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# Quantification of the Contribution of GLP-1 to Mediating Insulinotropic Effects of DPP-4 Inhibition With Vildagliptin in Healthy Subjects and Patients With Type 2 Diabetes Using Exendin [9-39] as a GLP-1 Receptor Antagonist

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**We quantified the contribution of GLP-1 as a mediator of the therapeutic effects of dipeptidyl peptidase 4 (DPP-4) inhibition (vildagliptin) by using the GLP-1 receptor antagonist exendin [9-39] in patients with type 2 diabetes and in healthy subjects. Thirty-two patients with type 2 diabetes and 29 age- and weight-matched healthy control subjects were treated in randomized order with 100 mg once daily vildagliptin or placebo for 10 days. Meal tests were performed (days 9 and 10) without and with a high-dose intravenous infusion of exendin [9-39]. The main end point was the ratio of the areas under the curve (AUCs) of integrated insulin secretion rates (total $AUC_{ISR}$) and glucose (total $AUC_{glucose}$) over 4 h after the meal. Vildagliptin treatment more than doubled responses of intact GLP-1 and glucose-dependent insulinotropic polypeptide and lowered glucose responses without changing $AUC_{ISR}/AUC_{glucose}$ in healthy subjects. Vildagliptin significantly increased this ratio by 10.5% in patients with type 2 diabetes, and exendin [9-39] reduced it (both $P < 0.0001$). The percentage reduction in the $AUC_{ISR}/AUC_{glucose}$ ratio achieved with exendin [9-39] was significantly smaller after vildagliptin treatment than after placebo treatment ($P = 0.026$) and was equivalent to 47 ± 5% of the increments due to vildagliptin. Thus, other mediators appear to contribute significantly to the therapeutic effects of DPP-4 inhibition.**

Inhibitors of dipeptidyl peptidase 4 (DPP-4) are used in the treatment of type 2 diabetes. Vildagliptin (1) and sitagliptin (2) have been shown to raise plasma levels of intact glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) after nutrient stimulation. GIP, however, has been shown to be ineffective in augmenting insulin secretion in type 2 diabetes (3–5). Thus, GLP-1 is assumed to be the main mediator of DPP-4 inhibition and its effects on stimulating insulin and suppressing glucagon secretion (1,2). Nevertheless, other potential substrates for DPP-4 include gastrointestinal hormones, neuropeptides, cytokines, and other biologically active peptides (6). Whether GLP-1 is the only or even the main mediator of DPP-4 inhibition is not clear (7,8).

Exendin [9-39] is a GLP-1 receptor antagonist (9,10) that can be used in humans to block effects of endogenously secreted GLP-1 (11,12). GLP-1–mediated (inhibitable by exendin [9-39]) and non–GLP-1–mediated portions of the clinical effects of the DPP-4 inhibitor sitagliptin have been

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**See accompanying article, p. 2124.**

found in glucose reduction and insulin stimulation after oral glucose in patients with type 2 diabetes (13). We used a similar approach but with the DPP-4 inhibitor vildagliptin and a mixed meal in both patients with type 2 diabetes and healthy subjects. We hypothesized that a different DPP-4 inhibitor and different nutrient stimulus, which may lead to a different release pattern of gut hormones (14) and may be emptied from the stomach with a different kinetic profile (15), would change the results as much as the choice of subjects with hyperglycemia versus normoglycemia (16). Preliminary data have been presented (17).

## RESEARCH DESIGN AND METHODS

The study protocol was approved by the ethics committee of the Medical Faculty, Georg-August-Universität Göttingen (Göttingen, Germany). Written informed consent was obtained from all participants.

### Subjects

Six healthy subjects participated in a pilot study examining the blockade of the insulinotropic effects of exogenous GLP-1 [7-36] amide by exendin [9-39]. Twenty-nine healthy subjects and 32 age-, sex-, and obesity-matched patients with type 2 diabetes participated in the main study.

Inclusion criteria for the patients with type 2 diabetes were treatment with either diet/exercise or metformin, age 30–75 years (inclusive), $HbA_{1c}$ 6.5–9.0 (normal range <6.1%), fasting plasma glucose 6.0–11.0 mmol/L, BMI 20.0–35.0 kg/m$^2$, and absence of significant heart, kidney (serum creatinine ≤123 μmol/L in women and ≤132 μmol/L in men), liver (transaminases less than twofold the upper limit of normal), and gastrointestinal disease. Metformin treatment was continued throughout the study. Healthy control subjects were required to have a normal oral glucose tolerance test (75 g) and no first-degree relatives with type 2 diabetes or personal history of gestational diabetes.

### Peptides (GLP-1 [7-36] amide and Exendin [9-39])

GLP-1 [7-36] amide of good manufacturing practice quality was Clinalfa brand (Bachem Distribution Service GmbH, Weil am Rhein, Germany). Exendin [9-39] was custom synthesized at Novartis Pharmaceuticals. Infusions were prepared with 0.9% NaCl in 1% human serum albumin (CSL Behring, Marburg, Germany).

After a screening examination, patients were treated with vildagliptin (100 mg once daily) or placebo and participated in meal tests (day 9 and 10, respectively) in a crossover design. Between the two treatment periods was a ≥5-week washout period. Details of the pilot study evaluating the ability of exendin [9-39] to inhibit insulinotropic actions of exogenous GLP-1 are presented in Supplementary Fig. 1.

### Procedure

#### *Meal Test and Determination of the Rate of Gastric Emptying*

On days 9 and 10 of treatment, study participants underwent a mixed meal (one scrambled egg, one slice of ham,

**Table 1—Characteristics of healthy control subjects and patients with type 2 diabetes participating in the pilot and main studies**

| | Pilot study: glucose-stimulated insulin secretion, effects of exogenous GLP-1 [7-36] amide ± exendin [9-39] | | Main study: meal-stimulated insulin secretion and effects of vildagliptin vs. placebo ± exendin [9-39] | | | |
|---|---|---|---|---|---|---|
| Characteristic | Healthy control subjects | | Healthy control subjects | | Patients with type 2 diabetes | |
| Sex, *n* | | | | | | |
| Female | 2 | | 3 | | 3 | |
| Male | 4 | | 26 | | 29 | |
| % female | 33.3 | | 10.3 | | 9.4 | |
| Age (years) | 45 ± 12 | 31–58 | 60 ± 9 | 41–72 | 61 ± 9 | 37–73 |
| BMI (kg/m$^2$) | 23.5 ± 1.9 | 20.7–26.1 | 27.1 ± 2.6 | 20.7–32.2 | 28.7 ± 3.3 | 23.1–34.8 |
| Fasting plasma glucose (mmol/L) | 4.7 ± 0.6 | 3.7–5.4 | 5.1 ± 0.5 | 4.2–6.2 | 7.8 ± 1.2 | 5.5–10.5 |
| 2-h glucose after 75-g OGTT (mmol/L) | 5.4 ± 1.2 | 4.4–7.1 | 6.3 ± 1.5 | 2.8–8.5 | NA | NA |
| $HbA_{1c}$ (%) | 5.3 ± 0.1 | 5.1–5.5 | 5.4 ± 0.3 | 4.8–5.9 | 7.2 ± 0.5 | 6.3–8.4 |
| Duration of diabetes (years) | NA | | NA | | 6 ± 6 | 0–24 |
| Pretreatment with metformin, *n* | NA | | NA | | | |
| Yes | | | | | 22 | |
| No | | | | | 10 | |
| % yes | | | | | 68.8 | |
| Metformin dose (mg/day) | NA | | NA | | 1,581 ± 548 | |
| Triglycerides (mmol/L) | | | 1.3 ± 0.6 | 0.5–3.1 | 1.9 ± 0.9 | 0.6–4.4 |
| Serum creatinine (μmol/l) | 80 ± 18 | 62–97 | 85 ± 10 | 67–116 | 83 ± 12 | 61–105 |

Data are mean ± SD or range unless otherwise indicated. NA, not applicable; OGTT, oral glucose tolerance test.

**Table 2—Integrated incremental response of glucose, parameters characterizing insulin secretory responses, incretin hormones, and selected gastrointestinal hormones in relation to vildagliptin treatment and to the administration of exendin [9-39] in healthy control subjects and patients with type 2 diabetes**

| | Condition | | | | Significance | | |
|---|---|---|---|---|---|---|---|
| | Vildagliptin no, exendin [9-39] no | Vildagliptin no, exendin [9-39] yes | Vildagliptin yes, exendin [9-39] no | Vildagliptin yes, exendin [9-39] yes | Vildagliptin | Exendin [9-39] | Interaction |
| Healthy control subjects | | | | | | | |
| Glucose (pmol · $L^{-1}$ · h) | 2.0 ± 0.2 | 2.6 ± 0.2 | 1.9 ± 0.2 | 2.4 ± 0.2 | 0.31 | <0.0001 | 0.94 |
| Insulin (pmol · $L^{-1}$ · h) | 386 ± 41 | 506 ± 55 | 352 ± 34 | 489 ± 43 | 0.21 | <0.0001 | 0.79 |
| C-peptide (ng · $mL^{-1}$ · h) | 8.5 ± 0.8 | 9.8 ± 1.0 | 8.2 ± 0.6 | 10.0 ± 0.7 | 0.93 | 0.0003 | 0.56 |
| Insulin secretion (pmol/kg) | 8.8 ± 0.8 | 10.2 ± 0.9 | 8.4 ± 0.6 | 9.9 ± 0.6 | 0.41 | 0.0002 | 0.86 |
| $GLP\text{-}1_{total}$ (pmol · $L^{-1}$ · h) | 11.3 ± 2.3 | 51.8 ± 5.6 | 8.0 ± 1.6 | 43.1 ± 4.9 | 0.11 | <0.0001 | 0.43 |
| $GLP\text{-}1_{intact}$ (pmol · $L^{-1}$ · h) | 3.4 ± 0.7 | 8.2 ± 1.0 | 5.7 ± 0.7 | 21.8 ± 2.6 | <0.0001 | <0.0001 | 0.0001 |
| $GIP_{total}$ (pmol · $L^{-1}$ · h) | 101 ± 8 | 49 ± 9 | 79 ± 9 | 21.8 ± 2.6 | 0.0008 | 0.014 | 0.90 |
| $GIP_{intact}$ (pmol · $L^{-1}$ · h) | 43.1 ± 4.5 | 55.4 ± 4.0 | 91.0 ± 9.4 | 110.9 ± 8.0 | <0.0001 | 0.015 | 0.56 |
| Patients with type 2 diabetes | | | | | | | |
| Glucose (pmol · $L^{-1}$ · h) | 3.9 ± 0.3 | 6.8 ± 0.5 | 3.9 ± 0.4 | 6.2 ± 0.4 | 0.28 | <0.0001 | 0.39 |
| Insulin (pmol · $L^{-1}$ · h) | 453 ± 58 | 514 ± 58 | 459 ± 40 | 558 ± 46 | 0.34 | 0.0024 | 0.46 |
| C-peptide (ng · $mL^{-1}$ · h) | 8.0 ± 0.7 | 8.8 ± 0.7 | 9.1 ± 0.5 | 10.4 ± 0.6 | 0.0002 | 0.0022 | 0.50 |
| Insulin secretion (pmol/kg) | 8.5 ± 0.7 | 9.5 ± 0.7 | 9.2 ± 0.5 | 10.9 ± 0.5 | 0.033 | 0.0001 | 0.35 |
| $GLP\text{-}1_{total}$ (pmol · $L^{-1}$ · h) | 13.8 ± 2.4 | 62.3 ± 6.5 | 7.4 ± 1.9 | 47.3 ± 6.5 | 0.016 | <0.0001 | 0.33 |
| $GLP\text{-}1_{intact}$ (pmol · $L^{-1}$ · h) | 3.2 ± 0.6 | 10.0 ± 1.5 | 7.9 ± 1.6 | 37.3 ± 6.5 | <0.0001 | <0.0001 | 0.0006 |
| $GIP_{total}$ (pmol · $L^{-1}$ · h) | 85.8 ± 9.8 | 111.0 ± 9.4 | 61.6 ± 8.4 | 85.8 ± 7.8 | <0.0001 | <0.0001 | 0.93 |
| $GIP_{intact}$ (pmol · $L^{-1}$ · h) | 35.0 ± 5.2 | 53.4 ± 4.6 | 77.6 ± 8.6 | 96.7 ± 7.8 | <0.0001 | 0.0010 | 0.93 |

Data are mean ± SE. Statistical calculations are ANCOVA (see RESEARCH DESIGN AND METHODS for details).

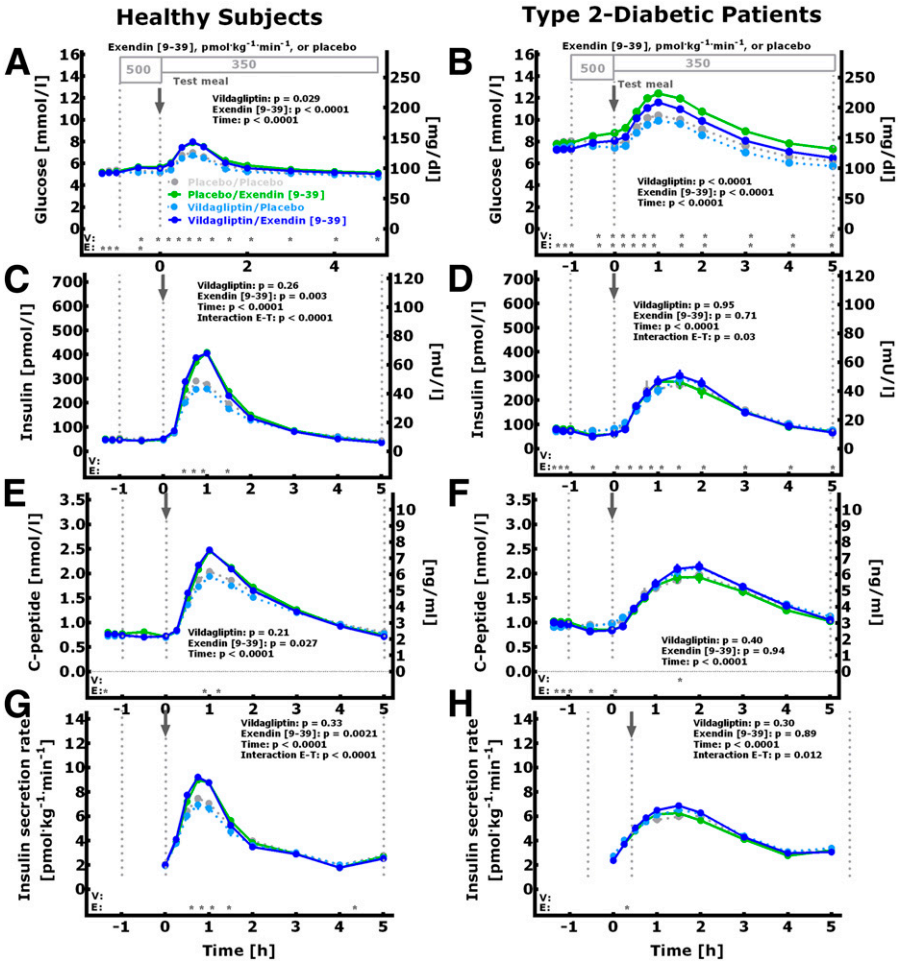


**Figure 1**—Capillary plasma concentrations of glucose (*A* and *B*), venous concentrations of insulin (*C* and *D*) and C-peptide (*E* and *F*), and insulin secretion rates (*G* and *H*) calculated by deconvolution in healthy control subjects and patients with type 2 diabetes. Tests were performed after treatment with placebo, with (day 10) or without (day 9) the administration of the GLP-1 receptor antagonist exendin [9-39], or with vildagliptin, with (day 10) or without (day 9) exendin [9-39]. Data are mean ± SEM. Statistical analyses were repeated-measures ANCOVA reporting *P* values for the independent variables vildagliptin (V), exendin [9-39] (E), time (T), and any significant interactions. Baseline concentrations and values with placebo were imputed as a covariate. Asterisks indicate time points when the independent variable in question (vildagliptin, exendin [9-39]) was associated with a significant ($P < 0.05$) difference in the dependent variable.

10 g of butter, two slices of toast, 20 g of strawberry jam, and 200 mL of unsweetened tea) test in the morning after fasting overnight. $^{13}$C-octanoic acid (110 μL/100 mg) was used as label (Supplementary Data).

### *Blood Specimens*

Blood was drawn from an indwelling Teflon cannula inserted into a forearm vein and processed as previously described (18).

### *Laboratory Determinations*

Glucose was measured (glucose oxidase method) with a Beckman Glucose Lab Analyzer 2 (Beckman Coulter, Munich, Germany). Insulin, C-peptide, glucagon, and total and intact GLP-1 and GIP were determined by specific immunoassays as previously described (18,19). Exendin [9-39] cross-reacted in the glucagon assay (by ~0.014%), thus not allowing interpretation of glucagon measurements. Exendin [9-39] was determined by using an antibody raised in rabbits against exendin-4, as previously described (20).

### Calculations

Integration (area under the curve [AUC]) was carried out by using the trapezoidal rule. Data are presented as incremental changes above baseline or total AUCs, as indicated. Insulin secretion rates were calculated from C-peptide concentrations by using ISEC software, version 3.4a, supplied by R. Hovorka (London, U.K.) (21).

### Statistical Analysis

Subject characteristics are reported as mean ± SD and results as mean ± SEM. Statistical calculations were carried out as repeated-measures ANCOVA using Statistica Version 5.0 software (StatSoft [Europe], Hamburg, Germany). Experimental conditions (vildagliptin vs. placebo; exendin [9-39] vs. placebo) were used as independent fixed variables, and the respective baseline values of the dependent

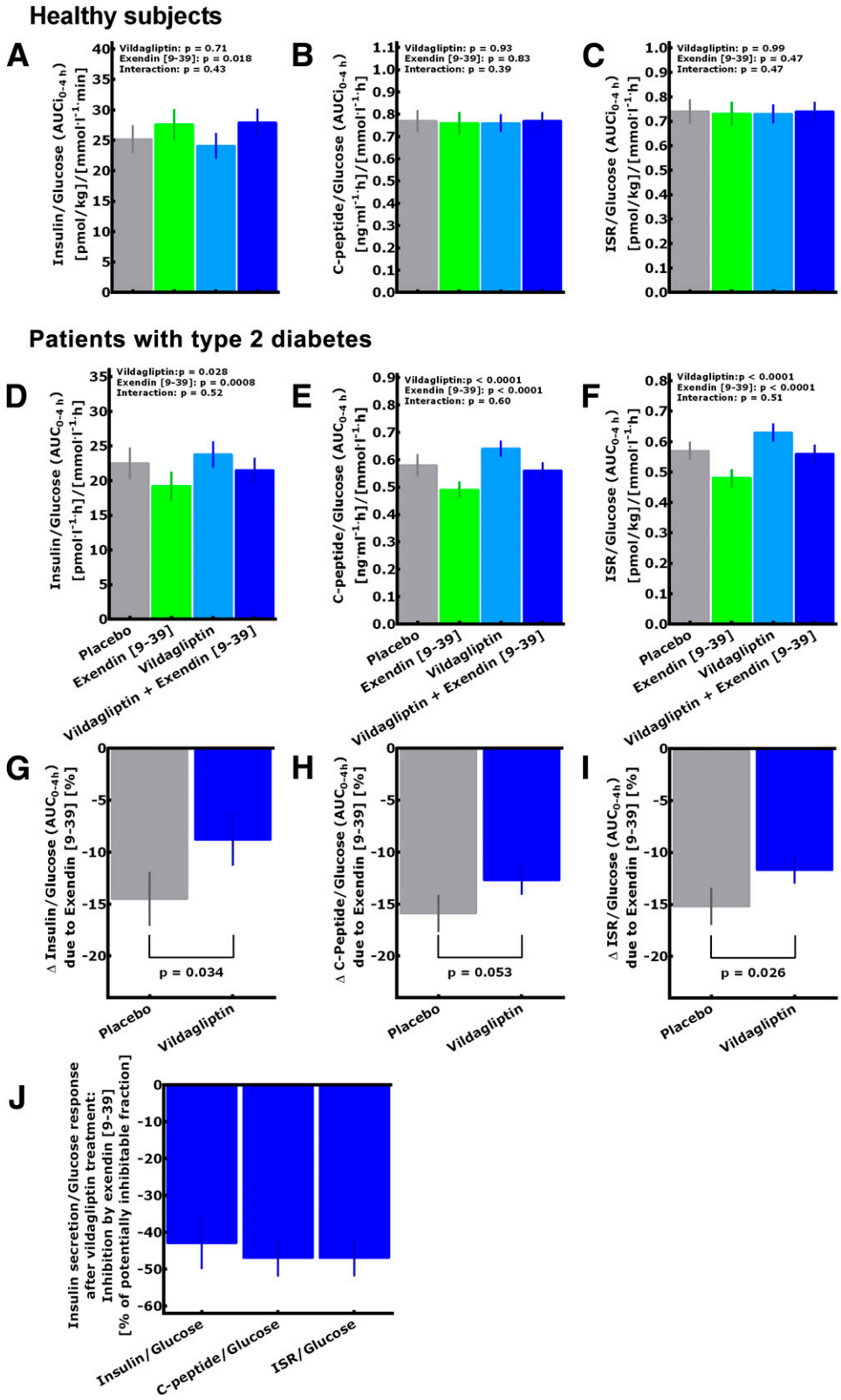


**Figure 2—**Insulinogenic indices (*A*–*F*) calculated as the ratio of insulin (*A* and *D*), C-peptide (*B* and *E*), and insulin secretory responses (ISRs) (*C* and *F*) over glycemic responses in healthy control subjects and patients with type 2 diabetes. The calculations are based on the total responses after meal stimulation from 0 h (beginning of meal ingestion) to 4 h. Meal tests were performed after treatment with placebo, with (day 10) or without (day 9) the administration of the GLP-1 receptor antagonist exendin [9-39], or with vildagliptin, with (day 10) or without (day 9) exendin [9-39]. Data are mean ± SEM. Statistical analyses are repeated-measures ANCOVA reporting *P* values for the independent variables vildagliptin, exendin [9-39], time, and any significant interactions. Baseline concentrations/values with placebo were imputed as a covariate. Asterisks indicate time points when the independent variable in question (vildagliptin, exendin [9-39], interaction of

variable with placebo treatment were used as a covariate. If a significant influence of vildagliptin treatment or of exendin [9-39] was documented by $P < 0.05$ or by interaction of treatment and time ($P < 0.05$), values at individual time points were analyzed by ANCOVA. A two-sided $P < 0.05$ was taken to indicate significant differences. The primary end point reported was the ratio of the AUCs (total response including baseline values) of insulin secretory responses (insulin secretion rates calculated by deconvolution) relative to glucose concentrations as a modified insulinogenic index based on total responses over 4 h after meal ingestion. The originally prespecified primary end point (glucagon) could not be used because of cross-reactivity of exendin [9-39] in the glucagon immunoassay (Supplementary Data). Secondary end points were a similar ratio of insulin and C-peptide concentrations over glucose and the responses of glucose, insulin, C-peptide, insulin secretion rates, and total and intact GLP-1 and GIP.

## RESULTS

### Patients and Healthy Control Subjects

Results from 32 of 34 patients with type 2 diabetes and 29 of 32 healthy control subjects originally recruited were analyzed (completers). Age, sex, and body mass were similar for both groups (Table 1).

### Treatment With Vildagliptin and Exendin [9-39]

Vildagliptin inhibited DPP-4 activity (Supplementary Table 1). In healthy subjects and patients with type 2 diabetes, vildagliptin treatment did not change the velocity of gastric emptying. However, exogenous exendin [9-39] (plasma concentrations shown in Supplementary Fig. 2) significantly accelerated gastric emptying in both groups (Supplementary Fig. 3 and Table 2).

### GLP-1 and GIP Concentrations

Vildagliptin treatment increased intact, biologically active GLP-1 and GIP by two- to threefold and reduced integrated incremental responses of total GLP-1 and GIP slightly (Table 2 and Supplementary Fig. 4) in both groups. Both total and intact GLP-1 increased three- to sixfold, and both total and intact GIP increased modestly with exendin [9-39] (Table 2).

### Glucose and Insulin Secretory Responses During Mixed Meal Stimulation

In healthy control subjects, neither glucose excursions nor meal-related insulin secretory responses were significantly influenced by vildagliptin treatment. Exendin [9-39] increased glycemic excursions and insulin secretory responses significantly (Fig. 1 and Table 2). In patients with type 2 diabetes, glucose concentrations were significantly lowered by vildagliptin treatment (Fig. 1, right panels), but integrated incremental glucose responses did not change significantly (Table 2). Exendin [9-39] administration induced a greater rise in glycemia after meal ingestion in patients with type 2 diabetes than in healthy control subjects. In patients, this was associated with a stimulation of insulin secretory responses with exendin [9-39] (Fig. 1 and Table 2).

### Insulin Secretory Responses Relative to Postmeal Glycemic Increments

In healthy control subjects, insulinogenic indices after meal ingestion were not changed significantly by vildagliptin treatment (Fig. 2). In contrast, in patients with type 2 diabetes, both vildagliptin treatment and exogenous exendin [9-39] had a significant influence on insulinogenic indices based on insulin, C-peptide, and insulin secretion rates after meal ingestion (Fig. 2). Insulinogenic indices significantly increased with vildagliptin treatment and decreased with exendin [9-39]. Expressing the reduction in insulinogenic indices due to exendin [9-39] as a percentage of the value determined without exendin [9-39] surprisingly indicated a lower contribution of GLP-1 after vildagliptin treatment (Fig. 2*G–I*).

With consideration that insulinogenic indices after placebo treatment with exendin [9-39] represent physiological responses minus any contribution of GLP-1 and that values after vildagliptin treatment in the absence of exendin [9-39] show the full DPP-4 inhibitor treatment effect, including actions mediated by GLP-1 (Table 2), the difference between these conditions defines the maximum that GLP-1 could contribute to the effects of DPP-4 inhibitor treatment. The true reduction in insulinogenic indices after vildagliptin treatment was thus expressed relative to this maximum estimate (= 100%): It was found to be ~50% of the potential maximum whether based on the measurement of insulin, C-peptide, or insulin secretion rates (Fig. 2*J*).

### Adverse Events

No adverse events emerged from treatment relative to either vildagliptin treatment or administration of exogenous exendin [9-39].

## DISCUSSION

The current study quantified the contribution of GLP-1 to the glucose-lowering effects of DPP-4 inhibition. Like

vildagliptin and exendin[9-39]) was associated with a significant ($P < 0.05$) difference in the dependent variable. *G–I*: For each patient with type 2 diabetes, the difference in insulinogenic indices (insulin, C-peptide, ISRs) after meal ingestion between experiments with vildagliptin without exogenous exendin [9-39] (i.e., the DPP-4 inhibitor treatment-related response, including potential contributions of GLP-1) and experiments without vildagliptin (oral placebo) but with exogenous exendin [9-39] (i.e., physiological response minus contribution of GLP-1) was taken as 100% of what could potentially be mediated by GLP-1. The true difference caused by exogenous administration of exendin [9-39] after vildagliptin treatment was expressed as a percentage of this potentially inhibitable fraction. Data are mean ± SEM. *J*: The percentage reduction as a result of the administration of exendin [9-39] in the insulinogenic indices calculated as the ratio of insulin, C-peptide, and ISR and glucose responses in patients with type 2 diabetes after treatment with vildagliptin relative to the sum of what exendin [9-39] was able to inhibit with placebo treatment plus what vildagliptin treatment added vs. placebo. AUCi, incremental area under the curve.

Aulinger et al. (13), we used exendin [9-39] (9,10) to estimate the proportion due to GLP-1. Aulinger et al. used higher doses of exendin [9-39] (900 pmol · $kg^{-1}$ · $min^{-1}$) than we did (500 pmol · $kg^{-1}$ · $min^{-1}$ from −1 to 0 h and 350 pmol · $kg^{-1}$ · $min^{-1}$ thereafter). A pilot experiment indicated that the present regimen blocked the insulinotropic effects of a pharmacological dose of exogenous GLP-1 (Supplementary Fig. 1). Different from Aulinger et al., we used vildagliptin instead of sitagliptin and a mixed meal rather than oral glucose. Aulinger et al. studied subjects with type 2 diabetes with an $HbA_{1c}$ of 6.2 ± 0.2% vs. 7.2 ± 0.5% in the present study; we also studied a nondiabetic control group. Aulinger et al. started DPP-4 inhibitor treatment 1 day before the experiments (after two daily doses), whereas we treated for 9–10 days. Nevertheless, the results are comparable.

Incretin hormone responses were similarly influenced by DPP-4 inhibition and exendin [9-39] in the study by Aulinger et al. (13) and the current study. In present study, no differences were found between healthy subjects and patients with type 2 diabetes (22).

In Aulinger et al. (13), gastric emptying of oral glucose (liquid) was mainly influenced by sitagliptin (no significant effect of GLP-1 receptor blockade), whereas in the current study, only exendin [9-39] significantly accelerated gastric emptying of solid components of a mixed meal without a significant influence of vildagliptin treatment, indicating a decelerating effect of endogenous GLP-1 on gastric emptying as previously described (23). This effect has an impact on the interpretation of the present results because accelerated gastric emptying led to greater glycemic excursions with exendin [9-39]. Aulinger et al. and the current study focused on the effects of DPP-4 inhibitor treatment on insulin secretion. Given the glucose-lowering effect of DPP-4 inhibitor treatment and the rise in glycemic excursions caused by GLP-1 receptor blockade, insulin secretory responses need to be interpreted relative to the glycemic rise after the stimulus (insulinogenic index). In both studies, the insulin/glucose ratio was significantly increased by DPP-4 inhibition. The GLP-1–mediated proportion was assessed as follows: The oral glucose/meal test with exendin [9-39] defines insulin secretion in the absence of GLP-1 effects. The experiments with DPP-4 inhibition define the therapeutic effect, including GLP-1– and non–GLP-1–mediated proportions; the difference between both defines the maximum range that could potentially be mediated by GLP-1. The experiment with DPP-4 inhibition and exendin [9-39] divides this range into the proportion mediated by GLP-1. The residual difference then represents the estimate of the non–GLP-1–mediated proportion (Supplementary Fig. 5). For both the glycemic excursions after oral glucose (13) or the mixed meal (Supplementary Fig. 5*A* and *B*) and the insulinogenic indices (Supplementary Fig. 5*C* and *D*), a proportion of the therapeutic effect is mediated by GLP-1, and another proportion cannot be inhibited by exendin [9-39]. Candidate mediators include GIP (24), oxyntomodulin (25), pituitary adenylate cyclase–activating peptide (26), and stromal cell-derived factor-1α (27).

The current study has some limitations. We cannot interpret the glucagon results because of some cross-reaction of exendin [9-39] in the assay. The differences in glycemic excursions and insulin secretory responses elicited by vildagliptin treatment and exendin [9-39] are small, so for an exact quantification, larger numbers of subjects may be necessary. Also of interest would be to examine longer treatment periods than 2 days as in Aulinger et al. (13) or 9–10 days, as in the present study.

In conclusion, this study suggests that not all insulinotropic effects introduced by DPP-4 inhibition (vildagliptin treatment) in patients with type 2 diabetes are mediated by GLP-1 as previously suggested by Aulinger et al. (13).

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**Author Contributions.** M.A.N. contributed to the data research, discussion, and writing, review/editing, and final approval of the manuscript. J.K. contributed to the discussion and writing, review/editing, and final approval of the manuscript. L.D.K., J.J.H., and C.F.D. contributed to the data research, discussion, and review/editing and final approval of the manuscript. M.B. contributed to the review/editing and final approval of the manuscript. Y.L.H. contributed to the data research and review/editing and final approval of the manuscript. L.K. and J.F. contributed to the discussion and review/editing and final approval of the manuscript. M.A.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## References

1. Ahrén 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 2004;89:2078–2084

2. Herman GA, Bergman A, Stevens C, et al. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 2006;91:4612–4619

3. Krarup T, Saurbrey N, Moody AJ, Kühl C, Madsbad S. Effect of porcine gastric inhibitory polypeptide on β-cell function in type I and type II diabetes mellitus. Metabolism 1987;36:677–682
4. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W. 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 1993;91:301–307
5. Vilsbøll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. Diabetologia 2002;45:1111–1119
6. Mentlein R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. Regul Pept 1999;85:9–24
7. Nauck MA, El-Ouaghlidi A. The therapeutic actions of DPP-IV inhibition are not mediated by glucagon-like peptide-1. Diabetologia 2005;48:608–611
8. Holst JJ, Deacon CF. Glucagon-like peptide-1 mediates the therapeutic actions of DPP-IV inhibitors. Diabetologia 2005;48:612–615
9. Göke R, Fehmann HC, Linn T, et al. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. J Biol Chem 1993;268: 19650–19655
10. Thorens B, Porret A, Bühler L, Deng SP, Morel P, Widmann C. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. Diabetes 1993;42:1678–1682
11. Schirra J, Sturm K, Leicht P, Arnold R, Göke B, Katschinski M. Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. J Clin Invest 1998;101:1421–1430
12. Edwards CMB, Todd JF, Mahmoudi M, et al. Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-39. Diabetes 1999;48:86–93
13. Aulinger BA, Bedorf A, Kutscherauer G, et al. Defining the role of GLP-1 in the enteroinsulinar axis in type 2 diabetes using DPP-4 inhibition and GLP-1 receptor blockade. Diabetes 2014;63:1079–1092
14. Carr RD, Larsen MO, Jelic K, et al. Secretion and dipeptidyl peptidase-4-mediated metabolism of incretin hormones after a mixed meal or glucose ingestion in obese compared to lean, nondiabetic men. J Clin Endocrinol Metab 2010;95:872–878
15. Lartigue S, Bizais Y, Des Varannes SB, Murat A, Pouliquen B, Galmiche JP. Inter- and intrasubject variability of solid and liquid gastric emptying parameters. A scintigraphic study in healthy subjects and diabetic patients. Dig Dis Sci 1994; 39:109–115
16. Schvarcz E, Palmér M, Aman J, Horowitz M, Stridsberg M, Berne C. Physiological hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus. Gastroenterology 1997;113:60–66
17. Nauck MA, Kind J, Deacon CF, et al. Blocking GLP-1 action with exendin [9-39] to determine the contribution of GLP-1 to the insulinotropic effects of the DPP-4 inhibitor vildagliptin (Abstract). Diabetologia 2011;54(Suppl. 1):S108
18. Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA. Effects of sitagliptin and metformin treatment on incretin hormone and insulin secretory responses to oral and "isoglycemic" intravenous glucose. Diabetes 2014;63:663–674
19. El-Ouaghlidi A, Rehring E, Holst JJ, et al. The dipeptidyl peptidase 4 inhibitor vildagliptin does not accentuate glibenclamide-induced hypoglycemia but reduces glucose-induced glucagon-like peptide 1 and gastric inhibitory polypeptide secretion. J Clin Endocrinol Metab 2007;92:4165–4171
20. Kielgast U, Asmar M, Madsbad S, Holst JJ. Effect of glucagon-like peptide-1 on alpha- and beta-cell function in C-peptide-negative type 1 diabetic patients. J Clin Endocrinol Metab 2010;95:2492–2496
21. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. Comput Methods Programs Biomed 1996;50:253–264
22. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? Diabetologia 2011;54:10–18
23. Deane AM, Nguyen NQ, Stevens JE, et al. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab 2010;95:215–221
24. Aaboe K, Knop FK, Vilsbøll T, et al. Twelve weeks treatment with the DPP-4 inhibitor, sitagliptin, prevents degradation of peptide YY and improves glucose and non-glucose induced insulin secretion in patients with type 2 diabetes mellitus. Diabetes Obes Metab 2010;12:323–333
25. Maida A, Lovshin JA, Baggio LL, Drucker DJ. The glucagon-like peptide-1 receptor agonist oxyntomodulin enhances beta-cell function but does not inhibit gastric emptying in mice. Endocrinology 2008;149:5670–5678
26. Filipsson K, Sundler F, Ahrén B. PACAP is an islet neuropeptide which contributes to glucose-stimulated insulin secretion. Biochem Biophys Res Commun 1999;256:664–667
27. Humpert PM, Neuwirth R, Battista MJ, et al. SDF-1 genotype influences insulin-dependent mobilization of adult progenitor cells in type 2 diabetes. Diabetes Care 2005;28:934–936