine the rate of fatty acid oxidation by using [U-$^{13}C_{16}$] palmitate. Ad-PDK4 and shPDK4 were delivered into diet-induced obesity mice to determine the role of PDK4 in hepatic gluconeogenesis.
Results: Knockdown of hepatic PDK4 in diet-induced obesity mice decreased hepatic glucose production. PDK inhibitor dichloroacetate also attenuated PKA-CREB signaling and gluconeogenesis. Mechanistically, inhibition of PDK4 decreased cAMP levels in hepatocytes. This correlated with lower ATP levels and an increase in phosphorylated AMP-activated protein kinase (AMPK), suggesting cAMP reduction was by the action of the AMPK-sensitive cyclic nucleotide phosphodiesterase 4B (PDE4B). Metabolic flux analysis showed that the reduction in ATP was a consequence of diminished rate of fatty acid oxidation (FAO). On the contrary, overexpression of PDK4 increased FAO and increased ATP which decreased phosphorylation of AMPK and allowed greater accumulation of cAMP. The latter were abrogated by the FAO inhibitor etomoxir, suggesting a critical role for PDK4 in FAO stimulation and the regulation of cAMP levels.
Conclusion: In this study, we suggest that PDK4 in the liver has a critical role in the regulation of hepatic gluconeogenesis by FAO stimulation and cAMP regulation.
Supported by: Korea Health Technology R&D Project, NRF of Korea
Disclosure: **J. Seo:** None.

1159

The increased flux of pyruvate cycling coupled with Krebs cycle is responsible for the pathogenesis of non-alcoholic liver disease

M.-J. Kim[1], Y.-H. Go[1,2], J.-H. Jeon[1,2], Y.-K. Choi[1], N.-Y. Kim[1], J. Seo[1], J.-G. Kim[1], K.-G. Park[1], E.-H. Kim[3], I.-K. Lee[1,2];
[1]Kyungpook National University School of medicine, Daegu, [2]Leading-edge Research Center for Drug Discovery and Development for Diabetes and Metabolic Disease, Daegu, [3]Internal Medicine, Fatima hospital, Daegu, Republic of Korea.

Background and aims: Non-alcoholic liver disease (NAFLD) is one of the most important chronic liver disorders worldwide and closely associated with obesity and insulin resistance, so called metabolic syndrome. The relationship between hepatic mitochondrial dysfunction and the pathogenesis of NAFLD is widely being investigated. However, the exact mechanism by which mitochondrial dysfunction affects the occurrence of NAFLD is hardly known. Pyruvate dehydrogenase kinase (PDK) inhibits mitochondrial pyruvate dehydrogenase complex (PDC) activity by phosphorylation. Excessive suppression of PDC activity might play a crucial role in the development of metabolic disease. Indeed, increase of PDK is observed in the context of mitochondrial dysfunction, encompassing diabetes and obesity. Thus, here we investigated the role of PDKs with regard to its role in the regulation of pyruvate cycling and Krebs cycle flux in NAFLD.
Materials and methods: Briefly, wild-type (WT) and PDK2 knockout (KO) mice (8-week-old male) were fed high-fat diet (HFD; 20% of calories were carbohydrate and 60% from fat). PDC activity assay was measured spectrophotometrically. Western blotting for PDK1, PDK2, PDK3, PDK4, phospho-PDHE1α (Ser293, Ser300), and β-tubulin was performed from hepatocytes. The mRNA expression was also detected by quantitative real-time RT-PCR. Hepatic metabolites were prepared from tissues of overnight-fasted mice. Hepatic pyruvate, ß-hydroxybutyrate, oxaloacetate (OAA), and citrate were measured by enzymatic methods. Hepatic succinate was measured with Succinic Acid Assay kit. Statistical analysis was determined by the unpaired Student's t-test when two groups were compared.
Results: In diet-induced obesity mice, the expression of pyruvate dehydrogenase kinase 2 (PDK2) in the liver was increased. In metabolic flux analysis, it was found pyruvate cycling as well as TCA cycle flux was augmented by chronic high fat feeding. This was a consequence of decreased PDC flux and corresponding increase of anaplerotic pyruvate carboxylase flux. As a result, increased phosphoenolpyruvate level in the hepatocyte increased the rate of gluconeogenesis and glyceroneogenesis. When PDK2 was knocked down, however, a flux of pyruvate cycling and Krebs cycle was attenuated. Mechanistically, decreased pyruvate cycling flux by PDK2 deficiency resulted in decreased level of OAA. Decreased rate of conversion of OAA to acetyl-CoA, in turn, increased the rate of ketogenesis. This result contributed to increased rate of lipolysis, fatty acid oxidation and depletion of intrahepatic triglyceride content. The PDK2-deficient mice showed decreased gluconeogenesis and improved NAFLD upon HFD compared with wild-type challenged with HFD.
Conclusion: We report the pathophysiology of NAFLD is strongly associated with the flux of pyruvate cycling. It depends on PDK2 activity which inhibits the PDC and increase hepatic Krebs cycle flux. On the other hands, inhibition of PDK2 results in decreased pyruvate recycling flux, thereby increasing ketogenesis and ß-oxidation. Modulation of pyruvate cycling might be a key pathway to improve NAFLD.
Supported by: Korea Health Technology R&D Project (KHIDI)
Disclosure: **M. Kim:** None.

1160

Insulin resistance and farnesoid X receptor expression in patients with non-alcoholic fatty liver disease and various disorders of carbohydrate metabolism

E. Mishina[1], A. Mayorov[1], A. Bogolyubova[2], P. Bogomolov[3], M. Matsievich[3], K. Kokina[3];
[1]Endocrinology Research Centre, Moscow, [2]Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, Moscow, [3]Moscow Regional Research and Clinical Institute, Moscow, Russian Federation.

Background and aims: Aim of the study was to assess the insulin resistance (IR) and expression of the farnesoid X receptor (FXR) in patients with non-alcoholic fatty liver disease (NAFLD) and various disorders of carbohydrate metabolism.
Materials and methods: The study included 20 patients aged 18–60 years with a body mass index more than 27.5 kg/m^2. All participants underwent clinical and laboratory examination, determination of IR (calculation by mathematical models of glucose homeostasis and hyperinsulinemic euglycemic clamp test); a biopsy of the liver was performed under ultrasound control with further morphological study of the biopsy specimen. The severity of NAFLD was estimated by the percentage of steatosis, the activity scale of NAFLD (NAS). When a liver biopsy

was performed, a part of the tissue was frozen at a temperature of −80°C with a further molecular biological study. Frozen liver tissue samples were used to isolate RNA and proteins with subsequent quantitative PCR analysis, the Western blot method. Statistical analysis was carried out using nonparametric statistical methods.

Results: In hyperinsulinemic euglycemic clamp test, it was found that in patients without NAFLD, IR was less pronounced (M-index 5,1 [3.3; 7.0] mg/kg/min) compared with patients with steatosis (M-index 3.1 [2.4; 3.5] mg/kg/min) or *nonalcoholic steatohepatitis (NASH) (*M-index 2.1 [1.8; 2.7] mg/kg/min). There was a negative correlation between the degree of NAFLD and the M-index (rs = −0.554, $p < 0.05$). A negative correlation was found between the M-index and the degree of a disorder of carbohydrate metabolism (rs = −0.532, $p = 0.01$). In a real-time PCR, the expression of FXR in liver tissues in groups of patients without NAFLD and with steatosis did not differ. The group of patients with NASH is divided depending on FXR expression level into two parts: in 4 out of 8 patients the expression was at the same level, as in patients with steatosis and without NAFLD (subgroup "NASH FXR low"), while in other patients the level of expression of FXR is much higher (subgroup "NASH FXR high"). Lower levels of immunoreactive insulin (23 vs 46 μU/ml) and HOMA-index (7 vs 13.5) were noted in the "NASH FXR high" subgroup assessing IR. Also, the "NASH FXR high" subgroup showed a lower percentage of steatosis (30 vs 56), and lower scores on the NAS scale (3.2 vs 4.2). However, all the above differences were not statistically significant due to the small number of patients in every group.

Conclusion: There is a negative correlation between the degree of NAFLD and the M-index, as well as between the M-index and the degree of a disorder of carbohydrate metabolism. Increased expression of FXR is probably associated with less pronounced steatosis and IR.

Supported by: Russian Science Foundation (grant No. 17-15-01475)

Disclosure: **E. Mishina:** Grants; grant from the Russian Science Foundation (project No. 17-15-01475).

1161

Nitric oxide (NO) contributes to the control of hepatic insulin response by promoting mitochondrial-endoplasmic reticulum interactions

A. Bassot[1], Y. Gouriou[1], M.-A. Chauvin[1], G. Vial[2], N. Bendridi[1], C. Cottet[3], J. Rieusset[1], B. Morio[1];
[1]CarMeN Laboratory, INSERM U1060, Lyon, [2]HP2 Laboratory, INSERM U1042, Grenoble, [3]LBFA Laboratory, INSERM U1055, Grenoble, France.

Background and aims: Under physiological conditions, NO produced by the endothelial NO synthase (eNOS) participates in the control of hepatic response to insulin. In addition, recent evidences have shown that the contact points (MAMs) between mitochondria and the endoplasmic reticulum (ER) are functional domains necessary for insulin signaling in the liver. Since mitochondria are targets of NO, we hypothesized that they could participate in the regulation of the hepatic response to insulin by regulating mitochondrial-ER (MAMs) interactions.

Materials and methods: The study was carried out on HuH7 cells and confirmed on primary rat hepatocytes and in the liver of C57B16J mice. NO concentration was modulated using the eNOS substrate (arginine) and inhibitor (L-Name), as well as an NO donor (Nonoate). MAMs were quantified using in situ Proximity Ligation Assay by targeting the interactions (<40 nm) of two main proteins at the MAM interface, VDAC1 (mitochondrial) and IP3R1 (RE). Involvement of cGMP pathway was explored with activators of soluble guanylate cyclase (sGC) (BAY 41-2272) and cGMP dependent protein kinase (PKG) (8Br-cGMP) and the PKG inhibitor (KT-5823). Insulin response was explored by Western Blot. Finally, proteins at the MAM interface that may be targets of NO (cyclophylin D: cypD, GRP75) were tested using siRNA approaches.

Results: Arginine (1 mM) and Nonoate (1 mM) enhanced MAMs by 37 and 165% vs. control ($p < 0.05$), whereas L-Name (1 mM) decreased them by 49% ($p < 0.05$). Those effects were prevented by inhibiting PKG (KT-5823, 1 μM) and mimicked by activating PKG (8Br-cGMP, 0.5 mM) and sGC (BAY 41-2272, 2 μM). Mitochondrial fusion (Mitotracker green®) was improved concomitantly with NO-induced MAMs, whereas eNOS inhibition caused mitochondrial fission. Mitochondrial oxygen consumption (oxygraphy) was not improved by increasing NO concentration but was reduced by 33% following eNOS inhibition ($p < 0.05$). In agreement with the regulation of MAMs, increasing NO concentration improved the response to insulin (100 nM) (+33% vs. control, $p < 0.05$) whereas eNOS inhibition diminished this response (−25%, $p < 0.05$). Finally, the decrease of cypD expression induced by the siRNA was the only condition that significantly reduced the effect of NO on MAMs (−54%, $p < 0.001$) and insulin response (−50% , $p < 0.05$) vs. treated control.

Conclusion: Under physiological conditions, NO participates in the control of the hepatic response to insulin by regulating mitochondrial-RE (MAMs) interactions. NO effects are mediated through the sGC/cGMP/PKG pathway and involve cypD. The mechanisms may involve the control of calcium exchanges between the two organelles, via the VDAC-1/Grp75/IP3R-1 complex with which cypD can interact.

Disclosure: **A. Bassot:** None.

1162

Adiponutrin (PNPLA3) rs738409 genotype influences the metabolic activity of non-alcoholic fatty liver disease

A. Nadasdi[1], V. Gál[2], K. Rosta[3], J. Harreiter[4], A. Kautzky-Willer[4], A. Somogyi[1], G. Firneisz[1,5];
[1]2nd Department of Internal Medicine, Semmelweis University, Budapest, Hungary, [2]Brain Imaging Centre, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary, [3]Department of Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria, [4]Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria, [5]MTA-SE Molecular Medicine Research Group, Hungarian Academy of Sciences, Budapest, Hungary.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) and T2DM are prevalent and closely associated to each other and to obesity, dyslipidaemia, insulin resistance (IR). The *PNPLA3* rs738409 *G/G* genotype is a risk factor for NAFLD development and progression. We aimed to measure the intrahepatic (IHCL) and intrapancreatic lipid contents (IPCL) quantitatively and to assess the correlations with metabolic parameters according to *PNPLA3* genotypes in a middle aged female population.

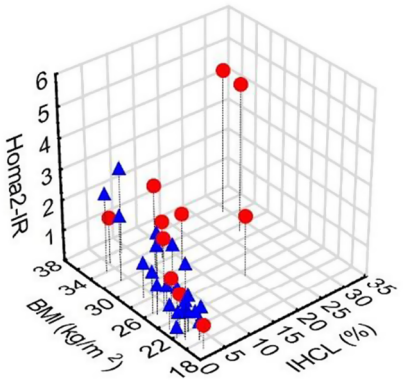
Materials and methods: IHCL and IPCL was measured with [1]HMRS and Cemical Shift Imaging in 34 non-pregnant women (mean: age = 37 yrs, BMI = 26.3 kg/m^2) with known *PNPLA3* rs738409 genotypes (*C/C* vs. *G/G*), pregnancy history (pGDM vs. pNGT) from our prior GDM genetic study. 75 g OGTT (0′–30′–120′), plasma glucose (PG), insulin, HbA_{1C} levels liver-tests were assessed and anthropometric data were recorded. Serum DPP4 activity (sDPP4) was measured in an enzyme-kinetic-assay using Gly-Pro-pNA as substrate. We analysed data according to prior GDM (pGDM/pNGT $n = 19/15$), *PNPLA3* rs738409 genotypes (*C/C*, *G/G* $n = 23$, 11), OGTT results (IFG+IGT+DM/NGT $n = 8/26$) and presence of NAFLD (IHCL >5.5%, $n = 9$). Statistics: T, MWU/Pearson, SRO correlation test and multiple regression.

Results: Women with *G/G* genotype (vs. *C/C*) had higher IHCL (median = 10.1 vs. 3.4%, $p = 0.01$), with pGDM (vs. pNGT) higher 120′PG (mean = 7.1 vs. 5.7 mmol/l, $p = 0.041$) levels and higher proportion of women had abnormal GT (abnGT/pGDM vs. abnGT/pNGT: $n = 6/19$ vs. 2/15, respectively). In the NAFLD group (vs. non-NAFLD) the 30′/120′ PG (mean = 10.1 vs. 7.5/8.1 vs. 5.8 mmol/l, $p = 2*10^{-5}/0.002$) levels were increased. There were different correlations among HOMA2-IR, IHCL and BMI according to the *PNPLA3* genotypes (Fig 1A). The correlation between the IHCL and 120′ PG (Fig 1B) was also influenced by the *PNPLA3* genotypes. Correlations between BMI-IHCL, IHCL-Uric

acid, IHCL-TG and IHCL-IPCL were also modified significantly by the *PNPLA3* genotype. We confirmed the correlation among seDPP4 activity and the liver tests (DPP4 vs. ASAT, ALAT and γGT: $p = 0.024$, 0.075 and 0.043, $r = 0.41$, 0.32 and 0.36 respectively).

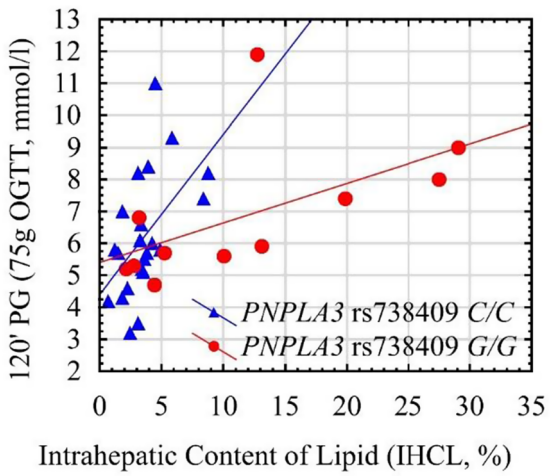
Conclusion: Both the intrahepatic lipid deposition and the history of GDM had significant metabolic consequences. Although an increased HOMA-IR and the abnormal OGTT were associated with NAFLD, the track of correlations between the IHCL and metabolic parameters were significantly modified by the *PNPLA3* rs738409 genotype. The rs738409 gene variant influences the metabolic activity corresponding to a given degree of liver fat accumulation.

Fig 1A: Correlation among BMI, IHCL and HOMA2-IR - by *PNPLA3* rs738409 genotypes



PNPLA3 rs738409*C/C* R(z/xy) = 0.863; $p<10^{-5}$
PNPLA3 rs738409*G/G* R(z/xy) = 0.859; p=0.005

Fig 1B: Correlation between IHCL and 120' PG values - by *PNPLA3* rs738409 genotypes



Supported by: EFSD/New Horizons
Disclosure: **A. Nadasdi:** Grants; EFSD/NH grant.

1163

Effect of the hepatic extracellular vesicles in inflammation-associated insulin resistance in non-alcoholic fatty liver disease

I. Garcia-Martinez[1,2], R. Alén[1], M. Izquierdo[1], Á. Valverde[1,2];
[1]Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, [2]Centro de Investigaciones Biomédicas En Red de Diabetes y Enfermedades Metabólicas (CIBERdem), Madrid, Spain.

Background and aims: Cell-cell communication by extracellular vesicles (EVs) is an emerging issue in the progression of non-alcoholic fatty liver disease (NAFLD). It has been shown that injured hepatocytes by lipotoxicity release EVs and, on the other hand, patients with NAFLD or non-alcoholic steatohepatitis (NASH) secrete increased levels of EVs. However, the role of EVs in cell-to-cell communications within liver cells remains uncertain. We previously analyzed the impact of the hepatocyte-macrophage cross-talk in inflammation-mediated insulin resistance in NAFLD. In order to better understand whether EVs may be novel non-invasive biomarkers to monitor liver damage in NAFLD we aimed to analyze first the EVs secretion profile in hepatocytes cultured under NAFLD conditions and, second, the impact of released EVs on the inflammatory responses triggered by macrophages and, ultimately, in insulin signaling in hepatocytes.

Materials and methods: Eight weeks old C57BL6j male mice were fed chow diet (control) or high fat diet (HFD, 60% kCal from fat) for 12 weeks. EVs were isolated from: hepatocytes from chow diet-fed mice stimulated with palmitic acid (PA) 1) or remained untreated as controls 2), hepatocytes from HFD-fed mice 3), and plasma from HFD-fed mice 4). EVs were characterized by detection of specific markers (CD81, Tsg101) by Western-blot and evaluation of size/concentration by Nanoparticle Tracking Analysis (NTA). Mouse peritoneal macrophages were stimulated for 8h with EVs isolated from Groups 1–4 and changes in iNOS, IL6, IL1b and TNFa levels were assessed by qPCR. In addition, hepatocytes were cultured for 24h with conditioned medium (CM) from these macrophages and insulin signaling was studied through the Akt (Serine 473) phosphorylation.

Results: EVs secretion was elevated by 3-4 fold in PA-stimulated hepatocytes or in hepatocytes isolated from HFD-fed mice compared to the levels of non-treated hepatocytes (controls). Likewise, EVs secretion was elevated in plasma from HFD-fed mice compared to those isolated from control mice fed a chow diet ($P < 0.05$). Addition of EVs isolated from both kinds of lipid overloaded hepatocytes (PA-stimulated or isolated from mice fed a HFD) to mouse peritoneal macrophages increased iNOS expression and proinflammatory cytokines levels ($p < 0.05$). Moreover, insulin resistance, manifested by decreased Akt (Serine 473) phosphorylation was induced in healthy hepatocytes pre-incubated with CM from macrophages stimulated with EVs released by lipid-overloaded hepatocytes.

Conclusion: Our results identified a novel hepatocyte-macrophage-hepatocyte cross-talk by which EVs secretion by hepatocytes under NAFLD conditions induced a pro-inflammatory response in macrophages which, in turn, generates insulin resistance in hepatocytes in a paracrine-manner. The study of the mechanisms of action of hepatocyte-derived EVs in hepatic and extra-hepatic insulin sensitive cells could provide new therapeutic targets against insulin resistance during NAFLD.

Disclosure: **I. Garcia-Martinez:** None.

1164

Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women

R. Burcelin, FLORINASH consortium FP7;
Inserm 1048 I2MC, Hopital Rangueil, Toulouse, France.

Background and aims: The role of molecular signals from the microbiome and their coordinated interactions with those from the host

in hepatic steatosis - notably in obese patients and as risk factors for insulin resistance and atherosclerosis - needs to be understood. Precisely, the early onset events triggering the hepatic lipid load have not be studied and could be linked to a specific gut microbiota profile characterized with a causal role.

Materials and methods: To this aim a cohort of 700 non diabetic obese women was recruited from Spain and Italy. 88 patients underwent a multi-omics analysis at baseline including metagenomics, liver transcriptomics, plasma and urine metabolomics and proteomics. Hundreds of clinical and biochemical parameters were recorded including NAFL scores (recorded by histological analyses), insulin resistance (as assessed by the hyperinsulinemic clamp), intima media thickness and oral glucose tolerance to cite a few. Through state of the art system biological analyses we here reveal molecular networks linking gut microbiome and host phenome to hepatic steatosis.

Results: We here show that steatotic patients have low microbial gene richness and increased genetic potential for processing of dietary lipids and endotoxin (LPS) biosynthesis (notably from Proteobacteria), hepatic inflammation and dysregulation of aromatic and branched-chain amino acid (AAA and BCAA) metabolism. We demonstrated that faecal microbiota transplants and chronic treatment with phenylacetic acid (PAA), a microbial product of AAA metabolism, successfully trigger steatosis and BCAA metabolism. Molecular phenomic signatures were predictive (AUC = 87%) and consistent with the gut microbiome making an impact on the steatosis phenome (>75% shared variation) and, therefore, actionable via microbiome-based therapies

Conclusion: Our data demonstrate that a specific fecal microbiota was causal of the early events triggering hepatic lipid accumulation. therefore, in addition to being a predictive biomarkers the molecular changes in gut microbiota metagenome could be considered as a putative targets for the control of NAFLD.

Clinical Trial Registration Number: 2009 046

Supported by: FP7 Health-F2-2009-241913 FLORINASH

Disclosure: **R. Burcelin:** None.

1165

A porcine placental extract alleviates lipotoxicity-induced steatohepatitis by suppressing activation of hepatic macrophages and stellate cells

T. Ota[1,2], L. Xu[2], G. Chen[2], M. Nagashimada[2];
[1]Asahikawa Medical University, Asahikawa, [2]Kanazawa University, Kanazawa, Japan.

Background and aims: Excessive hepatic lipid accumulation causes aberrant activation of liver macrophages and hepatic stellate cells (HSCs), resulting in the exacerbation of hepatic insulin resistance and nonalcoholic steatohepatitis (NASH). We previously developed a cholesterol- and saturated fatty acid-induced mouse model of lipotoxic NASH and revealed that hepatic oxidative stress and insulin resistance promotes hepatic inflammation and fibrosis, replicating the pathophysiological features of human NASH. Placental extracts have been used to treat various chronic diseases due to their anti-oxidative effect. However, the effects of the placental extracts on the development of NASH have yet to be elucidated. In the present study, we investigated the effect of porcine placental extract (PPE) in a lipotoxicity-induced NASH model.

Materials and methods: Eight-week-old C57BL/6 mice were fed a high-cholesterol and high-fat (CL) diet or a CL diet containing 0.3% PPE (CL+PPE) for 15 weeks. The liver histology, insulin sensitivity, inflammatory/stress signal, and fibrogenesis were examined. Intrahepatic immune cell numbers were quantified by flow cytometry.

Results: After 15 weeks of feeding, histological examination revealed hepatic steatosis, inflammation and fibrosis in mice fed CL diet. They showed hyperinsulinemia even though weight and adiposity were similar. The PPE significantly attenuated hepatic steatosis, and the increase in liver triglyceride and TBARS levels caused by the CL diet. The PPE improved glucose intolerance and hyperinsulinemia in the CL group and enhanced the insulin signal, assessed by IRβ and Akt phosphorylation in the liver, which was associated with the attenuation of MAPK (ERK/p38MAPK) and NF-κB activation. To quantify the numbers of total ($CD45^+CD11b^+F4/80^+$), pro-inflammatory M1-like ($CD11c^+CD206^-$), and anti-inflammatory M2-like ($CD11c^-CD206^+$) macrophages in the liver, we analyzed hepatic immune cells by flow cytometry. Although the PPE did not alter the total number of liver macrophages markedly, it decreased the number of M1-like macrophages by 44.2% In contrast, the PPE increased the number of M2-like macrophages by 1.4-fold, resulting in a predominance of M2 over M1 macrophage populations in the liver of NASH mice. Accordingly, the PPE suppressed lipopolysaccharide-induced M1 marker (*Tnfa*, *Il1b* and *Mcp-1*) mRNA expression in isolated murine peritoneal macrophages, whereas it facilitated interleukin 4-induced M2 marker (*Mrc2*, *Cd206* and *Mgl1*) mRNA expression in a dose-dependent manner. Importantly, the PPE markedly attenuated hepatic fibrosis by decreasing hepatic hydroxyproline, a marker of collagen fiber content by 62% in the liver of CL group. Furthermore, the PPE decreased TGFβ-induced phosphorylation of Smad, and α-SMA protein levels in the liver of NASH mice and in RI-T cells, a HSC line. Consistently, the PPE reduced mRNA expression of TGFβ-induced fibrogenic genes (*Col1a1* and *fibronectin*). Moreover, the PPE decreased mRNA expression of *Nox4* and intracellular reactive oxygen species levels in TGFβ-stimulated RI-T cells.

Conclusion: The PPE attenuated lipid accumulation and peroxidation, insulin resistance, inflammatory and stress signaling, and fibrogenesis in the liver of NASH. Thus, PPE may be a potential approach to prevent NASH by limiting lipid peroxidation, promoting M2 macrophage polarization, and attenuating HSC activation.

Supported by: MEXT, Japan

Disclosure: **T. Ota:** None.

PS 112 Unconventional aspects of cardiovascular disease in diabetes

1166

Insulin resistance and CVD risk: a time varying analysis in type 1 diabetes

T.J. Orchard, R.G. Miller, T. Costacou;
Epidemiology, University of Pittsburgh, Pittsburgh, USA.

Background and aims: We have previously demonstrated that baseline estimated glucose disposal rate (eGDR), an inverse measure of insulin resistance (IR) based on a prediction equation (waist hip ratio, hypertension and HbA1c) derived from hyperinsulinemic euglycemic clamp studies, is a strong predictor of coronary artery disease (CAD) in type 1 diabetes. Whether eGDR remains a strong independent predictor when risk factor assessments over time are accounted for, and whether it predicts total CVD including stroke, are unclear. We thus now report its role in predicting total cardiovascular disease (CVD) including stroke, in a time varying analysis.
Materials and methods: Data are from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study an ongoing, prospective cohort study of childhood-onset (<17 yrs) T1D diagnosed in 1950–80 with 25 yrs of follow up ($n = 658$, 49% women, mean age 27, T1D duration 18 yrs at 1986–88 baseline). In addition to biennial surveys throughout, follow-up exams occurred biennially for the first 10 yrs and again at 18 and 25 yrs. CVD was defined as the first occurrence of fatal MI, CAD, or stroke; nonfatal MI or stroke; or coronary revascularization (all validated by review of death certificates and/or medical records); ischemic ECG (Minnesota Codes 1.3, 4.1, 4.3, 5.1, 5.3, and 7.1) or EDC physician diagnosed angina. Those experiencing any CVD were compared to participants not developing any CVD during follow up. Baseline (BL), time varying updated mean (UM) and time varying most recent (MR) risk factors were assessed in Cox models (HRs per unit).
Results: A first CVD event was documented in 236 of the 604 participants without prevalent CVD at baseline. The final model was constructed using the independent predictors that emerged from models based on blocks of related variables (demographic, behavioral, family history, blood pressure, lipids, and diabetes specific). Independent predictors in the final model were diabetes duration (HR 1.099, $p < 0.0001$); current log albumin excretion rate (1.20, $p < 0.001$); mean log triglycerides (1.86, $p = 0.0005$); current eGDR (0.9, $p = 0.003$); baseline LDLc (1.005, $p = 0.012$); current eGFR (0.99, $p = 0.030$). An alternate model without eGDR but with its predictors, showed similar prediction with HbA1c and hypertension replacing eGDR and eGFR, and a similar fit (Akaike Information Criterion 2272.8 v 2270.2). Further models with three different outcomes (Major Atherosclerotic Cardiovascular Events - MACE i.e. stroke, MI or fatal CVD, $n = 107$); revascularization ($n = 38$) and soft endpoints (Ischemic ECG or angina, $n = 91$) suggest eGDR is a particularly strong predictor of soft endpoints (HR 0.817, $p < 0.001$).
Conclusion: We conclude that IR (eGDR) is an independent predictor of CVD (particularly "soft" CVD endpoints) in fully adjusted, time varying, analyses. The similarity of fit of models with either eGDR, or with its components, however, is consistent with the hypothesis that most of the prediction of IR (eGDR) is explained by blood pressure and glycemia.
Supported by: NIH/NIDDK 34818
Disclosure: **T.J. Orchard:** Employment/Consultancy; Consultant, Boehringer Ingelheim. Grants; NIH/NIDDK.

1167

Role of atherogenic dyslipidaemia in silent coronary heart disease of type 2 diabetes patients with LDL-cholesterol at therapeutic goal

M. Hermans[1], M. Nguyen[2], A. Sultan[3], I. Banu[2], E. Cosson[2], A. Avignon[3], P. Valensi[2];
[1]Cliniques universitaires St-Luc, Brussels, Belgium, [2]Hôpital Jean Verdier, Bondy, France, [3]Centre Hospitalier Universitaire, Montpellier, France.

Background and aims: Several studies have identified the role of atherogenic dyslipidaemia (AD), defined by the combination of low HDL-C and high TG, as a cardiovascular risk factor. AD is a well-established residual risk factor even in type 2 diabetes (T2DM) patients whose LDL-C reaches therapeutic target. Our goal was to evaluate the respective role of AD and of an LDL-C remained excessive in silent coronary disease of asymptomatic T2DM patients.
Materials and methods: Among T2DM patients screened (since 1991) for silent myocardial ischemia (SMI; by myocardial stress scintigraphy) and for coronary artery stenosis (CAS; in case of positive scintigraphy), we retained 1398 with LDL-C <130 mg/dl (58% male, 59.7 ± 10.3 years, BMI 29.3 ± 5.9 kg/m², hypertension 73%, albuminuria 59%, smoking 25%, lower extremity arterial disease 11%, receiving statins 39% and/or fibrate 8%). Rest echocardiography was also performed in 399 of these patients. The total population was divided into 665 patients with LDL-C between 100 and 130 mg/dl (group 1) and 733 patients with LDL-C <100 mg/dl (group 2). AD was defined as triglyceride levels ≥200 mg/dl and HDL-C ≤40 mg/dl (males) or ≤50 mg/dl (females).
Results: SMI was present in 24% of the total population, and significant CAS in 8%. In group 2, the proportion of patients on statins was higher ($p < 0.0001$). AD was present in 73 patients of group 1 (11%) and 73 patients of group 2 (10%). Patients with AD had lower diabetes duration, and higher values of BMI, HbA1c, and 10-year coronary events' risk (UKPDS) ($p = 0.02$ to <0.0001). Comparisons were made for patients in group 1 (with or without AD) and those in group 2 (with or without AD). The prevalence of SMI was slightly higher in patients in groups 1 and 2 with AD than in those without AD ($p = 0.139$). The prevalence of CAS was significantly higher in patients of groups 1 and 2 with AD (17.4% and 14.3%) than in patients of groups 1 and 2 without AD (8.2% and 6.1%) ($p = 0.002$). In a multivariate analysis model including AD, gender, hypertension, diabetes duration, BMI, HbA1c, nephropathy, and statins, AD remained significantly associated with coronary stenosis. Between the 4 groups there was no significant difference in left ventricular mass or ejection fraction, hypertension or smoking.
Conclusion: These data show that in T2DM patients the prevalence of silent coronary stenoses is higher in patients with AD, and even higher in the presence of AD with an LDL-C <100 mg/dl than in the presence of an LDL-C between 100 and 130 mg/dl without AD. They suggest that in patients with very high cardiovascular risk, in addition to lowering LDL-C below 100 mg/dl, management of atherogenic dyslipidaemia is necessary to reduce the residual risk of macrovascular disease.
Disclosure: **M. Hermans:** None.

1168

Effect of ADAMTS7 in left ventricular diastolic and systolic dysfunction in subjects with type 2 diabetes free of cardiovascular disease

A. Ganotopoulou[1], V. Lambadiari[2], A. Papazafiropoulou[1], A. Theodosis-Georgilas[3], A. Trikkalinou[1], N. Kassinos[3], V. Liossi[1], K. Anagnostopoulou[1], S. Iraklianou[1], G. Dimitriadis[2], A. Melidonis[1];
[1]Diabetes Center, Tzaneio General Hospital of Piraeus, Piraeus, [2]2nd Department of Internal Medicine-Propaedeutic, Research Institute and Diabetes Center, Attikon University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, [3]Cardiology Department, Tzaneio General Hospital of Piraeus, Piraeus, Greece.

Background and aims: ADAMTS7 belong to the family of metalloproteinases and contribute to the tissue morphogenesis, regulate cell proliferation and are important regulators of tissue regeneration.Recent studies have shown a pathogenetic role of ADAMTS7 to vascular remodeling caused by coronary atherosclerosis.However, its role to diabetic cardiomyopathy is still unknown. Therefore, the aim of the present study was to estimate the effect of ADAMTS7 to cardiac remodeling, expressed in terms of established ultrasound indicators of cardiac function, in subjects with type 2 diabetes mellitus (T2D) free of cardiovascular disease.

Materials and methods: 65 patients with T2D [mean age (± standard deviation, SD) of 57.5 ± 10.7 years, HbA1c: 6.9 ± 0.7%, body mass index (BMI) 30.8 ± 3.8 kg/m^2] without known cardiovascular disease were examined. All study patients underwent fully clinical examination and a blood sample was taken at fasting state for the measurement of ADAMTS7 (Elisa method) at baseline. Also an ultrasound examination of the heart was performed at baseline and 12 months after subject's enrollment into the study. The following ultrasound indicators of cardiac function were calculated: LVIDd, LVIDs, LVEF, LVFS, LVMASS, A′, A, D, E, DT, S, LA, E′ and E/E′.
Results: At baseline levels of ADAMTS7 were 0.32 ± 0.12 pg/ml. The majority of study patients were on metformin treatment (90%), 38,7% on DPP-4 inhibitors, 25,8% on GLP-1 analogs, 12.9% on sulfonylureas, 9.7% on SGLT-2 inhibitors, 3.2% on glitazones and 25.8% on insulin. 80.6% had hypertension and 61.3% dyslipidemia. Multivariate regression analysis (backward), after adjustment for the established ultrasound indicators of cardiac function, showed that ADAMTS7 levels were positive correlated with LVIDs (inner diameter of the left ventricle) (beta = 0.570, $p = 0.05$) and A′ (atrial contraction) (beta = 0.326, $p = 0.05$).
Conclusion: The results of the present study show that ADAMTS7 might be an indicator of early left ventricular diastolic (A′) and systolic dysfunction (LVIDs) in subjects with T2D. The above findings add information to the role of ADAMTS7 as a newly biomarker of cardiac remodeling.
Disclosure: **A. Ganotopoulou:** None.

1169

Subclinical ventricular dysfunction in young population with congenital generalised lipodystrophy detected by speckle-tracking echocardiography

V.O. Fernandes, C.B.R. Liberato, N.B.C. Olegario, A.D.R. Montenegro, G.E.C. Paiva, L.A.A. Batista, L.V. Martins, I.L.R. Liberato, A.B. Carvalho, C.B. d'Alva, R.M. Montenegro Junior;
Brazilian Group for the Study of Inherited and Acquired Lipodystrophies (BRAZLIPO), Universidade Federal do Ceara, Fortaleza, Brazil.

Background and aims: Congenital generalized lipodystrophy (CGL) is an autosomal recessive disorder characterized by absence of functional adipocytes and lipid stored in other tissues, including muscle and liver. Affected individuals develop severe insulin resistance (IR), dyslipidemia, hepatic steatosis and diabetes, usually with early cardiovascular mortality. Echocardiographic findings previously described in CGL used only the conventional technique. Thus, this study aimed to perform speckle-tracking echocardiography (STE), a recently developed technique for the characterization and quantification of myocardial deformation, in one of the largest casuistry of CGL in Brazil.
Materials and methods: A cross-sectional study with 22 CGL patients from 2013 to 2016. Clinical, biochemical and echocardiographic evaluation were conducted in CGL group and with 22 healthy subjects matched for sex and age. All patients undergone standard conventional transthoracic echocardiography and two-dimensional STE using Vivid 7 and 9 ultrasound system (GE Vingmed Ultrasound, Milwaukee, Wi, USA). This technique includes global longitudinal strain (GLS) measure, obtained by evaluating the mean of the strain value of the 18 segments of the left ventricle in the three standard apical incidences.
Results: The mean age was 14.6 ± 10.7 years old (yo): 40.9% (9) aged 0 to 9 years, 27.3% (6) aged 10 to 17 years and 31.8% (7) ≥18 years. There were 59% (13) females. All CGL patients had hypoleptinemia, 95.4% (21) low HDL-c, 86.36% (19) hypertriglyceridemia, 71.4% (15) severe insulin resistance, 50% (11) hepatic steatosis, 63.6% (14) diabetes, 40.9% (9) hyperinsulinemia, 41% (9) hypercholesterolemia and 18.2% (4) high blood pressure. When evaluated by conventional echocardiography, all CGL group presented normal systolic and diastolic function, 31.8% (7) left ventricular (LV) hypertrophy, 27.3% (6) left atrial diastolic volume increase, 18.2% (4) LV systolic diameter increase and 4.5% (1) LV diastolic diameter increase. Comparing CGL and control groups, the CGL subjects had higher LV volume, LV mass index and LV diastolic diameter. Although the normal ejection fraction (EF) in all sample, those with CGL under 18 yo had lower EF than control ($p < 0.05$). Evaluation by STE showed abnormal results of global longitudinal strain (GLS) in 68.2% (15) of CGL group ($p < 0.01$). The GLS was normal in all control group. CGL group also had worse results in the evaluation of global longitudinal strain and of all evaluated segments, except for the apical. In those with altered GLS, 93.3% (14) had hypertriglyceridemia, 93.3% (14) low HDL-c, 73.3% (11) diabetes, 60% (9) high HOMA-IR index, 53.3% (8) hyperinsulinemia and 46% (7) hypercholesterolemia. It was observed a positive correlation with GLS and insulin ($p = 0.007$), HbA1c ($p = 0.005$), blood glucose ($p = 0.018$) and HOMA-IR index ($p = 0.021$).
Conclusion: This young population with CGL presented subclinical ventricular dysfunction by speckle-tracking echocardiography, even with a normal conventional echocardiographic study.
Disclosure: **V.O. Fernandes:** None.

1170

Extracellular matrix turnover influences myocardial contraction behaviour in diabetic cardiomyopathy assessed by speckle tracking echocardiography

K. Pappritz[1,2], J. Grune[2,3], O. Klein[1,2], F. Dong[1], M. El-Shafeey[1], J. Lin[1], U. Kintscher[4,2], C. Tschöpe[1,5], S. Van Linthout[1,2];
[1]Berlin – Brandenburger Center for Regenerative Therapies (BCRT), Charité – Universitätsmedizin Berlin, Berlin, [2]German Center for Cardiovascular Research (DZHK), partner site Berlin, Berlin, [3]Institute of Physiology, Charité – Universitätsmedizin Berlin, Berlin, [4]Center for Cardiovascular Research (CCR), Charité – Universitätsmedizin Berlin, Berlin, [5]Department of Cardiology, Charité – Universitätsmedizin Berlin, Berlin, Germany.

Background and aims: Diabetic cardiomyopathy is referred to be an own clinical entity in the context of diabetes mellitus (DM). Experimental streptozotocin (STZ)-induced Type I diabetic cardiomyopathy is associated with cardiac collagen I deposition resulting in left ventricular (LV) dysfunction. LV contraction is determined by the shortening and thickening of the longitudinal and circumferential fibres, which can be investigated by two-dimensional speckle-tracking echocardiography (STE). The present study aimed to investigate the impact of LV matrix composition on global deformation behaviour in the pathogenesis of STZ-induced diabetic cardiomyopathy.
Materials and methods: Eight-week-old male C57BL6/j mice were intraperitoneally injected with 50 mg/kg BW STZ, dissolved in 0.1 mol/L sodium citrate, during 5 consecutive days. 6, 9, and 12 weeks (w) post DM induction, echocardiographic measurements were performed on a Vevo 3100 using a 30-MHz linear-frequency transducer. Two-dimensional STE-based analyses were assessed from B-mode images using the VevoStrain software package. In an additional set, the LV was harvested to analyze gene and protein expression.
Results: Global longitudinal strain (GLS) was impaired in mice at 6w (co 6 w: −21 ± 1.0% vs. STZ 6 w: −15 ± 0.43%; $p < 0.0001$), 9 w (co 9 w: −21 ± 0.93% vs. STZ 9 w: −12 ± 0.63%; $p < 0.0001$), and 12 w (co 12 w: −19 ± 0.81% vs. STZ 12 w: −13% ± 0.90%; $p < 0.0001$) post STZ application. Furthermore, the 9 w STZ group displayed a 3% ($p = 0.0287$) worsened GLS compared to the 6 w STZ group. In parallel, an enhanced global circumferential strain (GCS) was observed in the 6w (co 6 w: −23 ± 0.93% vs. STZ 6 w: −27 ± 1.2%; $p = 0.0054$) and 12w (co 12w: −24 ± 0.98% vs. STZ 12 w: −27 ± 0.69%; $p = 0.0988$) STZ mice vs. controls. Furthermore, 9 w STZ animals displayed a 5% ($p = 0.0003$) and 5% ($p = 0.0006$) impaired GCS vs. 6 w and 12 w STZ mice, respectively. The impairment in GLS in 9w STZ mice vs. controls was associated with a 1.7-fold ($p = 0.0005$) enhanced collagen I expression accompanied by a 2.9-fold ($p = 0.0059$) increased collagen I/III protein ratio compared to controls. Interestingly, collagen I protein, lysyl oxidase (Lox) and Lox-like (LoxL)-2 mRNA expression was 2.4-fold ($p < 0.0001$), 1.4-fold ($p =$

0.0284), and 1.4-fold ($p = 0.0170$) lower in the STZ 12 w vs. the STZ 9 w group, respectively. Quantitative segmental analysis further indicated decreased myocardial deformation behaviour of the anterior and posterior base at 9 w after STZ treatment compared to controls. This observation was supported by changes in protein expression assessed by imaging mass spectrometry.
Conclusion: Type I DM influences cardiac collagen deposition in a time-dependent manner, which finally results in changes of the endomyocardial deformation capacity indicated by altered GLS.
Supported by: the European 7th Framework Consortium REDDSTAR to C.T.
Disclosure: **K. Pappritz:** None.

1171

Diabetes-like environment impairs differentiation and induces senescence of epicardial adipose tissue-derived mesenchymal stem cells
S. Cabaro[1,2], V. D'Esposito[1,2], V. Parisi[1], M. Lecce[1,2], D. Liguoro[1,2], A. Liotti[1,2], I. Cimmino[1,2], F. Oriente[1,2], D. Leosco[1], F. Beguinot[1,2], P. Formisano[1,2];
[1]University of Naples "Federico II", Department of Translational Medical Sciences, Naples, [2]National Council of Research (CNR), URT GDD-IEOS, Naples, Italy.

Background and aims: Excess of visceral fat is a major culprit in the development of type 2 diabetes (T2D) and related disorders. A growing body of evidence indicates that epicardial adipose tissue (EAT), the visceral fat of the heart, may play an active role in dysregulation of cardiac function. Indeed, EAT thickness positively correlates with the release of inflammatory molecules and with the severity of heart pathologies, including heart failure, aortic valve stenosis and coronary artery disease (CAD). Moreover, EAT thickness is inversely associated with insulin sensitivity and positively correlates with metabolic parameters including postprandial glucose, HbA1c level, and HOMA-IR. In patients with diabetes, prolonged hyperglycemia damages several organs, including heart. Diabetic hyperglycemic microenvironment could regulate the balance between self-renewal and differentiation of stem cells in adipose tissue, but the involved mechanisms have not been thoroughly investigated. Thus, potent pro-inflammatory activation of EAT suggests a direct involvement of cardiac visceral fat in inflammatory phenomena occurring in patients with cardiovascular diseases. This study aims at investigating whether different glucose concentrations may impact on EAT functions.
Materials and methods: EAT biopsies were collected from diabetic ($n = 7$) and non-diabetic ($n = 7$) individuals with CAD. EAT-derived mesenchymal stem cells (MSCs) were isolated ($n = 5$) and cultured in high glucose (HG 25 mmol/l) or normal glucose (NG 5.5 mmol/l) concentration, as control. Cell surface markers were assessed by FACS analysis and gene expression levels by real time RT-PCR.
Results: EAT-MSCs cultured in NG and HG exhibited similar proliferation rate. Additionally, the expression of cell surface markers, characteristic of adipose tissue-derived MSCs ($CD90^+$, $CD31^-$, $CD45^-$, $CD73^+$), was similar in both conditions. Interestingly, HG-treated EAT-MSCs displayed a 50% decreased expression of the multipotent markers Oct-4 and Nanog. Moreover, mRNA levels of the senescence marker $p21^{CIP1/WAF1}$ were significantly increased in HG-cultured cells. HG-treated EAT-MSCs maintained their adipogenic and osteogenic potential, after the application of appropriate induction media, however either lipid or calcium accumulation seemed to be lower compared with NG-cultured cells. Consistent with these data, in EAT biopsies from diabetic patients there was a 1.7- and 2.2-fold reduction of mRNA levels of PPARgamma and BMP1, respectively master gene regulators of adipogenic and osteogenic differentiation.
Conclusion: Collectively, these data indicate that a diabetic-like environment directly influences EAT-derived MSC features and significantly affects their stemness and differentiation potential, driving towards accelerated senescence.
Supported by: SID/FO.DI.RI.-MSD ITALIA 2017 Research Fellowship
Disclosure: **S. Cabaro:** None.

1172

Continued administration of the hydroxyl fatty acid 9-PAHSA alleviates diabetic-induced cardiac dysfunctions by enhancing autophagic flux in the heart of db/db mice
H. Jin, Q.-L. Guo, H.-G. Zhou, J.-C. Guo;
Fudan University, Shanghai, China.

Background and aims: Diabetic cardiomyopathy (DCM) is one of the most common complications of type 2 diabetes mellitus (T2DM), leading to high mortality in patients. However, effective interventions for DCM therapy are still lack so far. Recently, a novel branched palmitic acid esters of hydroxyl stearic acid 9-PAHSA has been proved to be bioactive in T2DM, but whether it could protect cardiomyocytes in DCM pathology remains unclear.
Materials and methods: Thirty- two-week-old male C57BLKS/J db/db mice were used as the T2DM model mice. Age-matched male littermates C57BLKS/J wild type mice were used as the non-diabetic control. All the animals were purchased from Nanjing University Biological Center (Nanjing, China), and housed in colony cages with free access to water and regular diet in a 12h light/dark cycle and temperature-controlled environment. By using the iTRAQ approach and 1.5-fold change cut-off value, we analyzed the effect of 9-PAHSA on the myocardial protein profile of db/db diabetic mice, and evaluated the efficacy of 9-PAHSA against myocardium dysfunction.
Results: A total of 432 proteins were identified among these three groups. Of these 432 proteins, 80 were found to be differentially expressed in db/db+9-PAHSA group with respect to db/db+ ventricular hypertrophy(veh)group. Among these 80 proteins, the level of 37 proteins was elevated in the db/db+ veh group when compared with the control + veh group, and decreased after 9-PAHSA treatment. Other 43 proteins levels were decreased in the db/db+veh group with respect to the control +veh group, and reversed after the treatment. These identified 80 proteins were further functionally classified to evaluate the underlying metabolic processes altered after 9-PAHSA intervention. A total of 20 proteins could be classified into four main functional classes including cardiac lipid metabolism-related, mitochondrial-related, cardiomyopathy-related, and autophagy-related proteins. The expression level of serum 9-PAHSA levels was reduced in diabetic db/db mice. Continued administration of 9-PAHSA for four weeks could significantly improves cardiac functions and structure. Moreover, by using iTRAQ proteomics analysis, we found that 9-PAHSA could induce the expression changes in series of proteins, including autophagy-related BAG3 and HspB8.
Conclusion: Our results demonstrate that 9-PAHSA enhances the cardiac autophagy in db/db mice, as evidenced by electron microscopy and by western blotting analysis of LC3, P62, BECN1, PI3K III, and mTOR. Continued administration of 9-PAHSA could improve diabetic-induced myocardial dysfunction by promoting autophagic flux and reducing myocardial hypertrophy in db/db mice. Our results also showed that chronic treatment of 9-PAHSA could improve db/db diabetic-induced cardiac dysfunctions, possibly via enhancing autophagic flux. Considering the fact that 9-PAHSA levels are reduced in db/db diabetic mice, the exogenous supplementation of 9-PAHSA might be an effective means for the T2DM-related DCM. 9-PAHSA could also be a candidate drug for the clinical therapy of DCM.
Supported by: NSFC-81571361; NSFC-81671392
Disclosure: **H. Jin:** None.

1173

Retinal microvascular associations with blood pressure and arterial stiffness are modified by diabetes status: results from the UK Biobank
R.J. Tapp[1,2], C.G. Owen[1], S.A. Barman[3], D.P. Strachan[1], R.A. Welikala[3], P.J. Foster[4,5], P.H. Whincup[1], A.R. Rudnicka[1];
[1]Population Health Research Institute, St Georges, University of London, London, UK, [2]Melbourne School of Population and Global Health,

University of Melbourne, Melbourne, Australia, [3]Faculty of Science, Engineering and Computing, Kingston University, Surrey, UK, [4]Integrative Epidemiology Research Group, UCL Institute of Ophthalmology, London, UK, [5]NIHR Biomedical Research Centre at Moorfields Eye hospital, London, UK.

Background and aims: Retinal microvascular changes and arterial stiffness are both independent predictors of cardiovascular disease (CVD) and mortality. A growing body of evidence suggests an association between retinal microvascular changes and arterial stiffness. We examined the associations of retinal microvascular architecture with blood pressure (BP) and arterial stiffness, and examined whether associations were modified by diabetes.

Materials and methods: The UK Biobank eye study included 68,549 participants, aged 40–70 years, who underwent non-mydriatic retinal photography, BP and arterial stiffness measurement. Retinal vessel architecture was analyzed using the fully automated QUARTZ software, which measured vessel width and tortuosity. Blood pressure was measured using an Omron 705 IT BP monitor and the arterial stiffness index (ASI) was assessed using a photoplethysmography transducer. Diabetes status, blood pressure medication and previous CVD events were self-reported.

Results: A total of 53,094 participants were included in these analyses. Mean arteriolar and venular diameters were 10.0 pixels (SD 0.9) and 11.8 pixels (SD 1.6) and mean arteriolar and venular tortuosity were 4.4 (SD 1.6) and 3.2 (SD 1.4) respectively. Narrower arterioles were associated with higher systolic BP (beta coefficient (β)-0.09 per 10 mmHg, 95% CI −0.10 to −0.09, $p \leq 0.001$), mean arterial pressure (MAP) (β −0.16 per 10 mmHg, 95% CI −0.16 to −0.15, $p < 0.001$) mean pulse pressure (PP) (β −0.07 per 10 mmHg, 95% CI −0.07 to −0.06, $p < 0.001$) and ASI (β −0.06, 95% CI −0.08 to −0.05, $p < 0.001$). Increased arteriolar tortuosity was associated with higher systolic BP (β 1.14 per 10 mmHg, 95% CI 0.89 to 1.39, $p < 0.001$), MAP (β 1.22 per 10 mmHg, 95% CI 0.84 to 1.59, $p < 0.001$) and PP (β 1.81 per 10 mmHg, 95% CI 1.45 to 2.17, $p < 0.001$). Similar patterns of association were evident for venules (diameter and tortuosity). These associations were unaffected by adjustment for confounders and removal of participants with CVD events or on treatment for high BP. Furthermore, the presence of diabetes was strongly associated with arteriolar narrowing. However, the inverse association of arterial narrowing with systolic BP and MAP were significantly stronger in those without diabetes than in those with diabetes. Associations of venular tortuosity with MAP and ASI were also significantly modified by diabetes status.

Conclusion: This landmark study assessing the retinal microvasculature at scale, has shown clear associations between retinal microvascular architecture, BP and ASI. These observations may be useful in furthering our understanding of the interplay between microvascular and macrovascular disease at a population level.

Supported by: BHF

Disclosure: **R.J. Tapp:** None.

PS 113 Epidemiology of cardiovascular disease and diabetes

1174

Glycaemic control, cardiovascular disease and mortality in type 2 diabetes patients in a real life setting over time: population-based data from the Netherlands

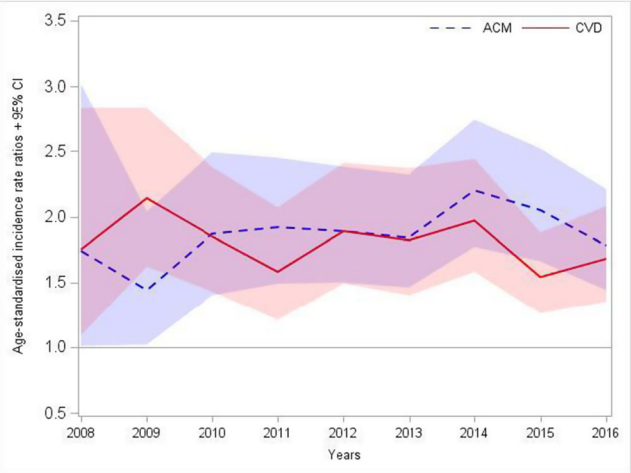
E. Heintjes[1], E. Houben[1], S. Cremers[2], W. Beekman[2], F. Penning-van Beest[1], C. Stehouwer[3], R. Herings[1,4];
[1]PHARMO Institute for Drug Outcomes Research, Utrecht, [2]AstraZeneca BV, Den Haag, [3]Department of Internal Medicine and Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre, Maastricht, [4]Department of Epidemiology & Biostatistics, VU Medical Center, Amsterdam, Netherlands.

Background and aims: According to the EURO Diabetes Index 2014, the quality of Dutch diabetes care is very good in terms of glycaemic control. The main criticism of the EURO Diabetes Index was the lack of data on short- and long-term outcomes. Dutch GP guidelines (revised in 2013) state that the goal of diabetes treatment is the prevention and treatment of cardiovascular and microvascular complications. The aim of this study was to assess trends in HbA1c levels, risk of cardiovascular disease (CVD) and all-cause mortality (ACM) among T2D patients in the Netherlands.

Materials and methods: A series of cohort studies were conducted among annual samples of adult T2D patients treated with glucose-lowering drugs in both primary and secondary care (2008–2016), using records from the PHARMO Database Network. January 1st of each year served as index date and inclusion was restricted to patients without type 1 diabetes, gestational diabetes and polycystic ovary syndrome. Most recent HbA1c levels were assessed in the year prior to index date. Annual incidence of CVD (acute myocardial infarction, ischemic stroke, angina pectoris or congestive heart failure) and ACM was assessed. Rate ratios (RRs) were calculated by comparing age-standardised incidence rates with a diabetes-free population matched on age, sex and general practitioner. Life years lost (LYL) to T2D was determined by comparing the life expectancy of the T2D patients with the matched diabetes-free patients.

Results: In total, 53,602 T2D patients were included in annual cohorts (mean age range: 66–69 years; proportion male range: 51–55%). Mean HbA1c increased from 52 ± 11 mmol/mol in 2008 (proportion <53 mmol/mol: 58%) to 54 ± 12 mmol/mol in 2016 (proportion <53 mmol/mol: 52%). In 2016, the RR (95% CI) was 1.68 (1.35–2.08) for CVD and 1.78 (1.44–2.21) for ACM (Figure). On average, RRs showed an 80% increased risk of CVD and 86% for ACM over all years. No trend was observed in the RRs for ACM and CVD. Overall, the LYL to T2D was approximately four years at the age of 50 years and decreased to approximately one year at the age of 80 years. At the (overall mean) age of 66 years this was approximately 2.5 years.

Conclusion: HbA1c levels increased slightly over time, in line with the introduction of revised GP guidelines for individualised HbA1c targets in the Netherlands. No trend was observed for the increased risk of ACM and CVD among T2D patients from 2008–2016, despite the introduction of several changes in the guidelines on diabetes care. Overall, T2D patients at the age of 66 years are expected to have about 2.5 years shorter life expectancy than the general Dutch population.



Supported by: This study was financially supported by AstraZeneca BV.
Disclosure: **E. Heintjes:** Employment/Consultancy; Edith Heintjes is an employee of the PHARMO Institute of Drug Outcomes Research. Grants; The PHARMO Institute of Drug Outcomes Research was financially supported by AstraZeneca for the conduct of this scientific work.

1175

Estimation of 4 year cardiovascular risk in Indian type 2 diabetic patients using the ADVANCE risk engine in daily clinical practice settings: results from DiaCRE study

G. Bantwal[1], A. Joshi[2];
[1]St. Johns Medical College and Hospital, Bengaluru, [2]Clinic, Mumbai, India.

Background and aims: Cardiovascular (CV) risk assessment is an imperative tool that directs clinical decision making on therapies and encourages patients for lifestyle changes and medication adherence. The ADVANCE (**A**ction in **D**iabetes and **V**ascular Disease: Pretar**A**x And Diamicro**N**Modified Release **C**ontrolled **E**valuation) risk engine is applicable in global real-world clinical settings owing to inclusion of participants from developing countries in the landmark ADVANCE study. The **DiaCRE** (**Dia**betes **C**ardiovascular **R**isk **E**valuation score card) study aimed at assessing the 4year CV risk using ADVANCE risk engine model in Indian type 2 diabetes (T2D) patients undergoing treatment in real-world clinical practice settings.
Materials and methods: DiaCRE was a prospective, non-interventional study that recruited T2D patients aged ≥18 years. General information and CV risk factors were collected at a single-visit. 4year CV risk was calculated using ADVANCE risk engine model. Data were analysed by bivariate and multiple logistic regression. Likelihood estimates corresponding to the impact of one unit change in each CV risk factor on 4 year CV risk score were calculated.
Results: 172 physicians enrolled 4648 patients at 69 cities across India. Mean age was 55.3 years, mean age at diagnosis of diabetes was 47 years and mean duration of diabetes was 8.3 years. The median 4 year CV risk score was 1.4% and a risk score ≥7% was estimated in 694 (14.9%) patients. The most common modifiable risk factor was HbA_{1c} ≥6% ($n = 4315$, 92.8%), followed by non-HDL cholesterol (≥116 mg/dL) ($n = 3556$, 76.5%), pulse pressure >50 mmHg ($n = 3277$, 70.5%), and hypertension ($n = 2742$, 59%) as defined in ADVANCE risk engine model. The estimated risk was significantly higher in male than female patients ($p < 0.0001$) and in obese than non-obese patients ($p = 0.0031$). The median 4-year CV risk score of patients with HbA_{1c} <6% was 0.5% which steadily increased to 1.4% and 4.3% in patients with HbA_{1c} 6–<9% and HbA_{1c} ≥9%, respectively. Presence of ≥2 risk factors in 3740 (80.4%) patients indicated high burden of risk factors in majority of Indian T2D patients in daily clinical practice settings. The percentage likelihood of 4 year CV risk increased with one unit change in all individual risk factors (as shown in table) ($p < 0.0001$). Multiple logistic regression analysis demonstrated that each CV risk factor significantly affected the 4 year CV risk independently ($p < 0.0001$).
Conclusion: The DiaCRE study using the ADVANCE risk engine model in daily clinical practice settings in India demonstrated high burden of risk factors. The estimated 4 year CV risk was significantly influenced by all risk factors and HbA_{1c} was the most common modifiable risk factor. These findings identify a need for measures (including modification of therapies) to control the risk factors. The ADVANCE CV risk engine may serve as a vital tool for patient awareness in addressing control of the risk factors in daily clinical practice.

Risk factor	Percentage likelihood of 4-year CV risk increase with one unit change in individual risk factors (95% CI)
HbA_{1c}	3.6 (3.1 - 4.1)
Age at diagnosis	1.1 (1.1 - 1.2)
Duration of diabetes	1.2 (1.2 - 1.3)
Gender (Male)	0.6 (0.5 - 0.7)
Atrial fibrillation	14.9 (8.7 - 25.5)
Retinopathy	15.0 (11.3 - 19.8)
Hypertension	9.3 (8.1 - 10.7)
Pulse pressure	4.7 (4.1 - 5.3)
Albuminuria	4.2 (3.8 - 4.6)
Non-HDL cholesterol	4.1 (3.6 - 4.6)

Disclosure: **G. Bantwal:** None.

1176

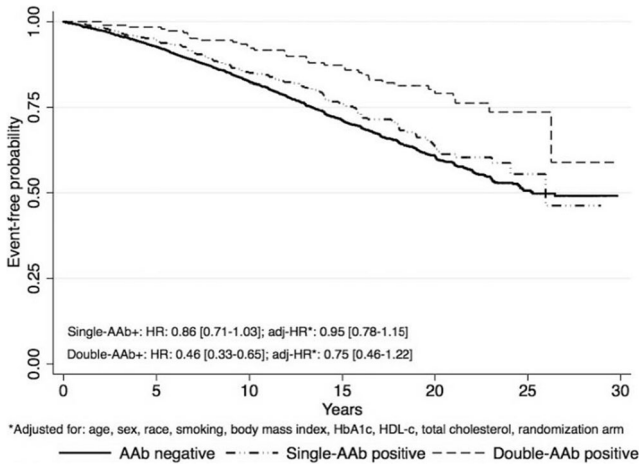
Risk of cardiovascular disease in individuals with latent autoimmune diabetes of adults: results from the UKPDS

E. Maddaloni[1], R.L. Coleman[2], P. Pozzilli[1], R.R. Holman[2];
[1]Dept of Medicine, Unit of Endocrinology and Diabetes, Campus Bio-Medico University of Rome, Rome, Italy, [2]Diabetes Trial Unit, University of Oxford, Oxford, UK.

Background and aims: Diabetes autoantibodies (AAb) to islet-cell cytoplasm (ICA), to glutamic acid decarboxylase (GADA) or to islet antigen-2 (IA-2A), are detectable in up to 12% of adults with a clinical diagnosis of type 2 diabetes (T2D). The presence of AAb identifies subjects with adult-onset autoimmune diabetes, who are mostly affected by a slowly progressive form known as latent autoimmune diabetes of adults. Subjects with detectable AAb tend to be leaner and to have a healthier cardiovascular (CV) risk profile than AAb-negative subjects, but it remains uncertain whether the risk of CV events differ between these two groups. We examined the long-term risk of CV disease in the large population with a clinical diagnosis of new-onset T2D enrolled in the United Kingdom Prospective Diabetes Study (UKPDS), according to their AAb status.
Materials and methods: ICA, GADA and IA-2A were measured in 5096 UKPDS participants at or soon after diagnosis of T2D. The incidence of major adverse CV events, a composite of cardiovascular death, nonfatal myocardial infarction or nonfatal stroke (MACE3), was compared between those with no antibodies (AAb-) and those with ≥1 AAb positive tests (AAb+). Hazard ratios (HR) were adjusted for pre-specified potential confounders (age, race, sex, metabolic profile and smoking status) and for therapy allocation (diet, insulin, sulfonylureas, metformin, non-randomized). The interaction between CV risk factors (age, sex, lipid profile, body mass index [BMI], HbA1c, systolic blood pressure [SBP], smoking status) and AAb status was also examined.
Results: The 557 AAb+ UKPDS participants were younger, with higher mean HbA_{1c} and HDL-cholesterol values but lower BMI, total

cholesterol and SBP values than AAb- subjects (all $p < 0.01$). Over a mean ± SD follow-up period of 16.3 ± 6.0 years a total of 1071 MACE3 events occurred with incidence rates/1000 person-years (95% confidence interval [CI]) of 17.1 (14.6–20.0) in AAb+ and 23.5 (22.4–24.7) in AAb- participants (HR 0.71, CI 0.60–0.84, $p < 0.001$). Following adjustment for pre-specified confounders, there was no significant difference in MACE3 risk between AAb+ and AAb- participants (adj-HR 0.90, CI 0.76–1.07, $p = 0.22$). The 186 UKPDS participants with ≥2 positive AAb tests (double-AAb+) had the lowest MACE3 risk (HR 0.46, CI 0.33–0.65, $p < 0.001$), but this difference became non-significant following adjustment for potential confounders (adj-HR 0.75, CI 0.46–1.22, interaction $p = 0.25$) (See Figure). There were no significant interactions between CV risk factors and AAb status.

Conclusion: In adults with newly-diagnosed diabetes the long-term risk of MACE3 does not differ between those with and without detectable AAb after adjustment for confounders. This suggests measurement of diabetes AAb does not aid in the stratification of CV risk among adults with a clinical diagnosis of new-onset T2D.



Supported by: EFSD Mentorship Programme supported by AstraZeneca
Disclosure: **E. Maddaloni:** None.

1177

Cardiovascular outcomes and mortality in type 2 diabetes with associated cardio-renal-metabolic comorbidities

D.Z.I. Cherney[1], D.C. Wheeler[2], M. Kosiborod[3], S.V. Arnold[3], S. MacLachlan[4], P.R. Hunt[5], H. Chen[6], E. Repetto[6], J. Vora[7];
[1]University Health Network, Toronto General Hospital, Toronto, Canada, [2]Centre for Nephrology, University College London, London, UK, [3]Saint Luke's Mid America Heart Institute, Kansas City, USA, [4]Evidera, London, UK, [5]Evidera, Waltham, USA, [6]AstraZeneca, Gaithersburg, USA, [7]University of Liverpool, Liverpool, UK.

Background and aims: Cardio-renal-metabolic comorbidities (CRMCs) in patients with type 2 diabetes (T2D) are associated with high morbidity and mortality rates. We evaluated the incremental contribution of various CRMCs to the risk of myocardial infarction, stroke, or cardiovascular death (MACE), heart failure (HF) and all-cause mortality in patients with newly diagnosed T2D.

Materials and methods: Using International Classification of Diseases (ICD)-9 codes and Read codes, CRMCs (hypertension [HTN], hyperlipidaemia [HPLD] and chronic kidney disease [CKD]) were identified at the time of T2D diagnosis using databases in the UK (Clinical Practice Research Datalink [CPRD]) and the US (Humedica/Optum). The CPRD database includes longitudinal primary care data from 714 real-life clinical practices and covers approximately 8% of the UK population while the Humedica/Optum databases are US electronic medical records that are claim based and derived mainly from hospitals and outpatient clinics. Humedica databases include approximately 18.5 million patients from 38 States. Both the UK and US (Humedica/Optum) databases are broadly representative of the demographic and geographic breakdown of their respective populations. Patients were followed post-diagnosis of T2D for the occurrence of MACE, HF and mortality and evaluated for the increase in relative risk due to CRMCs.

Results: Between 1 Jan 2011 and 31 Mar 2015, we identified 59,362 patients in the UK and 180,722 patients in the US with incident T2D (mean age [SD]: 61.8 [13.6] and 62.4 [13.5] years; 55.9% and 52% men, respectively). There were no significant differences in the effects of CRMCs on outcomes between countries. The risk of MACE, HF and mortality increased with the number of CRMCs (Table). CKD was associated with the highest incremental risk, with an HR (95% CI) of 2.23 (2.17, 2.49) for mortality compared with T2D alone.

Conclusion: In patients with a new diagnosis of T2D, the risk of MACE, HF and death increased with the number of CRMCs, with CKD being the largest driver of mortality. These results may have implications for risk stratification of patients with T2D, and highlight the importance of identifying novel renoprotection strategies among T2D patients with various CRMCs.

Table. Combined analyses of US and UK databases: MACE, HF and mortality rates in patients with incident T2D and cardio-renal-metabolic diseases

Outcomes	CRMCs at the time of T2D diagnosis	N	Events/100 PY (95% CI)	Incremental % increase in event rate	Adjusted HR (95% CI)
MACE	T2D only	31881	4.34 (4.21, 4.47)	Reference	Reference
	T2D + HTN + HPLD	73448	5.71 (5.61, 5.81)	31.57	1.18 (1.14, 1.23)
	T2D + HTN + HPLD + CKD	21570	8.30 (8.06, 8.54)	43.36	1.56 (1.47, 1.65)
HF	T2D only	31881	4.01 (3.88, 4.14)	Reference	Reference
	T2D + HTN + HPLD	73448	4.84 (4.75, 4.93)	20.70	1.11 (1.07, 1.16)
	T2D + HTN + HPLD + CKD	21570	7.34 (7.12, 7.57)	51.65	1.79 (1.69, 1.90)
Mortality	T2D only	31881	2.14 (2.05, 2.23)	Reference	Reference
	T2D + HTN + HPLD	73448	2.66 (2.60, 2.73)	24.30	1.0 (1.0, 1.1)
	T2D + HTN + HPLD + CKD	21570	5.44 (5.27, 5.62)	104.51	2.23 (2.17, 2.49)

CRMC, cardio-renal-metabolic comorbidity; CKD, chronic kidney disease; HF, heart failure; HPLD, hyperlipidaemia; HTN, hypertension; MACE, myocardial infarction, stroke, or cardiovascular death; N, number of patients; PY, patient-years; T2D, type 2 diabetes

Supported by: Funding for this analysis was supported by AstraZeneca Pharmaceuticals LP.
Disclosure: **D.Z.I. Cherney:** Employment/Consultancy; Janssen, Boehringer Ingelheim, Eli Lilly & Co., AstraZeneca, Merck, Sanofi. Grants; Janssen, Boehringer Ingelheim, Eli Lilly & Co., AstraZeneca, Merck.

1178

A contemporary Australian cardiovascular risk equation for type 2 diabetes: the Fremantle Diabetes Study Phase II

W.A. Davis[1], M. Hunter[2], T.M.E. Davis[1];
[1]Medical School, The University Of Western Australia, Fremantle, [2]Busselton Health Study, Busselton Population Medical Research Institute, Busselton, Australia.

Background and aims: Due to the lack of valid Australian cardiovascular disease (CVD) risk assessment for type 2 diabetes (T2D) we previously developed the Fremantle Diabetes Study (FDS) equation for 5-year risk of hospitalisation for myocardial infarction (MI)/stroke or CVD death. However, there have been substantial changes in the diagnosis, management and outcome of diabetes in the 20 years since the original FDS cohort was enrolled. When the equation was applied to the contemporary FDS Phase II (FDS2) T2D cohort, calibration was poor, while improved survival after acute CVD events has seen hospitalisation for heart failure (HF) become a key additional outcome. In light of these

considerations we have developed and validated a contemporary CVD risk equation.

Materials and methods: FDS2 is a community-based longitudinal observational study. 1551 participants with T2D were followed from baseline (2008–11) for 5 years or until a first CVD event (hospitalisation for MI/stroke/HF or CVD death) or death from other causes. Incident events were identified from hospital morbidity and death registries. Missing covariates were multiply imputed (x20), defining models that included the outcomes of CVD and competing risk of death. Competing risk regression modelling with backward elimination identified independent predictors of CVD within 5 years of study entry. The proportional subdistribution hazards assumption was checked using log-minus-log curves. Discrimination was assessed by the area under the receiver-operating characteristic curve (*c*-index), calibration using the Hosmer and Lemeshow test (Ĉ-statistic) and accuracy by the Brier score. Positive and negative predictive values (PPV, NPV), sensitivity and specificity were determined for a 10% 5-year CVD risk cut-off. The equation was validated in 174 adults with T2D from the Busselton Diabetes Study. StataIC 13 was used for statistical analyses.

Results: The average age of the cohort was 66 years, 52% were men and the median diabetes duration was 9 years. During 6,896 person-years' follow-up, 245 participants (15.8% (95% CI 14.0–17.7%)), had a CVD event. Variables in the final competing risk model comprised age, Australian Aboriginality, heart rate, diabetes duration, ln(gamma-GT), serum albumin, eGFR <45 ml/min/1.73 m^2, ln(urinary albumin:creatinine), left ventricular hypertrophy, anticoagulant use, peripheral arterial disease and history of CVD. The proportional hazards assumption was not violated. The mean 5-year predicted risk of CVD was 14.4%. Model discrimination was good (*c*-index: 0.81 (95% CI 0.78–0.84), $p < 0.001$, as were calibration (Ĉ-statistic = 0.12) and accuracy (Brier score (95% CI): 0.10 (0–0.83)). PPV, NPV, sensitivity and specificity for a 10% 5-year CVD risk cut-off were 31.5%, 95.3%, 82.4% and 66.2%, respectively. During 5-years' follow-up, 21.2% (37 cases) of the validation cohort had an incident CVD event compared with a mean predicted risk of 13.5% (24 cases). There was good discrimination (*c*-index: 0.80 (0.72–0.88), $p < 0.001$), calibration (Ĉ-test $p = 0.26$) and accuracy (Brier score: 0.14 (0–0.88)). At a 10% risk cut-off the sensitivity was 78.4%, specificity 65.0%, PPV 37.8% and NPV 91.8%.

Conclusion: A valid and more sophisticated 5-year CVD risk equation has been developed which includes HF as an outcome and reflects contemporary management and longer survival in Australians with T2D.

Supported by: NHMRC Grants 513781 and APP1042231; Spinnaker Health Research Foundation

Disclosure: **W.A. Davis:** Grants; Australian National Health and Medical Research Council project grants # 513781 and APP1042231, Spinnaker Health Research Foundation Award.

1179

Socio-economic inequalities in hospital admissions for major cardiovascular events in people with diabetes in England

E. Vamos, Z. Shather, A. Laverty, A. Bottle, H. Watt, A. Majeed, C. Millett;
Imperial College, London, UK.

Background and aims: While extensively studied in the general population, little is known about how people with diabetes from different socioeconomic groups have benefited from reductions in CVD over the last decade. This nationwide study aims to determine changes in absolute and relative socio-economic inequalities in hospital admissions for major cardiovascular events and procedures among people with diabetes in England between 2004–2005 and 2014–2015.

Materials and methods: We identified all patients with diagnosed diabetes aged above 16 years admitted to hospital in England between 2004–2005 and 2014–2015 for acute myocardial infarction (AMI), stroke, percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG). Socio-economic status was measured using Index of Multiple Deprivation. Diabetes-specific admission rates were calculated for each year by deprivation quintile. We assessed temporal changes relative inequalities in admissions for each outcome using negative binomial regression models, and we used linear regression models to assess changes in absolute inequalities between deprivation groups.

Results: Admission rates rose steadily with increasing levels of deprivation throughout the study period. People with diabetes from the most deprived quintile had 2.17-fold increased risk of AMI (95% CI 1.98–2.37), 2.04-fold risk of stroke (95% CI 1.88–2.22), 1.79-fold risk of CABG (95% CI 1.65–1.94), and 2.03-fold risk of PCI (95% CI 1.89–2.18) ($P \leq 0.001$ for all) compared with the least deprived group. Socio-economic gradients did not significantly change over the study period for any of the study outcomes. Absolute differences in admission rates between the least and most deprived quintiles did not significantly change for AMI ($P = 0.342$) and reduced for stroke, PCI and CABG admissions (by 14, 134 and 46 per 100,000 people with diabetes, respectively, $P \leq 0.005$ for all). From 2004–2005 to 2014–2015, there was a reduction in in-patient mortality rates for all outcomes except for PCI. Trends in in-patient mortality did not statistically significantly differ between the most affluent and other deprivation groups for the study outcomes.

Conclusion: Socio-economic inequalities persisted in hospital admissions for major CVD events in England among people with diabetes throughout the study period. Besides improved risk stratification strategies considering socio-economically defined needs, wide-reaching population-based policy interventions are required to reduce inequalities in diabetes outcomes.

Disclosure: **E. Vamos:** None.

1180

Ranking of cardiovascular impairments in impaired glucose tolerance

L. Lind;
Department of Medical Sciences, Uppsala, Sweden.

Background and aims: Impairments in several markers of the cardiovascular system have been described in impaired glucose tolerance, but the relative importance of those are not known. We aimed to investigate which cardiovascular markers that were most closely linked to an impaired glucose tolerance.

Materials and methods: In a population-based study of individuals all aged 50 years, the Prospective study of Obesity, Energy and Metabolism (POEM), 502 subjects were thoroughly investigated regarding endothelial function, arterial compliance, heart rate variability, arterial blood flow and atherosclerosis, performance at an exercise test with gas exchange (VO_2 and VCO_2), left ventricular structure and function, lung function and multiple measurements of blood pressure. Based on an oral glucose tolerance test (OGTT), the participants were grouped into: normal, impaired glucose tolerance and diabetes.

Results: Of all hemodynamic and structural variables analyzed, an impaired glucose tolerance was most closely related to resting heart rate (based on Chi^2-value at ANOVA). Heart rate was followed by maximal workload, VO_2-recovery 5 min following exercise, reflectance index at pulse wave analysis, manual systolic blood pressure, ambulatory pulse and pulse pressure, echolucency of carotid intima-media complex at ultrasound, and pulse wave velocity ($p < 0.0005$ for all variables).

Conclusion: Of multiple measured cardiovascular markers, resting heart rate was most closely related to an impaired glucose tolerance. Also exercise capacity and the recovery in VO_2 following exercise were amongst the top ranked cardiovascular impairments linked to an impaired glucose tolerance.

Supported by: University Hospital

Disclosure: **L. Lind:** None.

1181

Does sex influence the tolerance to ischaemia-reperfusion injury in a metabolic syndrome model?

N. Fourny, C. Lan, M. Bernard, M. Desrois;
Aix Marseille Univ, CNRS, CRMBM, Marseille, France.

Background and aims: Worldwide prevalence of Metabolic Syndrome (MetS) keeps increasing and becomes a real public healthcare problem. MetS is based on the presence of at least 3 risk factors including abdominal obesity, glucose intolerance, dyslipidemia and hypertension. MetS is a well-known risk factor of type 2 diabetes and cardiovascular (CV) complications such as myocardial infarction. Epidemic studies showed a higher prevalence and CV risk in men. However, few studies explore sex differences in this context. Consequently, the aim of this study was to compare the effects of a high-fat high-sucrose diet (HFHSD) on the sensitivity to ischemia-reperfusion injury of male and female Wistar rat hearts.

Materials and methods: Male and female Wistar rats were subjected to HFHSD (12 MHFSD and 10 FHFSD) or Normal Diet (12 MND and 10 FND) for 5 months. Then, rats underwent an intraperitoneal glucose tolerance test (IPGTT) to determine glycemic status, and *ex vivo* experiments on the isolated perfused heart were performed to study the tolerance to ischemia-reperfusion injury. Isolated hearts were perfused with a physiological buffer containing 0.4 mM palmitate for 24 minutes before switching to 1.2 mM palmitate for 32 minutes low-flow (0.5 mL/min/g wet wt) ischemia. Next, flow was restored with 0.4 mM palmitate buffer for 32 minutes. High-energy phosphates and intracellular pH were measured during the experimental course by ^{31}P magnetic resonance spectroscopy with simultaneous measurement of contractile function. Coronary flow was measured before and after ischemia. At the end of experiments, hearts were freeze-clamped for biochemical assays.

Results: After 5 months of HFHSD, body weight was increased in males ($p < 0.001$ vs. MND) but not in females and fat percent was higher in both HFHSD groups (respectively $p < 0.01$ and $p < 0.05$ vs. controls). IPGTT showed a significant glucose intolerance in male and female HFHSD ($p < 0.001$ vs. controls) which was more pronounced in females ($p < 0.05$ vs. MHFSD at T15min). HFHSD increased fasting blood glucose in both males and females compared with controls ($p < 0.05$) but increased plasma free fatty acids only in females ($p < 0.05$ vs. FND). Heart weight to tibia length ratio was higher with HFHSD only in males ($p < 0.001$ vs. MND). Interestingly, in male and female HFHSD, we found impaired myocardial function (respectively $p < 0.001$ and $p < 0.05$ vs. controls) and coronary flow (respectively $p < 0.01$ and $p < 0.05$ vs. controls) during reperfusion, with no difference between males and females. Energy metabolism was significantly altered in male and female HFHSD compared with controls. ATP was decreased only in MHFSD during reperfusion ($p < 0.01$ vs. MND). PCr was impaired during reperfusion in male and female HFHSD groups compared with their respective controls ($p < 0.01$ and $p < 0.05$) and in FND compared with MND ($p < 0.05$). Finally, intracellular pH was similar between groups during the whole protocol.

Conclusion: Five months of HFHSD induced metabolic syndrome in both male and female with sex differences in tolerance to glucose, body weight and heart weight to tibia length ratio. HFHSD also decreased the tolerance to ischemia-reperfusion in both sex, characterized by impaired energy metabolism, cardiac and endothelial function during reperfusion. In conclusion we found no effect of sex on the tolerance to ischemia-reperfusion in our model of MetS.

Supported by: Aix-Marseille Univ, CNRS, FLI

Disclosure: **N. Fourny:** None.

PS 114 Vascular complications

1182

Prolongation of the QTc interval is associated with an increased risk of cardiovascular diseases: the Hoorn study

S. Welten[1], A.A. van der Heijden[2], G. Nijpels[2], J.W.J. Beulens[1], P. Elders[2], J. Dekker[1];
[1]Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, [2]Department of General Practice and Elderly care Medicine, VU University Medical Center, Amsterdam, Netherlands.

Background and aims: Prolongation of the heart rate-corrected QT interval (QT/√heart-rate frequency=QTc), i.e. time from ventricular depolarization to complete repolarization, may predict cardiovascular diseases (CVD). There are several hypotheses about the underlying mechanisms, including a role for impaired glucose metabolism. We investigated whether prolongation of the QTc interval is associated with CVD in the general population, and whether this association is different within the glucose metabolism groups.

Materials and methods: We analyzed an age-, sex- and glucose tolerance-stratified sample from the Hoorn Study, a population-based cohort study which started in 1989. After exclusions, 447 participants aged 50–74 years, who had duplicate oral glucose tolerance tests (except for those using glucose lowering medication) and 12-lead electrocardiography (ECG) at baseline were eligible for analysis. Cox regression was used to investigate the association between sex-specific QTc quartiles and CVD incidence. All analyses were adjusted for age, sex, smoking status, systolic blood pressure, glucose tolerance status, hypertension and total cholesterol. Stratified analyses were conducted for glucose tolerance status. Sensitivity analyses were performed in participants without medication.

Results: During a mean follow-up of 10 years, 305 CVD events were observed. The age and sex adjusted hazard ratios (95% CI) of participants in the second (males; 370–388 ms, females; 386–401 ms), third (males; 389–408 ms, females; 402–420 ms) and fourth (males; >408 ms, females; >420 ms) sex-specific QTc quartiles were 1.17 (0.83–1.65), 1.41 (1.01–1.97), 1.70 (1.23–2.36) with the first quartile (males; <370 ms, females; <386 ms) as reference category. The multivariable adjusted hazard ratios for participants in the fourth sex-specific QTc quartile were 1.54 (1.10–2.14) compared with participants with a QTc interval in the first quartile. The same analyses in people without medication resulted in even stronger associations (HR: 1.75, 95% CI: 1.05–2.93). Stratified analyses showed that the association was stronger for participants with impaired glucose status (HR: 2.45, 95% CI: 1.13–5.30) and diabetes (HR: 2.19, 95% CI: 1.12 to 4.28).

Conclusion: Prolongation of the QTc interval on the ECG was significantly associated with increased risk of CVD in the general population. This association was stronger in participants with impaired glucose metabolism or type 2 diabetes. QTc duration may contribute to improved CVD risk stratification.

Supported by: Horizon 2020 grant number: 733381

Disclosure: **S. Welten:** None.

1183

Soluble urokinase plasminogen activator receptor predicts cardiovascular events, mortality and kidney function decline in patients with type 1 diabetes

V. Rotbain Curovic[1], S. Theilade[1], S.A. Winther[1], N. Tofte[1], J. Eugen-Olsen[2], F. Persson[1], T.W. Hansen[1], J. Jeppesen[3,4], P. Rossing[1,4];
[1]Steno Diabetes Center Copenhagen, Gentofte, [2]Clinical Research Centre, Hvidovre Hospital, Hvidovre, [3]Department of Medicine, Amager Hvidovre Hospital, Glostrup, [4]University of Copenhagen, Copenhagen, Denmark.

Background and aims: Soluble urokinase plasminogen activator receptor (suPAR) is an important inflammatory biomarker. The predictive qualities of suPAR in relation to complications in patients with type 1 diabetes (T1D) have not been determined. We investigated the prognostic ability of suPAR for the development of cardiovascular events (CVE), mortality and decline in renal function in T1D.

Materials and methods: 667 patients with T1D and various degrees of diabetic kidney disease were included in a prospective study. suPAR was measured with commercially available ELISA kits (suPARnostic kit). In 2016, patients were traced through the National Death Register and the National Health Register, from which data for CVE and mortality was gathered. Data for estimated GFR (eGFR) measurements, obtained at outpatient visits, were traced through electronic laboratory records. Endpoints were classified as: CVE (cardiovascular death, non-fatal myocardial infarction, non-fatal stroke and coronary or peripheral arterial interventions), mortality, and eGFR-decline of ≥30%. Median follow-up ranged from 5.2 to 6.2 years. Results were adjusted for known risk factors and confounders: sex, age, LDL cholesterol, HbA_{1c}, systolic blood pressure, BMI, smoking status, urinary albumin excretion rate, eGFR, prescribed renin-angiotensin-aldosterone system inhibitors, and c-reactive protein. Hazard ratios (HR) were calculated per doubling of suPAR and are presented with 95% confidence interval (CI). Furthermore, the relative integrated discrimination (rIDI) was calculated to assess the added predictive contribution of suPAR to the above described risk factors.

Results: Of the 667 participants, 368 (55%) were male; mean±SD age was 55 ± 13 years and eGFR 88 ± 25 ml/min/1.73 m^2. Median (interquartile range) of suPAR was 3.4 (2.7–4.5) ng/ml. There were 94 (14.2%) cases of CVE, 58 (8.7%) deaths and 93 (14.0%) cases of eGFR-decline ≥30%. The adjusted HR (95% CI) for the respective endpoints were 3.50 (2.09–5.87), 4.42 (2.15–9.07) and 2.91 (1.71–4.97). rIDI analysis showed discrimination slope contribution of 32.4% for CVE, 39.7% for mortality, and 14.8% for eGFR-decline. All presented results were highly significant ($p \leq 0.001$).

Conclusion: Higher suPAR level is consistently associated with an increased risk of CVE, mortality and eGFR-decline in patients with T1D, independent of classical risk factors. In addition, it is a sizeable and significant contributor in the risk stratification of the described complications based on rIDI. Our results suggest that suPAR may have an important role in identifying T1D patients at risk of severe complications at an early stage.

	Cardiovascular Events		Total Mortality		Decline in eGFR ≥30%	
Events, n (%)	94 (14.2)		58 (8.7)		93 (14.0)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Unadjusted	3.43 (2.52-4.66)	<0.001	4.37 (2.95-6.46)	<0.001	4.29 (3.14-5.84)	<0.001
Adjusted	3.50 (2.09-5.87)	<0.001	4.42 (2.15-9.07)	<0.001	2.91 (1.71-4.97)	<0.001
rIDI	32.4%	<0.001	39.7%	<0.001	14.8%	<0.001

Clinical Trial Registration Number: 2009-056
Disclosure: **V. Rotbain Curovic:** None.

1184

Coronary CT angiography improve discriminative performance of cardiovascular risk predictor in asymptomatic patient with type 2 diabetes

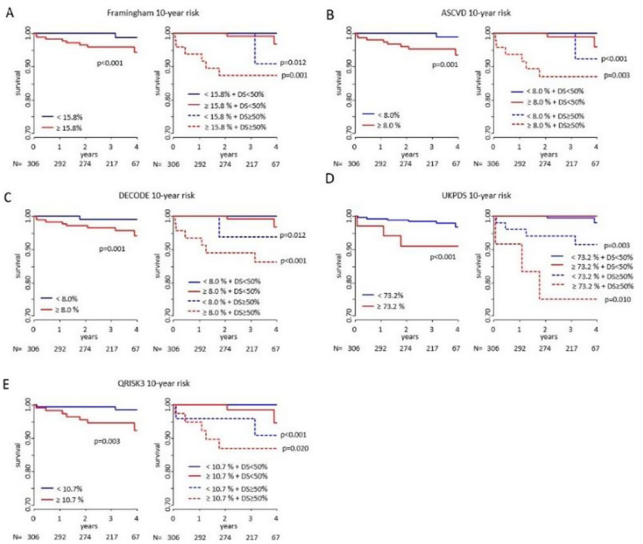
J. Ahn[1], K. Hur[1,2], Y. Choe[3], S.-A. Chang[4], J.-H. Choi[4,5], M.-K. Lee[1,2]; [1]Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, [2]Samsung Advanced Institute for Health Sciences & Technology, Seoul, [3]Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, [4]Division of Cardiology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, [5]Department of Emergency Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

Background and aims: Predicting cardiovascular risk in asymptomatic diabetic patients is unsatisfactory. Clinical guidelines advocate clinical risk predictors with various criteria and different approaches to predicting algorithms, which show poor calibration each other and typically overestimate the true risk. Considering the progressive nature of atherosclerosis, detection of subclinical atherosclerosis by non-invasive modalities such as coronary computed tomography angiography (CCTA) or carotid intima-media thickness measurement (CIMT) may improve risk stratification. We investigated whether non-invasive modalities could improve the prediction of cardiovascular event of asymptomatic patients with type 2 diabetes.

Materials and methods: In this prospective single center study, a total of 306 asymptomatic patients with type 2 diabetes without history of coronary artery disease were enrolled. Patients underwent coronary computed tomography angiography (CCTA), coronary calcium score, and carotid intima-media thickness (CIMT) measurement. Clinical risk predictors were calculated by Framingham, ASCVD, UKPDS, DECODE, and QRISK3 scores. The primary outcome was major adverse cardiac and cerebrovascular event (MACCE) consisting cardiovascular death, nonfatal myocardial infarction, stroke, and revascularization.

Results: A total of 12 MACCE developed during median follow-up time of 3.8 years. Clinical risk predictors was moderate in overall (c-statistics = 0.534 to 0.729) and overestimated the MACCE risk by 2- to 9-fold. The MACCE risk increased proportionally to the severity and extent of coronary atherosclerosis measured by CCTA ($p < 0.01$, all), but did not to coronary calcium score or CIMT. In time-dependent receiver operating characteristics, reclassification, and survival analyses, the performance of all clinical risk predictors improved by addition of the severity of stenosis measured by CCTA ($p < 0.05$, all). Subgroup analysis of survival data by maximal diameter stenosis ≥50% enables further discrimination of high- and low-risk subgroups ($p < 0.01$, all) (Figure).

Conclusion: CCTA could improve the predictive performance of the clinical risk predictors in asymptomatic type 2 diabetes patients, whereas coronary calcium score or CIMT did not.



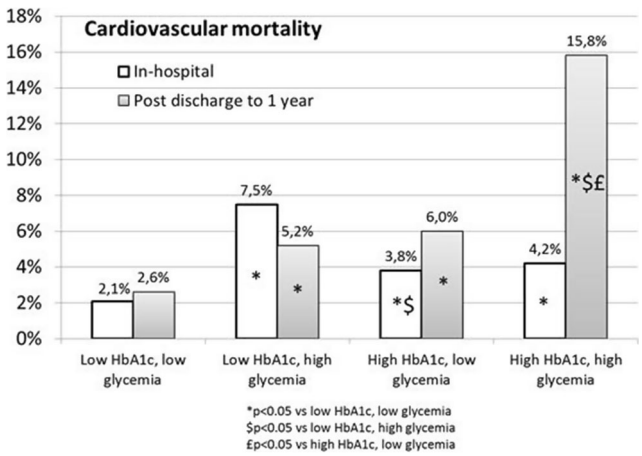
Disclosure: **J. Ahn:** None.

1185

Specific short and long term prognostic value of admission HbA_{1c} and plasma glucose in non-diabetic patients with acute myocardial infarction: data from the RICO survey

B. Vergès[1], B. Mouhat[1], M. Zeller[2], F. Chagué[1], J.-C. Beer[1], M. Maza[1], Y. Cottin[1];
[1]Hopital du Bocage, Dijon, [2]Université de Bourgogne Franche Comté, Dijon, France.

Background and aims: In non-diabetic patients with acute myocardial infarction (MI), acute hyperglycemia is associated with high risk of cardiovascular (CV) mortality. However, the prognostic value of HbA1c as a marker of chronically elevated plasma glucose remains unclear. From the large observational RICO survey, we aimed to identify the specific prognostic values of HbA1c and plasma glucose measured on admission for acute MI in non-diabetic patients regarding in-hospital and one year CV mortality.
Materials and methods: From the RICO survey database all the consecutive non-diabetic patients with acute MI ($n = 6884$) admitted in the cardiology intensive care unit of the university hospital of Dijon from January 2001 to June 2016 were included. Patients with diabetes were excluded. Cut off levels (high/low) were determined by ROC curve analysis for the prediction of CV death (HbA1c: 5.9% and glucose: 156 mg/dL). Patients were divided into 4 groups, based on levels of both HbA1c and plasma glucose: low HbA1c/low glucose (n = 3849), low HbA1c/high glucose ($n = 734$), high HbA1c/low glucose ($n = 1802$) and high HbA1c/high glucose ($n = 499$).
Results: Elevation of either glucose or HbA1c was associated with elevated risk of hospital mortality, when compared to group with low HbA1c and glucose levels (Figure). However, by multivariate logistic regression analysis, only high glucose remained a prognostic factor of hospital death (OR(95%CI): 1.59 (1.16–2.17) after adjustment for covariates (age, sex, risk factors, prior MI, creatinine clearance, chronic and acute treatments). In survivors at discharge, a marked increased risk of one-year CV mortality was found in the group with elevated levels of both glucose and HbA1c, when compared to all other groups ($p < 0.001$). Moreover, high HbA1c was an independent predictive factor of one year CV mortality, beyond high glucose (OR(95%CI): 1.75(1.35–2.27) and 1.98(1.49–2.61), respectively) and covariates (age, sex, risk factors, creatinine clearance, CRP, LVEF, acute and discharge treatments).
Conclusion: In our large population-based study, high levels of both HbA1c and glucose are in non-diabetic patients with acute MI, associated with increased risk of mortality at short or long term. Elevated admission plasma glucose, reflecting acute hyperglycemia and high HbA1c, reflecting chronic hyperglycemia, appear to give different prognostic information. Early mortality risk was mainly driven by acute hyperglycemia, and HbA1c was a strong predictive factor for one year mortality, independently of acute plasma glucose. Our findings may help identifying high risk patients to target for aggressive secondary prevention after MI.



Disclosure: **B. Vergès:** None.

1186

Association between serum cystatin C and vascular complications in type 2 diabetes without nephropathy

H. Park, J. Lee, H. Kim, D. Byun, K. Suh, M. Yoo;
Internal Medicine, Soonchunhyang University Hospital, Seoul, Republic of Korea.

Background and aims: Recent studies have shown associations between serum cystatin C levels and vascular complications; however, few have investigated these associations in diabetes without nephropathy. We evaluated the relationship between serum cystatin C and vascular complications in type 2 diabetes patients with normal renal function or mild renal impairment.
Materials and methods: A total of 806 consecutive patients with type 2 diabetes who were admitted to the diabetes center of Soonchunhyang University Hospital for blood glucose control were retrospectively reviewed. Patients with nephropathy were excluded. Subjects were categorized into quartiles of serum cystatin C levels (Q1, ≤0.65 mg/L; Q2, 0.66~0.79 mg/L; Q3, 0.80~0.94 mg/L; and Q4, ≥0.95 mg/L). Multivariate logistic regression was conducted for the risk of vascular complications.
Results: The proportion of patients with diabetic retinopathy (DR), coronary heart disease (CHD) and stroke increased across the serum cystatin C quartiles (p for trend <0.001). After adjustment for confounding factors, the highest serum cystatin C level remained a significant risk factor for DR (odds ratio [OR] 1.929, 95% confidence interval [CI] 1.030–3.614, $p = 0.040$). Compared with Q1, a significant positive association was observed between serum cystatin C and CHD in Q2 (OR 7.321, 95% CI 1.560–34.361, $p = 0.012$), Q3 (OR 6.027, 95% CI 1.324–27.435, $p = 0.020$) and Q4 (OR 8.122, 95% CI 1.755–37.577, $p = 0.007$). No associations were observed between cystatin C and stroke after additional adjustment for confounding variables.
Conclusion: Serum cystatin C levels are independently associated with DR and CHD, suggesting that cystatin C may be useful for identifying type 2 diabetes patients without nephropathy who are at high risk for vascular complications.
Disclosure: **H. Park:** None.

1187

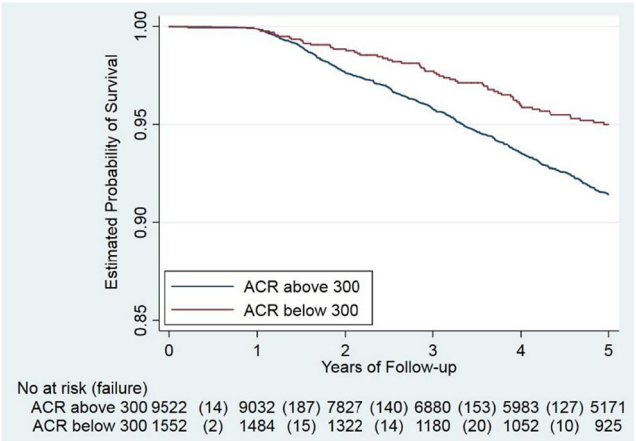
Relationship between albuminuria and total mortality among insulin treated patients with type 2 diabetes and nephropathy: analysis of a large UK primary care cohort

I. Idris, R. Donnelly, U. Anyanwagu;
Royal Derby Hospital Centre, University of Nottingham, Derby, UK.

Background and aims: Overt proteinuria (urinary albumin-creatinine ratio (ACR) >300 mg/g) is an established risk factor for progression of nephropathy and total mortality. However, whether, among insulin-treated patients with Type 2 diabetes in routine practice, a reduction in ACR translates into a mortality reduction remains far from clear.
Materials and methods: We obtained data on a large cohort of insulin users with T2D and nephropathy (baseline ACR ≥300 mg/g) from UK general practices between 2007–2014. Their corresponding ACR values after one year of follow up were thereafter categorised into: (1) <300 mg/g (i.e. proteinuria reduction) or (2) >300 mg/g (i.e. persistent or progressive proteinuria), and the cohort was followed up for 5 years for all-cause mortality. Cox proportional hazard models were fitted to estimate the risk of all-cause death.
Results: A total of 11,074 patients with insulin treated T2D met the inclusion criteria. Their mean age was 62.3(13.6) years; mean HbA1c: 8.7(1.8) %; and 53% were male. 682 deaths occurred after a follow-up period of 43,393 person-time with a mortality rate of 16 per 1000 person-

years. 5-year survival was markedly reduced in the group whose proteinuria persisted or progressed (91 vs 95%; log-rank p value <0.001). Compared to patients whose ACR levels remained above 300 mg/g, all-cause mortality was 27% lower compared with those whose proteinuria regressed to <300 g/g (aHR: 0.73; 95%CI: 0.56 to 0.97; $p = 0.028$) (Figure 1)

Conclusion: In patients with insulin treated T2D and nephropathy in routine practice, a reduction in proteinuria (e.g. via better BP or glycaemic control) is associated with a significant reduction in all-cause mortality. Thus, proteinuria is not simply a risk marker of renal and cardiovascular disease, but also an important target for therapy. Proteinuria reduction should be viewed as a goal for renal and cardiovascular protection.



Disclosure: **I. Idris:** Lecture/other fees; Novo Nordisk, Eli Lilly, AstraZeneca, MSD, Jansen, Sanofi Aventis.

1188

Role of glycaemic variability in increased cardiac output in the obese patients with impaired glucose tolerance

A. Rezki, M. Fysekidis, S. Chiheb, I. Banu, E. Cosson, P. Valensi;
AP HP Jean Verdier Hospital, Department of Endocrinology-Diabetology-Nutrition, CRNH-IdF, CINFO, Bondy, France.

Background and aims: In obese patients, cardiac output is increased and cardiac autonomic function often altered. This study examined the change in cardiac contractility and its determinants in obese patients with impaired glucose tolerance (IGT) compared with those with a normal glucose tolerance (NGT) and the influence of glycemic variability.

Materials and methods: We included 66 obese patients with normal blood pressure (BP) and without cardio-vascular history, separated according to OGTT: 38 NGTs and 28 IGTs, who were well matched for sex, age and BMI (38.4 ± 4.1 and 37.4 ± 4.3 kg/m²). Peripheral and central BP and carotid-to-femoral pulse wave velocity (PWV) were measured by applanation tonometry (SphygmoCor®), cardiac vagal and sympathetic control (HF-HR, LF-HR) by spectral analysis (Task Force Monitor® finger plethysmography), stroke volume (SV), indexed cardiac output (iCO) and thoracic fluid content (TFC) by thoracic impedance, cutaneous blood flow (CBF) and CBF response to acetylcholine (endothelial function) by laser doppler flowmetry (Periflux®). Glycemic variability was evaluated in IGTs by calculating standard deviation (SD-glucose), CONGA and J index from 24-h continuous glucose monitoring.

Results: IGTs had similar BP, PWV and sympatho-vagal activity but higher SV, iCO and TFC ($p < 0.04$ to < 0.002) than NGTs. In IGTs, SV and iCO correlated positively with CONGA, systolic BP ($p < 0.03$ to $p < 0.05$) and negatively with age and PWV ($p < 0.001$ and $p < 0.03$), and iCO also correlated negatively with HbA1c ($p = 0.04$). In multivariate analysis, SV and iCO remained correlated with CONGA independently of HbA1c, age, PWV and systolic BP. In IGTs TFC correlated negatively with mean basal CBF and CBF response to acetylcholine ($p < 0.03$ and < 0.04).

Conclusion: Among obese patients, IGTs exhibit higher left ventricle contractility and higher central blood volume than NGTs. Higher glycemic variability could contribute to increase cardiac work by increasing central blood volume secondarily to a reduction of peripheral microcirculation.

Disclosure: **A. Rezki:** None.

PS 115 Novel diagnostic tool for NAFLD

1189

Prediction of liver fat in people with and without type 2 diabetes: an IMI DIRECT study

N. Atabaki Pasdar[1], M. Ohlsson[2], A. Viñuela[3], F. Frau[4], R. Koivula[5,1], J. Fernandez[5], E. Thomas[6], A. Mari[7], R. Gupta[8], A. Kurbasic[1], E. Pearson[9], I. Pavo[10], J. Bell[11], P.W. Franks[1];
[1]Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Lund, Sweden, [2]Department of Astronomy and Theoretical Physics, Computational Biology and Biological Physics Unit, Lund University, Lund, Sweden, [3]Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland, [4]Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany, [5]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK, [6]Department of Life Sciences, University of Westminster, London, UK, [7]Institute of Biomedical Engineering, National Research Council, Padova, Italy, [8]Department of Bio and Health Informatics, Technical University of Denmark, Kongens Lyngby, Denmark, [9]Division of Molecular and Clinical Medicine, University of Dundee, Dundee, UK, [10]Eli Lilly, Vienna, Austria, [11]Metabolic and Molecular Imaging Group, MRC Clinical Science Centre, Imperial College Hammersmith Campus, London, UK.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is highly prevalent and causes serious health complications in type 2 diabetes (T2D) and beyond. In NAFLD, triglycerides accumulate in hepatocytes, promoting hepatic gluconeogenesis, and thereby raising risk of T2D or exacerbating the disease pathology. Liver biopsy, MRI scans, ultrasounds and liver enzyme tests are often used for NAFLD diagnosis, but the invasive nature of biopsies, the high costs of the MRI scans and ultrasounds and the low accuracy of liver enzyme tests are significant limitations. Here, we aim to derive a prediction tool for NAFLD by applying machine learning approaches to the extensive phenotypic data obtained in participants with pre-diabetes or diabetes cohorts from IMI DIRECT.
Materials and methods: Multi-omics (genetic, transcriptomic, proteomic, metabolomic) and clinical data including plasma biomarkers and measures of beta-cell function, insulin sensitivity, anthropometry and lifestyle comprised the key input variables. The outcome variable was MRI image-derived liver fat content (categorized into fatty ($\geq$5%) or normal (<5%) for 1514 participants. Random forest analysis with 5 × 5-fold cross-validation was used to develop the algorithms (2/3 of the data for training and the rest for validation). Feature selection of different layers of omics data was done using LASSO (least absolute shrinkage and selection operator). 'Advanced' (all available input variables) and 'pragmatic' (20 clinically accessible variables) prediction algorithms were developed.
Results: The 'pragmatic' algorithm yielded a cross-validated predicative accuracy (ROCAUC) of 81.4% in both cohorts combined (predictive accuracy was similar in the pre-diabetes and diabetes cohorts). For the 'advanced' algorithm, the ROCAUC was 86.7% in both cohorts combined. In the latter algorithm, measures of beta-cell function were amongst the highest-ranked input variables.
Conclusion: We have developed a new prediction tool for NAFLD as a safer and potentially more cost-effective alternative to MRI scans or biopsies in at-risk populations. In patients predicted by our algorithm to exceed the liver fat threshold, scans or biopsies would likely be performed for conformational diagnosis. Our analysis also highlights the important role of insulin sensitivity and beta cell function in liver fat accumulation, which are not features of conventional NAFLD risk algorithms.
Supported by: IMI Joint Undertaking under grant agreement n°115317 (DIRECT)
Disclosure: **N. Atabaki Pasdar:** Grants; Innovative Medicines Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), Financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies.

1190

Pooled cohort analysis of non-alcoholic fatty liver disease in patients with type 2 diabetes and the associated diagnostic performance of FibroScan

S. Czernichow[1], S.A. Harrison[2], M. Allison[3], E. Tsochatzis[4], Q.M. Anstee[5], D. Sheridan[6], I.N. Guha[7], J. Cobbold[8], V. Paradis[9], P. Bedossa[9], C. Barsamian[1], A.H. Paredes[10], P.N. Newsome[11];
[1]Department of nutrition, Hopital Européen Georges Pompidou, Paris, France, [2]Radcliffe Department of Medicine, Oxford, UK, [3]Addenbrookes Hospital, Cambridge, UK, [4]Royal Free Hospital, London, UK, [5]Institute of cellular medicine, Newcastle upon Tyne, UK, [6]Institute of Translational and Stratified Medicine, Plymouth, UK, [7]NIHR Nottingham Biomedical Research Centre, Nottingham, UK, [8]John Radcliffe Hospital, Oxford, UK, [9]Department of Pathology, Beaujon Hospital, Clichy, France, [10]Brooke Army Medical Center, Fort Sam Houston, USA, [11]NIHR Liver Biomedical Research Unit, Birmingham, UK.

Background and aims: We set out to establish the prevalence of non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes mellitus (T2DM) and then to evaluate the associated performance characteristics of FibroScan in determining the histological severity of NAFLD.
Materials and methods: Three cohorts were pooled for this analysis. *Screening cohort:* Patients referred for routine colon cancer screening in a single American centre were screened for evidence of NAFLD using FibroScan, Liver MultiScan (magnetic resonance imaging proton density fat fraction (PDFF), liver inflammation and fibrosis (LIF) score) and magnetic resonance elastography (MRE). Patients with PDFF ≥5% or LIF ≥2 or LSM ≥7 kPa on FibroScan or ≥3 kPa on MRE were offered a liver biopsy (LB). *Bariatric surgery cohort:* Patients with severe obesity defined as a BMI >35 kg/m² prospectively underwent a FibroScan examination before bariatric surgery in a single French centre. LB samples were collected during surgery. *NAFLD cohort:* Patients with suspected NAFLD prospectively underwent FibroScan and LB at seven British centres. All LB were scored by the same expert pathologists in a blinded manner using the NASH CRN system, and consensus achieved. All patients underwent a FibroScan examination which measured both liver stiffness and controlled attenuation parameter (CAP). The association between T2DM and prevalence of NAFLD-associated liver lesions assessed using risk ratio (95% CI). FibroScan diagnostic performance was assessed using AUC.
Results: 684 patients (186 in the screening cohort, 116 in the bariatric surgery cohort and 370 in the NAFLD cohort) had an interpretable LB and valid FibroScan examination. 38% had T2DM, 48% were female, and their median BMI was 34.5 [30.1–39.6] kg.m^{-2}. Prevalence of NAFLD and related histological features is presented in Table 1 with their associated risk ratios for T2DM. In patients with T2DM, the diagnostic performance (AUC) of FibroScan for identifying fibrosis ≥2, fibrosis = 4 and steatosis ≥1 was 0.77 [0.71–0.82], 0.89 [0.83–0.94] and 0.85 [0.73–0.96] respectively.
Conclusion: T2DM is a significant risk factor for all aspects of NAFLD-related liver pathology. FibroScan has good diagnostic accuracy to discern the presence of fibrosis and steatosis in patients with T2DM.

	All patients	Non-diabetic patients	T2DM patients	Risk ratio *[95% CI]*
N	684	423	261	-
Fibrosis stage ≥2	273 (**40%**)	111 (**26%**)	162 (**62%**)	**2.46** *[2.02-3.00]*
Fibrosis stage = 4 (cirrhosis)	35 (**5%**)	10 (**2%**)	25 (**10%**)	**1.96** *[1.55-2.48]*
Steatosis grade ≥1 (≥ 5% steatosis)	560 (**82%**)	316 (**75%**)	244 (**93%**)	**3.18** [2.02-4.99]
Ballooning grade ≥1	422 (**62%**)	213 (**50%**)	209 (**80%**)	**2.50** [1.92-3.24]
Lobular inflammation grade ≥1	429 (**63%**)	221 (**52%**)	208 (**80%**)	**2.33** [1.80-3.02]
Non-alcoholic steato-hepatitis (NASH)	350 (**51%**)	165 (**39%**)	185 (**71%**)	**2.32** [1.86-2.90]

Table 1. Prevalence of non-alcoholic fatty liver disease-associated liver lesions in all patients, non diabetic patients and type 2 diabetic (T2DM) patients and their associated risk ratios for T2DM.

Clinical Trial Registration Number: NCT03142867/NCT01985009/NA
Supported by: Echosens
Disclosure: **S. Czernichow:** Other; Study sponsored by Echosens.

1191

CcK18 detection as a reliable marker for NAFLD/NASH detection in type 2 diabetes

M. Hauber, B. Stratmann, D. Tschoepe;
Diabeteszentrum, Herz- und Diabeteszentrum NRW, Ruhr Universitaet Bochum, Bad Oeynhausen, Germany.

Background and aims: Liver disease (non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatosis hepatis (NASH) or liver fibrosis) are commonly detected in patients with type 2 diabetes mellitus (T2DM). We evaluated the laboratory marker Kreatin18Asp396 (ccK18) with respect to correctly identify non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatosis hepatis (NASH), determined by elevation in Alaninaminotransferase (ALAT>ULN).
Materials and methods: 603 consecutive non-selected patients with type 2 Diabetes mellitus were available for analysis; Kreatin18Asp396 (ccK18) (M30 Apoptosense-ELISA (VLVbio, Schweden) was analysed in EDTA-Plasma. Current diagnosis is made considering ALAT (women: ALAT >35 U/l, men: ALAT >50 U/L) activity.
Results: Patients (414m/189f, age 61.9 ± 12.3 years, average duration of T2DM 13.0 ± 9.5 years). HbA1c was 8.9 ± 2.0%, ASAT 35.7 ± 20.6 U/l, ALAT 42.0 ± 30.3 U/l, γGT 98.4 ± 161.8 U/l. CcK18 was 396.2 ± 530.7 U/l. Correlations of ccK18 to ALAT was 0.613, and to ASAT 0.629 (Pearson Correlation) for the whole cohort ($p < 0.0001$ for all). Weaker, but significant correlation were detected to HbA1c 0.162, BMI 0.118, Diabetes duration −0.190, ferritin 0.329 and γGT 0.346.
Separation by laboratory diagnosis of liver disease according to existing ALAT ULN: **Men ($N = 291$): ALAT ≤ ULN:** ccK18 = 242.1 ± 306.1 U/l. **Men ($N = 123$): ALAT > ULN:** ccK18 = 823.4 ± 815.6 U/l. **Women ($N = 118$): ALAT ≤ ULN:** ccK18 = 231.3 ± 243.4 U/l. **Women ($N = 71$): ALAT > ULN:** ccK18 = 563.1 ± 532.1 U/l. In a subgroup of 29 patients liver biopsy results were available and indicated an increased level of ccK18 correlating with NAFLD-Score: **NAS = 3 ($N = 7$):** ccK18 = 645.4 ± 686.1 U/l, ALAT = 53.0 ± 17.1 U/l, ASAT = 62.0 ± 22.6 U/l, HbA1c = 9.7 ± 1.4% **NAS = 4 ($N = 4$):** ccK18 = 453.2 ± 270.8 U/l, ALAT = 86.0 ± 26.0 U/l, ASAT = 93.5 ± 44.3 U/l, HbA1c = 9.3 ± 1.8% **NAS = 5 ($N = 8$):** ccK18 = 704.0 ± 411.7 U/l, ALAT = 50.0 ± 17.8 U/l, ASAT = 59.0 ± 16.3 U/l, HbA1c = 9.8 ± 1.6% **NAS = 6 ($N = 7$):** ccK18 = 836.7 ± 632.3 U/l, ALAT = 43.1 ± 17.7 U/l, ASAT = 56.1 ± 29.5 U/l, HbA1c = 9.2 ± 1.4% **NAS = 7 ($N = 1$):** ccK18 = 820.7 U/l, ALAT = 159 U/l, ASAT = 181 U/l, HbA1c = 10.4%
Conclusion: ccK18 might be useful tool to identify further patients being at risk for liver damage even after normalization of ALAT as results of liver biopsies indicate an increase in ccK18 with higher NAFLD-Score. CcK18 identifies further patients being at risk of liver disease presenting with ALAT-values within normal ranges. Dynamic course of ccK18 allows procedural observation of cellular liver regeneration after Intervention.
Supported by: unrestricted financial and material grant from TECOmedical AG, Buende
Disclosure: **M. Hauber:** Grants; supported by an unrestricted financial and material grant from TECOmedical AG, Buende, Germany.

1192

Frequency of fibrosis estimated by noninvasive scores in type 2 diabetic patients with nonalcoholic fatty liver disease

M. García Domínguez[1], I. Martin Timon[2], I. Moreno Ruiz[2], B. Ugalde Abiega[2], D. Varillas Delgado[3], F.J. del Cañizo Gómez[2];
[1]Sureste University Hospital, Madrid, [2]Infanta Leonor University Hospital, Madrid, [3]Francisco de Vitoria University, Madrid, Spain.

Background and aims: The prevalence of nonalcoholic fatty liver disease (NAFLD) is >70% in type 2 diabetes mellitus (T2DM). These patients are particularly susceptible to more severe forms of NAFLD, progression to esteatohepatitis and development of hepatocellular carcinoma. Moreover, the coexistence of NAFLD and T2DM results in worse metabolic profile and higher cardiovascular risk. Our aims were: 1) to study frequency of abnormal aminotransferase (AT) levels and fibrosis [by NAFLD Fibrosis Score (NFS) and Fibrosis 4 Calculator (FIB4)]; 2) to investigate variables associated with higher score, excluding those directly included in formulae.
Materials and methods: Patients with T2DM and NAFLD diagnosed by ultrasound attending our clinic were identified consulting diagnosis database in I. Leonor Hospital.
Results: 66 subjects; mean ± SD: 64.6 ± 13.9 y; 47% women; BMI 31.1 ± 6.8 kg/m^2; HbA1c 6.9 ± 1.1%. 77.3% had high AT. NFS score: −0.31 ± 1.30. 83.3% had intermediate or high probability of fibrosis (NFS ≥−1.5). FIB4 1.40 ± 0.70. 4.5% had FIB4 >3.25, meaning high probability of advanced fibrosis. Subset with normal AT: 84.3% had NFS ≥−1.5. No patients had FIB4 >3.25; and 43.1% had FIB4 1.45–3.25. Subset with high AT: 80% had NFS ≥−1.5. 20% had FIB4>3.25, and 13.3% had FIB4 1.45–3.25. Patients with high AT had lower IMC than those with normal AT (31.0 ± 5.0 vs 34.2 ± 6.9; $p = 0.05$); whereas glycemic control was worse in high-AT patients (A1c 7.6 ± 1.5 vs 6.7 ± 0.9; $p = 0.008$). Patients with hypercholesterolemia had higher NFS (−0.21 ± 1.31 vs −1.313 ± 0.69; $p < 0.05$). There was no significant association with BMI, fasting glucose, A1c, HDL, LDL, TG. Morbid obesity was associated with lower FIB4 (0.61 ± 0.15; grade 1 obesity 1.26 ± 0.65; overweight 1.62 ± 0.60; $p < 0.05$. This was explained because in our sample, older people had significantly lower BMI. Glycemic control was significantly worse in patients with higher FIB4 (low FIB4: mean A1c 7.1%; medium FIB 4: 6.5%; high FIB4: 8.5%; $p < 0.05$).
Conclusion: Our results confirm that patients with T2DM and NAFLD have high probability of fibrosis (estimated by noninvasive scores). The scores are as high in patients with normal AT levels as in normal-AT ones. NAFLD must be actively sought by ultrasound in every T2DM patient.
Disclosure: **M. García Domínguez:** None.

1193

Glutamate dehydrogenase and the hyperammonaemia in HI/HA syndrome: study on the contribution by the liver

K. Luczkowska, P. Maechler;
Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland.

Background and aims: The hyperinsulinism/hyperammonemia syndrome (HI/HA) is the second most common cause of congenital hyperinsulinism. Clinically, the phenotype shows severe hypoglycemia with neonatal and early infancy-onset, accompanied by elevated plasma ammonia levels. It is a rare genetic disease caused by gain-of-function mutations in *GLUD1*, a gene encoding for the enzyme glutamate dehydrogenase (GDH). Mammalian GDH is catalysing the reversible reaction of glutamate to alpha ketoglutarate plus ammonia. HI/HA syndrome gives rise to increased (3–5 times) plasma ammonia levels, presumably due to systemic expression of mutant GDH. This study aims to elucidate the contribution of the liver in the elevated circulating ammonia. Hepatocytes emerged as a main candidate playing a central role in amino acid catabolism and ammonia detoxification through urea cycle.
Materials and methods: To recapitulate the genetic background of the patients suffering from HI/HA (mostly heterozygous) we transduced primary mouse hepatocytes with adenoviruses carrying either human wild type hGDHwt or mutant $hGDH_{S445L}$ expressed along with the endogenous GDH. Mouse hepatocytes were isolated by liberase digestion and cultured in 6-well plates. After adenoviral transduction, hepatocytes were further cultured for 48 h. Then, cells were starved for 6 h and stimulated for 1 h with glucose-free KRBH containing either no substrate (non-

stimulated control) or 5 mM glutamine alone or combined with 5 mM alanine (mix). The supernatants were collected, ammonia and urea measurements were performed.

Results: The efficiency of transductions was controlled by immunoblotting in all the investigated conditions showing similar expression of the transgenes. Both alanine and glutamine are important substrates for gluconeogenesis. The ammonia production from glutamine is contributed by both deamidation by glutaminase, producing glutamate, and then its deamination by GDH generating alpha ketoglutarate. However, alanine solely relies on GDH for ammonia production. Upon amino acid challenge, both wild type and mutant hepatocytes increased ammonia production in comparison to endogenous GDH. When stimulated with the mix, ammonia generation was higher in the hepatocytes expressing $hGDH_{S445L}$ *vs.* hGDHwt (107 ± 8 *vs.* 88 ± 9 nmol/h per 10^6 cells, $n = 12$, $p < 0.0001$). In the liver, GDH provides ammonia to the first enzyme of the urea cycle, namely carbamoyl phosphate synthetase. Upon the mix challenge, the $hGDH_{S445L}$ higher activity was reflected by the increased urea production (334 ± 33 *vs.* 297 ± 32 nmol/h per 10^6 cells, $n = 12$, $p < 0.05$). Overall, the differences between $hGDH_{S445L}$ *vs.* hGDHwt were more pronounced in ammonia production hinting limited capacity of the urea cycle to compensate for pathological ammonia generation. In ongoing experiments, human wild type and mutant GDH are expressed by adenoviral intravenous delivery to mice before assessment of *in vivo* ammonia generation. Preliminary data show efficient expression of the respective transgenes in the liver.

Conclusion: Hepatocytes carrying mutant GDH produced significantly more ammonia upon amino acid exposure, which underscores hyperammonemia present in patients suffering from HI/HA syndrome.

Supported by: SNSF

Disclosure: **K. Luczkowska:** None.

1194

Plasmatic expression of miR-34a in non alcoholic fatty liver disease in a Mexican population

M. Velazquez[1], M.L. Lazo de la Vega[1], Y. Ruiz[1], L. Ibarra[1], M. Preciado[2], B. Jordan[3], S. Garnelo[3];
[1]Department of Medical Sciences, University of Guanajuato, Leon, Guanajuato, [2]Department of Medicine and Nutrition, University of Guanajuato, Leon, Guanajuato, [3]General surgery, Regional Genral Hospital of Leon, Leon, Guanajuato, Mexico.

Background and aims: Non alcoholic fatty liver disease (NAFLD) is caused by excessive accumulation of fatty acids (steatosis) in form of triglycerides in the liver, and it is one of the main causes of death by liver diseases in the world. There are several diagnostic methods for NAFLD. However, most of them are not sufficiently sensitive nor specific, and liver biopsy, the current gold standard, is invasive. Consequently, several molecules such as microRNAs (miRNAs) have been studied as potential biomarkers for diagnosing NAFLD. miRNAs participate in the post-transcriptional regulation of several biological processes, and can be found in most biological fluids. miR-34a participates in lipid metabolism, and it has been found to be upregulated in NAFLD. However, its expression appears to be dependent on the NAFLD studied population, and the diagnostic method used, with contradictory results between the studies. Prevalence of NAFLD has increased in the Mexican population, and there has been an increase in the incidence of the disease by 60%. The aim of the present study is to analyze whether plasmatic expression levels of miR-34a are associated with the presence or absence of NAFLD in a Mexican population.

Materials and methods: Seventy one subjects were recruited and classified in two groups: 30 subjects with NAFLD (NAFLD group) and 41 without NAFLD (Control group), diagnosed by histopathology. Anthropometric measurements were obtained: weight, height, body mass index (BMI), diastolic and systolic pressure, waist, hip, waist-hip ratio. Plasma and serum was used for lipid profile determination: total cholesterol (TC), triglycerides (TG), LDL, HDL, VLDL), and hepatic function tests: ALT, AST, GGT. Total RNA was isolated from plasma, using a spike-in of *C. elegans* miR-39 as extraction control. A histopathological analysis (Kleiner's index) was performed in which the diagnosis and degree of severity of NAFLD was subclassified by scoring from 0 to 6, taking 6 as the highest severity. Expression of miR-34a was determined by real time PCR using a specific Taqman MicroRNA Assay. Student's T test and U-Mann-Withney were used to analyze biochemical and anthropometric variables as well as for the comparison of miR-34a plasmatic expression between NAFLD group and control group.

Results: miR-34a expression was found five-fold higher in NAFLD compared to control group ($p = 0.009$).Similarly, when subclassifying by the degree of severity of the NASH, significant differences were observed between subgroup 3 *vs* subgroup 0 ($p = 0.009$) and *vs* group 2 ($p = 0.009$); between subgroup 4 *vs* 0 ($p = 0.03$) and *vs* subgroup 2 ($p = 0.03$); and between subgroup 4 *vs* 0 and *vs* subgroup 2. Differences were obtained when comparing NAFLD group *vs* control group in BMI ($p = 0.010$), diastolic pressure ($p = 0.047$), glucose ($p = 0.018$), TC ($p = 0.000$), LDL ($p = 0.044$), VLDL ($p = 0.047$), TG ($p = 0.002$), ALT ($p = 0.000$), AST (0.000) y GGT ($p = 0.012$).

Conclusion: In addition to the metabolic alterations demonstrated in the comparison of both groups, the expression levels of miR-34a were elevated in NAFLD group. This result supports the potential use of miR-34a as NAFLD biomarker in our population. More research must be done in order to elucidate the role of miR-34a in the molecular mechanisms of NAFLD.

Supported by: Secretariat for Innovation, Science and Higher Education

Disclosure: **M. Velazquez:** Non-financial support; No.

1195

The combination of Barberis Aristata, Elaeis Guineensis and Coffea Canephora extracts ameliorates the metabolic profile and hepatic miR-122 levels in a mouse model of NAFLD

C. Nigro[1], P. Mirra[1], V. Lembo[2], G. Mazzone[2], A. Rossi[2], G. D'Argenio[2], V. Cossiga[2], A. Leone[1], F. Beguinot[1], N. Caporaso[2], C. Miele[1], F. Morisco[2];
[1]URT-GDD of the IEOS-CNR & DiSMeT, University of Naples "Federico II", Naples, [2]Gastroenterology Unit, Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy.

Background and aims: Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disease affecting a large part of the world population. It is well documented that NAFLD is associated to metabolic syndrome. NAFLD and metabolic syndrome seem to have insulin resistance as a common pathophysiological mechanism. Recently, a number of studies have described microRNAs (miRNAs) as mediators in the pathogenesis of both insulin resistance and NAFLD. Therefore, the aim of this study is to evaluate whether and how a mixture of plant extracts consisting of Berberis Aristata, Elaeis Guineensis and Coffea Canephora may affect the development of obesity, hepatic steatosis and insulin resistance, conceivably associated to the modulation of miRNAs levels, in an animal model of NAFLD induced by a high fat diet.

Materials and methods: Three groups of C57BL/6 mice ($n = 8$ each) were randomized into one of the following 24 week diets: 1) standard diet (SD); 2) high fat diet (HFD, 60% fat); 3) HFD enriched with plant extract (HFD+E) (140 mg/Kg/die). Body weight was monitored during the diet protocol. At the end of the treatment all mice were fasted for 4 hours and intraperitoneally injected with a bolus of insulin (0.75 U/kg) to test the insulin tolerance by ipITT. Blood samples were used to measure glycaemia by a portable glucometer, insulinemia by ELISA assay, transaminases and lipid profile by a clinical chemistry benchtop analyzer. After sacrifice, liver biopsies were collected to examine the hepatic histology by H&E staining and to isolate total RNA and measure miRNA expression by q-PCR.

Results: The HFD+E mice show lower body weight (40 ± 3.6 vs 47.4 ± 6 g, HFD+E vs HFD, $p = 0.008$), the amelioration of insulin sensitivity (10138 ± 1620 vs 7529 ± 1469 mg/dl*120 min, iAUC of HFD+E vs HFD, $p = 0.008$) together with the reduction of insulinemia (725.8 ± 270 vs 1156.3 ± 307.4 pg/ml, HFD+E vs HFD, $p = 0.04$), total cholesterol (178 ± 17 vs 209 ± 19 mg/dl, HFD+E vs HFD, $p = 0.007$), low density lipoproteins (10.7 ± 3.2 vs 19.3 ± 1.6 mg/dl, HFD+E vs HFD, $p < 0.001$), triglycerides (86.3 ± 11 vs 130.3 ± 22 mg/dl, HFD+E vs HFD, $p < 0.001$) and alanine-aminotrasferase (38.7 ± 24 vs 81.3 ± 47 mg/dl, HFD+E vs HFD, $p = 0.03$) compared to HFD mice. H&E staining show macro- and microvesicular steatosis with ballooning degeneration in mice fed with HFD, which is improved in mice fed with HFD+E. Moreover, a down-regulation of miR-122 hepatic levels is observed in HFD mice compared to SD mice (0.95 ± 0.6 vs 2.17 ± 1.5 REU, HFD vs SD, $p = 0.03$). Interestingly, this effect on the miRNA levels is prevented by the combination of plant extracts with HFD (2.13 ± 1.39 vs 0.95 ± 0.6 REU, HFD+E vs HFD, $p = 0.03$).
Conclusion: In conclusion, the combination of Berberis Aristata, Elaeis Guineensis and Coffea Canephora added to HFD is able to ameliorate obesity, insulin resistance and hepatic steatosis, which are developed by C57BL/6 mice fed with a HFD. Moreover, this metabolic profile associates to the modulation of hepatic miR-122 levels, which are down-regulated in HFD mice and remain unchanged in mice fed with HFD+E compared to SD mice. These data suggest a possible role of miR-122 in the beneficial effects of plant extracts combination on metabolic syndrome.
Disclosure: **C. Nigro:** None.

1196

The characteristics of non-alcoholic fatty liver disease related hepatocellular carcinoma in a Chinese population: a retrospective study
H. Chen[1], X. Li[1], X. Gao[1,2];
[1]Zhongshan Hospital, Fudan University, Shanghai, [2]Institute of chronic metabolic disease, Fudan University, Shanghai, China.

Background and aims: The prevalence of hepatocellular carcinoma (HCC) among patients with non-alcoholic fatty liver disease (NAFLD) is increasing in recent years. The aim of this retrospective study was to evaluate the incidence and characteristics of patients with NAFLD-HCC compared with viral hepatitis B (HBV)-HCC in a Chinese population.
Materials and methods: Patients with newly diagnosed HCC at liver surgery department Zhongshan hospital from January 2011 to December 2015 were investigated in this study. They were divided into either HBV or NAFLD group based on underlying etiologies. Patients with other HCC etiologies, alcohol consumption history, or missing data were excluded. Demographic information, laboratory results, metabolic data and tumor behavior were assessed.
Results: Total 6402 patients were finally included in this study. The ratio of NAFLD related HCC were annually growing from 2011 (3.8%) to 2015 (6.4%). Male gender accounted for the majority part in both group. Patients with NAFLD-HCC were usually diagnosed at older age (63.7 ± 10.4 years) compared to HBV-HCC (53.6 ± 10.5 years, $p < 0.01$). Patients with NAFLD-HCC were observed as having significant higher BMI (24.6 ± 3.26 kg/m^2 in NAFLD-HCC vs. 23.3 ± 2.98 kg/m^2 in HBV-HCC, $p < 0.01$), total cholesterol (4.5 ± 0.97 mmol/L vs. 4.12 ± 0.92 mmol/L), triglycerides (1.69 ± 1.2 mmol/L vs. 1.09 ± 0.56 mmol/L), and HDL-C (1.13 ± 0.34 mmol/L vs. 1.2 ± 0.37 mmol/L). While fasting plasma glucose seemed to be no significant difference. As a key biomarker, alpha-fetoprotein was significantly higher in HBV group. The ratio of cirrhosis was a little higher in HBV-HCC (59.8% in HBV-HCC vs. 45.0% in NAFLD-HCC). Nodule size was larger in NAFLD-HCC (5.32 ± 0.14 cm vs. 4.61 ± 0.05 cm, $p < 0.01$).
Conclusion: This study indicates that the annual incidence rate of NAFLD related HCC is steady growing. These patients should be monitored carefully due to elder age at diagnosis, disorders of lipid metabolism, and worse tumor behavior.
Disclosure: **H. Chen:** None.

PS 116 Clinical aspects of NAFLD

1197

Influence of gender on progression of liver metabolic diseases in response to different nutritional challenges
S. Smati[1,2], A. Polizzi[1], M. Régnier[1], A. Marrot[1], C. Luzowicz[1], S. Ellero-Simatos[1], F. Lasserre[1], N. Loiseau[1], T. Al Saati[3], A. Montagner[2], P. Gourdy[2], H. Guillou[1];
[1]INRA, Toulouse, [2]INSERM, Toulouse, France, [3]Inserm, Toulouse, France.

Background and aims: Non Alcoholic Fatty Liver Disease (NAFLD) currently affects 30% of the population and is a pathology for which there is sexual dimorphism. Indeed, several epidemiological studies show protection against NAFLD in premenopausal women. The establishment of reliable animal models is essential for the study of this protection. The aim of our study was to evaluate the influence of gender in different mice models of steatosis and NASH and to identify a model that replicates at best the sexual dimorphism observed in humans.
Materials and methods: One hundred and twenty males and females C57BL/6J received different hypercaloric diets: High Fat Diet (HFD), Choline Deficient (CDHFD), enriched with cholesterol (Western Diet) and co-administered with drinking water containing glucose and fructose (Western Diet & sugar). These diets were given ad libitum for 15 weeks ($n = 12$ per group). The mice were sacrificed at the end of the follow-up, at the age of 6 months. This study was conducted under the EU guidelines for the use and care of laboratory animals and was approved by an independent ethics committee.
Results: The cholesterol enriched Western Diet (4.5 kcal/gram, lipids 42%, carbohydrates 42.7%, proteins 15.2%, cholesterol 0.2%) induces a strongly dimorphic phenotype for the onset of the NASH. Males have a major steatosis associated with severe inflammation and fibrosis. Females show much less steatosis (intrahepatic triglycerides measured at 305 versus 893 μg/mg in males, $p < 0.0001$). Both sexes develop obesity and have impaired glucose tolerance. In contrast, insulin resistance is more severe in males than in females (respectively HOMA-IR at 51.0 versus 13.1, $p = 0.0041$). Cytolysis in males is significantly higher than in females (ALAT at 115 versus 51 U/L, $p = 0.002$). Finally, the transcriptomic approach shows that the transcriptional responses induced by the diet are very contrasted between males and females.
Conclusion: The Western Diet induces NASH associated with more fibrosis only in males. These results suggest a possible effect of dietary cholesterol in the etiology of NASH. Obtaining this phenotype will allow to study the mechanisms involved in the protection of females and in particular the role of estrogen.
Disclosure: **S. Smati:** None.

1198

Neck circumference to height ratio is a reliable predictor of liver stiffness and nonalcoholic fatty liver disease in prediabetes
D. Dutta[1], M. Kumar[2], S.A. Mondal[3], S. Mukhopadhyay[2];
[1]Diabetes, Endocrinology & Metabolism, Venkateshwar Hospital, Dwarka, New Delhi, New Delhi, [2]Endocrinology & Metabolism, IPGMER & SSKM Hospital, Kolkata, [3]Biochemistry, IPGMER & SSKM Hospital, Kolkata, India.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) and dysglycemia are public health challenges. There is urgent need for anthropometric surrogates for NAFLD screening. This study evaluated role of neck circumference (NC) and neck-height ratio (NHtR) as predictors of liver stiffness measure (LSM) in individuals with prediabetes (IPD).
Materials and methods: In a cross-sectional study, 188 IPD from 1130 screened individuals underwent anthropometry, ultrasonography,

Fibroscan® for liver stiffness (LSM), dyslipidemia, insulin resistance (IR) and fetuin-A assessment.
Results: Hypertension, hypertriglyceridemia, low HDL-C, metabolic syndrome (MetS), NAFLD and significant liver stiffness (SLS) (LSM >8.5 kPa) were observed in 53.7%, 31.4%, 71.3%, 73.9%, 24.5% and 11.2% prediabetes individuals respectively. Of the 46-prediabetes individuals with NAFLD, 21 had significant liver stiffness on fibroscan. Prediabetes with NAFLD had significantly higher BMI, NC, NHtR, glycated haemoglobin, triglycerides, fatty liver index (FLI) and LSM. The median [25th–75th percentile] for NC was 36.5 cm [36–38] in males and 34 cm [32–36.75] in females ($P < 0.001$); the corresponding NHtR was 22.09 [21.32–22.96] and 21.70 [21.03–23.62] cm/m respectively ($P = 0.272$). Prediabetes in highest NHtR quartile had significantly higher BMI, hypertension, MetS, fasting glucose, glycated hemoglobin, HOMA-IR, NAFLD, LSM, SLS, and lower HDL-C. Stepwise forward linear regression revealed that NHtR, FLI and LDL-C were best predictors of LSM, at baseline (Model-1), after adjusting for age and sex (Model-2) and adjusting model-2 plus systolic and diastolic blood pressure (Model-3). Logistic regression, using presence of significant liver stiffness (LSM >8.5 kPa) as the dependent variable, showed relationship between NHtR and liver stiffness after adjusting for sex was statistically significant (odds ratio 1.421 [95% CI 1.111 −1.817]; $P = 0.005$). A similar logistic regression showed relation between NC and liver stiffness approached statistical significance (odds ratio 1.158 [95% CI 0.997–1.345]; $P = 0.056$). NHtR and NC (in females), and NHtR and BMI (in males) had largest AUCs for predicting LSM, NAFLD and MetS. NHtR (AUC: 0.816; $P = 0.001$) and NC (AUC: 0.744; $P = 0.014$) were best predictors of liver fibrosis in females. BMI (AUC: 0.695; $P = 0.025$) and NHtR (AUC: 0.619; $P = 0.061$) were the best predictor of liver fibrosis in males. NHtR ≥21.54 cm/m (sensitivity: 90%; specificity: 52.5%; females) and ≥21.62 cm/m (sensitivity: 80%; specificity: 49.4%; males) was best predictor of SLS. Nine out of the 10 females with SLS in this study had NHtR ≥21.54 cm/m. Nine out of 11 males with SLS in this study had NHtR of 21.62 cm/m. The occurrence of NAFLD in quartile-1 ($n = 47$) and quartile-4 ($n = 44$) of NC was 14.89% and 43.18% respectively. This evaluation achieved a power of 98%, keeping α (Type I error) at 0.05.
Conclusion: NHtR can be a good screening tool [good sensitivity (80–90%) with a relatively poor specificity (around 50%)] for detecting liver stiffness in prediabetes. The high sensitivity ensures we will not miss cases in community during screening. Patients thus detected can undergo confirmatory tests for NAFLD.
Supported by: DST WB INDIA
Disclosure: **D. Dutta:** None.

1199

Cardiorespiratory fitness is associated with markers of hepatic steatosis in patients with type 2 diabetes

J. Sokolovska[1], L. Sviklāne[1], K. Ostrovska[2], A. Kļaviņa[2], J. Stefanovičs[1], L. Selavo[1];
[1]University of Latvia, Riga, [2]Latvian Academy of Sport Education, Riga, Latvia.

Background and aims: several studies have shown that physical activity is effective for reduction of liver fat in patients with insulin resistance and type 2 diabetes. However, few studies address the question whether level of cardiorespiratory fitness (CF) is associated with the risk of liver steatosis. Fatty liver index (FLI), hepatic steatosis index (HSI), nonalcoholic fatty liver disease liver fat score (NAFLD-LFS) have been validated for evaluation of risk of hepatic steatosis in healthy subjects and patients with type 2 diabetes. The aim of this work was to study differences in the levels of hepatic steatosis markers FLI, HSI and NAFLD-LFS between groups of type 2 diabetic patients with different level CF.
Materials and methods: 63 previously untrained patients with type 2 diabetes aged 35–75 have been enrolled. The patients have been divided in two groups according to relative VO2 max status (low CF group: VO2 max males <29 ml/min/kg, females <25 ml/min/kg; high CF group: VO2max males ≥29 ml/kg/min, females ≥25 ml/min/kg). FLI, HSI, NAFLD-LFS were calculated by formulas, which are based on clinical features and blood biochemistry as previously published. Leisure time physical activity of participants was evaluated via Minnesota leisure time activity questionnaire and expressed in metabolic equivalent (MET).
Results: Clinical characteristics of the group were: mean age 58.6 ± 9.5 years, mean duration of diabetes 8.0 ± 9.7 years, mean BMI 33.5 ± 5.5 kg/m^2, mean HbA1c 6.9 ± 1.3%, mean FLI 79.8 ± 25.9, mean HSI 37.7 ± 5.6, mean NAFLD-LFS 1.73 ± 1.8, mean VO2 max 24.0 ± 95.1 ml/kg/min, mean leisure time physical activity 33.7 ± 25.2 (corresponds to moderate activity). HSI, FLI and NAFLD-LFS were higher in patients with low CF (FLI: Low CF group 82.0 ± 21.3 versus high CF group 66.5 ± 31.1, $p = 0.023$; HSI: Low CF group 39.1 ± 5.1 versus high CF group 34.9 ± 5.4, $p = 0.004$; NAFLD-LFS: Low CF group 2.2 ± 1.7 versus high CF group 0.9 ± 1.8, $p = 0.009$). Serum insulin concentration was higher in low CF group compared to high CF group (versus 11.7 ± 8.5 μV/ml versus 6.3 ± 8.6 μV/ml ($p = 0.045$). However, we did not observe differences between VO2 max groups in HbA1c, duration of diabetes, MET, microalbuminuria as well as cytokines associated with risk of diabetic complications (VEGF-A, Angiopoietin-2, MMP7, MMP2). There was a significant correlation between VO2 max and indices (VO2max and FLI $p = 0.029$, VO2max and HSI $p = 0.000$, VO2max and NAFLD-LFS $p = 0.005$) as well as insulin ($p = 0.012$) and waist ($p = 0.004$).
Conclusion: markers of hepatic steatosis HSI, FLI and NAFLD-LFS are associated with cardiorespiratory fitness in previously untrained subjects with type 2 diabetes. These results provide new data on association between hepatic steatosis and cardiac complications of diabetes
Supported by: MikroTik Donation administered by Foundation of University of Latvia
Disclosure: **J. Sokolovska:** Grants; Foundatoin of University of Latvia administering MikroTik donation.

1200

Association of serum vitamin D and insulin resistance with noninvasive markers among prediabetic individuals with nonalcoholic fatty liver disease

R. Zinnat, I.A. Hossain, L. Ali;
Biochemistry & Cell Biology, Bangladesh University of Health Sciences, Dhaka, Bangladesh.

Background and aims: Prediabetes and nonalcoholic fatty liver disease (NAFLD) both are independently known to be an insulin resistant condition. A number of noninvasive markers have been evaluated for the diagnosis of NAFLD where vitamin D deficiency due to impaired insulin action is a hallmark feature of the disorder. Data examining the association of serum vitamin D and insulin resistance with noninvasive markers are scare. In this context, the present study was assessed to investigate the association of serum vitamin D with noninvasive markers and to explore whether this association is mediated by insulin resistance among prediabetic individuals.
Materials and methods: We studied 277 prediabetic subjects (M/F, 167/110; age in years, 39.7 ± 9.3; BMI in kg/m^2, 25.4 ± 3.9; M ± SD) confirmed by OGTT following WHO Group Study criteria. NAFLD was diagnosed by upper abdomen ultrasonography comprising into 192 non NAFLD (120/72; 38.3 ± 8.9; 24.3 ± 4.1) and 85 NAFLD (47/38; 39.1 ± 9.6; 26.5 ± 3.3) groups. The noninvasive markers include the fatty liver index (FLI), hepatic steatosis index (HSI), NAFLD fibrosis score (NFS), fibrosis4 (FIB4) score, BARD score and BAAT score respectively. Serum insulin and vitamin D levels reflecting 25 hydroxyvitamin D [25(OH)D] were measured by ELISA techniques. Insulin resistance (HOMA-IR) was calculated by homeostasis model assessment (HOMA).

Results: In NAFLD subjects, the FLI index (>60), HSI (>36), NFS (−1.455–0.675), FIB4 index (>2.67), BARD score (2–4 points) and BAAT score (4 risk parameters) was 46.2% (66), 95.1% (136), 6.3% (9), 9.1% (13), 21% (30) and 40.6% (58) respectively. Compared to non NAFLD subjects, NAFLD subjects had significantly lower levels of [25(OH)D] (26.58 ± 6.46 vs. 35.89 ± 14.27 nmol/L, $p < 0.001$) while, significantly higher levels of HOMA-IR (2.37 ± 1.24 vs. 1.75 ± 0.55, $p < 0.001$). Pearson's correlation analysis showed a significant negative correlation of [25(OH)D] with HOMA-IR ($r = -0.289$, $p = 0.032$), fasting serum insulin ($r = -0.278$, p=0.046), postprandial serum insulin ($r = -0.210$, $p = 0.048$), FLI ($r = -0..342$, $p < 0.001$), HSI ($r = -0.214$, $p = 0.022$) and BAAT score ($r = -0.272$, $p = 0.026$) respectively in NAFLD subjects. Multiple linear regression analysis showed a significant negative association of HOMA-IR with [25(OH)D] ($\beta = -0.299$, $p = 0.035$) as well as significant positive association with FLI ($\beta = 0.525$, $p = 0.025$), BAAT score ($\beta = 0.279$, $p = 0.047$), NFS ($\beta = 0.458$, $p = 0.013$) and FIB4 score ($\beta = 0.396$, $p = 0.048$) after adjusting the effects of confounding variables of BMI and triglyceride respectively. On binary logistic regression analysis, [25(OH)D] (OR = 0.870, 95% CI: 0.821–0.921, $p < 0.001$), HOMA-IR (OR = 1.822, 95% CI: 0.901–3.684, $p = 0.034$), FLI (OR = 1.049, 95% CI: 1.007–1.094, $p = 0.023$), BAAT score (OR = 0.480, 95% CI: 0.242–0.951, $p = 0.035$) and FIB4 score (OR = 6.638, 95% CI: 1.322–33.321, $p = 0.021$) were found to be significant determinants of NAFLD when adjusted the effects of major confounders of BMI, triglyceride, HSI, BARD score and NFS respectively.

Conclusion: NAFLD subjects seem to have an association with [25(OH)D] deficiency and noninvasive markers and this relationship is mediated by insulin resistance which is considered the pathophysiological determinant of prediabetes.

Clinical Trial Registration Number: BADAS-ERC/13/00106

Supported by: Bangladesh Medical Research Council and NST (R&D)

Disclosure: **R. Zinnat:** None.

1201

Gamma-glutamyltransferase levels reflect glycaemic status in type 2 diabetic people treated with an SGLT2 inhibitor tofogliflozin

T. Takamura[1], K. Kaku[2], A. Yoshida[3], H. Kusakabe[3], H. Suganami[4]; [1]Department of Endocrinology and Metabolism, Kanazawa University Graduate School of Medical Sciences, Kanazawa, [2]Department of Internal Medicine, Kawasaki Medical School, kurashiki, [3]Medical Information and Product Advancement Department, Kowa Pharmaceutical Co., Ltd., Tokyo, [4]Clinical Data Science Department, Kowa Pharmaceutical Co., Ltd., Tokyo, Japan.

Background and aims: The circulating levels of gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) are used clinically as indices of fatty liver diseases and predictors of type 2 diabetes (T2D). However, contributions of hyperglycemia and obesity to levels of GGT and ALT have not been demonstrated clinically. Thus, we examined relationships between various clinical parameters and GGT and ALT levels during intervention for hyperglycemia and obesity with the SGLT2 inhibitor tofogliflozin in people with T2D.

Materials and methods: The pooled analysis included four tofogliflozin phase 2 and 3 trials (Supplemental Table 1), with durations of at least 24 weeks, that included 1046 participants with T2D who received either tofogliflozin (10, 20, or 40 mg) or placebo, either as a monotherapy or as an add-on to other antidiabetic agents. Differences in various parameters between tofogliflozin and placebo groups were analyzed by ANCOVA. Clinical parameters related to baseline and percent changes in hepatic enzymes were clarified using multivariate analysis.

Results: Study-group baseline characteristics were 67% men; mean age, 58 years; HbA1c, 8.1%; BMI, 26 kg/m^2; GGT, 48 IU/L; and ALT, 29 IU/L. In multivariate analyses, baseline GGT levels were positively correlated with FPG levels, but not body mass index (BMI). ALT levels were positively correlated with both HbA1c levels and BMI, but not FPG. Decreases in GGT and ALT levels during tofogliflozin therapy were distinct. GGT levels rapidly and significantly decreased as early as week 4 and plateaued after week 8, which was maintained until week 24, with a similar decrease in FPG. Conversely, ALT levels decreased gradually for 24 weeks, without a plateau, with a similar decrease in body weight. At weeks 4 and 24, overall GGT level reduction was significantly correlated with FPG and body weight reduction, whereas overall ALT reduction was correlated only with body weight reduction. Because the patterns of the decreases in GGT and ALT levels were distinct at the early and late phases; we analyzed the factors influencing the reduction in GGT and ALT levels individually at week 4 ($\Delta_{0\text{-}4}$) and week 24 ($\Delta_{0\text{-}24}$) by using a multivariate stepwise method. At both week 4 and 24, the overall reduction in GGT was significantly correlated with the reductions in FPG and body weight, whereas the overall reduction in ALT was correlated only with the reduction in body weight, and not FPG.

Conclusion: Our results indicate that GGT and ALT levels reflect two distinct pathologies of T2D, glycemic status and obesity, respectively, which may help understand mechanisms underlying liver enzyme elevation and establish GGT and ALT as surrogate markers for hyperglycemia and obesity, respectively, in clinical and epidemiological studies.

Disclosure: **T. Takamura:** Grants; Kowa.

1202

Hepatocellular carcinoma as a independent risk factor for post transplant diabetes in liver recipients

S. M. Bernardes[1], T. Gonçalez Bovi[2], F. Colitti Lemos[1], C. Minatel Righetto[2], A. Russo Fiore[2], L. Teixeira Lot[3], A. Moura Neto[2], I. de Fátima Ferreira Santana Boin[3]; [1]São Leopoldo Mandic Faculty of Medicine, Campinas, São Paulo, Brazil, Campinas, [2]Discipline of Endocrinology, Internal Medicine Department, Faculty of Medical Sciences, University of Campinas, Campinas, São Paulo, Brazil, Campinas, [3]Department of Surgery, Faculty of Medical Sciences, University of Campinas, Campinas, São Paulo, Brazil, Campinas, Brazil.

Background and aims: Non-alcoholic steatohepatitis (NASH), a condition tightly related to type 2 diabetes mellitus, is becoming increasingly common as a cause for end stage liverdisease and hepatocellular carcinoma (HCC). There are few data related specifically to theidentification of risk factors for post transplant Diabetes Mellitus (PTDM) in liverrecipients. The aims of this study were to investigate the risk factors for PTDM and therelation of HCC and PTDM in patients submitted to liver transplantation.

Materials and methods: Revision of medical records of adult patients submitted to liver transplantation. A total of 152 consecutive patients seen at the post transplantation clinic from February to June 2017 were included.

Results: Of all patients, 76.5% were men. The median (interquartile range) age was 59 (52.7–65) years, transplant time 6 (2.3–9) years, BMI 27.5 (24.4–29.8) kg/m^2 and diagnosis of DMPT was made at a median of 2 (1–6) years after thetransplant. Among the 152 patients, 43.2% had diagnosis of DM and 27% were classifiedas PTDM. As for comorbidities, 48.3% presented hypertension, 32.2%hypercholesterolemia and 31.5% hypertriglyceridemia. The major cause oftransplantation was hepatitis C virus (55%), followed by alcohol abuse (32.9%). Additionally, 40.3% were diagnosed with HCC before liver transplantation. The mostcommonly used immunosuppressants after liver transplantation were tacrolimus (78.4%), mycophenolate (62.8%), everolimus (24.5%) and prednisone (PDN) (23.8%).Comparisons using the Chi-Square test showed a predominance of males in patients withPTDM compared to those without PTDM (87.5% vs 72.2%, $p = 0.05$) and higher a frequencyof HepC (72.5% vs 42.1%, $p = 0.001$) and of tacrolimus use (92.5% vs 72.9%, $p = 0.01$) inthose with PTDM. There was no significant difference in rejection occurrence. The independent risk factors for PTDM according to multivariate logistic regression were HepC (OR =

3.8, 95% CI 1.3–10.9, $p = 0.013$), presence of HCC (OR = 3.7, 95% CI, 1.2–11 5, $p = 0.025$), the use of tacrolimus (OR = 6.3, 95% CI 1.2–32.5, $p = 0.029$) and PDN (OR = 7.2, 95% CI 2.4–21, 8, $p < 0.001$).

Conclusion: HCC is an independent risk factor for PTDM inliver recipients. Patients with NASH prior to liver transplantation could be more predisposed to both HCC and DM, thus possibly explaining this association. Patients with HepC and those exposed to tacrolimus and prednisone are other groups of high risk for PTDM. Liver recipients with history of HepC and/or HCC should be carefully evaluated for PTDM and more diabetogenic drugs avoided in these groups of patients, if possible.

	Without DM or Pre transplantation	PTDM	p
MALE SEX	72,20%	87,50%	0,05
HCC	41,70%	35,00%	0,571
HCC ALCOHOL	15,70%	22,50%	0,34
FHDM	54,50%	66,70%	0,68
ALCOHOLISM	31,50%	43,20%	0,231
SMOKING	38,10%	25,00%	0,363
HYPERTENSION	52,80%	37,50%	0,138
HYPERCHOLESTEROLEMIA	36,10%	22,50%	0,166
HYPERTRIGLYCERIDEMIA	36,10%	20,00%	0,074
HEPB	5,60%	5,00%	1
HEPC	42,10%	72,50%	0,001
REJECTION	13,90%	17,10%	0,593

HCC- hepatocellular carcinoma; HCC ALCOHOL- Carcinoma by alcohol; FHDM- Family History of Diabetes Mellitus; HEPB- Hepatitis B; HEPC- Hepatitis C

Disclosure: **S. M. Bernardes:** None.

1203

L-Carnitine supplementation effects on kidney damage in mice with nonalcoholic steatohepatitis

I. Terruzzi[1], F. Vacante[2], A. Montesano[3], P. Senesi[2], G. Mollica[2], R. Codella[3,2], L. Luzi[3,2];
[1]Diabetes Research Institute, Metabolism, Nutrigenomics and Cellular Differentiation Unit, San Raffaele Scientific Institute, Milan, [2]Metabolism Research Center, IRCCS Policlinico San Donato, Milan, [3]Department of Biomedical Sciences for Health, University of Milan, Milan, Italy.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is caused by an accumulation of fat in the liver, ranging from fatty infiltration plus inflammation (non-alcoholic steatohepatitis - NASH), to advanced fibrosis and, finally, to cirrhosis that can progress to hepatocellular carcinoma. It is now increasingly clear that NAFLD not only affects the liver but can also increase the risk of developing extra-hepatic diseases, including diabetes, cardiovascular disease and chronic kidney disease (CKD). An overabundance of common factors and pathways are implicated in the pathogenesis of NAFLD and CKD. Several studies, including our previous work, have examined the effectiveness of L-Carnitine (LC) in liver and heart function. If LC is properly prescribed, it is recognized to improve kidney function. The effects of LC administration on NAFLD development and kidney histological-functional failure association were investigated in mouse model of steatohepatitis, induced by a methionine-choline deficient diet (MCD).

Materials and methods: C57BL/6 male mice ($n = 18$, age: 12 weeks) were divided in three different groups and studied for 6 weeks: control group (CONTR) was fed normal diet while both MCD and LC groups received MCD diet. In LC group, from week 3 to week 6 MCD diet was enriched with 200 mg/kg/die LC (drinking water). To study if LC attenuates fat deposition, inflammatory infiltration tissue fibrosis and kidney histopathology were assessed by histochemical staining, western blot assay and immunofluorescence.

Results: There were no significant differences in body weight between MCD and LC, while, as described in literature, there was a significant weight loss in MCD and LC groups in respect with CONTR. MCD is strictly associated with inflammation and oxidative stress (ROS) onset: ROS production was significantly reduced and Ca^{2+}/calmodulin-dependent kinases II protein levels increased in LC group in respect to MCD group. This evidence seem to show that LC antioxidant action was linked to mitochondrial function and calcium signaling. Moreover, alpha smooth muscle actin protein content increased in MCD mice while decreased in LC mice. The trend of this fibrotic marker advocates for a LC anti-fibrotic action in kidney, further confirmed by Cytokeratin 18 (CK18) protein content decrease after LC supplementation. CK18 is also recently recognized as a marker of cellular stress and its decrease suggests an LC protective effect in renal tubular injury.

Conclusion: Taken together, these results suggest that LC preserves renal function probably by reducing ROS production and modulating Ca^{2+} homeostasis. Although the association between NAFLD and CKD is strong and consistent across different patient populations, the progression and the treatment of associated CKD-NAFLD remains an issue of intense debate. Our preliminary data could represent a new nutraceutical field of investigation.

Disclosure: **I. Terruzzi:** None.

PS 117 Treating NAFLD

1204

Modified mesenchymal stromal cells: a novel and safe therapy in type 2 diabetes and its complications

S. Sen, N. Kundu, Y. Kropotova, N. Ahmadi, C. Domingues;
George Washington University, Washington, USA.

Background and aims: Mesenchymal stromal cells (MSCs) are multipotent cells that can home-in to the sites of inflammation and may coalesce with the host tissue. Though systemic antioxidant delivery has been unsuccessful, delivery of antioxidants locally at the site of inflammation using antioxidant upregulated fat derived MSCs, delivered intraperitoneally, may reduce inflammation and improve glucose homeostasis and diabetes associated complications in diet-induced obese diabetic mouse model.
Materials and methods: GFP-containing adenoviral constructs were used to upregulate antioxidants Sod2 (mitochondrial), Catalase (cytosolic) or Null (control) individually, in adipose-derived MSCs, ex-vivo. Modified MSCs were delivered intraperitoneally (IP) into mice that received 45% and 60% high-fat diet for 8–16 weeks, $n = 4$ in each group
Results: Glucose tolerance was improved at week 4 in the antioxidant upregulated MSC-receiving groups with concomitant reduction in hyperplasia in omental fat. A reduction in plasma levels of TNFa, an well-known inflammatory marker, was noted in all treated animal groups in comparison to null-MSCs. RT-PCR analysis of omental and pericardial fat showed significant up-regulation in mRNA expression of brown fat marker, Ucp1 (~1000-fold and 10-100-fold, respectively) which was associated with PGC1A upregulation. Browning was confirmed by Ucp1-staining. Remarkably, the treatment showed a significant reduction in liver fat content (by histology) and triglyceride content measurement.
Conclusion: Delivery of Sod2 and Catalase upregulated MSCs improved glycemic control by reducing systemic inflammation, promotes browning of white adipose tissue in the peritoneum and reverses hepatic lipid accumulation. These results indicate that antioxidant upregulated MSCs can help to improve glucose homeostasis, improve adipocyte energetics and prevents development of fatty liver disease. Modified MSC therapy can be a promising therapy for type 2 diabetes, obesity and its complication such as fatty liver disease.
Supported by: GW Hot Topics in Diabetes Fund
Disclosure: **S. Sen:** None.

1205

Long term effects of liraglutide in type 2 diabetic patients with vs without steatosis at baseline: 5 years prospective real-world study

G. Castellino[1], A. Rizvi[2], D. Nikolic[1], R. Chianetta[1], A. Patti[1], R. Giglio[1], S. Speciale[1], C. Mannina[1], R. Citarrella[1], G. Montalto[1], N. Papanas[3], M. Rizzo[1];
[1]DIBIMIS, University of Palermo, Italy, Palermo, Italy, [2]Endocrinology, Diabetes and Metabolism, University of South Carolina, Columbia, USA, [3]Diabetes Centre, Second Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece.

Background and aims: Nonalcoholic fatty liver disease (NAFLD) is frequently seen in type 2 diabetes subjects (T2DM) and associated with increased cardio-metabolic risk. Several studies showed that liraglutide is safe, well tolerated, and has a certain benefit on NAFLD. We assessed if, after long-term treatment, the effects of liraglutide on glyco-metabolic parameters, including a marker of subclinical atherosclerosis, cIMT, could differ in T2DM subjects with steatosis versus those without steatosis at baseline.
Materials and methods: This prospective 5 years real-world study included 31 T2DM subjects (19 men and 12 women; mean age: 60 ± 17 years), naïve to incretin-based therapies, without prior history of a major CV event and treated with metformin only. Liraglutide (1.2 mg/day) was given as add-on to stabile dose of metformin (1500–3000 mg/day). cIMT was measured by B-mode real-time ultrasound, while the presence of steatosis was assessed by ultrasound. The cohort of patients was subdivided in those with steatosis ($n = 17$) and those without steatosis ($n = 14$).
Results: Paired t-test and ANOVA were performed. After 5 years of liraglutide treatment, anthropometric and glycemic parameters improved, as well as a good metabolic control was achieved in both groups (Table), although statistical significance was not reached for all parameters. On the other hand, cIMT reduced significantly in both groups.
Conclusion: The improvements of assessed glyco-metabolic parameters was seen in both groups with and without steatosis at baseline, including significantly reduced cIMT. However, no significant differences were found for all parameters in the inter-group analysis. These data indicate on an effective CV prevention by liraglutide treatment in T2DM subjects regardless of the presence of diabetic complications. Although it remains to be established by larger studies, these findings support the potential use of liraglutide in populations at increased CV risk and without diabetes.

	With steatosis at baseline (n=17)			Without steatosis at baseline (n=14)			
	before liraglutide therapy	after liraglutide therapy	p*	before liraglutide therapy	after liraglutide therapy	p*	p* (between groups)
BMI	34±18	31±6	*0.7467*	30±4	29±4	***0.1112***	*0.3144*
Weight	96±17	87±19	***0.0217***	83±15	81±14	***0.0547***	*0.3878*
Waist circumference (cm)	110±15	99±14	***<0.0001***	100±12	90±10	***<0.0001***	*0.3436*
Glycemia (mmol/l)	8.2±3.2	6.7±1.9	*0.0595*	9.7±3.1	6.9±1.5	***0.0006***	*0.6928*
HbA1c (%)	8.6±0.8	6.8±0.7	***<0.0001***	8.9±1.1	6.5±0.5	***<0.0001***	*0.8169*
Total cholesterol (mmol/l)	4.5±1.8	4.1±0.9	*0.5290*	4.8±1.2	4.2±1.1	*0.0903*	*0.3136*
HDL-cholesterol (mmol/l)	1.07±0.28	1.13±0.24	***0.0017***	1.14±0.28	1.12±0.35	***0.0005***	*0.7321*
LDL-cholesterol (mmol/l)	2.7±1.8	2.2±0.7	*0.1674*	3.0±1.0	2.5±0.8	*0.1248*	*0.2668*
Triglycerides (mmol/l)	1.6±1.0	1.4±0.6	*0.4773*	1.4±0.4	1.3±0.5	*0.6725*	*0.9179*
cIMT (mm)	1.0±0.2	0.8±0.1	***0.024****	1.0±0.2	0.9±0.1	***0.0155***	*0.2265*

Clinical Trial Registration Number: NCT01715428
Disclosure: **G. Castellino:** Other; I have participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

1206

Effects of a carbohydrate-reduced high-protein diet on liver, pancreas and muscle triglyceride content in patients with type 2 diabetes

A. Samkani[1], M.J. Skytte[1], A.D. Petersen[1], M.N. Thomsen[1], A. Astrup[2], E. Chabanova[3], J. Frystyk[4], J.J. Holst[5], H. Thomsen[3], S. Madsbad[6], T.M. Larsen[2], S.B. Haugaard[1], T. Krarup[1];
[1]Dept. of Endocrinology, Copenhagen University Hospital Bispebjerg, Copenhagen, [2]Dept. of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, [3]Dept. of Radiology, Copenhagen University Hospital Herlev, Herlev, [4]Dept. of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, [5]Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, [6]Dept. of Endocrinology, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark.

Background and aims: Nonalcoholic Fatty Liver Disease (NAFLD) is associated with obesity and insulin resistance and the prevalence of NAFLD is up to three times higher in individuals with type 2 diabetes (T2D) compared with the general population. We hypothesized that an iso-energetic carbohydrate-reduced high-protein diet (CRHP) reduces the liver triglyceride content within 6 weeks in T2D compared with a conventional diabetes diet (CD).
Materials and methods: An iso-energetic CRHP diet was compared with a CD diet (30/50 E% carbohydrate, 30/17 E% protein and 40/33 E% fat, respectively) in a randomized open label crossover trial with 12 weeks of continuous food provision. Each diet was consumed 6 weeks. Energy intake was adjusted continuously to reinforce weight stability. Twenty-eight participants with T2D treated with only oral glucose lowering medication were included in the trial; (mean±SD) age 64 ± 8 years, body mass index 30 ± 5 kg/m^2, daily caloric need 10.5 ± 1.6 MJ, HbA_{1c} 59.6 ± 8.4 mmol/mol, T2D duration 7.0 ± 5.4 years. Liver and psoas muscle fat were measured by Magnetic Resonance (MR) spectroscopy. Pancreas fat was measured by MR imaging. Subcutaneous and visceral

adipose tissue volumes were measured in a 1 cm axial slice through mid L3. All MR data were analyzed blinded to treatment. Differences from baseline and after 6 weeks on each diet were assessed using Wilcoxon signed rank tests, while differences between diets were assessed by employing a linear mixed model on logarithmized data to meet model assumptions.

Results: Six weeks of CRHP diet significantly reduced the liver fat content from 5.8 (2.1–13) % (median; IQR) to 1.0 (1–3.6) % ($p < 0.001$), while no change was found after 6 weeks of CD diet (from 3.3 (1.4–8.7) % to 2.6 (1.0–8.3) %, $p = 0.46$). The difference in liver fat content reduction between diets was significant ($p < 0.001$). Furthermore, 6 weeks of CRHP diet significantly reduced the pancreas fat content from 6.1 (4.5–13) % (median; IQR) to 5.1 (3.1–13.4) % ($p = 0.019$), while no change was found after 6 weeks of CD diet (from 4.5 (3.1–13) % to 6.3 (4.1–11) %, $p = 0.20$). The difference in pancreas fat content change between diets was significant ($p = 0.042$). Psoas muscle fat content, subcutaneous and visceral fat volumes and waist circumference did not change significantly on either diet.

Conclusion: The present findings are promising for the dietary management of nonalcoholic fatty liver disease in T2D as a CRHP diet significantly reduces liver and pancreas fat content within 6 weeks of treatment compared with a CD diet in weight stable subjects.

Clinical Trial Registration Number: NCT02764021
Supported by: AFH, CBMR, BFH, DC, RG, JIAS, NEXS, KU, ARLA
Disclosure: **A. Samkani:** None.

1207

The effect of semaglutide on liver enzymes in subjects with obesity and elevated alanine aminotransferase: data from a randomised phase 2 trial

T. Monk-Hansen[1], L. Van Gaal[2], S. Harrison[3], S. Rasmussen[1], P.N. Newsome[4];
[1]Novo Nordisk A/S, Søborg, Denmark, [2]Antwerp University Hospital, Edegem, Belgium, [3]University of Oxford, Oxford, UK, [4]Queen Elizabeth Hospital, Birmingham, UK.

Background and aims: The glucagon-like peptide 1 analogues semaglutide and liraglutide improve glycaemic control and reduce elevated liver enzymes in subjects with type 2 diabetes (T2D), and reduce body weight in subjects with or without T2D. Histological resolution of nonalcoholic steatohepatitis has also been seen for liraglutide in subjects with or without T2D. A randomised, placebo-controlled phase 2 trial of once-daily subcutaneous semaglutide (0.05, 0.1, 0.2, 0.3, or 0.4 mg following escalation every 4 weeks) in subjects with obesity without T2D, showed mean weight losses of −6.0% (0.05 mg) to −13.8% (0.4 mg) with semaglutide vs −2.3% with placebo at week 52. The effect of semaglutide on liver enzymes in subjects with elevated baseline alanine aminotransferase (ALT) was evaluated in a *post hoc* sub-analysis from this trial.

Materials and methods: Baseline fibrosis was categorised by the nonalcoholic fatty liver disease (NAFLD) fibrosis score (NFS) and Fibrosis-4 (FIB-4) score. Changes in ALT were estimated at week 52 (mixed model on log-transformed data) and semaglutide-to-placebo group ratios and 95% confidence intervals (95%CI) calculated from this model for subjects with high baseline ALT (>30 U/L [male]; >19 U/L [female]).

Results: Mean (range) baseline characteristics of the 957 treated subjects (35% male) were: age 47 (18–86) years, weight 111 (70–244) kg, body mass index 39 (30–80) kg/m^2, mean NFS −0.49 (−4.70–4.66) and mean FIB-4 0.72 (0.14–3.31); 52% ($n = 499$) had high ALT, 18% a high NFS (>0.676), and <1% a high FIB-4 (>3.25) at baseline. Semaglutide-to-placebo ALT ratios (95%CI) at week 52 in those with high baseline ALT were: 0.88 (0.76–1.01; 0.05 mg); 0.94 (0.82–1.08; 0.1 mg); 0.82 (0.71–0.95; 0.2 mg); 0.79 (0.68–0.91; 0.3 mg), and 0.82 (0.70–0.95; 0.4 mg). *P* values were <0.01 at doses >0.1 mg, unadjusted for multiple testing. Normalization of high baseline ALT was seen at week 52 in 29% (17/58; 0.05 mg), 25% (15/59; 0.1 mg), 38% (19/50; 0.2 mg), 43% (23/54; 0.3 mg), and 46% (21/46; 0.4 mg) of subjects on semaglutide, versus 18% (14/76) of subjects on placebo.

Conclusion: In this obese population 52% had elevated liver enzymes at baseline and 18% had a high NFS score. In subjects with obesity and high ALT, semaglutide 0.2–0.4 mg daily reduced ALT as compared to placebo and resulted in dose-related ALT normalization in up to 46% of subjects after 52 weeks. These data support a potential role for semaglutide in the treatment of NAFLD with elevated liver enzymes.

Clinical Trial Registration Number: NCT02453711
Supported by: Novo Nordisk
Disclosure: **T. Monk-Hansen:** Employment/Consultancy; Novo Nordisk.

1208

Dual chemokine receptor CCR2/CCR5 antagonist cenicriviroc prevents and reverses lipotoxicity-induced insulin resistance, steatohepatitis, and liver fibrosis in mice

G. Chen[1], M. Nagashimada[1], T. Ota[1,2];
[1]Kanazawa University, Kanazawa, [2]Asahikawa Medical University, Asahikawa, Japan.

Background and aims: Hepatic lipid accumulation drives innate immunity with recruitment of bone marrow-derived macrophages and activation of liver-resident Kupffer cells, leading to the development of insulin resistance and NASH. However, treatment options that target macrophage/Kupffer cells recruitment or activation with the aim of halting the insulin resistance and NASH remain limited. A C-C chemokine receptor 2 (CCR2) and its ligand, MCP-1, plays a central role in macrophage recruitment. In addition, we reported that a different C-C chemokine receptor, CCR5, promotes obesity-induced insulin resistance and hepatic steatosis by regulating macrophage M1/M2 polarization. Cenicriviroc (CVC), a dual CCR2 and CCR5 receptor antagonist, has been reported for its anti-fibrotic activity in a murine model of NASH and is currently evaluated in clinical trial in patients with NASH. In the present study, we investigated the effect of CVC in a lipotoxic model of NASH with hepatic fibrosis and insulin resistance, replicating the pathophysiological features of human NASH.

Materials and methods: Eight-week-old C57BL/6 mice were fed a high-cholesterol, high-fat (CL) diet or a CL diet containing 0.015% CVC (CL+CVC) for 12 weeks. The liver histology, insulin sensitivity, and fibrogenesis were examined. We next quantified intrahepatic immune cell numbers by flow cytometry.

Results: After 12 weeks of feeding, histological examination showed steatohepatitis with fibrosis in mice fed CL diet. They showed hyperinsulinemia without significant change in weight and adiposity. The CVC administration decreased the increase in plasma ALT levels as well as liver triglyceride, cholesterol, and TBARS levels caused by the CL diet. In addition, CVC improved glucose intolerance and hyperinsulinemia in the CL group and augmented the insulin signal, assessed by IRβ and Akt phosphorylation in the liver. Flow cytometry analysis revealed that the numbers of CD45$^+$CD11b$^+$F4/80$^+$ total macrophages were significantly reduced in CL+CVC group by 54%. Moreover, mice fed CL+CVC had 72% fewer CD11c$^+$CD206$^-$ M1-like macrophages, but 94% more CD11c$^-$CD206$^+$ M2-like macrophages than CL group. The hepatic total numbers of CD3$^+$, CD4$^+$, and CD8$^+$ T cells were lower in CL+CVC group than in CL group by 41%, 51%, and 48%, respectively (all $P < 0.05$), indicating that CVC caused an M2-dominant shift in macrophages/Kupffer cells and a subsequent reduction in CD4$^+$ and CD8$^+$ T cell recruitment in the liver. In parallel experiments *in vitro*, CVC decreased LPS-induced M1 marker (*Tnfa*, *F4/80* and *Il1β*) mRNA expression but enhanced IL-4-induced M2 marker (*Chi3l3*, *Mgl1* and *Mgl2*) mRNA expression dose-dependently in isolated murine peritoneal macrophages. Furthermore, CVC attenuated hepatic fibrosis by repressing the activation of hepatic stellate cells (HSCs) and decreasing hydroxyproline content by 32%. Accordingly, CVC suppressed mRNA

expression of TGFβ-induced fibrogenic genes (*a-SMA*, *Col1a1* and *fibronectin*) in RI-T cells, a HSC line. Importantly, CVC reversed insulin resistance, as well as hepatic steatosis, inflammation and fibrosis, in pre-existing advanced NASH.

Conclusion: The dual chemokine receptor CCR2/CCR5 antagonist, CVC prevented and reversed lipid accumulation and peroxidation, insulin resistance, inflammation and fibrogenesis in the liver of NASH by polarizing M2 macrophage and attenuating HSC activation.

Supported by: MEXT, Japan

Disclosure: **G. Chen:** None.

1209

The effect of the combination of dapagliflozin and liraglutide in non alcoholic fatty liver disease in patients with type 2 diabetes compared to sitagliptin and pioglitazone

A. Koutsovasilis, A. Sotiropoulos, D. Papadaki, M. Pappa, V. Kordinas, S. Bousboulas, T. Peppas;
3rd Internal Medicine Department & Diabetes Center, General Hospital of Nikaia-Piraeus, Athens, Greece.

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is now the most frequent chronic liver disease that occurs across all age groups and is associated with the presence and morphology of subclinical coronary atherosclerosis. Pioglitazone has several clinical evidence in the treatment of NAFLD in Type 2 Diabetes Mellitus (T2DM) patients. Since lifestyle modifications and weight loss remain difficult to achieve by most people, an effective pharmacologic treatment for NAFLD is sorely needed. Dapagliflozin and Liraglutide are agents which lower blood glucose, significantly reduce body weight and blood pressure. The aim of the study was to examine the effectiveness of the combination of dapagliflozin and liraglutide in NAFLD patients with T2DM compared with dapagliflozin, liraglutide, sitagliptin, and pioglitazone.

Materials and methods: 421 T2DM patients with NAFLD were included in the study. 85 patients under treatment with dapagliflozin and liraglutide, 89 patients were under treatment with dapagliflozin, 82 patients under treatment with liraglutide, 71 under treatment with pioglitazone and 94 under treatment with sitagliptin. All patients also received metformin. Mean follow up period was 52 weeks ± 2 weeks. The evaluation of liver fibrosis depended on the calculation of aspartase aminotransferase (AST) to platelet counts ratio (APRI) index. APRI over 1.5 was considered as bridging fibrosis and over 2.0 as liver cirrhosis. All patients went through an ultrasonography before being included in the study and after the end of the study.

Results: There was no difference in patients age (58.8 ± 9.6, $p = 0.288$), duration of T2DM (6.4 ± 2.9, $p = 0.312$), Body Mass Index (30.24 ± 2.98, $p = 0.362$) and HbA1c (8.05 ± 0.93%, $p = 0.196$) between study's groups. HbA1c values improved in all five groups with most patients maintain the therapeutic goal in the first group ($p < 0.001$) group after 52 weeks of treatment. APRI index's improvement was significant in the group with the combination of dapagliflozin and liraglutide (1.14 (0.52–1.24) vs 0.74 (0.41–0.95), $p = 0.001$), in the dapagliflozin group (1.10 (0.56–1.20) vs 0.96 (0.33–1.03), $p = 0.022$), in the liraglutide group (1.16 (0.52–1.24) vs 0.86 (0.45–1.05), $p = 0.010$) and in the pioglitazone group (1.18 (0.48–1.27) vs 0.88 (0.42–1.10), $p = 0.012$). There was no improvement in the sitagliptin group (1.08 (0.43–1.25) vs 1.06 (0.49–1.22) $p = 0.493$). APRI index's improvement was accompanied by a significant change of fatty liver in ultrasonography. Most of the patients (67) in the dapagliflozin and liraglutide group achieved a >5 kg decrease ($p < 0.001$).

Conclusion: Administration of dapagliflozin and liraglutide in combination led not only to good control of T2DM but also in significant improvement of liver inflammation, alteration of liver fibrosis, and reduction of body weight, particularly important factors in patients with T2DM. As far as we know, this study is the first to be carried out on the long-term effect of dapagliflozin and liraglutide in combination on liver fibrosis which indicates their positive impact in T2DM with NAFLD. Particularly, body weight reduction was a favorable outcome of applying dapagliflozin and liraglutide in NAFLD patients with T2DM.

Disclosure: **A. Koutsovasilis:** None.

1210

MEDI0382, a GLP-1/glucagon receptor dual agonist, improves NASH and reduces liver fibrosis in mice

J.L. Trevaskis[1], M.L. Boland[1], J. Conway[2], S. Guionaud[3], J. Grimsby[1], L. Jermutus[4], C.J. Rhodes[1];
[1]Cardiovascular, Renal and Metabolic Diseases, MedImmune, Gaithersburg, USA, [2]Translational Sciences, MedImmune, Gaithersburg, USA, [3]Global Pathology, AstraZeneca, Cambridge, UK, [4]Cardiovascular, Renal and Metabolic Diseases, MedImmune, Cambridge, UK.

Background and aims: Effective treatment of non-alcoholic steatohepatitis (NASH), characterized by hepatic steatosis, inflammation and fibrosis is an unmet medical need. MEDI0382, a balanced GLP-1/glucagon dual receptor agonist, is under development for the treatment of T2DM and NASH. Here we examined the effects of MEDI0382 on metabolic and NASH-related endpoints compared to liraglutide, a GLP-1 receptor agonist.

Materials and methods: Leptin-deficient *ob/ob* mice were maintained on low-fat (LFD) or high *trans*-fat, fructose and cholesterol diet for 8 weeks to induce NASH then randomized to four 6-week treatment groups: vehicle, MEDI0382 (30 nmol/kg), liraglutide (40 nmol/kg) or vehicle-treated and switched to LFD. Terminal liver sections were evaluated for NASH activity score (NAS), reflecting steatosis, lobular inflammation and ballooning. Fibrosis was assessed by pathologist score and collagen immunoreactivity. Gene expression was performed via RNAseq and qPCR. Mitochondrial function was assessed in primary mouse hepatocytes isolated from lean or *ob/ob* NASH mice.

Results: MEDI0382 and liraglutide reduced body weight and improved glucose tolerance to a similar extent relative to vehicle, with diet-switching having minimal impact. Hepatic lipid was reduced by 40% with MEDI0382 treatment ($p < 0.001$ vs. vehicle), which was more effective than liraglutide (20%) or switch to LFD (20%, both $p < 0.05$ vs. MEDI0382). The NAS was reduced by MEDI0382, more so than liraglutide or switch to LFD. Hepatic collagen increased 2.4-fold with NASH and was reduced by 40% in MEDI0382-treated mice ($p < 0.05$), but was not altered by liraglutide or diet-switch. Consistent with histopathology improvements, fibrotic (*Col1a1*, *Col3a1*, *Col4a1*) and inflammatory (*Tnf*, *Tgfb1*, *Il1b*, *Ccl2*) genes were increased with NASH and reduced with MEDI0382 treatment. An analysis of gene signatures demonstrated a NASH-related decrease in metabolic signaling which was reversed by MEDI0382. Conversely, inflammatory signatures increased with NASH were reduced by MEDI0382. Studies in primary hepatocytes revealed reduced mitochondrial function in *ob/ob* NASH mice that was significantly increased by MEDI0382, whereas liraglutide had no effect. Furthermore, MEDI0382, but not liraglutide, increased expression of *Ppargc1a* mRNA which could be blocked with an inhibitor of protein kinase A, but concomitantly increased the number of lysosomal-associated mitochondria.

Conclusion: MEDI0382 exerted similar metabolic control relative to liraglutide, but exhibited superior effects on primary NASH endpoints including improved NAS and reduced fibrosis. Increased mitochondrial biogenesis and degradation, leading to restored mitochondrial function, may underlie some of the observed improvement in NASH with MEDI0382.

Disclosure: **J.L. Trevaskis:** Employment/Consultancy; Employee of MedImmune/AstraZeneca. Stock/Shareholding; Stockholder of AstraZeneca.

PS 118 Cancer and diabetes

1211

Cancer incidence and mortality among 457,473 persons with type 2 diabetes compared to 2,287,365 matched controls in Sweden: an observational study

H.H. Bjornsdottir[1,2], S. Franzén[1], A. Rawshani[3,1], A. Rawshani[3,1], N. Sattar[4], A.-M. Svensson[1], S. Gudbjornsdottir[3,1];
[1]Swedish National Diabetes Register, Center of Registers in Region, Gothenburg, Sweden, [2]Faculty of Medicine, University of Iceland, Reykjavik, Iceland, [3]Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden, [4]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.

Background and aims: Studies suggest an increased risk for certain cancer types for type 2 diabetes (T2DM) and that these patients have an increased mortality from cancer. However, many of the studies in this area are limited by potential bias. Therefore, we set out to evaluate the incidence of all cancer and site-specific cancer, along with time-trends in cancer incidence as well as post-cancer mortality, among patients with T2DM compared to matched controls.
Materials and methods: We included patients defined by the epidemiological definition as T2DM in the Swedish National Diabetes Register (NDR) between 1998 through 2012 and followed them through 2014. Each diabetic person was matched to 5 controls based on age, sex and county. The cohort included 457,473 persons with diabetes and 2,287,365 matched controls. All individuals were followed until the first site-specific cancer occurrence, death or end of follow-up, whichever came first. Incidence, trends in incidence and post-cancer mortality for cancer were estimated with cox regression and standardised incidence rates.
Results: T2DM had a slightly increased risk for all cancer, HR 1.1 (95% CI, 1.09 to 1.12). For the most common cancer sites we observed the following for incidence rates: Increased risk for breast cancer, HR 1.05 (95% CI, 1.01 to 1.09) and colorectal cancer, HR 1.20 (95% CI, 1.16 to 1.23), decreased risk for prostate cancer, HR 0.82 (95% CI, 0.80 to 0.83), and risk of lung cancer, HR 1.01 (95% CI, 0.97 to 1.05) for T2DM compared to controls. Of these four cancer sites only lung cancer showed a significant difference in change of risk over time, with a 30% greater increase in incidence over a 10 year period for T2DM compared to controls. For post-cancer mortality we observed the following: Increased mortality after diagnosis of prostate cancer, HR 1.29 (95% CI, 1.25 to 1.35), breast cancer, HR 1.25 (95% CI, 1.18 to 1.33) and colorectal cancer HR 1.09 (95% CI, 1.05 to 1.13). in T2DM compared to controls. There was not a significant difference in post-cancer mortality between the groups for lung cancer, HR 1.02 (95% CI, 0.98 to 1.06). The cancer types that T2DM was most associated with increase in risk were the following: Liver (HR 3.31), corpus uterus (HR 1.78), penis (HR 1.56), kidney (HR 1.45), gallbladder and bile ducts (HR 1.32), stomach (HR 1.21) and bladder (HR 1.20). Of these sites, pancreas cancer and corpus uterus cancer showed a significant difference in change of risk over time, with a 38% greater increase in incidence over a 10 year period for pancreas cancer and a 26% greater decrease for corpus uterus cancer for T2DM compared to controls.
Conclusion: All cancer incidence was slightly elevated in patients with T2DM compared to controls. Patients with T2DM have an increased risk of certain cancers as well as lower post-cancer survival. Changes in cancer incidence over time were virtually the same in patients with diabetes compared to controls, with the exception of pancreas-, corpus uterus- and lung cancer. With rising incidence of diabetes these observations may be of importance and a challenge to improve future diabetes care.
Supported by: European Foundation for the Study of Diabetes
Disclosure: **H.H. Bjornsdottir:** None.

1212

Prospective cohort study of type 2 diabetes and the risk of cancer in Japan: 5-year interim report

K. Tsuneda[1], A. Yoshikawa[1], K. Okita[1], Y. Inui[1], S. Fujita[2], M. Takahara[2], H. Iwahashi[2], S. Kurebayashi[3], H. Konya[3], A. Otsuka[3], N. Handa[3], T. Fukui[3], H. Matsushima[3], N. Watanabe[3], S. Kawata[1];
[1]Hyogo Prefectural Nishinomiya Hospital, Nishinomiya City, Hyogo, [2]Osaka University Hospital, Suita City, Osaka, [3]Nishinomiya Study Group, Nishinomiya City, Hyogo, Japan.

Background and aims: It is reported that type 2 diabetes is associated with the increased risk of malignant neoplasms, mainly digestive organ cancer. Some clinical studies have also reported the association between antidiabetic medicines and the risk of malignant neoplasms, nevertheless sufficient conclusions have not been made yet. To investigate the association between diabetes and the risk of cancer, we initiated "Nishinomiya Study" in October 2012. As part of the study, we've followed up all cases including baseline characteristics and antidiabetic medicines taken before the onset of malignant neoplasms. The aim of the present study is to clarify the relation between the risk of cancer and type 2 diabetes including antidiabetic medicines.
Materials and methods: The subjects were recruited from currently registered type 2 diabetic patients receiving outpatient treatment at 51 clinical units. Participants consisted of 4516 cases (2711 male and 1805 female, aged 65.6 ± 11.7 years, body mass index (BMI) 25.1 ± 4.4 kg/m^2, glycated hemoglobin (HbA1c) 7.2 ± 1.0%, waist circumference 89.4 ± 10.9 cm). We prospectively investigated the association of baseline characteristics and the antidiabetic medicines with the risk of cancer. The Kaplan-Meier method with differences analyzed by the generalized Wilcoxon test and Cox proportional hazards models were used to evaluate the impact of the baseline characteristics and the antidiabetic medicines on cancer risk. The study protocol was approved by the ethics committee of Hyogo Prefectural Nishinomiya Hospital and registered with the University hospitalMedical Information Network.
Results: 168 cases (123 male and 45 female, aged 69.5% ± 9.0 years, BMI 24.9 ± 3.9 kg/m^2, HbA1c 7.2 ± 0.9%, waist circumference 89.2 ± 10.8 cm) were newly diagnosed with cancer. The majority of the malignant neoplasms were digestive tract cancers (35 cases of colon cancer and 30 cases of gastric cancer). As treatments for diabetes, the patients suffering from cancer were treated with sulfonylureas ($n = 70$, 41.7%), dipeptidyl peptidase-4 inhibitors ($n = 79$, 47.0%), insulin ($n = 36$, 21.4%), biguanides ($n = 56$, 33.3%), alpha-glucosidase inhibitors ($n = 27$, 16.1%), glinides ($n = 16$, 9.5%), thiazolidines ($n = 20$, 11.9%) or only diet ($n = 9$, 5.4%). Primary outcome is the onset of malignant neoplasm. Male's onset was significantly higher ($p < 0.01$) than female's with Kaplan-Meier analysis. Cox proportional hazards regression analysis including age, sex, sulfonylureas, dipeptidyl peptidase-4 inhibitors, insulin, biguanides, alpha-glucosidase inhibitors, glinides and thiazolidines showed that elder age (95% CI, 1.03 to 1.07; $p < 0.01$) and male (95% CI, 1.47 to 3.01; $p < 0.01$) significantly increase the risk of cancer. Furthermore restricted to digestive tract cancer or colon cancer, Kaplan-Meier analysis revealed that more subjects who take sulfonylureas, glinides or insulin suffered from colon cancer than subjects who take none of these medicines ($p < 0.05$).
Conclusion: Sex, age and some antidiabetic medicines may have a significant influence on the risk of cancer.
Clinical Trial Registration Number: UMIN000017309
Disclosure: **K. Tsuneda:** None.

1213

Insulin sensitivity in diabetes associated with pancreatic cancer

P. Skrha[1], P. Fric[2], J. Uhrova[3], M. Andel[1], J. Skrha[4];
[1]2nd Department of Internal Medicine, 3rd Faculty of Medicine, Charles University, Prague, [2]Department of Gastroenterology, General Military Hospital, Charles University, Prague, [3]Department of Medical

Biochemistry, Charles University, Prague, [4]3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University, Prague, Czech Republic.

Background and aims: Nearly 80% of patients with pancreatic ductal adenocarcinoma (PAC) have diabetes mellitus or prediabetes (DM). The insulin resistance and β-cell dysfunction were reported in the PAC patients previously. Lower leptin and higher adiponectin levels are associated with higher peripheral insulin sensitivity, whereas HOMA-IR reflects more the liver insulin resistance. Our aim was to compare adiponectin, leptin, leptin/adiponectin ratio and HOMA index (HOMA-IR) as markers of insulin sensitivity/resistance among patients with PAC, type 2 diabetes mellitus (T2DM) and healthy control persons and then in patients within the PAC group itself.
Materials and methods: Seventy seven patients with PAC, 34 T2DM patients without the cancer and 24 healthy control persons were enrolled in our study. The PAC group was subdivided according to the presence of diabetes. In 43 patients the new-onset diabetes was confirmed less than 1 year before the cancer diagnosis and this diabetes was evaluated as pancreatogenic caused by the cancer (PAC T3cDM). 17 PAC patients had long-term type 2 diabetes (PAC T2DM) and 17 patients had normal glucose tolerance (PAC NDM). Fasting plasma concentrations of adiponectin, leptin, insulin and glucose were measured in every subject. Leptin/adiponectin ratio and HOMA-IR were calculated, Kruskal-Wallis ANOVA test and Pearson's correlation were used for statistical evaluation.
Results: Basic results in the whole group of PAC patients were compared with T2DM and control groups (Table).In the PAC T3cDM group, the lowest leptin concentration (7.8 ± 2.3 ng/l) and the highest adiponectin concentration (14.7 ± 3.6 ng/ml) were observed. In the PAC patients a significant difference of the leptin/adiponectin ratio was found only between PAC T3cDM and T2DM or controls (0.8 ± 0.3 vs 2.4 ± 1.2 or 1.5 ± 0.5, $p = 0.0003$ and 0.02, respectively). In the PAC subgroups, HOMA-IR in the PAC T2DM (5.1 ± 2.3) corresponded to that in T2DM, and in the PAC NDM (2.1 ± 0.9) to controls. HOMA-IR in the PAC T3cDM (3.1 ± 0.9) was not significantly different from controls. Significant positive correlation between leptin/adiponectin ratio and HOMA-IR was observed only in T2DM and PAC T2DM ($r = 0.38$ and 0.63, respectively), but not in T3cDM.
Conclusion: Patients with pancreatic ductal adenocarcinoma-associated new-onset diabetes (T3cDM) are significantly more insulin sensitive than patients with long-term T2DM with or without pancreatic cancer. We conclude that there exists a significant heterogeneity of insulin sensitivity/resistance in patients with pancreatic cancer.

	PAC	T2DM	Controls	p-values
Adiponectin (ng/l)	12.8±1.3	9.6±2.5	10.3±4.3	NS
Leptin (ng/l)	8.2±1.7*	12.3±2.5*	10.6±2.5	*0.0006
Leptin/adiponectin	0.9±0.2*⁻	2.4±1.2*	1.5±0.5⁻	*0.0005, ⁻0.02
HOMA-IR	3.4±0.7*	4.6±1.1*°	2.5±0.9°	*0.009, °0.02

Supported by: Research project of Charles University P25
Disclosure: **P. Skrha:** None.

1214

Insulin resistance and colon epithelial proliferation

L. Kedenko[1], J. Mayböck[1], C. Kronberger[2], T. Kiesslich[1], I. Kedenko[1], B. Paulweber[1], G. Wolkersdörfer[1];
[1]University Clinic for Internal Medicine I, Regional Hospital of Salzburg/PMU, [2]Institute of Pathology, Regional Hospital of Salzburg/PMU, Austria.

Background and aims: Epidemiologic data showed an increased risk of colorectal cancer in subjects with type 2 diabetes and insulin resistance. Little is known about the actual effect of insulinemia and insulin resistance on proliferation and the underlying signaling in the intestinal mucosa. Therefore, the first objective of our work was to clarify the relation between hyperinsulinemia, a metabolic condition caused by insulin resistance, and its effect on colon epithelial proliferation. The second objective was to confirm the role of insulin signaling pathway in colon epithelial proliferation by conducting gene expression analysis of selected candidate genes from the insulin signaling pathway.
Materials and methods: Colonoscopy with mucosa biopsies was performed in 56 participants with high and low levels of insulin sensitivity based on the highest and lowest quartile of insulin sensitivity/resistance from short Insulin Tolerance Test (KITT). Evaluation of proliferative activity in various colonic subsites was performed immunohistochemically using Ki-67 as proliferation marker. Total RNA was isolated from the colon biopsies and was used for further gene expression analyses of the 84 genes using RT^2 Profiler PCR Array Human Insulin Signaling Pathway (Qiagen, Germany).
Results: Polypoid lesions were found in 29 subjects - 23 adenomas and 6 hyperplastic polyps. In 27 subjects no polypoid lesions were detected, resulting in a polyp detection rate of 0.52 and an adenoma detection rate of 0.41 in our cohort. Proliferation indices equally decreased from the right to the left colonic subsites in the insulin resistant as well as in the sensitive group. Surprisingly, insulin sensitive individuals showed generally higher proliferation activity than resistant ones in all examined localizations, though differences in mean proliferation indices between two groups were not significantly different between different colonic subsites. Independent Student's t-test revealed 6 out of 84 analyzed genes that showed significant difference ($p \leq 0.05$) in gene expression between insulin resistance and insulin sensitive groups. Most of these genes were overexpressed in insulin sensitive group compared to insulin resistance group. These genes showed also a negative relationship with proliferation index in correlation analysis with Pearson's correlation coefficient: −0.32 for *IGF2R*, −0.14 for *MAP2K1* and *PPARG*, −0.12 for *ANG* and *MAPK3*. Only expression levels of *NCK1* correlated positively with proliferation index (Pearson's correlation coefficient +0.24).
Conclusion: We demonstrated for the first time that in the insulin sensitive individuals proliferation indices and expression of the key genes from the insulin signaling pathway were lower in comparison with insulin sensitive individuals in all colon localizations. The general assumption, that hyperinsulinemia is a direct promotor of enhanced proliferative activity in colon epithelium of insulin resistant individuals can not be confirmed by our results. Rather, our results suggest a resistance of the intestinal mucosa towards insulin in insulin-resistant individuals.
Supported by: a project grant from PMU-FFG Nr. E-14/19/102-WOK
Disclosure: **L. Kedenko:** None.

1215

Obesity, glucose abnormalities and metabolic syndrome are hallmarks of well differentiated nets

A.P. Santos[1], C. Castro[2], I. Torres[1], R. Henrique[3,4], M.H. Cardoso[5,4], M.P. Monteiro[6,7];
[1]Endocrinology, IPO Porto, Porto, [2]Epidemiology, IPO Porto, Porto, [3]Pathology, IPO Porto, Porto, [4]ICBAS, Porto, [5]Endocrinology, CHP-UP, Porto, [6]Anatomy Department, ICBAS, Porto, [7]UMIB, Porto, Portugal.

Background and aims: Metabolic syndrome (MetS) and hypercholesterolemia had been previously described as risk factors for rectal NETs. An association between well-differentiated (WD) GEP-NETs and MetS has been described before by our group in a case control study**.** The aim of the present work was to compare different WD GEP-NETs focusing obesity and metabolic abnormalities characteristic of MetS, in a cohort of patients followed in a Portuguese tertiary centre.
Materials and methods: 135 patients (pts.) with WD (G1 and G2) GEP-NETs were recruited from the Endocrine Tumours Clinic of IPO Porto and were classified according to primary tumour localization (L),

hormonal secretion (F), extension of disease (Ext) and WHO 2010 grading (G). These different types of GEP-NETs were analysed according to BMI, obesity grade, waist circumference (WC), lipid and fasting glucose (FG) profile and MetS and MetS individual criteria, determined before treatment.

Results: Mean age and age at diagnosis was 62.1 y (30–85) and 58.6 y (29–85) respectively; 58.4% were male; 74.2% Gastrointestinal (GI) NETs; 69.5% G1; 55.4% non-functioning (NF) and 46.3% disseminated. Family history of type 2 DM (47.7%), excess weight and obesity (62.6%), abdominal obesity (52%); high blood pressure (HBP) (64%), metabolic abnormalities (MA) including FG abnormalities (41.5%) and MetS (57.1%) were frequent in the whole group. We found no differences between GI-NETs vs. pancreatic (pan) NETs; NF vs functioning (F); G1 vs G2 and localised vs loco-regional vs disseminated disease, concerning BMI: $p = 0.175$(L) , 0.308(F), 0.647(G) and 0.756(Ext); obesity grade: $p = 0.168$ (L), 0.281(F), 0.621(G) and 0.269(Ext); HBP: $p = 0.229$(L), 0.292(F), 0.427(G) and 1.0(Ext); dyslipidaemia: $p = 0.435$(L), 0.143(F) and 0.524(E), FG abnormalities: $p = 0.215$(L), 0.281(F), 0.575(G), 0.295(Ext) and MetS: $p = 0.838$ (L), 0.187(F) and 0.113(Ext). Concerning WHO grading, dyslipidaemia was significantly more frequent in G1 vs. G2 tumors ($p = 0.034$) and there was a trend for a higher association of G1 vs. G2 NETs with MetS ($p = 0.056$).

Conclusion: Although the association of GEP-NETs and metabolic risk factors was seldom described, it is traditionally stated that glucose abnormalities are associated with NF and some rare functioning pan-NETs. Our findings suggest that besides pancreatic and rectal NETs, metabolic abnormalities and MetS may be an hallmark of all WD-GEP-NETs, with a tendency to be more frequent in G1 tumours. These results reinforce the need of more studies exploring the association between obesity, metabolism and NETs in order to explain the recent burden of the disease, as it has already been described in other cancers.

Disclosure: **A.P. Santos:** None.

1216

Glucose enhances breast cancer cell aggressiveness via adipose-derived mesenchymal stem cells

V. D'Esposito[1], G. Mosca[1], M. Ambrosio[1], T. Migliaccio[1], S. Cabaro[1], N. Prevete[2], G. Piccolo[3], A. Maione[1], L. Albano[1], F. D'Andrea[3], F. Beguinot[1], P. Formisano[1];
[1]National Council of Research (CNR), URT GDD-IEOS & University of Naples "Federico II", Department of Translational Medicine, Naples, [2]University of Naples "Federico II", Department of Molecular Medicine and Medical Biotechnology, Naples, [3]University of Naples "Federico II", Department of Public Health, Naples, Italy.

Background and aims: Diabetes and cancer have been closely linked to each other both epidemiologically and biologically. Several studies indicate that hyperglycaemia increases breast cancer incidence and progression. However, the molecular mechanisms are still unclear. Glucose may exert its effects on both cancer cells and tumour microenvironment, including resident adipose-derived mesenchymal stem cells (MSCs). Both cancer cells and MSCs respond to metabolic insults changing their secretory pattern. Here, we analysed whether glucose could interfere on the crosstalk between mammary adipose tissue derived-MSCs (MAT-MSCs) and oestrogen positive-MCF7 breast cancer cells, thereby modifying MSC phenotype and affecting tumour progression.

Materials and methods: MSCs were isolated from mammary adipose tissue ($n = 6$) and characterized for mesenchymal stem cell markers ($CD90^+$, $CD29^+$, $CD106^-$, $CD45^-$) by FACS analysis. Adipocyte differentiation was tested by Oil Red O staining. MAT-MSCs were co-cultured with MCF7 in 25 mM glucose (high glucose, HG) or in 5.5 mM glucose (low glucose, LG) by using transwell systems. Upon 4 days, gene expression levels were analysed by real time RT-PCR. 3D co-cultures (spheroids) of MSC -MCF7 (1:5 ratio) were established in ultra-low attachment plates in HG or LG. Upon 10 days, spheroids were counted, collected and dissociated. Levels of specific proteins were determined by FACS analysis.

Results: MAT-MSCs stained positive for CD90 and CD29 (99.2% and 99.9% of cells respectively) and negative for CD106 and CD45 markers (99.9% and 98.1% of cells). Moreover, they were able to differentiate through the adipogenic lineage. When treated with HG for 4 days, the cells did not modify the expression of the multipotency gene OCT4 and of the fibrosis marker a-SMA, as compared with LG. However, when co-cultured with MCF7 in HG, MAT-MSCs displayed a 1.4 fold down-regulation of OCT4 and a 1.4-fold increase of a-SMA. No changes in the expression of genes involved in adipogenic commitment (ZNF423 and WNT10b) were detected. In HG 3D co-cultures, MAT-MSCs significantly increased the number of MCF7-derived spheroids either compared to MCF7 alone either to MCF7 - MSC co-cultured in LG. Spheroid dissociation showed a reduction of OCT4 and an increase of a-SMA proteins in MAT-MSCs and, interestingly, an increase of OCT4 protein in MCF7. This occurred more markedly in HG compared to LG.

Conclusion: Glucose modifies the complex relationship between cancer cells and MSCs contributing to loss of multipotency and acquisition of fibroblast-like features in MSCs (cancer associated fibroblasts). Interestingly, by acting through MSCs, glucose increases the stemness potential of breast cancer cells, thus suggesting a reduction of drug sensitivity and an increase of metastatic potential. These findings underline that hyperglycemia may contribute to cancer progression also acting on breast cancer surrounding MAT-MSCs.

Supported by: EFSD, AIRC

Disclosure: **V. D'Esposito:** None.

1217

The phosphorylation of Akt through mTORC2 a possible link between dietary AGEs, diabetes and colorectal cancer

A.I. Serban[1], O.I. Geicu[2], L. Stanca[1], A. Dinischiotu[2];
[1]Preclinical Sciences, University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Veterinary Medicine, Bucuresti sector 5, [2]Biochemistry and Molecular Biology, University of Bucharest, Faculty of Biology, Bucuresti sector 5, Romania.

Background and aims: Recent evidence suggested in type 2 diabetes the negative feedback from S6K is reduced, thus Akt-S473P by mTORC2 is enhanced. Activated Akt is associated with cell survival and proliferation, as well as angiogenesis. We explored the hypothesis that high dietary intake of advanced glycation end products (AGEs) may have a role in the correlation between type 2 diabetes and colorectal cancer risk.

Materials and methods: Total casein was glycated *in vitro* in the same conditions as those used in the production of sweetened UHT milk drinks (30 min 70°C, 4 sec at 140°C in the presence of 116 mM lactose and 55 mM glucose-fructose). C2BBe1 colorectal carcinoma cells with enterocyte morphology were treated with 200 μg/ml glycated or control casein for 3, 6, 9 and 24 h. Concurrently with AGEs treatment, TNF-α, RAGE (receptor of AGEs) and IL-1β were blocked using 1 μg/ml specific antibodies. The phosphorylation status of signaling proteins was assessed using Bio-Plex Pro Cell Signaling MAPK 9-plex panel and Akt 8-plex panel. IL-8 levels and matrix metalloproteinase (MMP) activity were evaluated from cell medium using the Bio-Plex Pro Human Cytokine 8-Plex Immunoassay and gelatin zymography.

Results: After 3 h, mTOR-S2448 and PTEN-S380 phosphorilation increased by 6.5 and 3-fold, followed by Akt (Ser473) (increasd by 4.4 fold), GSK-3α/β (Ser21/Ser9) and ATF-2 (Thr71) (both increased by about 3-fold) at the 6 h interval. Akt (Ser473) and ATF-2 (Thr71) were the only ones still phosphorilated at the 9 h interval, and ATF-2 (Thr71) persisted after 24 h. Although phosphorilation levels remained higher than controls, antibody blockade of RAGE was most efficient in diminishing AGEs induced phosphorilations, reducing them by almost half, while IL-1β blockade also had a significant effect, especially towards reducing mTOR-S2448 and PTEN-S380 phosphorilation. MMP-

2 and MMP-9 activity was induced by AGEs exposure in a time-dependent manner. MMP activation by AGEs exposure was diminished by both RAGE and IL-1β blockade. Our data show that AGEs exposure can mediate via RAGE, the phosphorylation of mTOR-S2448 and the formation of active mTORC2, thus increasing Akt-S473 phosphorylation and activation. The fact that p70 S6 kinase and IRS1were unphosphorylated indicated that the mTORC1 was probably inactive. The unphosphorylated form of IRS1 and the phosphorylated PTEN induced the stabilization of active Akt, suggesting that PI3K/Akt signaling pathway is active. In addition, Akt seems to phosphorylate Ser-136 inducing BAD inhibition, an anti-apoptotic mechanism. The activation of PI3K/Akt phosphorylated GSK-3β, leading to its ubiquitination and degradation; thus its inhibitory role in epithelial-mesenchymal transition, cell invasion and migration is diminished. The accumulation of IL-8 in a time dependent manner can be a consequence of both NF-κB and Akt activation inducing MMPs activation and cancer progression. MAPK 9-plex revealed that only ATF-2 is phosphorilated (Thr71) and activated by AGEs exposure. RAGE blockade effectively decreased ATF-2 activation by AGEs.

Conclusion: AGEs exposure may stimulate cancer cell survival by activating Akt signaling. Pro-metastatic conditions may also be encouraged, by sustained proliferation and MMP activation.

Supported by: CNCS – UEFISCDI, project no. PN-II-RU-TE-2012-3-0034

Disclosure: **A.I. Serban:** None.

1218

Endogenous insulin hypersecretion links diet-induced obesity to pancreatic cancer development in Ptf1a^{CreER};LSL-Kras^{G12D} mice

A. Zhang, X. Hu, J. Magrill, J.L. Kopp, J.D. Johnson;
Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada.

Background and aims: Obesity and type 2 diabetes (T2D) are risk factors for pancreatic ductal adenocarcinoma (PDAC), a cancer with a 5-year survival rate of only 6%. However, the mechanisms linking obesity and T2D to cancer remain unclear. Elucidating these mechanisms may help us target pathways that cause early lesions in at-risk patients before the disease progresses. Hyperinsulinemia is a cardinal feature of obesity and early-stage T2D. Elevated insulin is also strongly linked with many cancer types, and, even for non-obese people, cancer mortality is significantly higher in those with elevated insulin. However, although many epidemiological and clinical studies show the association between hyperinsulinemia and PDAC, no *in vivo* study has directly demonstrated a causal link between PDAC and insulin itself. Therefore, the aim of our study is to determine whether excess insulin contributes significantly to PDAC initiation and/or progression in the context of a high-fat diet (HFD).

Materials and methods: We have previously shown that partial knockout of the *Ins1* gene (*Ins1*$^{+/-}$) on a genetically stable *Ins2* null background can be used to reduce insulin production and circulating insulin in mice in the context of a HFD, when compared to mice with two alleles of *Ins1* gene (*Ins1*$^{+/+}$). To test our hypothesis that endogenous insulin production could modulate PDAC initiation, we employed a conditional mutant *Kras* allele, LSL-*Kras*^{G12D}, driven by a tamoxifen-inducible, acinar cell-specific *Ptf1a*^{CreER} knock-in allele. Thus, *Ins1*$^{+/-}$;*Ins2*$^{-/-}$;*Ptf1a*^{CreER};LSL-Kras^{G12D}(PK-*Ins1*$^{+/-}$;*Ins2*$^{-/-}$) mice, which are genetically incapable of sustained hyperinsulinemia on a HFD, were compared to littermate control *Ins1*$^{+/+}$;*Ins2*$^{-/-}$;Ptf1a^{CreER};LSL-*Kras*^{G12D} (PK-*Ins1*$^{+/+}$;*Ins2*$^{-/-}$) mice fed the same diet. After 1 year of HFD and tracking the body weight, fasting blood glucose (FBG), and insulin levels, we performed blind quantitative histopathological analysis of Hematoxylin & Eosin (H&E) stained pancreas sections to determine whether reducing insulin can limit HFD-promotion of *Kras*^{G12D}-driven PDAC via measuring the pre-neoplastic lesion pancreatic intraepithelial neoplasia (PanIN) and tumour area.

Results: PK-*Ins1*$^{+/-}$;*Ins2*$^{-/-}$ mice had modestly lower fasting insulin levels when compared with control PK-*Ins1*$^{+/+}$;*Ins2*$^{-/-}$ mice. Importantly, the modest reduction in circulating insulin did not affect glucose homeostasis. Blind quantification of the ratio of PanIN plus tumor area to the total pancreas area showed that PK-*Ins1*$^{+/-}$;*Ins2*$^{-/-}$ mice ($n = 9$) had a statistically significant ($p = 0.018$) reduction in PanIN area when compared with PK-*Ins1*$^{+/+}$;*Ins2*$^{-/-}$ controls ($n = 13$). Striking differences in histopathology were evident using H&E and Alcian blue staining. Quantitatively, reducing insulin production by limiting *Ins1* gene dosage reduced the percent PanIN by ~50% in average, an effect that was not significantly correlated with the difference in body weight or FBG.

Conclusion: Our data show that, in the context of a hyperinsulinemia-inducing HFD, modestly reducing endogenous insulin production by limiting *Ins1* gene dosage is sufficient to reduce the incidence of PanIN lesions without affecting glucose homeostasis. This suggests hyperinsulinemia is a causal factor linking obesity and T2D to PDAC. Lifestyle interventions or therapeutics with mild insulin suppressing actions may be useful in the prevention of some cancers.

Supported by: Pancreas BC/ VGH UBC/ CRS/ CCS

Disclosure: **A. Zhang:** None.

Author Index

D

S