Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid

Christine Feinle-Bisset, Michael Patterson, Mohammad A. Ghatei, Stephen R. Bloom, and Michael Horowitz. Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid. Am J Physiol Endocrinol Metab 289: E948–E953, 2005. First published July 5, 2005; doi:10.1152/ajpendo.00220.2005.—Stimulation of cholecystokinin and glucagon-like peptide-1 secretion by fat is mediated by the products of fat digestion. Ghrelin, peptide YY (PYY), and pancreatic polypeptide (PP) appear to play an important role in appetite regulation, and their release is modulated by food ingestion, including fat. It is unknown whether fat digestion is a prerequisite for their suppression (ghrelin) or release (PYY, PP). Moreover, it is not known whether small intestinal exposure to fat is sufficient to suppress ghrelin secretion. Our study aimed to resolve these issues. Sixteen healthy young males received, on two separate occasions, 120-min intraduodenal infusions of a long-chain triglyceride emulsion (2.8 kcal/min) without (condition FAT) or with (FAT-THL) 120 mg of tetrahydrolipstatin (THL, lipase inhibitor), followed by a standard buffet-style meal. Blood samples for ghrelin, PYY, and PP were taken throughout. FAT infusion was associated with a marked, and progressive, suppression of plasma ghrelin from t = 60 min (P < 0.001) and stimulation of PYY from t = 30 min (P < 0.01). FAT infusion also stimulated plasma PP (P ≤ 0.01), and the release was immediate. FAT-THL completely abolished the FAT-induced changes in ghrelin, PYY, and PP. In response to the meal, plasma ghrelin was further suppressed, and PYY and PP stimulated, during both FAT and FAT-THL infusions. In conclusion, in healthy humans, 1) the presence of fat in the small intestine suppresses ghrelin secretion, and 2) fat-induced suppression of ghrelin and stimulation of PYY and PP is dependent on fat digestion.

A NUMBER OF GASTROINTESTINAL PEPTIDES play a role in the regulation of energy intake in humans, including cholecystokinin (CCK) (19, 27), glucagon-like peptide-1 (GLP-1) (13, 17), peptide YY (PYY) (4), pancreatic polypeptide (PP) (5), and ghrelin (42). The secretion of CCK, GLP-1, and PYY from intestinal cells, and PP from the pancreas, is stimulated by meal ingestion or infusion of nutrients into the small intestine (10, 18, 21, 29). In contrast, ghrelin is secreted by the stomach, and plasma concentrations increase during fasting and are suppressed by a meal (7, 8).

Plasma ghrelin concentrations decrease rapidly following food ingestion, and there is evidence that ghrelin plays a role in meal initiation (7, 8). Intravenously administered ghrelin has been shown to stimulate appetite and increase food intake in humans (42). Both carbohydrate and fat, when ingested orally, suppress ghrelin secretion (9, 15), whereas protein may stimulate ghrelin secretion (9) or have no effect (15). Although until recently it has been unclear whether the suppressive effect of carbohydrate and fat on ghrelin secretion is mediated by the presence of nutrients in the stomach, the small intestine, and/or the circulation, recent animal (41) and human (30) studies indicate that the interaction of nutrients with the small intestine is important in the glucose-induced modulation of ghrelin secretion. In rats, the prevention of gastric emptying with a pyloric cuff abolished the suppression of ghrelin secretion by intragastric glucose (41). Moreover, in healthy older humans, both intragastric and intraduodenal glucose infusions suppress ghrelin secretion with no difference between them (34).

We (11) have recently established that the stimulation of both CCK and GLP-1 by a duodenal fat infusion is dependent on the interaction of the products of fat digestion with the gut. Inhibition of fat digestion by concomitant administration of the lipase inhibitor tetrahydrolipstatin (THL) completely abolished the increases in plasma CCK and GLP-1 concentrations induced by duodenal infusion of a long-chain triglyceride emulsion in healthy subjects (11). Intraduodenal infusion of the triglyceride emulsion was also associated with a reduction in perceptions of appetite as well as a decrease in energy intake at a buffet meal consumed immediately following the infusion compared with the condition in which fat digestion was inhibited (11). The stimulation of phasic and tonic pyloric pressures by intraduodenal triglyceride was also attenuated by lipase inhibition (11).

PYY and PP are both members of the pancreatic polypeptide family of peptides. PYY is secreted predominantly from endocrine cells in the ileum and colon and PP from endocrine cells in the pancreas, both in response to all three macronutrients, fat, carbohydrate, and protein, with fat being the most potent stimulus and carbohydrate the least potent or, possibly, impotent (2, 16, 29, 33). Enteral administration of free fatty acids, including dodecanoate (3) or oleate (22), are known to be potent stimuli of PYY secretion. Both PYY and PP, when infused intravenously, have been shown to reduce appetite and energy intake in healthy humans (4, 5), suggesting an important role for these peptides in the regulation of appetite. Because, as discussed, the appetitesuppressant effect of duodenal lipid is dependent on fat digestion (11), it is possible that fat digestion is also a prerequisite for fat-induced suppression of ghrelin and stimulation of PYY and PP. The suppression of

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ghrelin by fat is not mediated by an increase in plasma concentrations of free fatty acids (30). Although the effects on CCK and GLP-1 secretion in our previous study were striking (11), it has so far been unclear whether these findings could also be extrapolated to ghrelin, PYY, and PP. This hypothesis has, hitherto, not been evaluated.

We have now assayed the plasma samples from our previous study (11), in which we had evaluated the role of fat digestion on appetite and energy intake, antropyloroduodenal motility, and plasma CCK and GLP-1 concentrations. We hypothesized that, in response to duodenal fat infusion, 1) plasma ghrelin concentrations would be suppressed, and 2) the suppression of plasma ghrelin and stimulation of PYY and PP would be attenuated when triglyceride digestion is inhibited.

SUBJECTS AND METHODS

Subjects

16 healthy males, aged 21–39 yr, participated in the study, as described previously (11). All subjects were of normal body weight for their height [with a body mass index (BMI) of 19.5–27.6 kg/m^2 (mean: 24.1 kg/m^2)] and unrestrained eaters [score ≤ 12 on the eating restraint component of the Three Factor Eating questionnaire (40)]. The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, and all subjects provided written, informed consent prior to their inclusion.

Protocol

Each subject was studied on two occasions, separated by 3–10 days, when they received, in randomized, double-blind fashion, intraduodenal infusions of a long-chain triacylglyceride emulsion (LCT) without (condition FAT) or with (condition FAT-THL) 120 mg of the lipase inhibitor THL (F. Hoffmann-La Roche, Basle, Switzerland) for 120 min (11). After the duodenal infusion, the subjects received a standard, cold, buffet-style meal. Blood samples for measurement of plasma concentrations of ghrelin, PYY, and PP were taken at regular intervals throughout the infusion and after the meal.

On each study day, the subject arrived at the laboratory at 8:00 AM following an overnight fast. A small diameter manometric assembly was inserted through an anesthetized nostril into the stomach and allowed to pass through the pylorus into the duodenum by peristalsis (11). Fasting motility was observed until phase III of the interdigestive motor complex (MMC) occurred. Immediately after cessation of phase III activity, an intravenous cannula was placed in a left antecubital vein for blood sampling. A “baseline” blood sample was taken, and at t = 0 min (during phase I or II of the MMC), infusion of a long-chain triacylglyceride emulsion, either without (FAT) or with (FAT-THL) 120 mg of THL, was commenced and continued for 120 min (i.e., until t = 120 min). The preparation of the emulsions has been described previously (12) and was carried out by the Royal Adelaide Hospital pharmacy to allow a double-blind study design. The infusion rate was 1.4 ml/min, corresponding to an energy delivery of 2.8 kcal/min, so that the total volume infused in 120 min was 168 ml (a total of 2.52 g of soy lecithin, 1.96 g of ethanol, 33.6 g of soy oil, 130.2 g of 0.9% saline, and 120 mg of THL on 1 day). The emulsion containing THL results in ~75% lipase inhibition, as previously determined by stool fat analysis (unpublished data). Blood samples were taken at 15-min intervals throughout the infusion. At t = 120 min, the manometric assembly was removed. Fifteen minutes later, at t = 135 min, each subject was offered a cold buffet-style lunch and allowed 30 min to eat (11). At t = 165 min, the food was removed, and another blood sample was taken. After a further 30 min (t = 195 min), a final blood sample was taken. The intravenous cannula was then removed, and the subject was allowed to leave the laboratory.

Measurement of Plasma Ghrelin, PYY, and PP Concentrations

Venous blood samples were collected in chilled EDTA-treated tubes containing 400 KIU of aprotinin (Trasylol; Bayer Australia, Pymble, Australia) per liter of blood. Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and stored at −70°C until assayed. Ghrelin, PYY, and PP were measured on the blood samples obtained at t = 0, 15, 30, 45, 60, 75, 90, 105, and 120 min (during intraduodenal infusions) and at t = 165 and 195 min (after meal ingestion). All samples were assayed in duplicate and in one run, avoiding any influences of interassay variation.

Ghrelin-like immunoreactivity. Ghrelin-like immunoreactivity was measured with a specific and sensitive radioimmunoassay, as previously described (35). Briefly, the assay cross-reacted fully (100%) with both octanoyl and des octanoyl ghrelin and did not cross-react with any other known gastrointestinal or pancreatic hormone. The antiserum (SC-10368) was obtained from Santa Cruz Biotechnology and used at a final dilution of 1:50,000. 125I-Labeled ghrelin was prepared with Bolton & Hunter reagent (Amersham International, Little Chalfont, UK) and purified by high-pressure liquid chromatography using a linear gradient from 10 to 40% acetonitrile-0.05% trifluoroacetic acid over 90 min. The specific activity of ghrelin label was 48 Bq/fmol. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer, pH 7.2, containing 0.3% bovine serum albumin and was incubated for 3 days at 4°C before separation of free and bound antibody label by charcoal absorption. The assay detected changes of 20 pmol/l of plasma ghrelin with a 95% confidence limit. The intra-assay coefficient of variation was 5.5%.

PYY-like immunoreactivity. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay, as previously described (2, 4, 37). The assay measured both the hormone fragment [PYY-(3–36)] and the full-length hormone [PYY-(1–36)], both of which are biologically active. The antiserum (Y21) was produced in rabbits against synthetic porcine PYY 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 PYY but not with PP, neuropeptide Y, or other known gastrointestinal hormones. 125I-PYY was prepared by the iodogen method and purified by high-pressure liquid chromatography. The specific activity of the 125I PYY label was 54 Bq/fmol. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer, pH 7.2, containing 0.3% bovine serum albumin. The assay was incubated for 3 days at 4°C before separation of the free and antibody-bound label by sheep anti-rabbit antibody. The detection limit of the assay was 2.5 pmol/l, with an intra-assay coefficient of variation of 5.8%.

Plasma PP concentrations. Plasma PP concentrations were measured using a specific and sensitive radioimmunoassay (1). The assay cross-reacted fully (100%) with human PP and did not cross-react with any other member of the pancreatic polypeptide family or gastrointestinal hormone. Antiserum against human pancreatic polypeptide was produced in rabbits and used at a final dilution of 1:560,000. 125I-PP was prepared by the iodogen method and purified by high-pressure liquid chromatography. The specific activity of the 125I PP label was 54 Bq/fmol. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer, pH 7.2, containing 0.3% bovine serum albumin. The assay was incubated for 3 days at 4°C before separation of the free and antibody-bound label by charcoal absorption. The detection limit of the assay was 3.5 pmol/l, and the intra-assay coefficient of variation was 5.7%.

Statistical Analysis

Data were analyzed by repeated-measures analysis of variance (ANOVA), with time and treatment as factors, for both the infusion (t = 0–120 min) and postprandial (t = 165 and 195 min vs. t = 120 min) period, respectively. Post hoc paired comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when
ANOVA revealed significant effects. Statistical significance was accepted at \( P < 0.05 \), and data are presented as means ± SE.

**RESULTS**

**Plasma Ghrelin Concentrations**

*Effect of duodenal infusion.* There was a significant treatment \( \times \) time interaction \( (P < 0.001) \) for plasma ghrelin concentrations (Fig. 1A). Infusion of FAT was associated with a marked, and progressive, suppression of plasma ghrelin concentrations, which was significant from \( t = 60 \) min \( (P \leq 0.001) \); in contrast, FAT-THL had no effect on plasma ghrelin. Plasma ghrelin concentrations were lower during infusion of FAT compared with infusion of FAT-THL from \( t = 45 \) min \( (P < 0.01) \).

*Effect of meal.* During both conditions, plasma ghrelin concentrations decreased further following meal ingestion (time effects: \( P < 0.001 \)) but remained significantly lower following infusion of FAT compared with FAT-THL (treatment effect: \( P = 0.001 \)).

**Plasma PYY Concentrations**

*Effect of duodenal infusion.* There was a significant treatment \( \times \) time interaction \( (P < 0.001) \) for plasma PYY concentrations (Fig. 1B). Infusion of FAT was associated with a marked, and progressive, rise in plasma PYY concentrations, which was significant from \( t = 30 \) min \( (P < 0.01) \); in contrast, infusion of FAT-THL did not affect plasma PYY concentrations. Plasma PYY concentrations were higher during infusion of FAT compared with FAT-THL from \( t = 15 \) min \( (P < 0.01) \).

*Effect of meal.* Plasma PYY concentrations further rose during both conditions (time effects: \( P < 0.01 \)) at \( t = 165 \) min, and were significantly higher following infusion of FAT compared with FAT-THL (treatment effect: \( P < 0.01 \)).

**Plasma PP Concentrations**

*Effect of duodenal infusion.* There was a significant effect of treatment \( (P < 0.01) \) on plasma PP concentrations (Fig. 1C). During infusion of FAT, PP concentrations were higher from \( t = 15 \) min and then plateaued compared with infusion of FAT-THL; infusion of FAT-THL had no effect on plasma PP concentrations.

*Effect of meal.* There was a significant treatment \( \times \) time interaction \( (P = 0.01) \) for plasma PP concentrations. Plasma PP rose during both conditions (time effects: \( P < 0.001 \)) but was higher following infusion of FAT-THL compared with infusion of FAT \( (P < 0.01) \).

**DISCUSSION**

Our study establishes that, in healthy humans, 1) the presence of fat in the proximal small intestine is sufficient to suppress ghrelin secretion, and 2) the fat-induced suppression of ghrelin and stimulation of PYY and PP secretion are dependent on fat digestion.

Although it is known that meal ingestion suppresses ghrelin (8) and that this effect is induced by both fat and carbohydrate, but not protein (9, 15), it has been unclear whether the stimulus was the presence of nutrients in the stomach, small intestine, and/or postabsorptive factors. There is evidence that gastric distension per se does not reduce ghrelin (34, 39, 41) in that, although an oral glucose load suppressed ghrelin secretion in healthy humans, a water load of identical volume had no effect (39). The effect of oral glucose on ghrelin secretion is probably accounted for by the action of glucose in the small intestine rather than a “gastric” effect. In rats, a gastric glucose load did not suppress ghrelin secretion when gastric emptying was prevented by a pyloric cuff (41). A recent study from our laboratory (34) indicates that gastric and duodenal glucose infusions suppress ghrelin to a similar degree in healthy older subjects. In considering the potential role of postabsorptive
factors, intravenous infusion of glucose suppresses ghrelin in both rats (14) and humans (39), whereas intravenous infusion of a fat emulsion with subsequent elevation of free fatty acids had no effect in humans (30), but suppressed plasma ghrelin in rats (14). These latter observations argue against a role for postabsorptive factors in fat- but perhaps not glucose-induced suppression of ghrelin in humans. The current study is the first to demonstrate that duodenal infusion of a long-chain triglyceride emulsion potently suppresses ghrelin secretion in healthy young men, indicating that in humans ghrelin is sensitive to digested fats, an effect apparently not mediated by an increase in blood free fatty acids (30). The stimulation of PYY and PP by small intestinal fat is well documented (22, 26, 29).

Our study also provides additional insights into the role of fat digestion products in the modulation of gastrointestinal peptide secretion. Inhibition of fat digestion abolished the suppression of ghrelin and the stimulation of PYY and PP secretion, indicating that fat digestion products, i.e., free fatty acids, play an important role. Although the current findings in relation to the regulation of ghrelin, PYY, and PP secretion are novel, they are consistent with previous observations that fat digestion is a prerequisite for the slowing of gastric emptying (6, 36, 38), proximal gastric relaxation (12), stimulation of pyloric and suppression of antral pressures (11), stimulation of CCK, GLP-1, and GIP (glucose-dependent insulinotropic polypeptide) secretion in healthy subjects and type 2 diabetes (11, 32, 36, 38), suppression of appetite perceptions (11), induction of upper gastrointestinal symptoms (12), and suppression of energy intake (11, 31). Although our study did not include a formal control condition (i.e., administration of THL alone), there is no evidence that THL per se has any effects on the gastrointestinal tract, including gastrointestinal hormone secretion; furthermore, systemic absorption of THL is known to be very low (personal communication, Dr. Jacques Bailly, Hoffmann-La Roche, Basel, Switzerland). Although lipase inhibition had substantial effects on the antropyloroduodenal motor responses to duodenal lipid (11), it is most unlikely that these would account for the observed hormonal responses.

It is of interest that the pattern of suppression of ghrelin and stimulation of PYY and PP by the small intestinal triglyceride infusion differed markedly. The fall in ghrelin and rise in PYY appeared to be progressive between t = 0 and 120 min, whereas PP rose to a maximum within 15 min and did not change after that time. These discrepant responses provide insights into the potential small intestinal mechanisms that may modulate the secretion of these hormones. PYY-containing cells predominate in the distal small intestine and colon, and PP secretion is initiated either by direct luminal contact of nutrients with the endocrine cells (3, 22, 25) and/or indirectly through neurohumoral signals (23, 25). The rise in PYY was relatively prompt and, hence, most unlikely due exclusively to direct exposure of the distal small intestine. In dogs, CCK, which is released from enterendocrine cells located in the proximal small intestine, has been shown to modulate PYY release (23), and in our original report (11) we described that plasma CCK concentrations rose significantly within 15 min of commencing the duodenal triglyceride infusion but decreased after 30 min; this is consistent with the concept that the initial rise in PYY is due to a link between the proximal small intestine with PYY-releasing cells in the distal small intestine and that the continued rise of PYY throughout the infusion reflects the direct contact of lipid with the distal small intestine. The profile obtained for PYY plasma concentrations in our study closely resembles that observed after a meal (2). As the emulsion containing THL resulted in only ~75%, the observed total abolition of PYY secretion and ghrelin suppression suggests that a critical threshold concentration or load for luminal fatty acids is required for these effects. Conversely, there was a progressive suppression of ghrelin secretion over the infusion period. Hence, for both PYY and ghrelin, our data suggest that a critical nutrient load and/or exposure of a specific length or region of intestine to nutrient is required for their stimulation. This is not surprising, as studies in experimental animals have provided convincing evidence for a role of nutrient load, as well as the length of intestine exposed to nutrient, in the regulation of both gastric emptying and food intake (24, 28). In terms of the regulation of ghrelin secretion from the small intestine, it remains to be determined what factors and pathways may be involved in feeding back luminal signals to the ghrelin-secreting cells in the stomach. It has recently been demonstrated that intravenous PYY suppresses ghrelin secretion (4), suggesting that PYY and ghrelin may operate in a negative feedback relationship. In contrast to PYY and ghrelin secretion, there was a prompt rise in PP in response to the duodenal triglyceride infusion with no further increase over the course of the infusion. This pattern is consistent with that observed in response to meal ingestion (1) and suggests that regulation of PP secretion is confined to the proximal small intestine and that the critical nutrient load required for secretion had been exceeded. Alternatively, it is possible that a further increase in PP throughout the infusion was suppressed by negative feedback mechanisms induced by other peptides released from the distal small intestine.

In this study, THL was administered with the duodenal lipid infusion, which was ceased immediately before the meal. Because THL is known to only inhibit fat digestion from the meal it is ingested with (or in the case of this study, the duodenal lipid infusion), it was to be expected that meal ingestion would have an additional effect on hormone secretion. Although both PYY and ghrelin secretions were modified markedly by the duodenal lipid infusion, meal ingestion had only a moderate additional effect. PP secretion was, in contrast, stimulated markedly by the meal. This may suggest that, in contrast to PYY and ghrelin, gastric distension, enhanced by the greater amount of food eaten during the FAT-THL condition, may be a more important stimulus for PP. Indeed, moderate gastric distension with a balloon (volume: 600 ml) has been shown to cause a substantial (71%) increase in PP secretion in healthy volunteers (20). Alternatively, it is possible that the other macronutrients, carbohydrate and protein, are more important stimuli for PP secretion than fat. It remains perplexing that protein, the most satiating of the three macronutrients, does not decrease ghrelin (9). However, it needs to be recognized that interpretation of the meal-induced changes in plasma hormone concentrations in our study is limited by the fact that energy intake was variable between subjects and ~15% greater following lipase inhibition [as reported previously (11), energy intake after infusion of FAT-THL (5,999 ± 1,433 kJ) was greater than after infusion of FAT (5,177 ± 1,740 kJ, P < 0.05)] and that the changes in hormones are likely to be dependent on the premeal values, which were markedly different for ghrelin and PYY but not PP.
There is persuasive evidence that ghrelin, PYY, and PP play a role in the regulation of food intake, ghrelin as a stimulant (8, 42), and PYY and PP as suppressors (4, 5). Given that, as our data demonstrate, the secretion of all three hormones is modulated by fat and the inhibition of fat digestion abolishes this modulation, ghrelin, PYY, and PP may contribute to the suppression of energy intake by fat (11). Such a relationship has been established for CCK, in that the CCK-A receptor antagonist loxiglumide attenuated the inhibitory effects of an intraduodenally administered long-chain fatty acid, oleic acid, on energy intake (27).

In summary, our study demonstrates that, in healthy adults, 1) proximal small intestinal exposure to nutrients, in this case long-chain triglycerides, is sufficient to suppress ghrelin secretion; 2) fat digestion is required for the suppression of ghrelin and stimulation of PYY and PP secretion; and 3) the effects of small intestinal fat on ghrelin and PYY secretion occur progressively, indicative of modulation by mechanisms that depend on the nutrient load and length of intestine exposed to nutrient.

GRANTS
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