Brief Report

Effects of Dipeptidyl Peptidase-4 Inhibition on Gastrointestinal Function, Meal Appearance, and Glucose Metabolism in Type 2 Diabetes

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OBJECTIVE—We sought to determine whether alterations in meal absorption and gastric emptying contribute to the mechanism by which inhibitors of dipeptidyl peptidase-4 (DPP-4) lower postprandial glucose concentrations.

RESEARCH DESIGN AND METHODS—We simultaneously measured gastric emptying, meal appearance, endogenous glucose production, and glucose disappearance in 14 subjects with type 2 diabetes treated with either vildagliptin (50 mg b.i.d.) or placebo for 10 days using a double-blind, placebo-controlled, randomized, crossover design.

RESULTS—Fasting (7.3 ± 0.5 vs. 7.9 ± 0.5 mmol/l) and peak postprandial (14.1 ± 0.6 vs. 15.9 ± 0.9 mmol/l) glucose concentrations were lower ($P < 0.01$) after vildagliptin treatment than placebo. Despite lower glucose concentrations, postprandial insulin and C-peptide concentrations did not differ during the two treatments. On the other hand, the integrated (area under the curve) postprandial glucagon concentrations were lower (20.9 ± 1.6 vs. 23.7 ± 1.3 mg/ml per 5 h, $P < 0.05$), and glucagon-like peptide 1 (GLP-1) concentrations were higher (1,878 ± 270 vs. 1,277 ± 312 pmol/l per 5 h, $P = 0.001$) during vildagliptin administration compared with placebo. Gastric emptying and meal appearance did not differ between treatments.

CONCLUSIONS—Vildagliptin does not alter gastric emptying or the rate of entry of ingested glucose into the systemic circulation in humans. DPP-4 inhibitors do not lower postprandial glucose concentrations by altering the rate of nutrient absorption or delivery to systemic circulation. Alterations in islet function, secondary to increased circulating concentrations of active GLP-1, are associated with the decreased postprandial glycemic excursion observed in the presence of vildagliptin. ***Diabetes* 56:1475–1480, 2007**

Postprandial hyperglycemia in people with type 2 diabetes may be due to defects in insulin secretion, suppression of glucagon secretion, impaired glucose effectiveness (defined as the ability of glucose per se to stimulate its own uptake and suppress its own release), and impaired insulin action (defined as the ability of insulin to stimulate glucose uptake and suppress glucose release). Alteration in the rate of gastric emptying can also alter postprandial glucose concentrations (1).

Glucagon-like peptide-1 (GLP-1) enhances insulin secretion and inhibits glucagon release (2). In addition, GLP-1 delays gastric emptying (3) and may increase glucose effectiveness and insulin action under certain experimental conditions (4). While studies have shown that dipeptidyl peptidase-4 (DPP-4) inhibitors enhance glucose-induced insulin secretion and inhibition of glucagon secretion (5), prior studies have not simultaneously examined the effects of DPP-4 inhibition on the different mechanisms that determine postprandial glucose concentrations.

We hypothesized that DPP-4 inhibition increases circulating concentrations of active GLP-1 concentrations, which in turn delays gastric emptying and reduces postprandial glycemia. The aim of this study was to further understand the effects of DPP-4 inhibition on simultaneously measured gastric emptying, meal appearance, postprandial suppression of glucose production, and stimulation of glucose uptake. We report that at a dose sufficient to lower postprandial glucose concentrations, treatment with the DPP-4 inhibitor vildagliptin did not alter gastric emptying or the rate of systemic appearance of the ingested glucose. We conclude that DPP-4 inhibition lowers postprandial glucose concentrations via its effects on islet secretion rather than by delaying gastric emptying or reducing the rate at which ingested glucose enters the systemic circulation.

RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 14 subjects with type 2 diabetes gave written informed consent to participate in the study. Subjects were not taking medication known to alter gastric emptying. None of the subjects had a history of diabetes complications. At the time of screening,

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AUC, area under the curve; DPP-4, dipeptidyl peptidase-4; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1.

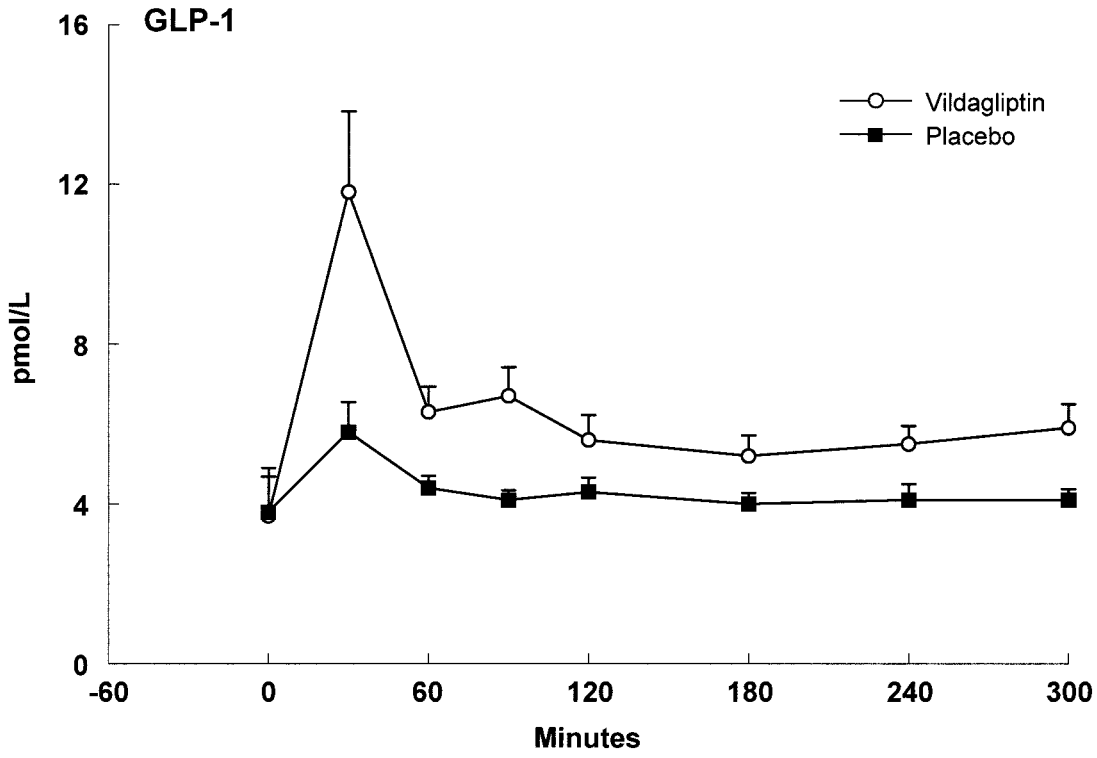

FIG. 1. Concentrations of active GLP-1 observed in the presence and absence of vildagliptin.

the validated Bowel Disease questionnaire revealed the absence of gastrointestinal symptoms (6). All agents used for the treatment of diabetes were discontinued 3 weeks before the study (subject characteristics can be found in an online appendix at http://dx.doi.org/10.2337/db07-0136).

We utilized a randomized, double-blind, placebo-controlled crossover design. Subjects received either 50 mg vildagliptin or placebo taken before breakfast and supper over a 10-day treatment period with the two treatment periods separated by a 2-week washout period. The order of treatment was randomized, and all participants and investigators were blinded to treatment; allocation was concealed. Vildagliptin and matching placebo were provided to the research pharmacy by the study sponsor. Subjects were admitted to the general clinic research center on the evening of the sixth day, and glucose turnover and gastric emptying were measured on the ninth day of each treatment period.

Glucose turnover and gastric emptying. Following an 8-h fast, a forearm vein was cannulated with an 18-gauge needle to allow infusions to be performed. A similar cannula was inserted retrogradely into a vein of the dorsum of the contra-lateral hand. This was placed in a heated Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood. At −180 min, a primed continuous infusion of [6,6-$^2H_2$]glucose was initiated. Subjects received the morning dose (50 mg vildagliptin or placebo) at −30 min. At time 0, subjects consumed a meal consisting of two scrambled eggs labeled with 0.75 mCi ^{99m}Tc (technetium-99m)-sulfur colloid, 55 g Canadian bacon, 240 ml water, and Jell-O containing 75 g glucose labeled with [1-^{13}C]glucose − (4% enrichment). This provided 510 kcal (61% carbohydrate, 19% protein, and 21% fat). An infusion of [6-^{3}H]glucose was started at this time, and the infusion rate varied to mimic the anticipated glucose appearance of the meal [1-^{13}C]glucose as previously described (7). At the same time, the rate of infusion of the [6,6-$^2H_2$]glucose was altered so as to approximate the anticipated pattern of fall in endogenous glucose production (7). Blood was collected at prespecified times. Anterior and posterior γ camera images were obtained immediately after meal ingestion and over the next 4 h for gastric emptying measurement (8).

Analytical techniques. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Plasma [6,6-$^2H_2$]glucose and [1-^{13}C]glucose enrichments were measured using gas chromatographic mass spectrometry (Thermoquest, San Jose, CA) to simultaneously monitor the C-1 and C-2 as well as C-3 to C-6 fragments, as described by Beylot et al. (9), and [6-^{3}H]glucose specific activity by liquid scintillation counting (supplemental appendix).

Calculation of glucose appearance and disappearance rates. The rates of systemic meal appearance, endogenous glucose production, and glucose disappearance were calculated using Steele's two-compartment model (10) as previously described (7) (supplemental appendix).

Statistical analysis. Values from −30 to 0 min were averaged and considered as basal levels. Area above basal and area under the curve (AUC) were calculated using the trapezoidal rule. All data are presented as means ± SE. Rates of glucose turnover are expressed as micromol per kilogram lean body mass. Paired comparisons between treatments were made using two-tailed Student's t test for paired samples. $P < 0.05$ was considered statistically significant.

Given the variance in the measurement of gastric emptying, $T_{1/2}$ (time to empty 50% of stomach contents) in people with type 2 diabetes in our laboratory (8), we estimated that 8 or 10 subjects would provide 80 or 90% power, respectively, to detect a 20% change in gastric emptying $T_{1/2}$ at $P < 0.05$. A 20% change in gastric emptying is deemed clinically significant as it approximates the degree of delay observed in diabetic patients (11).

RESULTS

Plasma GLP-1 concentrations. Fasting concentrations of active GLP-1 (3.7 ± 1.0 vs. 3.8 ± 1.1 pmol/l, $P = 0.68$) did not differ between groups (Fig. 1). However, in the presence of vildagliptin, after meal ingestion, concentrations rose (11.8 ± 2.0 vs. 5.8 ± 0.8 pmol/l, $P = 0.01$) and remained elevated for the duration of the study as shown by the AUC (1,878 ± 270 vs. 1,277 ± 312 pmol/l per 5 h, $P = 0.001$) than occurred during administration of placebo (Fig. 2).

Plasma glucose, insulin, C-peptide, and glucagon concentrations. Treatment with vildagliptin resulted in lower fasting glucose (7.3 ± 0.5 vs. 7.9 ± 0.5 mmol/l, $P = 0.005$), lower postmeal peak (14.1 ± 0.6 vs. 15.9 ± 0.9 mmol/l, $P = 0.0008$), and lower glycemic area above basal (954 ± 85 vs. 1,077 ± 94 mmol per 5 h, $P = 0.01$).

Insulin concentrations did not differ when subjects received vildagliptin or placebo before (54 ± 8 vs. 63 ± 8 pmol/l, $P = 0.11$) or after (63.1 ± 10.5 vs. 62.1 ± 10.0 nmol per 5 h, $P = 0.76$) meal ingestion. Fasting C-peptide concentrations did not differ in the fasting state (0.85 ± 0.08 vs. 1.00 ± 0.12 nmol/l, $P = 0.17$) or after meal ingestion (643 ± 55 vs. 657 ± 69 nmol per 5 h, $P = 0.73$). Treatment with vildagliptin resulted in lower postprandial

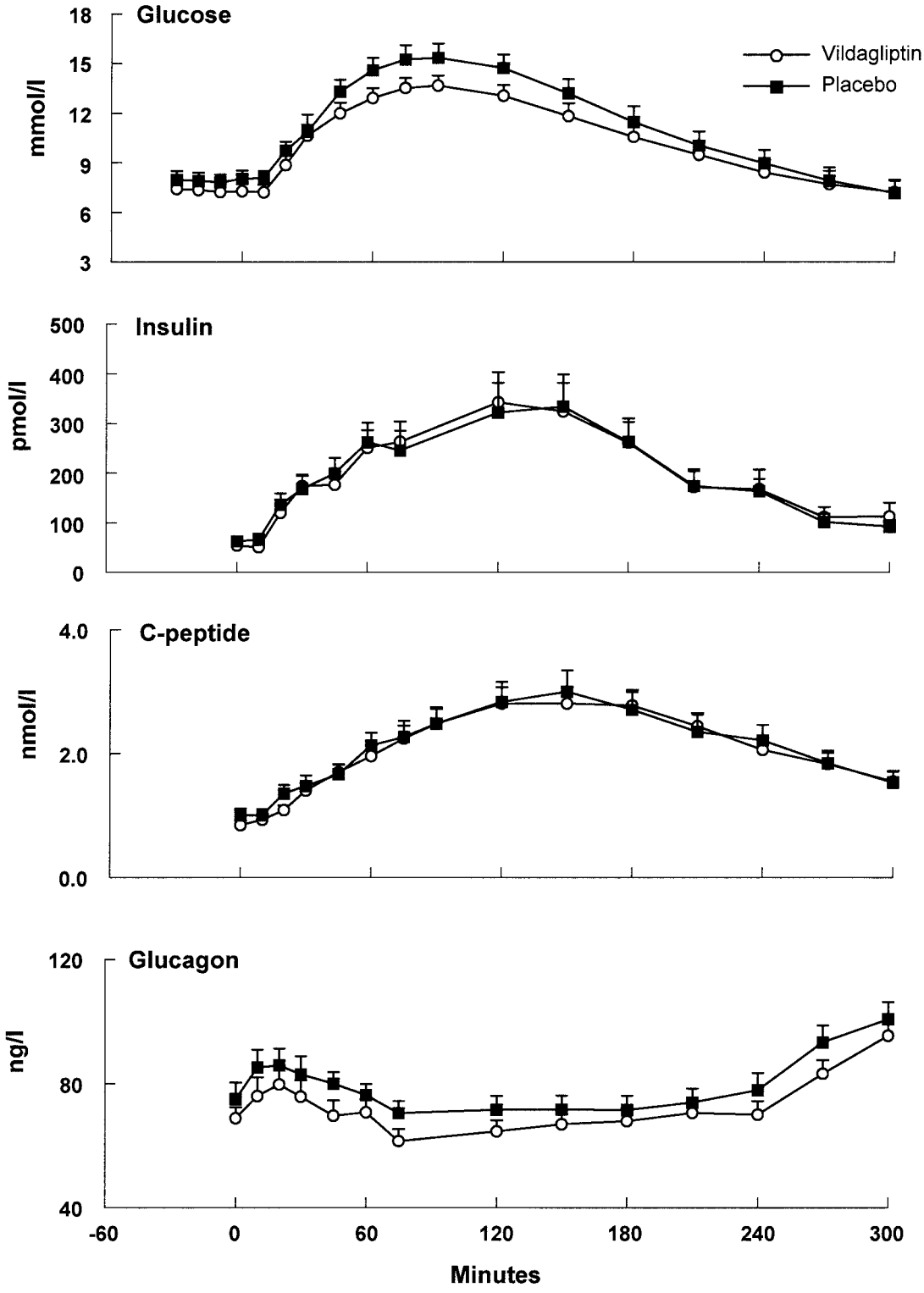

FIG. 2. Glucose, insulin, C-peptide, and glucagon concentrations observed in the presence and absence of vildagliptin.

glucagon concentrations (20.9 ± 1.6 vs. 23.7 ± 1.3 mg per 5 h, $P = 0.03$).

Gastric emptying. Gastric emptying did not differ between treatments. T_{lag} (the time to empty 10% of stomach contents) was 34.5 ± 4.3 on vildagliptin vs. 39.9 ± 6.2 min on placebo, $P = 0.46$ and $T_{1/2,}$ was 144.8 ± 7.3 vs. 143.3 ± 6.5 min, respectively ($P = 0.79$) (Fig. 3). Residual ^{99m}Tc counts at 4 h did not differ between vildagliptin and placebo (17.1 ± 4.8 vs. 15.0 ± 4.5%, respectively, $P = 0.36$).

Meal glucose appearance, endogenous glucose production, and glucose disappearance. The systemic rate of appearance of ingested glucose did not differ whether measured as maximum rate of appearance (90.8 ± 18.3 vs. 98.3 ± 15.6 μmol · kg^{-1} · min^{-1}, $P = 0.88$) or AUC (8,560 ± 557 vs. 8,711 ± 953 μmol/kg per 5 h, $P = 0.98$) (Fig. 4).

Fasting endogenous glucose production was slightly, but not significantly, lower with vildagliptin (19.8 ± 1.3 vs. 20.7 ± 1.3 μmol · kg^{-1} · min^{-1}, $P = 0.07$). Postprandial

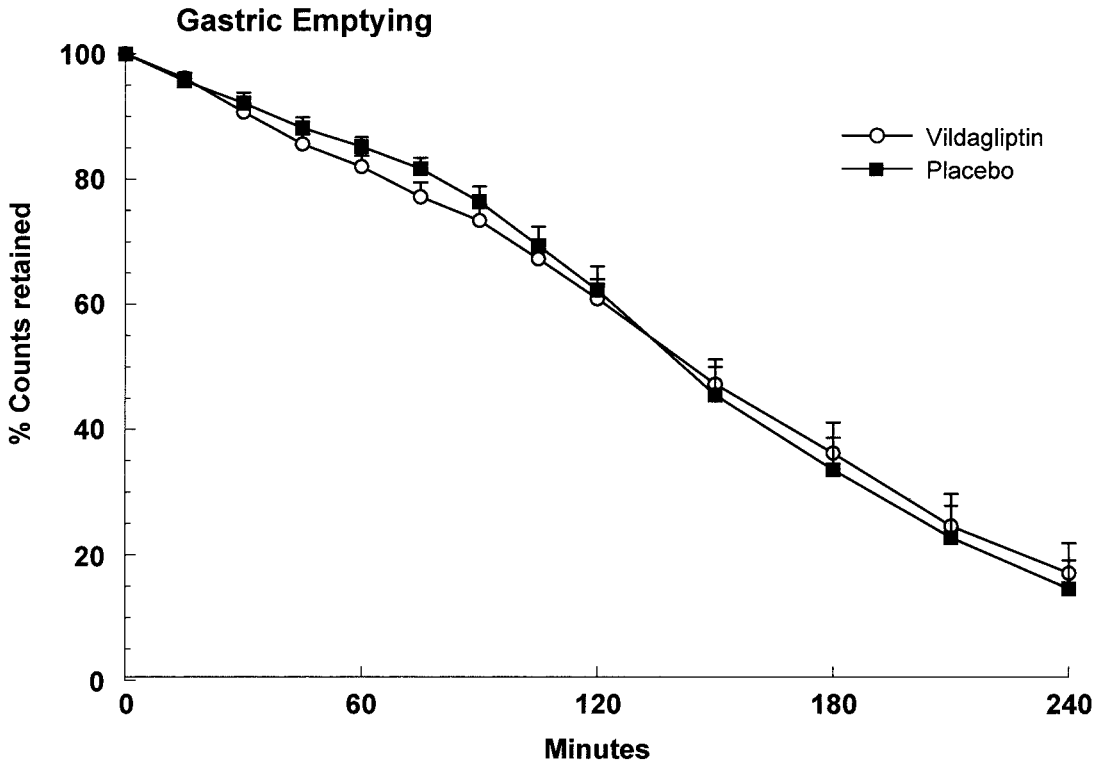

FIG. 3. Gastric emptying of solids in the presence and absence of vildagliptin.

suppression of endogenous glucose (area below basal) did not differ with vildagliptin or placebo ($-3{,}644 \pm 290$ vs. $-3{,}666 \pm 252$ μmol/kg per 5 h, $P = 0.90$).

Glucose disappearance did not differ in the fasting state (20.1 ± 1.5 vs. 21.1 ± 1.5 μmol/kg per 5 h, $P = 0.09$) or following meal ingestion ($5{,}085 \pm 771$ vs. $5{,}278 \pm 555$ μmol/kg per 5 h, $P = 0.68$) with vildagliptin or placebo.

DISCUSSION

GLP-1 and DPP-4 inhibitors both lower postprandial glucose concentrations in people with type 2 diabetes at least in part through their ability to enhance insulin secretion and to inhibit glucagon release (12,13). GLP-1 is also an inhibitor of gastric emptying (14). In contrast, the present experiments establish that DPP-4 inhibition, sufficient to double postprandial concentrations of active GLP-1 and to improve glycemic control in diabetes, does not alter gastric emptying. Moreover, the rate, pattern, or amount of glucose that enters the systemic circulation following ingestion of a mixed meal is unaltered by vildagliptin.

In this experiment, we used tracer methods (7) to simultaneously evaluate the effects of DPP-4 inhibition on gastrointestinal function and splanchnic handling of glucose. Glucose, ingested together with nonglucose nutrients, was labeled with ^{13}C-glucose (incorporated in the Jell-O), and its rate of appearance in the systemic circulation was measured using a second tracer. Since the ingested glucose has to be emptied from the stomach, absorbed by the intestine, and pass through the liver via the portal vein, the meal appearance represents a composite of all of these processes. Meal appearance did not differ during treatment with vildagliptin or placebo. In addition, direct measurement of gastric emptying showed that this was not altered by inhibition of DPP-4. These data indicate that DPP-4 inhibitors do not lower postprandial glucose concentrations by altering gastric emptying or the rate at which ingested glucose enters the systemic circulation.

When GLP-1 is infused at rates of 0.3–0.4 pmol · kg^{-1} · min^{-1}, resulting in sustained elevation of GLP-1, a slight, but significant, delay in gastric emptying (14,15) has been observed. The magnitude and duration of peak GLP-1 concentrations during DPP-4 inhibition may explain why DPP-4 inhibition does not alter gastric emptying and accommodation in people with type 2 diabetes (16). While a two- to threefold increase in postprandial GLP-1 concentrations are adequate to alter pancreatic islet secretion, higher and more sustained concentrations appear to be required to significantly delay gastric emptying.

Some authors have suggested that the actions of DPP-4 inhibitors are not mediated through GLP-1 (17). Indeed, inhibition of DPP-4 raises concentrations of other hormones such as glucose-dependent insulinotropic peptide (GIP), neuropeptide Y, and peptide YY(18). A conceivable explanation is that vildagliptin may raise the concentrations of hormones with gastric prokinetic activity in addition to raising hormones that delay gastric emptying thus resulting in no net change in gastric emptying rate. However, GIP, neuropeptide Y, and peptide YY all retard gastric emptying in humans (19), whereas the motility-stimulating hormone, motilin, is not a substrate of DPP-4. Hence, this explanation for the lack of effect of DPP-4 to inhibit gastric emptying appears untenable. DPP-4 inhibition in mice lacking GLP-1 and GIP receptors did not alter glucose concentrations implying that GLP-1 and GIP action are necessary for DPP-4 inhibitors to lower glucose (20). These observations support our data suggesting an important role of GLP-1 in mediating the effects of DPP-4 inhibitors.

Blood glucose concentrations per se alter gastric motility; hyperglycemia delays gastric emptying (21), while

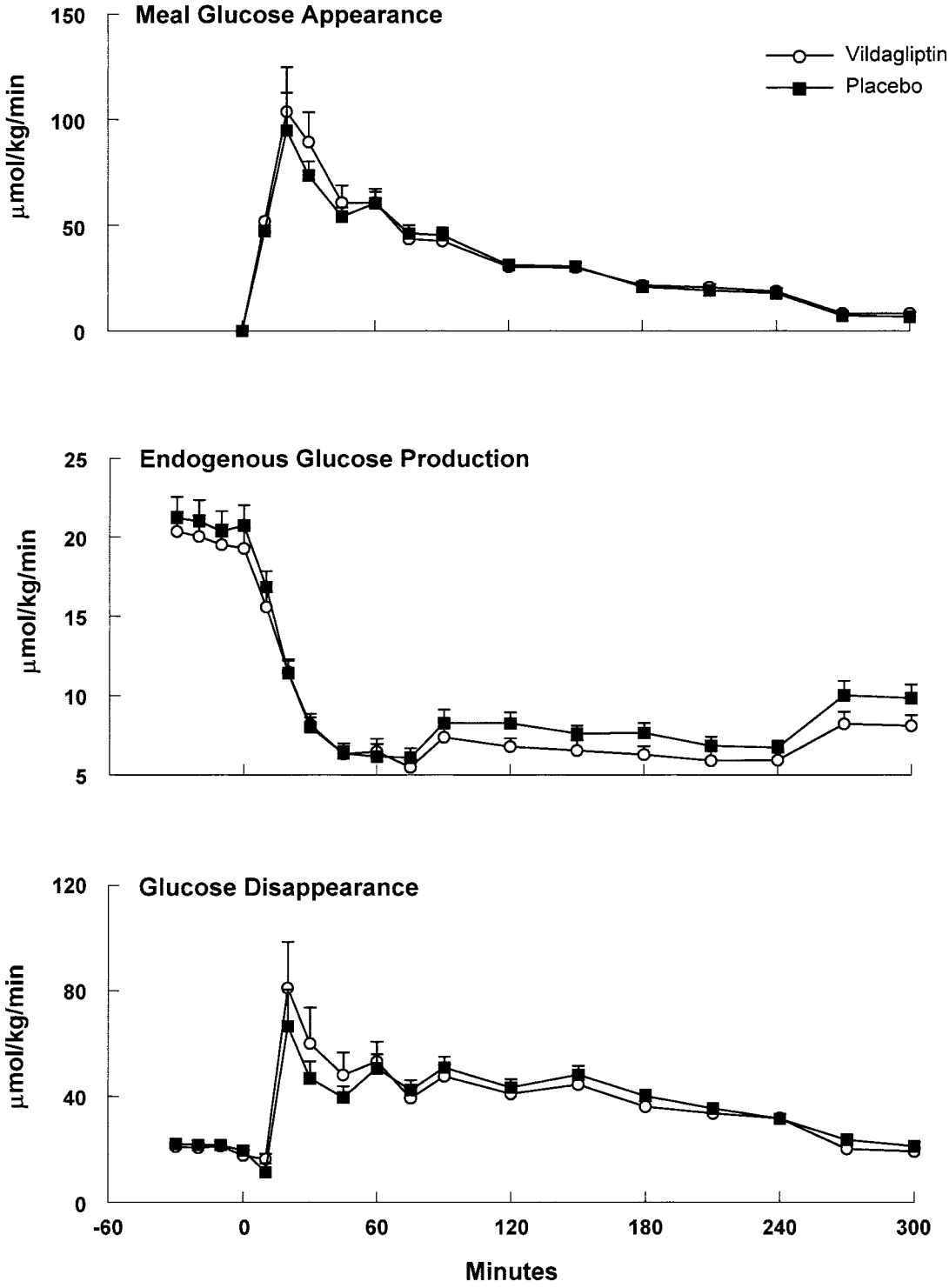

FIG. 4. Meal appearance, endogenous glucose production, and glucose disappearance in the presence and absence of vildagliptin.

hypoglycemia accelerates gastric emptying (22). It is possible that the lower glucose levels during vildagliptin administration may have overriden any inhibitory effect on gastric emptying. However, raising blood glucose concentrations from 4 to 8 mmol/l delayed $T_{1/2}$ of a liquid meal by ~10 min (21). It appears unlikely that the differences in glucose concentrations observed between study days could explain the absence of a difference in gastric emptying in this study.

Given the lack of a significant effect of DPP-4 inhibitors on gastric emptying, we need to ensure there is not a type II error. With the variance in gastric emptying $T_{1/2}$ on the placebo study day in this study, the 14 subjects studied provided 98% power to detect a 20% change in gastric emptying $T_{1/2}$ at $P < 0.05$. Therefore, the lack of effect on gastric emptying with vildagliptin is not the result of a type II error.

Vildagliptin lowered postprandial glucagon concentrations. Conversely, postprandial C-peptide concentrations did not differ between study days despite lower glucose

concentrations in the presence of vildagliptin. This pattern is consistent with previous studies (23) that have shown that, at a given glucose concentration, DPP-4 inhibition increases insulin and decreases glucagon release resulting in higher insulin and lower glucagon concentrations in portal blood (5). Despite this, postprandial endogenous glucose production and glucose disposal did not differ between the vildagliptin and placebo study days. It is known that glucose itself stimulates glucose uptake and decreases glucose production (24). Observation of comparable rates of stimulation of glucose uptake and suppression of glucose production (despite lower plasma glucose) are the corollaries of stimulation of insulin and suppression of glucagon secretion by vildagliptin.

One pitfall in our study was that it was underpowered to detect a significant effect of DPP-4 inhibitor on glucose disappearance. The observed variance in measured glucose disappearance suggests that 33 subjects would need to be studied to have 80% power to detect a 20% difference at $P < 0.05$ between the placebo and vildagliptin study days.

In summary, DPP-4 inhibition sufficient to double postprandial GLP-1 concentrations and lower glucose concentrations in people with type 2 diabetes does not alter gastric emptying or the rate of systemic appearance of ingested glucose. These data lend strong support to the concept that DPP-4 inhibitors improve glycemic control by stimulating insulin secretion and by inhibiting glucagon release rather than by altering nutrient absorption.

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