**PYY\textsubscript{3-36} and oxyntomodulin can be additive in their effect on food intake in overweight and obese humans.**

Short running title: Co-administration of PYY and oxyntomodulin

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Objective – PYY\textsubscript{3-36}, a Y2 receptor agonist, and oxyntomodulin (OXM), a GLP-1 receptor agonist, are co-secreted by intestinal L-cells after each meal. Separately each hormone acts as an endogenous satiety signal and reduces appetite in humans when infused intravenously. The aim of the current study was to investigate whether the anorectic effects of PYY\textsubscript{3-36} and OXM can be additive.

Research Design and Methods – Twelve overweight or obese human volunteers underwent a randomised, double-blinded, placebo-controlled study. An ad libitum test meal was used to measure energy intake during intravenous infusions of either PYY\textsubscript{3-36} or OXM or combined PYY\textsubscript{3-36}/OXM.

Results – Energy intake during co-administration of PYY\textsubscript{3-36} and OXM was reduced by 42.7\% in comparison to saline control, and was significantly lower than during infusions of either hormone alone.

Conclusions – The anorectic effects of PYY\textsubscript{3-36} and OXM can be additive in overweight and obese humans. Co-administration of Y2 receptor agonists and GLP-1 receptor agonists may be a useful treatment strategy for obesity.
Obesity is a major risk factor for the development of type 2 diabetes and its prevalence is increasing rapidly throughout the world (1;2). Weight loss reduces that risk substantially (3) but is difficult to achieve and sustain. Bariatric surgery is the only obesity treatment proven to reduce mortality (4;5). Furthermore, the rapid improvement in glucose homeostasis after Roux-en-Y gastric bypass (6;7) has led some to question whether type 2 diabetes, as well as obesity, should be considered a surgically curable disease (8;9). However, operative mortality and delayed complications are not uncommon (10;11). There is therefore a pressing need to develop safe, effective, non-surgical treatments for obesity.

The main proposed mechanism by which Roux-en-Y gastric bypass causes weight loss is through altering the secretion of gut hormones (12). Two important such hormones, peptide YY_3-36_ (PYY_3-36_) and oxyntomodulin (OXM), are physiologically co-secreted after meals (13;14). Post-prandial concentrations of both PYY_3-36_ and OXM are increased by Roux-en-Y gastric bypass (12;15-17). Intravenous infusion of each hormone individually has been shown to reduce appetite in humans (18-20). Furthermore, OXM causes weight loss in obese human volunteers when administered by repeated subcutaneous injection (21). However, one proposed physiological function of these hormones at higher concentrations is nausea (22). Thus if significant appetite reduction is attempted by giving a large dose of a single hormone, nausea or even vomiting may well result (20-23). We hypothesised that co-administered PYY_3-36_ and OXM would mimic the natural postprandial situation and have additive effects on appetite, but that neither hormone would reach concentrations associated with nausea.

RESEARCH DESIGN AND METHODS

Healthy male and female volunteers aged 18 years or over with a stable BMI of 25-40 kg/m^2_ were recruited by advertisement. Potential participants were screened and determined to be healthy by medical history, physical examination, routine blood tests and 12-lead electrocardiogram. The SCOFF questionnaire (24), the Dutch Eating Behaviour Questionnaire (25) and a three-day diet diary were used to exclude those with disordered eating and/or a high level of restrained eating. Palatability of the study meal was assessed using a nine-point hedonic scale. It was calculated that, for 90% power to detect a difference in energy intake of 10% between treatments, twelve participants would be required, assuming a within-subject standard deviation of 6% and a significance level of 0.05. Thus twelve volunteers were selected for the study (table 1). Women of child-bearing age were advised to avoid pregnancy during the study and underwent urine tests to exclude pregnancy prior to each infusion.

The study was approved by the Hammersmith & Queen Charlotte’s & Chelsea Research Ethics Committee (reference no. 06/Q0406/50). All participants gave written informed consent, and the study was planned and performed in accordance with the Declaration of Helsinki.

The study followed a randomised, double-blind, placebo-controlled crossover protocol, comparing the effect on energy intake of six different pairs of infusions, as shown in table 2. Each subject received two 110 minute intravenous infusions, A and B, simultaneously, at each visit. Infusion A consisted of either PYY_3-36_ or saline control. Infusion B consisted of either OXM or saline control. Infusion doses were based on previously established doses, with a two-fold difference between high and low doses for each peptide. The infusion rate for high dose...
Co-administration of PYY and oxyntomodulin

PYY$_{3-36}$ was based on previous work by Batterham et al. (26). The infusion rate for high dose OXM was similar to that used by Cohen et al. (19). The duration of infusion was chosen to allow steady state to be reached and sustained during test meals. Peptides were synthesised by Bachem UK and were sterile on culture and negative for pyrogen, as previously described (26). The amino acid content of representative peptide vials was measured independently (Alta Bioscience, UK). Control vials were prepared with sterile saline and were indistinguishable visually from those containing peptide. To reduce adsorption of peptide onto the walls of syringes and infusion lines, the contents of randomised vials were dissolved in Gelofusine (B. Braun Medical Ltd, UK).

Study visits were a minimum of 3 days apart. Subjects were asked to standardise their diet, abstain from alcohol and avoid strenuous exercise for 24 hours prior to each visit. Food diaries were used to monitor dietary compliance. Subjects fasted and drank only water from 9 p.m. on the night before each visit. After arrival at 9 a.m., peripheral venous cannulae were inserted in both forearms, one for infusions and one for blood sampling. A three-way tap with low internal volume (Becton Dickinson, NJ) was attached to the infusion cannula, to allow connection of two separate infusion lines. Subjects then relaxed for 30 minutes prior to the start of the infusions. All time cues were removed from the study room and subjects were encouraged to relax by reading or watching films on DVD.

Blood samples were collected at -30, 0, 30, 60, 75, 90, 120 and 135 mins, into lithium heparin-coated tubes containing 2000 kallikrein inhibitor units (0.2 ml) aprotinin (Bayer Schering Pharma, Germany). Samples were stored on ice until centrifugation at 4°C, after which, plasma was separated immediately and stored at -20°C until analysis. Immediately before each blood sample was taken, subjects completed visual analogue scales (VAS) rating hunger, satiety, prospective food consumption and nausea (27). Pulse and blood pressure were measured every 30 mins and at the end of each study visit.

Ninety minutes after the start of the infusions, subjects were offered a meal that was provided in excess, and were asked to eat until comfortably full. Water was freely available. Both food and water were weighed pre- and post-prandially, and energy intake was calculated. The test meal procedure was identical to that used in previous studies with PYY$_{3-36}$ and OXM (18;19;26). At the end of the meal, the infusions were discontinued and the subjects were asked to rate the palatability of the food using VAS.

**Hormone assays.** Plasma PYY and OXM-like immunoreactivity (OLI) concentrations were measured using established in-house radioimmunoassays. The PYY assay (28;29) could detect changes of 4.4 pmol/L (95% confidence limit) with an intra-assay variation of 11.5%. The OLI assay (14) could detect changes of 10 pmol/L (95% confidence limit) with an intra-assay variation of 5.7%. Because the radioimmunoassay technique is comparative and not absolute, all samples were assayed in duplicate and within a single assay to eliminate inter-assay variation. Plasma insulin and glucose concentrations at 0, 60 and 90 minutes were measured on an Olympus analyser in the Department of Clinical Biochemistry, Hammersmith Hospital.

**Statistical analysis.** Combined data are represented as the mean ± SEM. Comparisons of energy intake were by repeated measures ANOVA with Tukey’s multiple comparison post-test. Effects on changes in energy intake of subjects’ BMI and gender were analysed by linear regression and repeated measures two-way ANOVA respectively. VAS scores were adjusted for baseline and differences compared by
repeated measures non-parametric Friedman’s test with Dunn’s multiple comparison post-test. Comparisons at each time point of plasma insulin and glucose levels, and of cardiovascular parameters, were by one-way ANOVA with Tukey’s multiple comparison post-test. Analyses were performed using Prism version 4.03 software (Graphpad Software, San Diego, CA, USA).

RESULTS
Adverse effects. On one visit each, the first three participants experienced severe nausea and sweating. As a result, the randomisation code on this single occasion was examined by an independent medical colleague appointed for the purpose prior to the study and not directly connected with the investigation. It was identified that each case of nausea had occurred during high dose PYY infusion, with symptoms commencing approximately 50 mins after the start of the infusion. In each case, symptoms settled within 30 mins of stopping the infusions, and the participants were able to leave the investigation ward as normal after the last blood sample. Mean peak plasma PYY concentration achieved during these infusions was 156.5 ± 56.9 pmol/l (n = 3). It was not felt possible thereafter to continue the high dose PYY infusion arm and the study then proceeded as a 5-way crossover, maintaining the randomised, double-blind, placebo-controlled design. During the remainder of the study, four participants reported nausea, requiring early termination of their infusions, at one visit each. However, since the nausea was not associated with vomiting, the randomisation code was not examined again until the end of the study.

Effect of PYY and OXM infusions on energy intake and appetite. In comparison to saline control infusion, energy intake during combined PYY + OXM infusion was reduced by 42.7% at the study meal (p<0.001) and was also significantly lower than during infusions of either hormone alone (mean energy intake at buffet meal: 557 ± 88.9 kcal [saline]; 511 ± 85.2 kcal [low dose PYY]; 480 ± 80.0 kcal [low dose OXM]; 486 ± 86.2 kcal [high dose OXM]; 319 ± 61.9 kcal [combined PYY + OXM], p<0.001 vs. saline, p<0.01 vs. low dose PYY, p<0.05 vs. low dose OXM and high dose OXM, n = 12) (figure 1). There was no evidence that change in energy intake varied with either BMI or gender of subjects.

Neither the palatability of the buffet meal, nor other satiety-related VAS responses, was altered significantly by any infusion except high dose PYY. In particular, there were no significant differences in nausea scores between the five completed arms of the study at any time point (figure 2). However, four participants did report mild nausea, one during a high dose OXM infusion and the other three during combined PYY + OXM infusion. Even though the nausea settled rapidly in each case after the infusions were (prematurely) stopped, it may have reduced energy intake at the subsequent buffet meal. The energy intake data were therefore analysed further, excluding all data from the four affected participants. In this analysis (n = 8), the combined PYY + OXM infusion significantly reduced energy intake by 33% in comparison to saline control (p<0.05). However, the control low dose PYY, low dose OXM and high dose OXM infusions for just these subjects did not reduce food intake significantly (mean energy intake: 593 ± 132.7 kcal [saline]; 524 ± 129.9 kcal [low dose PYY]; 503 ± 121.3 kcal [low dose OXM]; 532 ± 113.6 kcal [high dose OXM]; 398 ± 77.3 kcal [combined PYY + OXM], p<0.05 vs. saline, n = 8).

Plasma concentrations of PYY, OLI, insulin and glucose. Basal plasma concentration of PYY was 22.2 ± 0.7 pmol/l. Infusion of low dose PYY caused a three-fold elevation in plasma PYY concentration to a peak of 62.9 ± 7.2 pmol/l and had no effect on plasma OLI
concentration. Basal plasma concentration of OLI was 83.6 ± 4.1 pmol/l. Infusion of low dose OXM caused a five-fold elevation in plasma OLI concentration to a peak of 381.2 ± 49.0 pmol/l, whereas infusion of high dose OXM caused a six-fold elevation to a peak of 505.3 ± 68.3 pmol/l (figure 3). Plasma PYY concentration remained at basal levels during low and high dose OXM infusions. There were no statistically significant differences between treatments in insulin or glucose concentrations prior to the meal. Plasma insulin and glucose were not measured postprandially since energy intake was not fixed.

Effect of PYY and OXM infusions on cardiovascular parameters. No statistically significant differences in pulse or blood pressure were detected between treatments at any time point.

DISCUSSION
Combined administration of PYY\textsubscript{3-36} and OXM at low dose resulted in a statistically significant reduction in energy intake of 42.7% in comparison to that on the saline control day. In contrast, mean energy intake during low dose infusion of either PYY\textsubscript{3-36} or OXM was 8.3% and 14% lower, respectively, than during saline infusion, but in neither case was this difference statistically significant from the food intake during saline infusion. In a separate analysis, that excluded all data from subjects who had experienced nausea at any point during the study, the reductions in energy intake achieved by low dose infusion of either hormone alone (12% and 15% for PYY\textsubscript{3-36} and OXM respectively) were again non-significant, but combined low dose infusions of PYY\textsubscript{3-36} and OXM reduced mean energy intake significantly by 33% compared to saline. This indicates that the combination of PYY\textsubscript{3-36} and OXM reduces food intake to a greater extent than either hormone infused separately at this same dose.

Although not directly comparable, since the procedure was different, the anorectic effect of low dose PYY\textsubscript{3-36} infusion in the current study was less than that observed by Batterham \textit{et al.} using a somewhat higher dose (26). The anorectic effects of both low and high dose OXM infusions were also less than those previously observed by Cohen \textit{et al.} (19). Furthermore, there was no difference in effect between these low and high dose OXM infusions, despite the two-fold difference in dose. However, the peak plasma OLI concentrations in the current study were substantially lower than those achieved previously, which may reflect differences in infusion preparation (19).

During high dose PYY\textsubscript{3-36} infusion, the mean peak plasma PYY concentration was sufficient to cause sweating and severe nausea in all subjects, in keeping with previous reports (20;22;23). In contrast, nausea did not occur with low dose PYY\textsubscript{3-36} infusion, during which the peak plasma PYY concentration was similar to that achieved in obese subjects by Batterham \textit{et al.} (26). High dose OXM infusion resulted in a mean plasma OLI concentration approximately 60% of that achieved previously by intravenous infusion (19) and considerably lower than that previously reported to cause nausea (21). Nevertheless, one of the twelve subjects experienced nausea during high dose OXM infusion, suggesting that the threshold for OXM-induced nausea may vary between individuals. There were no adverse effects with low dose OXM. However, combined low dose infusions of PYY\textsubscript{3-36} and OXM caused nausea in 3 of 12 subjects. Thus, although co-administration of PYY\textsubscript{3-36} and OXM can produce a robust reduction in energy intake, this combination may increase the incidence of side effects.

When satiety-inducing hormones that act via different receptors are administered in combination, it can be hypothesised that the effects on appetite should be additive. However, studies performed in lean human volunteers do not always support this. Neary
et al. reported that pancreatic polypeptide, a Y4 receptor agonist, and PYY$_{3-36}$, a selective Y2 receptor agonist, did not reduce food intake when infused together (30). Others have found that, although cholecystokinin and glucagon-like peptide-1 (GLP-1) synergistically reduced hunger sensations, the combination did not reduce energy intake to a greater extent than infusion of either hormone separately (31). It is possible that the absence of additive effects results either from duplication of the principal mode of action, or from unsuspected, mutually antagonistic actions, within each pair of hormones. In contrast, and in agreement with the current study, Neary et al. demonstrated that intravenous infusion of PYY$_{3-36}$ with GLP-1 reduced food intake to a greater extent than either hormone administered separately (32). Furthermore, exendin-4, which, like GLP-1 and OXM, is a GLP-1 receptor agonist, acts synergistically with PYY$_{3-36}$ to reduce food intake in mice (33). The current study thus supports the concept that Y2 receptor agonists and GLP-1 receptor agonists have distinct and additive effects on appetite.

The plasma concentrations of PYY and OLI during these infusions are within the range of those occurring after Roux-en-Y gastric bypass surgery. Measurement of OXM concentration in plasma presents particular difficulties because of cross-reactivity of total glucagon (enteroglucagon) antibodies with several circulating products of preproglucagon cleavage (14). This may account for the ten-fold difference in post-prandial levels reported after Roux-en-Y gastric bypass (16;17). Notwithstanding this discrepancy and differences in antibody specificity, the mean plasma OLI concentration achieved during the current study was similar to post-prandial enteroglucagon levels reported after Roux-en-Y gastric bypass (15). With regard to plasma PYY concentration, the mean peak level achieved during low dose infusion in the current study was approximately 50% higher than the peak concentration reported after a 420 kcal mixed meal (34) but slightly lower than that measured after a 398 kcal liquid meal (35), both studies being performed in patients who had undergone Roux-en-Y gastric bypass. Thus the results of the current study may throw light on the mechanism of food intake reduction after Roux-en-Y gastric bypass.

In summary, we have shown that combined infusion of PYY$_{3-36}$ and OXM appear to have an additive anorectic effect in overweight and obese humans. These results and data from other recent studies suggest that Y2 receptor agonists and GLP-1 receptor agonists may be particularly suited to co-administration for the treatment of obesity. However, further studies are required to establish whether chronic co-administration of gut hormones can increase the potential anorectic effect without inducing a parallel increase in nausea.

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**Statement of duality of interest:** BCTF, AMW, VP, KCRB, NMM, MP, SA, VA and KW have no duality of interest to declare. MAG and SRB are inventors of patents describing the use of gut hormones and their analogues and derivatives in the treatment of obesity. SRB is a consultant for Thiakis, a subsidiary of Wyeth Pharmaceuticals.

**Figure Legends**

**Figure 1:** Percentage reduction in energy intake at the buffet meal, with reference to the mean intake during saline infusion (all subjects included, n = 12). ***p<0.001 vs. saline. ‡p<0.05 vs. PYY + OXM. ‡‡p<0.01 vs. PYY + OXM.

**Figure 2:** Subjective rating of nausea, as measured by VAS response. Scores depicted as change from baseline value (mm) (n = 12 for each treatment except high dose PYY where n = 3). Black circles = saline; white circles = low dose PYY; black squares = low dose OXM; white squares = high dose OXM; black triangles = PYY + OXM; white triangles = high dose PYY. *p<0.05 vs. saline. Horizontal black bar: infusion duration. Dotted vertical line: meal time.

**Figure 3:** Plasma concentrations of (A) PYY and (B) OLI during study infusions. Black circles = saline; white circles = low dose PYY; black squares = low dose OXM; white squares = high dose OXM; black triangles = PYY + OXM. Horizontal black bar: infusion duration. Dotted vertical line: meal time.
REFERENCES
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**TABLE 1:** Baseline characteristics of participants

<table>
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<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Female / male</td>
<td>7 / 5</td>
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<tr>
<td>Height (m)</td>
<td>1.67 ± 0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.13 ± 3.48</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>30.94 ± 1.03</td>
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<tr>
<td>Fasting insulin (pmol/l)</td>
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</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.96 ± 0.05</td>
</tr>
<tr>
<td>Fasting PYY (pmol/l)</td>
<td>21.6 ± 0.98</td>
</tr>
<tr>
<td>Fasting OLI (pmol/l)</td>
<td>85.1 ± 5.94</td>
</tr>
</tbody>
</table>

Data presented as frequency or mean ± S.E.M.

**TABLE 2:** Summary of infusions

<table>
<thead>
<tr>
<th>Infusion pair designation</th>
<th>Infusion A</th>
<th>Infusion B</th>
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<tr>
<td>saline</td>
<td>saline</td>
<td>saline</td>
</tr>
<tr>
<td>low dose PYY</td>
<td>PYY$_{3-36}$ 0.25 pmol/kg/min</td>
<td>saline</td>
</tr>
<tr>
<td>low dose OXM</td>
<td>saline</td>
<td>OXM 1.5 pmol/kg/min</td>
</tr>
<tr>
<td>high dose PYY</td>
<td>PYY$_{3-36}$ 0.5 pmol/kg/min</td>
<td>saline</td>
</tr>
<tr>
<td>high dose OXM</td>
<td>saline</td>
<td>OXM 3.0 pmol/kg/min</td>
</tr>
<tr>
<td>PYY + OXM</td>
<td>PYY$_{3-36}$ 0.25 pmol/kg/min</td>
<td>OXM 1.5 pmol/kg/min</td>
</tr>
</tbody>
</table>
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