0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(8):4888–4894

doi: 10.1210/jc.2004-2460

Vildagliptin, a Dipeptidyl Peptidase-IV Inhibitor, Improves Model-Assessed β-Cell Function in Patients with Type 2 Diabetes

A. Mari, W. M. Sallas, Y. L. He, C. Watson, M. Ligueros-Saylan, B. E. Dunning, C. F. Deacon, J. J. Holst, and J. E. Foley

National Research Center, Institute of Biomedical Engineering (A.M.), Padova, Italy; Novartis Pharmaceuticals Corp. (W.M.S., M.L.-S., J.E.F.), East Hanover, New Jersey 07936; Novartis Institutes for Biomedical Research (Y.L.H., C.W.), Cambridge, Massachusetts 02139; PharmaWrite, LLC (B.E.D.), Princeton, NJ 08540; and Panum Institute, University of Copenhagen (C.F.D., J.J.H.), Copenhagen, Denmark

Aims/Hypothesis: The dipeptidyl peptidase IV inhibitor, vildagliptin, increases levels of intact glucagon-like peptide-1 (GLP-1) and improves glycemic control in patients with type 2 diabetes. Although GLP-1 is known to stimulate insulin secretion, vildagliptin does not affect plasma insulin levels in diabetic patients, suggesting that more sophisticated measures are necessary to ascertain the influence of vildagliptin on β-cell function.

Methods: This study examined the effects of 28-d treatment with vildagliptin (100 mg, twice daily; n = 9) *vs.* placebo (n = 11) on β-cell function in diabetic patients using a mathematical model that describes the insulin secretory rate as a function of glucose levels (β-cell dose response), the change in glucose with time (derivative component), and a potentiation factor, which is a function of time and may reflect the actions of nonglucose secretagogues and other factors.

Results: Vildagliptin significantly increased the insulin secretory rate at 7 mmol/liter glucose (secretory tone), calculated from the dose response; the difference in least squares mean (ΔLSM) was 101 ± 51 pmol·min^{-1}·m^{-2} ($P = 0.002$). The slope of the β-cell dose response, the derivative component, and the potentiation factor were not affected. Vildagliptin also significantly decreased mean prandial glucose (ΔLSM, −1.2 ± 0.4 mmol/liter; $P = 0.01$) and glucagon (ΔLSM, −10.7 ± 4.8 ng/liter; $P = 0.03$) levels and increased plasma levels of intact GLP-1 (ΔLSM, +10.8 ± 1.6 pmol/liter; $P < 0.0001$) and gastric inhibitory polypeptide (ΔLSM, +43.4 ± 9.4 pmol/liter; $P < 0.0001$) relative to placebo.

Conclusion: Vildagliptin is an incretin degradation inhibitor that improves β-cell function in diabetic patients by increasing the insulin secretory tone. **(*J Clin Endocrinol Metab* 90: 4888–4894, 2005)**

A PROMISING NEW approach to treating type 2 diabetes mellitus (T2DM) is to enhance and prolong the physiological actions of the endogenous incretin hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) by inhibiting dipeptidyl peptidase IV (DPP-4), the enzyme responsible for their degradation and inactivation (1). Both GIP and GLP-1 have been shown to stimulate insulin release in a glucose-dependent manner in humans (2), and studies in experimental animals have demonstrated that each of these incretins is necessary for the maintenance of normal glucose tolerance (3, 4). In addition, GLP-1 can exert several other beneficial metabolic effects, including inhibition of glucagon release (5), enhancement of glucose disposal (6), suppression of glucose production (7), slowing of gastric emptying (8), and reduction of food intake (9) and body weight (10). Moreover, animal studies suggest that chronic exposure to GLP-1 may increase β-cell mass by promoting growth and differentiation and inhibiting apoptosis (11).

First Published Online May 10, 2005

Abbreviations: AUC, Area under the curve; DPP-4, dipeptidyl peptidase IV; FPG, fasting plasma glucose; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; ISR, insulin secretory rate; ISR7, insulin secretory rate at 7 mmol/liter glucose; ΔLSM, difference in least squares mean; T2DM, type 2 diabetes mellitus.

Vildagliptin (formerly referred to by its code name LAF237) is an orally effective, selective inhibitor of DPP-4 that augments meal-stimulated levels of intact, biologically active GLP-1 and improves glucose tolerance in both experimental animals (12, 13) and patients with T2DM (14), but its effects on GIP levels have not yet been established. The insulinotropic effects of exogenous GLP-1 and GIP are well known, and numerous animal studies demonstrated that vildagliptin (13) and other DPP-4 inhibitors (15–17) augment postload insulin levels. However, significant increases in insulin levels have not been observed in clinical studies to date. Thus, in drug-naive patients with T2DM receiving vildagliptin (100 mg, twice daily) for 4 wk, mean postmeal glucose levels were significantly decreased, but postmeal insulin levels were unchanged (14). Similarly, in a 4-wk study of diet-controlled patients with T2DM, NVP DPP728 (150 mg, twice daily) decreased both glucose and insulin levels (18).

Although superficially such findings might be interpreted to suggest that DPP-4 inhibitors do not improve insulin secretion in patients with T2DM, it should be recognized, first, that circulating insulin levels are not a direct measure of insulin secretion and, second, that insulin secretion must be considered in the context of ambient glucose levels. Accordingly, β-cell function can be improved without appreciable changes in circulating insulin levels, particularly if glucose levels are reduced.

The purpose of the present study was to critically examine the influence of 4-wk treatment with vildagliptin (100 mg, twice daily) on insulin secretion *per se* in drug-naive patients with T2DM, using deconvolution of C peptide levels (19) and a mathematical model that derives multiple parameters of β-cell function relating insulin secretory rates (ISRs) and glucose levels (20, 21). Toward that end, the ISR was assessed during 24-h sampling comprising three standardized meals. The standardized meal challenges were performed at baseline and on d 1 and 28 of treatment to allow assessment of the time course with which vildagliptin exerts its hormonal and metabolic effects.

Patients and Methods

Study design and patient characteristics

This was a single-center, randomized, double-blind, placebo-controlled trial to compare the effects of 4-wk treatment with vildagliptin (100 mg, twice daily) and placebo in adult patients with T2DM not previously treated with antidiabetic agents. Patients, aged 30–65 yr, diagnosed with T2DM at least 6 months before screening were included. Females were required to be postmenopausal, surgically sterilized, or practicing a double-barrier method of contraception. Prerandomization hemoglobin A_{1c} was required to be between 6.5 and 10.0%, fasting plasma glucose (FPG) between 7.0 and 10.0 mmol/liter, and baseline body mass index between 22 and 35 kg/m^2, inclusive.

Patients were excluded if they had a history of type 1 or secondary forms of diabetes, significant diabetic complications, clinically significant cardiovascular abnormalities, liver disease, renal impairment, thyrotoxicosis, acromegaly, asthma or major skin allergies, or major gastrointestinal surgery. Patients with fasting triglyceride levels greater than 5.1 mmol/liter were excluded, as were those treated with thiazide diuretics, β-blockers, or any drugs that could affect the results or their interpretation.

Written informed consent was obtained from all participants, and the protocol was approved by the institutional review board/independent ethics committee at the study site. The study was conducted using good clinical practice in accordance with the Declaration of Helsinki.

Eleven patients were randomized to receive placebo, and 10 patients were randomized to receive vildagliptin (100 mg, twice daily). However, one patient randomized to vildagliptin withdrew consent before receiving the study drug. Accordingly, the intent to treat population consisted of 11 placebo-treated patients and nine patients treated with vildagliptin. All of these subjects completed the study. No adverse events suspected to be related to the study drug were observed; in particular, no hypoglycemic events were recorded. All observed adverse events (three patients treated with vildagliptin and two placebo-treated patients) were classified as mild.

The study consisted of a 21-d screening period, a baseline period (d −2 and −1), and a 28-d treatment period, with an end of study evaluation 7 d after the final dose of randomized treatment. Safety evaluations (hematology, biochemistry, urinalysis, vital signs, and electrocardiogram) were made on d −2 and 14 and at the end of the study visit. Standardized meal tests with 24-h sampling comprising three mixed meals were performed on d −1, 1, and 28.

Patients were domiciled at the study site for the standardized meal tests (d −2 to 2 and d 27–29). After an overnight fast, patients received placebo (d −1) or blinded medication (d 1 and 28) at 0700 h and consumed breakfast 30 min after treatment. Lunch and dinner were provided 4 and 10 h after the beginning of breakfast, respectively, and the evening dose of blinded medication was administered 30 min before dinner. The standardized breakfast contained 618 kcal (2588 kJ; 57% carbohydrate, 17% protein, and 26% fat); the lunch contained 692 kcal (2897 kJ; 66% carbohydrate, 16% protein, and 18% fat); the dinner contained 697 kcal (2918 kJ; 41% carbohydrate, 26% protein, and 32% fat).

During the standardized meal tests, blood samples for measurement of plasma glucose, C peptide, and insulin were obtained before the morning dose and at 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 5.0, 5.5, 6.0, 6.5, 7.5, 8.5, 10.5, 10.75, 11.0, 11.5, 12.0, 12.5, 13.5, 15.5, 18.5, 21.5, and 24 h after breakfast. Samples for determination of plasma glucagon were obtained before treatment and 0.5, 1, 2, 3.5, 11, and 13.5 h after the morning dose. Samples for measurement of intact GLP-1 were obtained before the morning dose and 0.5, 0.583, 0.666, 0.75, 1.0, 1.5, 2.0, 2.5, 5.0, 10.5, 10.583, 10.666, 10.75, 11.0, 11.5, 12.0, and 13.5 h after the morning dose. Samples for measurement of intact GIP were obtained before treatment and 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 10.75, 11.0, 11.5, 12.0, 12.5, and 13.5 h thereafter.

Analytical methods

Safety laboratory assessments, hemoglobin A_{1c}, glucose, insulin, C peptide and glucagon were analyzed at Medical Research Laboratories (Highland Heights, KY) using standardized procedures. Plasma levels of intact GLP-1 were measured by ELISA using an antibody specific for the N terminus (Linco Research, Inc., St. Charles, MO). This assay does not distinguish between GLP-1-(7–37) and GLP-1-(7–36) amide, but has no detectable cross-reactivity with GLP-1-(9–36) amide, GLP-2, or glucagon at a concentration of 100 nmol/liter. The limit of detection was 5 pmol/liter, and the intra- and interday precisions ranged from 1.0–14.3% and from 2.0–9.3%, respectively. Plasma levels of GIP were measured by RIA using antiserum 98171, which is specific for the intact N terminus of GIP (22). This assay has less than 0.1% cross-reactivity with GIP-(3–42) or GLP-1-(7–36) amide, GLP-1-(9–36) amide, GLP-2, or glucagon at a concentration of 100 nmol/liter. The intra- and interassay coefficients of variation were less than 6% and less than 10%, respectively. When GLP-1 or GIP levels were below the limit of detection of the assay, values were set at 50% the limit of quantification.

Modeling analysis

ISRs were calculated from plasma C peptide levels by deconvolution analysis (19) and were expressed per square meter of estimated body surface area. The dependence of ISR on glucose levels was modeled separately for each patient, on d −1, 1, and 28. The β-cell model used in the present study, describing the relationship between insulin secretion and glucose concentration, has been illustrated in detail previously (20, 21).

In short, ISR is the sum of two components: namely, $S_g(t)$ and $S_d(t)$. The first component, $S_g(t)$, represents the dependence of insulin secretion on the absolute glucose concentration (G) at any time point and is characterized by a dose-response function, f(G), which relates glucose and ISR. The dose-response is modulated by a time-dependent potentiation factor, P(t); thus, $S_g(t) = P(t)f(G)$.

Characteristic parameters of the dose-response are insulin secretion at a fixed glucose concentration of 7 mmol/liter (approximately the fasting glucose level in mildly diabetic subjects) and the mean slope in the observed glucose range. The potentiation factor, P(t), accounts for several modulators of insulin secretion (*e.g.* prolonged exposure to hyperglycemia, nonglucose substrates, gastrointestinal hormones, and neurotransmitters). The P(t) is set to be a positive function of time and to average 1 during the experiment. It thus expresses a relative potentiation of the secretory response to glucose.

The second insulin secretion component, $S_d(t)$, represents a dynamic dependence of insulin secretion on the rate of change in the glucose concentration and is termed the derivative component. $S_d(t)$ is proportional to the glucose time derivative [for a positive derivative, otherwise $S_d(t) = 0$]; the proportionality constant is termed rate sensitivity and is related to early insulin release (20, 21).

The model parameters (ISR at a fixed glucose level, the slope of the β-cell dose response, the rate sensitivity, and the potentiation factor) were estimated from glucose and C peptide concentration by regularized least squares, as previously described (20, 21). Regularization involves the choice of smoothing factors that were selected to obtain glucose and C peptide model residuals with SD values close to the expected measurement error (~2% for glucose and ~9% for C peptide). Estimation of the individual model parameters was performed blinded to the randomization of patients to treatment.

As previously shown (21), this parameter estimation procedure resulted in adequate reproducibility of the parameter estimates. Coefficients of variation were 16% for insulin secretion at fixed glucose concentration, 24% for the slope of the dose response, and 52% for the parameter of the derivative component. Because the potentiation factor is a function of time, a coefficient of variation was not computed.

An index of insulin sensitivity was calculated, applying to the break-

fast data a model-based method for assessing insulin sensitivity from a 3-h oral glucose tolerance test ($OGIS_{180}$), which has been validated against the hyperinsulinemic, euglycemic clamp (23). The validity of OGIS for a meal test was previously verified in a group of 43 normal subjects with an 8-fold span in insulin sensitivity assessed using the euglycemic insulin clamp technique. In this group, OGIS was well correlated with the M value from the clamp ($r = 0.59$; $P < 0.0001$) (Mari, A., O. Schmitz, and E. Ferrannini, unpublished observations).

Statistical analysis

Statistical analyses were conducted separately for d 1 and d 28 using an analysis of covariance model with the baseline (d −1) value as the covariate and treatment (vildagliptin or placebo) as the experimental factor. Analyses were performed with both original and log-transformed data. Unless otherwise stated, data are reported as the mean ± SE. Untransformed treatment effects [difference in least squares mean (ΔLSM)] are reported in the interest of clarity, but the statistical significance reported is based on log-transformed data.

Results

Characteristics of the study population

Table 1 reports the baseline characteristics of the intent to treat population. The groups were well balanced, and there were no statistically significant differences in any baseline characteristic.

Primary pharmacodynamics

Figure 1 depicts the 24-h profiles of glucose (A), insulin (B), C peptide (C), and ISR (D) during the standardized meal tests performed at baseline and after 28-d treatment with vildagliptin (100 mg, twice daily). As shown in Fig. 1A, glucose levels in the fasting state and throughout the 24-h sampling period were substantially reduced after 28-d treatment with vildagliptin. As shown in Fig. 1, B and C, insulin and C peptide were lower after treatment with vildagliptin than at baseline (d −1) during the morning and midday meals and were similar before and after treatment during the dinner meal and overnight. Similarly, the ISR was lower during the morning and midday meals on d 28 of treatment with vildagliptin than at baseline (Fig. 1D). In placebo-treated patients, 24-h glucose levels and C peptide and ISR profiles were similar on the 2 test days. These data are not depicted in a figure, but statistical comparisons of treatment effects adjusting for baseline values are reported subsequently.

TABLE 1. Baseline[a] demographic and background characteristics of the intent-to-treat population

Demographic variable	Vildagliptin 100 mg, b.i.d. (n = 9)	Placebo b.i.d. (n = 11)
Age (yr), mean ± SD	45.2 ± 9.9	44.7 ± 9.3
Sex		
Male n (%)	1 (11.1)	2 (18.2)
Female n (%)	8 (88.9)	9 (81.8)
Race		
Caucasian n (%)	5 (55.6)	5 (45.5)
Black n (%)	4 (44.4)	6 (54.5)
BMI (kg/m^2), mean ± SD	31.8 ± 3.9	32.3 ± 3.5
HbA_{1c} (%), mean ± SD	7.5 ± 1.0	7.5 ± 1.0
FPG (mmol/liter), mean ± SD	9.1 ± 0.3	8.7 ± 0.5
Systolic blood pressure (mm Hg), mean ± SD	124 ± 17	132 ± 18[b]
Diastolic blood pressure (mm Hg), mean ± SD	68 ± 10	73 ± 9

b.i.d., Twice daily.

[a] Baseline values assessed on day −2 during screening period, verifying eligibility, prior to randomization.

[b] Two patients in the placebo group were receiving antihypertensive medication.

Figure 2 depicts the 13.5-hr profiles of intact GLP-1 (A), intact GIP (B), and glucagon (C) during the standardized meal tests performed at baseline and after 28-d treatment with vildagliptin (100 mg, twice daily). As shown in Fig. 2A, before treatment, plasma levels of intact GLP-1 increased very modestly in response to breakfast and dinner. During treatment with vildagliptin, plasma levels of intact GLP-1 were increased throughout the 13.5-h sampling period. As depicted in Fig. 2B, during treatment with vildagliptin, basal levels of intact GIP were also increased, and there was a marked potentiation of the response to meals, particularly the dinner meal. As shown in Fig. 2C, in patients randomized to vildagliptin, plasma glucagon levels tended to be lower throughout the day on d 28 than on d −1. In placebo-treated patients, the GLP-1 profiles were very similar on d −1 and 28. Postbreakfast GIP levels were nearly identical on d −1 and 28, but postdinner levels of intact GIP tended to be higher on d 28 *vs.* d −1 in placebo-treated patients, and glucagon levels throughout the day tended to be higher on d 28 than on d −1. These data are not depicted graphically, but statistical comparisons of treatment effects, adjusting for baseline values, are reported below.

Table 2 summarizes and reports statistical analyses on FPG, 24-h mean [area under the curve (AUC)/time] plasma glucose and ISR, 13.5-h mean intact GLP-1 and intact GIP, and 3.5-h mean glucagon levels during tests performed before (d −1) and on d 1 and 28 of treatment with vildagliptin and placebo. The between-group ΔLSM represents the treatment effect assessed by analysis of covariance. FPG (measured 24 h after treatment dose) tended to decrease on d 1 and was significantly reduced relative to that in the placebo group ($P = 0.04$) on d 28 of treatment with vildagliptin. Treatment with vildagliptin significantly decreased 24-h mean glucose relative to placebo on both d 1 ($P = 0.022$) and d 28 ($P = 0.010$). Throughout the study, the 24-h mean ISR was somewhat lower in patients randomized to placebo than in patients randomized to vildagliptin, but there was no effect of treatment on 24-h mean ISR. Mean plasma levels of intact GLP-1 remained stable in patients receiving placebo and more than doubled during treatment with vildagliptin. Thus, vildagliptin significantly increased 13.5-h mean GLP-1 relative to placebo on both d 1 ($P < 0.001$) and d 28 ($P < 0.001$). Mean plasma levels of intact GIP increased marginally during treatment with placebo, but increased markedly during treatment with vildagliptin. Thus, vildagliptin significantly increased 13.5-h mean GIP, relative to placebo, on both d 1 ($P < 0.001$) and d 28 ($P < 0.001$). The 3.5-h mean postbreakfast glucagon level in patients randomized to vildagliptin decreased progressively with time and was significantly suppressed relative to that in the placebo group ($P = 0.030$) on d 1 of treatment. However, because the mean glucagon level on d 28 in placebo-treated patients was lower than that on d −1, the effect of vildagliptin to suppress mean glucagon levels relative to placebo failed to achieve statistical significance on d 28.

Insulin sensitivity, estimated by $OGIS_{180}$, was similar at

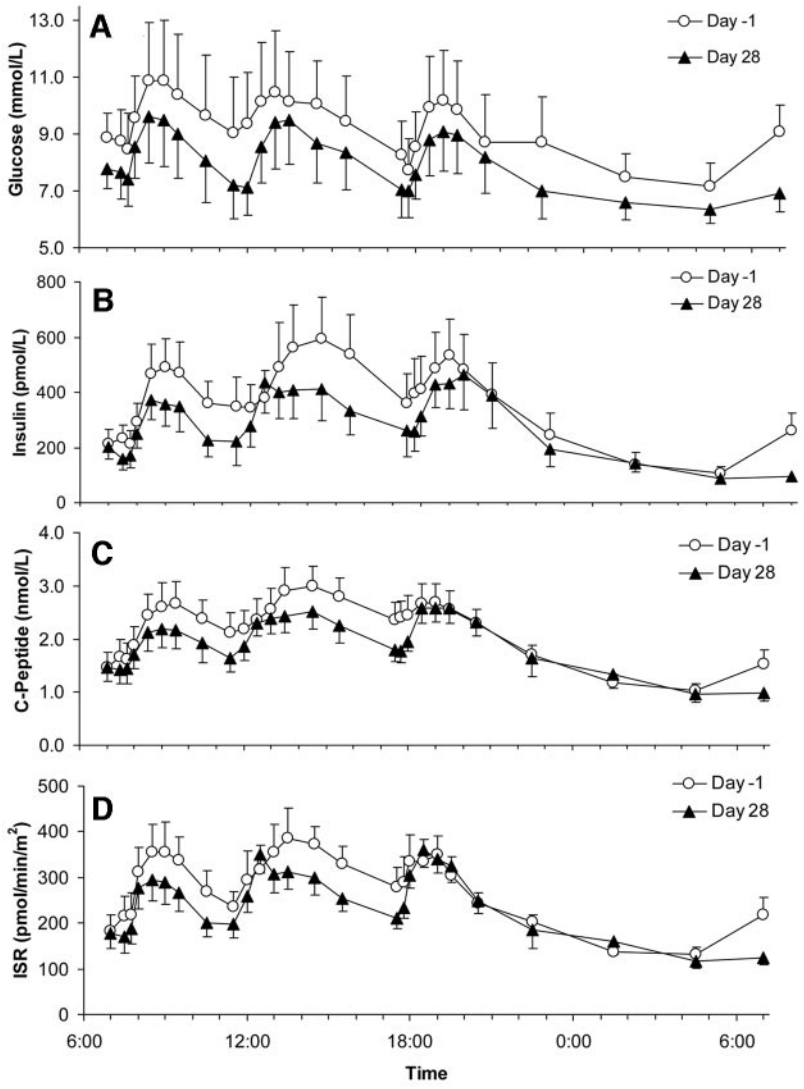

FIG. 1. Plasma glucose (A), insulin (B), and C peptide (C) and ISR (D) during 24-h sampling comprising three standardized meals before (d −1) and after 28-d treatment with vildagliptin (100 mg, twice daily) in drug-naive patients with T2DM. Values are the mean ± SE (n = 9 patients).

baseline in patients randomized to vildagliptin (312 ± 60 ml·min^{-1}·m^{-2}) and to placebo (327 ± 30 ml·min^{-1}·m^{-2}) and did not change in either group after 1 d of treatment. However, on d 28, the $OGIS_{180}$ was significantly increased in patients receiving vildagliptin (333 ± 40 ml·min^{-1}·m^{-2}) relative to that in patients receiving placebo (296 ± 21). The ΔLSM $OGIS_{180}$ averaged 46 ± 22 ml·min^{-1}·m^{-2} ($P = 0.015$).

Model-derived parameters of β-cell function

The model-derived parameters are reported in Table 3. The ISR at 7 mmol/liter glucose (ISR7) was significantly increased in vildagliptin-treated patients relative to placebo-treated subjects on both d 1 and 28 of treatment. The ISR at 8 mmol/liter glucose tended to be increased in vildagliptin-treated patients on d 1 of treatment, and the difference relative to placebo-treated subjects achieved full statistical significance on d 28. On d 28 there was also a significant treatment effect of vildagliptin to increase ISR at 9 mmol/liter glucose (ΔLSM, 177 ± 102; $P = 0.010$) and ISR at 10 mmol/l (ΔLSM, 210 ± 129; $P = 0.021$). However, the increase in the model-derived slope of the glucose dose-response with vildagliptin did not reach statistical significance. The derivative component of insulin secretion was associated with substantial intersubject variability and was not significantly affected by vildagliptin compared with placebo. Although the time course of the potentiation factor differed somewhat between the tests, there were no statistically significant differences attributable to treatment (data not shown).

Discussion

Despite the known insulinotropic actions of the incretin hormone, GLP-1, in both healthy subjects (24) and patients with T2DM (25, 26) and the role of DPP-4 in degrading and thereby limiting the actions of GLP-1 (27), the influence of DPP-4 inhibitors on β-cell function is poorly understood. The present modeling approach offers the first direct evidence that a DPP-4 inhibitor improves β-cell function in humans and that it does so by increasing insulin secretory tone.

This modeling-based demonstration of improved β-cell function in response to an inhibitor of DPP-4 is consistent with previous findings that indirectly suggested such an improvement. Thus, although insulin levels were signifi-

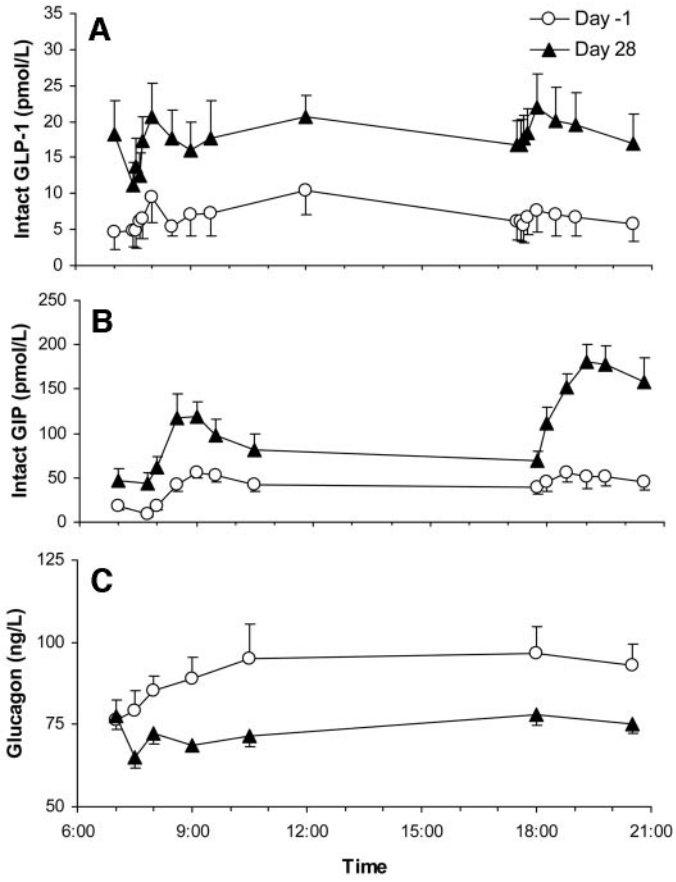

FIG. 2. Plasma levels of intact GLP-1 (A), intact GIP (B), and glucagon (C) during 13.5-h sampling comprising three standardized meals before (d −1) and after 28-d treatment with vildagliptin (100 mg, twice daily) in drug-naive patients with T2DM. Values are the mean ± SE (n = 9 patients).

cantly lower in patients receiving the DPP-4 inhibitor, NVP DPP728 (150 mg, twice daily, for 4 wk) than in placebo-treated patients, the insulinogenic index (postmeal incremental insulin AUC ÷ incremental glucose AUC) was significantly increased (18). In another recent study, drug-naive patients with T2DM received placebo or vildagliptin (100 mg, daily, for 4 wk), and it was found that glucose levels decreased substantially, but insulin levels were unchanged (14). This, too, could reflect enhanced β-cell responsiveness to glucose, perhaps with concomitant enhanced insulin sensitivity, as suggested by the researchers, who likened this effect to findings with 6-wk continuous infusion of exogenous GLP-1 (10).

Indeed, in the present study there was a significant increase in $OGIS_{180}$ after 28-d treatment with vildagliptin. This index of insulin sensitivity is calculated by modeling glucose and insulin data from OGTTs and is strongly correlated to several other measures of insulin sensitivity, including glucose clearance during hyperinsulinemic euglycemic clamps in lean and obese subjects with normal glucose tolerance as well as in subjects with impaired glucose tolerance and with T2DM (23). However, the $OGIS_{180}$ using data from meal tests has only been validated in nondiabetic subjects. Therefore, despite the present indirect evidence, the idea that vildagliptin improved insulin sensitivity in diabetic patients will require additional confirmation, optimally with a more direct approach, such as a two-step clamp.

In the current study, a significant augmentation of circulating levels of the intact forms of both GLP-1 and GIP was observed, and the magnitude of the effect on GLP-1 was similar to that reported previously (14). Other DPP-4 inhibitors have previously been found to augment the intact form of GIP in pigs (28) and dogs (29); however, the present findings are the first demonstration of the ability of a DPP-4 inhibitor to augment intact, biologically active GIP levels in humans.

Although both GLP-1 and GIP may have contributed to the observed effect of vildagliptin to improve β-cell function, it is by no means established that these incretin hormones are the only mediators, because, at least *in vitro*, DPP-4 can degrade many other biologically active peptides (30). Some of the potential substrates (*e.g.* pituitary adenylate cyclase activting polypeptide, gastrin releasing peptide, and vasoactive intestinal polypeptide) have been shown in animal studies to stimulate insulin release in a glucose-dependent manner (31), and thus may contribute to the therapeutic effects of a DPP-4 inhibitor such as vildagliptin. However, a recent demonstration that the insulinotropic and antihyperglycemic actions of a DPP-4 inhibitor, which are clearly apparent in wild-type mice, are absent in mice lacking receptors for both GLP-1 and GIP (double incretin receptor knockout mice) (32) would argue that substrates other than GLP-1 and GIP are not major mediators.

In this context it would appear that GLP-1 is more likely than GIP to mediate the effects of vildagliptin seen in the present study because the glucose-dependent insulinotropic effect of GLP-1 is maintained in patients with T2DM (2, 33), whereas GIP has markedly reduced effectiveness to acutely stimulate insulin release in patients with T2DM relative to healthy subjects (34, 35). It is also likely that GLP-1 (rather than GIP) contributed to the suppression of glucagon observed in response to vildagliptin [similar in magnitude to that reported previously (14)], because it is known that GLP-1 decreases glucagon levels (5, 33), whereas GIP, under certain conditions, may increase glucagon levels (36).

The model-derived parameter, ISR7, is considered an index of basal secretory tone, whereas, as mentioned previously, the slope of the glucose dose response is a measure of β-cell sensitivity to glucose. The lack of a statistically significant effect of vildagliptin on the slope, and the clear effect to increase ISR7 may suggest that vildagliptin exerts a proportionally greater impact at basal *vs.* postprandial glucose levels and help to explain the influence of this agent on FPG. It should be noted, however, that the main effect of vildagliptin on GLP-1 is to increase its mean levels rather than the postprandial peaks. This may explain a more marked effect on the basal secretory tone (ISR7) than on the dose-response slope.

In a recent modeling study of a small group of women with a broad range of glucose values, exogenous GLP-1 was found to increase the slope of the glucose dose response and the potentiation factor, as well as increasing ISR7 (25). The difference in outcomes of that study and the present findings with vildagliptin are probably attributable to important differences in experimental paradigms. In the earlier study, GLP-1 was given acutely as an iv infusion (0.75 pmol/kg·min

TABLE 2. FPG, mean (AUC/time; AUC computed on the time period shown) glucose, ISR, intact (N-terminally-detected) GLP-1 and GIP and glucagon (IRG) during the tests performed at before (day −1) and during treatment with vildagliptin (100 mg, twice daily) or placebo, together with the between-group difference in least squares mean (ΔLSM) derived from the ANCOVA model

Mean ± SE	d −1	d1	Δ LSM (d 1)	d 28	Δ LSM (d 28)
FPG (mmol/liter)[a]					
Vildagliptin	9.1 ± 0.9	7.2 ± 0.9	−1.4 ± 0.7	6.9 ± 0.7	−1.6 ± 0.7[b]
Placebo	8.1 ± 0.7	8.1 ± 0.9		8.1 ± 0.8	
24-h mean glucose (mmol/liter)					
Vildagliptin	8.9 ± 1.4	9.0 ± 1.5	−0.5 ± 0.2[b]	7.7 ± 1.0	−1.2 ± 0.4[c]
Placebo	7.7 ± 0.7	8.1 ± 0.9		8.0 ± 0.7	
24-h mean ISR (pmol·min^{-1}·m^{-2})					
Vildagliptin	248 ± 28	241 ± 24	11 ± 11	218 ± 24	−13 ± 16
Placebo	206 ± 17	195 ± 16		195 ± 20	
13.5-h mean GLP-1 (pmol/liter)					
Vildagliptin	7.7 ± 2.8	17.6 ± 3.3	9.4 ± 1.5[c]	18.5 ± 3.4	10.8 ± 1.6[d]
Placebo	7.4 ± 1.7	7.9 ± 1.7		7.4 ± 1.7	
13.5-h mean GIP (pmol/liter)					
Vildagliptin	41.0 ± 5.5	99.4 ± 11.1	49.3 ± 9.4[d]	94.0 ± 12.1	43.4 ± 9.4[d]
Placebo	33.7 ± 8.0	42.3 ± 9.0		42.4 ± 8.5	
3.5-h mean IRG (ng/liter)					
Vildagliptin	87.3 ± 6.3	81.8 ± 6.8	−10.7 ± 4.8[b]	70.1 ± 3.3	−6.7 ± 6.9
Placebo	82.6 ± 7.0	87.3 ± 9.0		74.7 ± 6.8	

[a] FPG is that measured 24 h after prebreakfast dosing on d −1, 1, and 28.
[b] $P < 0.05$; [c] $P \leq 0.01$, [d] $P < 0.001$ *vs.* placebo as assessed by ANCOVA performed on log-transformed data.

for 90 min) at the start of a mixed meal, raising plasma levels of intact GLP-1 approximately 10-fold. It is not surprising that acute iv administration of high doses of GLP-1 has additional influences on β-cell function not seen with a DPP-4 inhibitor, which enhances the physiological effects of endogenously released GLP-1.

With regard to the time course of effects of vildagliptin, a statistically significant effect to reduce postprandial glucose and glucagon and to increase active GLP-1 and GIP was seen on d 1 of treatment. Although there was a tendency for FPG to decrease on d 1, the significant reduction in FPG and the improvement in insulin sensitivity, as reflected by the $OGIS_{180}$, appeared to require chronic treatment. This observation highlights the importance of making serial determinations of each parameter of interest, particularly in any future studies to explore the mechanisms underlying the influence of vildagliptin on fasting glucose levels. The apparently slower onset of significant improvements of FPG may also suggest that a general improvement of the metabolic state, due possibly to amelioration of glucolipotoxicity may make a contribution (37–39). Interestingly, it was recently reported that GLP-1 prevents glucolipotoxicity in cultured human islets (40), possibly suggesting a novel mechanism by which a DPP-4 inhibitor could influence fasting glucose levels. It is also possible that chronically elevated GLP-1 levels can ameliorate glucolipotoxicity in peripheral insulin-sensitive tissues, and this effect could contribute to the apparent improvement of insulin sensitivity seen during treatment with vildagliptin. Such an effect of GLP-1 is consistent with reports that this peptide increases both insulin-mediated glucose uptake (41) and noninsulin-mediated glucose uptake (42). However, those effects were observed during acute administration of GLP-1, whereas the effect of vildagliptin on $OGIS_{180}$ was not apparent on d 1 of treatment, which argues for the effect being due to an overall improved metabolic state. As noted above, a detailed understanding of the many possible mechanisms by which vildagliptin exerts an antihyperglycemic effect will require additional study.

In summary, the data obtained in this study were consistent with previous clinical studies with vildagliptin or NVP

TABLE 3. Model-derived parameters of β-cell function before (d −1) and during treatment with vildagliptin (100 mg twice daily) or placebo, together with the between-group difference in least squares mean (ΔLSM) derived from the ANCOVA model

Mean ± SE	d −1	d 1	Δ LSM (d 1)	d 28	Δ LSM (d 28)
ISR at 7 mmol/liter glucose (pmol·min^{-1}·m^{-2})					
Vildagliptin	283 ± 71	338 ± 93	100 ± 71[a]	328 ± 77	101 ± 51[b]
Placebo	228 ± 38	197 ± 30		184 ± 29	
ISR at 8 mmol/liter glucose (pmol·min^{-1}·m^{-2})					
Vildagliptin	368 ± 96	471 ± 151	160 ± 127	427 ± 79	141 ± 76[b]
Placebo	295 ± 55	259 ± 42		233 ± 38	
Glucose sensitivity (pmol·min^{-1}·m^{-2}·mM^{-1})					
Vildagliptin	88 ± 26	137 ± 62	62 ± 57	101 ± 33	38 ± 26
Placebo	67 ± 18	63 ± 13		50 ± 9	
Rate sensitivity (pmol·m^{-2}·mM^{-1})					
Vildagliptin	983 ± 358	921 ± 355	293 ± 407	625 ± 263	−266 ± 327
Placebo	780 ± 292	552 ± 258		782 ± 290	

Parameters include ISR at glucose levels of 7 and 8 mmol/liter reflecting insulin secretory tone, the slope of the β-cell dose response between glucose levels of 7 and 9 mmol/liter, denoted as glucose sensitivity, and the parameter of the derivative component, reflecting the dependence of ISR on the glucose rate of change (early insulin release), denoted as rate sensitivity.
[a] $P < 0.05$, [b] $P < 0.005$ *vs.* placebo as assessed by ANCOVA performed with log-transformed data.

DPP728 and indicate that the DPP-4 inhibitor, vildagliptin, decreases day-long glucose levels, decreases glucagon levels, and augments plasma levels of the intact, biologically active forms of GLP-1 and GIP. The novel finding that this incretin degradation inhibitor improves β-cell function by increasing insulin secretion at any given glucose level (*i.e.* insulin secretory tone) may have important clinical implications regarding the effects of vildagliptin in both fasting and fed states.

Acknowledgments

Received December 14, 2004. Accepted May 2, 2005.

Address all correspondence and requests for reprints to: Dr. Andrea Mari, National Research Center, Institute of Biomedical Engineering, Corso Stati Uniti 4, 35127 Padova, Italy. E-mail: andrea.mari@isib.cnr.it.

References

1. **Holst JJ, Deacon CF** 1998 Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. Diabetes 47:1663–1670
2. **Kreymann B, Williams G, Ghatei MA, Bloom SR** 1987 Glucagon-like peptide-1 7–36: a physiological incretin in man. Lancet 2:1300–1304
3. **Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tahiro F, Miyazaki J, Seino Y** 1999 Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. Proc Natl Acad Sci USA 96:14843–14847
4. **Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ** 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 2:1254–1258
5. **Creutzfeldt WO, Kleine N, Willms B, Orskov C, Holst JJ, Nauck MA** 1996 Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7–36) amide in type I diabetic patients. Diabetes Care 19:580–586
6. **D'Alessio DA, Prigeon RL, Ensinck JW** 1995 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
7. **Prigeon RL, Quddusi S, Paty B, D'Alessio DA** 2003 Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. Am J Physiol 285:E701–E707
8. **Naslund E, Bogefors J, Skogar S, Gryback P, Jacobsson H, Holst JJ, Hellstrom PM** 1999 GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. Am J Physiol 277:R910–R916
9. **Gutzwiller JP, Drewe J, Goke B, Schmidt H, Rohrer B, Lareida J, Beglinger C** 1999 Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. Am J Physiol 276:R1541–R1544
10. **Zander M, Madsbad S, Madsen JL, Holst JJ** 2002 Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. Lancet 359:824–830
11. **Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U, Perfetti R** 2002 Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology 143:4397–4408
12. **Dardik B, Valentin M, Schwarztkopf C, Gutierrez C, Stevens D, Russel M, Villhauer E, Hughes T** 2003 NVP-LAF237, a dipeptidyl peptidase IV inhibitor, improves glucose tolerance and delays gastric emptying in obese insulin resistant cynomolgus monkeys. Diabetes 52(Suppl 1):A322
13. **Villhauer EB, Brinkman JA, Naderi GB, Burkey BF, Dunning BE, Prasad K, Mangold BL, Russell ME, Hughes TE** 2003 1-[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. J Med Chem 46:2774–2789
14. **Ahren B, Landin-Olsson M, Jansson P-A, Svenson M, Holmes D, Schweizer A** 2004 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
15. **Ahren B, Holst JJ, Martensson H, Balkan B** 2000 Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. Eur J Pharmacol 404:239–245
16. **Balkan B, Kwasnik L, Miserendino R, Holst JJ, Li X** 1999 Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7–36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. Diabetologia 42:1324–1331
17. **Mitani H, Takimoto M, Kimura M** 2002 Dipeptidyl peptidase IV inhibitor NVP-DPP728 ameliorates early insulin response and glucose tolerance in aged rats but not in aged Fischer 344 rats lacking its enzyme activity. Jpn J Pharmacol 88:451–458
18. **Ahren B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson PA, Sandqvist M, Bavenholm P, Efendic S, Eriksson JW, Dickinson S, Holmes D** 2002 Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. Diabetes Care 25:869–875
19. **van Cauter E, Mestrez F, Sturis J, Polonsky KS** 1992 Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 41:368–377
20. **Mari A, Tura A, Gastaldelli A, Ferrannini E** 2002 Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. Diabetes 51(Suppl 1):S221–S226
21. **Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E** 2002 Meal and oral glucose tests for assessment of β-cell function: modeling analysis in normal subjects. Am J Physiol 283:E1159–E1166
22. **Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ** 2000 Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. J Clin Endocrinol Metab 85:3575–3581
23. **Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ** 2001 A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 24:539–548
24. **Ritzel R, Orskov C, Holst JJ, Nauck MA** 1995 Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7–36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. Diabetologia 38:720–725
25. **Ahren B, Holst JJ, Mari A** 2003 Characterization of GLP-1 effects on β-cell function after meal ingestion in humans. Diabetes Care 26:2860–2864
26. **Kjems LL, Holst JJ, Volund A, Madsbad S** 2003 The influence of GLP-1 on glucose-stimulated insulin secretion: effects on β-cell sensitivity in type 2 and nondiabetic subjects. Diabetes 52:380–386
27. **Mentlein R** 1999 Dipeptidyl-peptidase IV (CD26): role in the inactivation of regulatory peptides. Regul Pept 85:9–24
28. **Larsen MO, Rolin B, Ribel U, Ribel U, Wilken M, Deacon CF, Svendsen O, Gotfredsen CF, Carr RD** 2003 Valine pyrrolidide preserves intact glucose-dependent insulinotropic peptide and improves abnormal glucose tolerance in minipigs with reduced β-cell mass. Exp Diabesity Res 4:93–105
29. **Deacon CF, Wamberg S, Bie P, Hughes TE, Holst JJ** 2002 Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. J Endocrinol 172:355–362
30. **Lambeir A-M, Durinx C, Proost P, Van Damme J, Scharpe S, De Meester I** 2001 Kinetic study of the processing by dipeptidyl-peptidase IV/CD26 of neuropeptides involved in pancreatic insulin secretion. FEBS Lett 507:327–330
31. **Deacon CF, Ahren B, Holst JJ** 2004 Inhibitors of dipeptidyl peptidase IV: a novel approach for the prevention and treatment of type 2 diabetes? Expert Opin Invest Drugs 13:1091–1102
32. **Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ** 2004 Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. Diabetes 53:1326–1335
33. **Ahren B, Larsson H, Holst JJ** 1997 Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 82:473–478
34. **Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W** 1993 Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 91:301–307
35. **Vilsboll T, Krarup T, Madsbad S, Holst JJ** 2002 Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. Diabetologia 45:1111–1119
36. **Meier JJ, Gallwitz B, Siepmann N, Holst JJ, Deacon CF, Schmidt WE, Nauck MA** 2003 Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. Diabetologia 46:798–801
37. **Boden G, Ruiz J, Kim CJ, Chen X** 1996 Effects of prolonged glucose infusion on insulin secretion, clearance, and action in normal subjects. Am J Physiol 270:E251–E258
38. **Boden G** 1997 Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3–10
39. **Rossetti L, Giaccari A, DeFronzo RA** 1990 Glucose toxicity. Diabetes Care 13:610–630
40. **Buteau J, El Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M** 2004 Glucagon-like peptide-1 prevents β cell glucolipotoxicity. Diabetologia 47:806–815
41. **Egan JM, Meneilly GS, Habener JF, Elahi D** 2002 Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. J Clin Endocrinol Metab 87:3768–3773
42. **Meneilly GS, McIntosh CH, Pederson RA, Habener JF, Gingerich R, Egan JM, Finegood DT, Elahi D** 2001 Effect of glucagon-like peptide 1 on non-insulin-mediated glucose uptake in the elderly patient with diabetes. Diabetes Care 24:1951–1956