Effects of PYY$_{3–36}$ and GLP-1 on energy intake, energy expenditure, and appetite in overweight men

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Schmidt JB, Gregersen NT, Pedersen SD, Arentoft JL, Ritz C, Schwartz TW, Holst JJ, Astrup A, Sjödin A. Effects of PYY$_{3–36}$ and GLP-1 on energy intake, energy expenditure, and appetite in overweight men. Am J Physiol Endocrinol Metab 306: E1248–E1256, 2014. First published April 15, 2014; doi:10.1152/ajpendo.00569.2013.—Our aim was to examine the effects of GLP-1 and PYY$_{3–36}$, separately and in combination, on energy intake, energy expenditure, appetite sensations, glucose and fat metabolism, ghrelin, and vital signs in healthy overweight men. Twenty-five healthy male subjects participated in this randomized, double-blinded, placebo-controlled, four-arm crossover study (BMI 29 ± 3 kg/m², age 33 ± 9 yr). On separate days they received a 150-min intravenous infusion of 1) 0.8 pmol·kg$^{-1}$·min$^{-1}$ PYY$_{3–36}$, 2) 1.0 pmol·kg$^{-1}$·min$^{-1}$ GLP-1, 3) GLP-1 + PYY$_{3–36}$, or 4) placebo. Abdominal energy intake was assessed during the final 30 min. Measurements of appetite sensations, energy expenditure and fat oxidation, vital signs, and blood variables were collected throughout the infusion period. No effect on energy intake was found after monoinfusions of PYY$_{3–36}$ (–4.2 ± 4.8%, P = 0.8) or GLP-1 (–3.0 ± 4.5%, P = 0.9). However, the coinfusion reduced energy intake compared with placebo (–30.4 ± 6.5%, P < 0.0001) and more than the sum of the monoinfusions (P < 0.001), demonstrating a synergistic effect. Coinfusion slightly increased sensation of nausea (P < 0.05), but this effect could not explain the effect on energy intake. A decrease in plasma ghrelin was found after all treatments compared with placebo (all P < 0.05); however, infusions of GLP-1 + PYY$_{3–36}$ resulted in an additional decrease compared with the monoinfusions (both P < 0.01). We conclude that coinfusion of GLP-1 and PYY$_{3–36}$ exerted a synergistic effect on energy intake. The satiating effect of the meal was enhanced by GLP-1 and PYY$_{3–36}$ in combination compared with placebo. Co infusion was accompanied by slightly increased nausea and a decrease in plasma ghrelin, but neither of these factors could explain the reduction in energy intake.

PYY; GLP-1; appetite; energy intake; energy expenditure

BARIATRIC SURGERY, particularly Roux-en-Y gastric bypass (RYGB), is currently the most effective treatment for severe obesity (2, 36, 37). Since malabsorption resulting from bypassing the duodenum and proximal jejunum has failed to explain the postsurgical weight loss (30), and because it remains controversial to what extent limitation of ingestive capacity by reduction of stomach size affects food intake (8, 29), focus has been drawn to specific gastrointestinal (GI) hormones secreted postprandially at considerably higher rates following RYGB (20, 22–25, 32). As recently reviewed, studies have shown that the short-term administration of peptide YY (PYY)$_{3–36}$ and glucagon-like peptide-1 (GLP-1) separately leads to a reduction in energy intake by mechanisms involving changes in central regulation of appetite (12). Delays in gastric emptying rates have also been proposed to play a role, but this is controversial (12, 41). As GLP-1 and PYY are cosecreted from intestinal L-cells upon feeding, there is great interest in investigating the satiating effect of these hormones in combination, with the goal being to more effectively mimic the physiology of the fed state.

In the two human studies where the effects of coinfusion of PYY$_{3–36}$ and GLP-1 were investigated, additive inhibitory effects on energy intake were reported compared with being given separately (13, 28). These findings were supported by reports of increased sensations of fullness and reduction in hunger following the combined administration of PYY$_{3–36}$ and GLP-1 but not after single administrations (13). In mice, coadministration of Ex-4, a long-acting GLP-1 analog, and PYY$_{3–36}$ decreased energy intake in a synergistic manner (i.e., an effect that was greater than the sum of their individual effects) (40), but synergistic effects have not been demonstrated in human studies. In addition, both PYY and GLP-1 have been suggested to affect hedonic regulation of energy intake (27), and an additive effect of GLP-1 and PYY$_{3–36}$ on activity in brain regions associated with reward-based eating has been reported (13).

The mechanisms behind potential synergistic effects are unknown, but nausea has been suspected to be involved, since nausea is a common side effect of supraphysiological plasma levels of both GLP-1 and PYY$_{3–36}$ (15, 38). However, the role of nausea in studies reporting a decrease in energy intake and appetite is not clear. If the anorexigenic effect of GLP-1 and PYY$_{3–36}$ is potentiated by coadministration, this may allow the use of lower doses, thereby avoiding excess nausea without simultaneously attenuating the wanted inhibitory effect on food intake.

The effect of GLP-1 and PYY$_{3–36}$ on caloric intake may also involve mediation through other hormones. Thus, studies have reported decreased levels of the orexigenic hormone ghrelin following administration of PYY$_{3–36}$ (5, 14) and a trend toward a greater effect when orally administered in combination with GLP-1 (39). These hormones may also be involved in the suppression of ghrelin reported after RYGB (11).

GLP-1 and PYY$_{3–36}$ have also been reported to affect energy metabolism. Thus, infusions of PYY$_{3–36}$ alone have been shown to increase energy expenditure and increase fat oxidation (38).
Whereas GLP-1-mediated insulin secretion is supported by a substantial body of literature (21), both negative and no effects of PYY$_{3-36}$ on insulin secretion have been reported (5, 42). To date, no studies have provided evidence supporting the suggestion that the effect of GLP-1 on glucose regulation is enhanced when given in combination with PYY$_{3-36}$.

In this randomized, controlled crossover study, we examined the acute effects of intravenous infusions of PYY$_{3-36}$ and GLP-1, as monoinfusions and in combination, on ad libitum intake in healthy overweight men. Second, we investigated the effect on subjective appetite sensation, nausea, energy expenditure, respiratory quotient (RQ), vital signs, and levels of ghrelin, insulin, glucose, and free fatty acids (FFA).

**METHODS**

**Study population.** Twenty-five moderately overweight (BMI $\geq 25$ kg/m$^2$), healthy Caucasian men, aged 18–50 yr, were included. Exclusion criteria were diabetes mellitus, weight changes of $>3$ kg within 2 mo prior to screening, hemoglobin concentration $<7.6$ mmol/l, a known monogenic or hypothalamic cause of obesity, cancer diagnosis, earlier incidence of thyroid dysfunction, or the use of thyroid medication. Individuals with mental illness, following special diets, smoking, substance abuse, use of prescription drugs, alcohol intake $>21$ drinks per week, high level of physical activity (structured activities $>10$ h/wk), or lack of ability or will to adhere to the protocol were also excluded.

The study was approved by the Municipal Ethics Committee of Copenhagen/Scientific Ethics Committee of the Capital Regions of Denmark (Journal no. H-1-2009-083) and carried out in accordance with the Helsinki II Declaration/Declaration of Helsinki and by the Danish Data Protection Agency (Journal no. 2007-54-0296). The study followed the guidelines of GCP and was registered at Clinical Trials (ID no. NCT00940134).

**Study design.** The study was a randomized, double-blinded four-arm crossover design. Subjects attended the Department of Nutrition, Exercise and Sports on four separate test days and received a 150-min intravenous infusion of 1) GLP-1, 2) PYY$_{3-36}$, 3) GLP-1 + PYY$_{3-36}$ or, 4) placebo. A washout period of at least 3 wk between each test day was included to minimize the risk of any carry-over effect and to ensure that the subjects regained the amount of blood taken on previous test days (Fig. 1).

**Infusions.** On the basis of a previous study (28) and a pilot study with six subjects, we aimed at doses giving a small effect of single infusions on energy intake, thereby allowing the potential to explore additive and synergistic effects of the coinfusion. We furthermore aimed at finding doses that could be given without unacceptable side effects in the form of nausea. Doses of 0.5 pmol·kg$^{-1}$·min$^{-1}$ GLP-1$_{1-36}$ amide and 0.4 pmol·kg$^{-1}$·min$^{-1}$ PYY$_{3-36}$ for the first 45 min, and 1.0 pmol·kg$^{-1}$·min$^{-1}$ GLP-1$_{1-36}$ amide and 0.8 pmol·kg$^{-1}$·min$^{-1}$ PYY$_{3-36}$ for the remaining 105 min were chosen. Synthetic GLP-1$_{1-7-36}$ amide and PYY$_{3-36}$ from Polypeptides (Wolfenbüttel, Germany) with a purity of $>97\%$ were used. Purity and structure were estimated by HPLC analysis, mass spectrometry, and sequence analysis. PYY$_{3-36}$ and GLP-1$_{1-7-36}$ amide, alone or in combination, were dissolved in saline with 2% human albumin to limit adhesion to infusion material, and the solutions were distributed in glass tubes. The solutions and placebo (saline and albumin only) were prepared according to Good Manufacturing Practice (GMP) and tested for sterility and pyrogenic content at the Capital Region Pharmacy (Herlev, Denmark). Treatments were given in random order, and both the subjects and the researchers involved with the subjects were blinded to the treatments.

**Prescreening.** Subjects were recruited from advertisements, and those contacting us underwent prescreening over the phone. If the subjects met the inclusion criteria and were interested in participating, they were invited to a screening visit.

**Screening visit.** Subjects received full written and verbal information about the study, and informed consent was obtained. Demographic information, medical history, and medication use were collected. A clinical examination was performed, and measurements of body weight, height, waist circumference, blood pressure (BP), heart rate (HR), body temperature, and hemoglobin concentration were collected.

**Test days (visits 1–4).** On each test day, the subject arrived at 7:30 AM after an overnight fast. To limit fluctuations in glycogen stores and energy balance, subjects received a standardized dinner and were instructed to refrain from alcohol and strenuous physical activities on the day prior to the test days. Anthropometric measurements and body temperature were recorded. Intravenous catheters were inserted into an antecubital vein in each arm, one for hormone infusion and the other for sampling of blood. The first blood sample was drawn 65 min prior to the start of infusion, and measurements of energy expenditure were initiated (baseline) (Fig. 1). Five minutes prior to start of the infusion, the remaining baseline measurements were collected and included BP, HR, and subjective appetite sensations and taste preferences, and baseline blood samples were drawn. Infusion began at time 0 and continued for a total of 150 min. Every 30 min, the same measurements as those collected at baseline were repeated. After 120 min, an ad libitum meal was served. Thirty minutes after initiation of the meal, a final evaluation of appetite was completed, and the last blood samples were drawn before the infusion was stopped, ending the test day.

**Anthropometric measurements.** Body weight was registered while the subjects were fasting, using an electronic scale with an accuracy to 0.1 kg. Waist circumference was measured twice to the nearest 0.5 cm at the midpoint between the lower rib margin and iliac crest, and

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**Fig. 1.** Overview of a test day. Infusion of glucagon-like peptide-1 (GLP-1), peptide YY (PYY)$_{3-36}$, GLP-1 + PYY$_{3-36}$, or placebo was initiated at time 0 and continued for 150 min while the ad libitum meal was served at 120 min. Baseline blood samples (droplet) and measurements of blood pressure (BP), heart rate (HR; $\heartsuit$), and subjective appetite sensation by visual analog scales (VAS) were collected at $-5$ min (baseline), during infusion in the fasted state (at 25, 55, 85, and 115 min) and postprandially (at 145 min). Energy expenditure was measured every 30 min for 20–25 min starting at $-60$ min (baseline) until the meal was served.
the average between the two measures was used. Height was measured to the nearest 0.5 cm. Bioelectrical impedance was used to estimate body composition.

Ad libitum meal. To measure spontaneous energy intake in an experimental setting an ad libitum meal was used. The meal was semihomogenized spaghetti Bolognese (11,536 kJ/1,200 g) consisting of 15 E% protein, 30 E% fat, and 55 E% carbohydrate, with 0.5 liter of water on the side. The subjects were instructed to finish the meal within half an hour and eat at a constant pace until they felt comfortably satiated.

Appetite sensation and taste preferences. Visual analog scales (VAS) of 100 mm were filled out at times −5, 25, 55, 85, 115, and 145 min (Fig. 1). Subjects were asked questions assessing hunger, satiety, fullness, prospective food intake, nausea, and desire to eat sweet and fatty foods. A mean appetite score, i.e., composite score, for each time point was calculated by the equation: composite score = {satiety (mm) + fullness (mm) + [100 – prospective food intake (mm)] + [100 – hunger (mm)]}/4. To determine the extent to which the food eaten during the eating episode reduced subjective appetite, an appetite quotient (AQ) was calculated for the same four appetite parameters by the equation: AQ = [rating pre-eating episode (mm) – rating post-eating episode(mm)]/energy intake of eating episode (MJ) (18).

Energy expenditure and substrate oxidation. Indirect respiratory calorimetry was carried out using a ventilated hood system to estimate energy expenditure and RQ. At each test day, the subjects took part in six respiratory measurements, each of 20 min duration, while lying awake but relaxed in a supine position; data from the last 15 min were used in the calculations. Energy expenditure and RQ were calculated by following the equations, assuming a fixed nitrogen excretion of 15 g/day: energy expenditure total (kJ) = [O2 (l) × 15,913] + [CO2 (l) × 5,207] – [urine nitrogen (g) × 4,646]; RQ = CO2 (l) – [urine nitrogen (g) × 4,97]/O2 (l) – [urine nitrogen (g) × 5,95] (16).

BP and HR. BP and HR were measured using an electric sphygmomanometer according to standardized procedures every 30 min after the subjects had been resting for at least 5 min (Fig. 1).

Biochemical measurements. Seven blood samples were collected during each test day, where the first blood sample (at −65 min) was used exclusively to determine hemoglobin concentration (Fig. 1). Blood samples were drawn into different tubes (fluoride tubes for glucose, chilled dry tubes for insulin, chilled EDTA tubes for active GLP-1, PYY3–36, total ghrelin, and FFA). Samples in dry tubes were left to coagulate for 30 min before being centrifuged, whereas the remaining samples were immediately cooled on ice and centrifuged at 4°C. All blood samples were frozen at −80°C until analyzed. Samples were analyzed for concentrations of glucose [enzymatic assay (APX Pentra Glucose HK CP)], insulin [solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite 1000 Insulin Kit, LKIN1)], total ghrelin [enzyme-linked immunosorbent assay (Millipore)], FFA [ABX Pentra 400 (Horiba ABX, Montpellier, France)], total GLP-1 [radioimmunoassay (antiserum no. 89390)](31), PYY3–36 [commercially available radioimmunoassay kit (Linco Research)].

Statistical analysis. All statistical analyses were carried out in R version 2.14.0 [www.r-project.org (1)]. Figures were produced using GraphPad Prism 5. Based on previous studies (28), a power analysis showed that the use of 16 subjects was sufficient to detect differences in ad libitum intake following placebo, PYY3–36, GLP-1, and PYY3–36 + GLP-1 (α = 0.05, β = 0.8). Due to previous findings of nausea with higher doses of PYY3–36, thereby increasing the risk of subjects dropping out (38), we decided to enroll 25 subjects.

Descriptive data are presented as means ± SD.

Ad libitum intake was analyzed in a linear mixed model with treatment, treatment order (to adjust for carryover effects), and body weight as fixed effects and with subject-specific random effects (to adjust for random variation between subjects).

Outcomes measured repeatedly at each visit (VAS scores and all biochemical parameters) were analyzed by means of a linear mixed model with a time × treatment interaction, treatment order, body weight, and baseline measurements (at −5 min) as fixed effects and with subject-specific random effects. Additionally, for energy expenditure, hood-specific random effects were used to capture differences between hoods. Postprandial measures (at 145 min) were analyzed separately using a linear mixed model similar to the one used for ad libitum intake.

If a treatment effect or a time × treatment interaction (termed overall effect) was found, pairwise comparisons between active treatments (GLP-1, PYY3–36, and GLP-1 + PYY3–36) and placebo were always performed, using post hoc model-based t-tests. Additionally, placebo-adjusted pairwise comparisons between active treatments were carried out for ad libitum intake, glucose, insulin, and ghrelin. For ad libitum intake, the mean reduction in intake (%) for the active treatments was estimated.

To explore mechanisms of potential effects of the interventions, supplementary analyses were carried out for ad libitum intake where ghrelin and nausea were included both separately and simultaneously as additional (fixed-effects) covariates. Figures are based on raw data, presented as means ± SE, whereas stated conclusions are based on the fitted models. P values of <0.05 were considered significant, and trends were defined as 0.05 < P < 0.01.

RESULTS

Subjects. Twenty-five subjects aged 33 ± 9 yr with a BMI of 29 ± 3 kg/m2, a body fat percentage of 28 ± 4%, and a waist circumference of 99 ± 10 cm were included. During the trial, no changes were found in these measurements. One subject only attended one test day with placebo (started smoking) and one attended two test days with PYY3–36 + GLP-1 (moved away). All available data were included in the linear mixed-model analyses, which provide unbiased estimation if missing observations are missing at random.

GLP-1 and PYY3–36. Infusions of GLP-1 and GLP-1 + PYY3–36 increased plasma levels of GLP-1 (time × treatment: both P < 0.0001), to peak concentration: 60 ± 13 and 64 ± 13 pmol/l, respectively (Fig. 2), and similar increases in plasma levels of PYY3–36 were found after infusion of PYY3–36 and GLP-1 + PYY3–36 (time × treatment: both P < 0.0001; peak concentration: 53 ± 19 and 55 ± 20 pmol/l, respectively).
found (both \( P < 0.05 \)), indicating that the insulin-enhancing effect is mediated by GLP-1 and not PYY\(_{3-36} \). Subsequently, plasma levels of glucose were lower following both infusions with GLP-1 at all time points during fasting (time \( \times \) treatment: \( P < 0.0001 \); pairwise comparisons: both \( P < 0.01 \)). When the active treatments were compared, only differences between PYY\(_{3-36} \) and treatments with GLP-1 were found (both \( P < 0.05 \)).

**Ghrelin.** A time \( \times \) treatment effect was found for plasma levels of ghrelin (\( P < 0.0001 \); Fig. 8). Pairwise comparisons showed that all active infusions decreased ghrelin levels compared with placebo from 90 min into the infusions (all \( P < 0.05 \)). A further reduction in ghrelin levels was found 120 min into the coinfusion compared with after monoinfusions (both \( P < 0.01 \)). However, ghrelin was found to explain less than 1\% of the decrease in ad libitum intake. When nausea was included in the model, in combination they still explained less than 1\% of the variation in energy intake, tentatively indicating that GLP-1 and PYY\(_{3-36} \) predominantly inhibit food intake through nausea- and ghrelin-independent mechanisms.

**Vital signs.** Confusion increased HR by 4 ± 7 beats/min (7\%) compared with placebo at 120 min (\( P < 0.01 \)), but monoinfusions did not affect HR (all \( P > 0.1 \)). No difference was found for systolic or diastolic BP (both \( P > 0.5 \)).

**Postprandial measurements.** After the ad libitum meal, a treatment effect was still present for nausea (\( P < 0.01 \); Fig. 5), insulin (\( P < 0.0001 \); Fig. 6), glucose (\( P < 0.0001 \); Fig. 7), and ghrelin (\( P < 0.01 \); Fig. 8). Additionally, a treatment effect was found for HR (\( P < 0.01 \)) by an increase after PYY\(_{3-36} \) infusion compared with placebo (\( P < 0.01 \)). AQs for prospective food intake and satiety were affected by the treatments (\( P < 0.05 \) and \( P < 0.01 \), respectively), in both cases resulting from a decrease in prospective food intake and increased satiety following coinfusion compared with placebo (both \( P < 0.05 \)). Near-significant treatment effects were found on AQ also for hunger (\( P = 0.07 \)) and fullness (\( P = 0.08 \)).

**DISCUSSION**

**Ad libitum intake.** In line with previous studies (13, 28, 39), we found that GLP-1 and PYY\(_{3-36} \) decreased energy intake...
more when administered together than when given as monoinfusions. However, for the first time we are able to show that this inhibitory effect on energy intake following coinfusion is larger than the summed effect of the single infusions, demonstrating a synergistic effect. As expected, monoinfusions only numerically reduced energy intake. These data support what has previously been shown in mice, with a significantly larger decrease in energy intake following combined systemic administration (31–67% reduction) compared with when Ex-4 and PYY3–36 were given separately (40). In a human study, Neary et al. (28) found a 27% reduction in ad libitum intake after coinfusion of GLP-1 and PYY3–36 compared with placebo, and no significant effect when GLP-1 and PYY3–36 were given as monoinfusions, suggesting a synergistic effect similar to our findings. In agreement, another study, from the same center, reported a 27% reduction in ad libitum intake after coinfusion of GLP-1 and PYY3–36 compared with placebo but no effect following monoinfusions (13). The findings of only additive effects of coadministration of GLP-1 and PYY3–36 on energy intake in these studies is most likely a result of inclusion of too few subjects (type II error), as they included only 10–16 subjects. Infusion doses in the studies by Neary et al. and De Silva et al. (12) were lower than those used in the present study (0.4 pmol·kg⁻¹·min⁻¹ GLP-1 and PYY3–36, 0.3 pmol·kg⁻¹·min⁻¹ PYY3–36, and 0.8 pmol·kg⁻¹·min⁻¹ GLP-1, respectively). However, this does not explain the larger effect of coinfusion on energy intake in the present study, since plasma levels of PYY3–36 in these studies were higher (peak value: >230 pg/ml after administration of PYY3–36 and GLP-1 + PYY3–36); likewise, levels of total GLP-1 were reported to be higher (peak value: >61 pmol/l) by Neary et al. Different assays used to measure GLP-1 and PYY3–36 concentrations may explain these discrepancies.

**Subjective appetite sensation.** The reduction in ad libitum intake was not reflected in effects on subjective rating of appetite in the fasting state. However, calculated AQ showed that the ad libitum meal decreased prospective food intake and increased satiety more when subjects received GLP-1 + PYY3–36 compared with placebo. Collectively, these data and the finding of a trend in AQ toward decreased hunger and increased sensation of fullness following coinfusion suggest a generally enhanced decrease in motivation to eat induced by a meal, beyond what can be explained by differences in energy intake itself. Lack of correlation between ad libitum intake and

### Table 1. *Ad libitum intake following infusions of GLP-1, PYY3–36, GLP-1 + PYY3–36, or placebo*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ad libitum intake, kJ</th>
<th>Δ energy intake, kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1</td>
<td>3,869.9 ± 224.9</td>
<td>GLP-1 + PYY3–36</td>
</tr>
<tr>
<td>PYY3–36</td>
<td>3,687.2 ± 312.1</td>
<td>GLP-1 + PYY3–36</td>
</tr>
<tr>
<td>GLP-1 + PYY3–36</td>
<td>2,843.0 ± 338.5</td>
<td>Placebo</td>
</tr>
<tr>
<td>Placebo</td>
<td>4,002.4 ± 300.7</td>
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Data are presented as means ± SE.

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**Fig. 5.** Subjective rating of nausea following infusion of (gray square), PYY3–36 (●), GLP-1 + PYY3–36 (♦), and placebo (○). All data are presented as means ± SE. At fasting (0–120 min), a time × treatment effect was found (P = 0.03). A treatment effect was also found postprandially (P < 0.01). Pairwise comparisons of active treatments vs. placebo: f highlights a difference (P < 0.01) between placebo and GLP-1 + PYY3–36.

**Fig. 6.** A: plasma levels of insulin following infusion of (gray square), PYY3–36 (●), GLP-1 + PYY3–36 (♦), and placebo (○). Data are presented as means ± SE. At fasting (0–120 min) and postprandially, a time × treatment/treatment effect was found (both P < 0.0001). Pairwise comparisons of treatments: g highlights a difference (P < 0.001) between placebo and GLP-1 + PYY3–36, m/o highlights a difference (P < 0.05/P < 0.001) between PYY3–36 and GLP-1, r highlights a difference (P < 0.001) between PYY3–36 and GLP-1 + PYY3–36, and u highlights a difference (P < 0.01) between GLP-1 and GLP-1 + PYY3–36. B: placebo subtracted area under the curve (AUC) values at the preprandial state (pmol/l × 120 min) are illustrated with bars of means ± SE for GLP-1 (gray), PYY3–36 (filled), and GLP-1 + PYY3–36 (open).
subjective premeal appetite feeling after GLP-1 administration has previously been addressed in a meta-analysis hypothesizing that large variations in energy intake might be the explanation (41). The same meta-analysis, however, concluded that GLP-1 reduced subjective ratings of motivation to eat, and similar results have been found after the administration of PYY3-36 (12). Measures of subjective ratings of appetite were also collected in two studies of coadministration and reported increased sensations of fullness and reduction in hunger following combined administration of PYY3-36 and GLP-1 but not after single administration (13, 39).

Recently, GLP-1 and PYY3-36 have also been suggested to be involved in hedonic regulation of food intake (6, 27). De Silva et al. (12) investigated the effect of both GLP-1 and PYY3-36 on brain regions implicated in food reward and found the summed reduction in blood oxygen level-dependent (BOLD) response following GLP-1 and PYY3-36 comparable to the reduction seen after infusion of the two hormones in combination, suggesting an additive effect. The fact that we found no effect on preferences for sweet and fatty foods, together with only a weak effect of subjective appetite sensations, may reflect the challenges associated with investigating complex behaviors, such as eating behavior, in experimental settings.

Side effects. Coadministration of GLP-1 and PYY3-36 was accompanied by an increase in nausea, which has been reported as a common side effect of PYY in some studies (14, 38) but not all (26), as well as following treatment with GLP-1 analogs (19, 43). Nausea is therefore a likely confounder for any effect found on energy intake and/or appetite. Neary et al. found no differences in nausea scores (28), but two of twelve subjects in the study by Steiner et al. experienced nausea, abdominal discomfort, and/or vomiting following coadministration, but not when GLP-1 and PYY3-36 were given separately (39). A delay of gastric emptying, which is known to cause nausea, is found acutely by GLP-1 administration in humans but most likely does not explain GLP-1-mediated reduction in energy intake (41). In mice treated with a combination of Ex-4 and PYY3-36, the reduction in gastric emptying was greater when animals received the hormones in combination. Those authors suggested that the inhibitory effect on energy intake was partly attributed to delayed gastric emptying but was not associated with significant behavioral changes associated with side effects (40). In agreement, including nausea as an explanatory covariate in the model for energy intake in the present study did not alter the significant effect on energy intake, supporting the hypothesis that the mechanisms behind the inhibitory effect on energy intake are not nausea-dependent. Likewise, De Silva et al. (13) found no correlation between nausea and energy intake. We cannot, however, exclude the possibility that nausea might alter eating behavior in the free-living situation, potentially by affecting food choices and meal frequency.

None of the infusions affected BP, but a 4 beats/min increase in HR was found after coinfusion compared with placebo. Similar effects have previously been reported after liraglutide.
treatment (a GLP-1 analog) (3), as well as following PYY3–36 given at comparable doses (38).

Ghrelin. Compared with placebo, all active infusions decreased ghrelin levels from 90 min until the infusion ended. Interestingly, 120 min into the infusion, a further reduction was found after coinfusion compared with both monoinfusions. Consistent with these findings, a PYY3–36-mediated suppression of ghrelin at fasting has been shown in previous studies (5, 6, 14), with two of these studies using doses of PYY3–36 that were comparable to those used in the present study. During the infusion of GLP-1 at doses of 0.9 pmol·kg\(^{-1}\)·min\(^{-1}\), Brennan et al. (9) also found a near-significant suppression of ghrelin. However, since only nine subjects were included in that study, only near-significant effects may reflect a type II error.

A reciprocal relationship between ghrelin and insulin has previously been shown (10). However, since the insulin response following monoinfusion is comparable to that of subjects receiving GLP-1 + PYY3–36, the further suppression of ghrelin following coinfusion must be ascribed to other mechanisms as well. Ghrelin-producing cells may also respond to plasma glucose concentrations (35), but we found no difference in plasma glucose when subjects received GLP-1 alone or coinusions, suggesting a smaller role for glucose in the observed ghrelin suppression. Although plasma ghrelin decreased by more than 15% after coinfusion compared with placebo, our analysis did not point toward an important role for ghrelin in the reduction in energy intake since it explained less than 1% of the variation. However, since ghrelin is known to be an important meal-initiator (10), its role in eating behavior might have been neglected by our study design, which primarily focused on termination of the meal/meal size. We cannot explain why ghrelin levels suddenly return to baseline levels after the ad libitum meal is finished, and these findings are in conflict with existing literature (34). In accord, Batterham et al. (5) found that concentrations of ghrelin decreased following PYY infusions, and a further reduction was observed after an ad libitum lunch (5).

Abnormally lower fasting plasma levels of ghrelin after RYGB have been suggested to play a role in a decreased motivation to eat reported after this procedure, but the mechanisms responsible for this are not known (11). Our data suggest that the lower levels of ghrelin after RYGB might be secondary to increased secretion of GLP-1 and PYY3–36. However, postoperative ghrelin levels normalize over time (17, 32). In addition, effects of RYGB on GLP-1 and PYY at a fasting state can be questioned (20), speaking for other mechanisms than an increase in GLP-1 and PYY involved in RYGB-mediated ghrelin suppression.

Insulin, glucose, and FFA. We found that infusions of GLP-1 and GLP-1 + PYY3–36 increased plasma insulin and decreased glucose concentrations. This GLP-1-induced insulin secretion is consistent with the existing literature (21). Our results, as well as those of others (28, 39), found no additional effect on plasma glucose or insulin secretion when GLP-1 was given in combination with PYY3–36, suggesting that PYY3–36 does not interfere with GLP-1-mediated insulin secretion. Although strong inhibitory effects of PYY alone on fasting plasma insulin and on glucose-stimulated insulin secretion have been demonstrated in PYY-deficient mice (42), no human studies have provided evidence for similar effects of PYY on fasting insulin and glucose concentrations. Thus, Batterham et al. found no alterations in fasting plasma insulin and glucose at plasma levels of PYY3–36 equivalent to those normally seen postprandially (6), and comparable results were reported by Sloth et al. at supraphysiological plasma levels (38). Inconsistent with our findings, the latter study also found that PYY3–36 increased FFA concentrations at fasting. Since the interpretation of post-prandial values of insulin, glucose, and FFA are complicated due to substantial variations in energy intake in the present study, no definitive conclusion will be drawn from these.

Energy metabolism. A stimulating effect of PYY3–36 on energy expenditure in combination with a decrease in RQ, indicating increased fat oxidation, was found by Sloth et al. (38), which is in contrast to the present study. The substantially higher plasma levels (peak levels: 770 ± 110 ng/ml) compared with the present study, and the use of different assays, may explain why an increase was not observed in this study.

Perspectives. Infusions resulted in at least a fourfold increase in plasma levels of PYY3–36 and GLP-1 compared with placebo, and treatment-induced plasma levels were somewhat comparable to those seen after RYGB (23). Although we found a substantial effect on energy intake, the effects of intravenously administered GLP-1 and PYY3–36 may not mimic the physiological appetite-suppressing action of endogenous GLP-1 and PYY3–36 (33). Moreover, alterations in secretion of several other hormones have been reported after RYGB, including ghrelin, leptin, adiponectin, and insulin (20), and these may together or in combination also contribute to the pronounced effects on appetite and glucose homeostasis found after this procedure. Such interactions between appetite hormones resulting in a greater inhibition of energy intake when administrated together have been demonstrated in animal models (4, 7). In agreement, our data support the suggestion that the total gut satiety response is mediated by actions of several hormones acting in an integrated and synergistic fashion. Understanding these complex interplays provides important knowledge for the development of pharmacological treatments mimicking the physiological fed state without the need for bariatric surgery and without causing intolerable side effects.

Conclusion. Coinfusion of GLP-1 and PYY3–36 decreased ad libitum food intake synergistically compared with monoinfusions in healthy overweight men. Compared with placebo, the ad libitum meal decreased motivation to eat more when subjects received GLP-1 + PYY3–36, but no effect was found on measures of appetite in the fasted state. Furthermore, we did not find effects on taste preferences or energy metabolism. Coinfusion was accompanied by increased nausea and a decrease in plasma ghrelin; however, none of these factors explained the effect on energy intake, indicating that GLP-1 and PYY3–36 predominantly inhibit food intake though other mechanisms.

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