Role of lipase in the regulation of postprandial gastric acid secretion and emptying of fat in humans: a study with orlistat, a highly specific lipase inhibitor

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Abstract

Background and aims—To investigate the importance of lipase on gastric functions, we studied the effects of orlistat, a potent and specific inhibitor of lipase, on postprandial gastric acidity and gastric emptying of fat.

Methods—Fourteen healthy volunteers participated in a double blind, placebo controlled, randomised study. In a two way cross over study with two test periods of five days, separated by at least 14 days, orlistat 120 mg three times daily or placebo was given with standardised daily meals. In previous experiments we found that this dose almost completely inhibited postprandial duodenal lipase activity. Subjects underwent 28 hour intragastric pH-metry on day 4, and a gastric emptying study with a mixed meal (800 kcal) labelled with ^{99m}Tc sulphur colloid (solids) and ^{111}In thiocyanate (fat) on day 5. Gastric pH data were analysed for three postprandial hours and the interdigestive periods. **Results**—Orlistat inhibited almost completely (by 75%) lipase activity and accelerated gastric emptying of both the solid (by 52%) and fat (by 44%) phases of the mixed meal ($p < 0.03$). Orlistat increased postprandial gastric acidity (from a median pH of 3.3 to 2.7; $p < 0.01$). Postprandial cholecystokinin release was lower with orlistat ($p < 0.03$).

Conclusion—Lipase has an important role in the regulation of postprandial gastric acid secretion and fat emptying in humans. These effects might be explained by lipolysis induced release of cholecystokinin.

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Keywords: lipase; orlistat; gastric secretion; gastric emptying; pH-metry

physiological role in the regulation of gastric acid secretion after ingestion of lipids.

Orlistat (tetrahydrolipstatin, THL), a product of *Streptomyces toxytricini*, has been demonstrated to be a potent and specific inhibitor of gastric lipase, carboxyl ester lipase, and human pancreatic lipase through covalent binding to serine, the active site of the enzyme.² Studies in animals³ and humans⁴ have demonstrated its marked inhibitory effect on lipolytic activity and consequently fat absorption. Previously it has been shown that the products of fat digestion in the small intestine inhibit gastric acid secretion⁵ and gastric emptying,^{6,7} probably by inducing cholecystokinin (CCK) release.⁸ Therefore, we hypothesised that inhibition of fat digestion by blocking lipase activity with orlistat would result in higher postprandial acid secretion and lower CCK levels. Furthermore, we assumed that inhibition of fat digestion would result in acceleration of gastric emptying. Recent data suggest an important action of orlistat on gastric emptying of an unphysiological liquid fatty meal.^{7,8}

The aim of the study was to clarify if specific inhibition of lipase by orlistat after ingestion of a regular meal increased postprandial gastric acidity during regular meals and altered gastric emptying of the fat phase compared with the protein phase. Such results would provide novel information on the specific role of lipase in the regulation of gastrointestinal functions and may help to better understand the mechanisms involved in the pathophysiology of pancreatic insufficiency.⁶ For this purpose we conducted a randomised, double blind, placebo controlled study and investigated the influence of orlistat on gastric acidity by intragastric pH measurements over 28 hours and gastric emptying of a meal, in particular fat, using a double tracer scintigraphy technique with a new fat phase marker (^{111}In -labelled olive oil).

Subjects and methods

The first part of the study consisted of pancreatic secretion studies. In the second part, pH-metry and gastric emptying studies were performed.

Abbreviations used in this paper: THL, tetrahydrolipstatin; HPL, pancreatic lipase; PEG, polyethylene glycol; CCK, cholecystokinin; AUC, area under the curve.

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Nutrient fat is known to be a potent inhibitor of gastric acid secretion. Digestion of dietary fat by gastrointestinal lipases results in activation of different neural and endocrine pathways which regulate gastric emptying and gastric secretion. Pancreatic lipase (HPL) is the key enzyme in the digestion of lipids in animals and humans.¹ It is not known if hydrolysis of fat by lipase represents a prerequisite for its action on gastric functions and therefore if lipase has a

Fourteen healthy volunteers (11 males, three females; age range 23–32 years) participated in two different studies. Subjects were of normal weight (BMI range 19.3–25.7; mean 22.6); none was receiving any medications or had a history of gastrointestinal symptoms or abdominal surgery. The studies were approved by the local ethics committee and all subjects gave written informed consent.

(1) EFFECT OF 120 MG OF ORLISTAT ON LIPASE ACTIVITY

In previous studies we administered 200 mg of orlistat orally and showed that it effectively inhibited postprandial lipase activity.⁸ As the dose of orlistat was changed to 120 mg by the producer,⁹ we had to demonstrate the efficacy of duodenal lipase inhibition for this lower dose.

Study design

Six healthy volunteers (five males, one female) participated in a double blind, placebo controlled, randomised, two way crossover study on two different days at least seven days apart. Pancreatic lipase (HPL) secretion and gastric emptying were measured using a double indicator technique after administration of a liquid fat meal with 120 mg of orlistat or placebo.

Experimental procedure

All studies were performed after an overnight fast. The experimental procedure has been described previously in detail.¹⁰ Briefly, subjects were intubated with a triple lumen tube, the tip being positioned at the ligament of Treitz and verified by fluoroscopy.¹⁰ Infusion of polyethylene glycol (PEG) 4000 (1.5 g PEG/100 ml 0.154 mol/l saline) was performed at a rate of 1.5 ml/min through a channel ending at the ampulla Vateri, 20 cm proximal to the tip, and 60 minutes were allowed for equilibration before administration of the meal. The test meal was a stable emulsion of long chain triglycerides (500 ml Intralipid 10%; Kabi Vitrum, Stockholm, Sweden). Orlistat is extremely hydrophobic and special care was required to incorporate orlistat (or placebo) into the test meal by continuous overnight stirring. After complete aspiration of gastric contents, the test meal was instilled through the proximal gastric port over 10 minutes. Duodenal samples (7–8 ml) were continuously siphoned onto ice from the distal sampling site at 10 minute periods from –20, –10, –1 minute before (baseline) and every 10 minutes up to 180 minutes after instillation of the test meal. Gastric samples (8 ml) were taken at identical times from the gastric aspiration site. All samples were immediately centrifuged and glycerol (50% v/v) was added to duodenal samples for later HPL analysis. All samples were frozen and stored at –20°C until assayed.

Determinations

Duodenal samples were analysed for PEG concentrations and HPL activity as described previously.⁸ Gastric samples were analysed for PEG concentrations. Measurement of PEG

concentrations of gastric aspirates showed that PEG reflux of duodenal contents into the stomach was either lacking or negligible.

Calculations

Secretory outputs of HPL passing the duodenal sampling site were calculated from the product of enzyme concentrations and flow rates.⁸

(2) EFFECT OF ORLISTAT ON POSTPRANDIAL GASTRIC 28 HOUR pH-METRY AND EMPTYING OF A MIXED MEAL

Study design

Eight healthy volunteers (six males, two females) participated in a double blind, placebo controlled, randomised, two way crossover study. Two test periods of five days each were separated by a washout period of at least four days (maximum 28 days). On days 1–4 during each test period, volunteers received 120 mg of orlistat or placebo three times daily, administered midway through three standardised meals containing 3000 kcal/day with 31% kcal as fat. On the morning of day 4 of each test period, subjects underwent a 28 hour pH-metry investigation. On the morning of day 5, gastric emptying of a test meal containing 120 mg of orlistat or placebo was assessed by scintigraphy. CCK, secretin, and gastrin were measured during the gastric emptying study on day 5.

Meals (days 1–4)

On days 1–4 volunteers were asked to eat three regular appetising meals containing a total of 110 g of proteins, 399 g of carbohydrates, and 101 g of fat at fixed times (8 am to 9 am, 11 30 am to 1 pm, and 6 pm to 7 pm). The meals were prepared by the hospital according to the dietitian to ensure stable caloric content. Compliance was assessed after each meal intake in the hospital restaurant. Breakfast consisted of milk (250 ml) with chocolate powder, two pieces of bread, 20 g of butter, 50 g of jam, and one yogurt with fruits and cream, containing 25 g of proteins, 122 g of carbohydrates, and 39.5 g of fat. Lunch consisted of freshly prepared hot dishes with different meats (veal, chicken), noodles or potatoes, salad and vegetables, a chocolate cream, and apple juice (300 ml), containing 42.5–48 g of proteins, 125–131 g of carbohydrates, and 30–32 g of fat. Dinner consisted of a ham and cheese sandwich, cream with fruits, fresh fruit, and orange juice (250 ml) and contained 36.5–41 g of proteins, 126–138 g of carbohydrates, and 32–33 g of fat. The total caloric contents of the meals were as follows: breakfast 943 kcal, lunch 944–964 kcal, supper 965–986 kcal, and daily fruit provided an additional 68–80 kcal. The total daily caloric content was 2920–2969 kcal.

Gastric 28 hour pH-metry (days 4 and 5)

Following an overnight fast two pH glass probes (Ingold Messtechnik, Urdorf, Switzerland) were passed transnasally after local anaesthesia and positioned under fluoroscopic control with the tips in the antrum and corpus, at 5 cm and 15 cm distal to the cardia, respec-

tively. The position was identical for the two experiments. Intragastric pH-metry was recorded using an ambulatory data logger (Medical Instrument Corporation, Solothurn, Switzerland) and stored digitally. pH-probes were calibrated at pH 7 and pH 1.7 before and after the experiments. On day 4 volunteers received a standardised breakfast, lunch, and dinner (as described above) and ingested the test meal on day 5 at fixed times (9.30 am, 1 pm, and 6.30 pm on day 4; 9.30 am on day 5). They were not allowed to eat between meals; only tap water was permitted. The start and end of each meal were marked by meal markers on the data logger.

Gastric emptying study (day 5)

The test meal for the gastric emptying study on day 5 consisted of a protein pancake (210 g; 350 kcal; 66.7 g of proteins, 2.6 g of carbohydrates, and 8.4 g of fat). The pancake was labelled with 18.5 MBq of ^{99m}Tc sulphur colloid topped with margarine (317 kcal; 0.1 g of carbohydrates, 0.1 g of proteins, and 35 g of fat) and jam (134 kcal; 32.7 g of carbohydrates). The margarine was mixed with labelled olive oil containing 3.5 MBq mCi of ^{111}In . The total calories in the test meal was 801 kcal. Volunteers started the test meal at 10 am (range 9 45 to 10 22 am). Subjects were allowed to eat the cake within 10–15 minutes. The observed range was 8–13 minutes. A total of 400 ml of water were consumed continuously after meal intake had started.

Labelling of the solid phase

For labelling of the solid phase, 18.5 MBq of ^{99m}Tc -SC and all ingredients were mixed and baked at 200°C for 35 minutes, resulting in a pancake of dense consistency. The stability of the labelling of the solid phase was examined by in vitro experiments ($n=10$) as described previously.¹¹ Briefly, 2.5 g of the baked pancake were homogenised in 5 ml of pooled gastric juice titrated to pH 2 ($n=5$) or pH 5 ($n=5$). In each experiment five cycles of homogenisation were performed over a two hour period with a magnetic vortex mixer, followed by centrifugation in gastric juice and washes with 10 ml of normal saline solution. Radioactivities of the final solid phase and supernatants were measured by a gamma counter and the counts of the supernatants were subtracted from the total radioactivity measured in each tube: 94 (0.5)% (mean (SEM)) of ^{99m}Tc -SC was retained at pH 5 and 67 (1.7)% at pH 2 after homogenisation.

Labelling of the fat phase

A new method of labelling of olive oil with ^{111}In was developed as the previously published protocol for ^{99m}Tc was not suitable for ^{111}In binding.¹² Olive oil was labelled with 3.7 MBq of $^{111}\text{In}(\text{III}) (\text{SCN})_3$ by direct extraction from dried sodium thiocyanate. The preparation of $\text{In}(\text{SCN})_3$ was performed by double decomposition between sodium thiocyanate and indium trichloride in dry ethanol and resulted in high yields of $\text{In}(\text{SCN})_3$.

Concentrated hydrochloric acid (50 ml) was added to a small volume (200 ml) of ^{111}In chloride (150 MBq) in a glass vial (10 ml). The content of the vial was evaporated to dryness at room temperature in a stream of nitrogen. The residue was dissolved in an alcoholic solution (0.3 ml) of sodium thiocyanate (5 mg/ml EtOH) and the content of the vial was stirred. The solution was again evaporated to dryness in a stream of nitrogen and the residue mixed with 20 ml of olive oil. After stirring (10 minutes), 10 ml of phosphate buffer (sodium hydrogen phosphate 2.2 M) were added and the mixture shaken for two minutes to remove excess thiocyanate. After centrifugation the oil was washed with phosphate buffer a second time and was ready to use. Residual thiocyanate, as determined by C-14-labelled SCN^- with a beta counter (Tri-Carb 2700, Packard, Meriden, USA), was negligible with a concentration of less than 3 mg of SCN^- in 6 g of the final ^{111}In labelled oil. The yield of radioactivity in the resulting labelled oil represented 15% of the initially added ^{111}In chloride. The radioactivity of the oil was measured and fresh inactive oil was added to adjust the radioactive concentration to 3.7 MBq/10 g oil per test meal and volunteer. Labelled olive oil was then mixed with 120 mg of orlistat and margarine at 35°C to avoid denaturation of orlistat at higher temperatures. Thereafter the mixture was cooled at -20°C for 20 minutes.

Validation of stability in dilute hydrochloric acid

Stirring of 4 g of labelled olive oil in 20 ml of dilute HCL (pH 1.4) over three hours demonstrated high stability (94.5% retention of labelling after 180 minutes, mean of two experiments).

Validation of stability in human gastric juice

The stability of labelling according to the same protocol as the solid phase showed a final labelling retention (after 180 minutes) in the fat phase of 89.4% at pH 2 and 90.8% at pH 5.

Measurement of gastric emptying

After ingestion of the meal, volunteers were seated between the two heads of a dual headed gamma camera fitted with a 270 keV parallel hole collimator and interfaced to a dedicated computer (Siemens, Erlangen, Germany). Radioactivities of ^{99m}Tc and ^{111}In were surveyed through 140 keV and 245 keV windows, respectively. For each radioisotope, anterior and posterior images were acquired simultaneously over 60 seconds every four minutes for the first 30 minutes, every six minutes for the next 90 minutes, and every 10 minutes for the last 60 minutes of the three hour test. Data were stored for subsequent analysis. For analysis, the gastric region of interest was outlined on the computer display. The total gastric region of interest was divided into proximal and distal regions, with the proximal corpus and the distal region. The proximal region included the fundus and proximal corpus and the distal region the distal corpus and antrum.¹³ For each time point total radioactivity for the respective radioisotope in the gastric

region was measured. The final values were obtained by calculating the geometric mean of the anterior and posterior counts. Background counts were subtracted and corrections for radionuclide decay and downscatter of ^{111}In into the $^{99\text{m}}\text{Tc}$ window were calculated based on phantom studies as previously published.¹⁴

RADIOIMMUNOASSAY OF CHOLECYSTOKININ AND GASTRIN

On day 5 of each test period blood samples were taken through an indwelling venous catheter at the following times: -30, -15, and -5 minutes before and 5, 10, 20, 30, 45, 60, 90, 130, and 180 minutes after intake of the test meal. Blood samples were collected in 10 ml tubes containing 16 mg of EDTA and 8000 U of aprotinin and were immediately centrifuged (4°C, 3000 rpm, 15 minutes). All samples were frozen on dry ice and stored at -20°C until assayed.

Plasma CCK concentrations were measured using a sensitive and specific radioimmunoassay.¹⁵ Antibody T204 binds to all CCK peptides containing the sulphated tyrosyl region of CCK. The antibody shows <2% cross reactivity to sulphated forms of gastrin and no binding to non-sulphated gastrins and does not cross react with structurally unrelated gastrointestinal peptides. CCK 33 coupled with [^{125}I]hydroxyphenylpropionic acid succinimide ester (Bolton-Hunter reagent) was used as a label and synthetic CCK 8 was used as a standard. The detection limit of the assay was 0.5 pmol/l of plasma and extraction recovery was 90 (2)% when sulphated CCK 8 was added to hormone free plasma. Intra-assay and interassay variations were 8% and 11%, respectively, at 2.6 pmol/l.

Plasma gastrin levels were measured using a sensitive and specific RIA as previously described in detail.¹⁶ In brief, antibody 2604, used in the gastrin assay, had equal affinity for component-1 gastrin and sulphated and non-sulphated forms of gastrin 34 and gastrin 17. Binding of gastrin 14 to the antiserum was 60% compared with gastrin 17. However, as gastrin 14 constitutes only a small percentage of total circulating gastrin, the serum concentration measured with antiserum 2604 closely reflects the total amount of gastrin present.

CALCULATIONS AND STATISTICAL ANALYSIS

Results are expressed as mean (SEM) or median (95% confidence intervals) (pH values). The mean of the two intragastric pH values was obtained to minimise intragastric pH variations due to localisation of the two pH probes. During the 28 hour pH-metry, the definition of the three hour postprandial periods was based on the event markers introduced by the volunteers on the data logger at the end of each meal. Accordingly, medians of the three hour postprandial pH recordings were calculated. Gastric emptying of the gastric region of interest was expressed at each time as percentage of the total counts measured in the first eight postcibal minutes. Emptying curves of the gastric region were fitted to a power exponential curve as described by Elashoff and

colleagues.¹⁷ The time period before 10% of the isotope had emptied from the stomach (lag period), the parameter β , the time at which 50% of the isotope had left the stomach ($t_{1/2}$), and the area under the emptying curve (AUC) were calculated. Increments in CCK and gastrin were computed by subtracting basal values from postprandial levels and the AUC were calculated. Groups of data were compared using the Kruskal-Wallis one way analysis of variance for non-parametric data and, consecutively if statistically different, by a Wilcoxon signed rank test for paired data or a Mann-Whitney U test for unpaired data, respectively. All tests were two-tailed. Differences were considered significant at $p < 0.05$.

Results

(1) EFFECT OF 120 MG OF ORLISTAT ON LIPASE ACTIVITY

HPL activity

Outputs of enzymatically active HPL (kU/10 minutes) before and after gastric instillation of the Intralipid mixed with orlistat or placebo are shown in fig 1. Basal secretory outputs of HPL were not different between experiments with orlistat and placebo (10.2 (2.1) kU/10 minutes *v* 7.7 (2.1) kU/10 minutes). HPL activity reached a maximum 30 minutes after ingestion of the meal during infusion of placebo; 120 mg of orlistat suppressed this early postprandial response almost completely ($p < 0.03$) resulting in values comparable with baseline. Cumulated outputs of HPL over 180 minutes were greatly decreased by orlistat (114.7 (13.9) kU) compared with placebo (446.5 (54) kU) ($p < 0.03$).

(2) EFFECT OF ORLISTAT ON POSTPRANDIAL GASTRIC 28 HOUR pH-METRY AND EMPTYING OF A MIXED MEAL

Gastric 28 hour pH-metry

Figure 2 shows the 28 hour medians for intragastric pH values during four meals and three interdigestive periods. The meal induced in-

Figure 1 Duodenal outputs of pancreatic lipase (HPL) after intragastric instillation of triglycerides (Intralipid) with placebo or orlistat. Values are mean (SEM) of six subjects.

Figure 2 Median intragastric pH during the 28 hour measurements in eight subjects who received placebo or orlistat. B, breakfast; L, lunch; D, dinner.

Figure 3 Box-whisker plots of postprandial gastric pH (three hour periods) in subjects who received placebo or orlistat. Boxes represent the medians and interquartile ranges, the error bars the 90% and the dots the 95% confidence intervals. $p < 0.01$.

crease in intragastric pH reached a postprandial maximum (pH 3.85–5.15) at 10–20 minutes followed by a decline in pH during the postprandial periods and returned to baseline (pH 1.17–1.93) after about three hours. Initial postprandial peak gastric pH values were similar after placebo and orlistat. The subsequent decline in postprandial pH values were markedly lower after orlistat compared with placebo (fig 2). Thus ingestion of 120 mg of orlistat resulted in lower (median 2.68 *v* 3.27 with placebo; $p < 0.01$) intragastric pH values during the three hour postprandial periods compared with placebo (fig 3).

Gastric emptying of the solid and fat phases

There was wide interindividual variation in intragastric distribution of the solid and fat phases (fig 4). Meal retention in the distal stomach was more pronounced for the solid phase compared with the fat phase (maximal meal contents in the distal stomach: 69.5 (4.1)% *v* 64.3 (4.3)%; $p < 0.001$) indicating redistribution and retention of fat towards the fundus (fig 4). Intragastric distribution of the two meal phases was not influenced by orlistat compared with placebo. The observed maximal meal retention in the distal stomach was 71.3 (5.4)% compared with 67.8 (6.4)% (NS, orlistat *v* placebo) for the solid phase and 65.1 (6.3)% compared with 63.5 (6.3)% (NS, orlistat *v* placebo) for the fat phase. Orlistat mixed to the fat phase considerably shortened half-emptying times of both the solid (by 52%) and fat (by 44%; $p < 0.03$ and $p < 0.02$) phases and decreased AUC values (by 13% and 12%; $p < 0.03$ and $p < 0.02$) for both meal phases compared with placebo (fig 5, table 1). The lag period of the fat phase was shortened by 33% with orlistat ($p < 0.05$) but was not different for the solid phase (table 1). The parameter β , describing the shape of the curve, was not statistically different between the experiments.

PLASMA CCK AND GASTRIN

Basal plasma values for CCK and gastrin concentrations were similar in experiments with placebo and orlistat. Postprandially, integrated

Figure 4 Gastric emptying of the proximal (left) and distal (right) stomach regions of the ^{111}In -labelled fat phase and $^{99\text{m}}\text{Tc}$ -labelled solid phase with placebo. Values are mean (SEM) of eight subjects.

Figure 5 Gastric emptying of the $^{99\text{m}}\text{Tc}$ -labelled solid phase (left) and ^{111}In -labelled fat phase (right) with placebo and orlistat. Values are mean (SEM) of eight subjects.

Table 1 Effect of orlistat on gastric emptying of the solid and fat meal phases

	Solid phase		Fat phase	
	Placebo	Orlistat	Placebo	Orlistat
Lag period (minutes)	57 (11)	34 (6)	33 (5)	22 (1)*
$t_{1/2}$ (minutes)	363 (89)	175 (19)**	255 (42)	144 (15)***
AUC (% counts $\times$ min)	14 542 (434)	12 842 (456)**	13 522 (441)	11 799 (299)***

Values are mean (SEM). * $p < 0.05$, ** $p < 0.03$, *** $p < 0.02$ orlistat *v* placebo.

CCK values (AUC) were markedly decreased by orlistat administration compared with placebo (91 (19) *v* 171 (26) pM $\times$ min; $p < 0.03$), while there was no difference in gastrin plasma concentrations during the 180 minute postprandial periods (2633 (498) *v* 3278 (414) pM $\times$ min; NS).

Side effects of orlistat were observed as follows: six of eight volunteers had intermittently soft, fatty, oily stools during orlistat treatment, and one volunteer had one episode of a flatus with discharge. One of eight volunteers had intermittent soft stools during placebo treatment. These were all expected side effects and volunteers were informed before study inclusion.

Discussion

This double blind, placebo controlled study demonstrates that specific lipase inhibition by orlistat increases postprandial gastric acid secretion after regular physiological solid-liquid meals. Furthermore, we found that orlistat accelerated a gastric meal, in particular fat emptying, and diminished postprandial CCK release. Thus our results suggest for the first time an important role for lipase and CCK in the regulation of postprandial gastric acid secretion and gastric emptying of fat and solids after physiological meals in humans.

In pH-metry studies we found a decrease in postprandial gastric acid secretion after inhibition of lipases in the small intestine by orlistat; to our knowledge this is the first time this has been demonstrated. Previous studies with intragastric aspiration of liquid meals in animals and humans showed that hydrolysed fat markedly inhibited acid secretion whereas undigested triglycerides were only weak inhibitors of gastric secretion.^{5 18 19} Shiratori and colleagues⁵ found in the rat complete loss of the inhibitory effect of fat on gastric acid secretion during intraduodenal triglyceride infusion if pancreatic juice was diverted. Studies in humans have shown that digested fat potently inhibited both gastrin and meal stimulated gastric acid secretion.^{20 21} Thus the increase in gastric acid secretions by orlistat may be explained by inhibition of triglyceride hydrolysis and consequent diminished release of free fatty acids, which are potent inhibitors of gastric acid secretion.⁵ In this study we have demonstrated that orlistat blunts fat induced inhibition of gastric acid secretion to a similar extent as its inhibitory effect on postprandial pancreatobiliary secretion.⁸ These findings indicate that common mediators and neurotransmitters of gastric and pancreatobiliary secretions, such as CCK and other putative enterogastrones (for example secretin, somato-

statin, peptide YY, GIP, and neurotensin) may be involved. Orlistat substantially decreased postprandial CCK release in our study indicating that CCK may play a major role as a mediator of postprandial intestinal inhibition of gastric acid secretion by lipolytic products. Similarly, we⁸ and others²² have previously found that CCK release after liquid fat meals is regulated by lipase dependent fat hydrolysis. Studies with specific CCK-A antagonists postulate CCK induced inhibition of gastric acid secretion involving paracrine somatostatin secretion from antral and fundic D cells.²³⁻²⁵ The presence of CCK-A and β receptors on the vagus nerve²⁶ and the finding that intraduodenal fat does not inhibit gastrin stimulated acid secretion in patients who had truncal vagotomy²⁷ suggest that neural mechanisms are also involved.

Although administration of orlistat increased postprandial gastric acid secretion we found similar gastrin release after orlistat and placebo. In accordance with these results, it has previously been demonstrated in duodenal ulcer patients that inhibition of gastric acid and pepsin secretion is largely independent of gastrin release.²⁰ Accordingly, studies with the selective gastrin receptor antagonist YM022 in rats showed no inhibition of bethanechol and histamine stimulated acid secretion.²⁸ Thus fat induced inhibition of gastric acid secretion does not appear to depend on circulating plasma gastrin.²⁶ Although it is known that postprandial acidity and gastric emptying are linked and both inhibited by free fatty acids in the small bowel, differences in intragastric pH with orlistat were similarly pronounced and occurred early during fast emptying breakfast meals and slow emptying test meals. Thus it seems improbable that the effect of orlistat on intragastric pH was caused only by acceleration of gastric emptying.

Orlistat moderately accelerated gastric emptying of a solid-liquid meal containing proteins and fat. Previously, we and others have shown that liquid meal emptying is accelerated by orlistat in animals⁹ and humans.⁸ The effects of orlistat in these earlier investigations using liquid fat emulsions were more pronounced compared with the findings of our study. This is probably because of the more homogeneous mixture of orlistat with the liquid meal resulting in more potent inhibition of lipolysis. However, liquid meals, as used in these previous studies, are unphysiological. Furthermore, emptying of fat has not been specifically tracked. Therefore, in this study we administered solid-liquid meals and labelled specifically the fat and solid phases. Monitoring of the fat compared with the solid phase is important as emptying of fat might be regulated differently than that of proteins and may depend on several factors such as layering, formation of emulsion droplets, and adherence to solid food.²⁹ Similarly, only 15–22% of extracellular fat was previously found to empty with the solid phase.^{29 30} Hence for the first time we showed that selective inhibition of lipase by orlistat has an equal accelerating effect on protein and lipid phase emptying, demonstrating a

critical role for lipase digestion of fat for regulation of emptying of both meal phases. Our findings are supported by studies in dogs with pancreatic fistulae⁷ showing that inhibition of triglyceride lipolysis accelerates gastric emptying of dietary fat in a dose related manner. The inhibitory action of lipase on gastric emptying is based on the release of free fatty acids by hydrolysis of fat as orlistat has no effect on emptying of meals containing already hydrolysed fat or exclusively proteins.⁸ Accordingly, non-hydrolysable fat was shown to empty rapidly, at the same rate as water.³¹ Cortot and colleagues³¹ showed with duodenal aspiration techniques that butter emptied very slowly (7% in the first hour) and sucrose polyester oil (a non-absorbable analogue of dietary fat) very rapidly (40% in the first hour). In contrast, our results indicate that margarine empties in the first hour similarly to solids (18% with placebo *v* 25% with orlistat) which may be explained by redistribution of fat. While emptying of butter was underestimated by the aspiration technique, the sucrose polyester meal of Cortot *et al* emptied more rapidly compared with our meals mixed with orlistat. This is due to gastric retention and layering of margarine in our experiments, probably resulting in delayed lipolysis and delayed inhibition of lipolysis by orlistat in the duodenum, respectively. The rather slow emptying of a moderate amount of margarine (35g of fat) in our experiments with and without orlistat is supported by previous studies showing that higher amounts of fat empty faster in patients with severe pancreatic insufficiency.^{6, 32} Thus it could be expected that meals with high fat contents (>70% ingested calories) and high fat loads entering the duodenum would result in a more pronounced effect of orlistat than shown in our experiments. Recently the chain length of free fatty acids was shown to be crucial, as only 12-carbon-long chain fatty acids or greater stimulate efficiently plasma CCK and reduce antral contractile amplitude.³³ Our analysis of intragastric distribution of fat showed only small differences compared with the protein phase. This was due to mixing of non-liquid fat to protein which results in more adherence and layering in contrast with a more marked difference in liquid oil and soup as published previously.³⁴ Furthermore, although gastric emptying was moderately accelerated, intragastric distribution was not changed significantly by orlistat. Interindividual variations in the proximal and distal areas of the stomach and overall slow emptying times in a limited number of subjects may explain why differences were not more pronounced.

In summary, our studies with the lipase inhibitor orlistat provide evidence for the importance of lipase in the feedback regulation of upper gastrointestinal functions, in particular gastric acid secretion and fat emptying during digestion of physiological solid-liquid meals. Our findings support the role of cholecystokinin as an enterogastrone involved in the regulation of postprandial gastric acid secretion and gastric meal emptying.

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- Borgström B. Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. *Biochim Biophys Acta* 1988;962:308-16.
- Hadvary P, Lengsfeld H, Wolfe H. Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. *Biochem J* 1988;256:357-61.
- Fernandez E, Borgström B. Effects of tetrahydrolipstatin, a lipase inhibitor, on absorption of fat from the intestine of the rat. *Biochim Biophys Acta* 1989;1001:249-55.
- Güzelhan C, Crijns HJM, Peeters PAM, Jonkman JHG, Hartmann D. Pharmacological activity (inhibition of fat absorption) and tolerability in healthy volunteers of tetrahydrolipstatin (THL)-specific lipase inhibitor (abstract). *Int J Obesity* 1991;15:29.
- Shiratori K, Watanabe S, Takeuchi T. Intestinal fat digestion plays a significant role in fat-induced suppression of gastric acid secretion and gastrin release in the rat. *Dig Dis Sci* 1993;38:2267-72.
- Carney BI, Jones KL, Horowitz M, Sun WM, Penagini R, Meyer JH. Gastric emptying of oil and aqueous meal components in pancreatic insufficiency: effects of posture and on appetite. *Am J Physiol* 1995;268:G925-32.
- Meyer JH, Elashoff JD, Domeck M, *et al*. Control of canine gastric emptying of fat by lipolytic products. *Am J Physiol* 1994;266:G1017-35.
- Schwizer W, Asal K, Kreiss Q, *et al*. Role of lipase in the regulation of upper gastrointestinal functions in humans. *Am J Physiol* 1997;273:G612-20.
- Sjöström L, Rissanen A, Andersen T, *et al*. Randomised placebo controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. *Lancet* 1998;352:167-73.
- Fried M, Erlacher U, Schwizer U, *et al*. Role of cholecystokinin in the regulation of gastric emptying and pancreatic secretion in humans. Studies with the cholecystokinin-receptor antagonist loxiglumide. *Gastroenterology* 1991;101:503-11.
- Wright RA, Thompson D, Syed I. Simultaneous markers for fluid and solid gastric emptying: new variations on an old theme: concise communication. *J Nucl Med* 1991;22:772-6.
- Cunningham KM, Baker RJ, Horowitz M, Maddox AF, Edelbrock M, Chatterton BE. Use of technetium-99m (V) thiocyanate to measure gastric emptying of fat. *J Nucl Med* 1991;32:878-81.
- Collins PJ, Horowitz M, Chatterton BE. Differential emptying rates of proximal and distal stomach in normal subjects. *Br J Radiol* 1988;57:689-95.
- Pires Jorge JA, Bischof Delaloye A, Delaloye B, Schwizer W, Fried M. Preliminary evaluation of technical parameters for dual isotope gastric emptying studies (abstract). *Eur J Nucl Med* 1993;20:773.
- Jansen JBMJ, Hopman WPM, Lamers CBHW. Plasma cholecystokinin concentrations in patients with pancreatic insufficiency measured by sequence specific radioimmunoassays. *Dig Dis Sci* 1984;29:1109-17.
- Jansen JBMJ, Lamers CBHW. Effect of changes in serum calcium on secretin-stimulated serum gastrin in patients with Zollinger Ellison syndrome. *Gastroenterology* 1982;83:173-8.
- Elashoff JD, Reedy TJ, Meyer JH. Analysis of gastric emptying data. *Gastroenterology* 1982;83:1306-12.
- Long JF, Brooks FP. Relation between inhibition of gastric secretion and absorption of fatty acids. *Am J Physiol* 1965;209:447-51.
- Shay H, Gershon-Cohen J, Fels SS. The role of upper small intestine in the control of gastric secretion; the effect of neutral fat, fatty acid, and soaps; the phase of gastric secretion influenced and the relative importance of the psychic and chemical phases. *Ann Intern Med* 1939;13:294-307.
- Gross RA, Isenberg JJ, Hogan D, Samloff IM. Effect of fat on meal-stimulated duodenal acid load, duodenal pepsin load, and serum gastrin in duodenal ulcer and normal subjects. *Gastroenterology* 1978;75:357-62.
- Maas MIM, Hopman WPM, Katan MB, Jansen JBMJ. Inhibition of gastrin stimulated gastric acid secretion by medium-chain triglycerides and long-chain triglycerides in healthy young men. *Regul Pept* 1996;66:203-10.
- Hildebrand P, Petrig C, Burckhardt B, *et al*. Hydrolysis of dietary fat by pancreatic lipase stimulates cholecystokinin release. *Gastroenterology* 1998;114:123-9.
- Borovicka J, Kreiss C, Asal K, *et al*. Role of CCK as a regulator of solid and liquid gastric emptying in man. *Am J Physiol* 1996;271:G448-53.
- Lloyd KCK, Maxwell V, Chuang CN, Wong HC, Soll AH, Walsh JH. Somatostatin is released in response to cholecystokinin by activation of type A CCK receptors. *Peptides* 1994;15:223-7.
- Schmidt WE, Schenk S, Nustede R, Hoist JJ, Fölsch UR, Creutzfeldt W. Cholecystokinin is a negative regulator of gastric acid secretion and postprandial release of gastrin in humans. *Gastroenterology* 1994;107:1610-20.
- Lin CW, Miller TR. Both CCK-A and CCK-B/gastrin receptors are present on rabbit vagus nerve. *Am J Physiol* 1992;263:R591-5.
- Johnston D, Duthie HL. Effect of fat in the duodenum on gastric acid secretion before and after vagotomy in man. *Scand J Gastroenterol* 1969;4:561-7.

- 28 Nishida A, Ayumi U, Miyata K. The role of endogenous gastrin in basal and stimulated acid secretion in rats (abstract). *Gastroenterology* 1995;**108**:A179.
- 29 Cortot A, Phillips SF, Malagelada JR. Gastric emptying of lipids after ingestion of a solid-liquid meal in humans. *Gastroenterology* 1981;**80**:922-7.
- 30 Meyer JH, Mayer EA, Jehn D, Gu Y, Fink AS, Fried M. Gastric processing and emptying of fat. *Gastroenterology* 1986;**90**:1176-87.
- 31 Cortot A, Phillips SF, Malagelada JR. Parallel gastric emptying of nonhydrolyzable fat and water after a solid-liquid meal in humans. *Gastroenterology* 1982;**82**:877-81.
- 32 Meyer JH, Hlinka M, Kao D, *et al*. Gastric emptying of oil from solid and liquid meals. Effect of human pancreatic insufficiency. *Dig Dis Sci* 1996;**41**:1691-9.
- 33 Mc Laughlin J, Luca MG, Jones MN, D'Amato M, Dockray GJ, Thompson DG. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology* 1999;**116**:46-53.
- 34 Horowitz M, Jones K, Edelbroek MA, Smout AJ, Read NW. The effect of posture on gastric emptying and intragastric distribution of oil and aqueous meal components and appetite. *Gastroenterology* 1993;**105**:382-90.