Plasma dipeptidyl peptidase-IV activity in patients with type-2 diabetes mellitus correlates positively with HbAlc levels, but is not acutely affected by food intake

Jakob Ryskjær, Carolyn F Deacon1, Richard D Carr2, Thure Krarup, Sten Madsbad3, Jens Holst1 and Tina Vilsbøll

Department of Internal Medicine F, Gentofte Hospital, University of Copenhagen, Niels Andersens Vej 65, DK-2900 Hellerup, Denmark, 1Department of Medical Physiology, The Panum Institute, Copenhagen, Denmark, 2Novo Nordisk A/S, Bagsvaerd, Denmark and 3Department of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark

(Correspondence should be addressed to T Vilsbøll; Email: t.vilsboll@dadlnet.dk)

Abstract

Objective: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide are incretin hormones, secreted in response to meal ingestion. The incretin hormones stimulate insulin secretion and are essential for the maintenance of normal plasma glucose concentrations. Both incretin hormones are metabolized quickly by the enzyme dipeptidyl peptidase-IV (DPP-IV). It is well known that type-2 diabetic patients have an impaired incretin effect. Therefore, the aim of the present study was to investigate plasma DPP-IV activity in the fasting and the postprandial state in type-2 diabetic patients and control subjects.

Design: The study included two protocols. Protocol one involved 40 fasting type-2 diabetic patients (28 men); age 61 ± 1.4 years; body mass index (BMI) 31 ± 0.6 kg/m²; HbAlc 7.2 ± 0.2%; and 20 matched control subjects (14 men) were studied. Protocol two involved eight type-2 diabetic patients (six men); age 63 ± 1.2 years; BMI 33 ± 0.5 kg/m²; HbAlc 7.5 ± 0.4%; eight matched control subjects were included.

Methods: In protocol one, fasting values of DPP-IV activity were evaluated and in protocol two, postprandial DPP-IV activity during a standard meal test (566 kcal) was estimated.

Results: Mean fasting plasma DPP-IV activity (expressed as degradation of GLP-1) was significantly higher in this patient group compared with the control subjects (67.5 ± 1.9 vs 56.8 ± 2.2 fmol GLP-1/h (mean ± S.E.M.); P = 0.001). In the type-2 diabetic patients, DPP-IV activity was positively correlated to FPG and HbAlc and negatively to the duration of diabetes and age of the patients. No postprandial changes were seen in plasma DPP-IV activity in any of the groups.

Conclusions: Plasma DPP-IV activity increases in the fasting state and is positively correlated to FPG and HbAlc levels, but plasma DPP-IV activity is not altered following meal ingestion and acute changes in plasma glucose.

European Journal of Endocrinology 155 485–493

Introduction

The two major incretin hormones, glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP, previously known as gastric inhibitory polypeptide), are secreted from the small intestine in response to meal ingestion and act on specific receptors on the β-cells. Both are metabolized by the enzyme dipeptidyl peptidase-IV (DPP-IV) (1, 2), which is primarily found not only in the brush-border membranes of the kidney and the small intestine, but also in other tissues (e.g. hepatocytes around bile canaliculi, epithelial cells of the pancreas, and in capillary endothelium), and in a soluble form in plasma (3). DPP-IV cleaves GLP-1 and GIP at the N terminus, resulting in the formation of metabolites, which lack insulinotropic activity (4). The cleavage can be blocked by specific DPP-IV inhibitors, resulting in increased plasma concentrations of the intact peptides (5, 6) and improved glucose tolerance (7). Therefore, DPP-IV inhibitors have been proposed as a possible pharmacological treatment of type-2 diabetes (8), and many compounds are presently in clinical development (9). The effect of GIP and GLP-1 is essential for the maintenance of normal glucose tolerance. In 1986, Nauck et al. showed that type-2 diabetic patients have a markedly impaired incretin effect, which may contribute to the relative insulin deficiency seen in these patients (10). Clinical studies suggest that a reduced postprandial secretion of GLP-1 (11, 12) combined with an
impaired insulinotropic effect of GIP (13, 14) contribute to the impaired incretin effect in type-2 diabetic patients. Pilot studies carried out in animal models of type-2 diabetes have demonstrated that plasma DPP-IV activity levels correlate positively with plasma glucose and HbA1c and negatively with plasma insulin levels, raising the possibility that endogenous DPP-IV activity may influence glucose homeostasis by altering the levels of intact (insulinotropic) incretin hormones. Few clinical studies examining plasma DPP-IV activity levels in patients with type-2 diabetes have reported unchanged (15), increased (16), and decreased (17, 18) plasma activity. Urinary DPP-IV activity has been shown to correlate positively with worsening glucose tolerance (19, 20). The aim of the present study was, therefore, to investigate plasma DPP-IV activity in the fasting state and evaluate whether plasma DPP-IV activity per se is acutely regulated during meal ingestion, both in patients with type-2 diabetes and in control subjects. Theoretically, acute changes in the concentrations of intact and total forms of incretin hormones during meal ingestion could be a result of either changes in hormone release or changes in the mechanisms responsible for their processing within, or removal from, the circulation. Furthermore, we evaluated whether a relationship between plasma DPP-IV activity and plasma glucose, insulin, incretin hormone concentrations and HbA1c could be found in the diabetic patients. Finally, since it has been suggested that metformin may have effects on both plasma DPP-IV activity (21, 22) and GLP-1 secretion (23, 24), the possible effects of metformin were also assessed.

Material and methods

Subjects

The study was divided into two protocols. Protocol one involved 40 fasting type-2 diabetic patients (28 men, 12 women; age (mean ± S.E.M.) 61 ± 1.4 years; body mass index (BMI) 30.8 ± 0.6 kg/m²; and HbA1c 7.2 ± 0.2%) and 20 control subjects, matched for age, gender, and BMI (14 men, 6 women; age 60 ± 2.0 years; BMI 30.9 ± 0.9 kg/m²; and HbA1c 5.4 ± 0.1%). Sixteen patients were treated with diet alone, and 24 treated with diet and oral antidiabetic medication (sulphonylurea and/or biguanides). Twenty-six patients had a history of hypertension and were treated with ACE inhibitors, thiazides, and/or calcium antagonists. In protocol two, eight type-2 diabetic patients (six men, two women; mean age 62.5 ± 1.2 years, BMI 32.7 ± 0.5 kg/m²; and HbA1c 7.5 ± 0.4%) and eight control subjects (six men, two women; mean age 60 ± 1.2 years, BMI 32.9 ± 0.7 kg/m²; and HbA1c 5.5 ± 0.1%) were selected from protocol one and studied during a standard breakfast meal test. The participants for protocol two were selected to have a BMI between 30 and 35 kg/m². Three patients were treated with diet alone, whereas five were treated with both diet and oral antidiabetic medication. All type-2 diabetic patients were diagnosed according to the criteria of the World Health Organization (25). No patient had albuminuria above 300 mg/day or proliferative retinopathy and all had normal liver function and were without history of gastrointestinal diseases. All control subjects were without family history of type-2 diabetes and all had a normal 75 g oral glucose tolerance test, which was carried out immediately before inclusion in the study. All agreed to participate and gave oral and written consent. The study was approved by the Copenhagen County Ethical Committee on 31 January 2002 (journal numbers in the committee: KA01175), and the study was conducted according to the Helsinki Declaration.

The subjects studied were chosen to represent typical obese type-2 diabetic outpatients and all were kept on their regular oral antidiabetic medication, which was continued until the evening before the study.

Methods

Protocol one: after an overnight fast, blood samples were collected in fluoride tubes for measurement of plasma glucose, in precooled glass tubes containing EDTA plus DPP-IV inhibitor (valine-pyrrolidide: 0.01 mmol/l, final concentration) and aprotonin (500 KIU/ml blood; Trasylo; Bayer) for incretin hormone measurements, and in precooled glass tubes containing heparin plus aprotonin for insulin, C-peptide, and DPP-IV activity measurements. Blood samples were immediately centrifuged at 4 °C and the plasma was stored at 20 °C (incretin hormones) and 80 °C (insulin, C-peptide and DPP-IV activity) until analysis.

Protocol two: After a 10-h overnight fast, a cannula was inserted into a cubital vein for blood sampling during the study. The test meal (34% fat, 47% carbohydrate, 19% protein, a total of 566 kcal (2730 kJ)), consisted 50 g white bread, 50 g black bread, 10 g butter, 40 g cheese, 20 g sugar-free jam, and 200 ml milk. The meal was consumed within 15 min. Blood samples were drawn 15, 10, and 0 min before ingestion of the meal and again 15, 30, 45, 60, 75, 90, 120, 150, and 180 min after ingestion. Blood samples were collected in accordance with the procedure described under protocol one.

Analysis

Plasma glucose concentrations were measured during the experiment using a glucose oxidase method in a glucose analyzer (Yellow Springs Instrument model: YSI 2300 STAT plus analyser; Yellow Springs, Ohio, USA). Plasma samples were assayed for total GLP-1 immunoreactivity, as previously described by Ørskov et al. (26), using a RIA (antisera no. 89 390), which is specific for the C-terminal of the GLP-1 molecule. Intact
GLP-1 was measured using an ELISA, as previously described (27). The assay is a two-site sandwich assay using two monoclonal antibodies; GLP-1F5 as the catching antibody (C-terminally directed) and Mab26.1 as the detecting antibody (N-terminally directed) (28). Total GIP was measured, as described previously, using the C-terminally directed antisera Mab26.1 as the catching antibody (C-terminally directed) (28). Total GIP and GLP-1 concentrations were similar in the type-2 diabetic patients compared with control subjects (Table 1). HOMA analysis revealed a higher IR in the type-2 diabetic patients compared with the control subjects (4.7 ± 0.4 vs 2.1 ± 0.2 (mean ± s.e.m.; P < 0.001).

**Incretin hormones** Significantly higher concentrations of both intact GIP and intact GLP-1 were seen in the type-2 diabetic patients compared with control subjects (Table 1). Total GIP and GLP-1 concentrations were similar in the two groups. No correlation between fasting levels of incretin hormones to plasma DPP-IV activity was found (data not shown). As a measure for DPP-IV degradation of the incretin hormones ratios between intact/total were calculated. However, there was no correlation between these ratios and plasma DPP-IV activity.

**DPP-IV activity** The mean fasting plasma DPP-IV activity was significantly higher in the patient group compared with the control subjects (67.5 ± 1.9 vs 56.8 ± 2.2 fmol GLP-1/h (mean ± s.e.m.) respectively; P = 0.001). In the patient group, a positive correlation was seen between DPP-IV activity and plasma glucose, HbAlc, insulin, and C-peptide concentrations (Fig. 1). A negative correlation was found between plasma DPP-IV activity and duration of diabetes, as well as the age of the patients (Fig. 1). No correlation was seen between DPP-IV activity and BMI. Multiple linear regression

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Fasting results in type-2 diabetic patients and control subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PG (mmol/l)</td>
</tr>
<tr>
<td>Type-2 diabetic patients</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>5.4 ± 0.1</td>
</tr>
</tbody>
</table>

PG, plasma glucose; p-DPP-IV, plasma dipeptidyl peptidase-IV; GIP, glucose-dependent insulinovertopic polypeptide; GLP-1, glucagon-like peptide-1.
analysis, with plasma glucose, insulin, C-peptide, HbAlc, age, and duration of diabetes as putative determinants of DPP-IV activity and GLP-1 levels were performed. Insulin and age were significant covariates for DPP-IV activity.

**Effects of metformin** The patient group was also subdivided according to whether they were treated with metformin or not (Table 2). Analysis of these sub-groups revealed no significant differences in DPP-IV activity or total and intact GLP-1 levels ($P=0.48$, $0.66$, $0.78$ respectively). However, both total and intact GIP concentrations were higher in the group receiving metformin ($P=0.002$, $0.004$ respectively). Multivariate analysis was performed in order to rule out the possibility that confounders (HbAlc, age, duration of diabetes, and BMI) prevented the observation of any significant difference between metformin-treated patients, but no differences were found.

**Protocol two – meal test**

**Plasma glucose, insulin, and C-peptide** Plasma glucose concentrations are shown in Fig. 2. In the patient group, the peak of postprandial insulin concentrations (mean ± S.E.M.) was seen at 60 min ($398 ± 94$ pmol/l) and peak C-peptide concentration occurred after 150 min ($2701 ± 339$ pmol/l). Corresponding results in control subjects were (peak values after 60 min) $469 ± 280$ and $2742 ± 355$ pmol/l respectively (Fig. 2). No significant differences in AUCl$_{insulin}$ or AUCl$_{C-peptide}$ were seen when comparing type-2 diabetic patients with control subjects. HOMA-analysis revealed a significantly higher IR in the

---

**Figure 1** Graphs showing associations between plasma DPP-IV activity and (A) plasma glucose, (B) insulin, (C) C-peptide, (D) HbAlc, (E) age, and (F) duration of diabetes.
type-2 diabetic patients compared with the control subjects (6.8 ± 1.7 vs 2.8 ± 0.7; P = 0.04).

**Incretin hormones** Baseline total and intact GIP concentrations (mean ± s.e.m.) were 8.6 ± 2.1 and 7.4 ± 0.8 pmol/l respectively in the patient group. Corresponding results for the control subjects were 7.4 ± 2.0 and 6.0 ± 1.2 pmol/l respectively (Fig. 3). Peak concentrations of total and intact GIP occurred at 60 and 45 min in the patient group respectively after meal ingestion and amounted to 138 ± 16 and 65 ± 10 pmol/l. In the control subjects, the corresponding results were 131 ± 11 and 61 ± 6 pmol/l respectively with peak concentrations also at 60 and 45 min. In the group of type-2 diabetic patients, the mean basal total GLP-1 was 23.4 ± 0.9 pmol/l compared to 19.9 ± 1.7 pmol/l in the matched control subjects (NS). Peak total GLP-1 concentrations occurred at 45 (38.8 ± 3.6 pmol/l) and 90 min (30.9 ± 2.4 pmol/l) in the patients and matched control subjects group respectively (Fig. 3). In both groups, both GLP-1 and GIP concentrations returned toward basal levels at the end of the experiment. Total and intact GIP AUC showed no significant differences between diabetic patients and control subjects. Total and incremental AUCGLP-1 (total), 0–180 min were not significantly different between the groups ((mean ± S.E.M., patients versus control subjects) 5950 ± 660 vs 4877 ± 285 min pmol per l (P = 0.158) and 1735 ± 560 vs 1296 ± 402 min pmol per l (P = 0.54) respectively). In both groups, both GLP-1 and GIP concentrations returned toward basal levels at the end of the experiment. Total and incremental AUCGLP-1 (intact), 0–180 min were not significant different when comparing the control subjects with the type-2 diabetics (706 ± 103 vs 437 ± 146 min pmol per l(P = 0.48) and 100 ± 83 vs 322 ± 89 min pmol per l (P = 0.33) respectively). No correlation between incremental AUC ratios (intact/total) and DPP-IV activity was found.

**DPP-IV activity** Plasma DPP-IV activity was slightly higher (though not statistically significant), both in the fasting state as well as postprandial, in the type-2 diabetic patients compared with the control subjects (Fig. 4). Significant changes in the DPP-IV activity after the ingestion of the breakfast meal were not seen in either of the groups. No difference in AUC was seen between the type-2 diabetics and the control subjects (NS).

**Discussion**

Previous studies have shown that the incretin hormones, GIP and GLP-1, stimulate insulin secretion and are essential for the maintenance of normal plasma glucose concentrations. It has also been shown that
type-2 diabetic patients have an impaired incretin effect. Since both incretin hormones are metabolized quickly by the enzyme DPP-IV, this raises the possibility that an increase in DPP-IV concentration and/or activity may contribute to the impaired incretin effect found in the patients. In the fasting study (protocol one), plasma DPP-IV activity was significantly higher in the patients compared with controls, and a similar tendency was seen before the meal test (protocol two), in agreement with the study of Mannucci et al. (16), but in contrast to those of Korosi et al. (17) and Meneilly et al. (18), who reported decreased levels. According to Mannucci et al. (16), plasma DPP-IV activity is related to the prevailing glycemic control, with significant elevations in activity first becoming apparent as HbAlc levels increase above 8.5%, which may, at least partially, explain some of the apparent conflicting results to be found in the literature, since the patients in the studies reporting decreased plasma DPP-IV activity had HbAlc levels of 8.0% or below (17, 18). However, in other patients with HbAlc levels below 8.5%, increased (present study) or unchanged (present study, 15, 16) DPP-IV activity compared to controls was found. These apparent differences may simply be coincidental, arising from the natural variability together with the use of different methodology for assessing plasma DPP-IV activity. Nevertheless, as glucose control worsens, it seems that there is a connection between glycemia and DPP-IV activity, as indicated by the finding of a positive correlation between plasma DPP-IV activity and HbAlc levels in type-2 diabetes (present study), protocol one and Ref. (16) and between plasma DPP-IV activity and fructosamine concentrations in type-1 diabetes (33). Whether this is a primary or secondary effect is at present unclear, but hyperglycemia does not appear to affect DPP-IV enzymatic activity per se, as indicated from experiments, where the purified enzyme was incubated under high glucose conditions for up to 1 week (16, 34). In contrast, exposure to elevated glucose can increase DPP-IV gene expression, which in turn results in changes in levels of DPP-IV activity. Thus, Pala et al. (34) showed that mRNA for DPP-IV in human glomerular endothelial cells cultured in vitro with
glucose increased dose-dependently with increasing glucose concentrations. This increased DPP-IV expression seemed, however, to be due to the associated hyperosmolarity of the incubation medium rather than the glucose per se, but nonetheless, hyperosmolarity could not account entirely for the effects of high glucose to increase endothelial DPP-IV activity, suggesting that additional mechanisms must also be involved (34).

Previous studies have suggested that some oral antidiabetic agents can alter soluble DPP-IV activity. Thus, both Lindsay et al. (22) and Lenhard et al. (21) reported that treatment with metformin was associated with reduced levels of DPP-IV activity in plasma or serum in vivo, and Lenhard et al. (21) also found that pioglitazone treatment resulted in similar reductions. The underlying mechanism of this action of metformin is controversial. Using in vitro activity assays, Lindsay et al. (22, 23) found that metformin inhibited DPP-IV activity, leading them to conclude that it behaves as a DPP-IV inhibitor by acting directly on the enzyme. In contrast, Hinke et al. (35), Yasuda et al. (24), and Lenhard et al. (21) were unable to influence the in vitro enzymatic activity, suggesting that neither metformin nor pioglitazone is a conventional competitive DPP-IV inhibitor. Metformin may, however, alter enzyme activity in a manner unrelated to competitive inhibition. For example, Lenhard et al. (21) suggested that these agents reduce the levels of the soluble form of the enzyme (and hence activity) by influencing secretion/release of the membrane-bound enzyme to the blood. Metformin-associated increased levels of GLP-1 (23, 24) have been attributed both to stimulation of GLP-1 secretion (24) and reduced GLP-1 degradation (23). In the present study (protocol one), metformin did not appear to influence plasma DPP-IV activity, and there were no differences in the proportion of intact GLP-1 or GIP, supporting the conclusions of Hinke (35), Yasuda (24) and Lenhard (21) suggested that metformin does not act as a DPP-IV inhibitor. Although fasting GLP-1 levels (both intact and total) were not affected by metformin, interestingly, both intact and total GIP were elevated in the patients on metformin treatment (Table 2), suggesting that metformin may somehow stimulate K-cell secretion.

Short-term changes in glycemia, such as those associated with meal ingestion, seem not to acutely affect plasma DPP-IV activity (protocol two), supporting previous observations that the enzymatic activity is unchanged during the oral glucose tolerance test (18, 36). However, long-term exposure to changes in glycemia can alter DPP-IV activity, as indicated by the positive correlation between changes in HbA1c levels and plasma DPP-IV activity over time (16). This could reflect that a long-term response is required to change tissue DPP-IV expression (34), leading to altered DPP-IV activity, raising the possibility that increased DPP-IV activity may be functionally related to poor metabolic control.

In light of the finding that plasma DPP-IV activity is increased in type-2 diabetes, it is tempting to speculate that this may result in increased degradation of the incretin hormones, leading to an impaired incretin effect and ultimately a reduced insulin secretion and hyperglycemia. However, in the present study, this does not appear to be the case. Under fasting conditions, intact incretin concentrations were actually higher in the diabetic patients than in the control subjects, leading to increases in the ratios (intact as a proportion of total) in the patients, despite their higher plasma DPP-IV activity levels. This observation illustrates the caveat that has previously been suggested, namely that measurement of DPP-IV activity in the plasma may not necessarily reflect the degree of in vivo degradation of the incretin hormones by DPP-IV (37), particularly as this relates to the endogenously released peptides. This is because the N-terminal truncation of both GLP-1 and GIP in vivo proceeds much more rapidly than can be explained solely by the activity of the soluble form of enzyme in the plasma. On the contrary, it is much more likely that the membrane-bound DPP-IV (particularly that associated with the capillary endothelium in close proximity to the intestinal endocrine cells (38)) is responsible. In support of this, the half-life of both incretin hormones in human plasma is much longer in vitro than that found in vivo in humans (2, 31, 39).

Thus, in the present study, intact incretin hormone concentrations were elevated in the patients, which may contribute to the hyperinsulinemia also present in these subjects. Previous studies from this laboratory showing that postprandial incretin concentrations, in particular GLP-1, are reduced in type-2 diabetes (11), seem at odds with the present observations of increased GLP-1 and GIP concentrations in the patients. However, this may reflect population and protocol differences between the two studies. Thus, in the study by Vilsbøll et al. (11), the patients were in poorer metabolic control (HbA1c of 9.2%) and underwent a washout period of 3 days of oral hypoglycemic agents prior to the meal test. In contrast, in the present
study, the patients had better metabolic control (HbA1c of 7.5%) and oral antidiabetic medication was continued until the evening prior to the study.

In summary, we found increased plasma DPP-IV activity in the fasting state of type-2 diabetic patients. This was not associated with a decrease in active incretin hormones, suggesting that measurement of plasma DPP-IV activity does not provide a predictive index of in vivo endogenous incretin hormone degradation. A positive correlation between DPP-IV activity and HbA1c suggests that metabolic control may influence the level of DPP-IV activity as found by others. This, however, may require long-term exposure to high glucose concentrations. Thus, we found that DPP-IV activity is not acutely regulated after ingestion of a meal, suggesting that incretin release, rather than processing in the plasma, is responsible for acute increases in incretin levels observed during meal ingestion.

Acknowledgements

We thank Jytte Purtoft and Susanne Reimer for technical assistance and Lene Hansen for statistical support.

References


15 Tolts-Nielsen M, Damholt M, Hilsted J, Hughes TE, Krarrup T, Madsbad S & Holst J. GLP-1 secretion is decreased in NIDDM patients compared to matched control subjects with normal glucose tolerance. *Diabetologia* 1999 42 143.
21 Lenhard JM, Croom DK & Minnick DT. Reduced serum dipeptidyl peptidase-IV after metformin and pioglitazone treatments. *Biochemical and Biophysical Research Communications* 2004 324 92–97.

Received 28 February 2006
Accepted 30 May 2006