Dipeptidyl Peptidase 4 inhibition by Vildagliptin and the effect on insulin secretion and action in response to meal ingestion in type 2 diabetes

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**Objective:** To determine the mechanism by which Dipeptidyl peptidase 4 (DPP-4) inhibitors lower postprandial glucose concentrations.

**Research Design and Methods:** We measured insulin secretion and action as well as glucose effectiveness in 14 subjects with type 2 diabetes who received vildagliptin (50mg bid) or placebo for 10-days in random order separated by a three-week washout. On day 9 of each period, subjects ate a mixed meal. Insulin sensitivity ($S_i$), Glucose effectiveness ($GE$) and β-cell responsivity indices were estimated using the oral glucose and C-Peptide minimal model. At 300 minutes 0.02 Units per Kg insulin was administered intravenously.

**Results:** Vildagliptin reduced postprandial glucose concentrations (905±94 vs. 1008±104 mmol per 6h, $p=0.02$). Vildagliptin did not alter net insulin sensitivity ($S_i$; 7.71±1.28 vs. 6.41±0.84 10⁻⁴ dl/kg/min/uU/ml, $p=0.13$) or glucose effectiveness, ($GE$, 0.019±0.002 vs. 0.018±0.002 dl/kg/min, $p=0.65$). However the net β-cell responsivity index ($\phi_{total}$) was increased (35.7±5.2 vs. 28.9±5.2 10⁻⁹ min⁻¹, $p=0.03$) as was DI$_{total}$ (381±48 vs. 261±35 10⁻¹⁴ dl/kg.min⁻² per pmol/L, $p=0.006$). Vildagliptin lowered postprandial glucagon concentrations (27.0±1.1 vs. 29.7±1.5 µg/L per 6h, $p=0.03$), especially after administration of exogenous insulin (81.5±6.4 vs. 99.3±5.6 ng/l, $p=0.02$).

**Conclusion:** Vildagliptin lowers postprandial glucose concentrations by stimulating insulin secretion and suppressing glucagon secretion but not by altered insulin action or glucose effectiveness. A novel observation is that vildagliptin alters α-cell responsiveness to insulin administration but the significance of this is as yet unclear.
Glucagon-like peptide-1 (GLP-1) is a peptide hormone produced by the enteroendocrine L cells of the intestinal mucosa and is released in response to caloric intake. The major form of secreted GLP-1, GLP-1(7,36)-amide, is a powerful insulin secretagogue which also suppresses glucagon secretion in a glucose-dependent fashion and may increase insulin action (1). This would theoretically make the hormone ideal therapy for use in type 2 diabetes – a disorder characterized by defective insulin secretion and action.

However, GLP-1 is rapidly inactivated by dipeptidyl peptidase-4 (DPP-4), a widely-distributed enzyme, which converts the intact peptide to the metabolite GLP-1-(9, 36)-amide. GLP-1 based therapy for type 2 diabetes has required the development of GLP-1 receptor agonists such as exenatide which are resistant to the action of DPP-4, or alternatively, compounds that inhibit DPP-4 and thereby raise endogenous concentrations of active GLP-1 (2). Administration of GLP-1 (3), GLP-1 receptor agonists (4) and DPP-4 inhibitors (2) all lower postprandial glucose concentrations.

GLP-1 and its analogues delay gastric emptying (5) whereas DPP-4 inhibitors do not (6) indicating that the effects of the latter on postprandial glucose concentrations must occur via other mechanisms. It is uncertain if the lack of gastrointestinal effects of DPP-4 inhibitors occurs because the resulting rise in peripheral active GLP-1 concentrations is not elevated or sustained, in marked contrast with concentrations observed during peripheral GLP-1 infusion. Another potential explanation is that DPP-4 inhibition may alter concentrations of other gut hormones with effects on appetite or motility (such as PYY) which neutralize the effect of GLP-1(7). DPP-4 inhibitors, GLP-1 and its analogues decrease postprandial glucagon concentrations (2). In contrast to GLP-1 and GLP-1 receptor agonists, the effect of DPP-4 inhibition on insulin secretion has been more uncertain – placebo-controlled studies have demonstrated similar insulin concentrations in the presence or absence of DPP-4 inhibition, despite lower glucose concentrations (6). This would imply that such compounds also increase insulin secretion for a given glucose concentration, as has been demonstrated previously using model-based parameters of β-cell function (8).

It is possible, however, that these agents lower postprandial glucose concentrations through changes in insulin action and glucose effectiveness. The direct effects of GLP-1 on the ability of glucose per se to stimulate its own uptake and suppress its own release (glucose effectiveness) are less clear (9). Some (10; 11) but not all (12) studies have suggested that when given in pharmacologic doses GLP-1 increases the ability of insulin and glucose to stimulate glucose uptake and to suppress glucose production. Similar controversy exists with regards to the effects of GLP-1 on insulin action (9). Given the known differences of DPP-4 inhibitors, in comparison to other GLP-1-based therapy, it is possible that these compounds also differ with regard to their direct effects on glucose metabolism.

In order to gain greater insight into the mechanism(s) by which DPP-4 inhibitors lower postprandial glucose concentrations we utilized a randomized, double blind, placebo-controlled crossover design where subjects received vildagliptin, a DPP-4 inhibitor, or placebo over a ten day period. The Disposition Index, a measure of insulin secretion for the prevailing insulin action, was measured using the oral glucose (13) and oral C-peptide minimal models (14). Glucose effectiveness was also measured simultaneously. We report that while vildagliptin stimulated insulin secretion and
enhanced suppression of glucagon, it had no effect on either insulin action or glucose effectiveness. Taken together with previous studies in the same subjects indicating that vildagliptin does not alter gastric emptying (6), these data indicate that DPP-4 inhibitors lower postprandial glucose concentrations solely by alterations of islet cell function.

RESEARCH DESIGN AND METHODS

Subjects—After approval from the Mayo Institutional Review Board, 14 subjects with type 2 diabetes gave written informed consent to participate in the study. All subjects were in good health and were at a stable weight and did not engage in regular vigorous exercise. Subjects were not taking medication known to alter gastric emptying such as narcotics or calcium channel blockers. None of the subjects had a history of microvascular complications of diabetes. All subjects were instructed to follow a weight maintenance diet containing ~55% carbohydrate, 30% fat, and 15% protein for the period of study. All oral agents used for the treatment of diabetes were discontinued three weeks before the study.

Experimental Design—We utilized a randomized, double blind, placebo-controlled crossover design. Subjects received either vildagliptin 50 mg or placebo taken before breakfast and the evening meal over a ten-day treatment period with the two treatment intervals being separated by at least a two-week washout period. The order of treatment was random. The trial was registered at www.clinicaltrials.gov. Identifier NCT00351507.

Subjects were admitted to the GCRC on the evening of the 6th day of the treatment period. Gastric accommodation was measured on the 7th day of the treatment period. The maximum tolerated volume of caloric or non-caloric liquids was measured on the 8th and 10th day of the treatment period to examine the effect of DPP-4 inhibition on satiety and postprandial gastrointestinal symptoms. Glucose turnover and gastric emptying were measured simultaneously on the 9th day of the treatment period; those results have been previously reported (6). Glucose, insulin and C-peptide concentrations measured prior to, and following, ingestion of a mixed meal on day 9 were analyzed using the oral and C-peptide minimal models are the subject of the current report.

In brief, following an 8 hour fast, a forearm vein was cannulated with an 18g needle to allow infusions to be performed. An 18g cannula was inserted retrogradely into a vein of the dorsum of the contra-lateral hand. This was placed in a heated Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood. At -180 minutes a primed continuous infusion of [6,6-2H2] glucose was initiated. Subjects received the morning dose (vildagliptin 50 mg or placebo) at -30 minutes. At time 0 subjects consumed a meal consisting of 2 scrambled eggs labeled with 0.75 mCi 99mTc-Sulfur colloid, 55g of Canadian bacon, 240ml of water and Jell-O containing 75g of glucose labeled with [1-13C] glucose – (4% enrichment). This provided 510 Kcal (61% carbohydrate, 19% protein and 21% fat). An infusion of [6-3H] glucose was started at this time and the infusion rate varied to mimic the anticipated glucose appearance of the meal [1-13C] glucose as previously described (15). At the same time, the rate of infusion of the [6,6-2H2] glucose was altered so as to approximate the anticipated pattern of fall in endogenous glucose production (15). Blood was collected at frequent intervals. To allow a model-independent assessment of the effect of vildagliptin on insulin action, five hours after the study start (300 minutes) subjects received 0.02 Units per Kg body weight of insulin intravenously (over a 5 minute period).

Analytical techniques—Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at -20°C until
assayed. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide were measured by RIA using reagents supplied by Linco Research (St. Louis, MO). Samples tubes utilized for measurement of GLP-1 had 100 µM of DPP-4 inhibitor (Linco Research, St. Louis, MO) added. Active GLP-1 concentrations were measured using an N-terminal immunoassay supplied by Linco Research (St. Louis, MO).

**Calculations**—Net insulin sensitivity (SI), and net glucose effectiveness (GE) were measured using the unlabeled oral minimal model whereas the effects of insulin (S_I*) and glucose (GE*) on glucose disposal were measured with the labeled oral minimal model as previously described (13). β-Cell responsivity indexes were estimated from the plasma glucose and C-peptide concentrations observed during the experiment by using the oral C-peptide minimal model (14), incorporating age-associated changes in C-peptide kinetics as measured by Van Cauter et al. (16). The model assumes that insulin secretion is comprised of a static and dynamic component. The dynamic component is likely to represent secretion of promptly releasable insulin and is proportional to the rate of increase of glucose concentrations through a parameter, φ_dynamics, which defines the dynamic responsivity index. The static component represents the provision of new insulin to the releasable pool and is characterized by a static index, φ_static, and by a delay time constant, T (14). Disposition indices were calculated as previously described to determine the appropriateness of insulin secretion for the prevailing degree of insulin action multiplying φ_total, φ_dynamics and φ_static, by SI.

**Statistical analysis**—All data are presented as means ± SEM. Paired comparisons between the treatment and placebo group were made using Student’s two-way t-test for paired samples. A p-value of less than 0.05 was considered to be statistically significant.

**RESULTS**

**Volunteer Characteristics**—Subject characteristics have been previously reported (6). Briefly, mean age was 53.1 ± 2.0 years, body mass index was 33.9 ± 1.5 Kg/M² and lean body mass was 73.8 ± 2.3 Kg. The mean HbA1c was 6.1 ± 0.2%. In 4 subjects diabetes was treated with diet alone, 7 had been treated with metformin monotherapy and the remaining 3 subjects were treated with a sulfonylurea and metformin combination.

**Plasma glucose, insulin, c-peptide and glucagon concentrations (figure 1)**—Administration of vildagliptin resulted in lower fasting glucose (7.3 ± 0.5 vs. 7.9 ± 0.5 mmol/l, p = 0.005), a lower post meal peak (14.1 ± 0.6 vs. 15.9 ± 0.9 mmol/l, p = 0.0008) and a lower glycemic area above basal (905 ± 94 vs. 1008 ± 104 mmol per 6h, p = 0.02) over the duration of study (Panel A).

Insulin concentrations (Panel B) did not differ when subjects received vildagliptin or placebo before (54 ± 8 vs. 63 ± 8 pmol/l, p = 0.11) or after (63.1 ± 10.5 vs. 62.1 ± 10.0 nmol per 5h, p = 0.76) meal ingestion. At 300 minutes subjects received 1.47 ± 0.04 IU of insulin intravenously over a 5 minute period. Subsequent peak insulin concentrations did not differ in the presence or absence of vildagliptin (367 ± 20 vs. 363 ± 36 pmol/l, p = 0.92).

Fasting C-peptide concentrations (Panel C) did not differ when subjects received vildagliptin or placebo before (54 ± 8 vs. 63 ± 8 pmol/l, p = 0.11) or after (63.1 ± 10.5 vs. 62.1 ± 10.0 nmol per 5h, p = 0.76) meal ingestion. At 300 minutes subjects received 1.47 ± 0.04 IU of insulin intravenously over a 5 minute period. Subsequent peak insulin concentrations did not differ in the presence or absence of vildagliptin (367 ± 20 vs. 363 ± 36 pmol/l, p = 0.92).

Fasting C-peptide concentrations (Panel C) did not differ in the fasting state (0.85 ± 0.08 vs. 1.00 ± 0.12 nmol/l, p = 0.17) or after meal ingestion (728 ± 60 vs. 746 ± 75 nmol per 6h, p = 0.69).

Fasting glucagon concentrations (Panel D) did not differ (70.2 ± 3.6 vs. 75.1 ± 5.4 ng/l, p = 0.1) in the presence or absence of vildagliptin. However, over the first 5 hours
after meal ingestion, treatment with vildagliptin resulted in lower postprandial glucagon concentrations \((20.9 \pm 1.6\text{ vs. } 23.7 \pm 1.3\text{ mg per 5h, } p = 0.03)\). Intriguingly, insulin administration (300 minutes) in the presence of vildagliptin was associated with suppression of glucagon to a nadir of \((81.5 \pm 6.4\text{ vs. } 99.3 \pm 5.6\text{ ng/l, } p = 0.02)\) at 315 minutes.

**Insulin Action**

Vildagliptin (upper panel) did not alter net insulin action \((S_I, 7.71 \pm 1.28\text{ vs. } 6.41 \pm 0.84 \times 10^{-4}\text{ dl/kg/min/uU/ml, } p = 0.13)\) or (lower panel) the effects of insulin on glucose disposal \((S_{I*}, 4.37 \pm 0.98\text{ vs. } 4.83 \pm 1.14\text{ dl/kg/min/uU/ml, } P = 0.53)\).

The effect of exogenous insulin on glucose concentrations over the last hour of the study was used as a model-independent estimate of insulin action. The change in glucose concentrations over the last hour of the study, after insulin administration, did not differ (-0.75 \pm 0.3\text{ vs. } -1.0 \pm 0.25\text{ mmol/l, } p = 0.22) in the presence or absence of vildagliptin (Figure 1, Panel A).

**Glucose Effectiveness**—Vildagliptin (upper panel) did not alter net glucose effectiveness, measured using the unlabeled oral minimal model \((GE, 0.019 \pm 0.002\text{ vs. } 0.018 \pm 0.002\text{ dl/kg/min, } p = 0.65)\) or (lower panel) the effect of glucose on glucose disposal measured with the labeled oral minimal model \((GE*, 0.011 \pm 0.001\text{ vs. } 0.010 \pm 0.001\text{ dl/kg/min, } P = 0.40)\).

**Insulin secretion and Disposition Indices** (figure 2)—Vildagliptin increased \((\phi_{\text{dynamic}}, 817 \pm 208\text{ vs. } 621 \pm 184\text{ }10^{-9}, p = 0.14)\), although this difference was not significant. On the other hand \((\phi_{\text{static}}, 30.4 \pm 4.3\text{ vs. } 24.5 \pm 4.2\text{ }10^{-9}\text{ min}^{-1, p = 0.03})\) and \((\phi_{\text{total}}, 35.7 \pm 5.2\text{ vs. } 28.9 \pm 5.2\text{ }10^{-9}\text{ min}^{-1, p = 0.03})\) were significantly increased compared to placebo. Expressing these indices as a function of prevailing insulin action (Disposition Index) demonstrated (upper, middle and lower right panels) increased insulin secretion in the presence of vildagliptin compared to placebo for \(DI_{\text{dynamic}}\) \((8654 \pm 1335\text{ vs. } 5904 \pm 1513\text{ }10^{-14}\text{ dl/kg/min per pmol/L, } p = 0.02)\), \(DI_{\text{static}}\) \((324.2 \pm 40.7\text{ vs. } 219.0 \pm 24.6\text{ }10^{-14}\text{ dl/kg.min}^{-2}\text{ per pmol/L, } p = 0.007)\) and \(DI_{\text{total}}\) \((381 \pm 48\text{ vs. } 261 \pm 35\text{ }10^{-14}\text{ dl/kg.min}^{-2}\text{ per pmol/L, } p = 0.006)\).

As we previously reported (6), fasting concentrations of active GLP-1 \((3.7 \pm 1.0\text{ vs. } 3.8 \pm 1.1\text{ pmol/l, } p = 0.68)\) did not differ between groups. After meal ingestion, in the presence of vildagliptin, concentrations rose \((11.8 \pm 2.0\text{ pmol/l vs. } 5.8 \pm 0.8, p = 0.01)\) and remained elevated for the duration of the study as shown by the area under the curve \((2224 \pm 330\text{ vs. } 1527 \pm 376\text{ pmol/l per 6h, } p = 0.001)\).

**CONCLUSION**

The present studies indicate that DPP-4 inhibitors increase insulin secretion and suppress glucagon concentrations. In contrast they do not alter hepatic insulin clearance, insulin action or glucose effectiveness. Taken together with the previous report in the same subjects indicating that DPP-4 inhibitors do not alter gastric emptying (6), these data demonstrate that DPP-4 inhibitors lower postprandial glucose concentrations solely by altering alpha and beta cell function. GLP-1’s effects on insulin action are controversial. Some, but not all, studies suggest that GLP-1, when given at pharmacologic concentrations, directly enhances glucose uptake and suppresses glucose production (9). The present data indicate that short-term (9 days) inhibition of DPP-4 does not alter insulin action in people with type 2 diabetes. Insulin action was measured using the labeled and unlabeled oral minimal model. In addition, the changes in glucose concentration over the last hour of the study, following insulin injection did not differ in the presence or absence of vildagliptin. Vildagliptin was only
administered for 9 days prior to the experiment, raising the possibility that an effect on insulin action might have been observed with longer periods of administration. Such an experiment would however have to account for the possibility that improved glycemic control per se might improve insulin action. The present data indicate that short-term administration of DPP-4 inhibitors does not lower postprandial glucose concentrations by increasing insulin action.

Our experiment differs from a recently published study (17) which utilized a similar double-blind, placebo-controlled, crossover design. However, vildagliptin (or placebo) was administered over a 42-day period. Insulin action was measured using a two-step insulin infusion-glucose clamp. Under euglycemic, hyperinsulinemic conditions a slight, but significant, increase in glucose disposal in the presence of vildagliptin was observed. This would imply that vildagliptin improves insulin action in people with type 2 diabetes. A potential explanation for this discrepancy is that improved glycemic control associated with DPP-4 inhibition alleviates glucose toxicity (18) or lipotoxicity (17) – phenomena more likely to occur over an extended rather than a brief period of administration. Another possible explanation is the greater imprecision of the model-dependent parameters of insulin action and the smaller sample size of our study meant that we were unable to detect a small change in $S_i$ produced by vildagliptin.

Some (10; 11), but not all (12), previous reports have suggested that GLP-1 can improve glucose effectiveness. The present data indicate that DPP-4 inhibitors do not improve glucose effectiveness. As with insulin action, glucose effectiveness was measured using both the unlabeled and labeled oral minimal models. The unlabeled model measures the net effect of glucose on suppression of glucose production and stimulation of glucose uptake. In contrast, the labeled minimal model only measures the ability of glucose to enhance its own uptake. Neither was altered by short-term treatment with vildagliptin. Therefore, as is the case for insulin action, Vildagliptin-mediated enhancement of glucose effectiveness cannot account for the lower postprandial glucose concentrations observed with DPP-4 inhibitors.

DPP-4 inhibition improved insulin secretion both when measured as global secretion ($\phi_{total}$) or relative to insulin action (Disposition index – DI). The increase was due to an increase in $\phi_{static}$ - a measure of insulin secretion at a given glucose concentration as well as an increase in $\phi_{dynamic}$ – a measure of insulin secretion in response to changing glucose concentrations. A prior experiment specifically designed to compare the effect of oral versus intravenous glucose on the β-cell responsivity to glucose in healthy subjects showed that oral glucose delivery increased both the $\phi_{static}$ and the $\phi_{dynamic}$ indices of insulin secretion (19). These results suggested that incretins modulate multiple steps in the process of insulin secretion in healthy subjects. Likewise, in this experiment the use of a DPP-4 inhibitor resulted in improvements in both static and dynamic indices of insulin secretion despite relatively small changes in incretin concentrations in the presence of vildagliptin (6). DPP-4 inhibition seems to improve multiple defects in the insulin secretory cascade (secretory granule priming or docking) in people with type 2 diabetes. These data are consistent with the previous reports examining the effect of DPP-4 inhibition on insulin secretion in people with type 2 diabetes (8; 17; 20-23).

Defective postprandial suppression of glucagon is an important contributor to postprandial hyperglycemia in people with type 2 diabetes (24), especially in the presence of defective insulin secretion (25).
Consistent with previous studies (17), postprandial glucagon concentrations were lower in the presence of vildagliptin. This could occur via multiple mechanisms including a direct effect of GLP-1 on α-cells, increased somatostatin secretion by islet δ-cells or a consequence of increased islet insulin concentrations. However, in this study we observed a novel effect of insulin injection at the start of the final hour of the study. This was undertaken in order to derive a model-independent measurement of insulin action. Surprisingly, in the presence of vildagliptin, this was accompanied by a greater suppression of glucagon than occurred in the absence of DPP-4 inhibition. This occurred in every subject studied so this observation is unlikely to be due to chance alone. Glucose concentrations were virtually identical at 300 minutes implying that inhibition of DPP-4, and the changes in incretin concentrations that this produces, enhances the ability of insulin to directly suppress glucagon release. Future studies will be required to determine the mechanism by which this occurs but this finding implies that incretin-induced increases in insulin secretion will result in an even greater suppression of glucagon release than that which would be observed for a comparable increase of intra-islet insulin in the absence of DPP-4 inhibition.

In summary, vildagliptin lowers glucose concentrations through its effects on insulin and glucagon secretion. In this study we also demonstrate through modeling and model-independent methodology that vildagliptin has no direct effect on insulin action, to increase glucose utilization or decrease glucose production. A novel observation is that vildagliptin alters α-cell responsiveness to insulin administration but the mechanism and significance of this is as yet unclear and requires further study.

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Figure Legends

Figure 1: Glucose, Insulin, C-Peptide and Glucagon concentrations in the presence and absence of Vildagliptin.

Figure 2: B-cell responsivity and Disposition Indices in the presence (solid bars - ■) and absence (open bars - □) of Vildagliptin (* = p < 0.05).
