Oral administration of glucagon-like peptide 1 or peptide YY 3-36 affects food intake in healthy male subjects

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ABSTRACT

Background: Peripheral infusion of glucagon-like peptide 1 (GLP-1) or peptide YY 3-36 (PYY3-36) reduces food intake in healthy, obese, and diabetic subjects. In vivo, both peptides are cosecreted from intestinal L cells; GLP-1 is subject to rapid breakdown by dipeptidyl peptidase IV, and together with PYY3-36 it is likely to be degraded in the liver before entering the systemic circulation. The largest concentrations are observed in the splanchnic blood rather than in the systemic circulation.

Objective: In contrast with peripheral infusion, oral delivery of sodium N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC) mimics endogenous secretion. We aimed to investigate how this affects food intake.

Design: Twelve healthy male subjects were studied in a randomized, double-blind, placebo-controlled, 4-way crossover trial. Each subject received in random order 2.0 mg GLP-1, 1.0 mg PYY3-36, or 2.0 mg GLP-1 plus 1.0 mg PYY3-36; the peptides were mixed with SNAC. The placebo treatment was the delivery agent alone. Food intake during an ad libitum test meal was measured.

Results: Both peptides were rapidly absorbed from the gut, leading to plasma concentrations several times higher than those in response to a normal meal. GLP-1 alone, but not PYY3-36, reduced total energy intake significantly, with marked effects on glucose homeostasis. Co-administration of both peptides reduced total energy intake by 21.5% and fullness at meal onset (P < 0.05) but not total 24-h energy intake.

Conclusion: The results show a marked effect of orally administered GLP-1 and PYY3-36 on appetite by showing enhanced fullness at meal onset and reduced energy intake. This trial was registered at clinicaltrials.gov as NCT00822705.

INTRODUCTION

Obesity has become one of the greatest public health challenges in the 21st century, with the epidemic prospect that >50% of the world’s adult population will be overweight or obese by 2030. Epidemiologic studies clearly indicate that overweight and obesity have serious consequences for human health, including diabetes, cardiovascular disease, and cancer (1). Detailed knowledge about the mechanisms that regulate appetite and body weight is therefore essential to develop effective obesity treatments. Meal initiation, frequency, and termination are controlled by complex processes, which necessitates a close communication between the gastrointestinal (GI) endocrine system and the brain (gut-brain axis). Satiety peptides are released in proportion to ingested calories and signal to the hypothalamus and brainstem via neural and endocrine mechanisms (2). Glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY) are 2 of these gut-derived hormones. Their endogenous plasma concentrations are low in the fasting state and rise during a meal, which is consistent with the satiety-inducing action shown in rats and humans (2–4). GLP-1 and PYY are cosecreted from intestinal L cells and enter the systemic circulation via intestinal capillaries draining into the hepatic portal vein (5). GLP-1 is subject to 1) rapid breakdown by dipeptidyl peptidase IV (DPP-IV) located on the luminal surface of endothelial cells, 2) high liver extraction, and 3) continued proteolytic activity of soluble DPP-IV present in the plasma (5, 6). Therefore, only 10–15% is estimated to enter the systemic circulation (5). PYY on the other hand is thought to be activated by DPP-IV from PYY1-36 into the predominant circulating Y2 agonist PYY3-36 (7). Similarly to GLP-1, we assume that a considerable amount of PYY3-36 is degraded in the liver before entering the systemic circulation. Largest concentrations of both peptides will therefore be found in the intestinal extra-cellular space and hepatic portal vein rather than in the systemic circulation. Assuming that GLP-1 and PYY3-36 act partly on afferent receptors very near the site of secretion, high local concentrations in the splanchnic blood could be potentially important for regulatory functions in the control of food intake. We recently showed that, using sodium N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC), GLP-1 and PYY3-36 can be delivered orally with a pharmacodynamic profile consistent with reported pharmacology (8, 9). In comparison with peripheral infusions, an oral enteric delivery system nearest mimics the physiologic path of endogenous secretion. We sought to determine to what extent...
orally administered GLP-1 and PYY3-36 affect food intake and appetite feelings in healthy male subjects.

SUBJECTS AND METHODS

Subjects

The study included 12 healthy, nonsmoking, male volunteers (mean age: 24.3 ± 1.5 y; range: 20–44). The body weight of all subjects was in the normal range for age, sex, and height and was stable for ≥3 months [mean body mass index (in kg/m²): 22.6 ± 0.5; range: 19.4–25.0]. The criteria for exclusion were smoking, substance abuse, chronic medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests. None of the subjects had a history of food allergies or dietary restrictions. Written informed consent was obtained from all participants. The State Ethical Committee of Basel approved the experimental protocol, and the study was carried out in accordance with the principles of the Declaration of Helsinki.

Study design

The study was performed as a randomized, placebo-controlled, double-blind, 4-way crossover trial (the treatment sequence was allocated by computer-generated random numbers in blocks of 4). The 4 treatments were separated by ≥5 d. The subjects were instructed to abstain from alcohol and strenuous exercise for 24 h before each treatment. Together with a standard diet, this should ensure equal glycogen stores and similar macronutrient balances (10). On each test day, the subjects reported to the research unit at 1200 after having consumed a standardized liquid breakfast before 0800 on each study day. Before this, they had consumed a restricted simple carbohydrate standard dinner the evening and then fasted for 10 h overnight. No additional food or fluid, except water, was allowed. After the subjects arrived at the research unit, a catheter was inserted into one antecubital vein of an arm for blood drawing. At time point \( t = 0 \) min, the subjects swallowed a test tablet (together with 50 mL tap water) formulated with 150 mg of the delivery agent SNAC mixed with 1) 2.0 mg GLP-1, 2) 1.0 mg PYY3-36, or 3) 2.0 mg GLP-1 plus 1.0 mg PYY3-36. The reference treatment (placebo) was 150 mg SNAC alone. The selection of oral doses for GLP-1 and PYY to achieve the maximum effect along with minimum side effects was based on 2 previous studies in healthy volunteers (8, 9).

An ad libitum test meal was served 15 min later, and the subjects were free to eat until comfortably satisfied. The test meal consisted of 1) orange juice as an appetizer (43 kcal/100 mL), 2) sandwiches [60 g bread, 10 g butter, and 25 g ham (pork); 245 kcal/sandwich] with optional additional orange juice, and 3) chocolate cream (134 kcal/100 g). The order of food intake had to follow the above schedule. Water consumption and toilet visits were allowed when necessary, but additional food was not allowed. To reduce the participants’ awareness of the amount of food being consumed, food was served in excess. Food intake during the test meal was quantified by measuring the quantity of food eaten, the quantity of fluid drunk, and the time for each subject to complete his meal. With these measurements, the following meal variables were calculated: 1) total energy intake, 2) total food quantity, 3) total fluid intake, and 4) meal duration. For hormone measurement, blood was drawn in the predose period \( (t = -1 \) min), the premeal period \( (t = 15 \) min; test meal started thereafter), and during or after the meal at regular time intervals \( (t = 30, 45, 60, 75, 90, 120, \) and 180 min). Blood samples were collected on ice into tubes containing EDTA (6 \( \mu \)mol/L) and aprotinin (500 kIU/L). After centrifugation at 4°C, plasma samples were processed into different aliquots and kept frozen at −70°C until analyzed. The appetite profile (hunger, satiety, and fullness) and gastrointestinal symptoms were assessed by using 100-mm visual analog scales (VAS), with words anchored at each end, expressing the most positive and most negative ratings (11). Subjects were allowed to talk, relax, and read, but they were not able to discuss or compare their ratings. After each test day, the subjects were instructed to eat ad libitum and complete a food diary for the following 24 h. Each participant was questioned after each experiment and after he had completed the 4 tests about whether he had experienced any adverse events.

Laboratory analysis

Active GLP-1 was measured as described recently (9) by using a commercially available enzyme-linked immunosorbent assay kit (Linco Research, St Charles, MO). This kit is for non-radioactive quantification of biologically active forms of GLP-1 (ie, 7-36 amide and 7-37) in plasma and other biological media. Total PYY, ghrelin, insulin, and glucagon were measured with commercially available radioimmunoassay kits (Linco Research; Cibisio International, Bagnols, France; and Siemens Medical Solution Diagnostics, Los Angeles, CA). Blood glucose concentrations were measured by using a commercial hexokinase-glucose-6-phosphate-dehydrogenase method (Roche AG, Basel, Switzerland).

Materials

GLP-1(7-36 amide) and PYY3-36, as well as the delivery agent SNAC, were obtained from Emisphere Technologies (Cedar Knolls, NJ). Drug tablets were prepared by mixing the peptides with 150 mg SNAC. Emisphere’s eligen technology is based on carrier molecules that possess hydrophobic moieties, which, in association with the drug, create a more lipophilic drug-carrier complex. This complex facilitates the transport of peptides with low oral bioavailability across biological membranes such as those of the gastrointestinal tract. Further characteristics of the delivery agent SNAC were described previously (8, 9).

Statistical analysis

Descriptive statistics were used for demographic variables such as age, weight, height, and BMI. Individual hormone concentrations versus time data were used to obtain GLP-1 and PYY metrics, including maximum plasma concentrations, the time of maximal peptide occurrence, and the area under the plasma concentration time curve. The general linear model procedure of repeated-measures ANOVA using simple contrast and Bonferroni correction of \( P \) values for multiplicity of comparison was used to test for significant differences between treatments. VAS were statistically analyzed by calculating the area under the concentration time curve (0–180 min) and differences from baseline (0 min) to 15 min (meal onset). These
data were compared between treatment groups by using the nonparametric Friedman test. In case of significant differences, pairwise comparison were performed by using the Wilcoxon’s signed-rank test followed by Bonferroni correction to account for multiplicity of comparisons. All statistical tests were 2-tailed, and differences were considered to be significant at $P < 0.05$. The statistical analysis was done using SPSS for Windows software (version 15.0). Data are presented as means ± SEMs ($n = 12$).

RESULTS

Effects of oral GLP-1 and PYY3-36 on meal variables

Oral administration of GLP-1 alone or PYY3-36 alone reduced total energy intake in 11 of 12 and in 10 of 12 subjects, respectively, compared with placebo (Figure 1, B and C). PYY3-36 caused a decrease in energy intake of $12.0 ± 6.7\%$ (NS), whereas GLP-1 decreased energy intake by $13.6 ± 3.6\%$ ($P < 0.05$) compared with placebo (Figure 2A, Table 1). During the combined administration of GLP-1 plus PYY3-36, 10 of 12 subjects had a lower spontaneous energy intake; the reduction averaged $21.5 ± 7.7\%$ ($P < 0.05$) compared with placebo (Figure 1A, Figure 2A, Table 1). The numerical higher reduction in energy intake was, however, not significantly different from single peptide administration (Figure 2A). Total food quantity (g) decreased significantly in subjects receiving GLP-1 only; in contrast, total fluid intake (mL) and meal duration (min) decreased significantly in subjects receiving GLP-1 plus PYY3-36 (Table 1). Cumulative energy intake over 24 h (including test meal) was unaffected by either treatment (Figure 2B).

Effects of oral GLP-1 and PYY3-36 on eating behavior

At meal onset ($t = 15$ min), oral peptide administration induced weak effects on hunger and satiety; when GLP-1 and PYY3-36 were coadministered, significantly increased fullness ratings were observed (Figures 3 and 4). Appetite profiles over the 180-min time course of the study, however, showed no
significant differences in satiety, fullness, or hunger between the groups (Figure 3). No significant differences in nausea scores were observed (data not shown).

### Plasma concentration of GLP-1, PYY3-36, and ghrelin

Both GLP-1 and PYY3-36 were rapidly absorbed from the GI tract, leading to peak plasma concentrations that were several times higher than the endogenous plasma concentrations in response to the test meal (Figure 5, Table 2). Interestingly when GLP-1 and PYY3-36 were coadministered, the absorption of PYY3-36 was slightly increased (albeit not significant) compared with single peptide administration. The plasma GLP-1 concentration returned to basal levels after 30 min, whereas circulating PYY3-36 remained elevated for 45 min (Figure 5).

Plasma ghrelin concentrations decreased in all treatment groups during meal consumption (see Supplemental Figure 1 under “Supplemental data” in the online issue). Oral administration of GLP-1 alone or GLP-1 plus PYY3-36 coadministration moderately suppressed plasma ghrelin concentrations in the premeal period, but the effects were not statistically significant (see Supplemental Figure 1 under “Supplemental data” in the online issue).

### Plasma concentrations of glucose, insulin, and glucagon

In both treatments with oral GLP-1, an early increase in fasting insulin was observed (ie, in the premeal phase); thereafter, insulin concentrations decreased slightly despite the start of food consumption (Figure 6A). Subsequently, insulin concentrations increased in a similar, but delayed magnitude compared with those of the placebo group, especially when GLP-1 and PYY3-36 were coadministered (Figure 6A). During placebo and PYY3-36 treatments, a normal insulin response was seen, with a rapid increase in plasma insulin concentrations after test meal ingestion. When GLP-1 was administered, blood glucose concentrations were reduced below fasting values until t = 30 min in both groups with GLP-1. The glycemic response was delayed compared with placebo, with lower initial plasma glucose concentrations during the meal (15–45 min) (Figure 6B). Glucagon secretion was reduced to below fasting values in the premeal phase when GLP-1 or GLP-1 plus PYY3-36 was administered (data not shown).

### Adverse events

At single administration, all the study subjects tolerated both peptides well, and no adverse events were recorded. When coadministered, one subject experienced brief phases of nausea and abdominal discomfort and one subject had a single episode of vomiting. Blood pressure and heart rate were not affected by oral GLP-1 and PYY3-36 (data not shown).

### DISCUSSION

In the present study, we investigated the effect of oral administration of GLP-1 or PYY3-36 or combined administration of both peptides on food intake and appetite in human volunteers. The experimental design is different from previous investigations, in which GLP-1 or PYY3-36 was peripherally infused. The study, therefore, addresses several important issues: 1) Is food intake suppressed under the present conditions with oral peptide administration, 2) Is there an interaction between the 2 peptides with respect to food intake, 3) What are the pharmacodynamic results of orally administered GLP-1 and PYY3-36, and 4) Is there a potential for therapeutic application of oral peptide delivery?

### Food intake inhibition with oral peptide administration

Previous investigations with intravenous infusions of either GLP-1 and/or PYY3-36 have documented dose-dependent inhibitory effects on food intake and appetite in humans; most studies concluded that the peptide concentrations obtained in these studies were in a supraphysiologic range and, therefore, were most likely pharmacologic rather than physiologic effects. Here, we obtained plasma peptide concentrations, which were several-fold higher than seen after regular meal intake, again suggesting pharmacologic conditions. Oral GLP-1 (2 mg) induced a significant 14% reduction in calorie intake; this is in keeping with early data by Flint et al (11), who reported that GLP-1 infusions enhance satiety and fullness and reduce spontaneous energy intake during a test lunch by 12% (12). A meta-analysis confirmed that intravenous GLP-1 suppresses food intake in the same order, with a mean reduction in calorie intake of 11.7% (13). Oral PYY3-36 (1 mg) was associated with a 12% reduction in energy intake compared with placebo administration, although this effect was not statistically significant. Several studies document that high plasma PYY3-36 concentrations potently trigger a significant satiety effect, with a marked reduction in food intake (3, 14). Infusions in these experiments were, however, ongoing (>60 min) before the test meal and/or during the meal, which suggests that a prolonged secretion rather than a single stimulus is important for an effect. PYY3-36 has also been shown to reduce cumulative 24-h calorie intake; the peptide is suggested to be involved in the intermediate term...
regulation of food intake across several meals. However, we did not observe an effect on 24-h calorie intake under the present experimental conditions.

Peripheral GLP-1 and PYY3-36 partly exert their appetite-reducing effects through receptors expressed on vagal afferent nerve fibers in the splanchnic region (15, 16); the anorectic effect of both peptides is abolished by subdiaphragmatic vagotomy or vagal transection of hindbrain hypothalamic pathways (2, 17).

Our findings indirectly support this suggestion: we showed that several appetite variables changed, despite the short lasting stimulus. GLP-1 and PYY3-36 might therefore act on afferent

FIGURE 3. Mean (±SEM) appetite profiles for satiety (A), fullness (B), and hunger (C) in healthy male volunteers during an ad libitum test meal (n = 12). Data indicate changes from baseline on a visual analog scale (VAS). No significant differences in satiety, fullness, or hunger over 180 min (area under the concentration time curve at 0–180 min) were observed between groups. All comparisons were performed by using the nonparametric Friedman test. GLP-1, glucagon-like peptide 1; PYY3-36, peptide YY 3-36.

FIGURE 4. Satiety (A), fullness (B), and hunger (C) ratings depicted as mean (±SEM) changes from baseline (0 min) to 15 min (after oral dosing at meal onset) in healthy male volunteers during an ad libitum test meal (n = 12). *Significantly different from placebo, P ≤ 0.05. No significant differences were detected between single administration and coadministration. All comparisons were performed by using the nonparametric Friedman test. In case of significant differences, pairwise comparisons were performed by using the Wilcoxon’s signed-ranks test followed by Bonferroni correction to account for multiplicity of comparisons. GLP-1, glucagon-like peptide 1; PYY3-36, peptide YY 3-36; VAS, visual analog scale.
Pharmacokinetics of oral glucagon-like peptide 1 (GLP-1) or peptide YY 3-36 (PYY3-36)

**TABLE 2**

<table>
<thead>
<tr>
<th>GLP-1 (2 mg) plus PYY3-36 (1 mg)</th>
<th>GLP-1 (2 mg)</th>
<th>PYY3-36 (1 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC (pmol · min/L)</strong></td>
<td>402.9 ± 49.9 (423.8)</td>
<td>776.5 ± 154.4 (688.5)</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt; (pmol/L)</strong></td>
<td>6.2 ± 0.8 (6.4)</td>
<td>30.3 ± 5.6 (26.2)</td>
</tr>
<tr>
<td><strong>T&lt;sub&gt;max&lt;/sub&gt; (min)</strong></td>
<td>78.8 ± 11.4 (82.5)</td>
<td>18.8 ± 3.8 (15.0)</td>
</tr>
</tbody>
</table>

**PYY3-36**

| **AUC (pg · min/mL)** | 7405 ± 1273 (5692) | 16890 ± 2759 (13807) | 8131 ± 1350 (6367) |
| **C<sub>max</sub> (pg/mL)** | 175.0 ± 17.0 (179.5) | 398.0 ± 62.2 (407.0) | 186.6 ± 17.2 (166.5) |
| **T<sub>max</sub> (min)** | 110.1 ± 11.4 (105.0) | 21.8 ± 3.2 (15.0) | 106.3 ± 11.7 (90.0) |

1 All values are means ± SEMs; medians in parentheses. *n = 12*. AUC, area under the concentration time curve (with baseline adjustment); C<sub>max</sub>, maximum peak plasma concentration; T<sub>max</sub>, time to peak plasma concentration.

2 Significantly different from placebo, *P* ≤ 0.05 (general linear model of repeated-measures ANOVA with simple contrast and Bonferroni correction of *P* values for multiplicity of comparison).

**Interactions between GLP-1 and PYY3-36 with respect to food intake**

Because GLP-1 and PYY3-36 are cosecreted from intestinal L cells (5), it is likely that they act in concert to terminate meal consumption. Additive and synergistic interactions between different satiety mechanisms have been described for several peptides (22–25), although not all combinations have been shown to interact in this way. Whereas infusions of CCK-33 or GLP-1 inhibited food intake individually, no enhanced reduction was observed when they were coadministered (26). Our data are consistent with recent observations showing an additive inhibitory effect on feeding in healthy subjects when intravenous GLP-1 and PYY3-36 were coadministered (24). Here, we document a numerical additive reduction in energy consumption with combined oral administration of both peptides; however, this additive effect was not statistically significant.

A significant increase in fullness feelings occurred at meal onset (*t* = 15 min), when oral GLP-1 and PYY3-36 were given together, which suggests a synergistic effect for fullness feelings. However, analysis of appetite profiles over the 180-min course of the study showed no significant differences in satiety, fullness, or hunger between the groups. Suppression of plasma ghrelin concentrations could be a potential explanation for early fullness feelings. It has been suggested that the rapid weight loss after gastric bypass is due to a dramatic loss of appetite triggered by low plasma ghrelin concentrations. An inverse relation between circulating concentrations of ghrelin and GLP-1 and PYY3-36 has been described (27).

**Pharmacodynamic effects of oral peptide administration**

When GLP-1 was administered, glucose, insulin, and glucagon profiles were consistent with reported pharmacology, as shown recently with orally administered GLP-1 before an oral-glucose-tolerance test (9). The postprandial glucose peak was delayed with GLP-1, which suggests an effect on gastric emptying; the pharmacodynamic profile after PYY3-36 administration does,
however, not suggest a delay in gastric emptying. Coadministration resulted in an increase in postprandial glucose concentrations, although insulin concentrations were not reduced. Sloth et al. (28) documented increased postprandial glucose and free fatty acid (FFA) responses after high doses of PYY3-36 infusions; however, mechanisms behind this effect remains elusive. Further research is needed to clarify the role of PYY3-36 in glucose homeostasis.

Therapeutic application of oral peptide delivery

The food intake reducing actions of GLP-1 and PYY3-36 are of great clinical interest, because the inhibitory effects are not only seen in healthy persons, but are preserved in obese and/or diabetic subjects. Several research groups and medical companies are therefore trying to convert GLP-1 and/or PYY3-36 into useful drugs. For GLP-1, long-acting analogs are already clinically available (exenatide and liraglutide) for the treatment of type 2 diabetes mellitus; interestingly subcutaneous injections of exenatide to patients with type 2 diabetes are associated with a gradual and linear weight loss and no signs of impaired efficacy with time (29). We showed that orally administered GLP-1 (alone or together with PYY3-36) leads to a reduction in calorie intake together with premeal increase in insulin secretion. Therefore, oral administration of both peptides might offer an attractive therapy for obese patients and patients with type 2 diabetes.

Study limitations

We used native peptides, the short half-life of which was a major hurdle. Also, the bioavailability of both peptides can be problematic when given with meals, as was recently shown with an orally available ghrelin agonist (30). The present study was a proof-of-concept trial with a limited number of subjects, not designed to meet the criteria for long-term clinical trials. Rather, it was focused on whether and to what extent native GLP-1 and PYY exert their inhibitory effect on food intake when administered orally. The results suggest that GLP-1 and PYY inhibit food intake additively with marked effects on glucose homeostasis.

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The authors’ responsibilities were as follows—RES, MCC, and CB: designed the research; BP: prepared the tablets; RES: conducted the research; RES and JD: performed the statistical analysis; RES and CB: wrote the manuscript; and CB: had primary responsibility for the final content. All authors read and approved the final manuscript. MCC is an employee of Emisphere Technologies, Cedar Knolls, NJ (Emisphere Technologies is a biopharmaceutical company that has developed a broad-based proprietary drug delivery platform called the Eligen Technology.) None of the other authors reported a conflict of interest.

REFERENCES