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Relationship between glycaemic variability and hyperglycaemic clamp-derived functional variables in (impending) type 1 diabetes

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Abstract

Aims/hypothesis We examined whether measures of glycaemic variability (GV), assessed by continuous glucose monitoring (CGM) and self-monitoring of blood glucose (SMBG), can complement or replace measures of beta cell function and insulin action in detecting the progression of preclinical disease to type 1 diabetes.

Methods Twenty-two autoantibody-positive (autoAb$^+$) first-degree relatives (FDRs) of patients with type 1 diabetes who were themselves at high 5-year risk (50%) for type 1 diabetes underwent CGM, a hyperglycaemic clamp test and OGTT, and were followed for up to 31 months. Clamp variables were used to estimate beta cell function (first-phase [$AUC_{5–10\ min}$] and second-phase [$AUC_{120–150\ min}$] C-peptide release)

combined with insulin resistance (glucose disposal rate; $M_{120–150\ min}$). Age-matched healthy volunteers ($n=20$) and individuals with recent-onset type 1 diabetes ($n=9$) served as control groups.

Results In autoAb$^+$ FDRs, $M_{120–150\ min}$ below the 10th percentile (P10) of controls achieved 86% diagnostic efficiency in discriminating between normoglycaemic FDRs and individuals with (impending) dysglycaemia. $M_{120–150\ min}$ outperformed $AUC_{5–10\ min}$ and $AUC_{120–150\ min}$ C-peptide below P10 of controls, which were only 59–68% effective. Among GV variables, CGM above the reference range was better at detecting (impending) dysglycaemia than elevated SMBG (77–82% vs 73% efficiency). Combined CGM measures were equally efficient as $M_{120–150\ min}$ (86%). Daytime GV variables were inversely correlated with clamp variables, and more strongly with $M_{120–150\ min}$ than with $AUC_{5–10\ min}$ or $AUC_{120–150\ min}$ C-peptide.

Conclusions/interpretation CGM-derived GV and the glucose disposal rate, reflecting both insulin secretion and action, outperformed SMBG and first- or second-phase AUC C-peptide in identifying FDRs with (impending) dysglycaemia or diabetes. Our results indicate the feasibility of developing minimally invasive CGM-based criteria for close metabolic monitoring and as outcome measures in trials.

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Abbreviations

autoAb$^+$	Autoantibody-positive
CGM	Continuous glucose monitoring
FDR	First-degree relative

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GV   Glycaemic variability
IA-2A   Insulinoma-associated protein 2 autoantibodies
IGT   Impaired glucose tolerance
ISI   Insulin-sensitivity index
IQR   Interquartile range
P10   Percentile 10
SMBG   Self-monitoring of blood glucose
ZnT8A   Zinc transporter 8 autoantibodies

Introduction

Prompt detection of type 1 diabetes enables patients to avoid diabetic ketoacidosis and start insulin treatment early in order to maximise beta cell preservation and minimise the risk of complications [1–3]. Moreover, secondary and tertiary prevention trials with immune interventions require the identification of individuals with impending or asymptomatic clinical onset of diabetes and relatively preserved beta cell function [4–6]. Virtually all first-degree relatives (FDRs) of patients with type 1 diabetes who have positivity for at least two diabetes-associated autoantibodies will develop diabetes within 20 years [7]. Those with dysglycaemia or low stimulated C-peptide release are likely to develop the disease rapidly (i.e. within 3 years) [8–12]. This subgroup can be detected by repetitive hyperglycaemic clamps or OGTTs, but these are not simple procedures to implement, especially in children [13, 14]. Moreover, OGTT data are subject to large inter- and intra-individual variability [13]. Elevated HbA_{1c} is a stable and specific indicator of impending diabetes, but occurs at a later phase of the disease and is therefore an insensitive detector [15, 16].

We hypothesised that glycaemic variability (GV), as measured by continuous glucose monitoring (CGM), can provide early and easily accessible detection of reduced beta cell function and impending type 1 diabetes in at-risk groups. An inverse relationship between crude measures of GV (as determined by self-monitoring of blood glucose [SMBG]) and residual beta cell function (as assessed by the hyperglycaemic clamp) has previously been demonstrated in islet graft recipients [17]. Here, we investigated this correlation in earlier disease phases (i.e. before and at clinical onset) [18].

We evaluated GV by CGM and SMBG in parallel with measurements of first- and second-phase C-peptide release, the glucose disposal rate and the insulin-sensitivity index (ISI) derived from hyperglycaemic clamps [10], the gold standard for assessing beta cell function [19, 20]. We examined whether GV above the range of healthy controls predicts the development of dysglycaemia or diabetes in autoantibody-positive ($autoAb^+$) FDRs of patients with type 1 diabetes and how this correlates with clamp-derived variables. This analysis could indicate whether minimally invasive CGM- or SMBG-derived data might serve as markers for the early

diagnosis of type 1 diabetes, and as surrogate endpoints in prevention and curative trials.

Methods

Participants

Informed consent was obtained from each participant, and also from their parents in the case of minors. The study protocol was approved by the ethics committees of the Belgian Diabetes Registry and the participating university hospitals, and was conducted in accordance with the Declaration of Helsinki as revised in 2013.

High-risk FDRs A total of 22 FDRs of patients with type 1 diabetes (12 siblings, nine offspring, one parent) were recruited by the Belgian Diabetes Registry and underwent an OGTT, followed by CGM for 5 days and finally a hyperglycaemic clamp test. All of the participants were positive for autoantibodies against insulinoma-associated protein 2 [IA-2A] or zinc transporter 8 [ZnT8A] and at least one other diabetes autoantibody, a profile that has previously been shown to confer about a 50% risk of developing diabetes within 5 years [18]. Seven FDRs carried the high-risk *HLA-DQ2/DQ8* genotype (Table 1).

The participants were followed (median [range]: 18 [3–31] months) for glucose tolerance and diabetes onset (OGTT), GV (CGM and seven-point profile with SMBG) and beta cell function (hyperglycaemic clamp) every 6 months. Impaired fasting glucose, impaired glucose tolerance (IGT) and diabetes were defined according to ADA criteria [21]. At diagnosis, FDRs were shifted to intensive insulin treatment and excluded from the study.

Patients with type 1 diabetes Six patients with recent-onset diabetes and fewer than 4 weeks of intensive insulin treatment and three patients in clinical remission (<0.5 U/kg/day insulin and HbA_{1c} <7% [<53 mmol/mol]; 5–15 months after diagnosis) [22] were recruited. These participants underwent a hyperglycaemic clamp, followed by 5 days of CGM.

Healthy volunteers CGM measurements were taken and hyperglycaemic clamps were performed in 20 healthy volunteers. None of these participants had a family history of type 1 diabetes or an FDR with type 2 diabetes. All tested negative for diabetes-associated autoantibodies (islet-cell cytoplasm autoantibodies, glutamate decarboxylase autoantibodies, IA-2A, insulin autoantibodies and ZnT8A) [18] and all had a normal OGTT (Table 1).

Table 1 Description of the study groups

Characteristic	Healthy volunteers	AutoAb[+] FDRs	Patients with T1D	Overall p value
Participants, n	20	22	9	NA
Age, years	18 (12–40)	19 (12–41)	20 (13–36)	NS
Males/females, n/n	12/8	9/13	6/3	NS
BMI, z score	0.5 (−3.1–2.0)	0.2 (−2.7–2.2)	0.6 (0.3–1.7)	NS
HLA-DQ2/DQ8 genotype, n (%)	1 (5)	7 (32)	1 (11)	NS
Basal C-peptide, pmol/l	572 (285–1,010)	487 (267–953)	193 (30–569)*	<0.001
High-risk autoantibody profile[a], n (%)	0 (0)	22 (100)**	8/9 (89)**	<0.001
HbA$_{1c}$, %	5.4 (4.7–5.7)	5.3 (4.7–5.9)	8.1 (5.4–11.3)*	<0.001
HbA$_{1c}$, mmol/mol	36 (28–39)	34 (28–41)	65 (36–100)*	<0.001
Dysglycaemic individuals				
At baseline, n (%)	0 (0)	5 (23)	9 (100)*	<0.001
Persistent IGT, n/n	NA	3/5[b]	NA	NA
Developed T1D, n/n	NA	2/5[b]	NA	NA
Normoglycaemic individuals				
At baseline, n (%)	20 (100)	17 (77)	0 (0)*	<0.001
Developed IGT, n/n	0/20	2/17[b]	NA	NA
Developed T1D, n/n	0/20	3/17[b]	NA	NA
Persistent NGT, n/n	20/20	12/17[b]	NA	NA

Data are medians (range), unless otherwise specified

[a] Positive for IA-2A or ZnT8A, plus at least one other autoantibody

[b] During a median (range) follow-up of 18 (3–31) months

*$p<0.001$ vs healthy volunteers and autoAb[+] FDRs; **$p<0.001$ vs healthy volunteers

Thresholds for significance were $p<0.05/k$ in the case of k comparisons: thus $p<0.05/10$ or $p<0.005$ for overall comparisons; $p<0.05/3$ or $p<0.017$ for comparison among groups in case of a significant overall p value

NA, not applicable; NGT, normal glucose tolerance; T1D, type 1 diabetes

OGTTs

Blood samples were collected for glucose analysis before and 2 h after an oral glucose load of 1.75 g/kg without exceeding the maximum of 75 g [8].

Hyperglycaemic clamps

A hyperglycaemic clamp was performed 1–2 weeks after the OGTT, as previously described, with the omission of a glucagon injection at 150 min [8, 19]. In patients treated with long-acting insulin, this insulin type was replaced by an intermediately acting insulin on the evening before the clamp.

Briefly, after an overnight fast, a 20% glucose solution (Baxter, Brussels, Belgium) was infused via the left antecubital vein at time 0. During the first 14 min, a priming glucose dose was administered and the blood glucose level was raised to reach the plateau of 10 mmol/l. Thereafter, this hyperglycaemic target was maintained by adjusting the glucose infusion rate upon assessment of bedside blood glucose levels every 5 min using the Accu-Chek Inform II glucose

monitor (Roche Diagnostics, Mannheim, Germany) calibrated as plasma glucose.

Blood samples for C-peptide and proinsulin determination were collected at 5, 7.5 and 10 min to derive the first-phase C-peptide release (AUC$_{5-10\ min}$ C-peptide), and at 120, 135 and 150 min for the second-phase release (AUC$_{120-150\ min}$ C-peptide). AUC C-peptide release was calculated using the trapezoidal rule and expressed per minute [8]. The glucose disposal rate between 120 and 150 min ($M_{120-150\ min}$, expressed as μmol/[kg×min]) corresponds to the amount of glucose metabolised during hyperglycaemia and was calculated as the average glucose infusion rate between 120 and 150 min minus a 'space correction' [19]. The ISI (ISI$_{120-150\ min}$) was calculated by dividing $M_{120-150\ min}$ (expressed as mmol/[kg×min]) by the average insulin concentrations (expressed as pmol/l) during the same period, multiplied by 100. The disposition index was computed by multiplying AUC$_{120-150\ min}$ C-peptide with the HOMA2-IR [23]. Percentile 10 (P10) of the healthy control group ($n=20$) was used to indicate low AUC$_{5-10\ min}$ C-peptide, AUC$_{120-150\ min}$ C-peptide and $M_{120-150\ min}$. No development of diabetes was reported in this reference population.

GV

An iPro2 CGM device (Medtronic, Northridge, CA, USA) was inserted following the OGTT (FDRs and volunteers) or hyperglycaemic clamp (patients). The median blinded CGM time was 120 h per participant, with a minimum of 96 h. During this period, participants performed a seven-point SMBG profile (preprandial, 2 h postprandial and pre-bedtime) using a Contour Link glucometer (Bayer, Leverkusen, Germany). The three preprandial and the pre-bedtime glucose values were used to calibrate each 24 h period. Participants were also asked to complete a simple log of their dietary intake, drug intake, activity, and sleep and wake times. Healthy volunteers ($n=20$), autoAb$^+$ FDRs ($n=22$) and patients with type 1 diabetes ($n=9$) did not differ in the percentages of missing CGM data (<6%). This study presents the baseline CGM and SMBG data; the collection of longitudinal data is ongoing.

Analytical methods

Plasma glucose was assessed using a glucose oxidase method (Vitros 5.1FS, Ortho Clinical Diagnostics, Rochester, NY, USA); C-peptide and insulin by electrochemiluminescence immunoassay (Cobas e411, Elecsys, Basel, Switzerland); proinsulin by time-resolved fluorescence immunoassay (AutoDELFIA, Perkin-Elmer, Waltham, MA, USA) [24]; and HbA$_{1c}$ by HPLC (HLC-723G7, Tosoh Bioscience, Tessenderlo, Belgium). Because of the 100% cross-reactivity of proinsulin in the C-peptide assay, free C-peptide levels were obtained by subtracting the proinsulin concentration from the total C-peptide result [8]. Diabetes autoantibodies (insulin autoantibodies, glutamate decarboxylase autoantibodies, IA-2A and ZnT8A) were determined by liquid-phase radiobinding assays and *HLA-DQ* polymorphisms by allele-specific oligonucleotide genotyping, as previously described [18].

Data analysis and statistical analysis

All GV variables are shown in the electronic supplementary material (ESM) Methods. SMBG-derived variables were obtained from at least five fingerstick measurements (two preprandial, two postprandial and one pre-bedtime) per 24 h for a minimum of 4 days. CGM-derived variables were calculated using the GlyVarT program, version 1.0 (Medtronic Bakken Research Center, Maastricht, the Netherlands). GV indices did not include the initial 2 h of monitoring, as this is an unstable calibration period. Daytime CGM measurements were calculated between awakening and bedtime (and night-time measurements between bedtime and awakening), as documented in the participants' logbooks. Data from a given day were excluded if more than 20% of CGM data were

missing or if fewer than three valid calibration values per 24 h period were available [14]. GV measures were considered to be elevated if they exceeded the maximum value found in healthy controls ($n=20$). Fasting C-peptide and glucose values before the start of the hyperglycaemic clamp were used to determine HOMA2-IR using the calculator available at www.OCDEM.ox.ac.uk [25].

Statistical analyses were performed two-tailed using SPSS version 22.0 for Windows (IBM SPSS Statistics, Chicago, IL, USA); figures were generated using GraphPad Prism version 5.00 for Windows (GraphPad, San Diego, CA, USA); and differences between groups were assessed using the Mann–Whitney U or Kruskal–Wallis tests for continuous variables, and by the χ^2 or Fisher's exact tests for categorical variables. $p<0.05$ or $p<0.05/k$ in the case of k comparisons was considered statistically significant. Correlations between variables were assessed using the Spearman rank test. Curve fitting was performed in GraphPad Prism version 5.00 according to the least-square ordinary fitting method. The extra sum-of-squares F test was used to compare fitting models. The more complicated model (H1; e.g. two-phase model) was chosen over the simpler model (H0; e.g. one-phase model) if $p<0.05$.
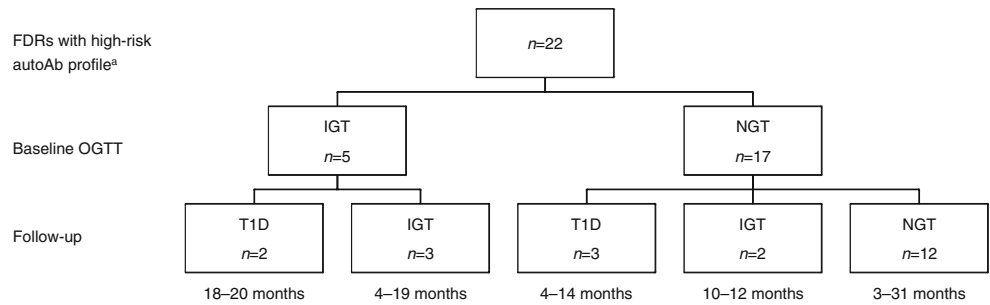
Results

Characteristics of the study groups

The healthy volunteer ($n=20$), autoAb$^+$ FDR ($n=22$) and type 1 diabetes ($n=9$) groups did not significantly differ in age, the ratio of males to females or BMI z score (Table 1). All FDRs and eight out of nine patients with type 1 diabetes were IA-2A or ZnT8A positive and carried at least one additional autoantibody among the four specificities tested. Healthy controls and FDRs did not differ in their HbA$_{1c}$ levels at baseline, with all values being below 6.0% (42 mmol/mol). Five of the 22 FDRs had IGT at baseline, with one of these participants also having impaired fasting glucose. Two of these five participants developed diabetes within 20 months, while the other three remained glucose intolerant during follow-up (4–19 months). Of the 17 initially normoglycaemic FDRs, two developed IGT after 10–12 months and three developed diabetes after 4–14 months (Table 1, Fig. 1).

Baseline hyperglycaemic clamp-derived and GV variables of study groups according to outcome

Figure 2 shows the average CGM profiles in healthy volunteers ($n=20$; Fig. 2a), FDRs with persistent euglycaemia ($n=12$; Fig. 2b), FDRs with euglycaemia at baseline but who became glucose intolerant or developed diabetes during follow-up ($n=5$; Fig. 2c), FDRs with dysglycaemia at baseline ($n=5$; Fig. 2d), patients with type 1 diabetes in remission

Fig. 1 Flow chart showing the development of IGT or type 1 diabetes (T1D) in FDRs at high autoantibody-inferred risk of the disease. [a]Positive for IA-2A or ZnT8A, plus at least one other autoantibody. AutoAb, autoantibody; NGT, normal glucose tolerance


FDRs with high-risk autoAb profile[a] — $n=22$

Baseline OGTT — IGT $n=5$ / NGT $n=17$

Follow-up — T1D $n=2$ (18–20 months) / IGT $n=3$ (4–19 months) / T1D $n=3$ (4–14 months) / IGT $n=2$ (10–12 months) / NGT $n=12$ (3–31 months)

($n=3$; Fig. 2e) and patients with recent-onset type 1 diabetes ($n=6$; Fig. 2f). From the CGM and SMBG measurements, we derived 201 GV variables (ESM Methods). Among these variables, we first investigated how simple baseline measures of GV during daytime (CGM-derived % CV_{day} and % $time_{day}$ >7.8 mmol/l glucose; SMBG-derived SD_{day}) were able to discriminate between the six above-mentioned groups of participants in comparison with hyperglycaemic clamp-derived variables of insulin secretion and action (Fig. 3).

Persistently euglycaemic autoAb[+] FDRs ($n=12$) did not differ from healthy controls for these variables (Fig. 3b,c), except

for a trend ($p=0.009$; threshold $p<0.005$; Bonferroni correction) towards a lower first-phase C-peptide release (Fig. 3a; ESM Fig. 1). FDRs who developed IGT or diabetes during follow-up ($n=5$) had lower first- and second-phase C-peptide release, and a lower glucose disposal rate ($M_{120-150\ min}$) during the hyperglycaemic clamp, compared with healthy controls ($p=0.006$ to $p<0.001$; Fig. 3a–c); they also tended to have lower clamp-derived values and higher GV variables than FDRs who remained euglycaemic (Fig. 3a–f).

$M_{120-150\ min}$ best discriminated between persistently normoglycaemic FDRs and FDRs with dysglycaemia at baseline ($p<0.001$; Fig. 3a–f). Overall, FDRs who developed

Fig. 2 Average CGM profiles of the different study groups. Each curve represents the average CGM profile over 4–5 days for one participant. (**a**) Healthy volunteers ($n=20$); (**b**) autoAb[+] FDRs with persistent euglycaemia ($n=12$); (**c**) autoAb[+] FDRs with euglycaemia at baseline and dysglycaemia during follow-up ($n=5$); (**d**) FDRs with dysglycaemia at baseline ($n=5$); (**e**) patients with type 1 diabetes in remission ($n=3$); (**f**) patients with recent-onset type 1 diabetes ($n=6$). Broken lines indicate the glycaemia range between 3.9 and 7.8 mmol/l [21]
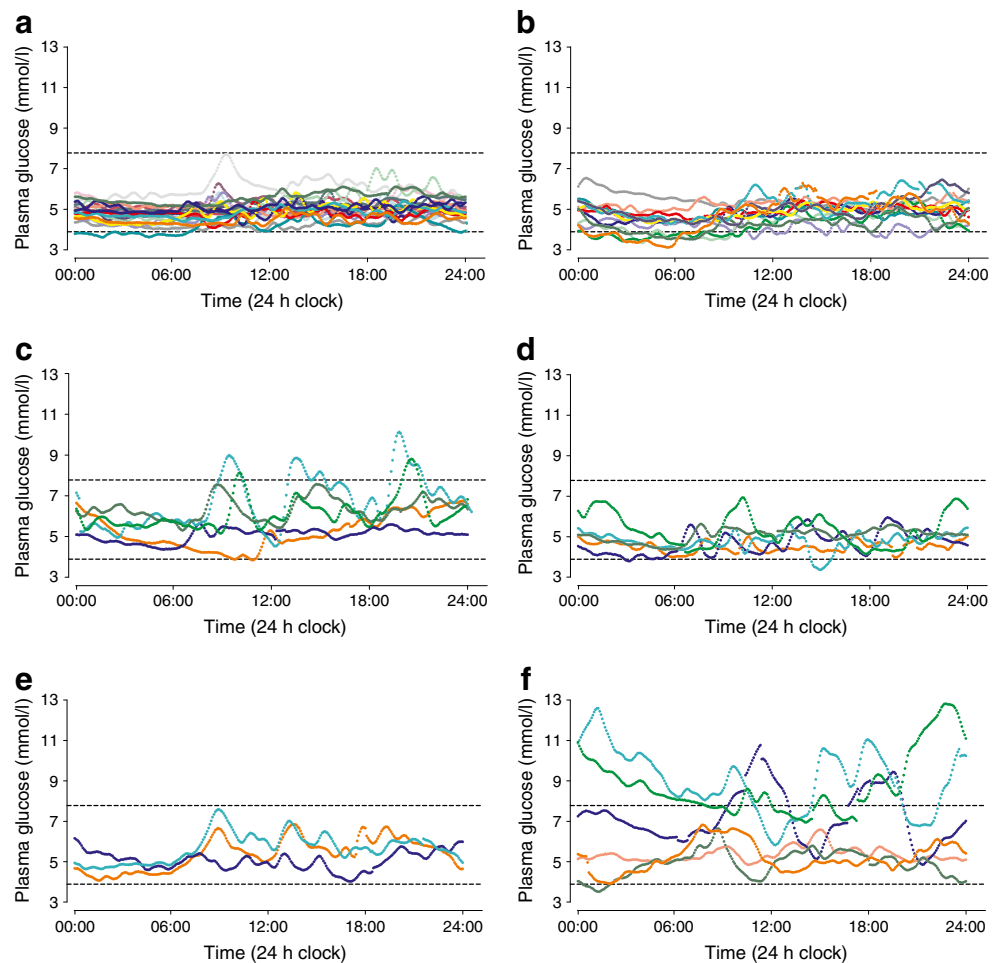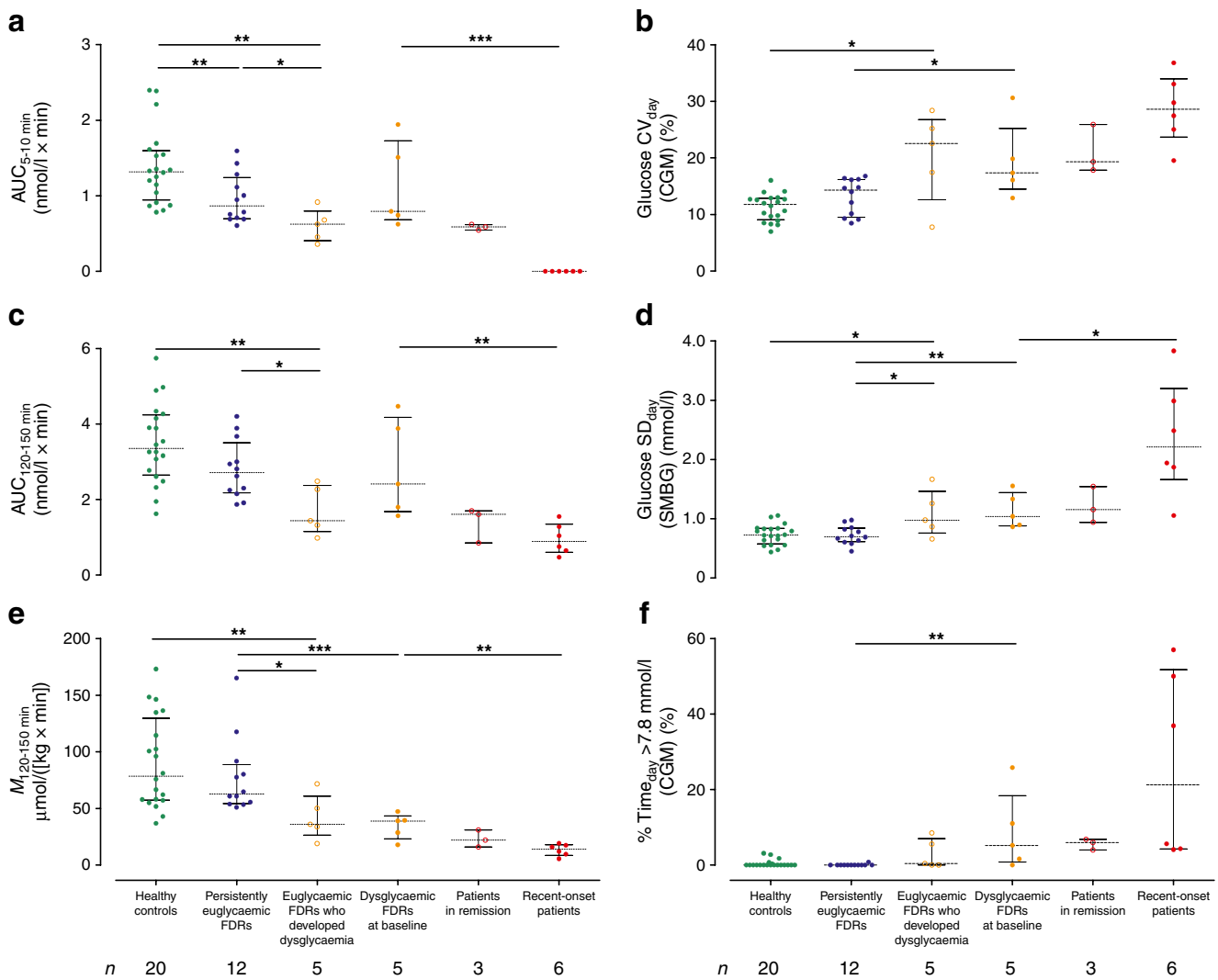
Fig. 3 Baseline hyperglycaemic clamp-derived and GV variables of the study groups. (**a**) First-phase AUC C-peptide release (AUC$_{5–10\ min}$); (**b**) second-phase AUC C-peptide release (AUC$_{120–150\ min}$); (**c**) glucose disposal rate ($M_{120–150\ min}$); (**d**) glucose CV$_{day}$ measured by CGM; (**e**) glucose SD$_{day}$ measured by SMBG; (**f**) % time$_{day}$ >7.8 mmol/l measured by CGM. Data are dot plots with indications of median values and IQR. *$p<0.05$, **$p<0.01$, ***$p<0.001$. In each panel, the overall significance was $p<0.001$ (the threshold for significance was $p<0.05/k$: $p<0.05/6$ or $p<0.008$). The threshold for significance of comparisons within each panel was $p<0.05/10$ or $p<0.005$

diabetes did not differ from those who remained glucose intolerant (data not shown). Patients with recent-onset diabetes had no measurable AUC$_{5–10\ min}$ C-peptide (Fig. 3a); all clamp-derived variables ($p=0.009$ to $p<0.001$) and some GV measures tended to differ from those of dysglycaemic autoAb$^+$ FDRs (Fig. 3b–f). Patients with type 1 diabetes in remission tended to have GV values more similar to those of dysglycaemic FDRs than of patients with recent-onset diabetes (no statistical test performed because of low numbers) (Fig. 3d–f). Other clamp-derived measures (e.g. ISI$_{120–150\ min}$; disposition index) [8], HOMA2-IR and more sophisticated CGM variables (e.g. continuous overall net glycaemic action [CONGA]) [26] performed less well in discriminating between the various groups (data not shown).

Diagnostic performance of hyperglycaemic clamp-derived variables and measures of GV to detect or predict dysglycaemia

Of the ten autoAb$^+$ FDRs who had ($n=5$) or developed ($n=5$) dysglycaemia, seven had a low first-phase C-peptide release, five a low second-phase release and seven a low glucose disposal rate ($M_{120–150\ min}$) when taking P10 of the healthy volunteers ($n=20$) as the cut-off. Only four FDRs had HbA$_{1c}$ levels ≥5.7% (39 mmol/mol). Because of its higher diagnostic specificity, $M_{120–150\ min}$ achieved 86% diagnostic efficiency, thereby tending to outperform AUC C-peptide.

CGM measurements (SD$_{day}$, interquartile range [IQR]$_{day}$ and CV$_{day}$) above the range of those from healthy controls

tended to achieve a higher diagnostic efficiency for detecting or predicting dysglycaemia than elevated SMBG variables (range$_{day}$ and SD$_{day}$) (77–82% vs 73%). Especially when considering multiple variables (e.g. elevated SD$_{day}$ and/or IQR$_{day}$), CGM achieved a diagnostic efficiency similar to that of $M_{120–150\ min}$ (86%) and higher than that of SMBG (73%) (Table 2).

Correlation of GV with beta cell function and glucose disposal rate

Among the 201 GV variables (ESM Methods), 31% were significantly and inversely correlated with beta cell function (AUC$_{120–150\ min}$ C-peptide) and with glucose disposal rate during this phase ($M_{120–150\ min}$) when considering all groups of participants together. ESM Table 1 shows the Spearman rank correlation coefficients and p values for the variables with $r \geq |0.700|$ for at least one of the two correlations. Overall, GV variables were more strongly correlated (higher $|r|$ values and all $p<0.001$) with clamp-derived $M_{120–150\ min}$ than with AUC$_{120–150\ min}$ ($p=0.017$ to $p<0.001$) (ESM Table 1) or AUC$_{5–10\ min}$ (data not shown). Some 24 h CGM variables, but none of the night-time indices, showed significant inverse correlations with AUC$_{120–150\ min}$ and $M_{120–150\ min}$ (ESM

Table 1). Good correlations were observed for postprandial variables, but these proved less practical because they required food intake to be logged and were more sensitive to interindividual variations.

The strongest correlations with clamp-derived variables were obtained for daytime GV measures derived from CGM (glucose CV$_{day}$; Fig. 4a–c) or SMBG (glucose SD$_{day}$; Fig. 4d–f). In addition, GV here was more closely related to $M_{120–150\ min}$ than to first- or second-phase C-peptide release. To allow comparison with data from Steck et al [14], we included correlations for CGM-derived % time$_{day}$ >7.8 mmol/l (Fig. 4g–i). The best curve-fitting model was a hyperbolic function (Fig. 4). For all patients (recent-onset type 1 diabetes: $n=6$; type 1 diabetes in remission: $n=3$), AUC$_{5–10\ min}$, AUC$_{120–150\ min}$ and $M_{120–150\ min}$ were below the P10 of healthy controls (Fig. 4), while CGM-derived glucose CV$_{day}$ (Fig. 4a–c) and % time$_{day}$ >7.8 mmol/l glucose (Fig. 4g–i) exceeded the range of controls. For SMBG-derived glucose SD$_{day}$, this was only the case in seven out of nine patients (Fig. 4d–f). Most autoAb$^+$ FDRs had clamp-derived values below the P50 of controls, and most of those with (impending) dysglycaemia scored below the P10 (Fig. 4a–i). Of note, two FDRs with persistent dysglycaemia and disproportionally high beta cell function,

Table 2 Diagnostic performance of HbA$_{1c}$, hyperglycaemic clamp-derived variables and measures of GV to detect or predict dysglycaemia as judged by OGTT in autoAb$^+$ FDRs

Variable at baseline	Sensitivity		Specificity	Efficiency[a]
	Euglycaemic FDRs who developed dysglycaemia	Dysglycaemic FDRs at baseline		
HbA$_{1c}$ ≥5.7% (39 mmol/mol)	1/5 (20)	3/5 (60)	12/12 (100)	16/22 (73 [54, 92])
Clamp-derived				
AUC$_{5–10\ min}$ C-peptide <P10	4/5 (80)	3/5 (60)	6/12 (50)	13/22 (59 [38, 80])
AUC$_{120–150\ min}$ C-peptide <P10	3/5 (60)	2/5 (40)	10/12 (83)	15/22 (68 [49, 88])
$M_{120–150\ min}$ <P10	3/5 (60)	4/5 (80)	12/12 (100)	19/22 (86 [72, 101])
GV				
CGM				
>2% glycaemia$_{day}$ >7.8 mmol/l	2/5 (40)	3/5 (60)	12/12 (100)	17/22 (77 [59, 95])
SD$_{day}$ >0.78 mmol/l (1)	3/5 (60)	4/5 (80)	10/12 (83)	17/22 (77 [59, 95])
IQR$_{day}$ >1.00 mmol/l (2)	4/5 (80)	4/5 (80)	10/12 (83)	18/22 (82 [66, 98])
CV$_{day}$ >16% (3)	4/5 (80)	3/5 (60)	11/12 (92)	18/22 (82 [66, 98])
(1) and/or (2)	4/5 (80)	5/5 (100)	10/12 (83)	19/22 (86 [72, 101])
(2) and/or (3)	4/5 (80)	5/5 (100)	10/12 (83)	19/22 (86 [72, 101])
SMBG				
Range$_{day}$ >5.78 mmol/l (4)	2/5 (40)	2/5 (40)	12/12 (100)	16/22 (73 [54, 92])
SD$_{day}$ >1.06 mmol/l (5)	2/5 (40)	2/5 (40)	12/12 (100)	16/22 (73 [54, 92])
(4) and/or (5)	2/5 (40)	2/5 (40)	12/12 (100)	16/22 (73 [54, 92])

Data are n/n (%) and n/n (% [95%] CI)

Cut-off values of GV were determined as the maximum values of healthy volunteers ($n=20$)

[a] Diagnostic efficiency is the fraction of correctly classified individuals (true positives and true negatives) among all FDRs studied (with and without dysglycaemia), expressed as a percentage

as judged from $AUC_{120-150\ min}$ C-peptide values (>P75), had high HOMA2-IR values (1.90 and 2.10, respectively) and high BMI z scores (2.06 and 2.18, respectively), yet low $M_{120-150\ min}$ (<P10) and elevated or borderline GV variables.

$M_{120-150\ min}$ correlated better with $AUC_{120-150\ min}$ ($p<0.001$) than with $AUC_{5-10\ min}$ ($p<0.05$; Fig. 5a,b). Unlike AUC C-peptide (data not shown), $M_{120-150\ min}$ was significantly and inversely correlated with HOMA2-IR ($r=-0.530$, $p=0.007$) (Fig. 5c); however, neither AUC C-peptide (data not shown) nor $M_{120-150\ min}$ were significantly correlated with $ISI_{120-150\ min}$ (Fig. 5d) or with the disposition index (data not

shown). Consistent with this, the GV variables analysed in Figs 3 and 4 were significantly correlated with HOMA2-IR ($r=0.505-0.740$, $p<0.005-0.001$), but not with $ISI_{120-150\ min}$ or with the disposition index (data not shown).

Discussion

This study assessed the relationship between GV variables, clinical outcomes and residual beta cell function in combination with insulin action in an early phase of
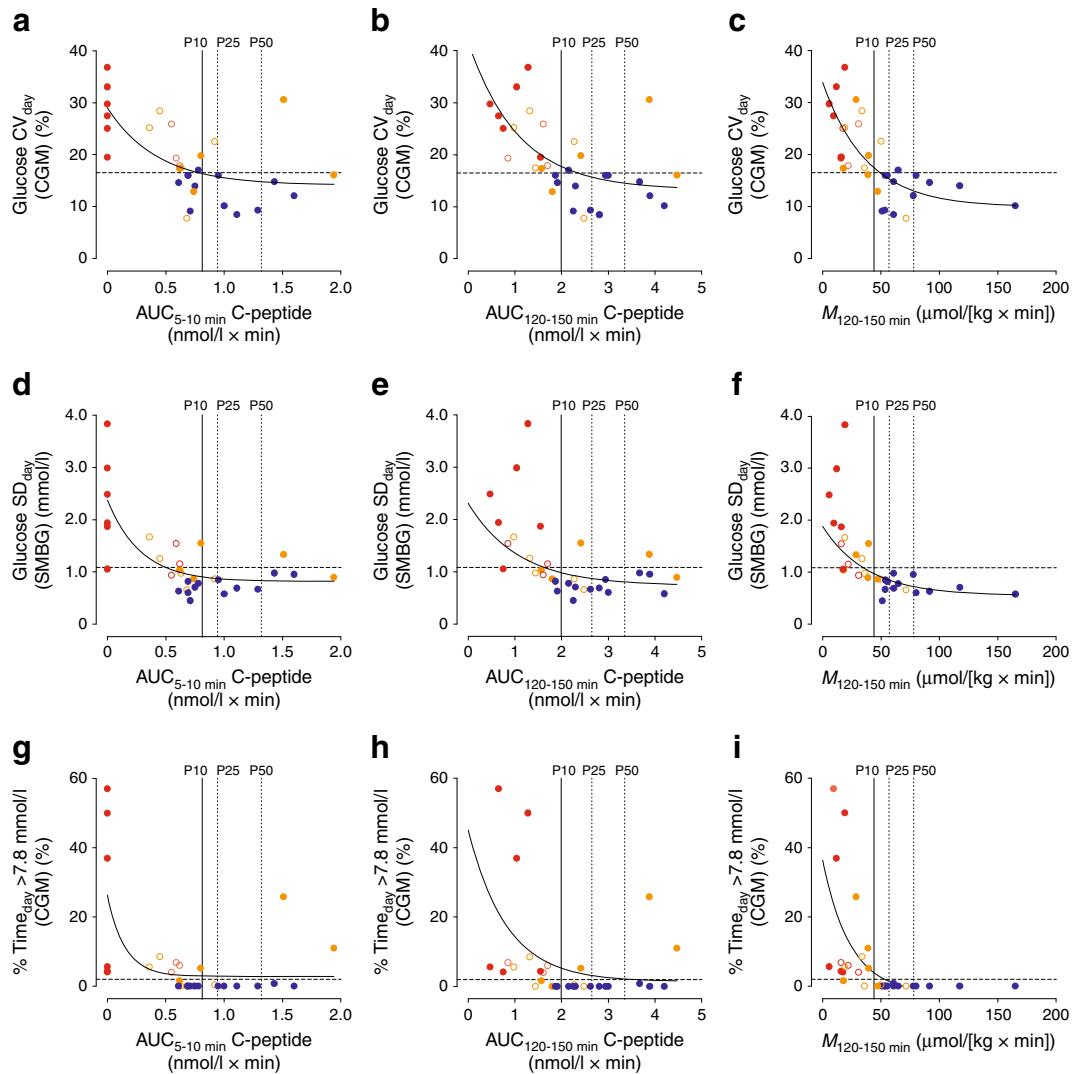
Fig. 4 Correlations between GV measures and clamp-derived variables. CGM glucose CV_{day} vs (a) $AUC_{5-10\ min}$ C-peptide release ($r_s=-0.581$; $p<0.001$), (b) $AUC_{120-150\ min}$ C-peptide release ($r_s=-0.632$; $p<0.001$) and (c) $M_{120-150\ min}$ ($r_s=-0.767$; $p<0.001$). SMBG glucose SD_{day} vs (d) $AUC_{5-10\ min}$ C-peptide release ($r_s=-0.775$; $p<0.005$), (e) $AUC_{120-150\ min}$ C-peptide release ($r_s=-0.631$; $p<0.001$) and (f) $M_{120-150\ min}$ ($r_s=-0.852$; $p<0.001$). CGM % $time_{day}$ >7.8 mmol/l vs (g) $AUC_{5-10\ min}$ C-peptide release ($r_s=-0.637$; $p<0.05$), (h) $AUC_{120-150\ min}$ C-peptide release ($r_s=-0.481$; $p<0.01$) and (i) $M_{120-150\ min}$ ($r_s=-0.807$; $p<0.001$). Red filled circles, patients with recent-onset diabetes ($n=6$); red open circles,

patients with diabetes in remission ($n=3$); orange filled circles, FDRs with dysglycaemia at baseline ($n=5$); orange open circles, FDRs with euglycaemia at baseline and dysglycaemia during follow-up ($n=5$); blue filled circles, FDRs with persistent euglycaemia ($n=12$). In each panel, vertical lines represent, from left to right, the P10, P25 and P50 of healthy controls ($n=20$). Horizontal dashed lines represent the cut-off values for GV variables, determined as the maximum value in healthy volunteers ($n=20$): glucose CV_{day}, 16% (a–c), glucose SD_{day}, 1.06 mmol/l (d–f) and % $time_{day}$ >7.8 mmol/l, 2% (g–i). r_s=Spearman rank correlation coefficient

Fig. 5 Correlation of $M_{120–150\ min}$ with (**a**) $AUC_{5–10\ min}$ C-peptide release (r_p=0.436; p<0.05), (**b**) $AUC_{120–150\ min}$ C-peptide release (r_p=0.622; p<0.001), (**c**) HOMA2-IR (r_s=−0.530; p<0.01) and (**d**) $ISI_{120–150\ min}$ (r_p=0.233; NS). Red filled circles, patients with recent-onset diabetes (n=6); red open circles, patients with diabetes in remission (n=3); orange filled circles, FDRs with dysglycaemia at baseline (n=5); orange open circles, FDRs with euglycaemia at baseline and dysglycaemia during follow-up (n=5); blue filled circles, FDRs with persistent euglycaemia (n=12). r_s, Spearman rank correlation coefficient; r_p, Pearson's correlation coefficient
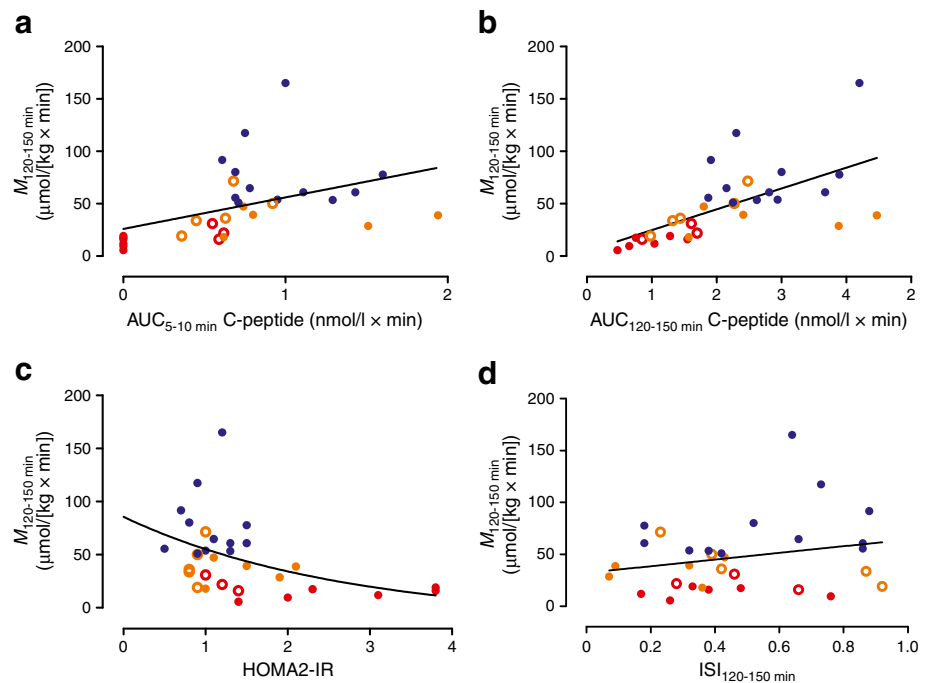
type 1 diabetes. It was conducted with the long-term perspective of identifying minimally invasive criteria for the impending clinical onset of disease and treatment goals for novel beta cell therapies. Comparison with healthy, normoglycaemic controls allowed the establishment of tentative cut-off values for the biomarkers tested.

GV variables were measured under real-life conditions (i.e. without dietary restrictions or compulsory meal times). Their use in predicting diabetes will, however, require studies of larger cohorts for longer periods of time in order to establish robust and harmonised cut-off values. The observed normal ranges for various CGM variables were in line with some previous studies [27–29], but lower than in others [14, 30, 31]. Apart from the limited number of participants in most studies, including the current study, these discrepancies might relate to differences in measuring devices, age ranges, ethnicities, dietary restrictions, risk levels for diabetes or criteria to ascertain normoglycaemia.

Our study provides the first demonstration that GV variables are equally as effective as a hyperglycaemic clamp, the gold standard for assessing beta cell function [19, 20], in discriminating normoglycaemic autoAb$^+$ FDRs from FDRs with (impending) dysglycaemia or diabetes. In this respect, a combination of CGM-derived indices was equally efficient as the glucose disposal rate ($M_{120–150\ min}$) and better than $AUC_{5–10\ min}$ or $AUC_{120–150\ min}$ C-peptide, or a combination of SMBG-derived variables.

Our study also provides the first demonstration of a correlation in (pre)type 1 diabetes between CGM data and clamp-derived variables. This correlation is present for different stages of glucose tolerance, ranging from healthy controls to

normoglycaemic and dysglycaemic risk groups and patients with recent-onset diabetes. Overall, a significant inverse and hyperbolic relationship was documented between GV variables, as assessed by CGM or SMBG, and measures of beta cell function and/or insulin sensitivity, as assessed by the hyperglycaemic clamp. GV variables demonstrated better correlation with $M_{120–150\ min}$, reflecting both insulin secretion and action [19, 20], than with first- or second-phase hormone discharge, and thus performed better in discriminating FDRs who were or who became glucose intolerant, or who developed diabetes within 20 months, from those who remained normoglycaemic.

We have previously validated the hyperglycaemic clamp for the accurate and precise determination of beta cell function in (pre)type 1 diabetes, which has proven useful as an inclusion criterion and outcome measure in beta cell therapy trials [4, 8, 9, 17]. Although the hyperinsulinaemic–euglycaemic clamp is the gold standard for determining insulin sensitivity [20], the hyperglycaemic clamp has also been validated for this purpose [32–35]. This therefore allows measurements of beta cell function relative to insulin sensitivity without the need to perform two clamp tests, which is undesirable because of an excessive burden on the patient, lack of participant or parent acceptability and inflated research costs [30]. We did not correct $M_{120–150\ min}$ for urinary glucose loss as it was assumed to be negligible for glycaemia clamped at 10 mmol/l [19, 33–35]. Likewise, we assumed that endogenous hepatic glucose production would be suppressed by high glucose levels during the procedure, but did not quantify this by administrating tritiated glucose [19]. Despite these limitations, $M_{120–150\ min}$ proved to be the best clamp-derived marker of (impending) glucose intolerance.

We recently observed that AUC C-peptide release during the hyperglycaemic clamp outperformed OGTT-derived variables in predicting diabetes within 3 years in autoAb$^+$ FDRs [8, 9]. Consistent with this, AUC$_{5-10 \, min}$ best distinguished healthy controls from autoAb$^+$ FDRs [9], but clamp-derived $M_{120-150 \, min}$ was more informative than AUC$_{120-150 \, min}$ C-peptide release with respect to the short-term metabolic outcomes of individuals at high autoantibody-inferred risk: this is supported by the better correlation of $M_{120-150 \, min}$ with GV variables and with the presence or development of dysglycaemia or diabetes within 20 months.

Glucose disposal and tolerance are determined not only by insulin secretion, but also by insulin action [36]. Consistent with this, $M_{120-150 \, min}$ was significantly correlated with HOMA2-IR and AUC C-peptide. Our results are in line with observations that the ratio of HOMA-IR to first-phase insulin release during the IVGTT test better predicted type 1 diabetes than first-phase insulin release alone, particularly in individuals with already-compromised insulin release [37–40], and with the strong inverse correlation between oral disposition index and GV variables in individuals at different stages of glucose tolerance, ranging from normoglycaemia to overt type 2 diabetes [41]. Indeed, while in our study euglycaemic FDRs and healthy controls tended to differ only in AUC$_{5-10 \, min}$, $M_{120-150 \, min}$ was the best discriminator between FDRs with or without (impending) dysglycaemia.

The fact that a combination of CGM variables was strongly correlated with $M_{120-150 \, min}$ and equally effective in predicting dysglycaemia or diabetes indicates their potential significance for defining selection criteria or intermediate therapeutic endpoints in secondary prevention trials [5, 42]. This is consistent with observations in recipients of beta cell allografts, where a relationship was seen between clamp-derived variables and a simple measure of GV—the per cent CV of fasting glucose—making the latter a clinically relevant readout [17]. In our group of participants with (pre)type 1 diabetes, such correlations could be demonstrated for several simple measures of GV obtained with CGM or even SMBG, such as SD$_{day}$, % CV$_{day}$ or % time$_{day}$ >7.8 mmol/l glucose. These variables have also the advantage of being easily understood by patients or their relatives [26]. CGM recordings seem preferable to SMBG measurements because they register all variability [26], as illustrated by the fact that combining different variables improved the efficiency of recognising dysglycaemia with CGM, but not with SMBG.

In conclusion, a decreased glucose disposal rate and simple measures of increased GV are closely associated with impending type 1 diabetes. Our results illustrate the feasibility of using these variables to develop entry criteria and therapeutic endpoints in secondary prevention trials. Larger prospective studies will be needed in order to achieve this.

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