Inhibition of DPP-4 with Vildagliptin Improved Insulin Secretion in Response to Oral as well as "Isoglycemic" Intravenous Glucose without Numerically Changing the Incretin Effect in Patients with Type 2 Diabetes

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Background and Aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors block the degradation of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide. The aim of the present study was to quantitatively assess the incretin effect after treatment with the DPP-4 inhibitor vildagliptin (V) or placebo (P) in patients with type 2 diabetes.

Materials and Methods: Twenty-one patients (three women, 18 men) with type 2 diabetes previously treated with metformin (mean age, 59 yr; body mass index, 28.6 kg/m^2; glycosylated hemoglobin, 7.3%) were studied in a two-period crossover design. They received 100 mg V once daily or P for 13 d in randomized order. The incretin effect was measured on d 12 (75-g oral glucose) and d 13 ("isoglycemic" iv glucose) based on insulin and C-peptide determinations and insulin secretion rates (ISR).

Results: V relative to P treatment significantly increased intact incretin concentrations after oral glucose and insulin secretory responses to both oral glucose and isoglycemic iv glucose (*e.g.* $AUC_{ISR\ oral}$, by 32.7%, $P = 0.0006$; $AUC_{ISR\ iv}$, by 33.1%, $P = 0.01$). The numerical incretin effect was not changed (IE_{ISR}, V *vs.* P, 35.7 $\pm$ 4.9 and 34.6 $\pm$ 4.0%, $P = 0.80$).

Conclusions: DPP-4 inhibition augmented insulin secretory responses both after oral glucose and during isoglycemic iv glucose infusions, with no net change in the incretin effect. Thus, slight variations in basal incretin levels may be more important than previously thought. Or, DPP-4 inhibitor-induced change in the incretin-related environment of islets may persist overnight, augmenting insulin secretory responses to iv glucose as well. Alternatively, yet unidentified mediators of DPP-4 inhibition may have caused these effects. **(*J Clin Endocrinol Metab* 96: 945–954, 2011)**

Insulin secretion after oral glucose is stimulated by rising glucose concentrations and, in healthy humans, also by a substantial contribution made by so-called incretin hormones like glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (1, 2). These insulinotropic gut hormones augment insulin secretion both in the fasting state and in response to elevations in glucose concentrations, but in a glucose concentration-dependent manner (3). Incretin hormones are released after nutrient ingestion (glucose, other carbohydrates, triglycerides, proteins, *etc.*), but not during the iv administration of glucose (4–6). Therefore, oral glucose elicits a higher insulin secretory response than does iv glucose, even if the same amount of glucose is given (7) or the

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Abbreviations: DPP-4, Dipeptidyl peptidase-4; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1.

glucose concentrations after both loads are comparable ("isoglycemia") (8, 9). This is called the incretin effect. In patients with type 2 diabetes, the incretin effect is reduced (9), *i.e.* the contribution of gut hormones to total insulin secretory responses is substantially smaller than in healthy subjects or even completely lost. This loss is associated with a lack of insulinotropic activity of GIP in patients with type 2 diabetes and hyperglycemia (10, 11). Furthermore, reduced β-cell sensitivity to GLP-1 (12) may also contribute, as may a reduced secretion of GLP-1 (13). Nevertheless, this can be overcome by stimulating insulin secretion with exogenous GLP-1 (10, 14). This is the reason that GLP-1 receptor agonists are used to treat type 2 diabetes (1, 15). Another approach to elevate GLP-1 concentrations is the inhibition of dipeptidyl peptidase-4 (DPP-4), the enzyme primarily responsible for the degradation of both GIP and GLP-1 (1, 16–18). A near-complete inhibition of DPP-4 causes GIP and GLP-1 concentrations to rise by 2- to 3-fold (16, 19) and is associated with reductions in fasting and postprandial glucose concentrations, stimulation of insulin secretion, and suppression of glucagon (16).

Because incretin hormones usually have low, basal concentrations in the fasting state and rapidly increase after nutrient ingestion (4, 6), DPP-4 inhibition supposedly has primarily postprandial effects, with some additional glucose-lowering effect in the fasting state, and is therefore thought to act as an "incretin enhancer" (20). Therefore, one might expect that the insulin secretory responses after oral glucose would be augmented more than after iv glucose, *i.e.* without the nutrient-stimulated release of incretin hormones, and it could be speculated that this may lead to an at least partial restoration of the reduced incretin effect (9). Therefore, we wanted to quantify the incretin effect by comparing insulin secretory responses to oral as well as isoglycemic iv glucose infusions in patients with type 2 diabetes with and without the administration of the DPP-4 inhibitor, vildagliptin, which has received approval as an antidiabetic drug, including in Europe and Japan. Preliminary results have been communicated in abstract form (21).

Subjects and Methods

Study protocol

The study protocol was approved by the ethics committee of the Board of Physicians of the State Niedersachsen (Ärztekammer Niedersachesen), Hannover, before the study (registration no., Bo/12/2006; date of approval, September 20, 2006). Written informed consent was obtained from all participants.

Subjects

Twenty-two patients with type 2 diabetes participated in the present study. The characteristics of the 21 completers are presented in Table 1. They were all treated with metformin monotherapy before entering the study. Key inclusion criteria were: 1) patients who had received metformin for at least 3 months and had been on a stable dose of at least 1500 mg daily for a minimum of 4 wk before visit 1; 2) agreement to maintain the same dose of metformin throughout the study; 3) age in the range of 30–78 yr inclusive; 4) body mass index in the range of 22–35 kg/m^2 inclusive at visit 1; 5) glycosylated hemoglobin of 6.5–9.0% inclusive at visit 1; and 6) fasting plasma glucose less than 200 mg/dl (11.1 mmol/liter) at visit 1. Patients were excluded if there was a diagnosis or a history of type 1 diabetes, diabetes that is the result of pancreatic injury, or secondary forms of diabetes or acute metabolic diabetic complications such as ketoacidosis or hyperosmolar state (coma) within the past 6 months. (Key inclusion and exclusion criteria are presented in more detail in Supplemental Data, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

TABLE 1. Patient characteristics of type 2 diabetic patients participating in the study of incretin effects after vildagliptin treatment

Parameter	Mean ± SD	Range
Gender (males/females)	18/3	n.a.
Age (yr)	59 ± 9	35–69
BMI (kg/m^2)	28.6 ± 2.6	23.0–32.5
Diabetes duration (yr)	6 ± 3	0.5–11
HbA_{1c} (%)	7.3 ± 0.5	6.6–8.5
Fasting glucose (mmol/liter)	9.3 ± 1.1	6.4–10.9
Serum creatinine (μmol/liter)	81 ± 13	66–111
Urea (mmol/liter)	5.8 ± 1.3	3.4–8.8
Uric acid (μmol/liter)	345 ± 82	252–529
Triglycerides (mmol/liter)	2.2 ± 1.1	0.7–4.8
LDL-cholesterol (mmol/liter)	3.3 ± 1.0	2.1–5.1
HDL-cholesterol (mmol/liter)	1.3 ± 0.5	0.7–2.6

n.a., Not applicable; HbA_{1c}, glycosylated hemoglobin; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

At a screening visit, blood was drawn in the fasting state for measurements of standard hematological and clinical chemistry parameters. Spot urine was sampled for the determination of albumin, protein, and creatinine by standard methods. Body height and weight were measured to calculate the body mass index (Table 1).

Study design

Patients were enrolled into a double-blinded, two-way crossover study (order randomized) quantifying the incretin effect both under treatment with vildagliptin (100 mg once daily by mouth) or matching placebo tablets. Under both conditions, (both in addition to their previous metformin medication), treatment was started on d 1. On d 12, an oral glucose load (75 g) was administered, and on d 13, an isoglycemic infusion of glucose was administered to copy glucose excursions after oral glucose. In both experiments, blood samples were drawn over 240 min. Between the treatment periods, there was a washout period of 4 wk.

Experimental procedures

The tests were performed in the morning after an overnight fast with subjects in a supine position throughout the experiments and the upper body lifted by approximately 30 degrees. One or two forearm veins were punctured with a Teflon cannula

(Moskito 123, 18 gauge; Vygon, Aachen, Germany) and kept patent using 0.9% NaCl. Both ear lobes were made hyperemic using Finalgon (nonivamid, 4 mg/g; nicoboxil, 25 mg/g; Boehringer Ingelheim, Ingelheim am Rhein, Germany).

On d 12, an oral glucose challenge (75 g of glucose and glucose oligomers; Accu-Chek Dextro O.G.-T., Roche Diagnostics, Mannheim, Germany) was given at 0 min. On d 13, 20% glucose was administered iv to copy the glycemic excursions after oral glucose (isoglycemic iv glucose infusion). This infusion was guided by determining capillary glucose concentrations every 5 min in blood taken from a hyperemic ear lobe. Glucose infusion rates were changed appropriately, and the infusion rates and time points, when it was changed, were recorded to be able to calculate the total amount of glucose administered as well as infusion rates averaged over 15-min periods.

After drawing basal blood specimens at −20 and −5 min, blood was taken at 15, 30, 45, 60, 75, 90, 105, 120, 180, and 240 min.

Blood specimens

Blood was drawn into chilled tubes containing EDTA and the DPP-4 inhibitor diprotin A at a final concentration of 0.1 mmol/liter for analysis of GLP-1 (intact and total), GIP (intact), insulin, and C-peptide. For analysis of glucagon, blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol, 20,000 KIU/ml, 180 μl per 9 ml blood; Bayer AG, Leverkusen, Germany) and kept on ice. A capillary sample taken from hyperemic ear lobes (approximately 100 μl) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate measurement of glucose. After centrifugation at 4 C, plasma for hormone analyses was divided into aliquots of 0.5 or 1 ml and stored frozen at −30 C.

Laboratory determinations

Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Munich, Germany).

Insulin and C-peptide were determined by specific immunoassays as previously described (22).

Plasma samples were assayed for total GLP-1 immunoreactivity, as previously described (4), using a RIA (antiserum no. 89390) that is specific for the C-terminal of the GLP-1 molecule and reacts equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. Intact GLP-1 was measured using an enzyme-linked immunosorbent assay, as previously described (23). The assay is a two-site sandwich assay involving two monoclonal antibodies: GLP-1F5 as the catching antibody (C-terminally directed), and Mab26.1 as detecting antibody (N-terminally directed) (17). For both assays, the detection limit was below 1 pmol/liter, and the intraassay coefficient of variation was below 5% at 20 pmol/liter.

Intact, biologically active GIP was measured as described (24) using an antiserum reacting with the N-terminal portion of GIP. The experimental detection limit was below 2 pmol/liter. Intra- and interassay coefficients of variation are below 6% and below 12%, respectively.

Pancreatic glucagon was measured using porcine antibody 4305 in ethanol-extracted plasma, as previously described (25). The detection limit was below 1 pmol/liter. Intraassay coefficients of variation were 6%, and interassay coefficients of variation were 16%.

All samples from the same individual were measured in the same assay run.

Calculations

Integration (area under the curve) was carried out using the trapezoidal rule. Integrated incremental responses describe changes above baseline.

Insulin secretion rates were calculated from C-peptide concentrations using software ISEC version 3.4a, kindly supplied by Dr. Roman Hovorka (London, UK) (26). Population-derived coefficients of transition between compartments were used as described (27, 28).

The incretin effect was calculated based on the integrated incremental responses (trapezoidal rule) of plasma insulin, C-peptide, or insulin secretion rates after oral and isoglycemic iv glucose administration. The difference was related to the respective response after oral glucose, which was taken as 100%. Therefore, incretin effects were expressed as the percentage contribution to the total β-cell secretory response after oral glucose as previously described (8, 9).

Statistical analysis

Results are reported as mean ± SEM. All statistical calculations were carried out using repeated-measures ANOVA with Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). Experiments (vildagliptin *vs.* placebo; oral *vs.* isoglycemic iv glucose) were used as fixed variables and subjects as random variable (to obtain an intraindividual comparison). This analysis provides P values for differences between groups/experiments (A), differences over time (B), and for the interaction of group/experiment with time (AB). If a significant interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by one-way ANOVA for repeated measurements and Duncan's *post hoc* test. A two-sided P value <0.05 was taken to indicate significant differences.

Results

Vlidagliptin led to a slightly reduced fasting glucose (vildagliptin, 8.8 ± 0.5 mmol/liter, compared with placebo, 9.2 ± 0.3 mmol/liter; $P = 0.19$). After oral glucose administration, the integrated incremental glucose concentration relative to placebo was reduced with vildagliptin from 1352 ± 78 to 1138 ± 103 mmol · liter^{-1} · min by 15.8% ($P = 0.053$) (further results for fasting values, for fasting secretion responses relative to glucose concentration, and for the difference between oral and iv stimulation are shown in Supplemental Table 1A). As proof of the clinical effectiveness of vildagliptin treatment, the ratios of integrated incremental responses of insulin, C-peptide, and insulin secretion rates over glycemic excursions were significantly enhanced by 91.9, 82.9, and 85.1% ($P = 0.003$, 0.002, and 0.0006) (Supplemental Table 1B).

It was possible to closely match the glucose excursion after oral and iv glucose administration (Fig. 1, C and D); some negligible differences were noted. Nevertheless, the conditions of isoglycemia necessary to accurately quantify the incretin effect were met. Both with placebo and with vildagliptin treatment, oral glucose elicited a higher insulin secretory response (whether based on insulin, C-peptide, or the calculation of insulin secretion rates; Fig. 1),

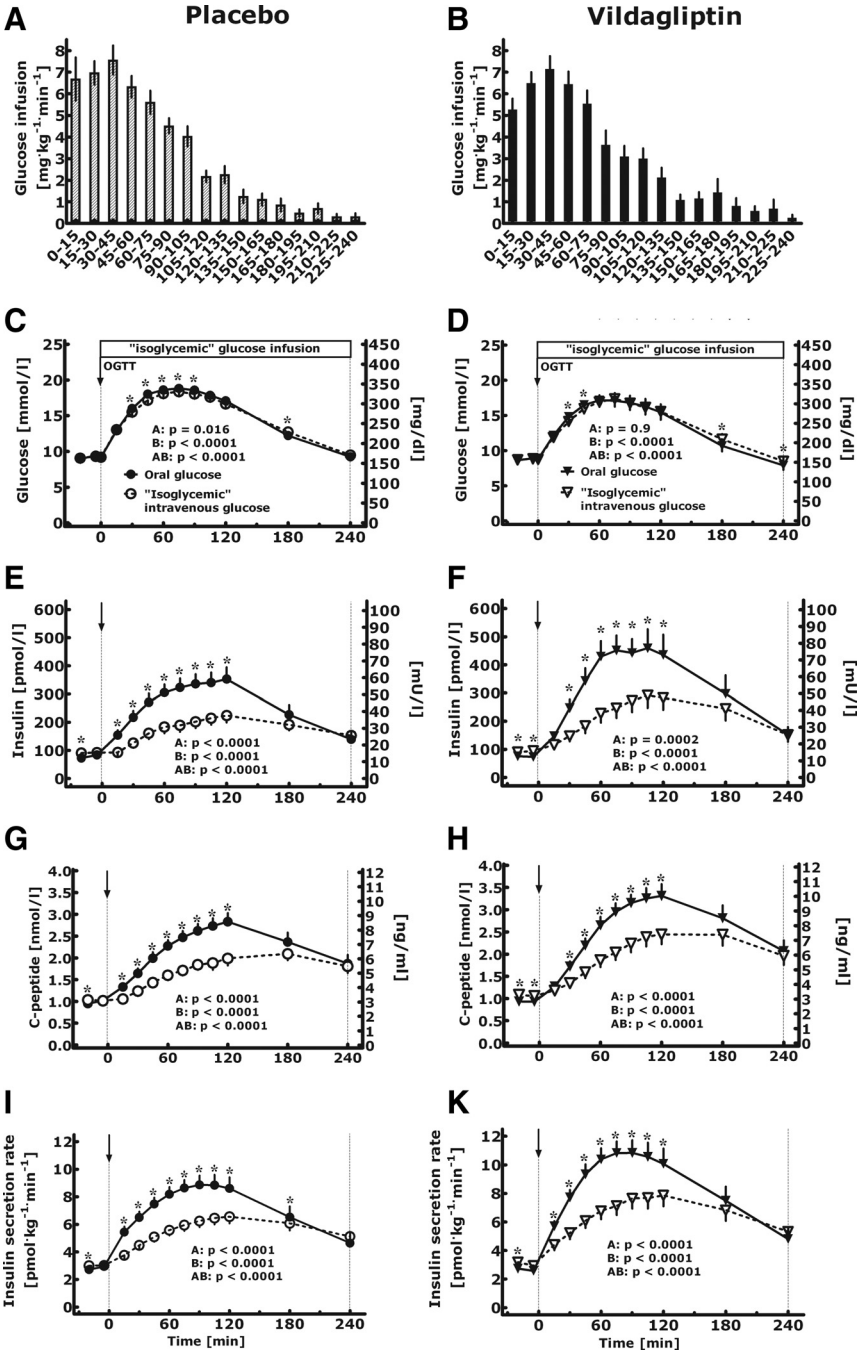

FIG. 1. Glucose infusion rates (A and B); concentrations of plasma glucose (C and D), insulin (E and F), and C-peptide (G and H); and insulin secretion rates (I and K) with placebo (A, C, E, G, I) and vildagliptin (B, D, F, H, K) after stimulation with oral glucose, 75 g (*filled symbols*) and isoglycemic iv glucose (*open symbols*), respectively, in patients with type 2 diabetes. Data are expressed as mean ± SEM. *P* values (A, B, and AB) are the result of repeated measures ANOVA and denote differences by experiment (A), differences over time (B), and differences due to the interaction of experiment and time (AB). *, Significant differences ($P < 0.05$) at individual time points (ANOVA). *Arrows* indicate the time of administration of oral glucose.

demonstrating a residual incretin effect in our type 2 diabetic patients. Quantitatively speaking, these results are compatible with previous publications describing a somewhat reduced incretin effect in patients with diabetes (9, 29).

Vildagliptin treatment significantly enhanced insulin secretion both under the condition of oral glucose stimulation and with the iv glucose stimulus (Table 2). This was the case despite the finding that, as expected, both intact GIP and GLP-1 concentrations were raised considerably more after oral glucose stimulation than during iv glucose infusions (Figs. 2 and 3 and Table 3). However, in experiments after vildagliptin and placebo treatment, a slight but significant increase in intact GLP-1 levels above baseline concentrations was noted after iv glucose administration (placebo, $P = 0.0002$; vildagliptin, $P < 0.0001$; Fig. 2). If the incretin effect was calculated as the contribution of gut factors to the insulin secretory responses after oral glucose, vildagliptin treatment did not change the numerical value, no matter whether this calculation was based on insulin, C-peptide, or insulin secretion rates. In line with the concept of gastrointestinally mediated glucose disposal (30), the total amount of glucose infused with and without vildagliptin treatment was similar (65.9 ± 2.1 *vs.* 63.6 ± 5.3 g per experiment; $P = 0.64$), indicating no significant change in the incretin effect (Table 2).

Glucagon concentrations were suppressed with both oral and iv glucose, and in a similar manner under vildagliptin and placebo treatment (Fig. 3). Intravenous glucose led to an earlier suppression of glucagon than did oral glucose, as previously observed in healthy subjects and type 2 diabetic patients (30, 31).

As is obvious from a nonsignificant difference between total GLP-1 response to oral glucose with vildagliptin and placebo (Table 3), we found no evidence of feedback inhibition of L-cell secretion in response to the administration of the DPP-4 inhibitor vildagliptin after 12–13 d of treatment.

Discussion

Inhibitors of the DPP-4 are thought to augment the activity of endogenously released GLP-1 (and GIP and, perhaps, other gut hormones that also are substrate to this peptidase) (32). The release of GLP-1 from L cells follows a typical temporal pattern, with fasting (basal) concentra-

TABLE 2. Integrated incremental responses of insulin, C-peptide, and insulin secretion rates (calculated by deconvolution) after oral glucose stimulation and during the isoglycemic iv infusion of glucose in type 2 diabetic patients treated with vildagliptin or placebo, and the amount of glucose administered per experiment

Parameter	Oral glucose	Isoglycemic iv glucose	Difference (oral − iv)	Significance (*P* value) oral *vs.* iv glucose	Incretin effect (%)
∫ Insulin (mU · liter^{-1} · min)					
Placebo	7064 ± 891	3497 ± 467	3568 ± 504	<0.0001	49.5 ± 3.4
Vildagliptin	9934 ± 1540	5156 ± 989	4778 ± 1255	0.001	44.2 ± 6.8
P value	0.009	0.044	0.30		0.48
∫ C-peptide (nmol · liter^{-1} · min)					
Placebo	304 ± 29	179 ± 18	125 ± 16	<0.0001	40.1 ± 3.7
Vildagliptin	397 ± 38	239 ± 32	158 ± 30	<0.0001	38.5 ± 5.2
P value	0.0004	0.028	0.27		0.82
∫ Insulin secretion rate (pmol/kg)					
Placebo	1008 ± 123	626 ± 69	382 ± 70	<0.0001	34.6 ± 4.0
Vildagliptin	1338 ± 146	829 ± 109	508 ± 98	<0.0001	35.7 ± 4.9
P value	0.0006	0.013	0.20		0.80
Glucose administration (g/experiment)					
Placebo	75 ± 0	65.9 ± 2.1	9.1 ± 2.1	0.0003	12.2 ± 2.7
Vildagliptin	75 ± 0	63.6 ± 5.3	11.4 ± 5.3	0.043	15.2 ± 7.1
P value	1.0	0.64	0.64		0.64

Differences between experiments with oral and isoglycemic iv glucose and the percentage contribution of the incretin effect to insulin secretory responses after oral glucose are shown. ∫, Integrated incremental responses (over baseline), mean ± SEM (n = 21).

tions being low and nutrient stimulation leading to substantial increases in plasma concentrations for a period of several hours (4–6). Therefore, it was reasonable to assume that DPP-4 inhibitors will lead to a greater augmentation of insulin secretion when given in association with oral nutrients, which increase both glucose concentrations and the release of gut hormones including GIP and GLP-1, than with iv glucose infusions, which raise glycemic levels to the same degree (isoglycemia) but do not substantially elicit a secretory response from either K cells (GIP) or L cells (GLP-1) (Figs. 2 and 3 and Table 3).

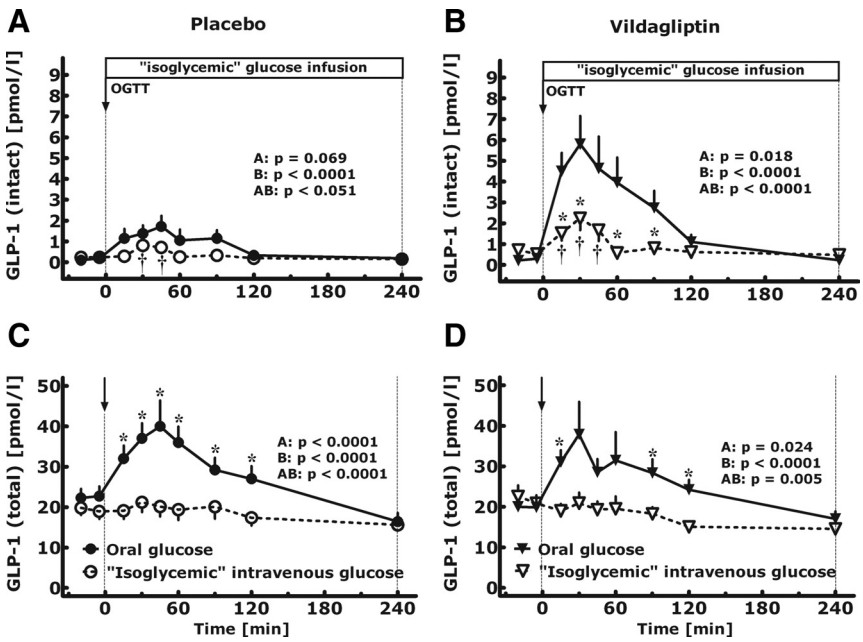

FIG. 2. Concentrations of GLP-1 (intact) and GLP-1 (total) with placebo (A and C) and vildagliptin (B and D) after stimulation with oral glucose, 75 g (*filled symbols*), and isoglycemic iv glucose (*open symbols*), respectively, in patients with type 2 diabetes. Data are expressed as mean ± SEM. *P* values are the result of repeated measures ANOVA and denote differences by experiment (A), differences over time (B), and differences due to the interaction of experiment and time (AB). †, Significant difference ($P < 0.05$) *vs.* baseline value of intact GLP-1. *, Significant differences ($P < 0.05$) at individual time points (ANOVA). *Arrows* indicate the time of administration of oral glucose.

Surprisingly, insulin secretion was augmented by vildagliptin treatment not only after oral glucose stimulation, but also during isoglycemic iv glucose infusions (Fig. 1 and Table 2), although the patterns of GIP and GLP-1 release were as expected (4–6), *i.e.* much more accentuated after oral glucose than during iv glucose administration (Fig. 2). However, in the present study, there was also a minor rise in the concentrations of intact GLP-1 accompanying iv glucose administration, especially after vildagliptin treatment. This may have contributed to the augmented insulin secretory responses after DPP-4 inhibitor treatment.

It could be argued that the reduction in glycemic excursions after oral glucose administration after vildagliptin treatment may have caused a subse-

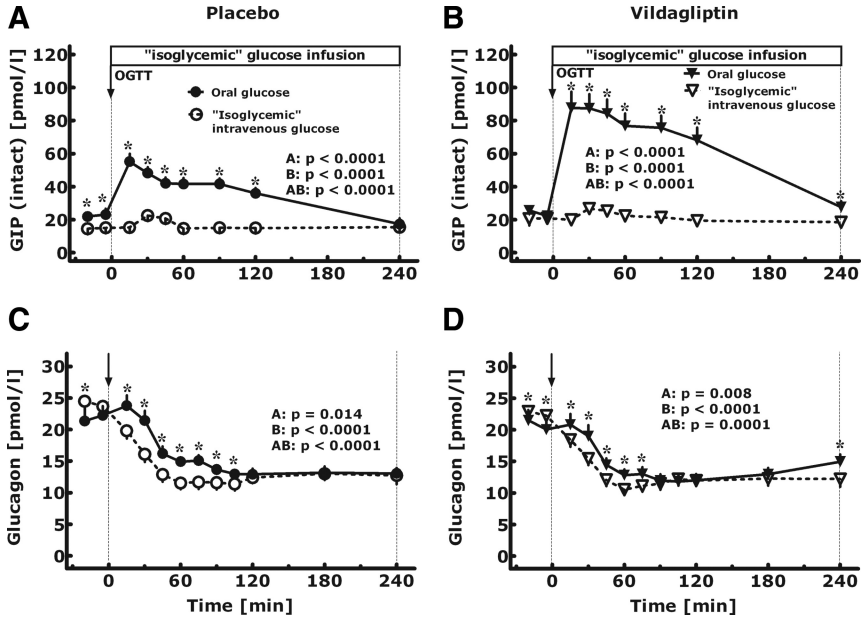

FIG. 3. Plasma concentrations of GIP (intact) and glucagon with placebo (A and C) and vildagliptin (B and D) after stimulation with oral glucose, 75 g (*filled symbols*), and isoglycemic iv glucose (*open symbols*), respectively, in patients with type 2 diabetes. Data are expressed as mean ± SEM. *P* values are the result of repeated measures ANOVA and denote differences by experiment (A), differences over time (B), and differences due to the interaction of experiment and time (AB). *, Significant differences ($P < 0.05$) at individual time points (ANOVA). *Arrows* indicate the time of administration of oral glucose.

quent reduction in insulin secretion due to a lower glycemic stimulus. This should, however, affect the insulin secretory responses during isoglycemic iv glucose infusions in the same manner because glucose infusions would aim at lower glucose concentrations in this case. Most likely, insulin secretion in response to oral and iv glucose would be affected to a similar degree, leaving the main parameter studied, the incretin effect, relatively uninfluenced.

Based on the methodology to measure the incretin effect, any reduction in glycemic levels after oral glucose will lead to a lower glucose level during isoglycemic iv glucose administration as well. So we assume that the net effect for the calculated incretin effect (percentage contribution of gut stimulation) should be negligible. Because patients with diabetes have a smaller incretin effect than metabolically healthy subjects (normal glucose tolerance), any reduction in glycemic levels might be assumed to be associated with an improved incretin effect. However, in cross-sectional studies, this is only the case below a fasting glucose of approximately 126 mg/dl (33). This again suggests that a minor reduction in hyperglycemia should not *per se* have affected the incretin effect as measured in this study.

It is interesting that a recent study (34) shows that exendin (9–39) reduced insulin responses to iv glucose, suggesting that this might be evidence for an effect of endogenous GLP-1 (at low, basal levels) to promote glucose-induced insulin secretion regardless of the mode of glucose administration (34). One might argue that with vildagliptin there was at least a minor increase in intact, biologically active GLP-1 (Fig. 2B) and GIP (Fig. 3B) that also followed iv glucose administration. However, this was not a statistically significant finding, and it is worth studying with a more adequate sample size. Even small increments may indicate much larger changes occurring in the portal vein (35).

Our findings do not support the presence of feedback inhibition of L cells in response to the administration of a DPP-4 inhibitor like vildagliptin, as previously described

TABLE 3. Integrated incremental responses (∫) after oral glucose stimulation and during the iv infusion of glucose of total (nonspecific assay) GLP-1, intact, biologically active GLP-1, and intact, biologically active GIP (for time courses, see Fig. 2) induced by DPP-4 inhibitor administration

Parameter	Oral glucose	Isoglycemic iv glucose	Significance (*P* value) oral *vs.* iv glucose
∫ GLP-1 (total) (pmol · liter^{-1} · min)			
Placebo	1750 ± 329	438 ± 131	0.0012
Vildagliptin	1819 ± 542	163 ± 110	0.0090
P value	0.92	0.13	
∫ GLP-1 (intact) (pmol · liter^{-1} · min)			
Placebo	133 ± 40	32 ± 13	0.0243
Vildagliptin	420 ± 99	83 ± 17	0.0044
P value	0.0048	0.02	
∫ GIP (intact) (pmol · liter^{-1} · min)			
Placebo	3370 ± 475	986 ± 202	<0.0001
Vildagliptin	9220 ± 1197	1197 ± 241	<0.0001
P value	<0.0001	0.58	

Data are expressed as mean ± SEM.

(22). The integrated incremental response of total GLP-1 (including DPP-4 breakdown metabolites) with vildagliptin administration (Fig. 2 and Table 3) was not significantly reduced after 12–13 d of treatment. This is in contrast to previous observations describing a significantly suppressed secretion of GLP-1 (postprandial response of total GLP-1 after oral glucose or a meal) after a single dose of vildagliptin (22) or sitagliptin (19). This may be more prominent after mixed meals, whereas in the present study oral glucose was used as the nutrient stimulus. Our present results may indicate that feedback inhibition of GLP-1 secretion by DPP-4 inhibitor is a transient phenomenon triggering compensatory mechanisms that reestablish normal GLP-1 release within periods shorter than 2 wk. It may be worth studying the time course of feedback inhibition in more detail because it could parallel the establishment of the full glucose-lowering activity of DPP-4 inhibitor treatment. A study comparing 1 and 12 wk of treatment with sitagliptin has recently been published (36). The longer term absence of such a feedback inhibition may contribute to the efficacy of DPP-4 inhibition in terms of antihyperglycemic activity.

It was expected that vildagliptin treatment, by inhibiting DPP-4 and leading to higher intact GLP-1 (and GIP) concentrations (Figs. 2 and 3), would augment insulin secretion in response to hyperglycemia. Most likely, GLP-1 interacted with the endocrine pancreas to help release insulin as part of its preserved incretin activity in patients with type 2 diabetes (1, 37). It is an open question whether GIP has made a significant contribution to this process because GIP is hardly insulinotropic in hyperglycemic patients with type 2 diabetes (10, 38). The ability of GIP to augment insulin secretion could only partly be rescued by intensive diabetes treatment aiming at near-normal glucose control in such patients (39, 40).

It may be argued that vildagliptin had only a weak antidiabetic effect in terms of lowering fasting glucose and postload glycemic excursions. However, vildagliptin, under the conditions of this study, clearly augmented insulin secretory responses relative to glycemic excursions. Thus, the present protocol was suitable for studying the role of incretins for insulin secretion after oral and iv glucose administration.

What might be the factors that— under the influence of DPP-4 inhibition—augment glucose-induced insulin secretion also during iv glucose infusions, when studied under treatment with vildagliptin? In the light of apparent biological activity of basal levels of incretin hormones, it is of interest that infusion of a GLP-1 receptor antagonist, exendin (9–39), raised glucagon concentrations in healthy subjects, indicating a tonic inhibition of glucagon secretion even by low, basal GLP-1 concentrations (41). A small overnight rise in intact, biologically active GLP-1 with DPP-4 inhibition (vildagliptin treatment; Fig. 2B) may have elicited an increased influence on both glucagon (suppression) and insulin secretion (augmentation), even with iv glucose not stimulating GLP-1 secretion from L cells. One might, in addition, speculate that there are additional peptides with biological activity, substrates to DPP-4 as well as GLP-1 and GIP, that are insulinotropic, but are present also or even predominantly in the fasting state (unlike typical incretins). Candidates might be neuropeptides like pituitary adenylate cyclase-activating polypeptide (42) that act locally rather than being transported to the target cells via the blood stream.

Glucagon was suppressed more readily with iv glucose than with oral glucose (Fig. 3). This is in line with recent observations (30, 31) and may indicate that GIP (released by oral glucose) may stimulate glucagon secretion (43).

It may be noted that in our cohort of type 2 diabetic patients, there was a significant incretin effect, which is in contrast to the previously described original cohort (9), who did not display a significant incretin effect at all. The sample size (n = 21) in the present study was greater than in the published study (n = 14), and the laboratory methods for determining insulin secretory products most likely have improved, which makes the detection of differences easier. As a result, our conclusion would be that the incretin effect in patients with type 2 diabetes is reduced (if compared with healthy subjects) but is preserved to some extent in the majority of subjects, in line with other recent studies (29). This may in part be the consequence of reduced glucotoxicity (44), which should affect insulin secretory responses to oral and iv glucose to a similar degree. It could, however, also be that metformin treatment in our subjects may have further stimulated L-cell secretion (45), potentially leading to an improvement in the incretin effect in comparison to less well-controlled, non-metformin-treated patients.

The fact that the numerical incretin effect was not changed by vildagliptin treatment is based on one commonly used approach to calculating/estimating the influence of incretins on post-nutrient-intake insulin secretory responses. With both the insulin secretory responses after oral and iv glucose administration being augmented, the difference between the two, in absolute terms, was increased (although not significantly; Supplemental Table 1A).

An interesting observation is the fact that the amount of glucose that has to be administered to obtain isoglycemia is a good surrogate measure of the incretin effect. The more glucose that needs to be infused to match glycemia to that after 75 g of oral glucose, the more the incretin effect is reduced if the difference in glucose administration between oral and iv route is expressed as the percentage of the 75-g oral glucose load. This significantly correlates

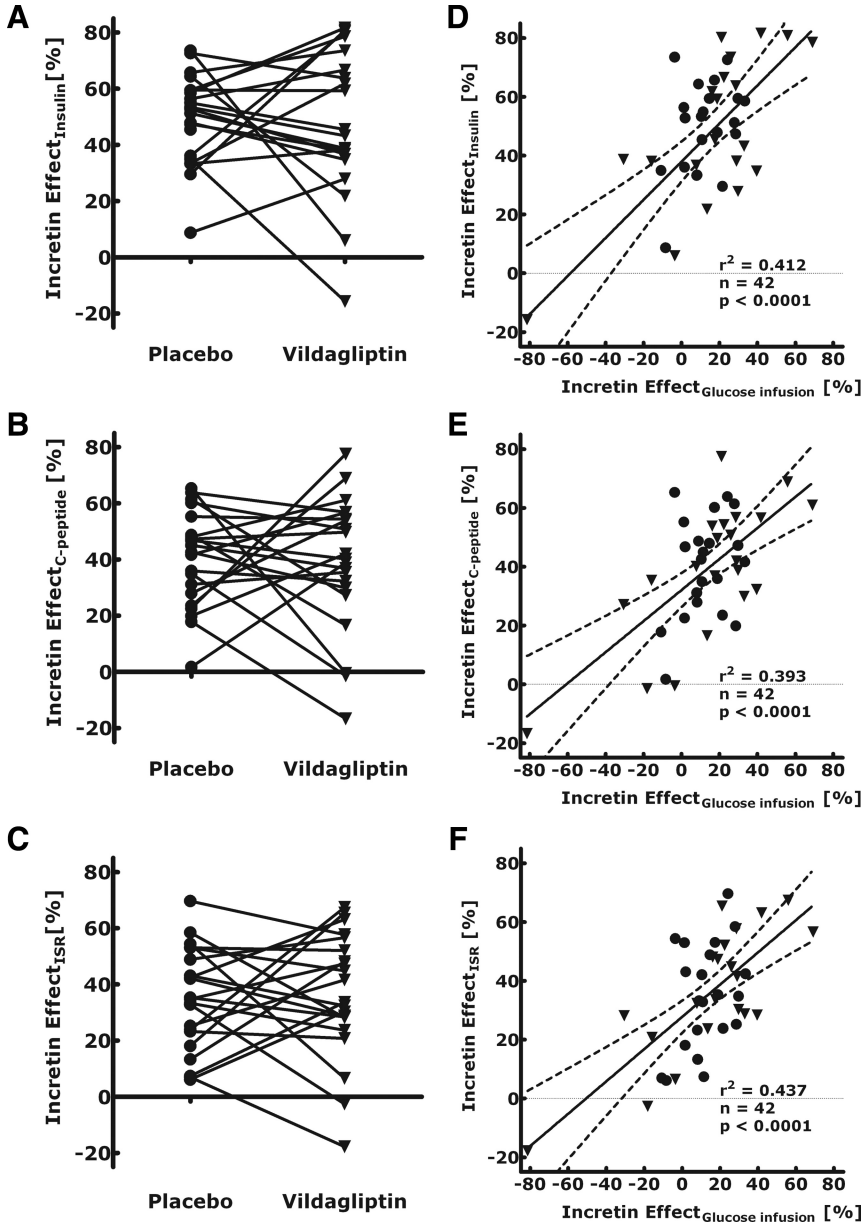

FIG. 4. *Left*, Scatter diagrams depicting individual incretin effects after treatment with placebo or vildagliptin, based on insulin (A), C-peptide (B), or insulin secretion rates (ISR) (C). *Right*, Regression analysis correlating incretin effects based on the amount of glucose administered during experiments with oral and isoglycemic iv glucose and incretin effects based on integrated incremental responses of insulin (D), C-peptide (E), and insulin secretion rates (F) are shown. For details, see *Subjects and Methods*.

with the incretin effect determined from insulin, C-peptide, and insulin secretion rates (r^2 = 0.393–0.437; $P <$ 0.0001 for all comparisons) (Fig. 4), indicating that with or without insulinotropic activity contributed by incretin hormones, the amount of insulin secreted per gram of glucose administered is more or less the same, as long as the glucose concentrations are comparable (isoglycemia). This is a remarkable aspect of the integrated regulation of glucose metabolism that has recently been emphasized by Knop *et al.* (30) in type 2 diabetic patients and by Hare *et al.* (46) even for type 1 diabetic patients.

In conclusion, vildagliptin augments insulin secretory responses both in response to the administration of oral glucose (accompanied by the release of incretin hormones) and during the iv infusion of glucose (*i.e.* without a major incretin response). Thus, against expectations, the incretin effect is not enhanced by DPP-4 inhibitor treatment, mainly due to a surprising augmentation of insulin secretory responses even with iv glucose infusions. The nature of this phenomenon needs to be further elucidated in mechanistic studies characterizing the mode of action of DPP-4 inhibitors, for example using GLP-1 receptor antagonists to more precisely define the role of GLP-1 as opposed to additional factors as mediators of DPP-4 inhibition.

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ployee and stockholder of Novartis Pharma AG, Basel, Switzerland. J.E.F. is an employee of Novartis Pharma Corporation and stockholder of Novartis Pharmaceuticals.

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