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Hyperglycaemia increases dipeptidyl peptidase IV activity in diabetes mellitus

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Abstract *Aims/hypotheses:* Chronic hyperglycaemia increases dipeptidyl peptidase IV (DPP-IV) activity in endothelial cells in vitro. The present study was designed to assess the effect of high glucose on circulating DPP-IV activity in patients with type 1 and type 2 diabetes. *Methods:* Plasma DPP-IV activity was measured in 29 patients with type 1 diabetes and 29 age-, sex- and BMI-matched control subjects. We also assessed DPP-IV activity in 31 type 2 diabetic patients with HbA_1c >8.5% and in plasma from matched groups of 31 newly diagnosed diabetic subjects with HbA_1c <7.5%, 31 subjects with IGT and 62 subjects with NGT. In a further sample of 66 type 2 diabetic patients, a longitudinal study was also performed to evaluate variations in DPP-IV activity and HbA_1c over 3 months. *Results:* DPP-IV activity in type 1 diabetic patients was not significantly different from that in control subjects; however, a significant correlation between DPP-IV and HbA_1c was observed in diabetic subjects (r=0.47; p<0.01). Type 2 diabetic patients with HbA_1c >8.5% showed significantly (p<0.05) higher DPP-IV activity (mean±SD 27.7±7.1 U/l) than newly diagnosed diabetic patients and subjects with IGT (22.1±6.0 and 18.8±8.8 U/l, respectively). Variations in DPP-IV activity over 3 months in type 2 diabetic patients showed a significant positive correlation with variations in HbA_1c (r=0.26; p<0.05). *Conclusions/interpretation:* Chronic hyperglycaemia induces a significant increase in DPP-IV activity in type 1 and type 2 diabetes. This phenomenon could contribute to the reduction in circulating active glucagon-like peptide-1 and to the consequent postprandial hyperglycaemia in type 2 diabetic patients with poor metabolic control.

Keywords Diabetes mellitus · Dipeptidyl peptidase IV

Abbreviations DPP: dipeptidyl peptidase · GIP: gastric inhibitory polypeptide · GLP-1: glucagon-like peptide 1

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Introduction

Glucagon-like peptide 1 (GLP-1) is a gastrointestinal hormone, mainly secreted after meals, which enhances glucose-induced insulin secretion [1, 2] and induces satiety [3]. It has been reported that GLP-1 levels after a mixed meal [4, 5] and after an oral glucose load [6, 7] are reduced in patients with type 2 diabetes. Reduction in oral glucose-stimulated active GLP-1 levels in patients with type 2 diabetes has also been observed in isoglycaemic and iso-insulinaemic conditions, i.e. during a euglycaemic–hyperinsulinaemic clamp [7]. This means that, although blood glucose and insulin levels immediately after the meal could affect GLP-1 secretion [8], differences in these parameters cannot explain the impairment of peptide response to oral glucose observed in diabetic patients. However, the reduction in postprandial circulating active GLP-1 in type 2 diabetic subjects could be the consequence of chronic hyperglycaemia. The impairment of the GLP-1 response to meals could contribute to the reduction in early postprandial insulin secretion in patients with type 2 diabetes; in fact, the administration of GLP-1 receptor antagonists to healthy volunteers elicits an impairment of meal-induced insulin secretion and an increase in postprandial glycaemia similar to that observed in type 2 diabetes [9, 10].

GLP-1 is rapidly inactivated in vivo, with the cleavage of an N-terminal dipeptide; inactivation is catalysed by dipeptidyl peptidase IV (DPP-IV), an enzyme which is produced by endothelial cells in different parts of the body and circulates in plasma [11]. It is still not clear whether the reduction in meal- or oral glucose-stimulated GLP-1 levels in type 2 diabetic patients is due to impairment of secretion, an increase in degradation, or both. GLP-1 gene expression and peptide synthesis have been reported to be unmodified with respect to controls in rodent models of type 1 and type 2 diabetes [12], whereas no data on the effects of high glucose on DPP-IV expression and activity are available in animal models. GLP-1 kinetics were reported to be unmodified with respect to control subjects in a small sample of type 2 diabetic patients [13], suggesting that the reduction in active GLP-1 in diabetic patients could be attributable to impairment of secretion rather than increased degradation. In fact, circulating DPP-IV levels were reported to be reduced in a small sample of elderly patients with type 2 diabetes [14]; in a study on another small sample of subjects, no significant difference in circulating enzyme activity was detected between type 2 diabetic patients and matched healthy controls [5]. On the other hand, DPP-IV mRNA expression and enzyme activity have been reported to be stimulated by hyperglycaemia in human endothelial cells in vitro [15]. In order to elucidate the relationships between chronic hyperglycaemia and DDP-IV in diabetes, enzyme activity was studied in blood samples from patients with type 1 and type 2 diabetes, compared with subjects with IGT or NGT. A longitudinal study of DPP-IV activity over 3 months was also performed in type 2 diabetic patients, in order to explore the possible correlation between variations in enzyme activity and metabolic control.

Subjects, materials and methods

DPP-IV activity in patients with type 1 diabetes Serum DPP-IV activity was measured in a consecutive series of 29 outpatients (16 women, 13 men) with type 1 diabetes, aged 34.3±6.1 years, with a duration of diabetes of 15.4±9.9 years, a BMI of 23.2±2.7 kg/m^2, HbA_1c of 7.5±1.3% and serum C-peptide <0.1 ng/ml. For comparison, a sex-, age- and BMI-matched sample of 29 healthy volunteers was also studied. Subjects in the control group had an age of 35.7±8.2 years and a BMI of 23.3±2.9 kg/m^2.

DPP-IV activity in patients with type 2 diabetes: cross-sectional study Serum DPP-IV activity was measured in a consecutive series of 31 outpatients (20 women, 11 men), aged 67.4±9.1 years, with type 2 diabetes of at least 1-year duration and HbA_1c >8.5%. Of the individuals enrolled, seven were treated with insulin, two with metformin, nine with combinations of two or more hypoglycaemic agents, and 12 with combinations of insulin and hypoglycaemic agents; one patient was not receiving any hypoglycaemic treatment. Their mean (±SD) BMI was 28.9±5.5 kg/m^2. These patients were compared with age-, sex- and BMI-matched subjects selected from individuals enrolled in a study assessing the prevalence of diabetes by oral glucose tolerance testing in a general population sample in a suburban township of Florence [16]. These control subjects consisted of: (1) 31 newly diagnosed type 2 diabetic subjects (fasting glucose >7 mmol/l and/or 2-h postload glucose >11.1 mmol/l), with HbA_1c <7.5%, aged 66.7±8.1 years and with BMI 28.8±2.9 kg/m^2, who were not receiving any hypoglycaemic treatment; (2) 31 subjects with IGT (2-h postload glucose >7.7 mmol/l, with fasting glucose ≤7 mmol/l), aged 65.9±7.8 years and with BMI 28.7±2.7 kg/m^2; and (3) 62 subjects with NGT and normal fasting glycaemia, aged 66.3±6.1 years and with BMI 28.7±2.8 kg/m^2.

For each of the diabetic outpatients enrolled, the first subject in the study database for each category listed (the first two for the NGT group) of the same sex, age (±2 years) and BMI (±1 kg/m^2) was enrolled.

DPP-IV activity in patients with type 2 diabetes: longitudinal study Variations in circulating DPP-IV activity over a period of 90±15 days were assessed in a further sample of 66 type 2 diabetic outpatients (36 women, 30 men) with normal liver function tests, aged 61.8±13.7 years, with duration of diabetes 11.8±9.2 years, BMI 29.0±5.9 kg/m^2 and HbA_1c 7.6±1.3%. Among these patients, those who underwent any variation in dose or type of hypoglycaemic drug prescribed during the 3-month follow-up were excluded from the study, so that all the patients enrolled maintained the same pharmacological treatment for the duration of observation.

Informed consent All the subjects enrolled provided their informed consent. The investigation was carried out in accordance with the Declaration of Helsinki, as revised in 2000.

Laboratory determinations Blood samples were drawn in the morning, after overnight fasting. In diabetic patients, HbA_1c was determined using an HPLC method (Menarini Diagnostics, Florence, Italy), with an upper limit of reference range for healthy subjects of 5.8%.

DPP-IV activity was measured with a colorimetric assay. Gly-Pro-4 *p*-nitroanilide, a chromogenic substrate of DPP-IV, is hydrolysed into the dipeptide Gly-Pro and the product 4-nitroaniline, the rate of appearance of which can be measured spectrophotometrically [17, 18]. To evaluate within- and between-run precision of the DPP-IV assay, the activities of serum samples with low (15 U/l), middle (30 U/l) and high (70 U/l) activity were assessed ten times in 5 days. The coefficients of variation were 2.6, 3.4 and 2.3%, respectively for within-run precision and 1.5, 4.8 and 4.7%, respectively for between-run precision [15].

In order to exclude interference of glucose with the assay, DPP-IV (Linco, St Charles, MO, USA) 100 U/l was incubated at 37°C in HEPES buffer without glucose, and with glucose 5.5 or 22 mmol/l, and activity was measured after 4 h, 24 h and 7 days. Measured activity with glucose 5.5 and 22 mmol/l, when compared with that without

glucose, was 103±3 and 101±3%, respectively after 4 h, 106±1 and 105±3% after 24 h and 104±4 and 102±4% after 7 days (all not significant, ANOVA).

Statistical analysis Because DPP-IV had a normal distribution, Student's unpaired *t*-test was used for comparisons between pairs of groups, ANOVA with the Bonferroni post hoc test for comparisons among more than two groups, and Pearson's methods for the assessment of correlations. Stepwise multiple linear regression was used for multivariate analysis. Significance was assumed at $p<0.05$.

Results

Circulating DPP-IV activity in type 1 diabetic patients DPP-IV activity in blood samples from type 1 diabetic patients was not significantly different from that of samples from matched control subjects (36.2±11.7 vs 34.5±11.8 U/l). In diabetic patients, DPP-IV activity showed a significant correlation with HbA_1c (Fig. 1); in both samples, the correlations of DPP-IV activity with age and BMI were not statistically significant.

Circulating DPP-IV activity in type 2 diabetic patients: cross-sectional study When DPP-IV activity was compared in type 2 diabetic patients with HbA_1c >8.5% and in matched subjects with (1) newly diagnosed diabetes and HbA_1c <7.5%, (2) IGT and (3) NGT, the difference among groups was statistically significant ($p<0.01$ at ANOVA); in post hoc analysis (Bonferroni), DPP-IV activity was significantly higher ($p=0.04$) in type 2 diabetic patients with HbA_1c >8.5% than in subjects with IGT or NGT. On the other hand, when newly diagnosed patients with mild hyperglycaemia were compared with those with IGT or NGT, no significant difference was observed; similarly, DPP-IV activity in subjects with IGT was not significantly different from that measured in individuals with NGT (Fig. 2). Among type 2 diabetic patients with HbA_1c >8.5%, a significant correlation of DPP-IV activity with HbA_1c ($r=0.42$, $p<0.01$), but not age ($r=-0.08$) or BMI ($r=-0.09$) was observed.

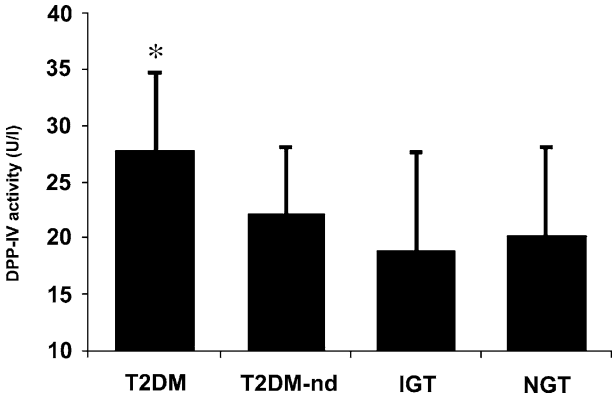

Fig. 2 Plasma dipeptidyl peptidase IV (*DPP-IV*) activity in matched samples of subjects with: (1) known type 2 diabetes and HbA_1c >8.5% (*T2DM*; $n=31$), (2) newly diagnosed type 2 diabetes and HbA_1c <7.5% (*T2DM-nd*; $n=31$), (3) *IGT* ($n=31$) and (4) *NGT* ($n=62$). Data are expressed as means±SD. ANOVA, $p<0.01$; Bonferroni test, *$p<0.05$ vs IGT and NGT

Circulating DPP-IV activity in type 2 diabetic patients: longitudinal study In a further sample of 66 patients with type 2 diabetes, serum DPP-IV activity at baseline (26.9±7.5 U/l) showed a significant inverse correlation with age ($r=-0.31$; $p=0.02$) and a significant direct correlation with HbA_1c ($r=0.25$; $p=0.04$), but not with BMI. After 3 months, no significant variation in mean HbA_1c, BMI or DPP-IV activity in the whole sample was observed (data not shown). Variations in DPP-IV activity in individual patients (Fig. 3) were directly correlated to variations of HbA_1c ($r=0.26$; $p=0.04$), but not in BMI ($r=0.20$; $p>0.05$); the same results were obtained using stepwise multiple linear regression with variation in DPP-IV as the dependent variable and variation in HbA_1c and of BMI as putative factors ($\beta=0.46$ and -0.12 respectively; $p=0.03$ for variation in HbA_1c only).

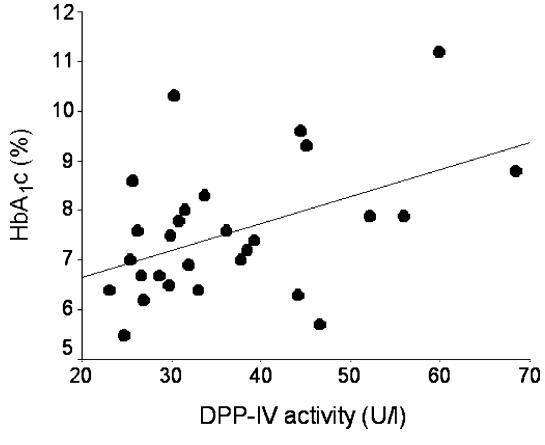

Fig. 1 Correlation between HbA_1c and plasma dipeptidyl peptidase IV (*DPP-IV*) activity in patients with type 1 diabetes and without residual insulin secretion ($r=0.47$, $p<0.01$)

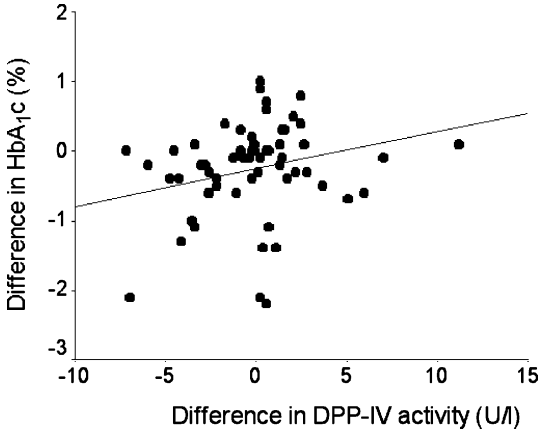

Fig. 3 Correlation between differences from baseline in HbA_1c and plasma dipeptidyl peptidase IV (*DPP-IV*) activity in individual type 2 diabetic patients after 3 months of longitudinal observation ($r=0.26$; $p<0.05$)

Discussion

The present data show for the first time that circulating DPP-IV activity has a direct correlation with the degree of hyperglycaemia in both type 1 and type 2 diabetic patients. Furthermore, longitudinal observation shows that variations in DPP-IV are correlated with variations in HbA_1c, meaning that impairment of metabolic control is associated with increased plasma enzyme activity. In fact, although the elevation of enzyme activity in type 2 diabetic patients with poor metabolic control could be due partly to interference from hypoglycaemic treatment [19], such a mechanism was unable to account for the correlation between variations in DPP-IV and HbA_1c in the longitudinal study. An increase in DPP-IV activity, leading to reductions in active GLP-1 and gastric inhibitory polypeptide (GIP), which stimulate early postprandial insulin secretion, could theoretically induce an elevation of HbA_1c in type 2 diabetic patients. On the other hand, the correlation between DPP-IV and HbA_1c in type 1 diabetic patients without residual insulin secretion cannot be attributed to an effect of enzyme activity on the degree of hyperglycaemia.

Taken together, these data suggest that chronic hyperglycaemia could lead to stimulation of DPP-IV activity. It should be observed that DPP-IV is expressed at the surface of endothelial cells [15] and that circulating enzyme accounts for only a fraction of total DPP-IV activity. Furthermore, differences in structure and function between circulating and endothelial DPP-IV, and the contribution of each component to incretin degradation, are not known in detail. For this reason, circulating DPP-IV cannot be assumed to be an accurate measure of total DPP-IV activity. The present data do not provide any information on the effect of hyperglycaemia on endothelial cell DPP-IV activity. However, our previous study [15] showed that prolonged exposure to high glucose is capable of increasing DPP-IV expression, confirming the stimulatory effect of chronic hyperglycaemia on enzyme activity. Further studies are needed to verify the impact of the hyperglycaemia-induced increase in endothelial and circulating DPP-IV on meal-stimulated active GLP-1 levels in diabetic patients.

On the other hand, type 2 diabetic patients with a mild to moderate degree of hyperglycaemia, as well as subjects with IGT, do not show significantly elevated plasma DPP-IV activity; this suggests that only a severe degree of hyperglycaemia is capable of inducing increased DPP-IV expression. The lack of a significant difference in DPP-IV activity between type 1 diabetic patients and matched controls is attributable to the fact that most diabetic subjects studied had good metabolic control, with a modest elevation of HbA_1c. In fact, in vivo studies have shown a relevant stimulation of endothelial cell DPP-IV mRNA expression and activity with exposure to glucose 11–22 mmol/l. The apparently contrasting results of previous studies reporting unmodified [5] or slightly reduced [14] circulating DPP-IV activity in type 2 diabetic patients could be due either to inadequate sample size or to the inclusion in the samples of some patients with mild or moderate hyperglycaemia.

DPP-IV catalyses the inactivation of several hormones and neuropeptides, including GLP-1 and GIP, another insulinotropic gastrointestinal hormone stimulated by meals. GLP-1 kinetics was reported to be unmodified with respect to control subjects in a sample of type 2 diabetic patients [13], apparently contradicting the present results. However, it should be observed that the small size of the samples or the inclusion of some patients with mild or moderate hyperglycaemia could have prevented the detection of differences. On the other hand, some studies reported a reduction in meal-stimulated circulating levels of intact GIP [5, 8]; furthermore, GIP clearance has been shown to be increased in patients with type 2 diabetes [20], which is consistent with increased DPP-IV activity.

GLP-1 levels after a mixed meal [4, 5] and after an oral glucose load [6, 7] have been reported to be reduced in patients with type 2 diabetes when compared with matched control subjects. Some of these studies were performed on samples of patients with a mild degree of hyperglycaemia [7], showing that the impairment of the GLP-1 response to meals is an early event in the natural history of diabetes. On the other hand, in the present study the increase in DPP-IV activity was observed only for a higher degree of hyperglycaemia or for chronic hyperglycaemia. The possibility that mild hyperglycaemia is capable of stimulating endothelial cell, but not circulating, DPP-IV activity, cannot be ruled out; however, the hypothesis of an impairment of GLP-1 secretion as an early event leading to glucose intolerance in type 2 diabetic patients appears more probable. This latter hypothesis should be tested in specifically designed studies assessing GLP-1 secretion in vivo in humans.

The mechanisms underlying the increase in circulating DPP-IV activity determined by hyperglycaemia are still unclear. Exposure to high glucose could determine an increase in endothelial DPP-IV mRNA expression and protein secretion, as suggested by previous studies in vitro [15]; alternatively, endothelial damage determined by hyperglycaemia could induce the release of vascular DPP-IV. Finally, hyperosmolarity and/or non-enzymatic glycation could modify circulating enzyme activity.

A limitation of our study arises from the fact that other peptidases, such as fibroblast activation protein α, DPP-7, DPP-8, DPP-9 and prolylcarboxypeptidase, could have interfered with the assay. However, these enzymes are present in plasma at much lower concentrations than DPP-IV [21]; furthermore, GLP-1 is a substrate for most of those peptidases as well as for DPP-IV.

In conclusion, mild hyperglycaemia does not appear to modify circulating DPP-IV activity, suggesting that the impairment of the GLP-1 response to meals in the earlier phases of type 2 diabetes could be due to a reduction in hormone secretion. However, once moderate or severe hyperglycaemia is established, high glucose induces an increase in DPP-IV activity, which could contribute to the reduction in active GLP-1 levels, and could therefore play a role in the pathogenesis of postprandial hyperglycaemia.

Furthermore, the increase in DPP-IV activity could determine an increase in circulating levels of the inactivated form of the hormone GLP-1(9-36)amide, which is a GLP-1 receptor antagonist [22, 23], possibly contributing to hyperglycaemia. The nature of the impaired GLP-1 response in type 2 diabetic patients with mild hyperglycaemia, as well as the relative contribution of the hyperglycaemia-induced elevation of DPP-IV to the reduction in active hormone levels in patients with poor metabolic control, needs further investigation.

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