

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

Accepted for publication 3 February 2007.

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Running title: Vildagliptin and Gastric Emptying

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Abstract Word Count: 244
Word Count (excluding abstract): 1994

Keywords: DPP-IV inhibition, meal appearance, gastric emptying, incretins

Abstract

Objective: To determine if alterations in meal absorption and gastric emptying contribute to the mechanism by which inhibitors of dipeptidyl peptidase-4 (DPP-4) lower postprandial glucose concentrations.

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 bid) 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 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 5h, $p < 0.05$) and GLP-1 concentrations were higher (1878 ± 270 vs. 1277 ± 312 pmol/l per 5h, $p = 0.001$) during vildagliptin administration compared to 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 the 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.

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 (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 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.

Methods

Subjects

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 complications of diabetes. At the time of screening, the validated Bowel Disease questionnaire revealed the absence of gastrointestinal symptoms (6). All agents used for the treatment of diabetes were discontinued three weeks before the study. For subject characteristics see Appendix.

Experimental Design

We utilized a randomized, double blind, placebo-controlled crossover design. Subjects received either vildagliptin 50mg or placebo taken before breakfast and supper each over a ten-day treatment period with the two treatment periods separated by a two-week washout period. The order of treatment was randomized, and all participants and investigators were blinded to treatment and allocation was concealed. Vildagliptin and matching placebo were provided to the research pharmacy by the study sponsor. Subjects were admitted to the GCRC on the evening of the 6th day and glucose turnover and gastric emptying were measured on the 9th day of each treatment period.

Glucose Turnover and Gastric Emptying

Following an 8 hour 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 minutes, a primed continuous infusion of [6,6-²H₂] glucose was initiated. Subjects received the morning dose (vildagliptin 50 mg or placebo) at -30 minutes. At time 0 subjects consumed a meal consisting of 2 scrambled eggs labeled with 0.75 mCi ^{99m}Tc-Sulfur colloid, 55g of Canadian bacon, 240ml of water and Jell-O containing 75g of glucose labeled with [1-¹³C] glucose – (4% enrichment). This provided 510 Kcal (61% carbohydrate, 19% protein and 21% fat). An infusion of [6-³H] glucose was started at this time and the infusion rate varied to mimic the anticipated glucose appearance of the meal [1-¹³C] glucose as previously described (7). At the same time, the rate of infusion of the [6,6-²H₂] glucose was altered so as to approximate the anticipated pattern of fall in endogenous glucose production (7). Blood was collected at pre-specified times. Anterior and posterior gamma camera images were obtained immediately after meal ingestion and over the next 4 hours for gastric emptying measurement (8).

Analytical techniques

Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Plasma [6,6-²H₂] glucose and [1-¹³C] glucose enrichments were measured using gas chromatographic mass spectrometry (Thermoquest, San Jose, CA) to simultaneously monitor the C-1 and C-2 and C-3 to C-6 fragments, as described by Beylot et al. (9) and [6-³H] glucose specific activity by liquid scintillation counting. Please see Appendix for details.

Calculation of glucose appearance and disappearance rates

The rates of systemic meal appearance ($R_{a_{meal}}$), endogenous glucose production (EGP) and glucose disappearance (R_d) were calculated using Steele's two compartment model (10) as previously described (7). Please see Appendix for details.

Statistical analysis

Values from -30 to 0 minutes were averaged and considered as basal levels. Area above basal and area under the curve was calculated using the trapezoidal rule. All data are presented as means \pm SEM. Rates of glucose turnover are expressed as μ mol per kilogram lean body mass. Paired comparisons between treatments were made using Student's two-tailed t-test for paired samples. A $p < 0.05$ was considered to be significant.

Given the variance in the measurement of gastric emptying $T_{1/2}$ in people with type 2 diabetes at 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 a $p < 0.05$. A 20% change in gastric emptying is deemed to be clinically significant as it approximates the degree of delay observed in diabetic patients (11).

Results

Plasma GLP-1 Concentrations (Figure 1)

Fasting concentrations of active GLP-1 (3.7 \pm 1.0 vs. 3.8 \pm 1.1 pmol/l, $p = 0.68$) did not differ between groups. However in the presence of vildagliptin, after meal ingestion, concentrations rose (11.8 \pm 2.0 pmol/l vs. 5.8 \pm 0.8, $p = 0.01$) and remained elevated for the duration of the study as shown by the area under the curve (1878 \pm 270 vs. 1277 \pm 312 pmol/l per 5h, $p = 0.001$).

Plasma glucose, insulin, C-peptide and glucagon concentrations (Figure 2).

Treatment with vildagliptin resulted in lower fasting glucose (7.3 ± 0.5 vs. 7.9 ± 0.5 mmol/l, $p = 0.005$), lower post meal peak (14.1 ± 0.6 vs. 15.9 ± 0.9 mmol/l, $p = 0.0008$) and lower glycemic area above basal (954 ± 85 vs. 1077 ± 94 mmol per 5h, $p = 0.01$).

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 5h, $p = 0.76$) 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 5h, $p = 0.73$).

Treatment with vildagliptin resulted in lower postprandial glucagon concentrations (20.9 ± 1.6 vs. 23.7 ± 1.3 mg per 5h, $p = 0.03$).

Gastric emptying (Figure 3)

Gastric emptying did not differ between treatments: T_{lag} , (the time to empty 10% of stomach contents), were 34.5 ± 4.3 on vildagliptin vs. 39.9 ± 6.2 min on placebo, $p = 0.46$, and the $T_{1/2}$, (time to empty 50% of stomach contents) were 144.8 ± 7.3 vs. 143.3 ± 6.5 min respectively, $p = 0.79$. Residual ^{99m}Tc counts at the 4 hours 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 (Figure 4)

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 $\mu\text{mol/kg/min}$, $p = 0.88$) or area under the curve (8560 ± 557 vs. 8711 ± 953 $\mu\text{mol/kg per 5h}$, $p = 0.98$).

Fasting endogenous glucose production was slightly, but not significantly, lower with vildagliptin (19.8 ± 1.3 vs. 20.7 ± 1.3 $\mu\text{mol/kg/min}$, $p = 0.07$). Postprandial suppression of endogenous glucose (area below basal) did not differ with vildagliptin or placebo (-3644 ± 290 vs. -3666 ± 252 $\mu\text{mol/kg per 5h}$, $p = 0.90$).

Glucose disappearance did not differ in the fasting state (20.1 ± 1.5 vs. 21.1 ± 1.5 $\mu\text{mol/kg/min}$, $p = 0.09$) or following meal ingestion (5085 ± 771 vs. 5278 ± 555 $\mu\text{mol/kg per 5h}$, $p = 0.68$) 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. The glucose, ingested together with non-glucose nutrients, was labeled with ^{13}C -glucose (incorporated in Jell-O) and its rate of appearance into the systemic circulation was measured using a second tracer. Since the ingested glucose has to be emptied from the stomach, absorbed by the intestine, 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/min, 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 2-3-fold 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 (NPY) and Peptide YY (PYY) (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, NPY and PYY 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 the 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 hypoglycemia accelerates gastric emptying (22). It is possible that the lower glucose levels during vildagliptin administration may have overcome 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 minutes (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 a $P < 0.05$. Therefore the lack of effect on gastric emptying with vildagliptin is not the result of a type II error.

Vildagliptin lowered postprandial glucagon concentrations. On the other hand, 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 a 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 a $P < 0.05$ 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 support to the concept that DPP-4 inhibitors improve glycemic control by stimulating insulin relative to the prevailing glucose concentration, and by inhibiting glucagon release rather than by altering nutrient absorption.

Acknowledgements

This research was supported by a research grant from Novartis Pharmaceuticals. and by the support of the nursing, GI physiological imaging and immunochemistry core of Mayo Clinic General Clinical Research Center (RR00585) from the National Institutes of Health.

Dr. Camilleri is supported by DK02638, NIH P01-DK068055

This paper was previously presented in oral form "The effect of the dipeptidyl peptidase IV inhibitor LAF237 on gastrointestinal function and glucose metabolism in type 2 diabetes" at the Annual meeting of the European Association for the Study of Diabetes, Copenhagen, Denmark, September 2006.

References

1. Vella A, Camilleri M, Rizza RA: The gastrointestinal tract and glucose tolerance. *Curr Opin Clin Nutr Metab Care* 7:479-484, 2004
2. Holst JJ, Toft-Nielsen MB, Orskov C, Nauck M, Willms B: On the effects of glucagon-like peptide-1 on blood glucose regulation in normal and diabetic subjects. *Ann N Y Acad Sci* 805:729-736, 1996
3. Schirra J, Wank U, Arnold R, Goke B, Katschinski M: Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans. *Gut* 50:341-348, 2002
4. Hansen PA, Corbett JA: Incretin hormones and insulin sensitivity. *Trends Endocrinol Metab* 16:135-136, 2005
5. Mari A, Sallas WM, He YL, Watson C, Ligueros-Saylan M, Dunning BE, Deacon CF, Holst JJ, Foley JE: Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed beta-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metab* 90:4888-4894, 2005
6. Talley NJ, Phillips SF, Melton J, 3rd, Wiltgen C, Zinsmeister AR: A patient questionnaire to identify bowel disease. *Ann Intern Med* 111:671-674, 1989
7. Basu R, Di Camillo B, Toffolo G, Basu A, Shah P, Vella A, Rizza R, Cobelli C: Use of a novel triple-tracer approach to assess postprandial glucose metabolism. *Am J Physiol Endocrinol Metab* 284:E55-69, 2003
8. Cremonini F, Mullan BP, Camilleri M, Burton DD, Rank MR: Performance characteristics of scintigraphic transit measurements for studies of experimental therapies. *Aliment Pharmacol Ther* 16:1781-1790, 2002
9. Beylot M, Previs SF, David F, Brunengraber H: Determination of the ¹³C-labeling pattern of glucose by gas chromatography-mass spectrometry. *Anal Biochem* 212:526-531, 1993
10. Steele R, Bjerknes C, Rathgeb I, Altszuler N: Glucose uptake and production during the oral glucose tolerance test. *Diabetes* 17:415-421, 1968
11. Bredenoord AJ, Chial HJ, Camilleri M, Mullan BP, Murray JA: Gastric accommodation and emptying in evaluation of patients with upper gastrointestinal symptoms. *Clin Gastroenterol Hepatol* 1:264-272, 2003
12. Holst JJ: Implementation of GLP-1 based therapy of type 2 diabetes mellitus using DPP-IV inhibitors. *Adv Exp Med Biol* 524:263-279, 2003
13. Drucker DJ: Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes. *Expert Opin Investig Drugs* 12:87-100, 2003
14. Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA: Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 88:2719-2725, 2003
15. Little TJ, Pilichiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, Wishart J, Horowitz M, Feinle-Bisset C: Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. *J Clin Endocrinol Metab* 91:1916-1923, 2006
16. Holst JJ, Deacon CF: Glucagon-like peptide-1 mediates the therapeutic actions of DPP-IV inhibitors. *Diabetologia* 48:612-615, 2005

17. Nauck MA, El-Ouaghlidi A: The therapeutic actions of DPP-IV inhibition are not mediated by glucagon-like peptide-1. *Diabetologia* 48:608-611, 2005
18. Mentlein R: Dipeptidyl-peptidase IV (CD26)--role in the inactivation of regulatory peptides. *Regul Pept* 85:9-24, 1999
19. Camilleri M: Integrated upper gastrointestinal response to food intake. *Gastroenterology* 131:640-658, 2006
20. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ: 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, 2004
21. Schvarcz E, Palmer 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* 113:60-66, 1997
22. Schvarcz E, Palmer M, Aman J, Berne C: Hypoglycemia increases the gastric emptying rate in healthy subjects. *Diabetes Care* 18:674-676, 1995
23. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A: Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 89:2078-2084, 2004
24. Butler PC, Caumo A, Zerman A, O'Brien PC, Cobelli C, Rizza RA: Methods for assessment of the rate of onset and offset of insulin action during nonsteady state in humans. *Am J Physiol* 264:E548-560, 1993

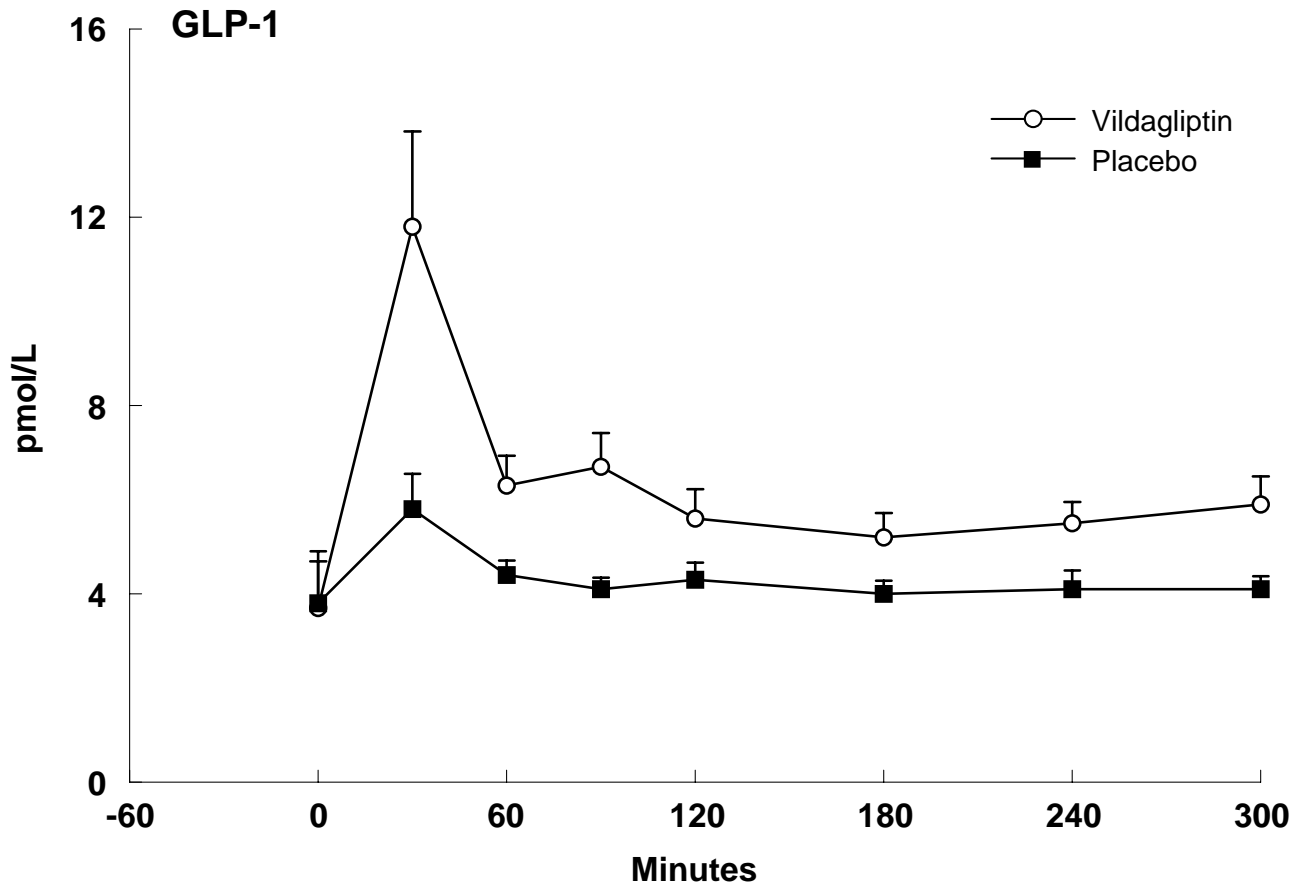


Figure 1: Concentrations of active GLP-1 observed during the experiment in the presence and absence of vildagliptin.

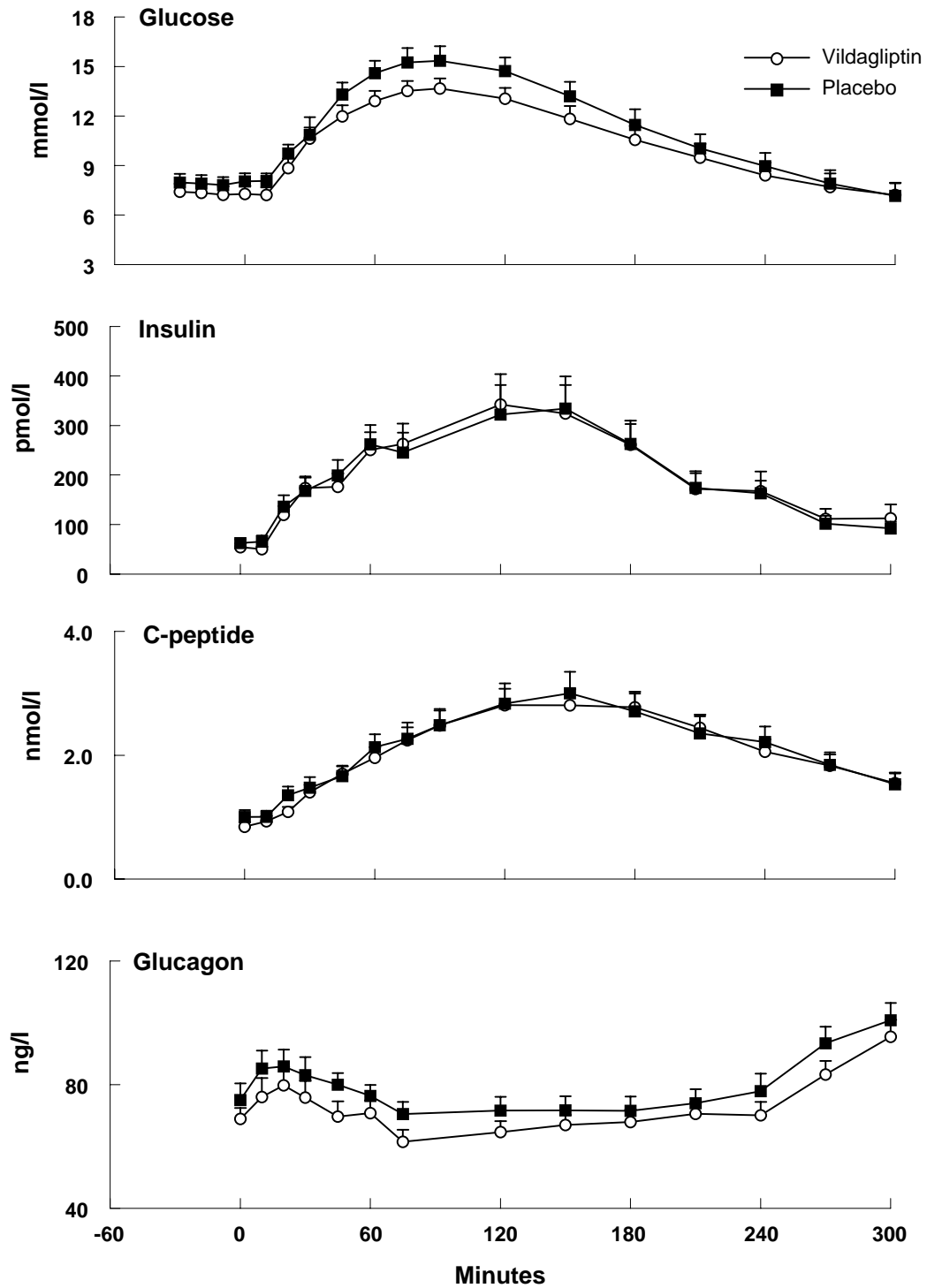


Figure 2: Glucose, Insulin, C-peptide and Glucagon concentrations observed in the presence and absence of vildagliptin

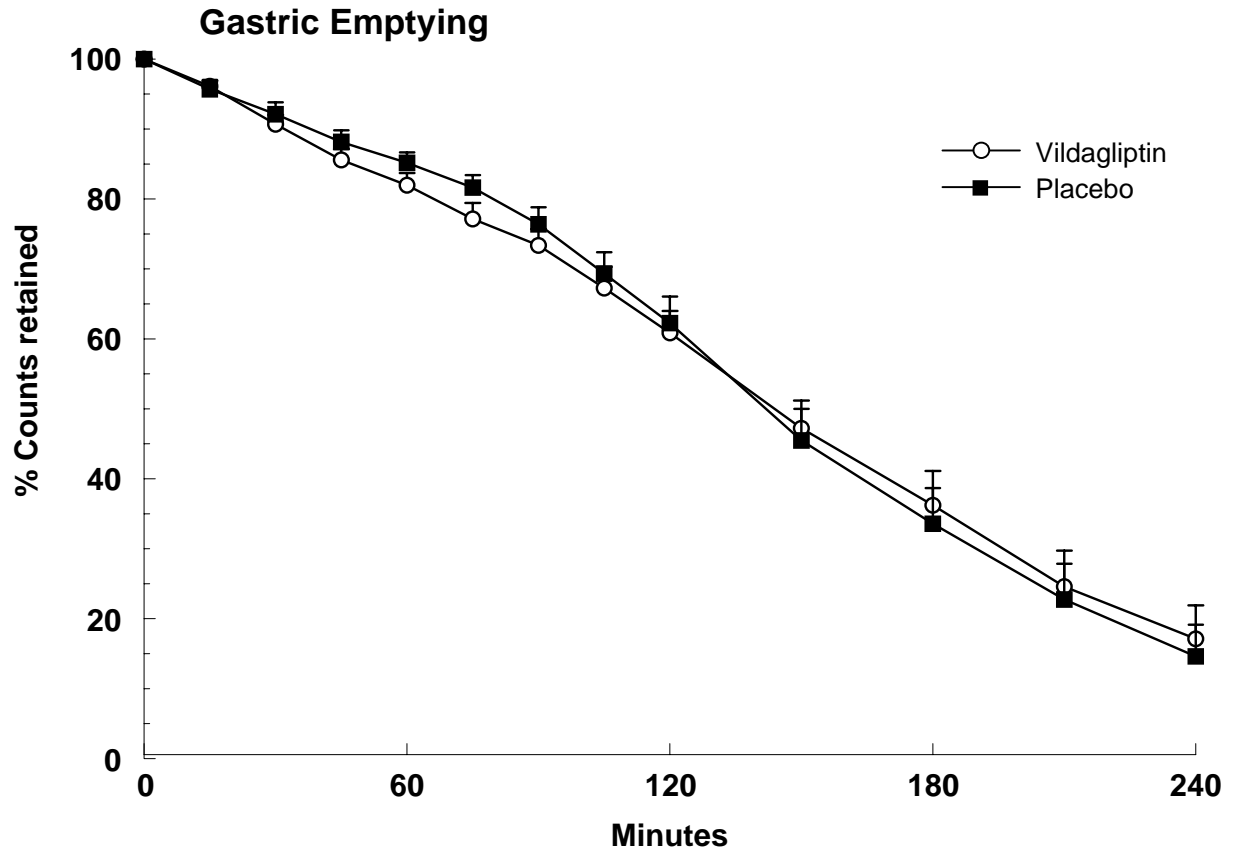


Figure 3: Gastric emptying of solids in the presence and absence of vildagliptin.

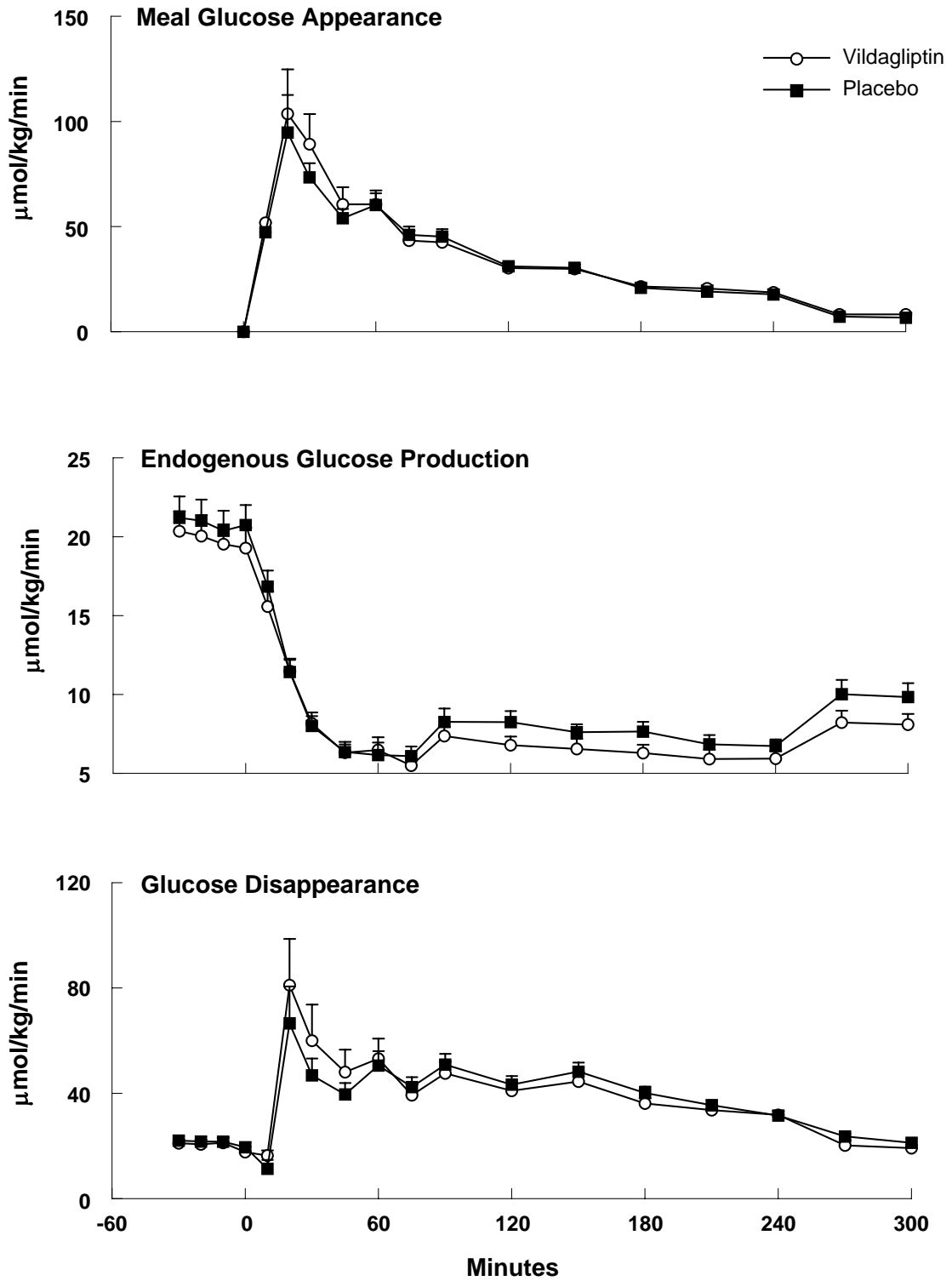


Figure 4: Meal appearance, endogenous glucose production and glucose disappearance in the presence and absence of vildagliptin.