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Orlistat accelerates gastric emptying and attenuates GIP release in healthy subjects

Feruze Yılmaz Enç,[1,9] Tunç Öneş,[2] H. Levent Akın,[3] Fuat Dede,[2] H. Turgut Turoğlu,[2] Gözde Ülfer,[4] Nural Bekiroğlu,[5] Goncagül Haklar,[4] Jens F. Rehfeld,[6] Jens J. Holst,[7] Nefise B Ulusoy,[1,8] and Neşe İmeryüz[1]

Departments of [1]Gastroenterology, [2]Nuclear Medicine, [4]Biochemistry, [5]Biostatistics Marmara University School of Medicine; [3]Department of Computer Engineering, Boğaziçi University, İstanbul, Turkey; [6]Department of Clinical Biochemistry, University of Copenhagen, Rigshospitalet; [7]Department of Medical Physiology, Panum Institute, University of Copenhagen, Copenhagen, Denmark; [8]Vehbi Koç Foundation American Hospital; and [9]Göztepe Research and Training Hospital, Istanbul, Turkey

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Enç FY, Öneş T, Akın HL, Dede F, Turoğlu HT, Ülfer G, Bekiroğlu N, Haklar G, Rehfeld JF, Holst JJ, Ulusoy NB, İmeryüz N. Orlistat accelerates gastric emptying and attenuates GIP release in healthy subjects. *Am J Physiol Gastrointest Liver Physiol* 296: G482–G489, 2009. First published December 24, 2008; doi:10.1152/ajpgi.90209.2008.—Orlistat, an inhibitor of digestive lipases, is widely used for the treatment of obesity. Previous reports on the effect of orally ingested orlistat together with a meal on gastric emptying and secretion of gut peptides that modulate postprandial responses are controversial. We investigated the effect of ingested orlistat on gastric emptying and plasma responses of gut peptides in response to a solid mixed meal with a moderate energy load. In healthy subjects, gastric emptying was determined using scintigraphy and studies were performed without and with 120 mg of orlistat in pellet form in random order. Orlistat shortened *t* lag and *t* half and decreased the area under the gastric emptying curve. Orlistat significantly attenuated the secretion of glucose-dependent insulinotropic polypeptide (GIP) but did not alter the plasma responses of cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), pancreatic polypeptide (PP), and insulin. There was no peptide YY (PYY) response. Area under the curve of gastric emptying was positively correlated with integrated secretion of GIP ($r = 0.786$) in orlistat and was negatively correlated with integrated plasma response of GLP-1 ($r = -0.75$) in control experiments, implying that inhibition of fat absorption modifies determinants of gastric emptying of a meal. Orlistat administered similar to its use in obesity treatment accelerates gastric emptying of a solid mixed meal with a moderate energy load and profoundly attenuates release of GIP without appreciably altering plasma responses of CCK, GLP-1, and PP. Since GIP is being implemented in the development of obesity, its role in weight control attained by orlistat awaits further investigation.

glucose-dependent insulinotropic polypeptide; glucagon like peptide-1; peptide YY; pancreatic polypeptide; cholecyctokinin; obesity; brain-gut axis

ORLISTAT (TETRAHYDROLIPOSTATIN), which is used in the treatment of obesity, is a covalent inhibitor of digestive lipases and prevents the hydrolysis of dietary triglycerides into free fatty acids and monoglycerides (17, 22). Carrière et al. (5) demonstrated that orlistat inhibits hydrolysis of fat by 70% of an intragastrically administered mixed meal. Nevertheless, in a meta-analysis of randomized controlled studies, weight loss for orlistat-treated obese patients at the end of 1 yr was quite modest at −2.89 kg (confidence interval: 2.27 to 3.51 kg; Ref. 22). Absorption of fat has been shown to modulate gastric, pancreatic, and biliary secretomotor functions (3, 4, 9, 23). Among the signaling mechanisms of fat absorption are secretions of gut peptides such as CCK (3, 9) peptide YY (PYY; Ref. 1), pancreatic polypeptide (PP; Ref. 34), glucose-dependent inhibitory polypeptide (GIP; Ref. 2), and glucagon-like peptide-1 (GLP-1; Ref. 19), which have central and peripheral receptors that are involved in the control of gastric emptying, pancreatico-biliary secretions, glucose homeostatis, and food intake as a part of the brain-gut axis.

Alterations of meal-induced gut peptide release and gastric motility by orlistat treatment may have important physiological as well as clinical implications. Schwizer et al. (36) in 1997 reported that orlistat inhibited pancreatico-biliary secretion, gastric emptying, and CCK secretion in response to an intragastrically administered meal in humans by its ability to inhibit fat hydrolysis. Almost concomitantly, Borovicka et al. (4) found that orlistat accelerated the gastric emptying of an ingested mixed solid meal, increased postprandial secretion of gastric acidity, and diminished the CCK response. Other investigators utilized either intraduodenal or intragastric route to study effect of orlistat on peptide hormone release (6, 7, 10, 11, 18) and gastric motility (6, 11). Accordingly, orlistat inhibits CCK (6, 11, 18), GLP-1 (11), PYY, PP, and ghrelin responses (7, 10, 12). The aforementioned studies (6, 7, 10, 11, 18, 36) were conducted by intraduodenal or intragastric administration of pure triglyceride emulsions with or without orlistat and thus do not replicate the physiological responses of ingested mixed meals. A recent study (15) conducted in healthy subjects showed that orlistat suppresses plasma CCK, GLP-1, and PYY responses and accelerates gastric emptying. However, the insulinotropic hormone GIP, which is implicated to play a role lipid metabolism and obesity (2), was not investigated in this study.

The aim of the study was to investigate the effect of orlistat on gastric emptying of a mixed solid-liquid meal that is similar in macronutrient composition to a physiological diet in conjunction with plasma responses of peptides that are implemented in the regulation of gastric motor activity.

Address for reprint requests and other correspondence: N. İmeryüz, Tonozlu sokak 11/4 Suadiye, Istanbul 34740, Turkey (e-mail: nimeryuz@yahoo.com).

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METHODS

Subjects. Seventeen healthy male volunteers (mean age: 23.7 ± 1.0 yr; range: 20–26; and mean body mass index: 23.0 ± 2.5 kg/m^2) participated in the study. None of the subjects had chronic diseases or prior abdominal surgery. Two subjects had earlier tonsillectomy and partial parathyroidectomy, respectively. The subjects were not on any chronic treatment and did not take any medications. The Institutional Ethics Committee approved the study protocol, and written informed consent was obtained. Seven subjects participated in both the gastric emptying studies and plasma peptide assays. Five of the subjects had gastric emptying studies only because their plasma was accidentally defrosted. From the remaining five subjects, blood was drawn for peptide assays only.

Study design. All studies were performed in the morning. Eating, smoking, and chewing gum were not allowed 12 h before or during the study. An antecubital vein was cannulated with an indwelling catheter to sample blood at specific time points. The meal consisted 100 g of egg, 10 g of butter, 30 g of low fat cheese, and 70 g of white bread providing 22 g of fat, 24.7 g of protein, 52.5 g of carbohydrate, and a total of 510 kcal. The proportion of fat, protein, and carbohydrate was 39.7, 19, and 41%, respectively, of the total energy load.

For determination of scintigraphic gastric emptying the egg component of the meal was mixed with 1 mmCi of 99mTc-tin colloid and, using 10 g of butter, an omelet was cooked until a firm consistency was achieved and was folded over. All subjects randomly underwent two sets of experiments, with and without orlistat, being unaware of the treatment given. In the experiments with orlistat, the contents of the orlistat capsule (120 mg Xenical; F. Hoffmann; La Roche, Basel, Switzerland) were sprinkled on top of the omelet, which was folded over so that the subjects were unaware of the treatment administered. Meals were ingested within 10 min along with 200 ml of sugar-free light tea (Twinnings of London, Ceylon Breakfast, UK), and immediately after scintigraphic acquisitions were obtained as reported previously (8) in 12 subjects.

In preparation for scintigraphic gastric emptying, anatomic markers labeled with low-activity 99mTcO4 were attached to the skin at the sternal notch and both anterior superior iliac spines. One-minute anterior and, immediately afterward, posterior scintigraphic acquisitions were obtained in the sitting position using a large field-of-view gamma camera fitted with a low-energy collimator and were interfaced with a dedicated computer system (GE XRT; General Electric Medical Systems, Milwaukee, WI). Technetium counts were obtained with a 20% energy window with peak set at 140 keV. The scintigraphic acquisitions were obtained immediately after ingestion of the test meal, every 5 min for the first 30 min, and every 10 min thereafter until ~10% of the counts remained in the stomach. Subjects were allowed to ambulate during the intervals between image acquisitions. A region of interest was manually outlined corresponding to the stomach for each scintigraphic image. Corrections were made for decay of the radioactivity. Geometric means of the counts obtained in the anterior and posterior projections were calculated for attenuation correction according to the following formula: geometric mean = square root of (anterior × posterior) counts. Data were normalized to 100% based on total gastric counts obtained immediately after ingestion of the radiolabeled meal. In vivo and in vitro radionuclide labeling stability tests were performed in two subjects as described previously (8).

The percent gastric retention radioactivity of each subject was analyzed using the modified power exponential function according to which $y(t)$ is the fractional meal retention at time t, k is the gastric emptying rate (in min^{-1}), t is the time interval (in min), and β is the extrapolated y-intercept from the terminal portion of the curve of the function $y(t) = 1 - (1 - e^{kt})^{\beta}$. With the use of fractional retention $y(t)$ vs. t, data as input in a hybrid algorithm were utilized to fit the data to the modified power exponential function as described previously (8). Hence, the unknown parameters k and β were determined and a time-activity curve was generated for each subject. Lag phase was calculated by the formula t lag = ln/k representing time t, at which the curve demonstrates an inflection point and after which the slope becomes constant. Gastric half-emptying time (t half) was defined as the time when scintigraphic counts decreased by 50% and was estimated by using data fitted to the modified power exponential function. The fitness of the emptying curve calculated according to modified power exponential model to the actual data has been proven before (8).

Blood specimens. Venous blood samples for GIP, CCK, GLP-1, and PYY analysis were obtained from an indwelling venous catheter at 15 min and immediately before the test meal (0 min) and after 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min in 12 subjects. Venous blood was collected into chilled tubes containing aprotinin (500 KIU/ml of blood; Trasylol, Leverkusen, Germany) and EDTA (1 mg/ml of blood; Merck, Darmstadt, Germany). The tubes were centrifuged at 4°C, and plasma was immediately stored at −20°C until assayed. Blood was collected into blank tubes and centrifuged at 4°C, and serum was stored at −20°C for insulin determinations and was collected into NaF (Merck)-containing tubes for glucose determination.

Analytical procedures. Plasma concentrations of CCK, GIP, GLP-1, PYY, and PP were all measured by highly specific RIAs: CCK using the antibody 92128 (31a), GIP using antibody R65 (8), GLP-1 using antibody 89390 (8), PYY using antiserum 8412–211 (8), and PP using antibody 146 (35), by methods as described in the cited references. Insulin concentrations were measured using a solid phase, two-site chemiluminescent enzyme-labeled immunometric assay (Immulite insulin; Diagnostic Products, Los Angeles, CA). Glucose was measured with the glucose oxidase method (glucose GOD-PAP, BM/Hitachi 917 analyzer; Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. Distribution of the data was analyzed by a one-sample Kolmogorov-Simirnov test. If the P value was >0.05, the distribution of the data was accepted as normal and represented as means ± SE. If the P value was <0.05, data were represented as median and ranges. Basal plasma hormone concentrations were calculated by taking an average of −15 min and 0 min values. Integrated responses of the area under curve (AUC) were calculated according to the trapezoidal rule. To determine whether meal ingestion altered basal plasma determinations, repeated-measures ANOVA with time as a main factor was applied to plasma hormone and glucose concen-

Table 1. *Gastric emptying characteristics of 510 kcal radiolabeled mixed meal*

	Control Experiments	Orlistat Experiments	P Value
t lag, min.	59 (36–96)	44.5 (16–73)	<0.05
t half, min	102.9 (68.4–156.9)	84.4 (49.3–113.2)	<0.05
AUC, min^{-1}	66.124 (49.52–77.7)	58.54 (40.39–69.21)	<0.02
k, min^{-1}	−0.0134 [(−0.02-(−0.0066)]	−0.013 [(−0.019)-(−0.0098)]	NS
β	2.14 (1.5–3.69)	1.7 (1.28–3.54)	NS

AUC, area under the curve; k, gastric emptying rate; t, time interval; β, extrapolated y-intercept from terminal portion of the curve of the function $y(t) = 1 - (1 - e^{kt})^{\beta}$. Data are represented as median (range).

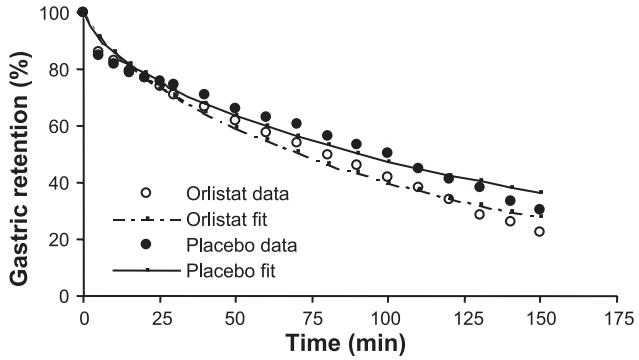

Fig. 1. Percent gastric retention of a radiolabeled 510 kcal mixed meal with and without 120 mg of orlistat. Dashed and solid lines represent data with and without orlistat, respectively, fit to the modified power exponential function. Orlistat accelerated the emptying of mixed meal significantly ($P < 0.02$).

trations, which were followed by Dunnet's test. To determine the effect of treatment on hormonal responses, two-way ANOVA was used, with time and treatment as main factors. Paired data at specific time points were compared by using the Wilcoxon signed-rank test or paired *t*-test, as appropriate. Correlations between gastric emptying data and hormonal responses were investigated by using Pearson's test. Multivariate stepwise regression analysis was used to determine the relationship between dependent and independent variables.

RESULTS

Scintigraphic gastric emptying. In control experiments, the median *t* lag was 59 (36–96) min and the median *t* half was 102.9 (68.4–156.9). The shape of the gastric emptying curve represented by β was 2.14 (1.5–3.69) consistent with solid gastric emptying. Administration of orlistat decreased both *t* lag and *t* half significantly ($P < 0.05$) and reduced β slightly (NS). Orlistat did not alter slope of the gastric emptying curve. The median area under the gastric emptying curve AUC (min^{-1}) was significantly reduced in the orlistat group ($P < 0.02$; Table 1; Fig. 1).

Insulin and glucose. In both experimental groups, blood glucose did not significantly increase except at 45 min in the orlistat group (Fig. 2*A*). Orlistat did not alter the AUC of glucose curve.

Serum insulin increased significantly between 30–90 min in both experimental groups ($P < 0.001$). Two-way analyses revealed that orlistat had no significant effect on the insulin response curve (Fig. 2*B*).

Peptide assays. A brisk increase of plasma PP levels was observed at 5 min after the ingestion of the test meal in both experiments compared with basal ($P < 0.0001$). After the first peak at 10 min, the PP curve had two plateaus at 15–30 and at 90–180 min and returned near to basal at 240 min. The shape and height of the PP curve were similar in both experiments (Fig. 3*A*).

In one-way analyses, plasma CCK levels increased significantly at 15 min in the orlistat group and at 30 min in the control group and remained elevated in both groups until 180 min ($P < 0.01$). Orlistat treatment attenuated the CCK response at 60 min compared with the control response ($P < 0.05$; Fig. 3*B*). However, in two-way analyses, there was no treatment effect and the overall integrated CCK response between 0–240 min was similar (Fig. 3*B*).

In response to test meals with and without orlistat, GIP levels increased significantly compared with basal values at 30 min and remained elevated until 150 min ($P < 0.001$). Orlistat profoundly attenuated the AUC of the GIP response ($P < 0.003$), and two-way analysis demonstrated that the GIP response was effected by both time ($P < 0.0001$) and treatment ($P < 0.0001$; Fig. 3*C*).

In two-way analyses, the plasma GLP-1 response increased significantly compared with basal values, although orlisat did not significantly modify the response. The overall AUCs of the GLP-1 responses were similar in both groups (Fig. 4*A*, *top inset*), but the AUC of the GLP-1 between 60–120 min as well as the plasma GLP-1 level at 90 min was significantly higher in the control experiments ($P = 0.03$ and $P < 0.01$; Fig. 4*A*, *bottom inset*).

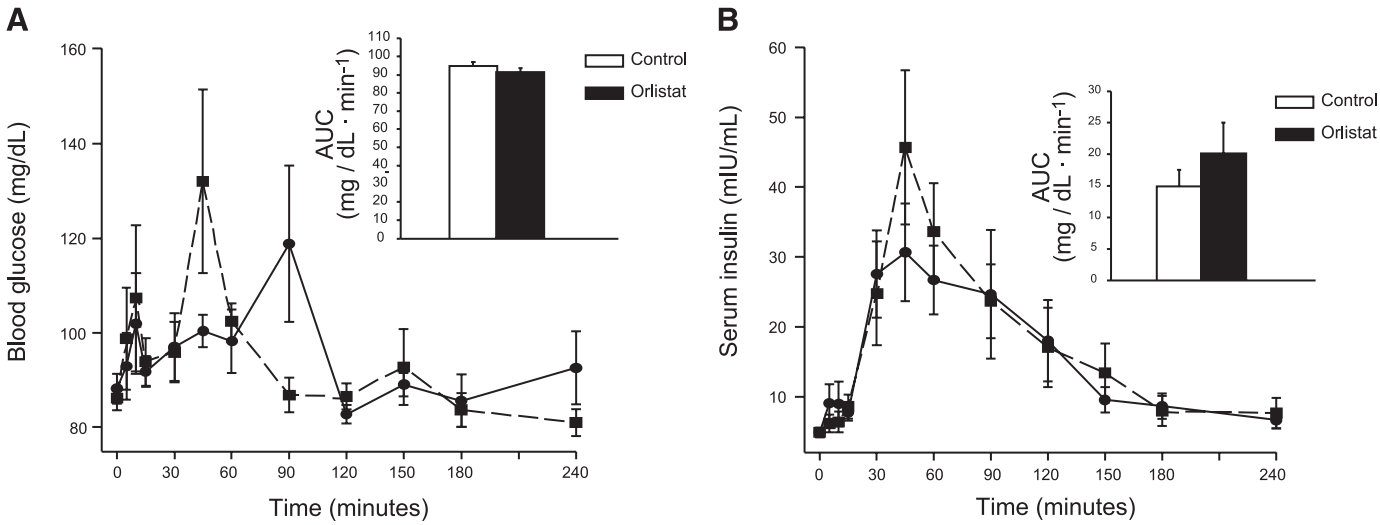

Fig. 2. Responses of blood glucose (*A*) and serum insulin (*B*) to an ingested 510 kcal mixed meal. *Insets*: integrated responses. Data are means ± SE. Meals were administered without (solid lines) or with orlistat (dashed lines). Blood glucose was unaltered compared with basal values in both experimental groups except at 45 min in the orlistat group. In both experimental groups, serum insulin was significantly increased between 30–90 min but orlistat did not alter the insulin response. AUC, area under the curve.

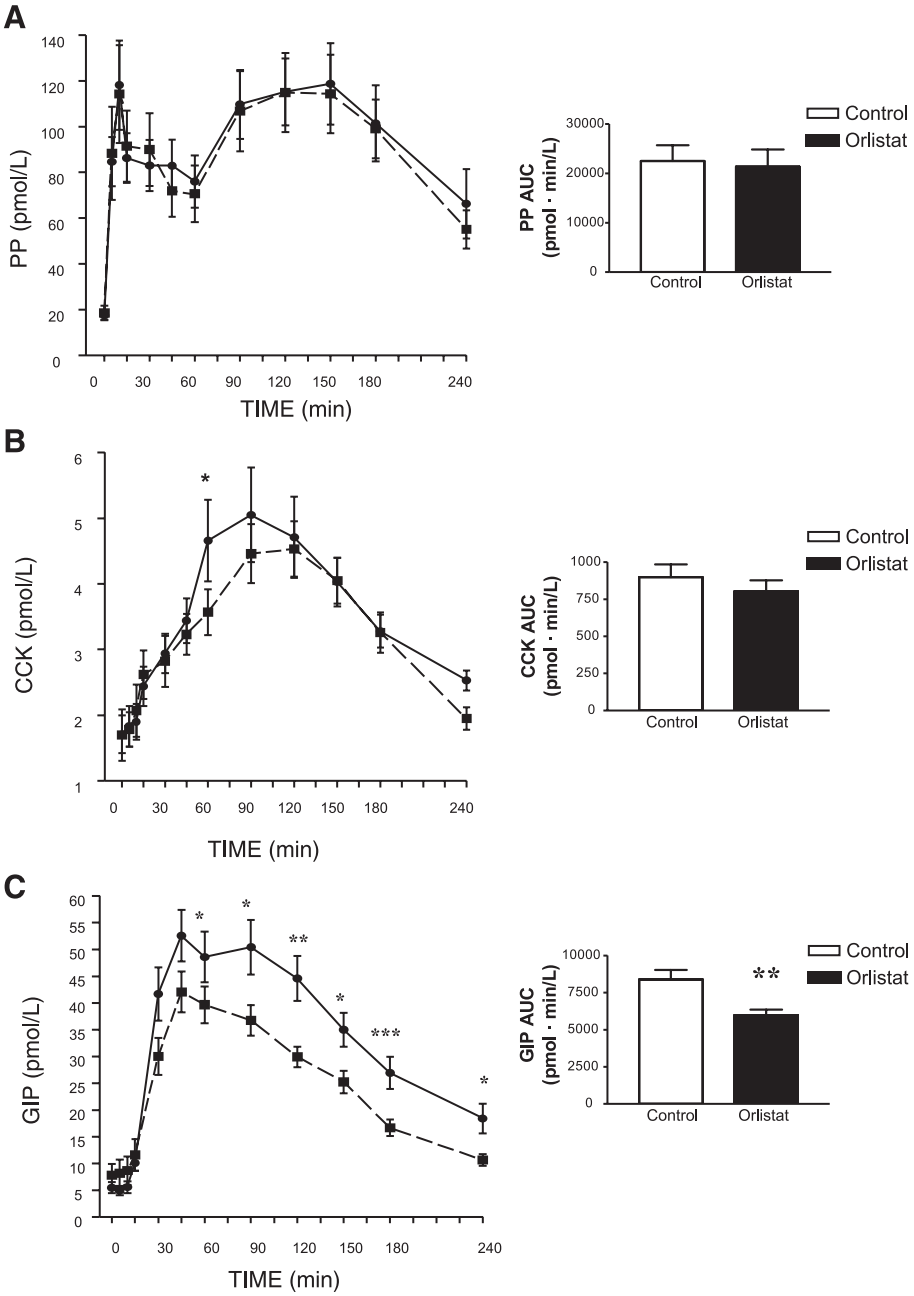

Fig. 3. Responses of plasma pancreatic polypeptide (PP; *A*), cholecystokinin (CCK; *B*), and glucose-dependent insulinotropic polypeptide (GIP; *C*) to an ingested 510 kcal mixed meal. Meals were administered without (solid lines) or with orlistat (dashed lines). *Insets*: integrated responses. Data are means ± SE. *A*: orlistat treatment had no effect on plasma PP response. *B*: orlistat blunted CCK response at 60 min significantly compared with control experiments ($*P < 0.05$) but did not alter overall meal-induced plasma CCK response. *C*: ingestion of the test meal resulted in a significant increase in plasma GIP response between 30–150 min in both groups. Orlistat treatment significantly attenuated GIP response ($*P < 0.0$, $**P < 0.01$, and $***P < 0.001$ compared with control experiments).

There was no significant plasma PYY response in both experiments with and without orlistat (Fig. 4*B*).

Correlation of peptide responses with gastric emptying plasma. Statistical correlations between gastric emptying variables and plasma/blood responses of gut peptides, glucose, and insulin were derived from seven subjects who simultaneously participated in all studies.

Area under the response curve of the humoral parameters was calculated both for 240 and 150 min, which corresponds to the time span of the gastric emptying studies. Because the characteristics of the gastric emptying curves were quite different in the two sets of experiments, correlation and regression analyses were performed separately in each set of experiments (Fig. 5.

Control experiments. AUC, *t* lag, and *t* half of th e gastric emptying curve were negatively correlated with integrated GLP-$1_{(240)}$ response ($r = -0.75$ and $P = 0.052$ for AUC; $r = -0.821$ and $P = 0.023$ for *t* lag and *t* half). When integrated peptide responses of the initial 150 min were used, GLP-$1_{(150)}$ negatively correlated with *t* lag ($r = -0.786$; $P = 0.036$). The *t* lag also weakly correlated with $GIP_{(150)}$ levels ($r = -0.714$; P 0.071). Regression analysis revealed that the most powerful determinant of the area under the gastric emptying curve is GLP-$1_{(150)}$ followed by insulin$_{(150)}$. The determinant of the *t* lag and *t* half is GLP-$1_{(150)}$ and GLP-$1_{(240)}$, respectively. Overall $GIP_{(240)}$ response was correlated with glucose ($r = 0.786$; $P = 0.036$) and PP curves ($r = 0.75$; $P = 0.052$).

Orlistat experiments. Ingestion of orlistat significantly changed the determinants of the gastric emptying curve. Integrated $GIP_{(240)}$ response was positively correlated with AUC

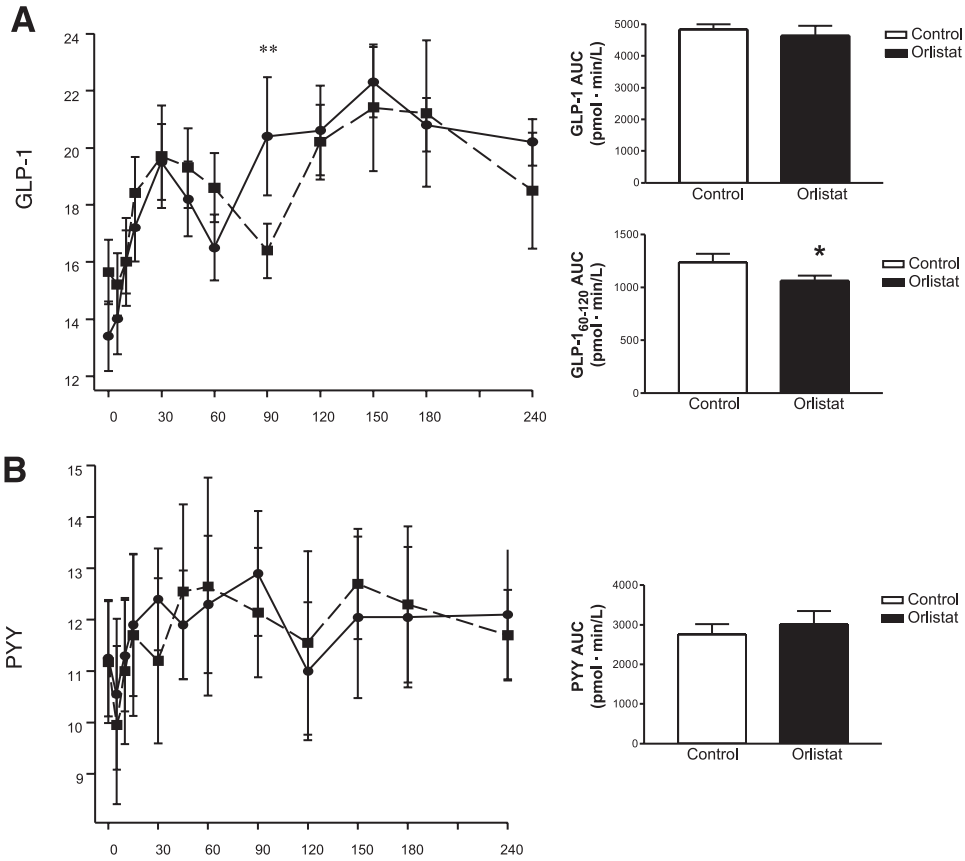

Fig. 4. Responses of plasma glucagon-like peptide-1 (GLP-1; *A*) and peptide YY (PYY; *B*) to an ingested 510 kcal mixed meal. *Insets*: integrated responses; GLP-1 response at the 0–240 min (*top*) and GLP-1 response between 60–120 min (*bottom*). Data are means ± SE. *A*: in the orlistat group plasma GLP-1 response at 90 min and AUC between 60–120 min was significantly atenauted ($P < 0.01$ and $P < 0.05$, respectively) but orlistat did not significantly alter the AUC (0–240 min). *B*: plasma PYY was unaltered by ingestion of the test meal in both experimental groups.

($r = 0.786$; $P = 0.036$), *t* half ($r = 0.75$; $P = 0.052$), and *t* lag ($r = 0.857$; $P = 0.014$) of the gastric emptying curve. $GIP_{(150)}$ was also correlated with the *t* lag ($r = 0.786$; $P = 0.036$).

Regression analysis demonstrated that the most important determinants of the AUC of the gastric emptying curve are $GIP_{(240)}$ followed by GLP-$1_{(240)}$. The *t* lag was determined by $GIP_{(240)}$, and the *t* half was determined by $GIP_{(240)}$ and GLP-$1_{(240)}$.

The integrated insulin response was negatively correlated with the CCK and PP responses ($r = -0.714$; $P = 0.071$ for both).

DISCUSSION

Our results demonstrate that in nonobese healthy subjects orlistat accelerates gastric emptying of an orally ingested ~500 kcal solid-liquid mixed meal and attenuates plasma GIP response, while it does not appreciably alter the plasma responses of CCK, GLP-1, and PP compared with control. Orlistat shortened *t* half and *t* lag and decreased the area under the gastric emptying curve without any significant alteration in the slope of the curve. Furthermore, with orlistat treatment, the gastric emptying parameters of *t* lag, *t* half, and AUC were positively correlated with plasma GIP response. Also, we did not demonstrate any significant effect of orlistat on blood glucose and serum insulin levels.

One of major regulators of gastric emptying is the lipolytic products of dietary fat, which are free fatty acids (FFAs) and 2-monoacylglycerols formed by luminal lipases acting on triglycerides. After their absorption into the enterocyte, FFAs with a chain length greater than C10 and 2-monoacylyglyserols are resynthesized into triglycerides and form cylomicrons. Exocytosis of cylomicrons into lymph triggers a series of neurohormonal/paracrine events involving vagal afferent signaling, all of which in turn induce suppression of food intake, gastrointestinal hormone release, and inhibition of gastric emptying (14, 23, 24, 32). Orlistat by covalently binding to gastric and pancreatic lipases inhibits the formation of FFAs and 2-monoacylglicerols from dietary fat (5). Although we did not measure gastric/duodenal content of products of lipid hydrolysis and the amount of absorbed fat, the most probable explanation for the accelerated gastric emptying is the impaired absorption of FFAs due to inhibition of lipases by orlistat.

Our finding of accelerated gastric emptying of a mixed meals by orlistat in healthy subjects confirms the findings of previous studies (4, 11, 15, 36) in which meals of various energy load and macronutrient composition were utilized and gastric motility was assessed using different methodologies. Contradictory to our results, Degen et al. (6) reported unaltered gastric emptying of an ingested mixed meal with 120 mg of orlistat. In their study, although the proportion of fat was similar to the meal in the present study, the total energy load was almost twice as high.

In studies that utilized intraduodenal/intragastric delivery of meals orlistat attenuated plasma responses of CCK (4, 6, 11, 18), GLP-1 (11), and PYY (7, 10), reflecting defective absorption of dietary fat. Since these peptides are known to participate in the inhibition of gastric emptying, their attenuation by orlistat was alluded to play a role in the mechanism by which orlistat accelerates gastric emptying. However, plasma levels of these gastric motility modifying peptides may not

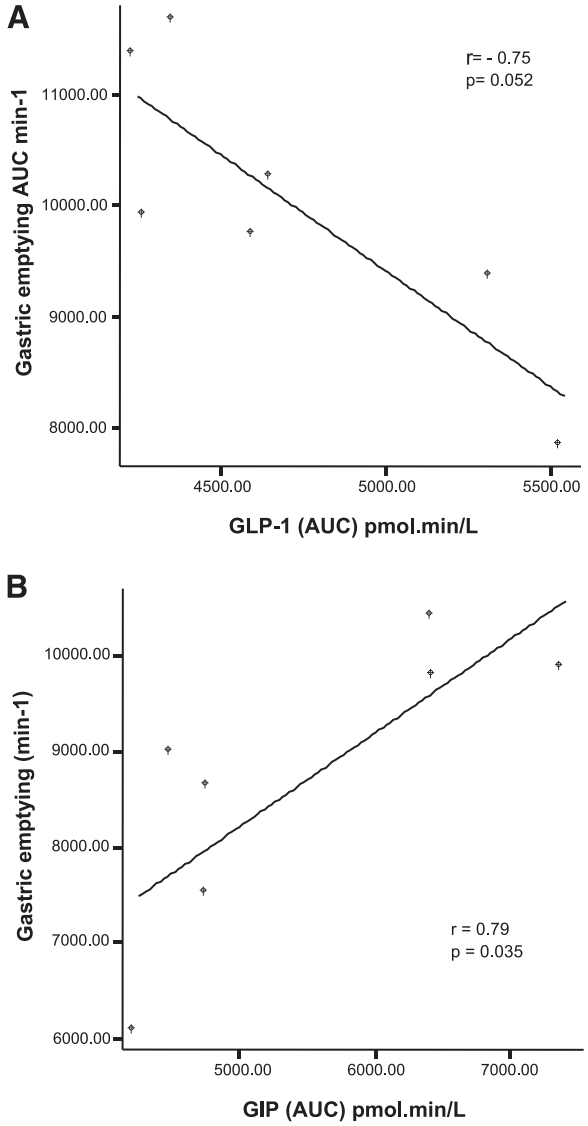

Fig. 5. Correlation between the AUC of the gastric emptying with integrated GLP-$1_{0\text{-}240}$ response in control experiments (*A*) and with integrated $GIP_{0\text{-}240}$ response in orlistat experiments (*B*). Gastric emptying was negatively correlated with GLP-1 ($r = -0.75$; $P = 0.052$) and positively correlated with GIP ($r = 0.786$; $P = 0.036$) in control and orlistat experiments, respectively.

necessarily reflect their capability in the inhibition of gastric emptying (see below).

We specifically aimed to use a test meal that mimicked physiological nutrient intake in terms of calorie load and macronutrient composition, and thus our test meal was composed of 39.7% fat (22 g), 41% carbohydrate (52.5 g), and 19% protein (24.7 g). In addition, orlistat was ingested together with the meal mimicking its use in obesity treatment. Therefore, our results and previous findings by others demonstrate that orlistat modifies the plasma responses of CCK, GLP-1, PP, and GIP, depending on the route of administration and macronutrient composition of meals as well as chemical state of the lipase inhibitor, while acceleration of gastric emptying is a more consistent finding but still modifiable depending on the fat load.

According to our results, the only gut peptide that demonstrated profound attenuation of plasma response by orlistat administration was GIP. With the ingestion of the control mixed meal, plasma GIP increased significantly at 30 min compared with basal concentrations and remained elevated throughout the experimental period and the temporally declining shape of the curve was similar to the previous studies utilizing similar test meals (38). GIP_{240} was positively correlated with *t* lag, *t* half, and AUC of the gastric emptying curve in the orlistat experiments surpassing GLP-1, which was the main predictor of gastric emptying in the control experiments. Therefore, in our experimental conditions, it is likely that orlistat by its ability to inhibit lipid absorption attenuated GIP release. To the best of our knowledge, this is the first study to evaluate the effect of orlistat on plasma levels of GIP in response to an orally ingested mixed meal in healthy subjects. Pilichiewicz et al. (30) evaluated the effect of orlistat on gastric emptying and plasma GIP response in type 2 diabetic subjects given a liquid high-fat meal and orlistat was mixed with the oil phase of the meal. This study, although not comparable to ours, also showed attenuated GIP secretion and accelerated gastric emptying by orlistat treatment. GIP is secreted from enteroendocrine cells of the K type in the upper gut in response to nutrients in a load-dependent manner, fat being the most potent secretagague in humans (2, 40). As also demonstrated in this study, the secretion of GIP has been shown to be very sensitive to acute and chronic changes in dietary fat (40). GIP similar to the incretin effect of GLP-1 stimulates glucose-dependent insulin secretion (2). In contrast to GLP-1, which is designated as an enterogastrone by its ability to inhibit gastric acid secretion and gastric emptying, GIP does not seem to have a physiological role as an enterogastrone (25). In turn, recent findings (2, 40) have attributed an important role for GIP in energy homeostasis, obesity, and diabetes. There are functional GIP receptors on adipocytes, which have insulin mimetic properties such as uptake of glucose (37), fatty acid synthesis, upregulation of lipoprotein lipase synthesis, and reduction in glucagon-induced lipolysis (2, 40). Thus GIP acting on its specific receptors and via insulin secretion promotes fat accumulation in adipocytes, obesity, and thus insulin resistance. GIP receptor knockout mice who are fed a high-fat diet are resistant to obesity (2). Additionally, *ob*/*ob* mice, which have K cell hyperplasia, elevated intestinal, and circulating levels of GIP, when treated with a GIP receptor antagonist attain improved glucose tolerance and amelioration of insulin resistance (2). GIP antagonism in mice fed a high-fat diet also protects against obesity, insulin resistance, and glucose intolerance (13). Therefore, GIP antagonism as a treatment option for obesity and insulin resistance in humans appears to be a promising research field. In this context, the role of attenuated GIP secretion in the weight-reducing effect of orlistat deserves further exploration.

Orlistat did not alter the integrated plasma responses of CCK compared with control although there was a significant attenuation of CCK response at 60 min ($P < 0.05$). In our experimental conditions, orlistat may not have critically inhibited FFA formation/absorption so that CCK release was unaltered. Alternatively, the rapid gastric emptying by orlistat may have resulted in augmented absorption of protein and carbohydrate components of the meal per unit of time, which compensated for the attenuated CCK secretion. In contrast to our findings, Borovikva et al. (4) and Ellrichmann et al. (15) reported attenuation of CCK release in response to an orally ingested ~

800 kcal solid liquid mixed meals. In the former study, after 4 days of priming with orlistat, 120 mg of the drug were mixed with the olive oil component of the meal before ingestion (4). In the latter study, liquid component of the test meal was dairy cream yielding 550 kcal (15). Therefore, the difference between our results and the aforementioned studies in CCK response may be due to the meal composition and particularly due to the liquid vs. solid phases in which fat was incorporated. On the other hand, our finding of unaltered plasma CCK response in response to an orally ingested meal along with orlistat is in agreement with the findings of Goedecke et al. (16), O'Donovan et al. (28), and Degen et al. (6). In these studies, meals with comparable energy content to ours with and without 120 mg of orlistat were utilized, whereas fat content was higher, 60 and 70%, respectively, in the studies by Goedecke et al. (16) and O'Donovan et al. (28).

CCK and its receptors are implemented as one of the major regulators of gastric emptying and particularly of dietary fat in humans (3, 9, 23) and laboratory animals (14, 31, 32). Recently, the inhibitory role of CCK in gastric emptying, particularly of lipid emptying, has been elucidated (14, 32). Accordingly, FFA absorption initiates CCK secretion from enteroendocrine cells, which, in turn, activates CCK1 receptors on vagal sensory nerves in a paracrine fashion to initiate vagovagal reflexes that mediate inhibition of gastric emptying (14, 21, 31). In rodents, immunoneutralization of endogenous CCK does not reverse the inhibition of gastric emptying induced by intraduonal infusion of peptone and lipids, implying that the paracrine/neural-mediated lipid sensing by CCK is the major determinant in its ability to inhibit gastric emptying (31, 32). Considering the above evidence, the role of CCK in the acceleration of gastric emptying by orlistat cannot be discarded, because attenuation of CCK-induced paracrine/neural signaling may be operative.

In healthy subjects, oral ingestion of orlistat with a mixed meal (15) and intraduodenal delivery of fat with orlistat (10) attenuate the plasma response of GLP-1. In type 2 diabetics, orlistat incorporated into an olive oil drink also attenuates GLP-1 (30). According to our results, orlistat attenuated GLP-1 response only between 60 and 120 min ($P < 0.03$) and the total response was unaltered. Our results are similar to the findings of O'Donovan et al. (29) in which type 2 diabetic subjects who were managed by diet alone were tested with a solid meal containing margarine in the absence and presence of orlistat. It is notable that in studies in which GLP-1 was suppressed by orlistat dietary fat was incorporated into the liquid phase of the meal, whereas in our study (butter) and also in the study by O'Donovan et al. (margarine) was incorporated into the solid phase of the meal. Therefore, similar to the discussion above held for CCK, the ability of orlistat to suppress GLP-1 is probably dependent on the meal composition and the solid vs. liquid compartment in which fat is incorporated. The gastric emptying parameters of AUC, *t* lag, and *t* half were negatively correlated with plasma GLP response in the control experiment, implying that GLP-1 was one of the major determinants of gastric emptying. There are several lines of evidence that demonstrate a physiological role for GLP-1 in its inhibitory action on gastric emptying (19, 26, 33). Because the influence of GLP-1 on gastrointestinal function depends on signaling via afferent sensory neurons relaying to the hypothalamus and regulating efferent parasymphatetic outflow, neural/paracrine effects rather than humoral ones are probably operative in the retardation of gastric emptying (20, 27, 33, 39). Hence, plasma GLP-1 levels may not represent the inhibitory interactions of peptide at the cellular level.

There was a significant plasma response of PP, and orlistat did not alter this response appreciably consistent with a previous study (12). The meal utilized in our study was effective in initiating significant plasma responses of CCK, GLP-1 ,GIP, PP, and insulin secretion, implying that nutrient-sensing mechanisms were operative. However, throughout the study period, basal plasma PYY remained unchanged in studies with and without orlistat consistent with a previous study that demonstrated that PYY secretion is nutrient load dependent (1). PYY is secreted postprandially from the enteroendocrine L cells, which are abundant in the distal intestine. In our experimental condition, it is probable that nutrient absorption was complete in the upper intestine so that distal gut failed to stimulate PYY irrespective of the presence of orlistat.

In conclusion, our results demonstrate that intake of a mixed meal with a modest energy load, orlistat, accelerates gastric emptying with profound inhibition of plasma GIP, while it has insignificant effects on CCK, GLP-1, and PP responses, which are considered to have inhibitory actions on gastric motility and appetite. It may be considered that orlistat by decreasing lipid absorptive signals may have attenuated the neural/paracrine responses of these peptides without appreciably altering their plasma responses. Alternatively, in the presence of orlistat, other mechanisms that modulate gastric motility are operative to accelerate gastric emptying. Profound inhibition of GIP by orlistat may have therapeutic implications in the treatment of obesity and glucose intolerance by attenuating fat accumulation in adipoctyes.

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