Physiology and pathophysiology of the ileal brake in humans

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Chapter 4

EFFECT OF THE ILEAL BRAKE ON SATIETY AND PROXIMAL GASTRIC FUNCTION: IS IT PEPTIDE YY?

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ABSTRACT

Recent studies indicate that peptide YY (PYY) influences satiety, but the role of PYY as a physiological regulator of satiety is under debate. This study was designed to compare the effects of ileal brake activation through ileal fat perfusion (endogenous PYY) versus exogenous PYY infusion on satiety and proximal gastric motor function. Two protocols were performed in both fasting and postprandial states. Protocol 1: ileal fat perfusion versus saline (control). Protocol 2: intravenous PYY_{3-36} infusion at low and high doses of 15 and 30 pM/kg/hr vs. placebo. Plasma PYY (RIA), satiety scores and proximal gastric motility (barostat) were measured. *Results:* Ileal fat significantly ($p<0.05$) increased plasma PYY from 15 ± 1 to 26 ± 2 pM. During PYY infusion plasma levels of 28 ± 3 pM (low dose) and 55 ± 10 pM (high dose) were reached. Both in the fasting and postprandial state ileal fat induced satiety ($p<0.05$) in contrast to PYY_{3-36}. Fasting gastric volume (barostat) increased significantly ($p<0.01$) in response to ileal fat (from 150 ± 14 ml to 433 ± 54 ml) but not in response to PYY_{3-36} infusion. In all experiments meal ingestion resulted in identical increments in proximal gastric volume. Only ileal fat, not PYY_{3-36} significantly ($p<0.01$) enhanced postprandial gastric relaxation. *Conclusions:* Ileal fat induces satiety and results in proximal gastric relaxation, in contrast to exogenous PYY_{3-36} at identical plasma levels. These data do not support a role for PYY as a physiological mediator in ileal brake induced satiety or ileal brake induced proximal gastric relaxation.

INTRODUCTION

Feelings of hunger and satiety are associated with gastrointestinal signals. Stimulation of gastric mechanoreceptors through balloon distension results in relaxation of the proximal stomach and is associated with feelings of fullness and a reduction in hunger and wish to eat (1-3). After meal ingestion the proximal stomach is able to accommodate a large volume meal. In patients with early satiety type dyspepsia the accommodation response of the proximal stomach is impaired (4). Not only mechanical but also chemical stimulation results in gastric relaxation and induces satiety. For instance, duodenal fat induces relaxation of the proximal stomach and results in feelings of fullness and satiation (5).

In the last decade evidence has become available indicating that not only the proximal gut but also the distal gut participates in the regulation of gastrointestinal motor and sensory functions and satiety. Perfusion of the ileum with nutrients delays gastric emptying, prolongs small intestinal transit time and inhibits pancreatico biliary secretion (6-8). This phenomenon, called the “ileal brake”, is a negative feedback loop from the distal to the proximal gut. There is evidence suggesting that the ileal brake is mediated through hormonal factors. Peptide YY (PYY) is considered an important mediator of the ileal brake (6,9). This 36-amino acid peptide first isolated from pig intestine, is localized in the endocrine cells of the ileal, colonic and rectal mucosa (10,11). The number of PYY secretory cells increases going more distally in the gut (11). PYY is released into the circulation in response to meals. The postprandial increase in plasma PYY levels is proportional to meal size with a peak response about an hour after food ingestion (11). In

both animals and humans intravenous infusion of PYY delays gastric emptying and inhibits pancreatico biliary secretion (12,13). Recently evidence has become available suggesting that PYY has an important role in satiety and eating behaviour. Batterham *et al* demonstrated that PYY_{3-36}, when infused intravenously reduced food intake in both humans and rodents (14,15). More recently Degen *et al* also found that intravenous infusion of PYY_{3-36} reduced food intake but only at supraphysiological plasma PYY levels (16). The physiological role of PYY in the regulation of satiety therefore remains to be defined. Furthermore, up till now, little is known about the effect of ileal brake activation and PYY release on proximal gastric motor function

The present study was therefore performed to compare the effects of ileal brake activation with ileal fat (endogenous PYY release) versus exogenous PYY_{3-36} infusion on satiety and on proximal gastric motor function. We therefore used two experimental protocols. In the first protocol we studied the effect of ileal fat and subsequent endogenous PYY release and in the second protocol we investigated the dose-response relationship of exogenous PYY. In both protocols satiety and motor and sensory function of the proximal stomach were monitored. PYY was infused at doses reaching plasma PYY levels comparable to those obtained during ileal fat perfusion.

SUBJECTS AND METHODS

Subjects

Fourteen healthy subjects (6 male; 8 female; mean (±SEM) age 29±3 year; mean (±SEM) BMI 22±3) without a history of gastrointestinal symptoms or

abdominal surgery participated in the study. For each experiment 8 subjects were studied. Two subjects participated in both experiments. None of them were taking any medication. Informed consent was obtained from each individual and the protocol had been approved by the ethics committee of the Leiden University Medical Center.

Gastric barostat

An electronic barostat (Medtronic Visceral Stimulator; Medtronic, Skovlunde, Denmark) was used to distend the stomach. A polyethylene bag (1000 mL maximum capacity) was tied to the end of a multilumen tube (16 French). This catheter was connected to the barostat. The barostat keeps the pressure in the intragastric bag at a preselected level. When the stomach relaxes, the system injects air. When the stomach contracts, the system aspirates air. Thus, the barostat measures gastric motor activity as changes in intragastric volume at a constant intragastric pressure (17).

Pressure (mmHg) and volume (ml) are constantly monitored and recorded on a personal computer connected to the barostat. On the day of the experiments, subjects were intubated with the barostat catheter through the mouth into the fundus. To unfold the bag, air was inflated to a volume of 200 ml and the catheter was carefully pulled back until its passage was restricted by the oesophageal sphincter. Then the tube was introduced another 2 cm. The correct position was checked under fluoroscopy at the start and the end of each experiment.

Study design

Experiment 1: ileal fat

Two experiments were performed in random order on two consecutive days in a double blind manner. The experiments started at 7:45 AM.

Day 0: Catheter intubation

In the morning subjects were intubated transnasally with an ileal catheter. The catheter (outer diameter 4 mm; length 350 cm) consists of a central perfusion port, a stainless steel tip weight and a distal inflatable balloon. Once the tip had passed the ligament of Treitz, the distal balloon was inflated with 10 ml air to facilitate further progression of the tube through the small intestine. Progression of the tube through the gut was monitored by fluoroscopy. The tip of the tube with the central perfusion port was located in the ileum. The time required for the tube to reach the distal ileum varied between 8-22 hours. Correct position was verified by fluoroscopy on day 0 and at the start and end of day 1 and 2.

Day 1 and day 2: ileal saline or fat

After an overnight fast subjects were intubated with the barostat catheter with bag through the mouth into the fundus, as described previously. An intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. During measurements subjects were seated in a comfortable lying chair in a semi-recumbent position with the lower extremities just above abdominal level.

A commercially available fat emulsion (Intralipid 20%; Pharmacia & Upjohn BV, Woerden, The Netherlands) was used to perfuse the ileum. Intralipid

20% consists of 20 g soybean oil, 1.2 g purified egg phospholipids, and 2.2 g glycerol anhydrous per 100 ml. The perfusion rate was 1 ml/min (2 kcal/min). In random order either Intralipid 20% or placebo (saline 0.9%), was given into the ileum on day 1 and day 2.

A 200 ml liquid meal (Nutridrink; Nutricia Zoetermeer, Holland) containing 10g protein, 36g carbohydrates and 13g fat was used as test meal (300 kcal).

The following procedures with the barostat were performed:

1- **Minimal distending pressure (MDP)** was determined by stepwise increasing pressures in steps of 1 mmHg every 90 sec from 0 mmHg until. MDP was defined as the first pressure level at which the intragastric bag volume was more than 30 ml.
2- **Barostat procedure**: The barostat was set at a pressure of MDP+2 mmHg. The basal intragastric bag volumes were measured during the first 15 min. Then ileal infusion of either saline or fat was started. The intragastric bag volumes were continuously measured for 60 min after the start of the ileal perfusion.
3- **Recovery period** of two hours. During this period the ileum was perfused with saline at a rate of 1ml/min.
4- **Isobaric distension**: Stepwise increasing bag pressures in steps of 1 mmHg every 90 sec from 0 mmHg to a maximum of 14 mmHg or when a maximum bag volume of 750 ml was reached. Thereafter the intragastric bag was deflated.
5- **Recovery period** of three hours. During this period the ileum was perfused with saline at a rate of 1ml/min
6- **Meal:** The barostat was set at a pressure of MDP+2 mmHg. After 15 min recording under fasting conditions, the liquid test meal was ingested

within 3 min. At the start of meal ingestion ileal infusion of either saline (1 ml/min) or fat was also started. Measurements were continued for 90 min after the start of the ileal perfusion and meal ingestion.

Experiment 2: PYY_{3-36} infusion

The three experiments were performed in double-blind randomized order. The experiments were separated by intervals of at least 7 days. Each subject received an intravenous infusion of: A) saline, B) PYY_{3-36} at a dose of 15 pM/kg/hour and C) PYY_{3-36} at a dose of 30 pM/kg/hour. PYY_{3-36} was purchased from Clinalfa, Switzerland. The doses of PYY_{3-36} we choose were based on results of previous studies (11,18,19). The low dose results in plasma PYY levels seen after ingestion of a regular meal, whereas the high dose results in plasma PYY levels that have been observed after meal ingestion in patients with malabsorptive disorders. On the day of the experiments, after an overnight fast, subjects were intubated with the barostat catheter with bag through the mouth into the fundus as described previously. A 200 ml liquid meal (Nutridrink; Nutricia Zoetermeer, Holland) containing 10g protein, 36g carbohydrates and 13g fat was used as test meal (300 kcal).
The following procedures with the barostat were performed (for details see experimental protocol 1):

1- **Minimal distending pressure (MDP)**.
2- **Barostat procedure**: The barostat was set at a pressure of MDP+2 mmHg. The basal intragastric bag volumes were measured during the first 15 min. Then intravenous infusion with either saline or PYY_{3-36} was started. The intragastric bag volumes were continuously measured for 60 min.

3- **Recovery period (15 min)**
4- **Isobaric distention**: Stepwise increasing pressures in steps of 1 mmHg every 90 sec from 0 mmHg to a maximum of 14 mmHg or when a maximum bag volume of 750 ml was reached.
5- **Recovery period (15 min)**
6- **Meal:** The barostat procedure was started at a pressure of MDP+2 mmHg. After 15 min recording under fasting conditions, the test meal was ingested within 3 min. At the start of meal ingestion intravenous infusion with either saline or PYY_{3-36} was also started. Measurements were continued for 90 min after the start of intravenous PYY infusion and meal ingestion.

Perception scores

Subjective feelings of fullness, hunger, desire to eat and nausea were scored at 15 min intervals throughout experiment 1 and 2 using 100 mm visual analogue scales (VAS).

Plasma PYY

Blood samples for measurement PYY were drawn at time t=-15, 0, 15, 30, 45, 60 min during the fasting barostat procedure and at time t=-15, 0, 15, 30, 45, 60, 75 and 90 min after meal ingestion. Plasma PYY was measured by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM Biochemica GmbH, Switzerland). PYY was labeled with ^{125}Iodine with chloramine T. The assay is highly specific. There is no cross-reactivity with PP or VIP. The detection limit is 10 pM/l. Both $PYY_{(1-36)}$ and $PYY_{(3-36)}$ bind to the antibody in dilutions up to

250000.

Data analysis

Intragastric volumes measured while pressure was set at MDP+2 mmHg are given as average values over 5 min periods. Intragastric volumes during the isobaric distension were determined as mean volumes during the last 60 sec of each pressure step. Perception scores during volume measurements at set pressure (MDP+2 mmHg) were calculated relative to the perception scores obtained at 0 min, immediately before the onset of the infusion. Perception scores obtained prior to the meal were used as zero reference points for the calculation of postprandial perception scores.

Statistical analysis

Results are expressed as mean±SEM. All data between and within groups were analysed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman Keuls analyses were performed to determine which values between or within groups differed significantly. Coefficient of linear correlation (Spearman) was used to calculate correlations between intragastric volumes, plasma PYY levels and perception scores. The significance level was set at $p<0.05$.

RESULTS

Experiment 1: ileal fat

Barostat, fasting

The MDP was not significantly different between the ileal saline (6.0±0.4 mmHg) and the ileal fat (6.1±0.9 mmHg) experiment. Intragastric volumes at MDP+2 mmHg before the start of ileal infusion at time 0 min were similar between the ileal saline (149±14 ml) and the ileal fat experiment (148±23 ml). Intragastric volumes increased significantly ($p<0.01$) during ileal fat perfusion compared to basal and compared to the ileal saline experiment from time t=10 min until the end of the procedure at time t=60 min (Figure 1, left panel).

Stepwise isobaric distension resulted in progressive increments in intragastric bag volume in both the saline and the ileal fat experiments. Intragastric volumes during pressure distension from level 6-11 mmHg were significantly ($p<0.01$) higher in the ileal fat compared to the ileal saline experiment (Figure 2; left panel).

Barostat, postprandial

Basal intragastric volumes at MDP+2 mmHg were not significantly different between the ileal saline (142±10 ml) and the ileal fat experiment (146±13 ml). After meal ingestion, intragastric volumes increased significantly ($p<0.01$) in both the experiments (Figure 3, left panel). In the ileal saline experiment, intragastric volumes reached a maximal volume at 15 min after meal ingestion and gradually returned to basal value at the end of the postprandial

period. On the other hand, intragastric volumes in the ileal fat experiment remained significantly ($p<0.01$) increased over basal during the 90 min postprandial period. Furthermore, intragastric volumes in the ileal fat experiment were significantly ($p<0.01$) higher compared to those in the ileal saline experiment during the period from 15 min until 90 min postprandially.

Satiety, fasting

During ileal fat perfusion, fullness increased significantly ($p<0.05$) over basal starting from 15 min while no significant changes were observed in the ileal saline experiment (Figure 4, upper left panel). Scores of hunger (Figure 4, lower left panel) and desire to eat (data not shown) were significantly ($p<0.05$) decreased in the ileal fat compared to the ileal saline experiment. Nausea scores were not affected.

Satiety, postprandial

In both the ileal saline and fat experiment, scores of fullness increased significantly ($p<0.01$) over basal value starting from 15 min after meal ingestion (Figure 5, upper left panel). In the saline experiment the perception of fullness gradually returned to basal values. The perception of fullness during ileal fat perfusion, on the other hand, remained significantly ($p<0.01$) increased compared to the basal value and compared to the saline experiment. Perception scores of hunger (Figure 5, lower left panel) and desire to eat (data not shown) were significantly ($p<0.05$) decreased in the ileal fat experiment compared to the saline experiment and compared to baseline values. Nausea was not affected (data not shown).

Plasma PYY, fasting

Basal plasma PYY levels were not significantly different between the ileal saline (13±1 pM) and the ileal fat experiment (15±1 pM). Plasma PYY levels gradually increased during ileal fat perfusion and were significantly ($p<0.05$) increased over basal from 30-60 min after the start of ileal fat perfusion (26±2 pM at 60 min). No significant changes compared to basal were observed in the ileal saline experiment (Figure 6, left panel).

Plasma PYY, postprandial

Plasma PYY levels before meal ingestion were not significantly different between the ileal saline (15±2 pM) and the ileal fat experiment (16±1 pM). Plasma PYY levels in response to meal ingestion were significantly ($p<0.05$) increased over basal from 15 min to 45 min in the ileal saline experiment. Ileal fat infusion resulted in significantly ($p<0.01$) higher plasma PYY levels compared to those in the ileal saline experiment during the period from 15 to 90 min (Figure 7, left panel).

Correlations

Perception of fullness at t= 60 min during fasting correlated significantly with gastric bag volume ($r=0.5$; $p=0.05$). In addition, postprandial fullness at t= 90 min also correlated significantly with postprandial gastric bag volume ($r=0.4$; $p=0.05$). Postprandial perception of hunger was significantly inversely correlated with gastric volume ($r=-0.6$; $p=0.04$). No significant correlations were found between satiety scores and plasma PYY levels neither in the fasting nor in the fed state. The correlation between plasma PYY and postprandial gastric volume ($r=0.4$; $p=0.09$) was not statistically significant.

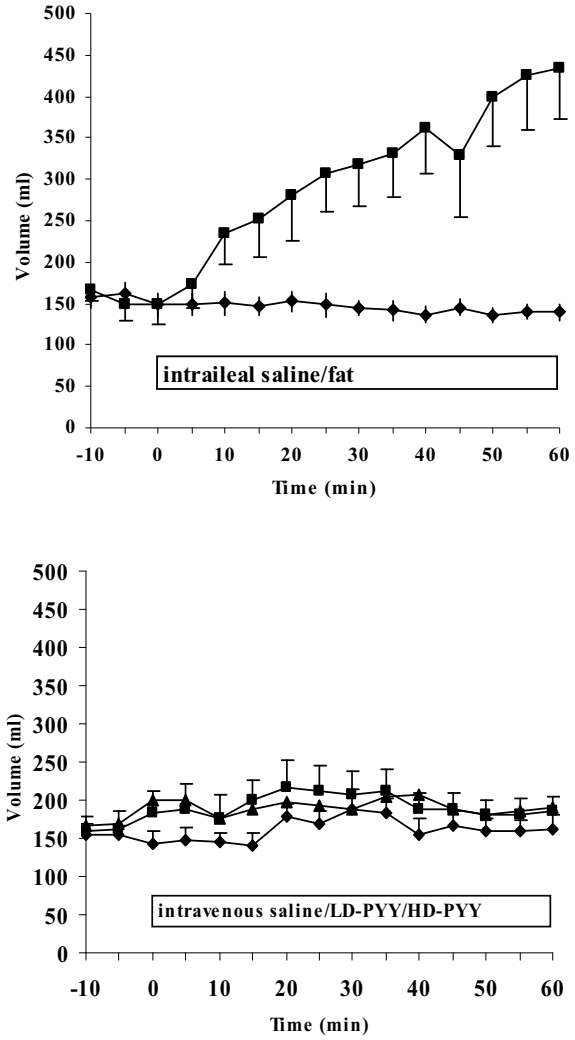

Figure 1*: Intragastric bag volume at MDP+2 mmHg. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

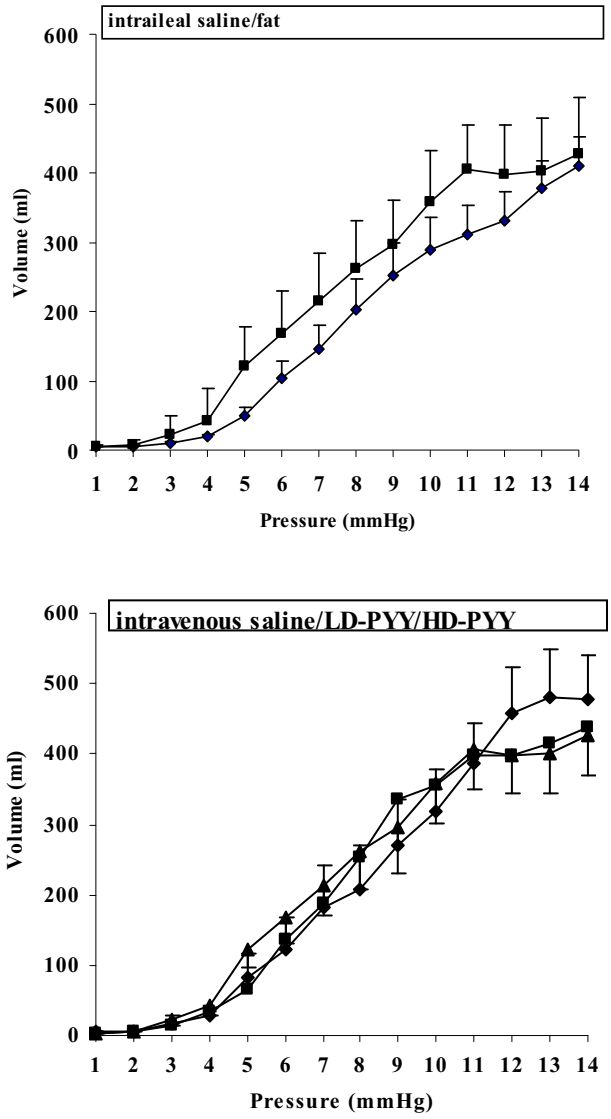

***Figure 2**: Intragastric bag volume in response to stepwise pressure distension. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

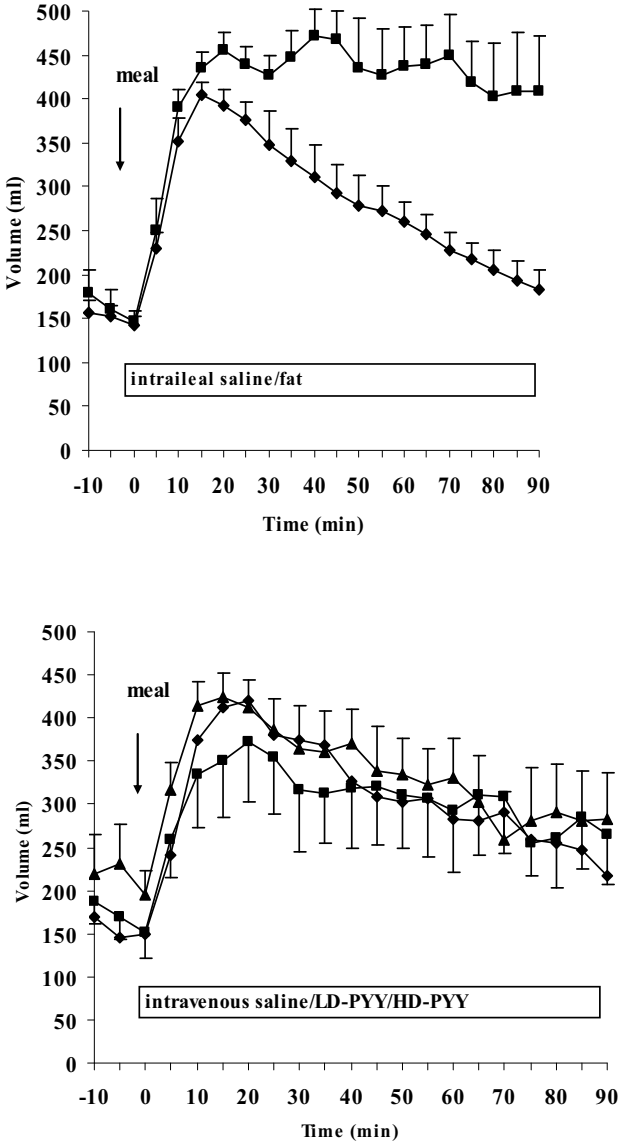

***Figure 3**: Intragastric volume at MDP+2 mmHg in response to meal ingestion. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

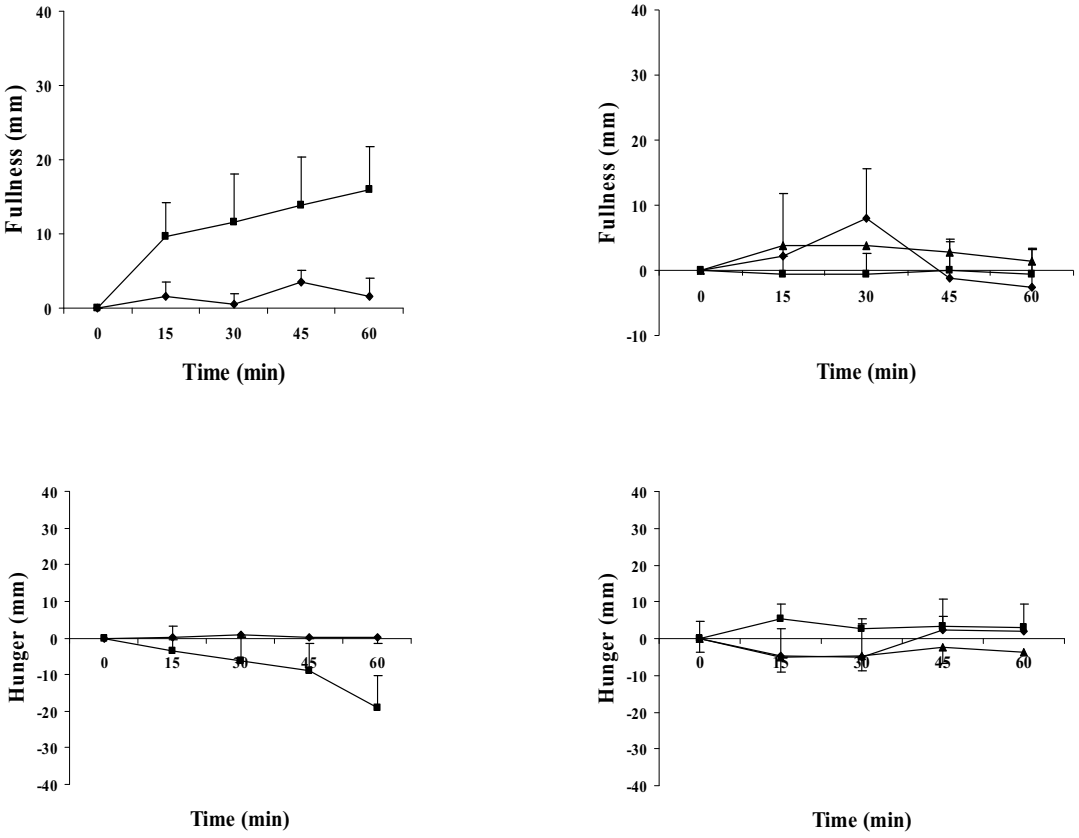

***Figure 4**: Satiety score of fullness and hunger during fasting. Left panel: during ileal perfusion of saline (diamonds) and fat (squares). Right panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

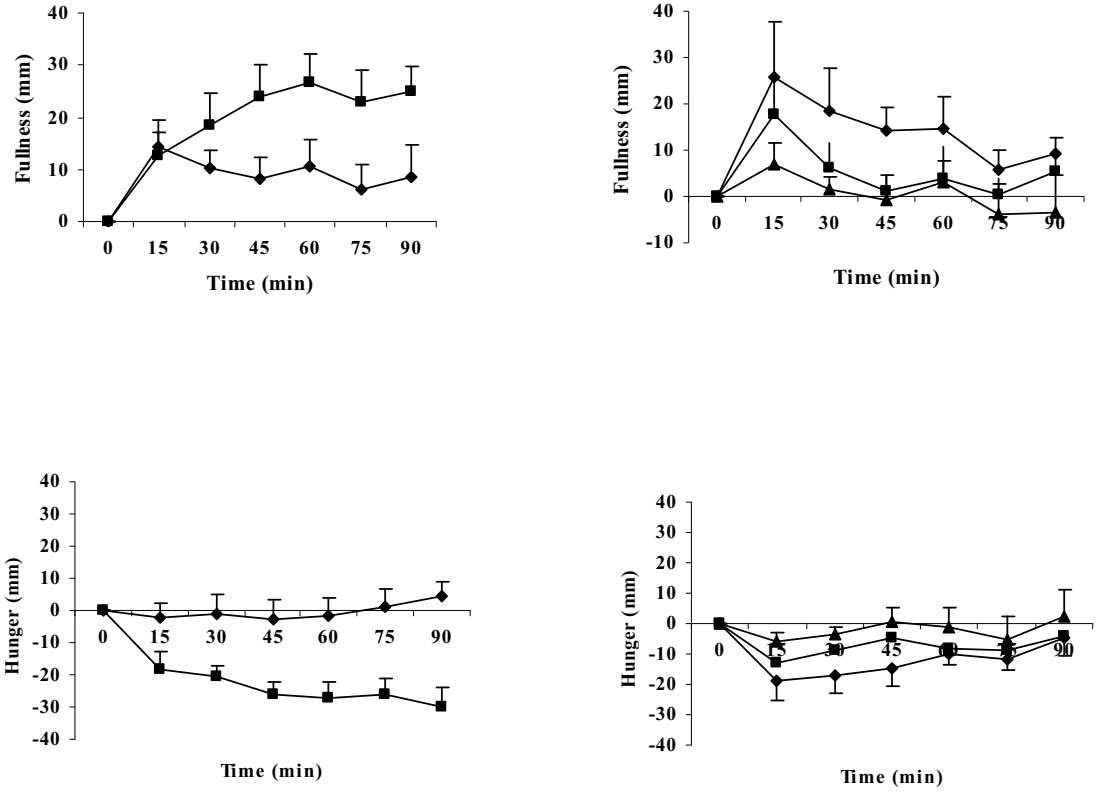

Figure 5*: Satiety score of fullness and hunger in response to meal ingestion. Left panel: during ileal perfusion of saline (diamonds) and fat (squares). Right panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

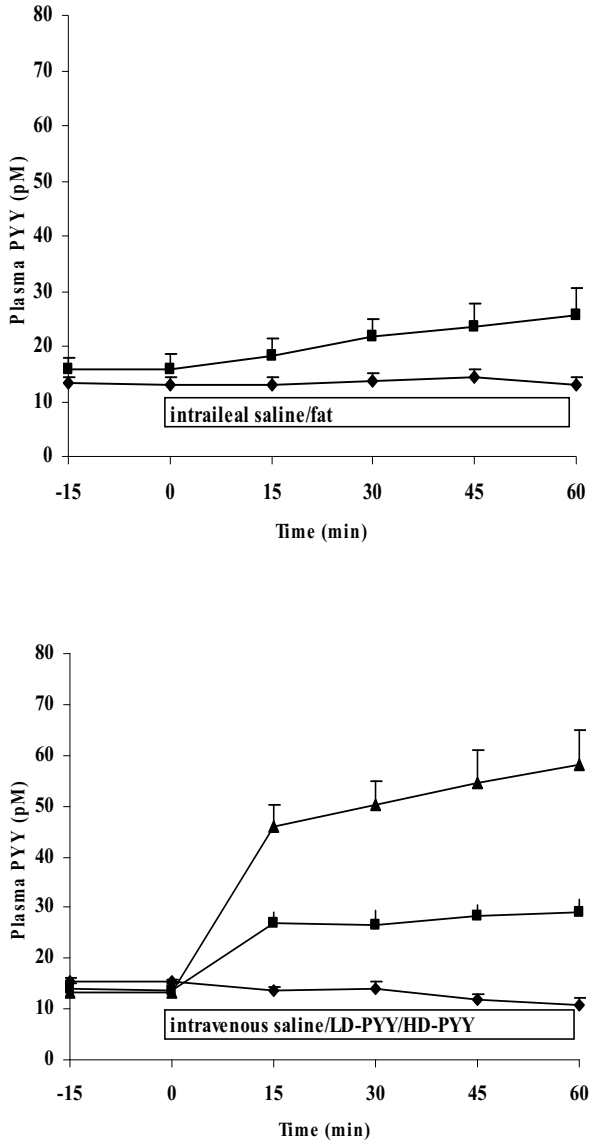

***Figure 6**: Plasma PYY level during fasting. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

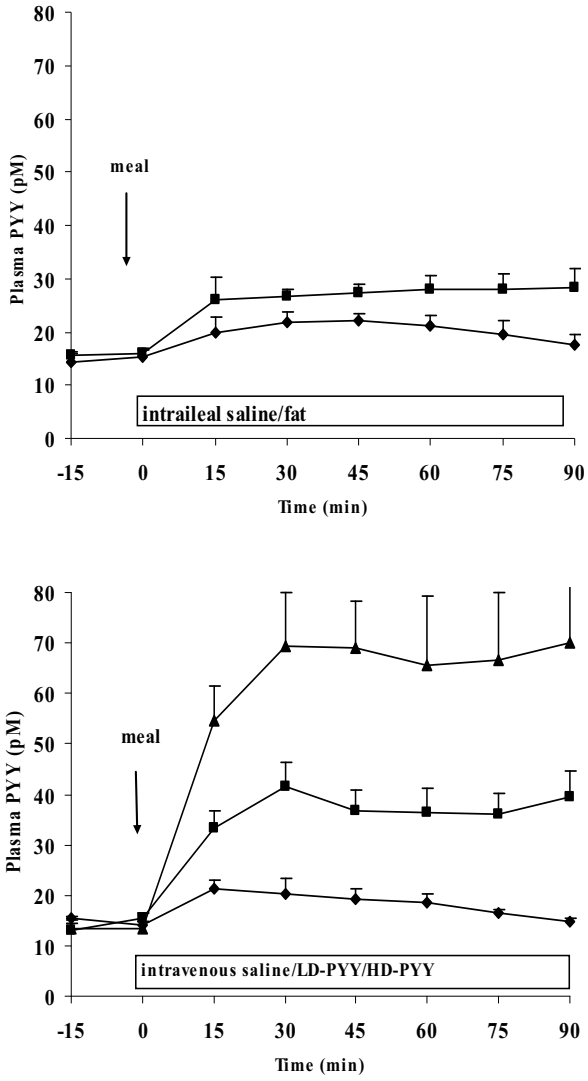

***Figure** 7: Plasma PYY level in response to meal ingestion. Left panel: during ileal perfusion of saline (diamonds) and fat (squares). Right panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean±SEM.*

Experiment 2: PYY_{3-36} infusion

Barostat, fasting

The MDP was not significantly different between the saline (6.4±0.6 mmHg), the low dose PYY (6.8±0.5 mmHg) and the high dose PYY experiment (6.5±0.7 mmHg). Intragastric volumes at MDP+2 mmHg before the start of the infusion were similar between the saline (155±14ml), the low dose PYY (159±18 ml) and the high dose PYY experiment (167±22 ml). During PYY infusion intragastric volume did not change significantly (Figure 1, right panel). Stepwise isobaric distension resulted in progressive increments in intragastric bag volume in all experiments. No significant differences in intragastric bag volume were observed between the saline, the low dose and the high dose PYY experiment (Figure 2, right panel).

Barostat, postprandial

Basal intragastric volumes at MDP+2 mmHg were not significantly different between the saline (148±24 ml), the low dose PYY (151±30 ml) and the high dose PYY experiment (195±27 ml). After meal ingestion, intragastric volumes increased significantly ($p<0.01$) in all experiments. No significant differences in intragastric bag volume were observed between the saline, the low dose PYY and the high dose PYY experiment (Figure 3, right panel).

Satiety, fasting

During PYY infusion scores of fullness, hunger, desire to eat and nausea did not change significantly over basal. No significant differences in perception scores were observed between the three experiments (Figure 4, right upper and lower panel).

Satiety, postprandial

After meal ingestion scores of fullness, hunger, desire to eat changed significantly ($p<0.05$) at time 15 min compared to basal. Nausea was not affected. No significant differences in fullness, hunger, desire to eat or nausea were observed between the three experiments (Figure 5, right upper and lower panel).

Plasma PYY, fasting

Basal plasma PYY levels before the start of infusion were not significantly different between the three experiments, respectively 16±1 pM in the saline experiment, 14±1 pM in the low dose PYY experiment and 13±2 pM in the high dose PYY experiment. During infusion of PYY, plasma PYY levels increased significantly ($p<0.01$) over basal and compared to the saline experiment (Figure 6, right panel). Note that the plasma PYY levels obtained during low dose PYY infusion are in the range of those reached with ileal fat (25-30 pM). The plasma levels obtained during high dose PYY infusion are in the range of those reached after meal ingestion in patients with maldigestion that is 50-60 pM.

Plasma PYY, postprandial

Meal ingestion resulted in significantly ($p<0.05$) increases in plasma PYY levels during saline infusion from 15 to 60 min (Figure 7, right panel). Plasma PYY levels during intravenous infusion of both the low dose PYY and high dose PYY were significantly higher compared to the control experiment. Plasma PYY levels of the high dose PYY experiment were significantly ($p<0.01$) higher compared to those of the low dose PYY experiment.

DISCUSSION

Recently much attention has been given to PYY as potential anorexogenic substance. PYY is mainly present in the ileocolonic region expressed by endocrine L cells and secreted in response to nutrient ingestion (11). PYY immunoreactivity has also been reported to be present in the central nervous system. Animal studies indicate that PYY can transmit signals via central Y2 receptors to which PYY binds with high affinity (20). Several animal experiments have shown that truncated PYY_{3-36} reduces food intake and impairs a gain in body weight (14,21). Recently, human studies with PYY infusion have obtained similar results: infusion of PYY_{3-36} reduced food intake and appetite not only lean but also in obese subjects (15). Subsequent studies failed to reproduce the anorexogenic effect of PYY_{3-36} (22). More recently, Degen et al again attempted to solve the issue by performing classical experiments with graded doses of PYY infusion reaching plasma levels in the physiological range (16). These authors clearly observed an anorexogenic effect of PYY because food intake was decreased by 30%. It should be noted that this effect was present only at supraphysiological plasma PYY levels.

The design we have chosen was based on the following research question: compare effects of exogenous PYY infusion and of ileal brake activation (endogenous PYY) by aiming at comparable plasma PYY levels with satiety (fasted, fed) and proximal gastric motor function as parameters. In doing so we have shown that in healthy volunteers: 1) ileal fat induces satiety in the fasting state and increases satiety in the fed state 2) ileal fat significantly increases proximal gastric volume and enhances postprandial proximal gastric relaxation and 3) exogenous PYY_{3-36} infusion did not affect satiety nor

proximal gastric motor and sensory function.

Our findings that ileal fat significantly increased gastric compliance and fasting gastric volume are in agreement with a previous study in dogs demonstrating that nutrients in the distal small bowel elicit a gastric relaxatory response (23). Several studies have shown that ileal fat activates the so called ileal brake with subsequent inhibition of gastric emptying and delay in intestinal transit (6,7). An increased postprandial fundic relaxation may contribute to the delay in gastric emptying induced by ileal fat since the proximal stomach accommodates food after meal ingestion and regulates the transfer of food to the distal stomach. It is not clear by which mechanism(s) the ileal fat induced gastric relaxatory response is mediated. Several distal gut hormones such as PYY, GLP-1 and enteroglucagon may be involved. PYY is considered to be an important hormonal mediator of the ileal brake. In humans, i.v. administration of exogenous PYY delays gastric emptying (12). In response to ileal fat, endogenous PYY is released resulting in higher plasma levels (6). In the present study infusion of PYY_{3-36}, to plasma levels reached during ileal brake activation did not result in changes in gastric volume neither in the fasting nor in the fed state. These findings do not support a role for PYY as mediator of ileal fat induced gastric relaxation and accommodation. Gastric emptying results from coordinated motor activities of different parts of the stomach, pylorus and duodenum. It is therefore conceivable that other factors apart from proximal gastric tone may contribute to delayed gastric emptying induced by PYY. To date, it is not known whether PYY affects antral, pyloric or duodenal motility. Not only hormonal but also neural factors may be involved in the gastric relaxatory response to ileal fat, for instance vagal afferent neural reflexes triggered by luminal osmo-

and chemosensitive receptors (24,25).

We have demonstrated that ileal fat induces satiety. We did not assess food intake, but quantified sensations related to eating behaviour such as fullness, hunger and desire to eat in both the fasting and the fed state. The finding of ileal fat induced satiety can be explained in a number of ways. First, satiety may have been induced by ileal fat through an increase in gastric volume. It is known that afferent fibers of the vagus nerve express mechanoreceptors (stretch receptors) which are sensitive to volume and to luminal pressure (26). The activated vagus then in turn activates centers in the brainstem, eliciting reflexes that control satiety and eating behaviour. In support of this concept is the significant correlation we observed between sensations of fullness, hunger and intragastric bag volumes. In addition, previous studies have shown that gastric distension is one of the most powerful triggers that decrease hunger (27,28). Penagini *et al* found a significant inverse correlation between postprandial hunger and proximal gastric volume and the increase in sensation of fullness paralled the increase in gastric volume in patients with reflux disease (29). Second, satiety induced by ileal fat can be mediated by gut peptides through endocrine, paracrine or neurocrine routes. Activation of the ileal brake stimulates release of PYY and Glucagon-like peptide-1 (GLP-1) from the endocrine L cells located primarily in the ileum and colon (6,30). Of these peptides, especially PYY is of interest because PYY reduces food intake and elicits satiety in humans and animals (14-16).

Concerning PYY and satiety, the satiating effect of ileal fat perfusion could not be reproduced during infusion of PYY (15 pM/kg/hr) which resulted in similar plasma PYY levels ranging from 25 pM to 30 pM. Neither did the high dose of PYY (30 pM/kg/hr) affect satiety. With this dose PYY levels

were reached in the range of 50-60 pM, comparable to postprandial levels in patients with malabsorption (18,19). In line with this observation is the finding that infusion of PYY neither at low nor at high doses did affect proximal gastric motor function.

The mechanisms that regulate gut-brain signalling are poorly understood. CCK, but also PYY_{3-36} may act as a neurocrine rather than as an endocrine substance. Animal studies indicate that gut PYY can transmit signals to the CNS but only via an intact vagus nerve (31). Previously several authors have clearly demonstrated the satiating effect of PYY with a marked reduction in food intake (14-16). An explanation for the discrepancy in results of studies may be related, for instance, to the doses of PYY given. Degen *et al* recently showed that exogenous PYY_{3-36} only at a high, pharmacological doses of 48 pM/kg/hr significantly reduced feelings of hunger and decreased food intake. These authors could not demonstrate any effect of PYY when infused at doses of 12 or 24 pM/kg/hour (16). In the studies of Batterham *et al*, a reduction in food intake was observed after PYY infusion at doses of above 35 pM/kg/hr (14,15). Taken together, we suggest that the doses of PYY required to produce a significant effect on satiety and food intake are in the supraphysiological range.

In conclusion, we have shown that ileal fat induces satiety and results in proximal gastric relaxation , in contrast to exogenous PYY at identical plasma levels. These data do not support a role for PYY as physiological mediator in ileal brake induced proximal gastric relaxation.

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