Inhibition of gastrointestinal lipolysis by Orlistat during digestion of test meals in healthy volunteers

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Obesity is a serious disease that predisposes one to numerous health problems including diabetes, hypertension, and atherosclerosis. It accounts for 2–7% of total health care costs in Western countries, and its prevalence is increasing rapidly in developing countries where it is already out of control (15). Dietary therapy is the first line of treatment for obesity, but because it has not proved to be sufficient in many cases, drug therapy also has to be envisaged. There were high hopes for the efficacy of drugs acting on the central nervous system as appetite suppressants. Serious cardiovascular side effects were reported, however, with drugs such as fenfluramine, and their clinical use was therefore severely restricted. Because excess fat consumption is widely thought to be one of the main causes of obesity, means of specifically inhibiting triglyceride (TG) digestion have been developed as a new approach to reducing fat absorption.

Orlistat (tetrahydrolipstatin) is a covalent inhibitor of digestive lipases (2, 16) derived from lipstatin, a natural product of Streptomyces toxytricini (31). It is an active site-directed inhibitor that reacts with the nucleophilic serine residue from the catalytic triad of pancreatic lipase (17, 23). By covalently blocking the lipase active site, Orlistat inhibits the hydrolysis of dietary TGs and thus reduces the subsequent intestinal absorption of the lipolysis products monoglycerides (MGs) and free fatty acids (FFAs). It also inhibits gastric lipase, cholesterol esterase, and various other lipases that are all serine hydrolases (22) and hormone-sensitive lipase (30) in vitro.

Many clinical trials have been performed with Orlistat during the last few years, and one formulation of the drug, Xenical, was licensed in the European Union...
in 1998 (6) and in the United States in 1999. The results of clinical studies have indicated that Xenical reduces fat absorption ≤30% (33) during a meal. Xenical has dose-dependent effects up to a dose of 400 mg/day after which a plateau is observed (33). When Xenical is administered in therapeutic doses (120 mg with the main meals) in conjunction with a mildly hypocaloric diet, obese patients have lost ~10% of their body weight over a 1-yr period (29).

In most clinical studies, the effects of Orlistat have been estimated indirectly from levels of fecal fat excretion, but its direct inhibitory effects on digestive lipases and lipolysis have not been quantified. The in vivo process of digestive lipase inhibition by Orlistat has received little attention up to now. Hildebrand and co-workers (19) observed that a dose-dependent inhibition of human pancreatic lipase (HPL) occurred concomitantly with a decrease in FFA levels at the angle of Treitz. Schwizer and co-workers (28) established that HPL activity in the duodenal contents was almost completely inhibited by Orlistat, whereas the total output of immunoreactive HPL remained unchanged compared with that recorded in control experiments without Orlistat. No data have been published on the in vivo inhibition of human gastric lipase (HGL), the second most important enzyme involved in dietary TG hydrolysis (7). Nor are the respective effects of Orlistat on gastric and duodenal lipolysis known. The aim of the present study was, therefore, to quantify the inhibition of HGL and HPL by Orlistat, as well as the decrease in lipolysis that occurs in response to Orlistat at the postpyloric and lower duodenal levels, in the course of test meals in healthy human volunteers. Two different formulations of Orlistat (Xenical pellets and micronized Orlistat powder) were studied in association with one complete liquid test meal and one mixed solid-liquid test meal.

MATERIALS AND METHODS

Volunteers for in vivo studies. Healthy male and female volunteers with a normal body weight, aged 20–50 yr, participated in this study. They had no history of gastrointestinal or endocrine disease and were not taking any medication. All the volunteers gave their written informed consent to the experimental procedure. The gastrointestinal lipolysis experiments were performed at the Centre de Pharmacologie Clinique et d’Etudes Thérapeutiques (CPCET), Hôpital d’adultes de la Timone (Marseille, France). The fat excretion experiments could thus be compared on the same basis, but its direct inhibitory effects on digestive lipases and lipolysis have not been quantified. The in vivo process of digestive lipase inhibition by Orlistat has received little attention up to now. Hildebrand and co-workers (19) observed that a dose-dependent inhibition of human pancreatic lipase (HPL) occurred concomitantly with a decrease in FFA levels at the angle of Treitz. Schwizer and co-workers (28) established that HPL activity in the duodenal contents was almost completely inhibited by Orlistat, whereas the total output of immunoreactive HPL remained unchanged compared with that recorded in control experiments without Orlistat. No data have been published on the in vivo inhibition of human gastric lipase (HGL), the second most important enzyme involved in dietary TG hydrolysis (7). Nor are the respective effects of Orlistat on gastric and duodenal lipolysis known. The aim of the present study was, therefore, to quantify the inhibition of HGL and HPL by Orlistat, as well as the decrease in lipolysis that occurs in response to Orlistat at the postpyloric and lower duodenal levels, in the course of test meals in healthy human volunteers. Two different formulations of Orlistat (Xenical pellets and micronized Orlistat powder) were studied in association with one complete liquid test meal and one mixed solid-liquid test meal.

Test drug dosage and formulations. For gastrointestinal lipolysis experiments, a single dose of 120 mg of Orlistat was used on two different occasions with each healthy volunteer. The first formulation was a capsule of Xenical (Ro-18-0647/090) containing 120 mg of Orlistat in a pelleted form (50% wt/wt Orlistat). The second formulation was a micronized powder of pure Orlistat (120 mg; Ro-18-0647/008). Micronized Orlistat powder was either suspended in the liquid test meal by stirring the mixture at 44°C for 15 min or was dissolved in 10 g of melted butter before it was mixed with the solid test meal. For fat excretion experiments, the same dosage and formulations were used, except for the experiments performed with the solid test meal and the micronized Orlistat powder. In this case, only 60 mg of Orlistat were dissolved in 10 g of butter before the meal was ingested by the volunteers. These experiments, initially scheduled for another clinical investigation, were included here because they clearly illustrate the high level of fat excretion that occurs when Orlistat is given with a solid test meal even though the dosage is reduced (see RESULTS and Table 6).

In vivo study protocols. The aims of the study were to 1) quantify the inhibitory effects of Orlistat on HGL in the stomach and on HGL and HPL in the upper and lower parts of the duodenum and 2) measure the levels of lipolysis in the stomach and upper and lower parts of the duodenum in the presence and absence of Orlistat in the course of test meals. This open randomized, three-way crossover study was conducted in two consecutive series in which the test meals differed: a liquid test meal was used in the first series, and a mixed liquid-solid test meal was used in the second. Initially, 18 volunteers were included: subjects 1–6 in the first series and subjects 7–18 in the second. Two additional volunteers (subjects 19 and 20) were included in the second series after the experimental procedure used to collect the samples had been modified (see Experimental devices for collecting in vivo samples in the course of the solid test meal).

Each volunteer was given the test meal on three occasions, at least 8 days apart, by intragastric intubation (for a period of 5 min), with Xenical pellets administered midway through
the meal ingestion, with micronized Orlistat powder pre-
mixed with the meal, or without lipase inhibitor (controls).
Additional experiments were performed to measure the
level of fecal fat excretion in volunteers fed the same liquid
test meal (Shak Iso). Five volunteers were given Xendical pellets and five other volunteers were given micronized Or-
listat powder suspended in the liquid meal for 30 min at
38°C. Two further groups of seven and six volunteers were
given a solid test meal with either Xendical pellets adminis-
tered at the beginning of the last quarter of the meal ingestion
or with micronized Orlistat powder dissolved in butter.

Experimental devices for collecting in vivo samples in the
course of the liquid test meal. Six volunteers (subjects 1–6)
were intubated with a 4-lumen duodenal tube and with a
single-lumen gastric tube. The duodenal tube was placed
with the use of fluoroscopy in the position shown in Fig. 1A.
We also checked that the tubes were properly placed by
analyzing the pH of basal aspirates. An inflatable balloon
was used to occlude the upper part of the duodenum close to
the pylorus, where no HPL is present. Continuous aspiration
of the postpyloric output was performed upstream of the
balloon to determine the gastric lipolysis products and HGL
output. After being sampled, the postpyloric aspirate was
reinjected downstream of the occluding balloon. A second
continuous system of aspiration was set up at the ligament of
Treitz to assess duodenal lipolysis products and HPL output.
The contents of the upper and lower parts of the duodenum
(postpyloric and duodenal collections) were continuously as-
pirated for 15-min periods. The total volume of both aspirates
was measured, and 5-ml samples were collected. The single-
lumen gastric tube was used to administer the meal and to
collect 5-ml gastric samples every 15 min as well as the total
residual gastric contents at the beginning of the last quarter of the meal ingestion or with micronized Orlistat powder dissolved in butter.

Experimental devices for collecting in vivo samples in the
course of the liquid test meal. Six volunteers (subjects 1–6)
were first intubated with the same system of tubing as that used for the liquid meal. With this experimental device (Fig. 1A), a satisfactory level of postpyloric recovery of

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Handling of samples. The 5-ml samples of gastric postpy-
loric or duodenal contents were immediately mixed with
protease inhibitors (final concentrations: 1 mM benzamidine,
1 mM phenylmethylsulfonfyl fluoride, 1 μM leupeptin, and 1
μM pepstatin) to prevent the proteolytic inactivation of lipases from occurring. One milliliter of each sample was
immediately acidified with 0.1 N HCl and mixed with 5 ml of
hexane-1-butanol to stop the lipolysis. This mixture was
further used for the lipid extraction and analysis procedures.
Another fraction (0.5 ml) of each sample was diluted twofold
with glycerol and frozen at −20°C for ELISA measurements
of HGL and HPL. The rest of the sample was kept on ice
and subsequently used to measure the pH, HGL, and HPL activities and the PEG concentration.

PEG measurements and volume corrections. The PEG concen-
tration was measured with Hyden’s turbidimetric method
(20) as modified by Malawer and Powell (24). In the experi-
ments with the solid test meal, the presence of solid particles
contributing to the total volume of the samples was taken
into account. One milliliter of each sample was centrifuged
at 1,000 rpm for 1 min, and the upper liquid phase was collected
to estimate the liquid volume percentage (vol/vol) and mea-
sure PEG concentration. When calculating the PEG recovery,
only the volume of the liquid phase was taken into account.

The volumes of the postpyloric fractions were corrected by
a factor that accounted for the partial recovery of the PEG

Fig. 1. Experimental device for the in vivo study of meal fat digestion in healthy volunteers. A: gastric and 4-lumen
duodenal tubes were used with the liquid test meal to collect gastric, postpyloric, and duodenal samples. The same
duodenal tube was also used with the solid test meal for the postpyloric collection of gastric contents. B: gastric and
2-lumen duodenal tubes were used with the solid test meal to collect gastric and duodenal samples. PEG, polyethylene glycol.
(3). This correcting factor was calculated for the whole digestion period by dividing the total amount of PEG initially present in the meal (5 g) by the total amount of PEG recovered at the end of the experiment in all the postpyloric fractions, gastric samples, and residual gastric contents. A similar corrective procedure was applied to the volumes of the duodenal fractions, but in this case, the correction factor was calculated on the basis of the total amount of PEG recovered in all the duodenal fractions, postpyloric and gastric samples, and residual gastric contents.

**ELISA measurements of HGL and HPL.** The mass concentrations of HGL and HPL were measured in all the samples with two specific ELISAs developed at our laboratory (11).

**Lipase activity measurements and estimation of lipase inhibition levels.** The assays of HGL and HPL activity were performed using the pH-stat technique with tributyrin as the substrate as previously described (7). In the samples containing both HGL and HPL, it was possible to distinguish between the activities of the two lipases by measuring the HGL activity at pH 4.5 in the presence of 0.6 M NaCl (no HPL activity) and by measuring the HPL activity at pH 8.0 (no HGL activity). We checked whether purified carboxyl ester lipase (CEL) from pancreatic juice could hydrolyze tributyrin, the substrate used when measuring HPL activity in the duodenal contents. As previously reported by Lombardo et al. (21), its specific activity was found to be very low compared with that of HPL (1–2% of HPL activity). The contribution of CEL to tributyrin hydrolysis was therefore ignored. The fact that the contribution of CEL was very low was further confirmed by the very good correlation found to exist between the HPL output (in mg) estimated from the activity measurements and those based on the ELISA (see Table 3).

The active lipase concentrations were expressed either in international units per milliliter (IU/ml; 1 IU = 1 μmol of butyric acid released per minute) or in micrograms per milliliter based on the specific activities of pure HGL (1,300 IU/mg) and pure HPL (8,000 IU/mg) as determined under the assay conditions mentioned above.

In both the HGL and HPL assays, the release of butyric acid from tributyrin was found to be linear with time, and the lipase activity was usually estimated from the slope of the corresponding curve (micromoles of butyric acid released per minute). When measuring the residual HPL activity from samples containing Orlistat, we observed that a partial reactivation of HPL occurred in the course of the pH-stat assay, and the amount of butyric acid released with time was found to increase continuously (data not shown). The residual HPL activity was therefore estimated from the slope at time 0 (initial velocity of butyric acid release). We checked that the HPL reactivation occurred only in the pH-stat vessel and not in the duodenal samples. For this purpose, the amounts of lipolysis products present in several duodenal samples were determined at various times after the complete inhibition of HPL was estimated from the pH-stat assay. The amount of lipolysis products was not found to increase with time (data not shown).

In the case of HGL, the above-mentioned reactivation phenomenon was not observed in the present study. Contrary to what has often been reported in the literature, the inhibitory effects of Orlistat on digestive lipases are not irreversible, and some precautions therefore have to be taken when measuring the residual activity with the pH-stat method.

The HGL and HPL inhibition levels were estimated by comparing the amount of active lipase (pH-stat measurements) with the total amount of lipase determined by ELISA.

**Lipid extraction and analysis.** Lipid extraction was performed with a two-step extraction procedure (8). A 1-ml fraction of each sample was acidified to a pH value of ~1–2 by adding 200 μl of 0.1 N HCl. Neutral lipids were extracted in two steps by first adding 5 ml of a hexane-1-butanol (3:2 vol/vol) mixture and then 5 ml of a hexane-isopropanol (3:2 vol/vol) mixture. The samples from the experiments performed with the solid test meal were homogenized with the organic solvents with an Ultraturrax mixer before the phase separation was performed, and this procedure was repeated at both extraction steps. The organic phases were sprayed bandwise onto an 10 × 20-cm silica gel 60 thin-layer plate (Merck) with a Linomat IV apparatus (CAMAG). The sample migration was performed with heptane-diethyl ether-acetic acid (55:45:1 vol/vol/vol). After the plates were sprayed with a cupric acetate-phosphoric acid solution, the neutral lipid bands were revealed by heating the plate at 180°C for 10–15 min. The cupric acetate-phosphoric acid solution was prepared by mixing a saturated aqueous solution of cupric acetate with 85% phosphoric acid in a 1-to-1 volume ratio. The lipids were quantified by scanning densitometry with a BioRad imaging densitometer (GS-700). The optical densities were converted to mass using standard curves established as follows: various amounts (1,25, 2,5, 5, 10, and 20 μg) of standard compounds [triolein, (1,2- and 1,3-diolein, monoolein, and oleic acid] were loaded on each TLC plate to determine the variation in the optical density as a function of the mass of each compound. The masses of the lipids obtained from the scanning densitometry data were then converted into moles using the following mean molar masses: TGs, 821.9 Da; diglycerides (DGs), 578.6 Da; MGs, 353.4 Da; FFAs, 261.3 Da.

**Calculation of lipolysis levels.** On hydrolysis, one molecule of TG can release a maximum of three molecules of FFA. The hydrolysing (or lipolysis) level (L%) is usually defined as the percentage of acyl chains released from the meal TGs (TG0): $L\% = \frac{100 \times (FFA + MG)/(3 \times TG_0)}{100 \times (FFA + MG)/(3 \times TG_0) + 100 \times (DG + MG + FFA)}$. According to this new definition, 100% lipolysis corresponds to the conversion of one TG molecule into one MG and two FFA molecules. This definition of the level of lipolysis does not take the possible hydrolysis of MGs into account, the latter process not being essential for fat absorption.

The gastric lipolysis level and the overall lipolysis level were calculated from the postpyloric and duodenal recovery of TGs, DGs, MGs, and FFAs, respectively. The duodenal lipolysis level was calculated by subtracting the gastric lipolysis from the overall lipolysis. When the liquid test meal was used, the three lipolysis levels could be calculated in the case of each experiment. When the solid test meal was used, gastric and overall lipolysis levels were obtained from separate experiments and the duodenal lipolysis level was calcu-
lated by subtracting the mean level of gastric lipolysis from the mean level of overall lipolysis.

Fat excretion measurements. The volunteers collected stool quantitatively for 30 h before and 100 h after the test meal. The amount of fat excreted was quantified gravimetrically after stool homogenization and extraction from aliquots with chloroform-methanol (1). The fat excretion levels recorded after the test meal were corrected in each individual experiment based on the baseline fat excretion determined during the premeal stool collection period. The fat excretion levels were expressed as the percentage of fat ingested with the respective meals.

In vitro experiments. Each experiment was performed in a 50-ml vessel equipped with a thermostat (37°C), a pH electrode, and a 1-cm magnetic rod rotating at 1,000 rpm. At time 0, the meal (15 ml), with or without Orlistat, and gastric juice (3 ml) were mixed, and the pH value was adjusted to 5.5. One-milliliter samples were collected at 5, 15, and 29 min for analysis. The pancreatic juice and bile mixture (11 ml) was then added at time 30 min, and the pH value was adjusted to 6.25. One-milliliter samples were collected at 35, 40, 45, 60, and 90 min. The 1-ml samples were used immediately after being collected to measure the lipase activities and to extract the lipids. The meal-to-juice ratios, the lipase concentrations (16 μg/ml HGL at t = 0; 250 μg/ml HPL at t = 30), and the pH values corresponded to the mean values observed in vivo at 50% meal gastric emptying. Similar experiments were carried out with purified lipases and colipase instead of juices.

The meal volume-to-drug dose ratio was kept identical to the ratio used for the in vivo experiments with the liquid test meal (500-ml meal with 120 mg of Orlistat). Before the gastric juice was added, a 7.2 mg Xenical pellet (containing 3.6 mg of Orlistat) or 3.6 mg of pure Orlistat (micronized powder) were premixed with the meal. Control experiments were performed without Orlistat.

Digestive juices and purified lipases. Several samples of human gastric juices were collected from healthy volunteers under pentagastrin stimulation. The HGL concentration in these samples was variable, and they were pooled to obtain an average value of 100 μg/ml of HGL, which is the mean HGL concentration in basal human gastric juices (7). Three-milliliter aliquots of the gastric juice mixture were kept frozen at −20°C.

In each in vitro experiment with digestive juices, we used an 11-ml mixture of human pancreatic juice and bile that was prepared as follows: 400 mg of lyophilized human pancreatic juice containing 1.7% HPL wt/wt (i.e., 6.75 mg of HPL) were mixed with 2 ml of human bile containing 60 mM bile salts and water to obtain a total volume of 11 ml. After adding the pancreatic juice-bile salt mixture to the reaction vessel, the final HPL and bile salt concentrations were 250 μg/ml and 4.4 mM, respectively. The bile salt concentration was greater than the critical micellar concentration, as previously observed in intestinal contents (3, 18).

HGL and HPL were purified at our laboratory as described by Moreau et al. (25) and De Caro et al. (12). Porcine procolipase was purified as described (9). To replace the gastric juices with pure HGL, we prepared a 100 μg/ml HGL solution in 0.15 M NaCl. To replace the pancreatic juice with pure HPL plus colipase, we prepared the following solution before each experiment: 6.75 mg of HPL, 1.35 mg of porcine procolipase, and 2 ml of human bile containing 60 mM bile salts mixed with a 0.15 M NaCl solution to obtain a total volume of 11 ml. HPL (50 kDa) and procolipase (10 kDa) were added in a 1-to-1 molar ratio that has been found to occur in the human duodenal contents (4).

Statistical analysis. All data are expressed as means ± SD unless stated otherwise. The data obtained in the various series of experiments were compared with paired and unpaired Student’s t-tests.

RESULTS AND DISCUSSION

Gastrointestinal lipolysis experiments in healthy volunteers. Healthy volunteers were intubated with a gastric and a duodenal tube as depicted in Fig. 1, and on three occasions they were given either a complete and preemulsified liquid test meal (Shak Iso) or a solid test meal as a suspension of various ingredients (hamburger, french fries, string beans, and butter). The meals were given twice with a lipase inhibitor (Xenical pellets or micronized Orlistat powder) and once without drug treatment (control group). When the solid test meal was used, the Orlistat powder was predissolved in butter. After the meal had been injected into the stomach, samples were collected every 15 min at the gastric, postpyloric, and lower duodenal levels to measure the lipase and lipolysis product concentrations and output, as well as the recovery of the nonabsorbable marker (PEG) initially present in the meal. The lipase inhibition levels were estimated by comparing the total amount of secreted lipase (ELISA measurements) with the residual amount of active lipase (pH-stat activity measurements). In parallel, the levels of lipolysis were estimated from the recovery of FFAs, MGs, DGs, and residual meal TGs.

Twenty volunteers were included in this part of the study. Some experiments were interrupted, mainly because the tubes were not tolerated, and only 49 of 60 experiments were successfully completed. Once the PEG and the acyl chain (FFA, MG, DG, and TG) balances at the postpyloric and duodenal levels had been estimated, the results of the experiments were either used to estimate the lipase inhibition and lipolysis levels (43 experiments) or withdrawn (6 experiments) from the calculations because the PEG and/or acyl chain recovery levels were too low. The experiments were selected on the basis of two main criteria: a high level of PEG recovery (60–80%) and an acyl chain recovery level >80% (see Table 1). To calculate the lipolysis level (see MATERIALS AND METHODS), all the lipolysis products had to be recovered. This was expected to be the case in the postpyloric samples used to estimate the gastric lipolysis but not in the duodenal samples because FFAs and MGs are known to be absorbed in the small intestine. In the present study, it was observed, however, that a high acyl chain recovery was achieved at the duodenal level in most of the experiments performed (see Table 1), probably as a result of the continuous aspiration of duodenal juice, which limited the residence time of FFAs and MGs in the duodenum.

Effects of Orlistat on the overall level of HGL secretion. The overall level of HGL secretion was estimated from ELISA measurements of the postpyloric samples. No significant differences in HGL secretion were observed among the various groups of experiments (Table 2) with either the liquid or the solid test meals. The
difference in HGL secretion between the liquid and the solid test meal was also nonsignificant (Table 2). The mean HGL secretion value obtained in all the groups (17.3 ± 4.8 mg of HGL) was found to be in the same range as the values published in previous studies [22.6 ± 8.1 mg (7) and 24.7 ± 9.1 mg (5)].

**HGL inhibition by Orlistat.** Some HGL inactivation was observed in the control experiments (29.4% with the liquid meal; 12.3% with the solid meal; Table 2). Partial inactivation of HGL was found to occur mostly in the samples with the lowest pH values (1–2), which were collected at the end of the meal gastric emptying process (data not shown).

In the presence of a lipase inhibitor, the HGL inhibition was always very high (Table 2), regardless of the mode of Orlistat administration (Xenical pellets or micronized Orlistat powder) or the type of meal ingested (liquid or solid). The differences observed in comparison with the control experiments were significant ($P < 0.05$) for both Xenical pellets and micronized Orlistat powder given with the liquid meal and were highly significant for Xenical pellets ($P < 0.005$) and for micronized Orlistat powder ($P < 0.001$) given with the solid meal. The mean inhibition level of HGL was found to be higher with micronized Orlistat powder than with Xenical pellets (Table 2), but this difference was not statistically significant. Micronized Orlistat powder premixed with the meal fat was therefore as efficient as the Orlistat present in the Xenical pellets. It was observed that HGL inhibition was also very high in the duodenal samples (data not shown).

**Effects of Orlistat on the overall level of HPL secretion.** The overall level of HPL secretion was estimated from ELISA measurements performed on the duodenal samples. With both the liquid and solid test meals, a decrease in the HPL secretion level was found to occur in all the groups treated with the two forms of Orlistat (Table 3), but the differences from the HPL secretion level observed in control experiments were not found to be significant by both the paired and unpaired $t$-tests. A decrease in the level of HPL secretion was found to be induced by Orlistat in previous studies by Schwizer et al. (28) and Hildebrand et al. (19). These authors also observed that Orlistat decreased the postprandial plasma CCK levels. These findings support the idea that the release of CCK is controlled by FFA. The inhibition of lipolysis by Orlistat would reduce the amount of FFAs in the small intestine and thereby the release of CCK. The decrease in CCK release would in turn reduce the pancreatic exocrine secretory response.

In the control experiments, the average HPL output was $253.5 ± 95.5$ and $202.9 ± 96.1$ mg with the liquid and solid test meals, respectively. The difference between the two types of meals was not found to be significant. For comparison with our results, various HPL outputs during the digestion of test meals have been reported in previous studies: $245 ± 22$ (5) and $88 ± 25$ mg (7) with a complete liquid test meal, $442 ± 87$ mg with a high-fat meal containing 500 ml of Intralipid (28), 645 ± 96 mg with a meal containing large amounts of FFAs (28), and 263 ± 21 mg with an albumin-glucose meal (28).

**HPL inhibition by Orlistat.** The results obtained with the liquid and solid test meals are listed in Table 3. A high level of HPL inhibition was observed in all the groups treated with the two forms of Orlistat and with both types of meals. With the liquid meal, the difference from control values was highly significant in the Xenical pellet group ($P < 0.001$) and the micronized Orlistat powder group ($P < 0.01$). With the solid test meal, the difference from control values was highly significant in the Xenical pellet group ($P < 0.001$) and significant in the micronized Orlistat powder group ($P < 0.05$). The difference between the two treated

<table>
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<tr>
<th>Meal</th>
<th>Treatment</th>
<th>Amount of HGL Secreted, mg</th>
<th>Active HGL</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Amount, mg</td>
<td>%Control</td>
</tr>
<tr>
<td>Liquid meal: Shak Iso</td>
<td>Xenical pellets</td>
<td>17.6 ± 6.7</td>
<td>5.4 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Orlistat powder</td>
<td>17.2 ± 11.6</td>
<td>1.4 ± 1.0</td>
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<tr>
<td></td>
<td>Control</td>
<td>21.6 ± 14.5</td>
<td>14.5 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>Xenical pellets</td>
<td>9.1 ± 3.2</td>
<td>4.8 ± 1.7</td>
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<td></td>
<td>Orlistat in butter</td>
<td>16.3 ± 9.6</td>
<td>5.4 ± 3.4</td>
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<tr>
<td></td>
<td>Control</td>
<td>15.2 ± 5.1</td>
<td>13.2 ± 4.6</td>
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Values are means ± SD; see Table 1 for no. of experiments in each group of volunteers. HGL, human gastric lipase.
The half-inhibition time was estimated to be 6.2 min. using all the data. A logarithmic model for HPL inhibition was obtained by regression of the sample and the known specific activity of HPL (in IU/mg). A value means the percentage of total possible HPL activity deduced from the ELISA measurement of HPL amounts (in mg) present in the sample. In the absence of Orlistat, the HPL activity (in IU/ml; 100% value) present in each sample was calculated from the ELISA measurement of the HPL concentration (in mg/ml) and the known specific activity (in IU/mg) of HPL. A duodenal sample collected in the absence of Orlistat was used as a control. In the presence of protease inhibitors, the HPL activity of this sample remained stable during a 6-h period of storage at 4°C. In the presence of Orlistat, the HPL residual activity was already <50% of the maximum HPL activity in most of the samples tested only a few minutes after they were collected (Fig. 2). The rate of HPL inhibition was then reduced but not completely stopped by storage at 4°C. The residual HPL activity was found to be ~10–30% after 2 h of storage. The inhibition level remained fairly constant during the next hour. The time required for HPL half-inhibition to be reached was estimated to be 6.2 min.

It was observed, however, that the level of HPL inhibition measured immediately after the sampling was lower than that measured later in the same samples. The time course of the inhibition of HPL was therefore examined during the storage of several duodenal samples collected during experiments with Orlistat (Fig. 2). Because HPL was already partly inactivated at the time of the sampling, the initial HPL activity (in IU/ml; 100% value) present in each sample was calculated from the ELISA measurement of the HPL concentration (in mg/ml) and the known specific activity (in IU/mg) of HPL. A duodenal sample collected in the absence of Orlistat was used as a control. In the presence of protease inhibitors, the HPL activity of this sample remained stable during a 6-h period of storage at 4°C. In the presence of Orlistat, the HPL residual activity was already <50% of the maximum HPL activity in most of the samples tested only a few minutes after they were collected (Fig. 2). The rate of HPL inhibition was then reduced but not completely stopped by storage at 4°C. The residual HPL activity was found to be ~10–30% after 2 h of storage. The inhibition level remained fairly constant during the next hour. The time required for HPL half-inhibition to be reached was estimated to be 6.2 min.

Lipolysis levels in control experiments. Gastric lipolysis was significantly lower (P < 0.02) with the solid meal than with the liquid meal (Table 4). Duodenal lipolysis was also found to be lower with the solid meal than with the liquid meal (Table 4). The TGs of the liquid test meal therefore provided a better substrate for HGL and HPL, probably because they were pre-emulsified and stabilized in the form of a fine emulsion by the large numbers of phospholipids present in the Shak Iso test meal. In the solid meal, the physicochemical state of the lipids is more heterogeneous, and most of the TGs have to be emulsified in the course of the digestive process.

Although the lipolysis levels were found to increase in the duodenal contents, it is worth noting that the overall lipolysis levels at the angle of Treitz reached only 59.4 ± 5.6% with the liquid meal and 27.7 ± 6.8% with the solid meal. The lipolysis could not be complete because the duodenal contents were collected continuously at the angle of Treitz for 15-min periods, and at the end of each period, the lipolysis of the aspirate was stopped to analyze the lipolysis products. The mean residence time of the meal lipids in the small intestine, and hence their contact with lipases, was therefore shortened artificially compared with what occurs under normal physiological conditions. The present experimental procedure was nevertheless suitable for observing the effects of Xenical pellets, Orlistat powder, or Orlistat in butter compared with control experiments.

Effects of Orlistat on gastric lipolysis. With both types of test meal and all drug formulations, the level of gastric lipolysis was highly and significantly (P < 0.05) reduced in vivo by Orlistat (10.9–33.4% of control values; Table 4), and a good correlation was always observed with the high level of HGL inhibition (46.6–91.4%; Table 2).
Effects of Orlistat on duodenal lipolysis. With the liquid meal, duodenal lipolysis (Table 4) decreased only slightly when micronized Orlistat powder was pre-mixed with the meal (74.5% of controls) and did not decrease at all when Xenical pellets were added in the course of the administration of the meal (115.7% of controls). The differences between treated and control groups were not significant. This lack of effect of Orlistat as a means of reducing duodenal lipolysis was not correlated with the high level of HPL inhibition observed in the duodenal contents (Table 3). These paradoxical results were, however, supported and explained by the results of further in vitro experiments.

With the solid meal, in vivo duodenal lipolysis was highly and significantly reduced by both forms of Orlistat (32.6% to 37.6% of controls; Table 4), and in this case, a good correlation was observed with the high level of HPL inhibition (51.2 to 82.6%; Table 3).

Effects of Orlistat on overall lipolysis. Generally speaking, the overall level of lipolysis (gastric + duodenal lipolysis) of the liquid test meal did not decrease significantly in response to Xenical pellets (81.9% of controls). The decrease in overall lipolysis was, however, found to be significant (P < 0.05) in the group treated with micronized Orlistat powder (48.4% of controls). The difference between the two treated groups was mainly a result of the very high level of inhibition of gastric lipolysis observed in the group treated with micronized Orlistat powder (Table 4).

With the solid test meal, the overall level of lipolysis was highly and significantly reduced by the double action of Orlistat on gastric and duodenal lipolysis (28.8% of controls for Xenical pellets, P < 0.005; 33.4% of controls for micronized Orlistat powder in butter, P < 0.001; Table 4). The difference between the two treated groups was not significant.

Specific observations during experiments with duodenogastric reflux. A duodenogastric reflux occurred in two experiments with the liquid meal and Xenical pellets. These experiments were therefore analyzed separately. The first evidence of a duodenogastric reflux obtained in these experiments was the yellow color of the gastric samples containing bile. The fact that a reflux of the duodenal contents into the stomach occurred was further confirmed by measuring the HPL activity in these samples. The secretion (24.1 ± 12.1 mg) and the inhibition (77.9 ± 1.6%) of HGL were stable throughout the experiments with both the liquid and solid meals and with both Xenical pellets and micronized Orlistat powder. The same situation was also observed in the duodenal contents (Table 3). These paradoxical results were, however, supported and explained by the results of further in vitro experiments.

In vitro lipolysis of test meals. The experimental conditions used here to simulate meal fat digestion in vitro were chosen based on the in vivo data obtained during this study, as well as on previous in vivo studies (7). The pH values, lipase concentrations, and meal-to-digestive juice ratios used were those observed at 50% gastric emptying of a liquid test meal, both in the stomach and the lower duodenal contents.

The liquid or solid test meal, with or without Orlistat, was first mixed with human gastric juice for 30 min and then with pancreatic juice and bile for a further 60 min (Fig. 3). Samples were collected at various times to measure the concentrations of lipolytic products and the residual HGL and HPL activity.

In the control experiments without the drug, we checked that the HGL and HPL activities remained constant for 90 min, the final level of lipolysis was 88.9 ± 1.4%.

The rate of HGL inhibition by Orlistat was found to be higher with the liquid meal (Fig. 4A and B) and the solid (Fig. 4, D and E) test meals, regardless of the source of enzyme used. The lipolysis levels were found to be higher with the liquid meal (Fig. 4C) than with the solid meal (Fig. 4F) as previously observed in vivo (Table 4). Gastric lipolysis (t = 0–29 min) reached 7.4 ± 0.7% with the liquid meal (Fig. 4C) and 2.2 ± 0.4% with the solid meal (Fig. 4F). When pancreatic juice and bile were added (t = 30 min) to the liquid meal and gastric juice mixture, the level of lipolysis increased drastically (Fig. 4C) and reached 77.5 ± 0.5% after only 5 min of incubation (t = 35 min). The rate of lipolysis then became very slow, probably as a result of the limiting amount of substrate. At t = 90 min, the final level of lipolysis was 88.9 ± 1.4%.

With the solid meal, a slower increase in the level of lipolysis was observed when pancreatic juice and bile were added, and the rate of lipolysis remained fairly constant for ~30 min (Fig. 4F). The level of hydrolysis reached 7.9 ± 2.9% at t = 35 min and 69.7 ± 4.5% at t = 90 min.

Effects of Orlistat on in vitro lipolysis of test meals incubated with human gastric and pancreatic juices. The rate of HGL inhibition by Orlistat was found to be similar with both the liquid (Fig. 4A) and solid (Fig. 4D) meals and with both Xenical pellets and micronized Orlistat powder. The same situation was also

<table>
<thead>
<tr>
<th>Table 4. Effects of Orlistat on gastrointestinal lipolysis during digestion of test meals in healthy volunteers</th>
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<tbody>
<tr>
<td>Meal</td>
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<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Liquid meal: Shak Iso</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Solid meal: hamburger, fries, butter</td>
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Values are means ± SD; see Table 1 for no. of experiments. Nos. in brackets, percentage of respective control values.
observed in the case of HPL inhibition by Xenical pellets and micronized Orlistat powder (Fig. 4, B and E, respectively), but the rate of HPL inhibition was found to be slower than that of HGL. The half-inhibition times were found to be ~5 min with HPL and <1 min with HGL.

With the liquid meal, the level of gastric lipolysis was drastically reduced in the presence of the lipase inhibitor (Fig. 4C; Table 5). At $t = 29$ min, the lipolysis level was reduced to 7% of control values by Xenical pellets and to 0% by micronized Orlistat powder. The level of duodenal lipolysis also decreased in the presence of the lipase inhibitor but less than the level of gastric lipolysis. At $t = 35$ min, the lipolysis levels were $31.9 \pm 4.2\%$ with Xenical pellets (41% of controls) and $10.3 \pm 5.2\%$ with micronized Orlistat powder (13% of controls). At $t = 90$ min, the lipolysis levels were $61.3 \pm 2.7\%$ with Xenical pellets (69% of controls) and $50.8 \pm 5.7\%$ with micronized Orlistat powder (57% of controls). A significant level of lipolysis of the liquid meal TGs was, in fact, reached before HPL was significantly inhibited by Orlistat. A good correlation was observed between the variation in the level of lipolysis (Fig. 4C) and the variation in the residual HPL activity (Fig. 4B). With the solid meal, both gastric and duodenal lipolysis were drastically reduced in the presence of Orlistat (Fig. 4F; Table 5).

**Effects of Orlistat on in vitro lipolysis of test meals incubated with pure HGL and HPL.** In vitro experiments with and without micronized Orlistat powder were also performed with purified lipases instead of digestive juices (Fig. 5). No significant differences were observed between the values obtained with the juices and those obtained with the purified lipases (Table 5).

**An explanation for weak effects of Orlistat on duodenal lipolysis of liquid test meal.** In vivo, the duodenal lipolysis of the liquid meal was only slightly decreased by Xenical pellets and micronized Orlistat powder (Table 4), whereas a strong HPL inhibition was recorded in the duodenal samples (>77%; Table 3). An explanation for these paradoxical results was obtained on the performance of in vitro lipolysis of the test meals, with or without Orlistat. On the one hand, it was observed that the half-inhibition time of HPL was ~5 min with both types of test meals and both forms of the drug (Fig. 4, B and E, and Fig. 5, B and E). This value was found to be similar to that estimated from in vivo experiments (6.2 min; Fig. 3). On the other hand, the variations in the lipolysis levels of the liquid and solid test meals with time were found to be strikingly different. When pancreatic juice or pure HPL was added to the meal-gastric juice mixture, the TGs of the liquid test meal were hydrolyzed to a large extent before the enzyme was significantly inhibited by Orlistat (Figs. 4C and 5C). In contrast, no significant hydrolysis of the solid meal TGs occurred before the enzyme had been significantly inhibited by Orlistat (Figs. 4F and 5F). The liquid meal TGs are a much better substrate for HPL than the solid meal TGs. It can therefore be concluded that the duodenal lipolysis of the liquid meal TGs is poorly reduced by Orlistat because the rate of TG hydrolysis by HPL is faster than the rate of HPL inhibition by Orlistat. With the solid meal, the opposite situation occurs, and Orlistat is an efficient inhibitor of duodenal lipolysis. No such problem was encountered in the case of gastric lipolysis because the rate of HGL inhibition by Orlistat is extremely fast (half-inhibition time < 1 min), and gastric lipolysis was always stopped.
rapidly, regardless of the type of test meal and the drug formulation. One exception was observed, however, when a duodenogastric reflux occurred in the presence of Orlistat with the liquid test meal. In the latter case, the gastric level of lipolysis was found to be higher than the gastric level of lipolysis observed in the control experiments without Orlistat. The lipolysis was in fact due to a reflux of HPL into the stomach, and this particular experiment confirmed that the action of HPL on the liquid meal TGs was not significantly reduced by Orlistat.

Table 5. Effects of Orlistat on in vitro lipolysis of test meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Treatment</th>
<th>%Lipolysis</th>
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<tr>
<td></td>
<td></td>
<td>£29 min</td>
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<tr>
<td></td>
<td></td>
<td>t = 29 min</td>
</tr>
<tr>
<td>Liquid meal: Shak Iso</td>
<td>Xenical pellets</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Orlistat powder</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>£29 min</td>
</tr>
<tr>
<td>Solid meal: hamburger, fries, butter</td>
<td>Xenical pellets</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Orlistat in butter</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Lipolysis with purified HGL</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>£29 min</td>
</tr>
<tr>
<td>Liquid meal: Shak Iso</td>
<td>Orlistat powder</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>Solid meal: hamburger, fries, butter</td>
<td>Orlistat in butter</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.0 ± 0.5</td>
</tr>
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</table>

Values are means ± SD; nos. in parentheses, lipolysis in %controls.
Effects of Orlistat on fat excretion. Additional in vivo experiments were performed to evaluate whether Orlistat inhibited fat absorption in volunteers fed the liquid meal (Shak Iso). Fat absorption was indirectly measured from fecal fat excretion. As predicted from the previous in vivo and in vitro gastrointestinal lipolysis experiments, it was observed that Xenical pellets poorly inhibited the absorption of fat from the finely preemulsified liquid test meal. Only 4.2% fat excretion was achieved compared with 40.5% fat excretion with the solid test meal ingested with Xenical pellets (Table 6). These results are in good agreement with previous in vivo data (Table 4) showing that the overall level of lipolysis undergone by the liquid meal was not significantly reduced by Xenical pellets compared with what occurred in the control experiments. Because it was observed in the same experiments that the level of gastric lipolysis was significantly reduced, the data on fat excretion indicate that duodenal lipolysis by HPL compensates for the decrease in gastric lipolysis during the whole digestion process.

When the liquid meal was given with micronized Orlistat powder instead of Xenical pellets, the mean level of fat excretion reached 18.8% but was very variable and remained significantly lower than the fat excretion level determined after ingestion of the solid meal with micronized Orlistat powder (57.4% fat excretion; Table 6). Here again, the results are in good agreement with previous in vivo data (Table 4) showing that micronized Orlistat powder significantly decreased the overall lipolysis level of the liquid meal versus what occurred in the control experiments. This decrease was mainly a result of the drastic drop in the

<table>
<thead>
<tr>
<th>Meal</th>
<th>Treatment</th>
<th>Dose, mg</th>
<th>Fat Excretion, % of ingested fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid meal:</td>
<td>Xenical</td>
<td>120</td>
<td>4.2 ± 6.8 (n = 5)</td>
</tr>
<tr>
<td>Shak Iso (990 ml)</td>
<td>Orlistat powder</td>
<td>120</td>
<td>18.8 ± 31.7 (n = 5)</td>
</tr>
<tr>
<td>Solid meal:</td>
<td>Xenical</td>
<td>120</td>
<td>40.5 ± 26.1 (n = 7)</td>
</tr>
<tr>
<td>hamburger, fries,</td>
<td>Orlistat in butter</td>
<td>60</td>
<td>57.4 ± 16.8 (n = 6)</td>
</tr>
<tr>
<td>butter</td>
<td></td>
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</table>

Values are means ± SD; n, no. of volunteers.
level of gastric lipolysis (10.9% of controls). Interestingly, the level of fat excretion (18.8% of ingested fat) was found to be similar to the decrease in the level of gastric lipolysis (from 24.4% in controls to 2.7% in the presence of Orlistat powder). It therefore emerged from the latter experiments that duodenal lipolysis by HPL does not suffice to compensate for the decrease in gastric lipolysis. One possible explanation for these results focuses on the synergy existing between the action of HGL and that of HPL. It has been established by Gargouri et al. (14) that HPL hydrolyzed a TG emulsion covered by phospholipids only after partial hydrolysis by HGL had occurred. The FFAs generated by HGL were found to trigger the action of HPL by changing the “quality” of the oil-water interface. One can therefore envision a further indirect inhibitory effect of Orlistat on HPL when gastric lipolysis is highly reduced.

Conclusions. Because the contribution of HPL to the overall level of lipolysis is much greater than that of HGL, HPL has always been the main target in the development of lipase inhibitors for use as antiobesity agents. The inhibition of HGL by Orlistat had been investigated previously in vitro under optimized assay conditions (2, 13, 26), but it had not been studied so far in the course of test meal lipolysis. The present clinical study provides the first comprehensive overview of test meal lipolysis by HGL and HPL in healthy human volunteers in the presence and absence of a lipase inhibitor (Orlistat). The results obtained show that Orlistat, administered as Xenical pellets, micronized powder, or melted in butter, is a powerful HGL inhibitor that strongly reduces the level of gastric lipolysis of liquid and solid test meals. The effects of Orlistat on duodenal lipolysis depend on the type of meal ingested. This study highlights the fact that lipase inhibition and lipolysis are two competitive processes. The balance between these processes depends on the physicochemical properties of the dietary TGs. Improving the lipase inhibition rate versus the lipolysis rate will be a key issue in the future development of lipase inhibitors for obesity treatment. Before new inhibitors and formulations are available, an alternative approach to improve the drug efficiency will probably be to associate the prescriptions of Xenical with more specific diets in which fat is poorly premulsified and is therefore not a good substrate for gastrointestinal lipases.

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