Effect of glucagon-like peptide-1 receptor antagonism on appetite and food intake in healthy men1–3

Robert E Steinert, Joerg Schirra, Anne C Meyer-Gerspach, Philipp Kienle, Heiko Fischer, Felix Schulte, Burkhard Goeke, and Christoph Beglinger

ABSTRACT

Objective: The GLP-1 receptor antagonist exendin(9–39)NH2 (ex9–39) was used to further explore the role of GLP-1 as an endogenous satiation signal.

Design: Two double-blind, 4-way crossover studies were performed, each of which included 10 healthy men. In study A, subjects received an intravenous infusion of ex9–39 or saline plus an oral glucose preload and an intraduodenal infusion of saline or glucose for 60 min. In study B, intravenous infusions were identical, but an oral mixed-liquid meal preload and a 60-min intraduodenal infusion of saline or oleic acid were administered. Thirty minutes after oral preloads, subjects ate and drank ad libitum, and amounts ingested and the time to meal completion were quantified. In addition, appetite and plasma GLP-1, peptide YY (PYY), insulin, glucagon, and blood glucose concentrations were measured.

Results: In both studies, GLP-1, PYY, and glucagon were substantially higher with intravenous ex9–39 than with intravenous saline (P ≤ 0.001). Insulin was lower with intravenous ex9–39 during intraduodenal glucose (P ≤ 0.05). The decrease in prospective food consumption and desire to eat during ad libitum eating after glucose ingestion was slightly attenuated (P ≤ 0.05 and P ≤ 0.01, respectively) with ex9–39. However, with intravenous ex9–39, food and fluid intakes and eating duration were not changed in either study.

Conclusions: GLP-1 receptor antagonism slightly modulates appetite during ad libitum eating, but food and fluid intakes and meal duration remain unchanged, suggesting that endogenous GLP-1 is a weak satiation signal. However, concomitant substantial increases in plasma PYY and glucagon may counteract a desatiating effect of ex9–39. The effect of ex9–39 on PYY secretion supports an autoinhibitory feedback mechanism that controls L cell secretion; the effect on insulin and glucagon confirms the role of GLP-1 in glycemic control through its action on pancreatic α and β cells. This trial was registered at clinicaltrials.gov as NCT01900340. Am J Clin Nutr doi: 10.3945/ajcn.114.083246.

INTRODUCTION
The ingestion of food activates a neuroendocrine reflex that controls nutrient digestion, absorption, and assimilation as well as postprandial glycemia and eating. The process involves luminal chemosensing for nutrients and other food components, nutrient mixing and progression along the gut by changes in motor activity, secretions by the stomach and exocrine pancreas, gallbladder contraction, and signaling to peripheral organs, such as the endocrine pancreas or the central nervous system. Different hormonal and neural mediators have been implicated in these events, and there are many examples of overlapping functions. However, in recent years, glucagon-like peptide-1 (GLP-1) has emerged as one of the most important mediators of several of these events. The exogenous administration of GLP-1 or its potent, long-acting analogs slow gastric emptying, improve postprandial glycemia, inhibit eating, and reduce body weight in many species, including healthy, overweight, and type 2 diabetic humans (1–3).

Exendin(9–39)NH2 (ex9–39), which is a highly specific GLP-1 receptor (GLP-1R) antagonist (4), has been used to further characterize whether the effects of exogenous GLP-1 are of a pharmacologic rather than physiologic nature. With the use of clamp techniques, Schirra et al (5) documented in humans that endogenous GLP-1 modulates antropyloroduodenal motility and is involved in the regulation of postprandial glycemia by its effects on the endocrine pancreas. Studies with ex9–39 that evaluated the role of GLP-1 in the control of gastric emptying have generated mixed results, which presumably reflected methodologic issues and the failure to control for concomitant increases in the secretion of peptide YY (PYY) (6, 7). Deane et al (8) reported a small but significant acceleration of gastric emptying with ex9–39; in contrast, gastric emptying was not changed by GLP-1R blockade in 2 other independent studies (9, 10). To our knowledge, whether GLP-1R antagonism modulates appetite and food intake in humans has not been investigated.

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4Abbreviations: used: ex9–39, exendin(9–39)NH2; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; PYY, peptide YY; VAS, visual analog scale.
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leaving its physiologic role as an endogenous satiation signal unresolved.

With the use of an experimental paradigm in which subjects received an oral nutrient preload (study A: glucose; study B: mixed-liquid meal) in combination with an intraduodenal infusion of saline or nutrient (study A: glucose; study B: oleic acid), we sought to evaluate the effect of GLP-1R antagonism on ad libitum eating in healthy men. We hypothesized that, if GLP-1 acts as a physiologic satiation signal, ex9–39 should lessen a nutrient-induced eating-inhibitory effect by modulating appetite and increasing food intake. We used a combination of gastric and intestinal nutrient stimuli because it has been shown that this combination is necessary to elicit optimal satiation, whereby 1) oral preloads together with intraduodenal nutrient infusion decrease food intake more than individual manipulations (11, 12), 2) intragastric nutrient administration is more potent in stimulating GLP-1 than are comparable intraduodenal infusions (13, 14), and 3) the eating-inhibitory effect of intravenous infusion of GLP-1 is increased by oral preloads (15). Moreover, in rats (16), ex9–39 showed no effect on feeding after a fast but increased the meal size in satiated animals.

SUBJECTS AND METHODS

Subjects

In total, 22 healthy, male volunteers (mean ± SEM age: 25.7 ± 1.0 y; age range: 18–35 y) of normal body weight for their heights [BMI (in kg/m²): 22.8 ± 0.4; BMI range: 18.1–24.9] were recruited. Exclusion criteria were the use of medications and the presence of any medical or psychiatric illnesses or food allergies. Baseline demographics for studies A and B are shown in Table 1. Written informed consent was obtained from all participants. The State Ethics Committee of Basel approved the experimental protocol (protocol EKBB25/11), and the study was carried out in accordance with the principles of the Declaration of Helsinki of 1975 as revised in 1983.

Protocol

Study A

The experiment was performed as a randomized, placebo-controlled, double-blind, 4-way crossover trial that included 12 subjects (Figure 1). The treatment sequence was allocated by computer-generated random numbers in blocks of 4. Visits were separated by≥3 d. Before each visit, subjects were instructed to abstain from alcohol and strenuous exercise for 24 h, consume a standardized meal (450 g pork ravioli (Bischosfzell Nah-

![FIGURE 1](image-url)

rungsmittel AG); 150 mL vegetable soup and 32 g cereal bar (both from HACO AG); total energy: 773.5 kcal) the night before at 1900 and to fast overnight from any additional solids and liquids until they arrived at the laboratory at 0800 the next morning.

On arrival, a radiopaque polyvinyl feeding tube (external diameter: 8 French) was inserted through an anesthetized nostril into the duodenum by using a guide wire. After correct positioning was ascertained by fluoroscopy, the guide wire was removed, and the tube was firmly attached to the skin behind the ear, thereby preventing additional progression during the experiment. During the next 60 min, subjects grew accustomed to the feeding tube. At 0900, an antecubital vein catheter was inserted into a forearm vein of both arms. At 0930 (t = −60 min) and 1300 (t = 150 min), iv sal (control) or ex9–39 (600 pmol · kg⁻¹ · min⁻¹) infusion between 0950 (t = −40 min) and 1230 (t = 120 min). In study A, at 1030 (t = 0 min), ID sal or ID gluc (caloric load: 2.0 kcal/min; total: 120 kcal) infusion until 1130 (t = 60 min). Also at 1030, subjects received an oral glucose preload (50 g glucose in 250 mL H₂O; caloric load 200 kcal), consumed in ≤5 min. In study B, at 10:30 (t = 0 min), ID sal or ID oa (caloric load: 0.2 kcal/min; total: 12 kcal) infusion until 1130 (t = 60 min). Also, at 1030, subjects received an oral mixed-liquid meal preload (17% protein, 30% fat, and 53% carbohydrate; vol: 250 mL; caloric load: 200 kcal) consumed in ≤5 min. At 1100 (t = 30 min), subjects ate and drank ad libitum but not for longer than 90 min or when the experimenter observed that the subject had not eaten for >5 min. At regular time intervals, participants scored their subjective appetite, thirst, and nausea by using VAS. ex9–39, exendin(9–39) (30 g glucose in 150 mL H₂O; caloric load: 2.0 kcal/min; total: 120 kcal) was infused intraduodenal at 2.5 mL/min for 60 min until 1330 (t = 120 min). At 1300 (t = 0 min), saline or glucose (30 g glucose in 150 mL H₂O; caloric load: 2.0 kcal/min; total: 120 kcal) was infused intraduodenal at 2.5 mL/min for 60 min until 1330 (t = 60 min). In addition, at time 0 (1030), each

<table>
<thead>
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<th>Variable</th>
<th>Study A</th>
<th>Study B</th>
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<tr>
<td>n</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Age (y)</td>
<td>24.4 ± 1.2</td>
<td>26.9 ± 1.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78.5 ± 2.0</td>
<td>73.9 ± 2.7</td>
</tr>
<tr>
<td>Body height (m)</td>
<td>1.86 ± 0.1</td>
<td>1.80 ± 0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 0.5</td>
<td>22.7 ± 0.7</td>
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1Mean ± SEM (all such values).
subject received an oral glucose preload (50 g glucose in 250 mL H₂O; caloric load: 200 kcal), which they had to consume in ≤5 min. At 1100 (t = 30 min), subjects were invited to eat and drink as much as they wished but not for longer than 90 min or when the experimenter observed that the subject had not eaten for >5 min. The test meal consisted of ham sandwiches [50 g bread, 10 g butter, and 29 g ham (pork); 247 kcal/sandwich] and tap water. To reduce the participant’s awareness of the amount of food eaten, sandwiches were presented in small samples and in excess. With the use of visual analog scales (VASs), participants scored their subjective appetite and nausea at regular time intervals.

Study B

The experiment was performed as a randomized, placebo-controlled, double-blind, 4-way crossover trial that included 10 subjects (Figure 1). The treatment sequence was allocated by computer-generated random numbers in blocks of 4. The experimental design was identical to that of study A except that at 1030 (t = 0 min), saline or oleic acid (1.21 g sodium oleate plus 0.29 g lecithin in 150 mL H₂O; caloric load: 0.2 kcal/min; total: 12 kcal) was infused intraduodenally at 2.5 mL/min for 60 min until 1130 (t = 60 min). In addition, at time 0 (1030), each subject received an oral mixed-liquid meal preload [17% protein, 30% fat, and 53% carbohydrate; vol: 250 mL; caloric load: 200 kcal (Ensure Plus; Abbott AG)], which they had to consume in ≤5 min. As previously stated, at 1100 (t = 30 min), subjects were invited to partake ad libitum of a standardized meal and, again, assessed their appetite and nausea with the use of VASs. Oleic acid at 0.2 kcal/min was chosen on the basis of previous studies by ourselves (17, 18) and others (19), which documented that free fatty acids potently stimulate GLP-1 at caloric loads <0.5 kcal/min.

Intravenous ex9–39 infusions

Synthetic ex9–39 was obtained from Bachem (lot 1011910). The peptide content was 100% with a purity of 99%. High-performance liquid chromatography showed a single peak for ex9–39. The peptide was dissolved in 1% human serum albumin and stored at −20°C until use. The dose administered (600 pmol/ kg/min) was calculated that, with 10 subjects, we would be able to detect a 12% difference in food intake at ≥0.05 with a power of 80%. Two subjects withdrew from study A and were excluded from the analysis because of incomplete data sets.

Hormones and glucose

With the use of in-dwelling antecubital catheters, blood was drawn into Monovette tubes kept on ice. Monovette tubes contained EDTA (6 μmol/L; Sarstedt), a protease inhibitor cocktail (1 tablet/25 mL; Roche Diagnostics GmbH) and a dipeptidyl peptidase intravenous inhibitor (50 μmol/L; Merck KGaA).

Hormones measured were GLP-1, insulin, PYY, and glucagon. Active GLP-1 was measured as previously described (21) by using a commercially available ELISA kit (Millipore Corp). The intraassay and interassay CVs were <9% and <13%, respectively. The analytic sensitivity was 0.5 pmol/L. Insulin was measured with a commercial radioimmunoassay (Cisbio International). Intraassay and interassay CVs were <12.2% and <9%, respectively. The lowest concentration of insulin that could be detected was 4.6 μU/mL. Glucagon was measured by using a commercially available radioimmunoassay (Siemens Medical Solution Diagnostics.). Intrassay and interassay CVs were <6.5% and <11.9%, respectively. The analytic sensitivity was >13 pg/mL. Blood glucose concentrations were measured by using a glucoseoxidase method (Bayer Consumer Care). Total PYY was measured by using a commercially available radioimmunoassay (Millipore Corp). Intrassay and interassay CVs were <9.5% and <8.4%, respectively. The lowest concentration of PYY that could be detected was 20 pg/mL.

Appetite and eating variables

Appetite (hunger, fullness, satiety, desire to eat, and prospective food consumption), thirst, and nausea were measured by using validated VAS questionnaires as previously described (22). The amount of food eaten, volume of fluid imbibed, and time for each subject to complete the ad libitum test meal was quantified. From these observations, energy intake, fluid intake, and eating duration were calculated.

Statistical analysis

Plasma hormone and blood glucose concentrations as well as appetite ratings (VASs) were analyzed by calculating AUCs (calculated by using the trapezoidal rule). Significant differences between treatment groups were assessed by using a 2-way repeated-measures ANOVA with intravenous and intraduodenal infusions as factors. Post hoc tests were performed if ANOVAs showed significant effects. All post hoc tests were adjusted for multiple comparisons by using Bonferroni correction. All values were reported as means ± SEMs. All tests were 2-tailed, and differences were considered statistically significant at P ≤ 0.05. All statistical analyses were done with the statistical software package SPSS for Windows (version 19.0; SPSS). The number of subjects was determined by power calculations performed with the GPower 3 program (University of Düsseldorf) with an effect size estimate that was based on Verdich et al (23) and our own previous studies with GLP-1 infusions (24, 25). We calculated that, with 10 subjects, we would be able to detect a 12% difference in food intake at α = 0.05 with a power of 80%. Two subjects withdrew from study A and were excluded from the analysis because of incomplete data sets.

Results

Effects of intravenous ex9–39 on food intake

Study A

There was no intravenous treatment effect. Compared with intravenous saline, intravenous ex9–39 did not affect energy intake, fluid intake, or eating duration (Table 2). However, there was a significant intraduodenal treatment effect. Energy intake was lower with intraduodenal glucose than intraduodenal saline (intraduodenal treatment effect, P = 0.002; Table 2), and the eating duration was shorter (P = 0.01; Table 2).

Study B

There was no intravenous or intraduodenal treatment effect (Table 2).
Effects of intravenous ex-9–39 on appetite, thirst, and nausea

Study A

There was an intravenous treatment effect. Intravenous ex-9–39 slightly attenuated the decrease in prospective food consumption and desire to eat during ad libitum eating with intraduodenal saline and intraduodenal glucose (intravenous treatment effect, \( P = 0.041 \) and \( P = 0.010 \), respectively; Figure 2, A and C). No effect on ratings of fullness, satiety, and hunger was noted (data not shown). There was also no intraduodenal treatment effect on appetite ratings.

Study B

There was no intravenous or intraduodenal treatment effect on appetite ratings (Figure 2, B and D). No intravenous or intraduodenal treatment effect on thirst or nausea was seen in either study A or B (data not shown).

Effects of intravenous ex-9–39 on plasma GLP-1 and PYY concentrations

Study A

There was an intravenous treatment effect. The increase in plasma GLP-1 and PYY was substantially higher with intravenous ex9–39 than with intravenous saline (intravenous treatment effect, \( P < 0.001 \) and \( P = 0.010 \), respectively; Figure 3, A and C; Table 3). There was also an intraduodenal treatment effect for GLP-1. The increase in plasma GLP-1 was higher with intraduodenal glucose than intraduodenal saline (intraduodenal treatment effect, \( P < 0.001 \); Figure 3A, Table 3).

Study B

Similar to study A, there was an intravenous treatment effect. Compared with intravenous saline, intravenous ex9–39 markedly increased glucagon secretion (intravenous treatment effect, \( P = 0.004 \); Table 3, Figure 4B). There was no intraduodenal treatment effect.

Effects of intravenous ex-9–39 on plasma glucagon concentrations

Study A

There was an intravenous and intraduodenal treatment effect. The increase in plasma glucagon was markedly higher with intravenous ex9–39 than intravenous saline (intravenous treatment effect, \( P < 0.001 \); Figure 4A, Table 3). Moreover, the increase in plasma glucagon was higher with intraduodenal glucose than intraduodenal saline (intraduodenal treatment effect, \( P = 0.004 \); Figure 4A, Table 3). There was no intravenous or intraduodenal treatment effect.

Study B

There was an intravenous treatment effect. The increase in plasma glucagon was substantially higher with intravenous ex9–39 than with intravenous saline (intravenous treatment effect, \( P < 0.001 \); Table 3, Figure 4B). There was no intraduodenal treatment effect.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Energy intake, fluid intake, and eating duration during the ad libitum test meal in response to iv saline (control) or ex9–39 after an oral glucose preload plus ID saline or glucose infusions (study A) or after an oral mixed-liquid meal preload plus ID saline or oleic acid infusion (study B)</th>
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<tbody>
<tr>
<td>Study A: oral glucose preload plus ID saline or glucose</td>
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<tr>
<td>Energy intake (kcal)</td>
<td>967.9 ± 138.5</td>
</tr>
<tr>
<td>Fluid intake (mL)</td>
<td>642.4 ± 125.3</td>
</tr>
<tr>
<td>Eating duration (min)</td>
<td>22.6 ± 2.7</td>
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<tr>
<td>Study B: oral mixed-liquid meal preload plus ID saline or oleic acid</td>
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</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>739.8 ± 124.7</td>
</tr>
<tr>
<td>Fluid intake (mL)</td>
<td>590.1 ± 86.9</td>
</tr>
<tr>
<td>Eating duration (min)</td>
<td>19.3 ± 1.6</td>
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</table>

All values are means ± SEMs. \( n = 10 \). Significant differences between treatment groups were assessed by using a 2-way repeated measures ANOVA with iv and id infusions as factors. ex9–39, exendin(9–39)NH2; ID, intraduodenal; iv, intravenous.
Effects of intravenous ex9–39 on plasma insulin concentrations

Study A

There was an intravenous-intraduodenal interaction effect for plasma insulin. The increase in plasma insulin was lower with intravenous ex9–39 than intravenous saline during intraduodenal glucose. Moreover, the increase in plasma insulin was higher with intraduodenal glucose than intraduodenal saline during intravenous saline administration (intravenous-intraduodenal interaction, \( P = 0.025 \); Figure 4C, Table 3).

Study B

There was no intravenous or intraduodenal treatment effect (Figure 4D, Table 3).

Effects of intravenous ex9–39 on blood glucose concentrations

Study A

Although blood glucose was numerically higher with intravenous ex9–39 than intravenous saline and during intraduodenal glucose than intraduodenal saline, there was no intravenous or intraduodenal treatment effect (Figure 4E, Table 3).

Study B

There was an intravenous treatment effect. The increase in blood glucose concentrations was higher with intravenous ex9–39 than intravenous saline (intravenous treatment effect, \( P = 0.013 \); Figure 4F, Table 3). There was no intraduodenal treatment effect.

Side effects

With the exception of 2 subjects who experienced a single episode of vomiting in study A, no nausea or abdominal discomfort was reported, and the protocol was well tolerated. Blood pressure and heart rate were not affected. No other adverse events were observed.

DISCUSSION

We hypothesized that, if GLP-1 acts as an endogenous satiation signal, GLP-1R antagonism should lessen a nutrient-induced, eating-inhibitory effect by modulating appetite and increasing food intake during ad libitum eating. We showed that,
after glucose-stimulated GLP-1 secretion (study A), ex9–39 attenuated the decrease in prospective food consumption and desire to eat but evoked no changes in food and fluid intakes or eating duration. No effect of ex9–39 on appetite, food and fluid intakes, or eating duration was observed after the mixed-liquid meal/oleic acid–stimulated GLP-1 secretion (study B).

A number of empirical criteria for assessing whether a hormone truly functions as an endocrine-satiation signal were first introduced by Gibbs et al (26) with the classic demonstration that cholecystokinin inhibits eating by truly eliciting satiation. This approach has become paradigmatic in the study of gut peptide controls of eating (27, 28). With the application of these criteria to GLP-1, the following information can be deduced:

1) GLP-1 is released in response to food ingestion with a close temporal link between GLP-1 secretion and eating inhibition (29).

2) Intravenous infusions of GLP-1 at physiologic doses increase fullness and reduce energy intake (24, 30). This effect occurs without evoking nausea; however, it is rather modest and, for reasons not yet understood, does not occur under all test conditions (23, 31).

3) In rats, the administration of ex9–39 blocks the eating-inhibitory effect of exogenous GLP-1 (16, 32).

Only a few animal studies have examined the role of GLP-1 in eating by antagonizing endogenous GLP-1, and results have been conflicting. Although Williams et al (33) and other authors (16) showed that ex9–39 alone stimulated eating under certain conditions when food intake was normally low, other researchers (34) have reported that GLP-1R antagonism did not affect food intake. Rüttimann et al (32) even described decreases in the meal duration with ex9–39 and concluded that endogenous intestinal GLP-1 is not required for the control of spontaneous meal size in rats under their conditions.

The failure of ex9–39 to affect food intake in the current study is in line with findings in rats and argues against a major physiologic role of endogenous GLP-1 in the control of eating. There are several explanations for the failure of ex9–39 to affect food intake as follows:

1) The pleiotropic effect of ex9–39 on PYY and glucagon: both hormones were substantially increased to pharmacologic plasma concentrations (up to ~1.8-fold above normal prandial concentrations for PYY and ~1.5-fold for glucagon), which may have counteracted a possible desatiating effect of ex9–39. In healthy men, intravenous infusion of PYY or glucagon that produced similarly high PYY or even lower glucagon plasma concentrations significantly reduced energy intake by ~12% (35) or ~20% (36), respectively.

2) The redundancy of the satiating system: other gut peptides, such as cholecystokinin or amylin, or central...
TABLE 3
AUCs for GLP-1 and PYY glucagon, insulin, and glucose in response to iv saline (control) or ex9–39 after an oral glucose preload plus ID saline or glucose infusions (study A) or after an oral mixed-liquid meal preload plus ID saline or oleic acid infusion (study B)

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<tbody>
<tr>
<td>GLP-1 AUC</td>
<td>876.9 ± 47.5</td>
<td>1651.9 ± 151.5</td>
<td>1276.1 ± 181.8</td>
<td>2210.5 ± 192.32</td>
<td>0.001</td>
<td>2210.5 ± 192.32</td>
<td>0.001</td>
<td>NS</td>
<td></td>
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<tr>
<td>(pmol · kg⁻¹ · min⁻¹)</td>
<td>10,335.9 ± 3268.5</td>
<td>18,148.7 ± 5759.2</td>
<td>11,592.9 ± 3419.9</td>
<td>20,145.2 ± 5953.4</td>
<td>0.010</td>
<td>20,145.2 ± 5953.4</td>
<td>0.010</td>
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<td></td>
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<tr>
<td>PYY AUC</td>
<td>9265.1 ± 465.2</td>
<td>13,950.5 ± 398.9</td>
<td>11,663.6 ± 761.2</td>
<td>15,717.9 ± 1207.8</td>
<td>0.001</td>
<td>15,717.9 ± 1207.8</td>
<td>0.001</td>
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<tr>
<td>(pg · mL⁻¹ · min⁻¹)</td>
<td>15,608.8 ± 1365.0</td>
<td>15,291.2 ± 1875.3</td>
<td>18,908.9 ± 1347.8</td>
<td>15,946.8 ± 1154.6</td>
<td>0.008</td>
<td>15,946.8 ± 1154.6</td>
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<td>Glucagon AUC</td>
<td>1199.4 ± 53.6</td>
<td>1242.77 ± 35.3</td>
<td>1199.20 ± 31.0</td>
<td>1235.44 ± 28.5</td>
<td>NS</td>
<td>1235.44 ± 28.5</td>
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<tr>
<td>(pg · mL⁻¹ · min⁻¹)</td>
<td>1044.9 ± 98.3</td>
<td>1852.0 ± 120.2 3</td>
<td>—</td>
<td>865.5 ± 49.0</td>
<td>NS</td>
<td>865.5 ± 49.0</td>
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<tr>
<td>Insulin AUC</td>
<td>12,458.1 ± 3797.4</td>
<td>15,683.4 ± 4735.5</td>
<td>—</td>
<td>12,471.6 ± 3971.1</td>
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<td>12,471.6 ± 3971.1</td>
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<tr>
<td>(µU · mL⁻¹ · min⁻¹)</td>
<td>6575.5 ± 482.5</td>
<td>7945.2 ± 386.7</td>
<td>—</td>
<td>6086.9 ± 265.9</td>
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<td>6086.9 ± 265.9</td>
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<td>Glucose AUC</td>
<td>13,303.3 ± 1565.1</td>
<td>13,468.2 ± 1452.0</td>
<td>—</td>
<td>13,812.8 ± 1270.9</td>
<td>NS</td>
<td>13,812.8 ± 1270.9</td>
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<tr>
<td>(mmol · L⁻¹ · min⁻¹)</td>
<td>1127.1 ± 36.8</td>
<td>1184.8 ± 46.0</td>
<td>—</td>
<td>1128.8 ± 43.0</td>
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</table>

1 All values are means ± SEMs. n = 10. Significant differences between treatment groups were assessed by using a 2-way repeated-measures ANOVA with iv and ID infusions as factors. Post hoc tests were performed if ANOVAs showed significant effects. Post hoc tests were adjusted for multiple comparisons by using Bonferroni correction. a iv saline/ID glucose compared with iv saline/ID saline, P < 0.05 b iv ex9–39/ID glucose compared with iv saline/ID glucose, P < 0.05 ex9–39, exendin(9–39)NH₂; GLP-1, glucagon-like peptide-1; ID, intraduodenal; iv, intravenous; PYY, peptide YY.
mechanisms were not antagonized and, thus, may have overridden a possible desatiating effect of ex9–39.

3) The experimental design: to stimulate endogenous GLP-1, we used glucose in study A and a mixed-liquid meal preload plus ID sal or ID gluc infusions [study A (A, C, and E)] or after an oral mixed-liquid meal preload plus ID sal or ID oa infusions [study B (B, D, and F)]. Significant differences between treatment groups were assessed by using a 2-way repeated measures ANOVA with iv and ID infusions as factors. Post hoc tests were performed if ANOVAs showed significant effects. All post hoc tests were adjusted for multiple comparisons by using Bonferroni correction. n = 10. A: iv treatment effect, \( P \leq 0.001 \); ID treatment effect, \( P = 0.004 \). B: iv treatment effect, \( P = 0.004 \). C: significant iv/ID interaction, \( P \leq 0.025 \). D: No effect. E: No effect. F: iv treatment effect, \( P = 0.013 \). Also see Table 3. ex9–39, exendin(9–39)NH2; gluc, glucose; ID, intraduodenal; iv, intravenous; oa, oleic acid; sal, saline.

FIGURE 4. Mean (±SEM) plasma glucagon, insulin, and blood glucose concentrations in response to iv sal (control) or iv ex9–39 after an oral glucose preload plus ID sal or ID gluc infusions [study A (A, C, and E)] or after an oral mixed-liquid meal preload plus ID sal or ID oo infusions [study B (B, D, and F)].
showed that intraduodenal glucose stimulated GLP-1 and decreased food intake beyond what was observed with the oral glucose preload plus intraduodenal saline. However, in study B, intraduodenal oleic acid did not increase GLP-1 or decrease food intake beyond the effect of the mixed-liquid meal preload and intraduodenal saline. Thus, additional studies are warranted to investigate whether different loads, a combination of different nutrients, or a different experimental setup to stimulate GLP-1 can reveal a desatiating effect of ex9–39.

The significant effect of ex9–39 on VAS measures of appetite in the absence of a reliable effect on meal size in study A was interesting but not surprising. VAS measures of appetite generally show sensitivity to experimental manipulations and good reproducibility under controlled conditions but often do not predict the actual meal size (41, 42).

Consistent with the literature, ex9–39 substantially increased the secretion of GLP-1 and PYY (5, 6, 9). An increased delivery of nutrients to the duodenum secondary to accelerated gastric emptying may be one explanation. However, although exogenous GLP-1 slows gastric emptying (43), 3 other studies reported little or no acceleration of gastric emptying with ex9–39 (8–10), arguing against such a mechanism. In consideration of the substantial increase in PYY with ex9–39, the most likely explanation is, thus, a negative auto-feedback mechanism that controls entire L cell secretion (ie, both GLP-1 and PYY release) that would be interrupted by GLP-1R antagonism (5). This explanation would concur also with a study that showed that GLP-1 infusion reduced plasma PYY (44); moreover, a similar phenomenon has been observed for intestinal I cells and cholecystokinin receptor antagonism (45).

Ex9–39 has been used predominantly in clamp studies to establish a role for endogenous GLP-1 in stimulating β cell secretion and tonically inhibiting glucagon release (5, 9, 10). In the current article, we add new evidence, to our knowledge, for the digestive state showing that endogenous GLP-1 contributes to glucose homeostasis through its action on both α and β cells. However, we did not measure gastric emptying and, thus, could not provide additional information on the involvement of gut motor function in the control of postprandial glycemia. However, the fact that ex9–39 substantially increased plasma PYY (which slows gastric emptying when exogenously administered) suggested that this effect may counteract ex9–39’s accelerating effect on gastric emptying (9, 10).

In conclusion, the current study shows that GLP-1R antagonism modulates appetite (ie, it slightly attenuates the decrease in prospective food consumption and desire to eat during ad libitum eating after the stimulation of endogenous GLP-1 by glucose). The appetite effect is not accompanied by changes in food and fluid intakes or eating duration, thereby suggesting that GLP-1 is a weak satiation signal. However, ex9–39, substantially increases plasma PYY and glucagon, which may counteract a desatiating effect of ex9–39. The effect of ex9–39 on PYY secretion supports an autoinhibitory feedback mechanism that controls L cell secretion; the effects on insulin and glucagon confirm that endogenous GLP-1 contributes to glycemic control through its action on α and β cells under ad libitum eating conditions.

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and plasma CCK and GLP-1 in humans vary with their chain length.


