Effects of fat digestion on appetite, antropyloroduodenal motility, and gut hormones in response to duodenal fat infusion in humans

Short title: Lipase inhibition and antropyloroduodenal motility

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Abbreviations: APD, antropyloroduodenal, GLP-1, glucagon-like peptide-1, IPPW, isolated pyloric pressure wave, PW, pressure wave, THL, tetrahydrolipstatin, TMPD, transmucosal potential difference, VAS, visual analogue scale

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Abstract

Background & Aims: The presence of nutrients in the small intestine slows gastric emptying and suppresses appetite and food intake; these effects are partly mediated by the release of gut hormones, including cholecystokinin. We investigated the hypothesis that the modulation of antropyloroduodenal motility, suppression of appetite, and stimulation of cholecystokinin and glucagon-like peptide-1 secretion by intraduodenal fat are dependent on triglyceride hydrolysis by lipase.

Methods: 16 healthy, young, lean men were studied twice in double-blind, randomised, cross-over fashion. Ratings for appetite-related sensations, antropyloroduodenal motility and plasma cholecystokinin and glucagon-like peptide-1 concentrations were measured during a 120 min duodenal infusion of a triglyceride emulsion (2.8 kcal/min), on one day with, on the other without, 120 mg tetrahydrolipstatin, a potent lipase inhibitor. Immediately following the duodenal fat infusion, food intake at a buffet lunch was quantified.

Results: Lipase inhibition with tetrahydrolipstatin was associated with reductions in tonic and phasic pyloric pressures, increased numbers of isolated antral and duodenal pressure waves and stimulation of antropyloroduodenal pressure wave sequences (all P<0.05). Scores for prospective consumption and food intake at lunch were greater, and nausea scores slightly less, and the rises in plasma cholecystokinin and glucagon-like peptide-1 were abolished (all P<0.05).

Conclusion: Lipase inhibition attenuates the effects of duodenal fat on antropyloroduodenal motility, appetite and cholecystokinin and glucagon-like peptide-1 secretion.

Key words: Fat digestion, food intake, gastrointestinal manometry, cholecystokinin, glucagon-like peptide-1
Introduction

The interaction of nutrients with small intestinal receptors regulates both gastric emptying and appetite, and stimulates the release of gastrointestinal hormones, including cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). In healthy subjects, intraduodenal infusion of fat slows gastric emptying (20) and reduces hunger and subsequent food intake (6,29-31); these effects are, at least in part, mediated by CCK (16,30). The slowing of gastric emptying by small intestinal nutrients is associated with a reduction in proximal gastric tone (10), suppression of antral pressure waves (20,33) and stimulation of tonic and phasic pyloric pressures (13,21). The increase in pyloric motility may be the most important of these mechanisms, as the stimulation of phasic and tonic pyloric pressures is associated with cessation of transpyloric flow (46).

Studies in animals (36,37,40) and humans (5,11,24,32,39) suggest that the slowing of gastric emptying, suppression of appetite and stimulation of CCK secretion by fat are dependent on lipolysis of triglyceride to fatty acids. In particular, in animals, lipase inhibition accelerates gastric emptying of fat (34,35). In humans, it has also been established that both pharmacological inhibition of lipase (3,24) and pancreatic exocrine insufficiency (5) are associated with more rapid gastric emptying of fat. Recent studies also suggest that fat digestion is required for the triggering of gastrointestinal sensations, such as fullness and nausea, as induced by concurrent gastric distension and duodenal infusion of a triglyceride emulsion (11).

Recent studies (11,32,39) used the lipase inhibitor tetrahydrolipstatin (THL), a specific and potent inhibitor of gastric and intestinal lipases (19). When ingested with a meal in the recommended dose of 120 mg, THL inhibits fat digestion by approximately 30 % (48). However, the weight loss in obese subjects may be less than would be predicted by the degree of inhibition of fat absorption (7,25). While the cause(s) of the latter are unknown, it is possible that some patients may increase their energy intake in compensation for the unabsorbed fat; this hypothesis is supported by two studies, which indicate that lipase inhibition may attenuate the suppression of subsequent food intake by fat (32,39). Immediately following a 60 min duodenal infusion of olive oil, healthy subjects ate significantly more from a test meal when the olive oil contained 120 mg THL, compared to olive oil alone (32). Furthermore, when healthy
subjects were given 120 mg THL orally with a high-fat yoghurt preload at breakfast, their energy intake during the remainder of the day, was greater compared to the yoghurt alone (39). The secretion of CCK in response to a meal or intestinal fat infusion is known to be attenuated by lipase inhibition (3,11,32), providing a potential causal link between lipase inhibition and the increase in food intake. Glucagon-like peptide-1 (GLP-1) is also released by meal ingestion or intestinal nutrient administration (23). Moreover, there is persuasive evidence that GLP-1 plays an important role in the regulation of appetite (12,17) and the modulation of gastrointestinal motility and gastric emptying (38,43). The effect of lipase inhibition on GLP-1 secretion is unknown.

The motor mechanisms associated with the accelerated gastric emptying of fat induced by lipase inhibition have not been evaluated. Furthermore, no studies have assessed the impact of lipase inhibition on antropyloroduodenal motility and appetite concurrently. It is conceivable that diminished small intestinal feedback inhibition by fat will result in decreased phasic and tonic pyloric pressure activity and increased ‘propulsive’ antroduodenal motor patterns. Such changes may potentially be associated with more rapid small intestinal transit, so that the interaction of fat with small intestinal receptors is reduced, leading to gastrointestinal side effects as well as a reduction in feedback signals to slow gastric emptying and decrease appetite. The potential impact of accelerated gastric emptying on appetite is uncertain, but it has been suggested that a slowing of gastric emptying may decrease food intake by enhancing gastric distension (8).

The aims of this study were to evaluate in healthy subjects the broad hypothesis that the stimulation of tonic and phasic pyloric pressure activity, inhibition of propulsive antropyloroduodenal motor patterns, suppression of appetite, and secretion of gastrointestinal hormones (CCK, GLP-1) by intraduodenal fat are attenuated by inhibition of lipase. We used an intraduodenal infusion to bypass gastric mechanisms (and potential effects of lipase inhibition on gastric emptying) involved in the regulation of food intake.
Methods

Subjects

16 healthy male subjects aged 21 - 39 years took part in the study. Male subjects were studied because they are most sensitive to dietary manipulation (41). The subjects were of normal body weight for height (with a body mass index (BMI) of 19.5 - 27.6 kg/m² [mean: 24.1 kg/m²]), unrestrained eaters (score < 12 on the restraint section of the three factor eating questionnaire (45)), were not taking medication that could affect appetite, body weight or gastrointestinal function and had no history of gastrointestinal disease. Subjects with a consumption of alcohol > 20 g/day, or who smoked, were also excluded. Prior to entry into the study, each subject underwent a pre-study ‘screening’, which included a medical history, a physical examination, clinical laboratory tests, 12-lead ECG, and measurement of vital signs. The study protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital and performed in accordance with principles laid out by the Declaration of Helsinki; each subject gave informed, written consent prior to inclusion. The subjects were informed that the primary purpose of the study was to assess the effect of nutrient digestion on gastrointestinal motor activity and gastrointestinal hormone secretion (ie the subjects were not aware that appetite and food intake were major endpoints).

Experimental protocol (Figure 1)

The studies were carried out in randomised order according to a double-blind, two-period cross-over design. On each study day, the subject arrived at the laboratory at 8.00 am following an overnight fast. A small diameter manometric assembly (Dentsleeve, Wayville, Australia), incorporating a pyloric sleeve sensor, was inserted through an anaesthetised nostril into the stomach and allowed to pass through the pylorus into the duodenum by peristalsis. The correct position of the sleeve was verified by continuously monitoring the antroduodenal transmucosal potential difference (TMPD) gradient across the pylorus (2,31). Fasting motility was observed until phase III of the interdigestive migrating motor complex (MMC) occurred (31). Immediately after cessation of phase III activity, an intravenous cannula was placed in a left antecubital vein for blood sampling, and the first ‘baseline’ blood sample (for subsequent measurement of plasma cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1)) was
taken, and a visual analogue scale (VAS) to evaluate appetite-related sensations administered (t = -30 min). Two more baseline blood samples and VAS scores were obtained at t = -15 min and at t = 0 min. 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 the lipase inhibitor tetrahydrolipstatin (THL, F Hoffmann-La Roche, Basle, Switzerland), was commenced and continued for 120 min (ie until t = 120 min). Blood samples were taken and VAS administered at 15 min intervals throughout the infusion. Antropyloroduodenal (APD) motility was recorded continuously from t = -30 min until t = 120 min, at which time the manometric assembly was removed. 15 min later, at t = 135 min, each subject was offered a cold buffet-style lunch, and allowed 30 minutes to eat (31). At t = 165 min, the food was removed, another blood sample was taken and a VAS administered. After a further 30 minutes (t = 195 min), a final blood sample was taken and a VAS completed. The intravenous cannula was then removed and the subject allowed to leave the laboratory.

The occurrence of lower gastrointestinal side effects was not assessed formally. However, subjects were informed of this possibility and asked to report any side effects, as well as their severity.

Intraduodenal fat infusion

The preparation of the emulsions has been described in detail previously (11) 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 minutes was 168 ml (a total of 2.52 g soy lecithin, 1.96 g ethanol, 33.6 g soy oil and 130.2 g 0.9% saline, and 120 mg THL on one day). In our previous study (11), in which stool collections were performed, lipase inhibition during intraduodenal administration of this particular emulsion was shown to result in an inhibition of absorption of ~75% of the fat (unpublished data).
Measurement of antropyloroduodenal pressures

Intraluminal pressures were recorded with a perfusion manometry system and a 17 channel manometric assembly (outer diameter: 4.0 mm). Sixteen channels (lumen diameter: 0.5 mm) were used to record pressures, and one (lumen diameter: 1.0 mm) for intraduodenal nutrient infusion (Figure 2). Six manometric channels, spaced at 1.5 cm intervals, were located in the antrum (channels 1-6), a 4.5 cm sleeve sensor straddled the pylorus (channel 7) and 2 channels, 1.5 and 3 cm from its orad end, were situated on the back of the sleeve (channels 8 and 9). Seven more distal channels, spaced at 1.5 cm intervals, were situated in the duodenum (channels 10 – 16); the infusion port was situated a further 2.25 cm beyond this chain of sideholes, 11.25 cm from the pylorus. All manometric channels were perfused with distilled, degassed water at 0.08 ml/min, except channels 6 and 10 (on either side of the sleeve), which were perfused with 0.9 % saline for monitoring TMPD (31).

Data were recorded at 10 Hz using a computer-based system (PowerMac 7100/75; Apple Computer, Cupertino, CA) running commercially available software (MAD; Prof C Malbert, Unité de Flux Digestifs, Institut National de la Recherche Agronomique, Saint Gilles, France, in Labview 3.0.1 (National Instruments)). Data were digitised, logged to disk for subsequent analysis, and only analysed when the assembly was positioned correctly, according to previously described TMPD criteria (22).

Analysis of basal pyloric pressure activity (‘tone’) was performed, as previously described (31). Basal pyloric pressure was quantified for each minute, using custom written software (MAD), by subtracting the mean pressure (excluding phasic pressures) recorded at the most distal antral side hole (channel 6) from the mean pressure recorded at the sleeve (channel 7) (22); mean values were then calculated for each 15 minute time interval. Data were then imported into a novel custom-designed computer programme (Trace! 1.1, A/Prof GS Hebbard, Royal Melbourne Hospital, Australia) for the analysis of APD pressures. In Trace! data are displayed as “3D” colour pressure contour plots (2), with time on the x axis, distance along the assembly on the y axis, and pressure encoded by colour. This allows for a better visual interpretation of the pressure profile along the assembly. APD pressures were analysed for (i) number and
amplitude of isolated pressure waves in the antrum, pylorus and duodenum and (ii) number and length of propagation of pressure wave sequences. A phasic pressure wave (PW) in the antrum or pylorus was defined as a pressure lasting between 1 and 20 s with an amplitude of \( \geq 10 \) mmHg. In the duodenum a PW was defined as lasting between 0.8 and 7 s with an amplitude of \( \geq 6 \) mmHg (1). PWs in adjacent channels were regarded as temporally related if they had onsets within \( \pm 3 \) s (in the duodenum) or \( \pm 5 \) s (in the antrum) of each other. A PW sequence was defined as 2 or more temporally related PWs. Data were analysed in 15 min segments, including the baseline period (t = -15 min to 0 min) and the first 45 min of the lipid infusion (t = 0 min to 45 min), as we and others have previously established that the major effects of intraduodenal lipid infusion on APD pressures occur during this time (21,22). All motility analyses were jointly performed by two blinded observers (ie both were unaware of the study conditions).

**Assessment of appetite/food intake**

Subjective sensations of appetite/hunger, fullness, desire to eat, prospective consumption (“how much food do you think you could it if you were given a meal now?”) and nausea were rated by each subject at regular intervals (Figure 1) on validated visual analogue scales (VAS) (44). The buffet meal, given at the end of the duodenal infusion, contained food in excess of what a subject would normally eat, and each subject was invited to eat as much as he wished until he felt comfortably full (29,31). The meal consisted of four slices each of brown and white bread, cheese and cold meat, 20 g margarine, 24 g mayonnaise, sliced cucumber and tomato, lettuce leaves, an apple, a banana, 150 g fruit salad, 200 g fruit yoghurt, 150 g custard, 300 ml orange juice, 300 ml iced coffee and 600 ml mineral water. Total energy intake (kcal), the amount eaten (g), and macronutrient composition (% energy and g) were calculated subsequently using commercially available software (Foodworks Version 2.10, Xyris Software (Australia) Pty Ltd, Highgate Hill, QLD, Australia).

**Measurement of plasma cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1)**

For plasma CCK and GLP-1 determinations, 10 ml samples of venous blood were collected in ice-chilled EDTA dipotassium–treated tubes containing 400 KIU/ml blood aprotinin (Trasylol;
Plasma was separated by centrifugation (3000 rpm for 15 min at 4°C) within 30 min of collection and stored at -70°C until assayed.

Plasma CCK (pmol/l) was determined by a radioimmunoassay as previously described (31). A commercially available antibody (C2581, Sigma Chemical, St Louis, MO, USA) raised in rabbits against synthetic sulphated CCK-8 was employed. The intra-assay coefficient of variation was 9.5% at 50 pmol.

Plasma GLP-1-(7-36) (pmol/l) was determined by radioimmunoassay after ethanol extraction of plasma samples (47). The antibody used was provided by Prof SR Bloom (Hammersmith Hospital, London) and had been raised in rabbits immunised with GLP-1_{7-36} conjugated to bovine serum albumin by carbodiimide. The detection limit was 2 pmol/l. The intra-assay coefficient of variation was 17%.

**Statistical analysis**

Visual analogue scores, plasma concentrations of CCK and GLP-1 (as obtained during the entire study period) and manometry data (baseline, ie t = -15 min to 0 min, and t = 0 min to t = 45 min during the infusions) were analysed by repeated measurement analysis of variance (ANOVA) with time and treatment as factors. A two-tailed Student’s paired t-test was used to test for differences between study conditions in food intake (amount and energy consumed and macronutrient distribution). An error probability of P < 0.05 was assumed to indicate statistically significant differences. The relationship between scores for both prospective consumption and nausea with food intake were assessed using Pearson’s product moment correlation test, stating the correlation coefficient r^2 and assuming P < 0.05 to indicate a significant linear relationship.
Results

All subjects tolerated the study procedures, including nasoduodenal intubation and fat infusion, well. Nine of the 16 subjects did not experience any side effects at all. The other seven subjects reported transient side effects including loose, oily stools (4 subjects following FAT-THL and 1 subject following FAT) and abdominal bloating (2 subjects following FAT-THL).

Antropyloroduodenal pressures

The manometric assembly was positioned correctly for 98 % of the recording time. Infusion of FAT resulted in a typical fed motor pattern in which isolated antral and duodenal pressure waves were inhibited, and isolated pyloric pressure waves stimulated (Figure 3, left). In contrast, inhibition of lipase activity (condition FAT-THL) resulted in pronounced ‘propulsive’ antropyloroduodenal activity and reduced tonic and phasic pyloric pressures (Figure 3, right).

Isolated antral pressure waves. The FAT infusion tended to decrease both the number and amplitude of pressure waves (time effect: n.s.), while the FAT-THL infusion resulted in an increase in both the number and amplitude of pressure waves (time effect: n.s.), when compared with baseline. Hence, the FAT-THL infusion resulted in a greater number (treatment effect: P = 0.005, treatment x time interaction: P = 0.014) and amplitude (treatment effect: P < 0.001) of pressure waves, when compared with the FAT infusion (Table 1).

Pyloric pressure waves. The FAT infusion stimulated both the number and amplitude of IPPWs (time effects: n.s.), with a maximum effect occurring between 15 – 30 min during the infusion, while the FAT-THL infusion was not associated with any changes in the number or amplitude of IPPWs (time effects: n.s.). Overall, the FAT-THL infusion resulted in a smaller number (treatment effect: P = 0.006, treatment x time interaction: P = 0.024) and amplitude (treatment effect: P = 0.023, treatment x time interaction: P = 0.004) of IPPWs, when compared with the FAT infusion (Figure 4A and B, Table 1). The FAT infusion was associated with a marked increase in basal pyloric pressure (time effect: P = 0.011) peaking between 15 – 30 min. In contrast, during condition FAT-THL, basal pyloric pressure declined steadily (Figure 4C). The differences between the two conditions were significant (treatment effect: P = 0.005).
Isolated duodenal pressure waves. The FAT infusion was associated with a slight decrease in the number (with a minimum number between 15 – 30 min during the infusion) (time effect: \( P = 0.038 \)) and the amplitude (time effect: \( P = 0.079 \)) of isolated duodenal waves. During the FAT-THL infusion, the number of pressure waves was variable, while the amplitude increased, with a maximum between 15 – 30 min (time effect: \( P = 0.078 \)). Overall, the amplitude (treatment effect: \( P < 0.001 \)), but not the number (treatment effect: n.s.), of isolated duodenal pressure waves was greater during the FAT-THL infusion when compared with the FAT infusion (Table 1).

Pressure wave sequences. The number of APD pressure wave sequences was greater during the FAT-THL condition when compared with FAT (Figure 5A). Specifically, a greater number of sequences originated in the antrum, pylorus and duodenum (Table 2). Furthermore, the length of these sequences was greater (Figure 5B).

Appetite/food intake
Scores for ‘prospective consumption’ declined progressively during the FAT infusion to levels that were lower than during the FAT-THL condition (\( P = 0.037 \), Figure 6A). During the FAT-THL condition, scores for ‘prospective consumption’ did not change from baseline during the infusion period (Figure 6A). ‘Nausea’ scores were very low throughout both infusion periods, yet statistical analysis showed that nausea was marginally higher during the FAT condition (maximum score (mm): FAT: 10.6 \( \pm \) 3.6, FAT-THL: 7.8 \( \pm \) 2.4, \( P = 0.045 \), Figure 6B). As expected, consumption of the buffet meal was associated with a reduction in scores for ‘prospective consumption’, but there were no differences between the two study conditions. Nausea scores were very low following the meal, with no differences between the two study conditions (data not shown).

Energy intake (FAT: 1237 \( \pm \) 104 kcal, FAT-THL: 1433 \( \pm \) 86 kcal, \( P = 0.04 \)) and the amount of food consumed (FAT: 1165 \( \pm \) 104 g, FAT-THL: 1335 \( \pm \) 87 g, \( P = 0.032 \)) at the buffet meal were greater after the FAT-THL compared with the FAT condition, with a mean difference in
energy intake of ~200 kcal. Greater absolute amounts of carbohydrate (FAT: 141.4 ± 11.9 g, FAT-THL: 161.9 ± 12.1 g, P = 0.045) and protein (FAT: 57 ± 5 g, FAT-THL: 67.6 ± 4.4 g, P = 0.029) were consumed. Fat consumption did not differ (FAT: 49.6 ± 5.2 g, FAT-THL: 57.4 ± 3.5 g, n.s.) and the percentage of energy derived from the three macronutrients remained unchanged between the two study conditions.

**Relationship between ‘prospective consumption’ and ‘nausea’ scores with subsequent energy intake.** The score for ‘prospective consumption’ immediately preceding the buffet meal was positively related with (r² = 0.284, P < 0.001), while there was no relationship between the score for ‘nausea’ and (r² = 0.009, P > 0.1), subsequent energy intake.

**Plasma CCK and GLP-1 concentrations**

**Plasma CCK.** Baseline CCK did not differ between the study days. During FAT infusion there was an increase in plasma CCK within 15 min of the start of the infusion to levels that were higher than during the FAT-THL condition (P = 0.001, Figure 7A). Inhibition of lipase activity (condition FAT-THL) prevented the FAT-induced increase in plasma CCK concentrations. Ingestion of the buffet meal increased plasma CCK concentrations during both study conditions, but levels were higher during the FAT condition (P = 0.001, data not shown).

**Plasma GLP-1.** Baseline GLP-1 did not differ between the study days. FAT infusion was associated with an increase in plasma GLP-1, when compared with FAT-THL. This was evident within 30 min of starting the infusion (P = 0.001, Figure 7B). During FAT-THL, the FAT-induced increase in plasma GLP-1 was prevented. Ingestion of the buffet meal increased plasma GLP-1 concentrations during both study conditions, but levels were higher during the FAT condition (P = 0.001, data not shown).
Discussion

Our study confirms that lipase inhibition reduces the inhibitory effect of duodenal fat on appetite and subsequent food intake (32) and diminishes fat-induced CCK secretion (3,11,32). The novel observations are that lipase inhibition abolishes the stimulation of GLP-1 secretion and modifies antropyloroduodenal pressure activity in response to intraduodenal fat infusion. In particular, lipase inhibition reduced tonic and phasic pyloric pressures and was associated with a greater number of isolated pressure waves in the antrum, as well as more and longer antropyloroduodenal pressure wave sequences, all of which are thought to represent a more propulsive motor pattern.

Input from small intestinal receptors (activated by nutrients and/or neuropeptides) is transmitted to centres in the brainstem, such as the nucleus tractus solitarius and the area postrema, which are both involved in the control of gastrointestinal motility (26), and also conveyed to the hypothalamus (42), which plays a major role in appetite regulation. Our data suggest that lipase inhibition ‘interrupts’ activation of these pathways by triglyceride. The observed effects of lipase inhibition on hormone secretion and antropyloroduodenal pressure activity potentially have important implications for the use of tetrahydrolipstatin in the treatment of obesity.

In considering the doses of intraduodenal fat and THL used, the infusion rate approximates the rate at which fat is emptied from the stomach into the small intestine after a high-fat meal (35), and the THL dose of 120 mg is that recommended for ingestion with meals to achieve weight loss (7,25). However, the degree of inhibition of fat absorption in our study (approximately 75 %) was substantially greater than that achieved in previous weight loss studies (approximately 30 %) (7,25); this should be taken into consideration in interpreting our data. The fat emulsion was based on soy bean oil, which contains exclusively long-chain triglycerides with a chain length \( \geq 16 \), and a large percentage of oleic acid (24 %, monounsaturated fatty acid) and linoleic acid (53 %, polyunsaturated fatty acid). Both the chain length and the degree of saturation have been shown to modify the effects of fat on gastric motility, appetite and CCK secretion. Long chain fatty acids with a chain length \( \geq 12 \), but not short chain fatty acids (chain length \( \leq 11 \)), induce gastric relaxation (11,33), inhibit food intake and stimulate CCK secretion (11,32,33); fat
emulsions containing a large percentage of polyunsaturated fatty acids appear to inhibit food intake more than fat emulsions containing monounsaturated fatty acids (15).

It is well established that the presence of nutrients in the small intestine slows gastric emptying. The motor mechanisms associated with the slowing of gastric emptying by intraduodenal fat include proximal gastric relaxation (10,33), inhibition of antral contractile activity (20,33), stimulation of phasic and tonic pyloric pressure waves (13,21) and conversion of small intestinal activity into a pattern of irregular contractions (1,4,27). While proximal gastric relaxation was not measured in the current study, the other responses were evident. The temporal and spatial organisation of antropyloroduodenal pressure patterns is a major determinant of gastric emptying and small intestinal transit - changes in gastrointestinal motility in response to food ingestion serve to optimise the interaction of nutrients with small intestinal receptors by slowing gastric emptying and transport of chyme along the gut. Studies in dogs, combining measurement of small intestinal motility using strain gauge transducers with fluoroscopy to monitor movement of intestinal contents, have demonstrated that nutrient ingestion shortens the length of pressure wave sequences in the duodenum when compared with a nutrient-free cellulose meal; this is associated with slower transport of intestinal content (4,27). In contrast to the fat infusion, antropyloroduodenal activity during inhibition of lipase activity was characterised by an increased number of pressure wave sequences, inhibition of tonic and phasic pyloric pressures and stimulation of antral and duodenal pressures. While the effects of lipase inhibition on small intestinal transit have not been evaluated in humans, lipase inhibition markedly accelerates gastrocolonic transit in rats (36), hence, the demonstrated stimulation of antropyloroduodenal pressure wave sequences is likely to be associated with increased propulsion of intestinal content (4,36). In addition, other studies have shown that increased propagating gastrointestinal motor activity, as induced by the prokinetic drug, cisapride (14), is associated with acceleration of gastric emptying (14,18). Therefore, it is likely that these motor effects (together with a decreased relaxation of the proximal stomach (11)) are responsible for the acceleration of gastric emptying of fat induced by lipase inhibition (35) or in patients with exocrine pancreatic insufficiency (5). Our study also indicates that duodenally administered fat requires about 30 – 45 minutes to exert its maximal modulating effects on APD motility (9). It
could, therefore, be postulated that the latency for stimulation of IPPWs during the lipid infusion is accounted for by the time required for digestion, although it is also possible that a threshold ‘dose’ of nutrient (12) or stimulation of a minimum length of intestine (37) is required to interrupt the fasting motor pattern.

Lipase inhibition also prevented the reduction in scores for prospective consumption induced by the duodenal fat infusion and increased energy intake at the buffet meal in our study. These findings are consistent with previous observations that the decrease in hunger and increase in fullness induced during gastric distension and duodenal lipid infusion are diminished by lipase inhibition (11). Moreover, the data are consistent with the findings of two other recent studies that the suppression of food intake by duodenal infusion of olive oil (32) or a high-fat yoghurt preload (39) are attenuated when fat digestion is inhibited, underlining the importance of the products of fat digestion in the regulation of appetite perception and food intake by fat. It may be argued that nausea, which was greater in the absence of lipase inhibition, may be responsible for a reduction in food intake. However, while statistically different, nausea scores were very low during both study conditions and unlikely to be relevant. A lack of an influence of nausea on food intake is also supported by the fact that there was no relationship between nausea scores at the end of the lipid infusion and the amount eaten at the buffet meal. Furthermore, food intake was substantial in all subjects, ie they all ate what would be considered to be a ‘normal’ meal.

Our data suggest that by increasing energy intake at a subsequent meal, subjects largely compensated for the deficit generated by the inhibition of fat digestion. The duodenal infusion administered contained 336 kcal, and it may be assumed that as a result of 75 % inhibition of lipase activity, ~250 kcal were not available for digestion and absorption during the FAT-THL condition. Hence, by ingesting an additional ~200 kcal during this condition, subjects compensated for approximately 80 % of the energy deficit.

The regulation of appetite, gastrointestinal motor function and hormone secretion is complex and inter-related and modulated by a large number of factors, including gut hormones. It has been established that the slowing of gastric emptying by fat is dependent on CCK mechanisms (16);
CCK is also regarded as one of the most important satiety hormones (30) and has been shown to be involved in mediating the effects of intestinal fat on perceptions of hunger and fullness (10). Furthermore, fat digestion is required for the stimulatory effects of fat on CCK secretion (11,32,33,40). GLP-1 has received considerable attention in recent years; it is known that, like CCK, GLP-1 when infused intravenously, is a potent regulator of gastric emptying and gastrointestinal motility (38,43) and also inhibits food intake (12,17). Our data illustrate that lipase inhibition completely abolishes the stimulation of both CCK and GLP-1 by small intestinal fat, and this may potentially account for the increase in food intake observed with lipase inhibition.

THL is effective in both the induction and maintenance of modest weight loss in overweight and obese subjects (7,25). As a result of inhibition of gastric and pancreatic lipase (19), THL in a dose of 120 mg with a meal decreases dietary fat absorption by ~30 % (48). However, although the inhibitory effect of THL on lipase activity is maintained during chronic administration, the mean weight loss is less than would be predicted by the degree of inhibition of fat absorption; this was despite the fact that in these studies subjects were also asked to consume an energy-restricted diet (7,25). Therefore, it is possible that some patients taking THL increase their energy intake (28), although the latter has not been formally evaluated in these chronic studies (7,25,28). There are a number of potential mechanisms by which THL could affect appetite, and the relative importance of these is likely to vary between individuals, so that some people may reduce their food intake, while others increase it. Our data suggest that the observed increase in food intake in the present study is attributable to the reduction in stimuli (such as free fatty acids, CCK, GLP-1) that act to inhibit food intake. However, a contribution of other factors, eg of humoral, psychological, environmental or psychosocial nature, cannot be excluded. A role for the rate of gastric emptying as a factor per se was eliminated in our study by infusing fat directly into the duodenum.

In summary, our data illustrate how apparently different systems (gastrointestinal motility and appetite regulation) which are commonly studied separately are, at least in part, governed by the same mechanisms and interact intimately. Lipase inhibition prevents the release of free fatty
acids from triacylglycerides which in turn affects motility patterns in the antropyloroduodenal region, hormone release and food intake.
References


Acknowledgements

Christine Feinle is supported by a Florey Research Fellowship from the Royal Adelaide Hospital. The authors are grateful to Hoffmann-La Roche, Basle, Switzerland, for financial support of the study and the supply of tetrahydrolipstatin. The authors would also like to thank Ms Virginia Sharley from the Royal Adelaide Hospital pharmacy for producing the fat emulsions and Assoc Prof Geoff Hebbard, Royal Melbourne Hospital, for providing Trace! and his input into the analysis of the pressure data.
Figure legends

Figure 1: Study protocol. Each subject received a duodenal infusion of a fat emulsion at a rate of 2.8 kcal/min for 120 min, on one day with, and on the other day without, 120 mg of the lipase inhibitor tetrahydrolipstatin (THL). Appetite-related sensations, plasma cholecystokinin and glucagon-like peptide-1 concentrations and antropyloroduodenal pressure activity were measured during the infusion. Immediately following the infusion, food intake at a buffet meal was quantified.

Figure 2: Schematic drawing of the manometric assembly. Six manometric channels were located in the antrum at 1.5 cm intervals (channels 1-6); a 4.5 cm sleeve sensor straddled the pylorus (channel 7) and 2 channels, 1.5 and 3 cm from its orad end (channels 8 and 9), were situated on the back of the sleeve; seven more channels were situated in the duodenum at 1.5 cm intervals (channels 10 – 16). The infusion port for the duodenal infusion was situated ~13 cm beyond the pylorus.

Figure 3: Example of antropyloroduodenal pressure patterns during duodenal infusion of FAT (A, triglyceride emulsion) and FAT-THL (B, triglyceride emulsion containing 120 mg of the lipase inhibitor THL). Infusion of FAT resulted in a ‘fed’ motor pattern (A), characterised by isolated pyloric pressure waves and inhibition of antral and duodenal phasic pressures. In contrast, during condition FAT-THL, there was pronounced propulsive antropyloroduodenal pressure activity (B).

Figure 4: Phasic and tonic pyloric pressure activity. Both the number (A) and amplitude (B) of isolated pyloric pressure waves and basal pyloric pressure (C) were less during FAT-THL when compared with FAT. Data are means ± SEM, n = 16 subjects. * P = 0.0059, # P = 0.0234, ♦ P = 0.005, significantly different from FAT. FAT: triglyceride emulsion, FAT-THL: triglyceride emulsion containing 120 mg of the lipase inhibitor THL.
Figure 5: Antropyloroduodenal pressure wave sequences. A greater number of pressure wave sequences (A) with a greater length (B) occurred in the antropyloroduodenal region during condition FAT-THL when compared with FAT. Data are means ± SEM, n = 16 subjects. * P = 0.0013, # P = 0.0003, significantly different from FAT. FAT: triglyceride emulsion, FAT-THL: triglyceride emulsion containing 120 mg of the lipase inhibitor THL.

Figure 6: Scores for ‘prospective consumption’ (A) and nausea (B). ‘Prospective consumption’ scores declined progressively during the FAT infusion, while remaining at baseline levels throughout the infusion period during the FAT-THL condition. Nausea scores were very low during both infusions. Data are means ± SEM, n = 16 subjects. * P = 0.037, # P = 0.045, significantly different from FAT. FAT: triglyceride emulsion, FAT-THL: triglyceride emulsion containing 120 mg of the lipase inhibitor THL.

Figure 7: Plasma concentrations of CCK (A) and GLP-1 (B). The rise in plasma CCK and GLP-1 levels in response to the FAT infusion was absent during the FAT-THL infusion. Data are means ± SEM, n = 16 subjects. * = significantly different from FAT, P = 0.000. FAT: triglyceride emulsion, FAT-THL: triglyceride emulsion containing 120 mg of the lipase inhibitor THL.
**Table 1:** Characteristics of isolated pressure waves in the antrum, pylorus and duodenum

<table>
<thead>
<tr>
<th></th>
<th>Total number (0 – 45 min)</th>
<th>Amplitude (mmHg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FAT</td>
<td>FAT-THL</td>
</tr>
<tr>
<td>Antrum</td>
<td>2.8 ± 1.9</td>
<td>17.2 ± 5.0</td>
</tr>
<tr>
<td>Pylorus</td>
<td>50.2 ± 10.1</td>
<td>25.9 ± 5.2</td>
</tr>
<tr>
<td>Duodenum</td>
<td>64.5 ± 10.4</td>
<td>91.5 ± 9.8</td>
</tr>
</tbody>
</table>

Data are means ± SEM, n = 16 subjects.
Table 2: Characteristics of pressure wave sequences

<table>
<thead>
<tr>
<th>Origin</th>
<th>FAT</th>
<th>FAT-THL</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Antrum</td>
<td>2.7 ± 1.7</td>
<td>9.7 ± 3.0</td>
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<tr>
<td>Pylorus</td>
<td>4.6 ± 2.1</td>
<td>14.9 ± 3.3</td>
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<tr>
<td>Duodenum</td>
<td>27.0 ± 7.8</td>
<td>49.8 ± 6.3</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Data are means ± SEM, n = 16 subjects.
Figure 1

Antropyloroduodenal motility

Intraduodenal fat +/- THL

Buffet meal

-30 -15 0 15 30 45 60 75 90 105 120 135 165 195

Time (min)

Blood sample

Visual analogue scale
Figure 3
Feinle et al

Figure 4

A. Number of IPPWs

B. Amplitude of IPPWs

C. Basal pyloric pressure

* * # **
Figure 5

**Number of pressure wave sequences**

A  

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No./15 min</th>
<th>FAT</th>
<th>FAT-THL</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15 - 0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 - 15</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 - 30</td>
<td>35</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>30 - 45</td>
<td>25</td>
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</tbody>
</table>

**Length of pressure wave sequences**

B  

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Length (cm)</th>
<th>FAT</th>
<th>FAT-THL</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15 - 0</td>
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<td></td>
</tr>
<tr>
<td>0 - 15</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>15 - 30</td>
<td>5</td>
<td></td>
<td>#</td>
</tr>
<tr>
<td>30 - 45</td>
<td>3</td>
<td></td>
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</tr>
</tbody>
</table>
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Figure 6

Prospective consumption

**A**

![Graph showing prospective consumption](image)

**B**

Nausea

![Graph showing nausea](image)
Figure 7

A. Plasma CCK concentration

B. Plasma GLP-1 concentration