

ORIGINAL ARTICLE

## Inhibition of Food Intake in Obese Subjects by Peptide YY<sub>3-36</sub>

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### ABSTRACT

#### BACKGROUND

The gut hormone fragment peptide YY<sub>3-36</sub> (PYY) reduces appetite and food intake when infused into subjects of normal weight. In common with the adipocyte hormone leptin, PYY reduces food intake by modulating appetite circuits in the hypothalamus. However, in obesity there is a marked resistance to the action of leptin, which greatly limits its therapeutic effectiveness. We investigated whether obese subjects were also resistant to the anorectic effects of PYY.

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#### METHODS

We compared the effects of PYY infusion on appetite and food intake in 12 obese and 12 lean subjects in a double-blind, placebo-controlled, crossover study. The plasma levels of PYY, ghrelin, leptin, and insulin were also determined.

#### RESULTS

Caloric intake during a buffet lunch offered two hours after the infusion of PYY was decreased by 30 percent in the obese subjects ( $P < 0.001$ ) and 31 percent in the lean subjects ( $P < 0.001$ ). PYY infusion also caused a significant decrease in the cumulative 24-hour caloric intake in both obese and lean subjects. PYY infusion reduced plasma levels of the appetite-stimulatory hormone ghrelin. Endogenous fasting and postprandial levels of PYY were significantly lower in obese subjects (the mean  $[\pm SE]$  fasting PYY levels were  $10.2 \pm 0.7$  pmol per liter in the obese group and  $16.9 \pm 0.8$  pmol per liter in the lean group,  $P < 0.001$ ). Furthermore, the fasting PYY levels correlated negatively with the body-mass index ( $r = -0.84$ ,  $P < 0.001$ ).

#### CONCLUSIONS

We found that obese subjects were not resistant to the anorectic effects of PYY. Endogenous PYY levels were low in the obese subjects, suggesting that PYY deficiency may contribute to the pathogenesis of obesity.

**O**BESITY AND ITS ASSOCIATED PATHOLOGIC features are major causes of illness and death worldwide. In the United States, obesity accounts for 280,000 deaths annually, and at current rates of increase it will supplant smoking as the primary cause of preventable death.<sup>1</sup> However, despite the recognition that even moderate weight loss confers significant health benefits,<sup>2</sup> to date there have been few effective treatments for obesity, although surgery has been shown to be of use in selected patients.<sup>3</sup>

We recently showed that infusions of the gut hormone fragment peptide YY<sub>3-36</sub> (PYY) that produced typical naturally occurring postprandial levels reduced 24-hour food intake in human subjects of normal weight.<sup>4</sup> Furthermore, long-term administration of PYY to rodents decreased weight gain. These observations suggest that PYY may be a treatment for obesity. Given, however, that the majority of obese subjects are resistant to the effects of the adipocyte hormone leptin, limiting its effectiveness as an antiobesity treatment,<sup>5,6</sup> we undertook to compare the effects of PYY infusion on appetite and food intake in obese and lean subjects.

## METHODS

### STUDY SUBJECTS

Healthy obese and lean subjects were recruited by advertising in local newspapers and on the Hammersmith Hospital campus in London. The mean ( $\pm$ SE) body-mass index (the weight in kilograms divided by the square of the height in meters) was  $33.0 \pm 0.9$  in the obese group and  $20.5 \pm 0.1$  in the lean group. The inclusion criteria were a body-mass index of 27 to 40 for the obese group and 17 to 23 for the lean group. All subjects were between the ages of 18 and 50 years (mean,  $29.0 \pm 2.4$  for the obese group and  $27.3 \pm 0.4$  for the lean group) and had had a stable body weight for at least three months. The criteria for exclusion were smoking, substance abuse, pregnancy, use of medications (except for oral contraceptives), medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, fasting glucose, and liver function).

Twelve subjects (six men and six women) were recruited for each group. The subjects gave written informed consent for the study, and approval was obtained from the Hammersmith Hospital research ethics committee. The study was carried out in ac-

cordance with the principles of the Declaration of Helsinki. The subjects were screened by a dietitian who assessed their eating behavior with the Dutch Eating Behavior Questionnaire<sup>7</sup> and the Eating Attitudes Test questionnaire.<sup>8</sup> They also completed a three-day diet diary to permit us to assess their usual eating habits before acceptance into the study. Food preferences were assessed at screening with a nine-point hedonic scale to ensure that the food offered at the buffet lunch was acceptable.

### STUDY PROTOCOL

The study was performed in a randomized, double-blind, placebo-controlled, crossover manner, with each subject studied on two occasions one week apart. The subjects' food intake for the 48 hours before each study day was standardized, and during this period they completed food diaries to confirm compliance. In addition, they consumed an identical meal between 7 p.m. and 8 p.m. on the night before each study. The subjects refrained from alcohol and strenuous exercise for the 24 hours before and after each study day. They fasted and drank only water from 8 p.m. the night before the study. They arrived at 8:30 a.m. on each study day. Cannulas were inserted into veins in both forearms, one for the collection of blood and the other for the infusion of PYY or saline. After venous cannulation, the subjects relaxed for 30 minutes before the start of the study protocol. All time cues were removed from the study room, so that the subjects were unaware of the time. Throughout the study, the subjects were encouraged to relax by reading or watching videos.

Blood was collected every 30 minutes throughout the study into heparin-coated tubes (LIP) containing 5000 kallikrein inhibitor units (0.2 ml) of aprotinin (Bayer). Plasma was separated immediately by centrifugation at 4°C and then stored at -70°C until it was analyzed. Basal samples were taken 30 minutes before and at the beginning of the infusion. The subjects received a 90-minute infusion of either saline or PYY (total dose, 2 nmol per square meter of body-surface area). Two hours after the termination of the infusion, the subjects were offered a buffet lunch with food in such excess that all appetites could be satisfied. The amounts of food and water were quantified preprandially and postprandially, and the caloric intake was calculated. Appetite ratings were made on 100-mm visual-analogue scales (higher values indicate greater appetite), with the text expressing the most positive and the most negative ratings anchored at each end.<sup>9</sup> Visual-analogue

scores were used to assess hunger, nausea, and meal palatability. The subjects remained in the study room for six hours after the beginning of the infusion. They completed a food diary until 1 p.m. the following day to allow continued assessment of food intake. The food diaries were analyzed by a dietitian who was unaware of study assignments, and energy intake was calculated with the aid of Dietplan (Forestfield Software).

#### PYY

PYY was obtained from Bachem. The limulus amoebocyte lysate assay test for pyrogen was negative, and the peptide was sterile on culture. PYY was dissolved in 0.9 percent saline (Bayer) containing Haemacel (Beacon) (5 percent by volume) to reduce adsorption to the syringe and tubing.

#### HORMONE ASSAYS

All samples were assayed in duplicate and in one assay to eliminate the effects of interassay variation. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay, as previously described.<sup>10-12</sup> The assay measured both the hormone fragment (peptide hormone YY<sub>3-36</sub>) and the full-length hormone (peptide hormone YY<sub>1-36</sub>); both are biologically active. The antiserum (Y21) was produced in a rabbit against synthetic porcine PYY (Bachem) coupled to bovine serum albumin by glutaraldehyde and used at a final dilution of 1:50,000. This antibody cross-reacts fully with the biologically active circulating forms of human PYY, but not with pancreatic polypeptide, neuropeptide Y, or other known gastrointestinal hormones.

Iodine-125-labeled PYY was prepared by the iodogen method and purified by high-pressure liquid chromatography. The specific activity of the iodine-125-labeled PYY was 54 Bq per femtomole. The assay was performed in a total volume of 700  $\mu$ l of 0.06 M phosphate buffer, pH 7.3, containing 0.3 percent bovine serum albumin. The sample was incubated for three days at 4°C before the separation of free and antibody-bound label by sheep antirabbit antibody. Two hundred microliters of unextracted plasma was assayed. Two hundred microliters of PYY-free, charcoal-stripped plasma was added to standards and other reference tubes to negate any effects of nonspecific assay interference. The assay detected changes of 2 pmol per liter, with an intraassay coefficient of variation of 5.8 percent.

Plasma pancreatic polypeptide, insulin, and glucagon-like peptide 1 (GLP-1) were measured in du-

PLICATE by established in-house radioimmunoassays. The pancreatic polypeptide assay detected changes of 3 pmol per liter, with an intraassay coefficient of variation of 5.4 percent.<sup>13</sup> The GLP-1 assay detected changes of 7.5 pmol per liter, with an intraassay coefficient of variation of 6.1 percent.<sup>14</sup> The insulin assay detected changes of 6.2 pmol per liter, with an intraassay coefficient of variation of 5.4 percent. Plasma ghrelin was measured in duplicate with a Phoenix Pharmaceutical assay kit, and plasma leptin was measured in duplicate with a commercially available assay (Linco Research).

#### STATISTICAL ANALYSIS

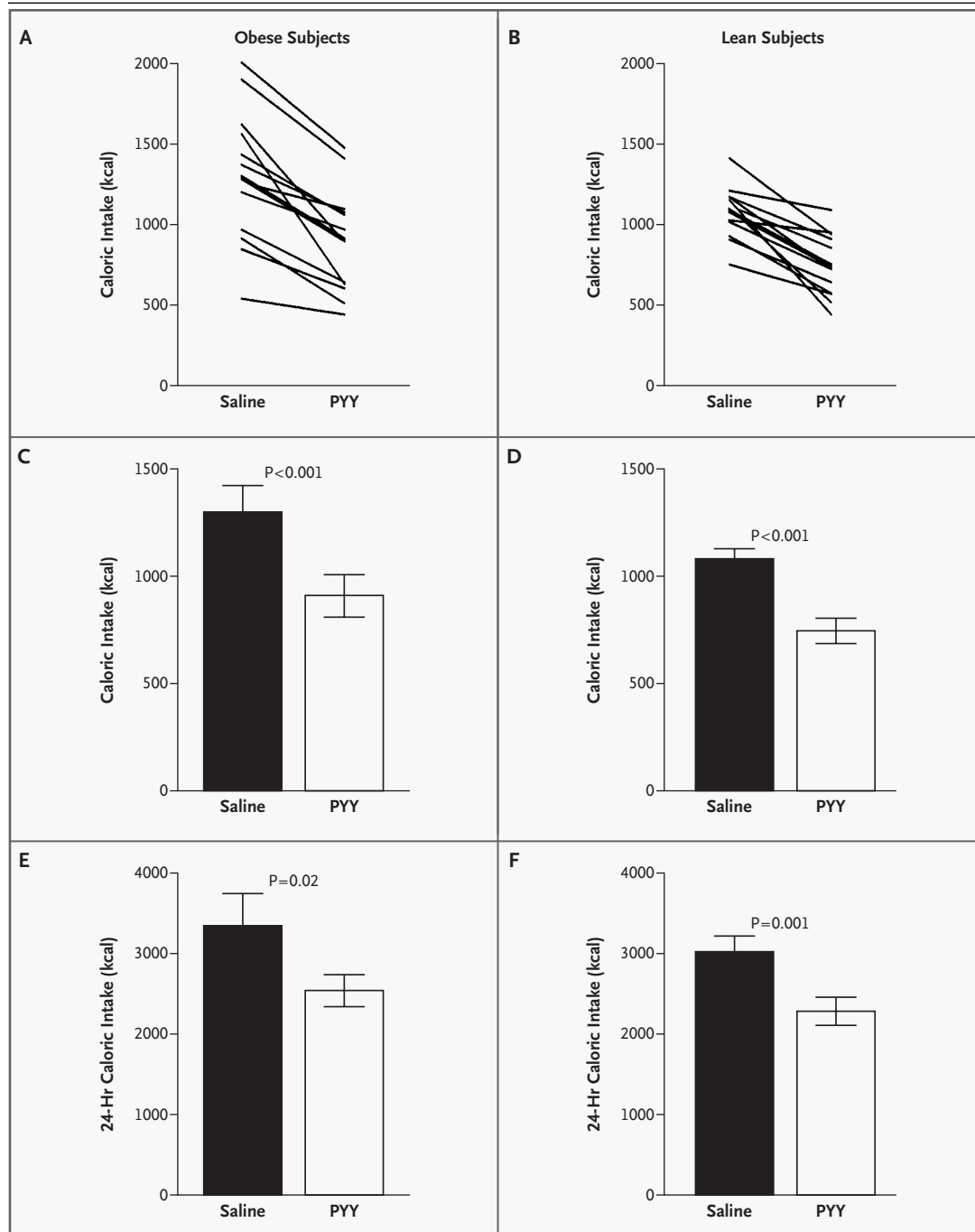
Caloric intake and plasma hormone levels are expressed as means  $\pm$ SE. The integrated area under the curve was calculated with use of the trapezoid rule. Caloric intake and visual-analogue scores within groups were compared by the Wilcoxon signed-rank, matched-pairs test. Areas under the curve and plasma hormone levels in the lean and obese groups were compared by Wilcoxon rank-sum analysis.

## RESULTS

#### EFFECT OF INFUSION OF PYY ON APPETITE AND FOOD INTAKE

PYY infusion reduced the caloric intake of all subjects at the buffet lunch, as compared with their intake after the infusion of saline (Fig. 1A and 1B). In the obese subjects, PYY caused a 29.9 $\pm$ 4.4 percent reduction ( $P < 0.001$ ) (Fig. 1C), and a similar decrease of 31.1 $\pm$ 4.5 percent was observed in the lean subjects ( $P < 0.001$ ) (Fig. 1D). There was no change in the proportion of calories obtained from carbohydrate, protein, or fat. Furthermore, analysis of the food diaries showed a significant inhibition of food intake in the 12-hour postinfusion period in both the obese and the lean subjects. In the obese group, on the day when the saline infusion was given, subjects consumed 2456.0 $\pm$ 256.1 kcal, and on the day when the PYY infusion was given, they consumed 1810.3 $\pm$ 142.6 kcal — a reduction of 26.3 $\pm$ 6.8 percent. In the lean group, on the day when the saline infusion was given, subjects consumed 2312.2 $\pm$ 167.7 kcal, and on the day when the PYY infusion was given, they consumed 1533.0 $\pm$ 140.0 kcal — a reduction of 33.7 $\pm$ 5.6 percent.

However, food intake during the period from 12 to 24 hours after infusion was unaffected by PYY in either group. In the obese group, on the day when they received saline, the subjects consumed 884.9 $\pm$



**Figure 1. Caloric Intake by Obese and Lean Subjects after Infusion of Peptide YY<sub>3-36</sub> (PYY) or Saline.**

Panel A shows the caloric intake by individual obese subjects, and Panel B shows the intake by individual lean subjects, during a buffet lunch two hours after the infusion of PYY or saline. Panel C shows the mean ( $\pm$ SE) caloric intake by obese subjects, and Panel D shows the intake by lean subjects, during a buffet lunch two hours after infusion of saline or PYY. Panel E shows the mean ( $\pm$ SE) cumulative 24-hour caloric intake by obese subjects, and Panel F shows the intake by lean subjects, after infusion of saline or PYY. In all panels, the lean and obese groups each consisted of 12 subjects: 6 women and 6 men.

170.4 kcal, and on the day when they received PYY, they consumed 723.7±118.6 kcal. In the lean group, on the day when they received saline, the subjects consumed 706.5±84.9 kcal, and on the day when they received PYY, they consumed 712.2±84.9 kcal. Overall, PYY significantly reduced 24-hour caloric intake (Fig. 1E and 1F) in both the obese and the lean groups. The reduction was 16.5±6.6 percent in the obese group (P=0.02) and 23.5±5.8 percent in the lean group (P=0.001). PYY infusion reduced hunger, as assessed by visual-analogue scores (Fig. 2), but there was no effect on the palatability of the meal or feelings of nausea (data not shown). The subjects reported no other side effects during or after the PYY infusion.

#### PLASMA LEVELS OF PYY

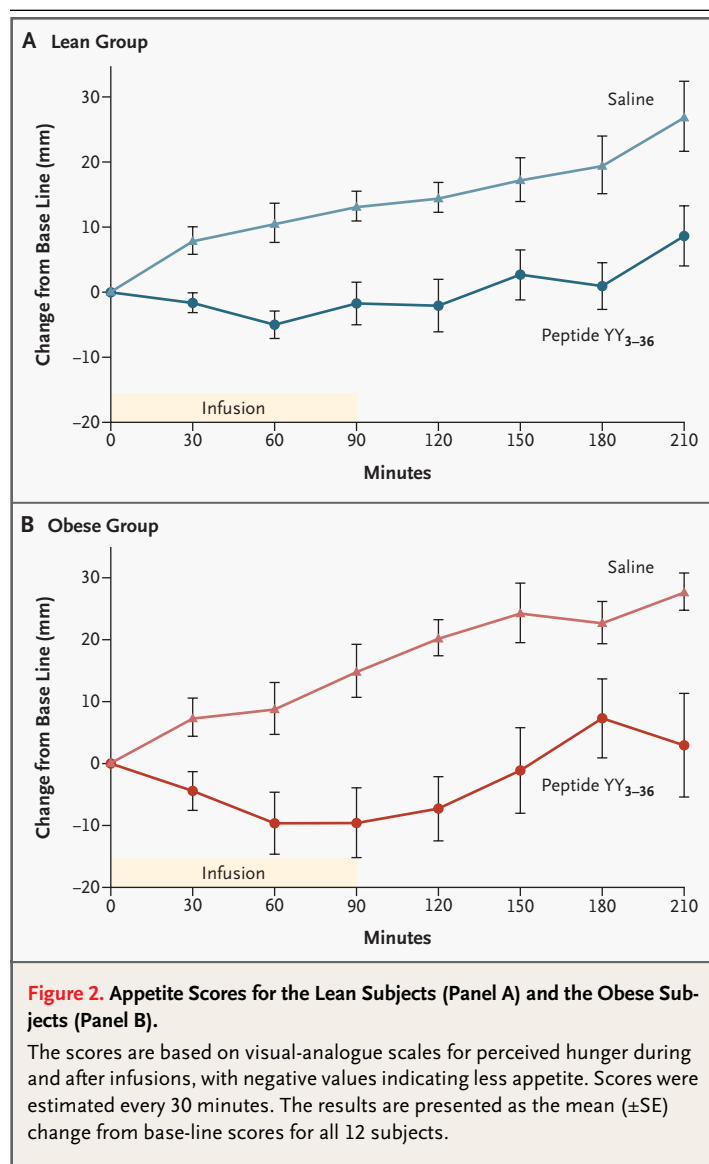
PYY infusion produced similar plasma PYY profiles in the obese and lean groups. The peak PYY level was 57.1±3.8 pmol per liter in the obese group and 53.7±3.5 pmol per liter in the lean group.

Plasma PYY levels were measured in subjects receiving saline to make possible the assessment of fasting and postprandial levels (Fig. 3). The fasting PYY levels were significantly lower in obese than in lean subjects. The base-line PYY levels at the beginning of the infusion were 10.2±0.7 pmol per liter in the obese group and 16.9±0.8 pmol per liter in the lean group (P<0.001). Furthermore, the area under the curve for PYY during the fasting period (from the beginning of the infusion until 210 minutes later) was significantly lower in the obese group than in the lean group: 558.6±43.0 vs. 929.8±47.6 pmol per hour per liter (P<0.001). The fasting PYY levels were negatively correlated with body-mass index ( $r=-0.84$ , P<0.001) (Fig. 4).

The postprandial PYY levels increased in both lean and obese subjects. However, even though they consumed more calories at the buffet lunch, the obese subjects showed a diminished postprandial PYY response as compared with that in the lean subjects. The peak PYY value was 14.4±1.2 pmol per liter in the obese group and 23.5±0.9 pmol per liter in the lean group. In addition, the postprandial area under the curve for PYY (from 210 to 360 minutes after the beginning of the infusion) was significantly less in obese subjects than in lean subjects (562.0±44.6 vs. 841.4±34.9 pmol per hour per liter, P<0.001).

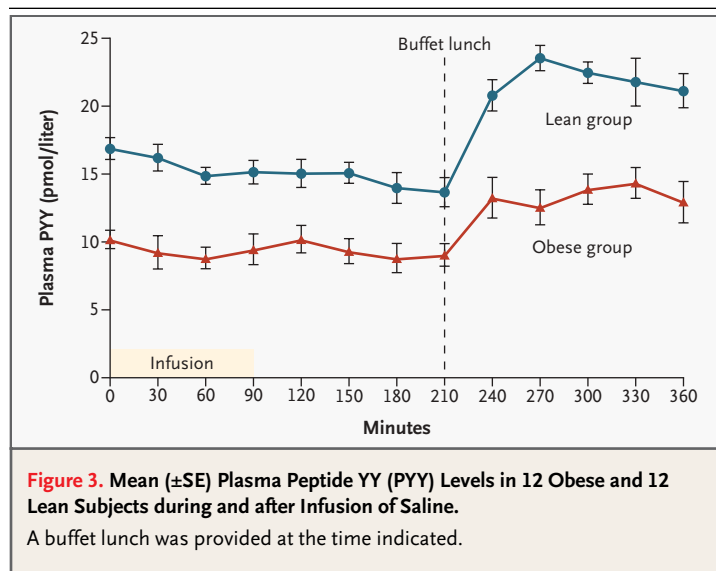
#### PLASMA LEVELS OF GHRELIN

In the lean group, the levels of the appetite-stimulating hormone ghrelin increased throughout the



fasting period on the day the subjects received saline (from 207.7±12.6 pmol per liter at the beginning of the infusion to 247.4±13.8 pmol per liter 210 minutes later) and then fell postprandially to 170.2±13.2 pmol per liter 30 minutes after the meal began (Fig. 5). PYY infusion significantly decreased ghrelin levels during the fasting period and abolished the preprandial rise: the area under the curve for ghrelin from the beginning of the infusion to 210 minutes later was 13,510.6±813.5 pmol per hour per liter on the day they received saline and 11,272.0±724.3 pmol per hour per liter on the day they received PYY (P=0.001).

The ghrelin levels were markedly lower in obese



subjects and, in contrast to the lean subjects, they had no significant preprandial rise in ghrelin levels during the infusion of saline: the ghrelin level was  $87.7 \pm 14.1$  pmol per liter at the beginning of the infusion and  $108.4 \pm 18.7$  pmol per liter 210 minutes later. However, as in the lean subjects, the ghrelin levels decreased postprandially to  $78.2 \pm 12.6$  pmol per liter 30 minutes after the start of the meal, and PYY infusion reduced the fasting ghrelin levels. The area under the curve for ghrelin in the obese subjects from the beginning of the infusion to 210 minutes later was  $5973.2 \pm 1051.4$  pmol per hour per liter on the day they received saline and  $4418.5 \pm 743.0$  pmol per hour per liter on the day they received PYY ( $P=0.02$ ).

#### PLASMA LEVELS OF LEPTIN, INSULIN, PANCREATIC PEPTIDE, AND GLP-1

The fasting plasma leptin levels were significantly higher in the obese group ( $1.98 \pm 0.3$  and  $0.5 \pm 0.1$  nmol per liter for obese and lean women, respectively;  $0.71 \pm 0.2$  and  $0.2 \pm 0.1$  nmol per liter for obese and lean men, respectively) and were unaffected by PYY infusion. Likewise, the fasting insulin levels were higher in obese subjects ( $61.3 \pm 8.5$  pmol per liter in the obese group and  $19.2 \pm 4.3$  pmol per liter in the lean group), and there was no effect of PYY infusion. The fasting plasma levels of GLP-1 were similar in obese and lean subjects ( $14.0 \pm 1.1$  and  $13.9 \pm 1.4$  pmol per liter, respectively) and were not affected by PYY infusion. The fasting levels of pancreatic peptide were similar in obese and lean subjects ( $14.4 \pm$

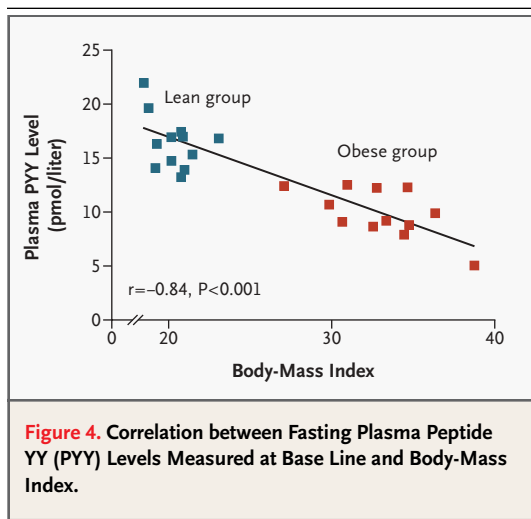
$1.4$  and  $15.6 \pm 1.8$  pmol per liter, respectively) and were unaffected by PYY infusion.

#### DISCUSSION

We have previously found that PYY is released from the gut in proportion to the calories ingested<sup>15</sup> and signals food intake to the appetite-regulating circuits of the brain.<sup>4</sup> Furthermore, PYY infusion reduces food intake in subjects of normal weight, and repeated administration to rodents reduces weight gain.<sup>4</sup> These findings suggest that PYY may be a useful treatment for obesity. PYY acts on the same hypothalamic neural circuits as leptin to regulate food intake.<sup>16,17</sup> However, obesity is associated with resistance to the action of leptin, which greatly limits its therapeutic effectiveness in this condition.<sup>5,6</sup> We therefore studied whether obese subjects responded to the anorectic effects of PYY.

Infusion of PYY caused an equivalent inhibition of appetite and food intake in the obese and lean groups, resulting in reduced cumulative 24-hour food intake. These findings indicate that obesity is not associated with substantial resistance to PYY. We found that fasting PYY levels were lower in the obese group than in the lean group and that there was a negative correlation between fasting PYY levels and body-mass index. Furthermore, postprandial PYY release was lower in obese than in lean subjects, despite the fact that the obese subjects consumed more calories at the buffet lunch. These findings are consistent with the hypothesis that a deficiency in circulating PYY may be involved in the pathogenesis of obesity. It is unclear whether low PYY levels initiate the development of obesity or whether PYY levels are reduced as a result of obesity. PYY appears to be a major factor limiting appetite after meals.<sup>17,18</sup> If obesity is the cause of low levels of circulating PYY, impaired postprandial satiety signaling would result, leading to increased appetite and food intake, which would act to perpetuate the condition.

PYY is synthesized and released from specialized endocrine cells (L cells) that are found primarily in the distal gastrointestinal tract. In response to the ingestion of nutrients, plasma PYY levels increase within 15 minutes, peak at around 60 minutes, and remain elevated for up to 6 hours.<sup>15</sup> The initial increase occurs before nutrients have reached the L cells, implicating a neural or endocrine mechanism. The sustained release is thought to be due to the direct effects of the intraluminal gut contents

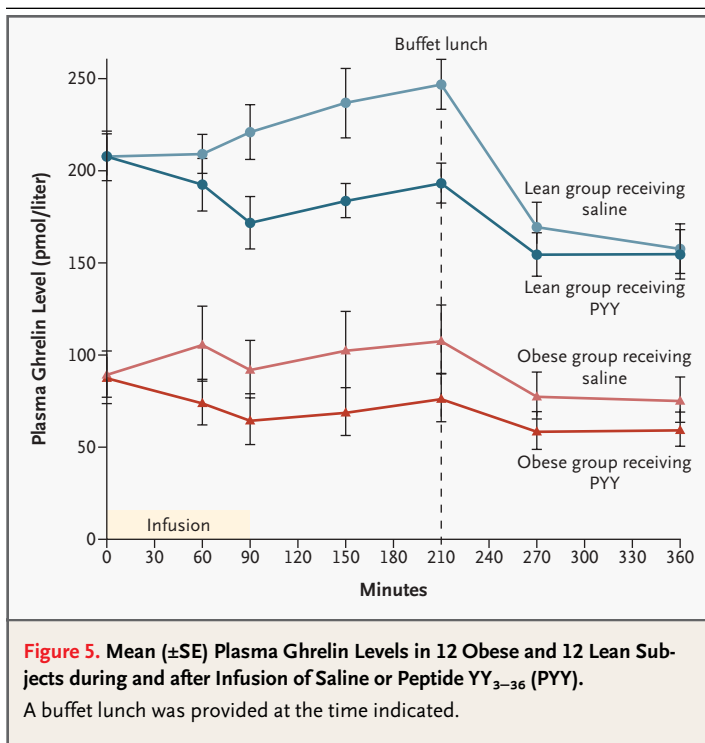


**Figure 4.** Correlation between Fasting Plasma Peptide YY (PYY) Levels Measured at Base Line and Body-Mass Index.

on the L cells.<sup>19</sup> The reduced PYY levels in obesity may result from abnormalities in its synthesis, release, or clearance. Increased clearance is unlikely to have a major role, since after exogenous administration the rate of elimination was similar in the lean and the obese groups. However, the precise factors controlling PYY synthesis and release and the effect of obesity on these remain to be determined.

Our current studies give further insights into the potential mechanisms by which PYY inhibits food intake. Plasma levels of the gut hormone ghrelin rise preprandially,<sup>20</sup> and administration of ghrelin increases food intake in rodents and humans.<sup>21-23</sup> These findings suggest that ghrelin has a role in the regulation of meal initiation.<sup>20</sup> In our current study of both lean and obese subjects, PYY infusion significantly decreases plasma ghrelin levels. This suppression may add to the effects of PYY on appetite reduction.

Currently, surgery is the only effective treatment for morbid obesity (body-mass index, >40).<sup>3,24</sup> Recently, gastric-bypass surgery has been shown to be associated with low ghrelin levels, which may be re-



**Figure 5.** Mean ( $\pm$ SE) Plasma Ghrelin Levels in 12 Obese and 12 Lean Subjects during and after Infusion of Saline or Peptide YY<sub>3-36</sub> (PYY).

A buffet lunch was provided at the time indicated.

sponsible for the reduced appetite associated with this procedure.<sup>25</sup> Interestingly, PYY levels have been shown to be elevated in patients after jejunioleal-bypass surgery, suggesting that increased PYY levels may also contribute to the reduced appetite and food intake observed in these patients.<sup>26</sup>

In summary, we have demonstrated that PYY levels are low in obesity, suggesting that PYY may be involved in the pathogenesis of this condition. Furthermore, the administration of PYY reduces appetite and food intake in normal and obese subjects. Thus, the administration of exogenous PYY or stimulation of the release of endogenous PYY may be an attractive therapeutic option for obesity.

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