Effects of sitagliptin on glycemia, incretin hormones, and antropyloroduodenal motility in response to intraduodenal glucose infusion in healthy lean and obese humans, and patients with type 2 diabetes treated with or without metformin

Running title: actions of sitagliptin and metformin

Key words: DPP-4, metformin, incretin hormones, glycemia, type 2 diabetes

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Abstract
The impact of variations in gastric emptying, which influence the magnitude of GIP and GLP-1 secretion, on glucose-lowering by DPP-4 inhibitors is unclear. We evaluated responses to intraduodenal glucose infusion (60g over 120min, ie. 2kcal/min – a rate that predominantly stimulates GIP, but not GLP-1) after sitagliptin vs. control in 12 healthy lean and 12 obese subjects, and in 12 type 2 patients taking metformin 850mg bd vs. placebo. As expected, sitagliptin augmented plasma intact GIP substantially, and intact GLP-1 modestly. Sitagliptin attenuated glycemic excursions in healthy lean and obese, but not type 2 subjects, without affecting glucagon or energy intake. In contrast, metformin reduced fasting and glucose-stimulated glycemia, suppressed energy intake, and augmented total and intact GLP-1, total GIP and glucagon in type 2 subjects, with no additional glucose-lowering when combined with sitagliptin. These observations indicate that in type 2 diabetes (i) the capacity of endogenous GIP to lower blood glucose is impaired, (ii) the effect of DPP-4 inhibition on glycemia is likely to be dependent on adequate endogenous GLP-1 release, requiring gastric emptying above 2kcal/min, and (iii) the action of metformin to lower blood glucose is not predominantly via the incretin-axis.
Inhibition of dipeptidyl peptidase 4 (DPP-4) lowers glycemia by increasing intact glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) concentrations (1). In type 2 diabetes, the insulinotropic effects of GIP and GLP-1 are diminished, although the effect of GLP-1 is better preserved (2; 3). GLP-1 also suppresses glucagon secretion (4), appetite and energy intake (5), and slows gastric emptying (6; 7). Therefore, the glucose-lowering effect of DPP-4 inhibitors in this disorder is likely to be dependent primarily on the actions of GLP-1 rather than GIP.

Postprandial incretin secretion is regulated by the rate of nutrient delivery to the small intestine (8; 9) – GIP secretion increases linearly with increasing rates of intraduodenal (ID) glucose infusion, whereas the GLP-1 response is minimal at 1-2 kcal/min, but substantially greater at 4 kcal/min (8; 9). In both obesity (10; 11) and type 2 diabetes (12; 13), postprandial GLP-1 responses have inconsistently been reported to be reduced after oral nutrients, and those of GIP to be intact. However, even in health, the overall rate of gastric emptying varies from 1 to 4 kcal/min (14), and patients with long-standing type 2 diabetes frequently have accelerated or delayed gastric emptying (15). Therefore, evaluation of incretin responses to nutrients and characterization of the effects of DPP-4 inhibition should be controlled for the rate of gastric emptying.

The majority of studies have reported the lack of effect of DPP-4 inhibitors on gastric emptying for unclear reasons (16-22). Although endogenous GLP-1 slows gastric emptying via suppression of antral motility and stimulation of pyloric contractions (6; 7), the effect of DPP-4 inhibitors on these motor mechanisms has not been assessed. DPP-4 inhibitors appear weight-neutral (23), but reports on their effect on appetite are also limited.
Recently, it was shown that the combination of metformin and a DPP-4 inhibitor is more beneficial than either alone for optimization of glycemic control in type 2 diabetes (24). Metformin augments GLP-1 concentrations by uncertain means (25; 26) and can, in mice, increase expression of GLP-1 and GIP receptors in pancreatic islets (25). Although plasma DPP-4 activity was reportedly reduced in type 2 patients treated with metformin (27), in vitro studies failed to show any direct effect of metformin on the catalytic activity of DPP-4 (27; 28). Regardless, co-administration of metformin and sitagliptin resulted in an additive increase in plasma intact GLP-1 concentrations and improvement in postprandial glycemia in both health and type 2 diabetes (26; 29).

In the present study, we hypothesized that glucose-lowering by DPP-4 inhibitors would be diminished in type 2 diabetes compared to health in response to ID glucose infused at 2 kcal/min – a rate at which GIP appears to be the dominant incretin (8; 9). We evaluated acute effects of sitagliptin on glycemia, incretin hormones, antropyloroduodenal (APD) motility, and appetite in healthy lean and obese subjects, and in patients with type 2 diabetes treated with metformin or placebo.
Research design and methods

Subjects

12 healthy lean subjects, 12 obese subjects without diabetes, and 12 patients with type 2 diabetes (Table 1), all Caucasian males, were studied in double-blind, randomized fashion after providing written, informed consent. Type 2 subjects maintained HbA1c < 7.5% (58 mmol/mol) on diet alone, and had no micro- or macro-vascular complications. The protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee, and conducted in accordance with the Declaration of Helsinki.

Protocol

Lean and obese subjects were studied twice (sitagliptin or control), separated by 3-14 days. Type 2 subjects were studied four times, during therapy with metformin 850 mg bd or placebo for 7 days each (first dose in evening of day 1; sitagliptin or control on days 5 and 8), with 14 days ‘washout’ between.

After a standardized beef lasagne meal (McCain, Victoria, Australia) the evening before each study (~1900h), subjects fasted until they attended the laboratory at ~0800h. A manometric assembly (Dentsleeve International, Ontario, Canada) was inserted transnasally and positioned in the duodenum by peristalsis, with monitoring of antral and duodenal transmucosal potential difference (30). The assembly incorporated 7 antral and 6 duodenal channels, spaced at 1.5 cm intervals, and a 4.5 cm pyloric sleeve sensor, each perfused with 0.9% saline (30). An additional infusion channel opened 12 cm beyond the pylorus.
An intravenous cannula was inserted for blood sampling. 100 mg sitagliptin or matching control (with 850 mg metformin or matching placebo in type 2 subjects) was administered orally with 30 mL water (t = -30 min), followed after 30 min by an ID glucose infusion (60 g glucose dissolved in water to a volume of 240 mL) over 120 min (t = 0 to 120 min; 2 kcal/min). The catheter was subsequently removed, and subjects ate ad libitum from a cold buffet-style meal (t = 120 to 150 min) from which energy intake was calculated using Foodworks 3.01 (Xyris Software, Highgate Hill, Queensland, Australia) (30). Venous blood was sampled frequently into ice-chilled EDTA tubes for plasma glucose and hormone measurements, with DPP-4 inhibitor (DPP4-010; Linco Research, MO, USA; 10 µL/mL) added to tubes for intact incretin measurements. Plasma was separated within 15 min and stored at –70 °C for subsequent analysis.

**Measurements**

Plasma glucose concentrations were measured by the glucose oxidase technique (YSI 2300 STAT Plus, Yellow Springs Instruments, OH, USA). GLP-1, GIP and glucagon analyses were performed as previously described (31; 32). Intact GLP-1 was measured using a two-site ELISA (C-terminally directed GLP-1F5 catching antibody; N-terminally directed Mab26.1 detecting antibody) (31). Total GLP-1 was assayed using antiserum 89390, requiring the intact amidated C-terminus of the molecule, and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite (31). Intact and total GIP were analyzed with the N-terminally and C-terminally directed antisera, 98171 (31) and 80867 (32), respectively. Glucagon immunoreactivity was determined using the C-terminally directed antiserum 4305, which measures glucagon of pancreatic origin (31). Insulin was measured by ELISA (10-1113, Mercodia, Uppsala, Sweden).
Manometric pressures were digitally recorded (Flexisoft, Oakfield Instruments of Oxford, UK), and analyzed using custom-designed software (Prof AJ Smout, Academic Medical Center, Amsterdam, The Netherlands) to determine the number of isolated pyloric pressure waves (IPPWs), and antral and duodenal pressure waves over successive 15 min periods (30).

**Statistical analysis**

Area under the curve (AUC) for plasma glucose and hormones was calculated using the trapezoidal rule. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin sensitivity (33). The $\text{AUC}_{\text{insulin}}/\text{AUC}_{\text{glucose}}$ ratio was calculated to compare insulin concentrations whilst correcting for differences in blood glucose (33). Data were analyzed using paired Student’s $t$-test in healthy lean and obese groups and two-factor repeated measures ANOVA, with sitagliptin and metformin as factors, in type 2 patients. Repeated measures ANOVA, with treatment and time as factors, was also employed for intra-group comparisons. Inter-group comparisons were performed using one-factor ANOVA. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed, if ANOVAs revealed significant effects. Relationships between variables were assessed using Pearson correlation analysis. All analyses were performed using SPSS 19 (IBM Corporation, Armonk, NY, USA). A sample size of 12 subjects was calculated to have 80% power (at $\alpha = 0.05$) to detect a difference in the AUC for blood glucose of 73 mmolL$^{-1}$min with a SD of 82 mmolL$^{-1}$min between sitagliptin and control in healthy lean and obese subjects (19), and to detect an additive glucose-lowering effect between metformin and sitagliptin in type 2 subjects.
(29). Data are presented as means ± standard error; $P < 0.05$ was considered statistically significant.
Results

All subjects tolerated the study well.

Plasma glucose

In healthy lean and obese subjects, fasting plasma glucose concentrations (t = -30 min) did not differ between control and sitagliptin, and remained unchanged immediately before ID glucose infusion (t = 0 min). During ID glucose infusion (t = 0-120 min), plasma glucose concentrations increased, and were lower after sitagliptin than control (lean: \( P = 0.001 \); obese: \( P = 0.004 \)) (Figure 1A and 2A; Table 2).

In type 2 subjects, fasting glucose (t = -30 min) did not differ between control and sitagliptin during either placebo or metformin treatment, but was reduced with metformin (ANOVA, metformin effect: \( P < 0.001 \)). Plasma glucose remained unchanged at = 0 min, but increased during ID glucose infusion, and was lower with metformin (ANOVA, AUC: \( P < 0.001 \)), without any effect of sitagliptin or interaction between metformin and sitagliptin (Figure 3A; Table 3).

Both fasting plasma glucose (Table 1), and the AUC after ID glucose with sitagliptin and control (Table 2), were greater in type 2 patients during placebo treatment than healthy lean and obese subjects (\( P < 0.001 \) for each), without any difference between the latter two groups.

Plasma total and intact GLP-1

In healthy lean and obese subjects, neither total nor intact GLP-1 concentrations differed between control and sitagliptin prior to ID glucose. During ID glucose infusion, GLP-1
responses were minimal, but total GLP-1 was lower after sitagliptin than control in healthy lean subjects ($P = 0.067$ for AUC; $P < 0.05$ for ANOVA), without any difference in obese subjects (Figure 1C and 2C; Table 2). In contrast, intact GLP-1 was higher after sitagliptin than control in both healthy lean and obese subjects ($P = 0.016$ and $P < 0.001$ for AUC) (Figure 1E and 2E; Table 2).

In type 2 subjects, neither total nor intact GLP-1 concentrations differed between control and sitagliptin prior to ID glucose, but were greater with metformin than placebo ($P = 0.016$ and $P < 0.001$). During ID glucose infusion, GLP-1 responses were minimal, but total GLP-1 was greater with metformin than placebo ($P = 0.001$ for AUC), without any effect of sitagliptin or interaction between metformin and sitagliptin. In contrast, intact GLP-1 was greater with both metformin ($P = 0.001$ for AUC) and sitagliptin ($P = 0.007$ for AUC), without any interaction between them (Figure 3C and 3E; Table 3).

During fasting, neither total nor intact GLP-1 differed between the three groups. On the control days, total GLP-1 during ID glucose infusion was less in obese than healthy lean subjects ($P < 0.05$ for AUC), and intact GLP-1 was less in obese than type 2 subjects ($P < 0.05$) (Table 2), while the AUC for total (but not intact) GLP-1 after ID glucose in all groups combined was inversely related to BMI ($r = -0.41$, $P = 0.04$). After adjusting for BMI, the AUC for total GLP-1 did not differ between the groups. Neither total nor intact GLP-1 AUC was related to age. However, after adjusting for BMI, the AUC for intact GLP-1 tended to be positively related to age ($r = 0.31$, $P = 0.06$).
Plasma total and intact GIP

In healthy lean and obese subjects, neither total nor intact GIP concentrations differed between control and sitagliptin prior to ID glucose (Table 2). During ID glucose infusion, GIP increased substantially, but total GIP concentrations were lower after sitagliptin than control in healthy lean subjects ($P = 0.019$ for ANOVA), without any differences in obese subjects (Figure 1D and 2D; Table 2). In contrast, intact GIP was higher after sitagliptin than control in both healthy lean and obese subjects ($P < 0.001$ for both AUC and ANOVA) (Figure 1F and 2F; Table 2).

In type 2 subjects, neither total nor intact GIP concentrations differed between control and sitagliptin prior to ID glucose, and neither was altered by metformin. During ID glucose infusion, GIP increased substantially. Total GIP was greater with metformin ($P = 0.014$ for AUC), without any effect of sitagliptin, or interaction between metformin and sitagliptin. In contrast, intact GIP was greater for sitagliptin than control ($P = 0.008$ for AUC), without any effect of metformin, or interaction between metformin and sitagliptin (Figure 3D and 3F; Table 3).

During fasting, neither total nor intact GIP differed between the three groups (Table 1), nor did total GIP during ID glucose infusion (Table 2). Intact GIP during ID glucose was greater in type 2 subjects, without any difference between healthy lean and obese subjects ($P < 0.01$ for AUC). The AUC for total or intact GIP was not related to BMI. However, the AUC for intact GIP was positively related to age on the placebo, but not sitagliptin, days ($r = 0.65$, $P < 0.001$). This relationship remained significant when adjusting for the presence of type 2 diabetes ($r = 0.43$, $P = 0.01$).
**Plasma glucagon**

In healthy lean and obese subjects, glucagon concentrations did not differ between control and sitagliptin prior to ID glucose. After ID glucose infusion, glucagon declined comparably in both groups, without any effect of sitagliptin (Figure 1G and 2G; Table 2).

In type 2 patients, glucagon concentrations before ID glucose were not affected by sitagliptin, but were elevated with metformin ($P = 0.025$). During ID glucose infusion, glucagon declined from $t = 30$ min, but was greater with metformin ($P = 0.003$ for AUC), without any effect of sitagliptin, or interaction between metformin and sitagliptin (Figure 3G; Table 3).

Glucagon concentrations were higher in type 2 than healthy lean subjects, both during fasting ($P = 0.034$, Table 1) and after ID glucose with control ($P = 0.023$ for AUC) and sitagliptin ($P = 0.019$ for AUC, Table 2). Fasting glucagon was positively related to plasma intact GIP ($r = 0.529$, $P = 0.001$), even after adjusting the presence of type 2 diabetes ($r = 0.47$, $P = 0.005$).

**Serum insulin**

In healthy lean and obese subjects, neither fasting insulin nor HOMA-IR differed between control and sitagliptin. During ID glucose infusion, insulin increased more with sitagliptin than control (lean: $P = 0.002$, obese: $P = 0.088$ for AUC) and the $\text{AUC}_{\text{insulin}}/\text{AUC}_{\text{glucose}}$ ratio was greater after sitagliptin than control in both groups (lean: $P < 0.001$, obese: $P = 0.032$) (Figure 1B and 2B; Table 2).
In type 2 patients, neither fasting insulin nor HOMA-IR differed between the control and sitagliptin days, and neither was altered by metformin. During ID glucose infusion, insulin was greater after sitagliptin than control ($P = 0.049$ for AUC), without any effect of metformin, or interaction between metformin and sitagliptin. However, the $\text{AUC}_{\text{insulin}}/\text{AUC}_{\text{glucose}}$ ratio was increased by metformin ($P = 0.019$), and tended to increase with sitagliptin ($P = 0.065$), without any interaction between them (Figure 3B; Table 3).

Fasting insulin was greater in obese than lean subjects ($P = 0.005$), and tended to be greater for type 2 than lean subjects ($P = 0.07$), without any difference between obese and type 2 subjects (Table 1). HOMA-IR was greater in obese and type 2 than lean subjects ($P < 0.05$ for each), without any difference between obese and type 2 subjects (Table 1). During ID glucose infusion, serum insulin on control days was greater for obese than type 2 subjects ($P = 0.020$), and tended to be greater for obese than lean subjects ($P = 0.073$), without any difference between lean and type 2 subjects (Table 2). Both fasting insulin and the AUC during ID glucose were positively related to BMI ($r = 0.67$ and $0.62$, $P < 0.001$ for each). The increase in the $\text{AUC}_{\text{insulin}}/\text{AUC}_{\text{glucose}}$ ratio was positively related to the rise in intact GIP ($r = 0.33$, $P < 0.05$), but not GLP-1.

**APD pressure waves**

The numbers of antral waves (AWs), duodenal waves (DWs), and isolated pyloric pressure waves (IPPWs) in response to ID glucose ($t = 0-120$ min) did not differ between the groups on either the control or sitagliptin days (Table 2). However, the number of AWs was less in healthy lean subjects ($P = 0.032$), and tended to be less in obese subjects ($P = 0.10$), after sitagliptin than control (Figure 4; Table 2). The number of DWs was also less after sitagliptin in healthy lean
subjects \((P = 0.018)\) (**Figure 4**; **Table 2**). The number of IPPWs did not differ between control and sitagliptin in healthy lean or obese subjects (**Figure 4**; **Table 2**). There was no effect of metformin or sitagliptin on any parameter in type 2 subjects (**Figure 5**; **Table 3**).

**Energy intake**

There was no effect of sitagliptin on energy intake in healthy lean or obese subjects (**Table 2**), but metformin suppressed energy intake in type 2 subjects \((P = 0.040)\), without any effect of sitagliptin, or interaction between metformin and sitagliptin (**Table 3**). On both the control and sitagliptin study days, energy intake was greater in obese than type 2 subjects \((P < 0.05\) for each), without differing between the other groups (**Table 2**).
Discussion

There are substantial inter-individual variations in the rate of gastric emptying and resultant stimulation of incretin hormones in both health and type 2 diabetes (8; 9; 14; 15), but the impact of this on glucose-lowering by DPP-4 inhibitors has not been evaluated. Here, we standardized glucose entry to the small intestine at 2 kcal/min, a rate known to induce predominantly GIP, rather than GLP-1, secretion (8; 9). This experimental model allowed comparison of incretin stimulation and the effects of DPP-4 inhibition between the groups in response to an identical small intestinal glucose stimulus.

As expected, sitagliptin increased plasma intact GIP substantially and intact GLP-1 minimally. However, glycemic excursions were attenuated with sitagliptin in healthy lean and obese, but not type 2 subjects, without any effect on plasma glucagon. In contrast, in type 2 patients, metformin reduced fasting and glucose-stimulated glycemia, associated with modest augmentation of total and intact GLP-1, total GIP and glucagon concentrations, but the addition of sitagliptin did not reduce glycemia further. These observations support the concepts that in type 2 diabetes (i) the capacity of GIP to lower blood glucose is markedly impaired, (ii) the glucose-lowering efficacy of DPP-4 inhibitors is dependent on the rate of nutrient delivery into the small intestine and the resultant magnitude of GLP-1 stimulation, and (iii) glucose-lowering by metformin does not predominantly involve the incretin-axis.

GIP secretion was preserved in both obesity and type 2 diabetes compared to lean healthy subjects, whereas the GLP-1 response was diminished in obesity, but not in type 2 diabetes. The latter is consistent with our previous report in BMI-matched healthy and type 2 subjects (9).
Plasma intact GLP-1 was less in obese than type 2 subjects, and tended to be less than in healthy lean subjects, while plasma intact GIP was greatest in type 2 subjects without any difference between the healthy lean and obese. This discrepancy is likely to reflect differences in DPP-4 activity, which is reportedly increased in obesity (34) and decreased with aging (35). In support of this, we observed positive relationships of plasma intact GLP-1 and GIP levels with age on the ‘control’ study days, particularly for GIP, probably since its concentrations were greater. The mechanism by which GLP-1 secretion is attenuated in obesity is unclear. Leptin resistance may play a role; in mice made leptin resistant by a high-fat diet, both basal and oral glucose-stimulated GLP-1 concentrations were decreased (36). Alternatively, the apparent volume of distribution of GLP-1 could be greater in obesity, resulting in greater dilution.

Sitagliptin suppressed total GLP-1 and GIP concentrations in healthy lean subjects, consistent with negative feedback on incretin secretion by the intact peptides (37-39). This effect was diminished in obesity and type 2 diabetes, which might be detrimental, since GIP acts on adipocytes to enhance fat deposition and impair insulin sensitivity (40).

The very modest increase in intact GLP-1 after sitagliptin in each group, together with the lack of any glucagonostatic effect, suggests that GLP-1 is unlikely to play a major glucoregulatory role in the current model. The lack of glucose-lowering with sitagliptin in type 2 subjects therefore supports the hypothesis of a defective glucoregulatory capacity of endogenous GIP in this group, and is consistent with the marked impairment of glucose-lowering by sitagliptin in type 2 patients after oral glucose during GLP-1 antagonism with exendin (9-39) (20). Although insulin response to exogenous GIP can be partly restored with strict glycemic control in type 2
diabetes, its insulinotropic effect is not associated with improvement in glucose disposal during hyperglycemic clamp studies (41). In a group of relatively well-controlled type 2 patients, exogenous GIP was shown to exert a modest effect on glucose disposal when blood glucose is clamped at ~12 mmol/L, but had no effect on fasting hyperglycemia (~8 mmol/L), suggesting a glucose-dependent effect of GIP to lower glycemia (42). That the insulinotropic effect of GIP deteriorates with progression of glycemic control in type 2 diabetes does not support major contribution of GIP to reduce glycemia with DPP-4 inhibitors. Both the obese subjects and type 2 patients displayed comparable insulin resistance, so the failure of the type 2 patients to achieve a compensatory insulin response to ID glucose indicates impaired β-cell function despite augmented intact GIP (2; 3). The fact that DPP-4 inhibitors do lower blood glucose concentrations in type 2 diabetes in other settings highlights the importance of endogenous GLP-1; when the latter is specifically stimulated by dietary strategies, such as consuming a D-xylose preload before a meal (18), the glucose-lowering capacity of DPP-4 inhibitors is augmented. To further establish this concept, it would be of interest to modify the current model to investigate the effects of DPP-4 inhibition at a rate of ID glucose infusion (eg. 4 kcal/min) that is known to stimulate a much greater endogenous GLP-1 response (9).

Consistent with previous reports (25; 26), plasma total and intact GLP-1 increased during metformin treatment in type 2 patients. Metformin might increase preproglucagon gene expression in the intestine (26), but this has not been a consistent observation (25). Metformin appears not to stimulate L-cells directly (43), but could do so via neural pathways (43) or changes in intestinal glucose or bile acid transport (44). Our observation that GIP was modestly increased with metformin has been reported (45), but not consistently (25; 26), although the
mechanism remains to be established. As expected, metformin lowered both fasting and post-glucose glycemia. The fact that its stimulation of the incretins was modest, and that sitagliptin did not potentiate its effects in our model, suggests that non-incretin mechanisms predominate, and indeed, the glucose-lowering effect of metformin persists in GLP-1 and/or GIP receptor knockout mice (25). Alternative actions of metformin include enhancement of insulin-mediated peripheral glucose disposal, suppression of hepatic glucose production (46), or antagonism of hepatic glucagon signaling (47). The increase in plasma glucagon that we observed after metformin could reflect a reactive response to the latter (48). The increase in the 
\[ \text{AUC}_{\text{insulin}} / \text{AUC}_{\text{glucose}} \] ratio after metformin is likely due to improved β-cell responsiveness, resulting from attenuation of glycemia (49).

Sitagliptin was associated with suppression of antral and duodenal motility in the lean, with a tendency for fewer antral waves in the obese, but no effect in patients with type 2 diabetes. Gastric emptying is driven by antral and duodenal contractions acting against pyloric resistance. Since pyloric motility was not altered in any group, the modest suppression of antroduodenal motility in the lean by sitagliptin may not have been sufficient to affect gastric emptying (19). Although sitagliptin might have a larger effect on IPPWs if endogenous GLP-1 levels were greater, we recently demonstrated that gastric emptying was not affected by sitagliptin in type 2 patients even in the setting of augmented endogenous GLP-1 secretion (18). This might relate to the effects of peptide YY (PYY), whose degradation from PYY 1-36 to PYY 3-36, which has more potent effects to slow gastric emptying, is prevented by DPP-4 inhibition (50). Our data are thus in keeping with the majority of evidence that DPP-4 inhibition does not influence gastric emptying (17-19; 21; 22), although a recent study reported slowing of oral glucose emptying
after sitagliptin in type 2 patients (20). Metformin was reported to slow gastric emptying in mice (25), but we did not observe any effect on APD motility in the current experimental setting.

Sitagliptin had no effect on energy intake, consistent with its neutral effect on weight during long-term trials (23). Again, this could be due to the stimulation of GLP-1 being modest and/or to the potential concomitant decrease in PYY 3-36, which also has satiating effects (51). In contrast, metformin suppressed energy intake in type 2 patients, consistent with its known anorectic effect and capacity to induce weight loss (52). This reduction was not associated with nausea, and is probably GLP-1-independent, since the increase in plasma GLP-1 after metformin was modest, and moreover, metformin suppresses food intake comparably in GLP-1 receptor knockout and wild type mice (25).

Our study has several limitations. The number of subjects was relatively small; however, effects were consistent between subjects, so increasing the sample size is unlikely to change the outcomes substantially. The type 2 subjects were older than the other groups; age may not influence incretin concentrations (53) or insulinotropic actions (3), but it may impact on insulin sensitivity and incretin metabolism (35).

In conclusion, our observations are consistent with defective glucoregulatory capacity of endogenous GIP in type 2 diabetes, and indicate that the effect of DPP-4 inhibition on glycemia is likely to be dependent on the release of GLP-1, which may require a threshold of gastric emptying of glucose above 2 kcal/min.
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TW was involved in study design and coordination, subject recruitment, data collection and interpretation, statistical analysis, and drafting of the manuscript; JM, MJB, and HC assisted data collection; CFD performed glucagon and incretin hormone assays and was involved in data interpretation; KLJ and MH were involved in conception of the study and data interpretation; CKR was involved in conception and design of the study, data interpretation, and had overall responsibility for the study. All authors critically reviewed the manuscript, and have approved the publication of this final version of the manuscript.

CKR is the guarantor of this work.

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Table 1. Demographic and biochemical variables in the three study groups.

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<td>Age (yr)</td>
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<td>BMI (kg/m²)</td>
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<td>HbA1c (mmol/mol)</td>
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<td>3.3 ± 0.5</td>
<td>10.6 ± 2.0</td>
<td>8.4 ± 1.8</td>
<td>0.005</td>
</tr>
<tr>
<td>HOMA-IR (pmol.mmol.L⁻²)</td>
<td>4.8 ± 0.7</td>
<td>17.7 ± 3.8</td>
<td>18.5 ± 4.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Fasting total GLP-1 (pmol/L)</td>
<td>17.6 ± 1.2</td>
<td>17.1 ± 1.5</td>
<td>17.5 ± 1.3</td>
<td>0.952</td>
</tr>
<tr>
<td>Fasting intact GLP-1 (pmol/L)</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>0.237</td>
</tr>
<tr>
<td>Fasting total GIP (pmol/L)</td>
<td>6.8 ± 1.1</td>
<td>7.5 ± 1.3</td>
<td>7.3 ± 1.5</td>
<td>0.911</td>
</tr>
<tr>
<td>Fasting intact GIP (pmol/L)</td>
<td>7.9 ± 0.3</td>
<td>8.6 ± 0.4</td>
<td>9.2 ± 0.6</td>
<td>0.109</td>
</tr>
</tbody>
</table>

One factor ANOVA was used to determine the statistical significance. Post hoc comparisons were adjusted by Bonferroni’s correction. *P < 0.001 and δP < 0.05 for comparisons of lean vs. obese, or T2DM; #P < 0.01 for comparisons of obese vs. T2DM. Data are means ± SEM.
Table 2. Basal values and areas under the curves (AUCs) for plasma glucose, GLP-1 and GIP (total and intact), glucagon and serum insulin, antropyloroduodenal (APD) pressure waves, and energy intake in response to intraduodenal glucose infusion (2 kcal/min, during t = 0-120 min) after control (C) or sitagliptin (S) in healthy lean and obese subjects, and type 2 diabetic subjects treated with placebo (P) (n = 12 for each group).  

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>S</td>
<td>C</td>
</tr>
<tr>
<td>Basal glucose (mmol/L)</td>
<td>4.7 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Glucose AUC (mmol·L⁻¹·min)</td>
<td>967.8 ± 29.7</td>
<td>887.7 ± 35.9</td>
<td>1033.4 ± 42.0</td>
</tr>
<tr>
<td>Basal total GLP-1 (pmol/L)</td>
<td>18.1 ± 1.5</td>
<td>17.2 ± 1.1</td>
<td>17.7 ± 2.1</td>
</tr>
<tr>
<td>Total GLP-1 AUC (pmol·L⁻¹·min)</td>
<td>2924.2 ± 238.2</td>
<td>2532.7 ± 148.9</td>
<td>2290.2 ± 141.0</td>
</tr>
<tr>
<td>Basal intact GLP-1 (pmol/L)</td>
<td>0.4 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Intact GLP-1 AUC (pmol·L⁻¹·min)</td>
<td>79.1 ± 21.1</td>
<td>156.4 ± 31.1</td>
<td>16.9 ± 4.5</td>
</tr>
<tr>
<td>Basal total GIP (pmol/L)</td>
<td>6.3 ± 1.6</td>
<td>7.2 ± 1.6</td>
<td>7.4 ± 2.2</td>
</tr>
<tr>
<td>Total GIP AUC (pmol·L⁻¹·min)</td>
<td>3207.9 ± 319.1</td>
<td>2900.6 ± 411.5</td>
<td>3247.1 ± 392.2</td>
</tr>
<tr>
<td>Basal intact GIP (pmol/L)</td>
<td>7.8 ± 0.4</td>
<td>8.0 ± 0.4</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>Intact GIP AUC (pmol·L⁻¹·min)</td>
<td>1611.9 ± 67.1</td>
<td>2435.6 ± 113.7</td>
<td>1608.8 ± 68.8</td>
</tr>
<tr>
<td>Basal glucagon (pmol/L)</td>
<td>6.3 ± 0.8</td>
<td>7.2 ± 0.9</td>
<td>10.3 ± 1.4</td>
</tr>
<tr>
<td>Glucagon AUC (pmol·L⁻¹·min)</td>
<td>547.3 ± 67.0</td>
<td>544.6 ± 84.3</td>
<td>921.6 ± 128.7</td>
</tr>
<tr>
<td>Basal insulin (mU/L)</td>
<td>3.2 ± 0.6</td>
<td>3.4 ± 0.4</td>
<td>10.1 ± 1.9</td>
</tr>
<tr>
<td>Insulin AUC (mU·L⁻¹·min)</td>
<td>3040.7 ± 401.6</td>
<td>3713.3 ± 475.0</td>
<td>4944.4 ± 824.8</td>
</tr>
<tr>
<td>AUC_insulin/AUC_glucose (mU/mmol)</td>
<td>3.1 ± 0.4</td>
<td>4.2 ± 0.5</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>Total AWs (number)</td>
<td>86.4 ± 22.1</td>
<td>31.8 ± 6.3</td>
<td>164.1 ± 36.9</td>
</tr>
<tr>
<td></td>
<td>1179.6 ± 168.8</td>
<td>779.9 ± 137.5</td>
<td>1193.2 ± 208.2</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Total DWs (number)</td>
<td>84.4 ± 15.6</td>
<td>73.2 ± 20.6</td>
<td>87.0 ± 30.1</td>
</tr>
<tr>
<td>Total IPPWs (number)</td>
<td>5005.6 ± 615.3</td>
<td>4446.3 ± 521.6</td>
<td>5809.3 ± 619.7</td>
</tr>
</tbody>
</table>

1. Data are means ± SEM; paired Student’s *t*-test for intra-group comparisons: *P < 0.05, C vs. S;
2. One-factor ANOVA for inter-group comparisons, adjusted by Bonferroni’s correction for post hoc comparisons: #P < 0.05 for lean vs. obese; δP < 0.05 for lean vs. T2DM; εP < 0.05 for obese vs. T2DM.
Table 3. Basal values and areas under the curves (AUCs) for plasma glucose, GLP-1 and GIP (total and intact), glucagon and serum insulin, antropyloroduodenal (APD) pressure waves, and energy intake in response to intraduodenal glucose infusion (2 kcal/min, during t = 0-120 min) after placebo (P) + control (C), P + sitagliptin (S), metformin (M) + C, or M + S in patients with type 2 diabetes (n = 12).

<table>
<thead>
<tr>
<th></th>
<th>P + C</th>
<th>P + S</th>
<th>M + C</th>
<th>M + S</th>
<th>S effect</th>
<th>M effect</th>
<th>MS interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose (mmol/L)</td>
<td>6.6 ± 0.3</td>
<td>6.8 ± 0.4</td>
<td>6.0 ± 0.3</td>
<td>6.2 ± 0.3</td>
<td>0.101</td>
<td>0.000</td>
<td>0.987</td>
</tr>
<tr>
<td>Glucose AUC (mmolL⁻¹min)</td>
<td>1353.2 ± 45.4</td>
<td>1334.8 ± 51.1</td>
<td>1227.2 ± 44.4</td>
<td>1247.4 ± 40.4</td>
<td>0.961</td>
<td>0.000</td>
<td>0.253</td>
</tr>
<tr>
<td>Basal total GLP-1 (pmol/L)</td>
<td>17.7 ± 1.5</td>
<td>17.3 ± 1.5</td>
<td>21.9 ± 2.2</td>
<td>19.8 ± 2.1</td>
<td>0.277</td>
<td>0.016</td>
<td>0.472</td>
</tr>
<tr>
<td>Total GLP-1 AUC (pmolL⁻¹min)</td>
<td>2556.5 ± 204.8</td>
<td>2599.2 ± 193.0</td>
<td>3062.1 ± 240.1</td>
<td>2906.5 ± 240.1</td>
<td>0.566</td>
<td>0.001</td>
<td>0.216</td>
</tr>
<tr>
<td>Basal intact GLP-1 (pmol/L)</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>0.201</td>
<td>0.000</td>
<td>0.534</td>
</tr>
<tr>
<td>Intact GLP-1 AUC (pmolL⁻¹min)</td>
<td>108.0 ± 30.2</td>
<td>169.9 ± 32.4</td>
<td>191.3 ± 33.2</td>
<td>256.8 ± 41.0</td>
<td>0.007</td>
<td>0.001</td>
<td>0.904</td>
</tr>
<tr>
<td>Basal total GIP (pmol/L)</td>
<td>6.8 ± 1.6</td>
<td>7.8 ± 1.5</td>
<td>8.5 ± 2.2</td>
<td>7.1 ± 2.0</td>
<td>0.746</td>
<td>0.634</td>
<td>0.233</td>
</tr>
<tr>
<td>Total GIP AUC (pmolL⁻¹min)</td>
<td>2865.4 ± 247.1</td>
<td>2919.6 ± 351.2</td>
<td>3551.3 ± 407.9</td>
<td>3351.5 ± 284.1</td>
<td>0.694</td>
<td>0.014</td>
<td>0.477</td>
</tr>
<tr>
<td>Basal intact GIP (pmol/L)</td>
<td>9.6 ± 0.9</td>
<td>8.8 ± 0.7</td>
<td>9.8 ± 0.9</td>
<td>9.6 ± 0.9</td>
<td>0.312</td>
<td>0.384</td>
<td>0.646</td>
</tr>
<tr>
<td>Intact GIP AUC (pmolL⁻¹min)</td>
<td>2865.4 ± 247.1</td>
<td>2919.6 ± 351.2</td>
<td>3551.3 ± 407.9</td>
<td>3351.5 ± 284.1</td>
<td>0.008</td>
<td>0.107</td>
<td>0.777</td>
</tr>
<tr>
<td>Basal glucagon (pmol/L)</td>
<td>12.4 ± 2.4</td>
<td>12.5 ± 2.8</td>
<td>15.5 ± 2.9</td>
<td>16.3 ± 3.6</td>
<td>0.525</td>
<td>0.025</td>
<td>0.626</td>
</tr>
<tr>
<td>Glucagon AUC (pmolL⁻¹min)</td>
<td>1282.1 ± 287.3</td>
<td>1309.8 ± 309.6</td>
<td>1677.1 ± 351.8</td>
<td>1661.0 ± 406.6</td>
<td>0.938</td>
<td>0.003</td>
<td>0.681</td>
</tr>
<tr>
<td>Basal insulin (mU/L)</td>
<td>8.6 ± 1.9</td>
<td>8.2 ± 1.7</td>
<td>7.9 ± 1.5</td>
<td>7.6 ± 1.3</td>
<td>0.250</td>
<td>0.522</td>
<td>0.924</td>
</tr>
<tr>
<td>Insulin AUC (mUL⁻¹min)</td>
<td>2607.0 ± 472.6</td>
<td>3500.4 ± 816.8</td>
<td>2779.4 ± 473.8</td>
<td>3697.0 ± 832.7</td>
<td>0.049</td>
<td>0.142</td>
<td>0.827</td>
</tr>
<tr>
<td>AUC_insulin/AUC_glucose (mU/mmol)</td>
<td>1.9 ± 0.4</td>
<td>2.7 ± 0.7</td>
<td>2.3 ± 0.5</td>
<td>3.1 ± 0.8</td>
<td>0.065</td>
<td>0.019</td>
<td>0.718</td>
</tr>
<tr>
<td>Total AWs (number)</td>
<td>102.3 ± 18.0</td>
<td>93.3 ± 34.1</td>
<td>142.7 ± 21.8</td>
<td>118.0 ± 27.9</td>
<td>0.863</td>
<td>0.083</td>
<td>0.676</td>
</tr>
<tr>
<td>Total DWs (number)</td>
<td>1163.1 ± 115.5</td>
<td>956.5 ± 102.9</td>
<td>1204.5 ± 111.1</td>
<td>959.8 ± 129.8</td>
<td>0.100</td>
<td>0.594</td>
<td>0.673</td>
</tr>
<tr>
<td>Total IPPWs (number)</td>
<td>65.2 ± 7.4</td>
<td>59.7 ± 8.7</td>
<td>66.3 ± 11.5</td>
<td>38.8 ± 10.1</td>
<td>0.090</td>
<td>0.418</td>
<td>0.123</td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>4184.6 ± 465.9</td>
<td>3900 ± 488.6</td>
<td>3497.0 ± 472.3</td>
<td>3735.0 ± 392.8</td>
<td>0.913</td>
<td>0.040</td>
<td>0.307</td>
</tr>
</tbody>
</table>

1. Data are means ± SEM; two-factor repeated ANOVA was used to determine statistical significance, with S and M as factors.
Figure 1. Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) (total and intact), plasma glucagon, and serum insulin in response to intraduodenal glucose infusion (2 kcal/min, during t = 0 to 120 min) after control (C) and sitagliptin (S) in healthy lean subjects (n = 12). Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni’s correction, were performed, if ANOVAs were significant. *P < 0.05 for each. Data are means ± SEM.

Figure 2. Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) (total and intact), plasma glucagon, and serum insulin in response to intraduodenal glucose infusion (2 kcal/min, during t = 0 to 120 min) after control (C) and sitagliptin (S) in healthy obese subjects (n = 12). Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni’s correction, were performed, if ANOVAs were significant. *P < 0.05 for each. Data are means ± SEM.

Figure 3. Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) (total and intact), plasma glucagon, and serum insulin in response to intraduodenal glucose infusion after placebo (P) + control (C), P + sitagliptin (S),
metformin (M) + C, or M + S in patients with type 2 diabetes (n = 12). Repeated measures
ANOVA was used determine the statistical significance, with treatment and time as factors. Post
hoc comparisons, adjusted by Bonferroni’s correction, were performed, if ANOVAs were
significant. Results of ANOVA are reported as $P$-values for (A): differences by experiment, (B)
differences over time and (AB): differences due to the interaction of experiment and time. $^\alpha P <
0.05$, P + C vs. P + S; $^* P < 0.05$, P + C vs. M + C; $^\# P < 0.05$, P + C vs. M + S; $^\delta P < 0.05$, P + S
vs. M + S; $^\varepsilon P < 0.5$, M + C vs. M + S. Data are means ± SEM.

Figure 4. The frequency of antral waves (AWs), duodenal waves (DWs), and isolated pyloric
pressure waves (IPPWs) in response to intraduodenal glucose infusion (2 kcal/min, during t = 0-
120 min) after sitagliptin (S) or control (C) in healthy lean and obese subjects (n = 12 for each
group). Repeated measures ANOVA was used determine the statistical significance, with
treatment and time as factors. Results of ANOVA are reported as $P$-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni’s correction, were
performed, if ANOVAs were significant. $^* P < 0.05$ for each. Data are means ± SEM.

Figure 5. The frequency of antral waves (AWs) (A), duodenal waves (DWs) (B), and isolated
pyloric pressure waves (IPPWs) (C) in response to intraduodenal glucose infusion (2 kcal/min,
during t = 0-120 min) after placebo (P) + control (C), P + sitagliptin (S), metformin (M) + C, or
M + S in patients with type 2 diabetes (n = 12). Repeated measures ANOVA was used determine
the statistical significance, with treatment and time as factors. Results of ANOVA are reported as
\(P\)-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Data are means ± SEM.
Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (total and intact), plasma glucagon, and serum insulin in response to intraduodenal glucose infusion (2 kcal/min, during t = 0 to 120 min) after control (C) and sitagliptin (S) in healthy lean subjects (n = 12).

Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni’s correction, were performed, if ANOVAs were significant. * P < 0.05 for each. Data are means ± SEM.
Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (total and intact), plasma glucagon, and serum insulin in response to intraduodenal glucose infusion (2 kcal/min, during t = 0 to 120 min) after control (C) and sitagliptin (S) in healthy obese subjects (n = 12).

Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni's correction, were performed, if ANOVAs were significant. * P < 0.05 for each. Data are means ± SEM.
Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino tropic polypeptide (GIP) (total and intact), plasma glucagon, and serum insulin in response to intraduodenal glucose infusion after placebo (P) + control (C), P + sitagliptin (S), metformin (M) + C, or M + S in patients with type 2 diabetes (n = 12). Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Post hoc comparisons, adjusted by Bonferroni’s correction, were performed, if ANOVAs were significant. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. α P < 0.05, P + C vs. P + S; * P < 0.05, P + C vs. M + C; # P < 0.05, P + C vs. M + S; δ P < 0.05, P + S vs. M + S; ε P < 0.5, M + C vs. M + S. Data are means ± SEM.
The frequency of antral waves (AWs), duodenal waves (DWs), and isolated pyloric pressure waves (IPPWs) in response to intraduodenal glucose infusion (2 kcal/min, during t = 0-120 min) after sitagliptin (S) or control (C) in healthy lean and obese subjects (n = 12 for each group). Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni’s correction, were performed, if ANOVAs were significant. * P < 0.05 for each. Data are means ± SEM.
The frequency of antral waves (AWs) (A), duodenal waves (DWs) (B), and isolated pyloric pressure waves (IPPWs) (C) in response to intraduodenal glucose infusion (2 kcal/min, during t = 0-120 min) after placebo (P) + control (C), P + sitagliptin (S), metformin (M) + C, or M + S in patients with type 2 diabetes (n = 12).

Repeated measures ANOVA was used to determine the statistical significance, with treatment and time as factors. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Data are means ± SEM.

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