Dipeptidyl Peptidase-4 Inhibition by Vildagliptin and the Effect on Insulin Secretion and Action in Response to Meal Ingestion in Type 2 Diabetes

Chiara Dalla Man, phd[1]
Gerlies Bock, md[2]
Paula D. Giesler, rn[2]
Denise B. Serra, pharmd[3]
Monica Ligueros Saylan, md[3]
James E. Foley, phd[3]

Michael Camilleri, md[4]
Gianna Toffolo, phd[1]
Claudio Cobelli, phd[1]
Robert A. Rizza, md[2]
Adrian Vella, md[2]

OBJECTIVE — The purpose of this study was to determine the mechanism by which dipeptidyl peptidase-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 (50 mg b.i.d.) or placebo for 10 days in random order separated by a 3-week washout. On day 9 of each period, subjects ate a mixed meal. Insulin sensitivity (S_I), glucose effectiveness, and β-cell responsivity indexes were estimated using the oral glucose and C-peptide minimal models. At 300 min 0.02 unit/kg insulin was administered intravenously.

RESULTS — Vildagliptin reduced postprandial glucose concentrations (905 ± 94 vs. 1,008 ± 104 mmol/6 h, $P = 0.02$). Vildagliptin did not alter net S_I (7.71 ± 1.28 vs. 6.41 ± 0.84 10^{-4} dl·kg^{-1}·min^{-1}·μU^{-1}·ml^{-1}, $P = 0.13$) or glucose effectiveness (0.019 ± 0.002 vs. 0.018 ± 0.002 dl·kg^{-1}·min^{-1}, $P = 0.65$). However, the net β-cell responsivity index was increased (35.7 ± 5.2 vs. 28.9 ± 5.2 10^{-9} min^{-1}, $P = 0.03$) as was total disposition index (381 ± 48 vs. 261 ± 35 10^{-14} dl·kg^{-1}·min^{-2}·pmol^{-1}·l^{-1}, $P = 0.006$). Vildagliptin lowered postprandial glucagon concentrations (27.0 ± 1.1 vs. 29.7 ± 1.5 μg·l^{-1}·6 h^{-1}, $P = 0.03$), especially after administration of exogenous insulin (81.5 ± 6.4 vs. 99.3 ± 5.6 ng/l, $P = 0.02$).

CONCLUSIONS — 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 action is as yet unclear.

Diabetes Care **32:14–18, 2009**

G lucagon-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 that also suppresses glucagon secretion in a glu-cose-dependent fashion and may increase insulin action (1). These characteristics 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). GLP-1 (3), GLP-1 receptor agonists (4), and DPP-4 inhibitors (2) all lower postprandial glucose concentrations.

GLP-1 and its analogs 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 whether 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 peptide YY), which neutralize the effect of GLP-1 (7). DPP-4 inhibitors, GLP-1, and its analogues decrease postprandial glucagon concentrations (2). In contrast with 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 result implies 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 effec-

tiveness) are less clear (9). Some (10,11) but not all (12) studies have suggested that, when given in pharmacological doses, GLP-1 increases the ability of insulin and glucose to stimulate glucose uptake and to suppress glucose production. Similar controversy exists with regard to the effects of GLP-1 on insulin action (9). Given the known differences in DPP-4 inhibitors, in comparison with other GLP-1–based therapy, it is possible that these compounds also differ with regard to their direct effects on glucose metabolism.

To gain greater insight into the mechanism(s) by which DPP-4 inhibitors lower postprandial glucose concentrations, we used a randomized, double-blind, placebo-controlled crossover design in which subjects received vildagliptin, a DPP-4 inhibitor, or placebo over a 10-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 whereas 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 — After approval from the Mayo Clinic 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 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 3 weeks before the study.

We used a randomized, double-blind, placebo-controlled crossover design. Subjects received either 50 mg vildagliptin or placebo taken before breakfast and the evening meal over a 10-

day treatment period with the two treatment intervals being separated by at least a 2-week washout period. The order of treatment was random.

Subjects were admitted to the General Clinical Research Center 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 volumes of caloric or noncaloric liquids were 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 reported previously (6). Glucose, insulin, and C-peptide concentrations measured before and after ingestion of a mixed meal on day 9 and analyzed using the oral and C-peptide minimal models are the subject of the current report.

In brief, after an 8-h fast, a forearm vein was cannulated with an 18-gauge needle to allow infusions to be performed. An 18-gauge cannula was inserted retrogradely into a vein of the dorsum of the contralateral hand. This was placed in a heated Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood. At −180 min a primed continuous infusion of [6,6-^{2}H$_2$]glucose was initiated. Subjects received the morning dose (50 mg vildagliptin or placebo) at −30 min. At time 0 subjects consumed a meal consisting of two scrambled eggs labeled with 0.75 mCi ^{99m}Tc-sulfur colloid, 55g of Canadian bacon, 240 ml of water, and Jell-O containing 75 g glucose labeled with [1-^{13}C]glucose (4% enrichment). This provided 510 kcal (61% carbohydrate, 19% protein, and 21% fat). An infusion of [6-^{3}H]glucose was started at this time, and the infusion rate varied to mimic the anticipated glucose appearance of the meal [1-^{13}C]glucose as described previously (15). At the same time, the rate of infusion of the [6,6-^{2}H$_2$]glucose was altered 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, 5 hours after the study start (300 min) subjects received 0.02 unit/kg body weight of insulin intravenously (over a 5-min 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 (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide were measured by radioimmunoassay using reagents supplied by Linco Research (St. Louis, MO). Sample tubes used for measurement of GLP-1 had 100 μmol/l of DPP-4 inhibitor (Linco Research) added. Active GLP-1 concentrations were measured using an N-terminal immunoassay supplied by Linco Research.

Calculations
Net insulin sensitivity (S_I) and net glucose effectiveness were measured using the unlabeled oral minimal model, whereas the effects of insulin and glucose on glucose disposal were measured with the labeled oral minimal model as described previously (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 comprises 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, $\phi_{dynamic}$, 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 indexes were calculated as described previously to determine the appropriateness of insulin secretion for the prevailing degree of insulin action by multiplying β-cell responsivity indexes (ϕ_{total}, $\phi_{dynamic}$, and ϕ_{static}) by S_I.

Statistical analysis
All data are means ± SEM. Paired comparisons between the treatment and placebo group were made using a two-way Student's t test for paired samples. $P <$ 0.05 was considered to be statistically significant.
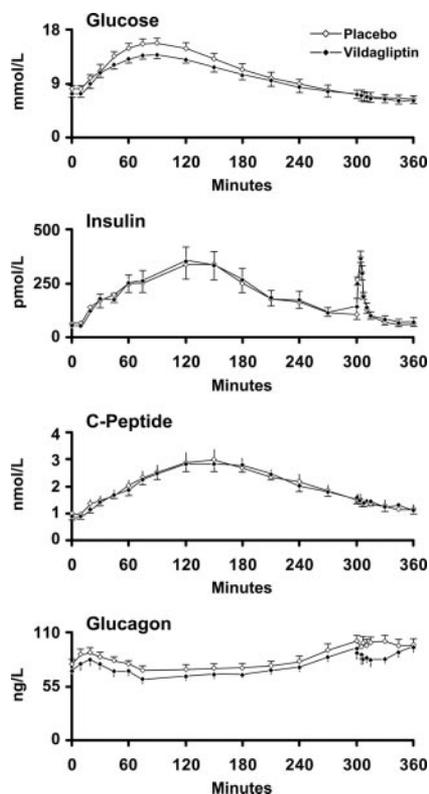
Figure 1—*Glucose, insulin, C-peptide and glucagon concentrations in the presence and absence of vildagliptin.*

RESULTS — Subject characteristics have been reported previously (6). Briefly, mean age was 53.1 ± 2.0 years, BMI was 33.9 ± 1.5 kg/m², and lean body mass was 73.8 ± 2.3 kg. A1C was 6.1 ± 0.2%. In four subjects diabetes was treated with diet alone, seven subjects had been treated with metformin mono-therapy, and the remaining three subjects were treated with a sulfonylurea and met-formin combination.

Plasma glucose, insulin, C-peptide, and glucagon concentrations
Administration of vildagliptin resulted in lower fasting glucose (7.3 ± 0.5 vs. 7.9 ± 0.5 mmol/l, $P = 0.005$), a lower postmeal 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. 1,008 ± 104 mmol/6 h, $P = 0.02$) over the duration of the study (Fig. 1*A*). Insulin concentrations (Fig. 1*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/5 h, $P = 0.76$) meal ingestion. At 300 min subjects received 1.47 ± 0.04 IU of insulin intravenously over a 5-min period. Subsequent peak insulin concen-

trations did not differ in the presence or absence of vildagliptin (367 ± 20 vs. 363 ± 36 pmol/l, $P = 0.92$).

C-peptide concentrations (Fig. 1*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/6 h, $P = 0.69$). Fasting glucagon concentrations (Fig. 1*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 h after meal in-gestion, treatment with vildagliptin re-sulted in lower postprandial glucagon concentrations (20.9 ± 1.6 vs. 23.7 ± 1.3 mg/5 h, $P = 0.03$). Intriguingly, insulin administration (300 min) in the presence of vildagliptin was associated with sup-pression of glucagon to a nadir of 81.5 ± 6.4 vs. 99.3 ± 5.6 ng/l ($P = 0.02$) at 315 min.

Insulin action
Vildagliptin did not alter net insulin ac-tion (S_I 7.71 ± 1.28 vs. 6.41 ± 0.84 10^{-4} dl·kg^{-1}·min^{-1}·μU^{-1}·ml^{-1}, $P = 0.13$) or the effects of insulin on glucose dis-posal (4.37 ± 0.98 vs. 4.83 ± 1.14 dl·kg^{-1}·min^{-1}·μU^{-1}·ml^{-1}, $P = 0.53$). The effect of exogenous insulin on glu-cose 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 insu-lin administration, did not differ (−0.75 ± 0.3 vs. −1.0 ± 0.25 mmol/l, $P = 0.22$) in the presence or absence of vildagliptin (Fig. 1*A*).

Glucose effectiveness
Vildagliptin (Fig. 2, *top panel*) did not al-ter net glucose effectiveness, measured using the unlabeled oral minimal model (0.019 ± 0.002 vs. 0.018 ± 0.002 dl·kg^{-1}·min^{-1}, $P = 0.65$), or (Fig. 2, *bottom panel*) the effect of glucose on glucose dis-posal, measured with the labeled oral minimal model (0.011 ± 0.001 vs. 0.010 ± 0.001 dl·kg^{-1}·min^{-1}, $P = 0.40$).

Insulin secretion and disposition indexes
Vildagliptin increased $\phi_{dynamic}$ (817 ± 208 vs. 621 ± 184 10^{-9}, $P = 0.14$) (Fig. 2, *top left panel*), although this difference was not significant. On the other hand, ϕ_{static} (30.4 ± 4.3 vs. 24.5 ± 4.2 10^{-9} min^{-1}, $P = 0.03$) (Fig. 2, *center left panel*) and ϕ_{total} (35.7 ± 5.2 vs. 28.9 ± 5.2 10^{-9} min^{-1}, $P = 0.03$) (Fig. 2, *bottom left*

panel) were significantly increased com-pared with placebo. Expressing these in-dexes as a function of prevailing insulin action (disposition index [DI]) demon-strated (Fig. 2, *top, center, and bottom right panels*) increased insulin secretion in the presence of vildagliptin compared with placebo for DI$_{dynamic}$ (8,654 ± 1,335 vs. 5,904 ± 1,513 10^{-14} dl·kg^{-1}·min^{-1}·pmol^{-1}·l^{-1}, $P = 0.02$), DI$_{static}$ (324.2 ± 40.7 vs. 219.0 ± 24.6 10^{-14} dl·kg^{-1}·min^{-2}·pmol^{-1}·l^{-1}, $P = 0.007$), and DI$_{total}$ (381 ± 48 vs. 261 ± 35 10^{-14} dl·kg^{-1}·min^{-2}·pmol^{-1}·l^{-1}, $P = 0.006$).

As we reported previously (6), fasting concentrations of active GLP-1 (3.7 ± 1.0 vs. 3.8 ± 1.1 pmol/l, $P = 0.68$) did not differ between groups. After meal inges-tion, in the presence of vildagliptin, con-centrations rose (11.8 ± 2.0 vs. 5.8 ± 0.8 pmol/l, $P = 0.01$) and remained elevated for the duration of the study as shown by the area under the curve (2,224 ± 330 vs. 1,527 ± 376 pmol·l^{-1}·6 h^{-8}, $P = 0.001$).

CONCLUSIONS — The present studies indicate that DPP-4 inhibitors in-crease insulin secretion and suppress glu-cagon concentrations. In contrast, they do not alter hepatic insulin clearance, insulin action, or glucose effectiveness. Taken to-gether with the previous report in the same subjects indicating that DPP-4 in-hibitors do not alter gastric emptying (6), these data demonstrate that DPP-4 inhib-itors lower postprandial glucose concen-trations solely by altering α- and β-cell function.

The effects of GLP-1 on insulin action are controversial. Some, but not all, stud-ies suggest that GLP-1, when given at pharmacological concentrations, directly enhances glucose uptake and suppresses glucose production (9). The present data indicate that short-term (9 days) inhibi-tion of DPP-4 does not alter insulin action in individuals with type 2 diabetes. Insu-lin action was measured using the labeled and unlabeled oral minimal model. In ad-dition, the changes in glucose concentra-tion over the last hour of the study after insulin injection did not differ in the pres-ence or absence of vildagliptin. Vildaglip-tin was only administered for 9 days before the experiment, raising the possi-bility that an effect on insulin action might have been observed with longer pe-riods of administration. Such an experi-ment would, however, have to account for the possibility that improved glycemic control per se might improve insulin ac-
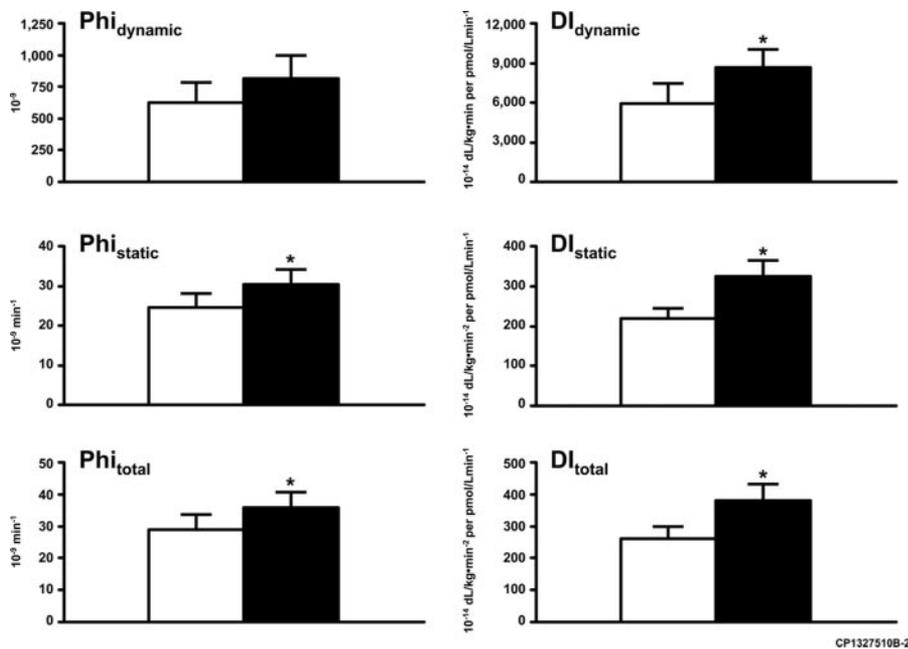
Figure 2—*β-Cell responsivity (Phi) and disposition indexes (DI) in the presence (■) and absence (□) of vildagliptin.* *P < 0.05.

tion. 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) that used 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 result implies that vildagliptin improves insulin action in individuals 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 that 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 (ϕ_{total}) or relative to insulin action (disposition index). The increase was due to an increase in ϕ_{static} (a measure of insulin secretion at a given glucose concentration) as well as to 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 ϕ_{static} and the $\phi_{dynamic}$ indexes 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 indexes 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 individuals with type 2 diabetes. These data are consistent with the previous reports examining the effect of DPP-4 inhibition on insulin secretion in individuals with type 2 diabetes (8,17,20–23).

Defective postprandial suppression of glucagon is an important contributor to postprandial hyperglycemia in individuals 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 lowering could occur via multiple mechanisms including a direct effect of GLP-1 on α-cells, increased somatostatin secretion by islet δ-cells, or 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 injection was given to derive a model-independent measurement of insulin action. Surprisingly, in the presence of vildagliptin, it was accompanied by a greater suppression of glucagon than in the absence of DPP-4 inhibition, which occurred in every subject studied. Thus, this observation is unlikely to be due to chance alone. Glucose concentrations were virtually identical at 300 min, implying that inhibition of DPP-4 and the changes produced in incretin concentrations enhance the ability of insulin to directly suppress glucagon release. Further studies will be required to determine the mechanism by which this suppression occurs, but this finding implies that incretin-induced increases in insulin secretion will result in an even greater suppression of glucagon release than would be observed for a comparable increase of intraislet 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 finding are as yet unclear and require further study.

References

1. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744, 1993
2. Drucker DJ, Nauck MA: The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 368:1696–1705, 2006
3. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1(7–36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316–1322, 1992
4. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD: Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 28:1092–1100, 2005
5. Schirra J, Wank U, Arnold R, Goke B, Katschinski M: Effects of glucagon-like peptide-1(7–36)amide on motility and sensation of the proximal stomach in humans. *Gut* 50:341–348, 2002
6. Vella A, Bock G, Giesler PD, Burton DB, Serra DB, Saylan ML, Dunning BE, Foley JE, Rizza RA, Camilleri M: Effects of dipeptidyl peptidase-4 inhibition on gastrointestinal function, meal appearance, and glucose metabolism in type 2 diabetes. *Diabetes* 56:1475–1480, 2007
7. Vella A, Bock G, Giesler PD, Burton DB, Serra DB, Saylan ML, Deacon CF, Foley JE, Rizza RA, Camilleri M: The effect of dipeptidyl peptidase 4 inhibition on gastric volume, satiation and enteroendocrine secretion in type 2 diabetes: a double blind, placebo-controlled crossover study. *Clin Endocrinol (Oxf)* 69:737–744, 2008
8. Mari A, Sallas WM, He YL, Watson C, Ligueros-Saylan M, Dunning BE, Deacon CF, Holst JJ, Foley JE: Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed β-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metab* 90:4888–4894, 2005
9. Vella A, Rizza RA: Extrapancreatic effects of GIP and GLP-1. *Horm Metab Res* 36:830–836, 2004
10. Prigeon RL, Quddusi S, Paty B, D'Alessio DA: Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. *Am J Physiol Endocrinol Metab* 285:E701–E707, 2003
11. D'Alessio DA, Prigeon RL, Ensinck JW: Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes. a physiological role of glucagon-like peptide I. *Diabetes* 44:1433–1437, 1995
12. Vella A, Shah P, Basu R, Basu A, Holst JJ, Rizza RA: Effect of glucagon-like peptide 1(7–36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. *Diabetes* 49:611–617, 2000
13. Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C: Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. *Am J Physiol Endocrinol Metab* 289:E909–E914, 2005
14. Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C: Oral glucose tolerance test minimal model indexes of β-cell function and insulin sensitivity. *Diabetes* 50:150–158, 2001
15. Basu R, Di Camillo B, Toffolo G, Basu A, Shah P, Vella A, Rizza R, Cobelli C: Use of a novel triple-tracer approach to assess postprandial glucose metabolism. *Am J Physiol Endocrinol Metab* 284:E55–E69, 2003
16. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
17. Azuma K, Radikova Z, Mancino J, Toledo FG, Thomas E, Kangani C, Dalla Man C, Cobelli C, Holst JJ, Deacon CF, He Y, Ligueros-Saylan M, Serra D, Foley JE, Kelley DE: Measurements of islet function and glucose metabolism with the dipeptidyl peptidase 4 inhibitor vildagliptin in patients with type 2 diabetes. *J Clin Endocrinol Metab* 93:459–464, 2008
18. LeRoith D: β-Cell dysfunction and insulin resistance in type 2 diabetes: role of metabolic and genetic abnormalities. *Am J Med* 113 (Suppl. 6A):3S–11S, 2002
19. Campioni M, Toffolo G, Shuster LT, Service FJ, Rizza RA, Cobelli C: Incretin effect potentiates β-cell responsivity to glucose as well as to its rate of change: OGTT and matched intravenous study. *Am J Physiol Endocrinol Metab* 292:E54–E60, 2007
20. Ahren B, Hughes TE: Inhibition of dipeptidyl peptidase-4 augments insulin secretion in response to exogenously administered glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide in mice. *Endocrinology* 146:2055–2059, 2005
21. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A: Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 89:2078–2084, 2004
22. Balas B, Baig MR, Watson C, Dunning BE, Ligueros-Saylan M, Wang Y, He YL, Darland C, Holst JJ, Deacon CF, Cusi K, Mari A, Foley JE, DeFronzo RA: The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. *J Clin Endocrinol Metab* 92:1249–1255, 2007
23. Brazg R, Xu L, Dalla Man C, Cobelli C, Thomas K, Stein PP: Effect of adding sitagliptin, a dipeptidyl peptidase-4 inhibitor, to metformin on 24-h glycaemic control and β-cell function in patients with type 2 diabetes. *Diabetes Obes Metab* 9:186–193, 2007
24. Shah P, Vella A, Basu A, Basu R, Schwenk WF, Rizza RA: Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 85:4053–4059, 2000
25. Shah P, Basu A, Basu R, Rizza R: Impact of lack of suppression of glucagon on glucose tolerance in humans. *Am J Physiol* 277:E283–E290, 1999