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The effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects.

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Short running head: PYY, energy balance, glucose and fat metabolism

Abstract

Background: Peptide YY (PYY) 3-36 has been shown to produce dramatic reductions in energy intake (EI), but no human data exist regarding energy expenditure (EE), glucose and fat metabolism. Nothing is known regarding PYY1-36.

Objective: To compare effects of PYY1-36 and PYY3-36 on appetite, EI, EE, insulin, glucose and free fatty acids (FFA) concentrations.

Design: Twelve lean and 12 obese males participated in a blinded, randomised, crossover study with 90 min infusions of saline, 0.8 pmol/kg/min PYY1-36 and PYY3-36. Only 4 participants completed PYY3-36 infusions because of nausea. Subsequently 6 lean and 8 obese participants completed 0.2 pmol/kg/min PYY3-36 and 1.6 pmol/kg/min PYY1-36 infusions.

Results: 0.8 pmol/kg/min PYY3-36 produced reduced EI, lower ratings of well-being, increases in FFA, postprandial glucose (only 0.8 pmol/kg/min PYY3-36) and insulin concentrations, as well as heart rate and EE (only 0.8 pmol/kg/min PYY3-36). 1.6 pmol/kg/min PYY1-36 produced increased heart rate and postprandial insulin response. Ratings of appetite were opposite with infusions of 0.8 pmol/kg/min and 1.6 pmol/kg/min PYY1-36, and seemed to depend on subjects being lean or obese.

Conclusion: PYY3-36 caused increased thermogenesis, lipolysis, postprandial insulin and glucose responses, suggestive of increased sympathoadrenal activity. PYY1-36 had no effect on EI and no clear effects on appetite, but resulted in increased heart rate and postprandial insulin response. However, highest tolerable dose of PYY1-36 was probably not reached in the present study.

Keywords: Peptide YY, free fatty acids, insulin sensitivity, arcuate nucleus, NPY receptors.

Introduction

The imbalance between EI and EE that produces the obese state may partly be a consequence of an inadequate appetite regulation. Hence scientific interest in recent years has concentrated on both the secretion and function of different gastrointestinal hormones, and on the appetite regulatory centers of the brain, including the arcuate nucleus which is considered a key brain area (22).

PYY is a 36-amino acid peptide released postprandially from the endocrine L-cells in the distal gastrointestinal tract in proportion to the calorie and fat content of the ingested meal (1; 8; 18). PYY is part of the neuropeptide Y (NPY) protein family and exists in human blood in two forms: the intact PYY1-36, and PYY3-36, in which the N-terminal Tyr-Pro dipeptide has been removed by dipeptidyl aminopeptidase IV (DPP IV) cleavage (12; 13). PYY1-36 binds to and activates the Y1, Y2 and Y5 NPY receptor subtypes, whereas PYY3-36 preferentially binds to the inhibitory presynaptic Y2 receptor, which is highly expressed in NPY neurons in the appetite regulatory centre in the arcuate nucleus (27).

Infusion of PYY3-36 at doses producing supraphysiologic plasma concentrations has been shown to result in a dose dependent decrease in subsequent food intake (8; 18). The highest suppression of food intake has been demonstrated with doses of 0.8 pmol/kg/min and amounted to a decrease of between 25% and 34%, with the highest reduction in lean subjects (4; 5; 8; 18) and a somewhat lower reduction in obese subjects (4). The differences in EI were observed either during the subsequent *ad libitum* meal or during the first 12 hours after the infusion. In the context of appetite suppressant effect in humans the quantitative effect of PYY is of a magnitude not previously reported in connection with anorectic drugs (sibutramine etc.), and PYY receptors therefore constitute a very promising drug target for obesity management. Stimulation of PYY receptors may cause nausea, and it is possible that the reduction in EI is not solely due to a reduction in hunger or increased satiety, but that it might be confounded by nausea.

Under normal physiological circumstances both PYY1-36 and PYY3-36 are found in the circulation and both hormone moieties would therefore be expected to be part of the physiological satiety signal, but so far the effects of PYY1-36 on appetite have not been investigated. Neither have the effects of PYY1-36 and PYY3-36 on EE, glucose and fat metabolism in humans been investigated.

This study aimed to compare the effects of peripheral infusions of saline, PYY1-36 or PYY3-36 on appetite, EI, EE, glucose and fat metabolism in obese and normal weight males.

Subjects and methods

Subjects

Twelve normal weight (Mean±SD: BMI: 22.7±1.4 kg/m^2; Age: 25.9±2.5 y) and 12 overweight/obese (BMI: 31.6±2.8 kg/m^2; Age: 34.9±8.7 y) healthy, weight stable, Caucasian men participated in a randomised, blinded, placebo-controlled, crossover study with infusion of saline, 0.8 pmol/kg/min PYY1-36, or PYY3-36. The choice of dosages were base on findings in previous human studies (4; 5). Minimum washout period was 3 weeks. Five of the first 9 participants did not complete their PYY3-36 infusion day due to severe malaise and the PYY3-36 infusions had to be terminated. Therefore only data from the 4 completers (2 lean and 2 obese subjects) are available for 0.8 pmol/kg/min PYY3-36 infusions, whereas all 24 subjects completed the saline and 0.8 pmol/kg/min PYY1-36 infusions. Due to the side effects of PYY3-36 infusions and the lack of effect of PYY1-36 infusions it was decided to add two more arms to the study with a lower dose of PYY3-36 (0.2 pmol/kg/min) and a higher dose of PYY1-36 (1.6 pmol/kg/min). Of the total 24 participants, 6 lean and 8 obese participants completed these 2 arms of the study. For comparison the data on PYY1-36 (0.8 pmol/kg/min) infusions already presented for the 24 subjects were included again in the data analysis for the 14 subjects. The study was approved by the Municipal Ethical Committee of Copenhagen, Frederiksberg and Zealand (Registration no. 01-091/04) and was carried out in accordance with the Helsinki-II declaration. Informed consent was obtained from all participants.

Protocol

Subjects received a standardized meal between 1900 h and 2000 h the night before each test day and they fasted thereafter (including no water intake after midnight). Subjects were instructed to refrain from drinking alcohol and from strenuous exercise 48 hours before each test day. On each

test day subjects arrived at the department at 0800 h having used non-strenuous transportation. Two venflon cathethers were inserted into opposite antecubital veins, one for infusion and one for blood sampling. The pre-infusion measurements and blood sampling were performed after a minimum of 15 min of rest. Two hours after termination of the 90 min infusion an *ad libitum,* homogeneous, casserole lunch meal was served. An *ad libitum* buffet dinner was served 5 hours later. One hundred mm visual analogue scales (VAS) (10) were employed for ratings of well-being and appetite sensations. EE was measured by indirect calorimetry using a ventilated hood system (described in detail elsewhere (3)). EE was calculated using a formula assuming a fixed protein catabolism (26) as the error introduced by omitting the exact correction from urinary nitrogen is negligible and EE cannot be reliably estimated from protein combustion on the basis of short term urinary nitrogen outputs. The precision of the ventilated hood system was validated by an alcohol burning test on a weekly basis; CV was <1.5%. The measurements were performed with intervals of approximately 25 min measurement and 5 min break for blood sampling and VAS questionnaires. Heart rate was monitored by a digital wrist blood pressure monitor (UB-401, A&D Instruments LTD, Oxon, UK).

Infusions

The PYY peptides were purchased from Polypeptide (Wolfenbüttel, Germany), dissolved to a concentration of 8 nmol/ml saline (sodium chloride 9 g/l; acetic acid 2.3 g/l), and filled into 2R glass vials (Apodan 712026, ApodanNordic A/S, Copenhagen, Denmark) with bromobutyl rubber stoppers (Apodan 712638, ApodanNordic A/S, Copenhagen, Denmark) after sterile filtration under aseptic conditions at the Central Pharmacy, Herlev Hospital, Denmark. Vial content was tested for sterility and bacterial endotoxins (Ph.Eur. 2.6.14, Method C. Turbidimetric kinetic method), and content was verified by sequence, mass and amino acid analysis. Vials were kept at -20°C until preparation of the infusion, where the peptide solution was diluted further with saline (sodium

chloride 9 g/l) to which was added the subjects' own plasma in a concentration of 1 vol%. Final peptide concentration in the infusion fluid was 32 pmol/ml, which was confirmed by radioimmunoassay analysis.

Blood samples and analysis

Venous blood was drawn without stasis through an indwelling antecubital cannula into iced chilled syringes (Vacuette, Greiner Labortechnic, Austria). Syringes for PYY and FFA contained EDTA. All samples were centrifuged for 10 min at 2800 *g* at 4°C within 30-60 min of sampling, and stored at – 20°C until analysis.

Plasma concentrations of FFA, glucose, and TG were all determined by use of enzymatic colorimetric method using a Cobas Mira Plus spectrophotometer (Roche Diagnostic Systems, Basel, Switzerland). Plasma FFA concentrations were determined using NEFA-C test kit (ACS-ACOD Method, Wako chemicals GmbH, Neuss, Germany) with intra- and inter-assay CV <4.5% and <4.2%, respectively. Plasma glucose concentrations were determined using gluco-quant® Glucose/HK kit (Roche Diagnostics, GmbH, Mannheim, Germany) (7) with intra- and interassay CV <0.7% and <1.5%, respectively. Serum TG concentrations were determined by use of Triglycerides GPO-PAP kit (Roche Diagnostics GmbH, Mannheim, Germany) (25) with intra- and interassay CV <0.6% and <3.3%, respectively.

Serum insulin concentrations were determined by chemiluminescense immunometric assay (IMMULITE 1000 Insulin kit, Diagnostic Products Corporation, Los Angeles, US) usingImmulite 1000 analyzers (Diagnostic Products Corporation, Los Angeles, US) (2) with intra- and interassay CV <2.5% and <4.9%, respectively.

Radioimmunoassay of PYY in plasma was performed using antiserum code no. 8412-2II (9), which reacts equally with PYY1-36 and PYY3-36. Synthetic human PYY1-36 or PYY 3-36 (Peninsula

Laboratories, UK) were used as standards where appropriate. ^{125}I-PYY1-36 (code no. IM259) was from Amersham Biosciences (UK). Assay buffer was 0.05 mol/l sodium phosphate, pH 7.5, containing in addition 400 KIE/ml Trasylol-aprotinin, 0.1 mol/l sodium chloride, 10 mmol/l EDTA, 0.6 mmol/l merthiolate. 150 µl unknown plasma samples + 150 µl assay buffer or 150 µl charcoal treated plasma + 150 µl standards were pre-incubated with antiserum, 100 µl, diluted 1:20.000 (final concentration), for 48 hours at 4°C. Then 100 µl tracer (5 fmol, specific activity 70 MBq/nmol) was added and the mixture incubated for 24 hours before bound and free peptide moieties were separated by plasma-coated charcoal (15). Detection limit of the assay was <2.5 pmol/l and 50% inhibition was obtained with 23 pmol/l PYY. Recovery of PYY added to plasma in concentrations between 5 and 50 pmol/l deviated less than 15% from expected values, with intra-assay CV <5%. The antiserum showed no cross-reaction with human neuropeptide Y (NPY) or human pancreatic peptide (PP) in concentrations up to 500 pmol/l.

Statistics

Data were analysed using repeated measurements testing the effect of group (lean versus obese, only for analysis with n=24 and n=14), time and infusion, as well as interactions of these factors with baseline value as cofactor and subject as random factor. When interactions were not significant the model was reduced successively. When a significant interaction between group and infusion was found, separate analyses of lean and obese participants were performed. For n=14 VAS data, the analyses were divided into pre- and post-meal data, due to opposite effects in these two periods. Non-repeated data were analysed by one-way ANOVA with subject as random factor. Normal distribution of data was assessed by Shapiro-Wilk test, box plot and normal probability plot. Where necessary data were Log transformed. All statistical analyses were performed using Statistical Analysis Package version 9.1 (SAS Institute, Cary, NC). Results are reported as Mean±SE

Results

Saline, PYY1-36 and PYY3-36 infusions (0.8 pmol/kg/min) in the 4 subjects completing the PYY3-36 infusions

Sixty min after start of the infusion, PYY levels reached a plateau with a mean concentration of 214±48 pmol/l after PYY1-36 infusion and 190±27 pmol/l after PYY3-36 infusion, compared to a baseline value of 28±5 pmol/l. Following termination of the infusion, PYY levels fell rapidly approaching basal levels within 30-60 min. Five of the 9 subjects did not complete their PYY3-36 infusion day due to severe malaise or nausea. The nausea was in some instances accompanied by abdominal pain and heat flushes. The 4 subjects who were able to complete the PYY3-36 infusions showed a 36% decrease in VAS ratings of well-being progressing from the beginning towards the end of the infusion ($P_{time*infusion} = 0.02$; data not shown). After termination of the infusion the ratings of well-being gradually recovered and were back to normal before the *ad libitum* lunch. PYY3-36 infusion resulted in decreased EI in all 4 subjects, with mean decreases in EI at lunch of 19.4% and 22.4% compared to saline and PYY1-36 infusions, respectively (Placebo: 4189±478 kJ; PYY1-36: 4348±186 kJ; PYY3-36: 3375±341 kJ; P=0.03). No effects of infusions were seen on EI, macronutrient composition or energy-density at the *ad libitum* buffet dinner (data not shown). There was a significantly lower rating of perceived ability to eat after both PYY1-36 and PYY3-36 compared to saline infusions (P<0.0001), but no significant differences were found in other appetite ratings (data not shown).

In the fasting state PYY3-36 infusions produced significantly higher FFA concentrations compared to saline and PYY1-36 infusions (Fig. 1A). Significantly higher postprandial glucose and insulin responses were produced by PYY3-36 infusions compared to PYY1-36 and saline (Fig. 1B&C). No significant differences were observed in TG concentrations ($P_{infusion} = 0.58$; data not shown). Heart rate was significantly higher with PYY3-36 compared to PYY1-36 and placebo (Fig. 1D).

There was a near-significant infusion effect (P=0.056) on EE, with ~292 kJ/day higher EE following PYY3-36 compared to PYY1-36 infusion, and ~154 kJ/day higher EE following PYY3-36 compared to saline infusion, where only data for 3 subjects exist (Fig. 2A). There was a significant time*infusion effect in RQ with lower levels, indicating greater fat oxidation, following PYY3-36 compared to saline infusion, especially towards the end of the measuring period (Fig. 2B).

Saline and PYY1-36 (0.8 pmol/kg/min) in all 24 subjects completing PYY1-36 infusions

Sixty min after start of the infusion, PYY levels reached a plateau with a mean concentration of 152±14 pmol/l after PYY1-36 infusion compared to a baseline value of 22±3 pmol/l. Following termination of the infusion, PYY levels fell rapidly approaching basal levels within 30-60 min. No side effects were reported with the PYY1-36 infusions and there was no difference in ratings of well-being between the PYY1-36 and placebo test days (data not shown). The infusion of PYY1-36 compared to placebo did not result in any reduction in EI at the *ad libitum* lunch (Placebo: 3998±284 kJ; PYY1-36: 3822±259 kJ; P=0.30) or at the *ad libitum* buffet dinner (Placebo: 6934±306 kJ; PYY1-36: 7332±283 kJ; P=0.17).

The analysis of ratings of hunger resulted in a significant group*infusion interaction, meaning that the lean group tended to be less hungry during the morning until the *ad libitum* lunch after the PYY1-36 infusions compared to saline infusions, whereas there was no difference in the obese group (Fig. 3A). When analysing the data from the obese and lean group separately, the lean group ($P_{infusion}$=0.0003) showed a significantly lower rating of hunger following PYY1-36 compared to saline infusion, whereas no difference was seen in the obese subjects ($P_{infusion}$=0.87).

The same pattern was observed for ratings of satiety, where a significant group*infusion*time interaction was found, with the lean group feeling more satiated during the mornings until the *ad*

libitum lunch and vice versa for the obese group in the same time period (Fig. 3B). Analysing the data from the obese ($P_{infusion}$=0.81) and lean groups ($P_{infusion}$=0.68) separately, no significant difference between PYY1-36 and saline infusion could be found. Although a similar picture as that for hunger and satiety ratings was seen for perceived ability to eat, these ratings resulted in a non-significant infusion*group interaction (P=0.15; data not shown), but in significant main effects with lower ratings in the obese group compared to the lean group and with lower ratings following PYY1-36 compared to placebo infusion. Analysing the data from the obese and lean groups separately, the lean group showed a significantly lower rating of perceived ability to eat following PYY1-36 compared to saline infusion ($P_{infusion}$=0.001), whereas no difference was seen in the obese group ($P_{infusion}$=0.64). No significant difference between PYY1-36 and saline infusions in ratings of fullness was found, neither when analysing the lean and obese subjects together ($P_{group*infusion}$=0.93; data not shown), nor when doing separate within-group analysis (Lean: $P_{infusion}$=0.55; Obese: $P_{infusion}$=0.58).

FFA, glucose, insulin, and TG concentrations over time were not different comparing PYY1-36 infusions with saline infusions (data not shown). A significant difference ($P_{time*infusion}$=0.003; data not shown) was found in heart rate, with the post hoc test showing significantly higher mean value at min 90, and lower mean value at min 300 and 480, following PYY1-36 compared to saline infusion. Neither EE ($P_{infusion}$=0.08) nor RQ ($P_{infusion}$=0.49) differed following PYY1-36 compared to saline infusion (data not shown).

Saline, PYY 3-36 (0.2 pmol/kg/min), and PYY1-36 (1.6 pmol/kg/min) infusions in the 14 subjects completing all 3 protocols.

Sixty min after start of the infusion, PYY levels reached a plateau with mean concentrations of 76±23 pmol/l, 157±22 pmol/l, and 182±20 pmol/l after 0.2 pmol/kg/min PYY3-36, 0.8 and 1.6

pmol/kg/min PYY1-36 infusions, respectively, compared to a baseline value of 17±1 pmol/l. Following termination of the infusion, PYY levels fell rapidly approaching basal levels within 30-60 min. One participant reported mild nausea and a feeling of coldness during the infusion of PYY3-36. Another participant reported heat flushes and abdominal pain towards the end of the PYY3-36 infusion. There was a significant difference in VAS ratings of well-being between the different infusions (Fig. 4A) with lower rating for well-being following the PYY3-36 infusion compared to all other infusions, and a tendency to lower rating following the higher compared to the lower PYY1-36 dose. There were no effects of PYY infusions on EI at the *ad libitum* lunch (Placebo: 3790±328 kJ; 0.2 pmol/kg/min PYY3-36: 4028±326 kJ; 0.8 pmol/kg/min PYY1-36: 3894±284 kJ; 1.6 pmol/kg/min PYY1-36: 4216±294 kJ; P=0.40) or at the *ad libitum* buffet dinner (Placebo: 6980±352 kJ; 0.2 pmol/kg/min PYY3-36: 6503±528 kJ; 0.8 pmol/kg/min PYY1-36: 7275±440 kJ; 1.6 pmol/kg/min PYY1-36: 7389±364 kJ; P=0.45). However, there were significant differences in appetite, with a pattern of higher pre-meal ratings of hunger and lower ratings of satiety following the high dose PYY1-36 infusion, and a compensatory opposite rating in the late postprandial period (Fig. 4B&C). Ratings of perceived ability to eat and fullness, and all appetite ratings following the 0.2 pmol/kg/min PYY3-36 infusion, showed no clear pattern (data not shown). Infusion of PYY3-36, but not PYY1-36, produced a significant elevation of plasma FFA (Fig. 5A). Baseline glucose concentrations were higher on the placebo and low dose PYY1-36 infusion days compared to high dose PYY1-36 and PYY3-36 days (Placebo: 5.34±0.10 mmol/l; 0.2 pmol/kg/min PYY3-36: 5.12±0.12 mmol/l; 0.8 pmol/kg/min PYY1-36: 5.41±0.13 mmol/l; 1.6 pmol/kg/min PYY1-36: 5.14±0.12 mmol/l; P=0.0009), but no significant difference was found in the repeated measurement analysis of glucose concentration (Fig. 5B). No significant difference was found in the repeated measurement analysis of insulin concentrations (Fig. 5C), but the postprandial iAUC values were significantly higher following PYY3-36 and high dose PYY1-36 infusions compared

with low dose PYY1-36 and placebo (Placebo: 27179±3369 pmol*min/l; 0.2 pmol/kg/min PYY3-36: 39526±6476 pmol*min/l; 0.8 pmol/kg/min PYY1-36: 27465±3665 pmol*min/l; 1.6 pmol/kg/min PYY1-36: 36960±5717 pmol*min/l; P=0.004). No differences were observed in TG concentrations ($P_{infusion}$=0.97; data not shown). PYY3-36 and high dose PYY1-36 produced significantly higher increases in heart rate compared to placebo and low dose PYY1-36 (Fig. 5D). Baseline values of heart rate were not significantly different (Placebo: 55.7±2.5 beats/min; 0.2 pmol/kg/min PYY3-36: 54.2±2.5 beats/min; 0.8 pmol/kg/min PYY1-36: 55.7±2.3 beats/min; 1.6 pmol/kg/min PYY1-36: 56.1±2.4 beats/min; P=0.73). No differences between infusions were observed for EE ($P_{infusion}$=0.28, data not shown), but a tendency to lower RQ following PYY3-36 and high dose PYY1-36 infusions compared to placebo was observed (Fig. 6).

Discussion

PYY3-36

Our study confirms previous findings (4; 5; 8; 18) of a suppression of food intake, i.e. lower *ad libitum* EI, following PYY3-36 infusions at a rate of 0.8 pmol/kg/min, but the picture is less clear for subjective appetite sensations (VAS ratings), probably due to the statistical power being too low, with only 4 subjects completing the high dose PYY3-36 infusions. We found that 5 out of 9 subjects experienced adverse effects, mainly in the form of nausea, but also in some instances accompanied by abdominal discomfort and heat flushes. These effects were of a severity that precluded continuation of the infusions. Subjective ratings of well-being were lower during the infusion period in the 4 completing subjects who did not report nausea. Although these ratings were back to normal before the *ad libitum* lunch meal it cannot be ruled out that discomfort, at least partly, can account for the lower *ad libitum* EI. The infusions of 0.2 pmol/kg/min PYY3-36 on the other hand were generally well tolerated with fewer and milder reported side-effects, but in

accordance with other studies (8; 18) this infusion rate did not result in significant lowering of energy intake or in differences in appetite ratings.

If we compare our plasma concentrations of PYY we find that they were somewhat higher than in the 3 previous studies reporting infusion of 0.8 pmol/kg/min (5; 8; 18), but also among these studies great differences are seen in plasma peak levels of PYY. Since the differences among studies in baseline values are generally lower than differences in peak levels it is likely due to different preparation of the infusion solution. In the present study, the subjects' own plasma was used as carrier in the preparation of the infusion solution compared to Hemaccel (4; 5; 18; 19) and human serum albumin (8) in other studies, probably resulting in different adsorption of the peptide to infusion bag and tubing. Since the actual concentrations of the hormone in the infusion liquid after passage trough the tubing are generally not reported it is difficult to compare doses across studies, and differences in assays used to analyse plasma PYY concentrations may add to these discrepancies. Plasma levels closest to the ones in the present study were found in the study by Degen et al. (8) who also reported side effects in the form of nausea, vomiting, abdominal discomfort, fullness, and sweating, with plasma levels in the order of >74 pmol/l leading to nausea. Our observations with regards to side effects are also in accordance with a previous study with nasal administration of PYY3-36, in which doses resulting in a 4-fold increase in plasma PYY levels from baseline to peak were associated with dizziness and nausea (20). Furthermore, PYY has been shown to have emetic effects in dogs, both as regards endogenous PYY induced by cisplatin treatment (21) and as regards exogenous PYY infused at doses below 100 pmol/kg (14). This corresponds well with our findings, where a 90 min infusion amounting to a total dose of 72 pmol/kg caused nausea in 5 out of 9 subjects. Both Degen et al. (8) and le Roux et al. (18) have demonstrated a dose dependent decrease in EI, and their data suggest that plasma levels should reach at least 60 pmol/l to have significant effects on EI. Neary et al. (19) found that doses of 0.4

pmol/kg/min, giving rise to plasma concentrations around 60 pmol/l, resulted in a borderline significant reduction of food intake, whereas our infusion with 0.2 pmol/kg/min resulting in mean plasma PYY concentrations of 76 pmol/l had no effect on EI. Consequently,the therapeutic window for reduction of food intake without nausea is narrow, and PYY therapy will require methods of administration that produce a stable plasma level within this therapeutic range.

PYY3-36 infusions produced progressive increases in FFA until a peak was reached 30 min after serving the ad libitum test meal with 182% and 95% increases from baseline following high and low dose PYY3-36, respectively. The PYY3-36 infusions moreover resulted in increased postprandial insulin responses and as for the high dose infusion increased postprandial glucose response. The only other PYY3-36 infusion study where insulin and glucose were measured is that of Neary et al. (19) who, in accordance with our results, found no differences in fasting glucose and insulin, but they did not measure postprandial values. The increased FFA and postprandial insulin and glucose concentrations could be caused by a PYY induced impairment in insulin sensitivity, resulting in higher release of FFA from adipose tissue but without any obvious effects on glucose and insulin levels before a challenge is presented in the form of a meal. However, we find it more likely that increased lipolysis was the primary event, with the higher levels of FFA producing insulin resistance with resulting higher postprandial insulin and glucose concentrations. An activation of the sympathetic nervous system could be the cause of an increased lipolysis, which is supported by the data indicating higher heart rate for both low and high dose PYY3-36 infusions, and higher EE, during the high dose PYY3-36 infusions. Measurements of catecholamine concentrations could help clarify this picture, but such samples were not taken in the present study. The decrease in RQ during the PYY3-36 infusions was expected, as the higher FFA concentrations result in higher fat oxidation, but the increased EE also indicates sympathoadrenal activation. It remains unclear whether the reduced ratings of well-being associated with the PYY3-36 infusions

could be the reason for the hypothesized increased sympathoadrenal activity.

PYY1-36:

In the present study PYY1-36 infusions had no effect on subsequent EI. However, subjective appetite ratings suggested an effect of low dose PYY1-36 in lean individuals with lower ratings of hunger and perceived ability to eat, whereas this effect was not present in the obese participants. In contrast with this, high dose PYY1-36 resulted in increased hunger and decreased satiety in the period before the ad libitum meal, where after a compensatory opposite rating was observed. The difference between low and high dose PYY1-36 in VAS ratings of hunger could be speculated to be due to different study groups with more obese subjects in the high dose PYY1-36 part of the study. Besides effects on appetite, the only other significant effects seen with PYY1-36, were a higher postprandial insulin response and increases in heart rate following high dose PYY1-36. Since PYY1-36 is thought to be cleaved by DPP-IV, the infusion of PYY1-36 should give rise to higher circulating concentrations of PYY3-36, and thereby similar effects should be seen with administration of the two different peptide fractions. Our data therefore suggests that this cleavage process is not a complete and rapid process such as is seen with the cleavage of GLP-1, which has a half-life of 1-2 min (24).

The difference between lean and obese subjects in appetite ratings could be speculated to be due to different expression of the Y1, Y2 and Y5 receptors, which have opposite effects on appetite, with the Y2 receptor signalling anorexigenic effects (5), and the Y1 and Y5 orexigenic effects (11; 16; 17; 23). Another possibility is lower activity of DPP-IV in obese compared to lean humans, thereby giving rise to higher circulating levels of PYY3-36 in the lean compared to obese participants, but this theory is not supported by previous data showing an equal ratio between PYY1-36 and PYY3-36 in obese compared to lean individuals (18). Furthermore, the appetite regulation in obese may be speculated to be affected more by other factors, such as psychological

factors, which could tend to blur the assessment of the effects of a physiological factor like PYY. Finally, a previous infusion study in rats comparing effects on EI of PYY1-36 and PYY3-36 found that PYY1-36 seemed to be around 10-fold less potent than PYY3-36. This suggests that higher doses than those used in the present study could be needed in order to find significant effects on EI, and on fat and glucose metabolism, in humans after PYY1-36 administration (6). The present study might not have reached highest tolerable dose of PYY1-36, although the highest dose of PYY1-36 elicited significant increases in e.g. heart rate and postprandial insulin concentrations.

In summary, PYY1-36 infusions had no effects on EI, and no clear dose dependent effect was observed in relation to appetite following PYY1-36 infusions, but data indicate that effects might differ between lean and obese individuals. Low dose PYY1-36 had no effects on EE, FFA, glucose, insulin or TG concentrations, whereas high dose PYY1-36 resulted in a somewhat increased heart rate and postprandial insulin response. The anorectic effects of PYY3-36 were confirmed in this study, but may be due to discomfort and it seems that the therapeutic window, resulting in decreased EI without side-effects, is narrow. PYY3-36 caused increased thermogenesis, lipolysis, and increased postprandial insulin and glucose responses. The mechanisms behind these findings are unknown and call for further studies.

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Disclosures

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Legends to Figures

Figure 1: Mean±SE FFA, glucose, insulin concentrations and heart rate before (0 min), during (0-90 min) and after infusion of saline, PYY1-36 and PYY3-36. An *ad libitum* meal was served at 210 min. Differences between infusions were tested by ANCOVA repeated measurement, with baseline values as cofactor.

FFA: $P_{time*infusion} < 0.0001$

Glucose: $P_{time*infusion} = 0.004$

Insulin: $P_{time*infusion} = 0.004$

Heart Rate: $P_{time*infusion} = 0.02$

Figure 2: Mean±SE energy expenditure (EE) and respiratory quotient (RQ) before (-30 min), during (0-90 min) and after infusion of saline, PYY1-36 and PYY3-36. Differences between infusions were tested by ANCOVA repeated measurement, with baseline values as cofactor.

EE: $P_{infusion} = 0.056$

RQ: $P_{time*infusion} = 0.003$

Figure 3: Mean±SE VAS ratings of hunger and satiety before (0 min), during (0-90 min) and after infusion of saline and PYY1-36. An *ad libitum* meal was served at 210 min. Differences between infusions and group (lean vs. obese) were tested by ANCOVA repeated measurement, with baseline values as cofactor.

Hunger: $P_{group*infusion} = 0.01$

Satiety: $P_{group*infusion*time} = 0.02$

Figure 4: Mean±SE VAS ratings of well-being, hunger and satiety before (0 min), during (0-90 min) and after infusion of saline, PYY1-36 and PYY3-36. An *ad libitum* meal was served at 210 min. Differences between infusions were tested by ANCOVA repeated measurement, with baseline values as cofactor.

Well-being: $P_{infusion} < 0.0001$

For hunger and satiety the analysis was divided in preprandial (min 0-210) and postprandial (min 240-480).

Hunger: $P_{infusion}$ (preprandial) = 0.006; $P_{infusion*group}$ (postprandial) = 0.02.

Satiety: $P_{infusion}$ (preprandial) = 0.0004; $P_{infusion}$ (postprandial) = 0.03.

Figure 5: Mean±SE FFA, changes in glucose concentrations, insulin concentrations and changes in heart rate before (0 min), during (0-90 min) and after infusion of saline, PYY1-36 and PYY3-36. An *ad libitum* meal was served at 210 min. Differences between infusions were tested by ANCOVA repeated measurement, with baseline values as cofactor.

FFA: $P_{infusion} = 0.02$

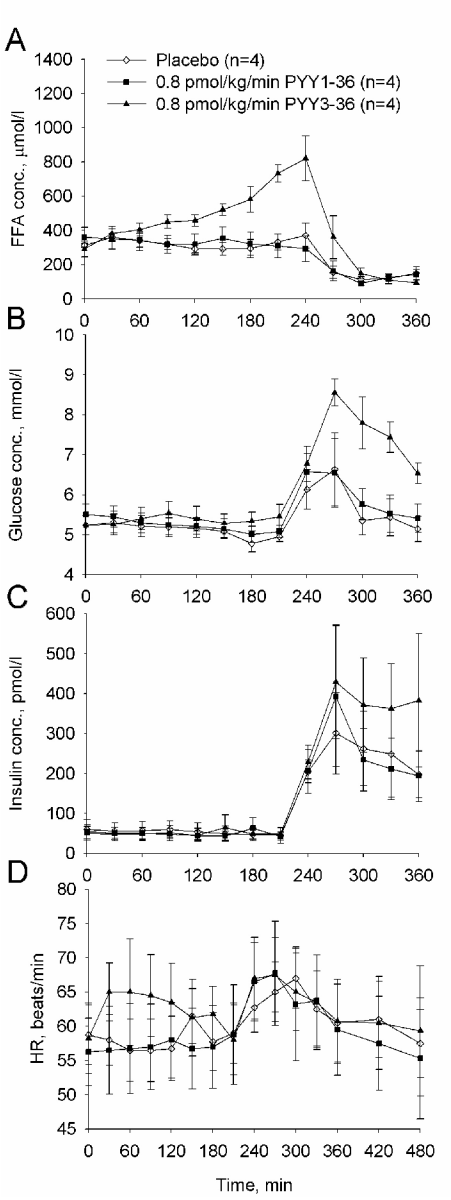
Changes in glucose: $P_{infusion} = 0.27$

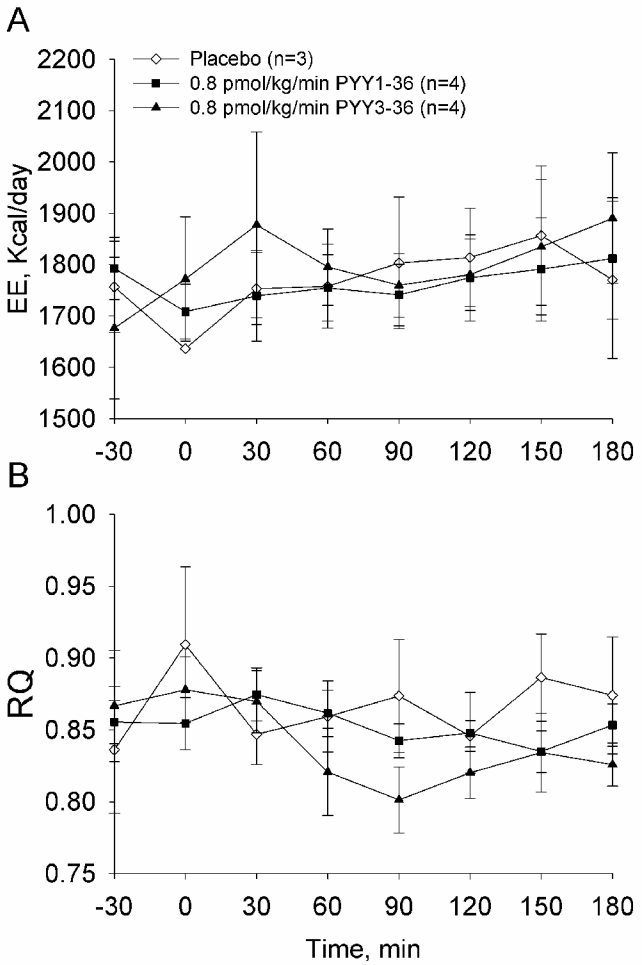
Insulin: $P_{infusion} = 0.23$

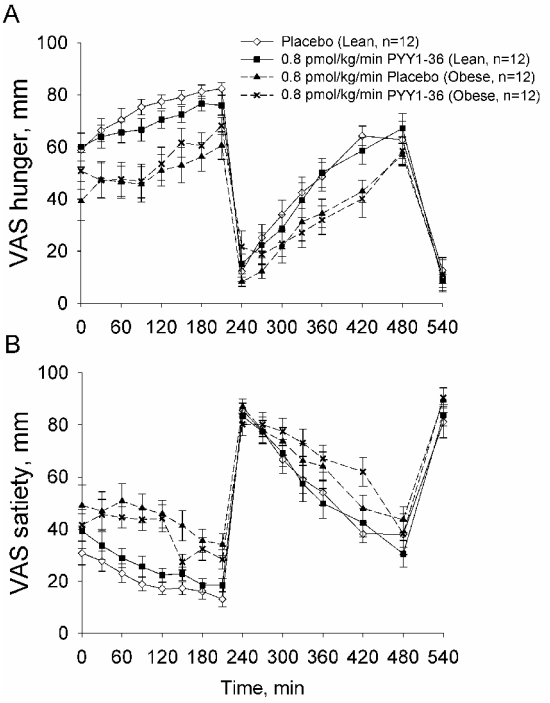
Changes in heart rate: $P_{infusion} < 0.0001$

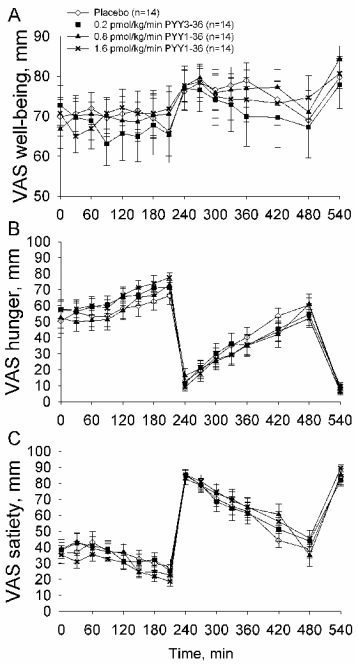
Figure 6: Mean±SE respiratory quotient (RQ) before (-30 min), during (0-90 min) and after a 90 mins infusion of saline, PYY1-36, and PYY3-36. Differences between infusions were tested by ANCOVA repeated measurement, with baseline values as cofactor.

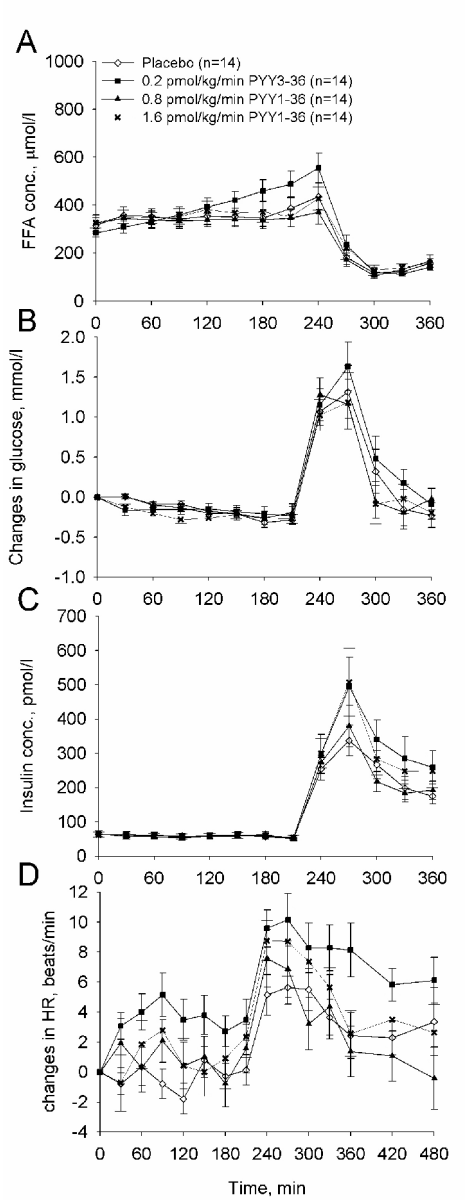
$P_{infusion} = 0.06$

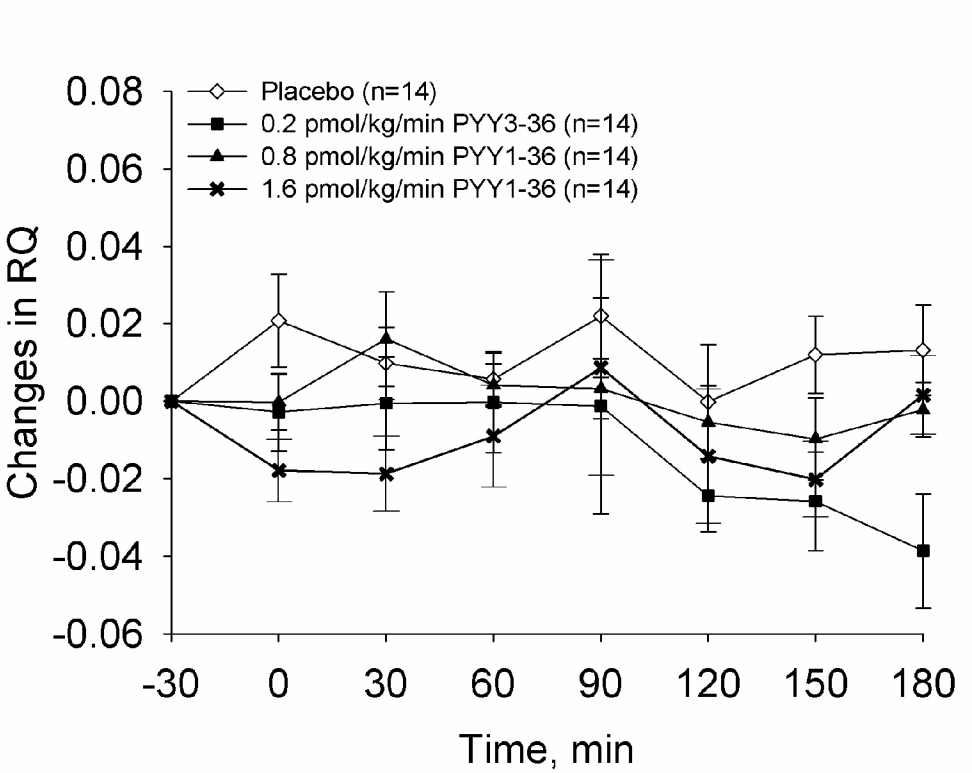

A
Placebo (n=4)
0.8 pmol/kg/min PYY1-36 (n=4)
0.8 pmol/kg/min PYY3-36 (n=4)
FFA conc., µmol/l
B
Glucose conc., mmol/l
C
Insulin conc., pmol/l
D
HR, beats/min
Time, min


A
Placebo (n=3)
0.8 pmol/kg/min PYY1-36 (n=4)
0.8 pmol/kg/min PYY3-36 (n=4)
EE, Kcal/day
2200
2100
2000
1900
1800
1700
1600
1500
-30
0
30
60
90
120
150
180
B
RQ
1.00
0.95
0.90
0.85
0.80
0.75
Time, min


A
Placebo (Lean, n=12)
0.8 pmol/kg/min PYY1-36 (Lean, n=12)
0.8 pmol/kg/min Placebo (Obese, n=12)
0.8 pmol/kg/min PYY1-36 (Obese, n=12)
VAS hunger, mm
100
80
60
40
20
0
0 60 120 180 240 300 360 420 480 540
B
VAS satiety, mm
100
80
60
40
20
0
0 60 120 180 240 300 360 420 480 540
Time, min


A
Placebo (n=14)
0.2 pmol/kg/min PYY3-36 (n=14)
0.8 pmol/kg/min PYY1-36 (n=14)
1.6 pmol/kg/min PYY1-36 (n=14)
VAS well-being, mm
B
VAS hunger, mm
C
VAS satiety, mm
0 60 120 180 240 300 360 420 480 540
Time, min


A
Placebo (n=14)
0.2 pmol/kg/min PYY3-36 (n=14)
0.8 pmol/kg/min PYY1-36 (n=14)
1.6 pmol/kg/min PYY1-36 (n=14)
FFA conc., µmol/l
B
Changes in glucose, mmol/l
C
Insulin conc., pmol/l
D
changes in HR, beats/min
Time, min


Placebo (n=14)
0.2 pmol/kg/min PYY3-36 (n=14)
0.8 pmol/kg/min PYY1-36 (n=14)
1.6 pmol/kg/min PYY1-36 (n=14)
Changes in RQ
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-30
0
30
60
90
120
150
180
Time, min