Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract[1–3]

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ABSTRACT

Background: The extent of fat emulsification affects the activity of digestive lipases in vitro and may govern digestion and absorption of dietary fat.

Objective: We investigated the effect of the fat globule size of 2 enteral emulsions on fat digestion and assimilation in humans.

Design: Healthy subjects received intragastrically a coarse (10 μm) and a fine (0.7 μm) lipid emulsion of identical composition in random order. Gastric and duodenal aspirates were collected throughout digestion to measure changes in fat droplet size, gastric and pancreatic lipase activities, and fat digestion. Blood lipids were measured postprandially for fat assimilation.

Results: Despite an increase in droplet size in the stomach (2.75–6.20 μm), the fine emulsion retained droplets of smaller size and its lipolysis was greater than that of the coarse emulsion (36.5% compared with 15.8%; $P < 0.05$). In the duodenum, lipolysis of the fine emulsion was on the whole higher (73.3% compared with 46.3%). The overall 0–7-h plasma and chylomicron responses given by the areas under the curve were not significantly different between the emulsions, but the triacylglycerol peak was delayed with the fine emulsion (3 h 56 min compared with 2 h 50 min).

Conclusions: Fat emulsions behave differently in the digestive tract depending on their initial physicochemical properties. A lower initial fat droplet size facilitates fat digestion by gastric lipase in the stomach and duodenal lipolysis. Overall fat assimilation in healthy subjects is not affected by differences in initial droplet size because of efficient fat digestion by pancreatic lipase in the small intestine. Nevertheless, these new observations could be of interest in the enteral nutrition of subjects suffering from pancreatic insufficiency. *Am J Clin Nutr* 1999;70:1096–106.

KEY WORDS Fat droplet size, fat, lipolysis, digestion, assimilation, gastric lipase, pancreatic lipase, postprandial lipemia, humans, pancreatic insufficiency, emulsion, enteral nutrition

INTRODUCTION

Gastrointestinal lipid digestion consists of several sequential steps that include physicochemical and enzymatic events (1–3). In humans, digestion of dietary triacylglycerols starts in the stomach with the action of gastric lipase at the lipid-water interface (2) and continues in the duodenum with the synergetic action of gastric and colipase-dependent pancreatic lipases (3). Then, the lipolysis products generated and accumulated at the fat globule surface are transferred into structures made of phospholipids or bile salts, forming multi- or unilamellar vesicles and mixed micelles in the aqueous phase (1, 4–8), and are then absorbed by the enterocytes. Good synergetic action of all these physicochemical and enzymatic steps is necessary for optimal bioavailability of dietary lipids and other lipid nutrients such as fat-soluble vitamins or drugs (1, 9–11).

In healthy humans, the importance of gastric lipase is still debated. Results of a few experiments have been reported in which lipolysis in the stomach catalyzed by gastric lipase reached 10–30% of the ingested triacylglycerols, generating fatty acids and a mixture of diacylglycerols and monoacylglycerols (2, 12–15). However, gastric lipase facilitates subsequent hydrolysis by pancreatic lipase by promoting fat emulsification (14, 15) and by generating fatty acids and diacylglycerols (2, 12–16). Furthermore, in physiologic (pre- or full-term infants) and pathologic pancreatic (eg, cystic fibrosis and pancreatitis) insufficiency, gastric lipolysis is assumed to play a key role in the digestion of dietary fat (17–19).

The characteristic feature of lipases is their specificity for insoluble, emulsified substrates (20). Thus, lipid emulsification, which occurs in the stomach (14, 15), must be considered a fundamental step in fat digestion by generating a lipid-water interface essential for the interaction between water-soluble lipases and insoluble lipids (1, 14, 15). The properties of this interface modulate fat digestion and consequently the bioavailability of lipid nutrients. These properties are directly dependent on the physicochemical properties of lipid, such as fat droplet size (which governs the lipid interface area), fat globule organization, and the molecular struc-

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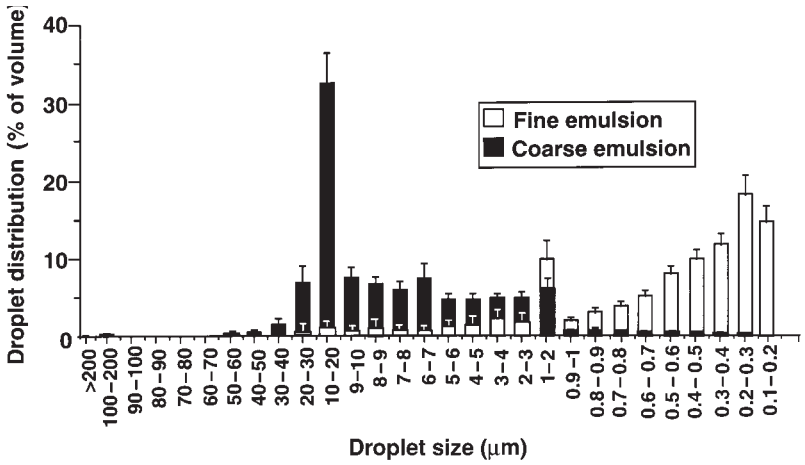

FIGURE 1. Mean (±SE) droplet size distribution of the fine and coarse emulsions before digestion calculated by particle-sizer software (Capa-700; Horiba, Kyoto, Japan) as the percentage of the total volume occupied by lipid droplets in a given sample; n = 8 determinations.

ture of the triacylglycerols constituting the fat droplet. Without consideration of the emulsion droplet size, the hydrolytic activity of gastric lipase was measured previously on pure short-chain, medium-chain, and long-chain triacylglycerols (21). Furthermore, in vitro experiments have raised the notion that the extent of lipid emulsification affects the activity of digestive lipases (20, 22, 23). Nevertheless, little attention has been paid thus far to the physicochemical properties of the emulsions used for enteral nutrition. In fact, because there are no potential direct health complications caused by the presence of large lipid droplets in enteral formulas, a wide spectrum of particle sizes can be found in the different commercially available formulas (from 0.3 to 10 μm). However, a first study conducted in rats in our laboratory suggested that emulsions with different droplet sizes behave differently in the digestive tract and, during tube feeding, these different emulsions are digested and then metabolized differently (24).

In light of these data, the aim of this study was to determine the physicochemical properties of emulsions with different initial droplet sizes in the digestive tract (stomach and duodenum) of healthy adults and to test the hypothesis that differences in droplet size could affect the extent of fat digestion and fat assimilation as determined by postprandial blood lipids.

SUBJECTS AND METHODS

Subjects

The study protocol was approved by the local Medical Ethics Committee (CCPPRB, Marseille, France). Eight healthy male volunteers 24.0 ± 1.4 y of age, 174 ± 3 cm in height, and 66 ± 5 kg in weight were enrolled in the study after giving written, informed consent. None had a history of gastrointestinal or metabolic disorders, as checked by medical history and fasting blood measures, or was taking drugs that might alter gastrointestinal function or lipid metabolism. Fasting blood concentrations of triacylglycerols (0.92 ± 0.09 mmol/L), total cholesterol (4.19 ± 0.29 mmol/L), phospholipids (2.36 ± 0.18 mmol/L), and retinyl palmitate (0.32–2.10 nmol/L) were in the normal range.

A 3-d diet record was used to determine the usual diet of each subject and dietary analyses were done by using the GENI software package (Micro 6; Nancy, France). The subjects consumed a typical Western diet with moderate energy contents (10 810 ± 707 kJ/d, or 2586 ± 169 kcal/d) and 16.1 ± 1.1% of energy as protein, 43.6 ± 2.7% as fat, and 40.3 ± 3.4% as carbohydrates. The subjects were asked to have a light dinner the day before each experiment.

Emulsions

Each subject was given 2 formulas (Clintec Technologies, Velizy, France) differing only in the droplet size of the fat globules, 1 wk apart, and in a random order. Both formulas contained (by wt) fish oil (4.8%), olive oil (4.8%), soybean lecithin (0.46%), glycerol stearate (0.7%), proteins from milk and whey (6.75%), carbohydrates (10%), and retinyl palmitate (29.6 and 34.7 retinol equivalents/L for the fine and coarse emulsions, respectively). Lipids were mainly composed of triacylglycerols and phospholipids, and the triacylglycerol-phospholipid ratio (by wt) was ≈27:1. One formula contained small-sized fat globules with a median droplet diameter of 0.7 ± 0.2 μm (fine emulsion) and the other contained fat globules sized 10.1 ± 0.9 μm (coarse emulsion) (**Figure 1**). The specific surface areas were 20.3 and 1.4 m^2/g emulsified fat for the fine and coarse emulsions, respectively ($P < 0.05$). Each bolus (500 mL) provided 3290 kJ (787 kcal): 57% as fat, 26% as carbohydrate, and 17% as protein. The amount of triacylglycerol given (48 g/bolus) was an unusual fat intake for most industralized countries. The fatty acid blend of both formulas was myristic acid (4.1%), palmitic acid (13.7%), palmitoleic acid (4.9%), margaric acid (0.7%), stearic acid (8.2%), oleic acid (45.7%), linoleic acid (7.1%), α-linoleic acid (0.8%), arachidic acid (1.2%), arachidonic acid (0.5%), eicosapentaenoic acid (8.2%), docosapentaenoic acid (0.9%), and docosahexaenoic acid (3.3%).

The nonabsorbable marker polyethylene glycol (PEG 4000; Merck, Darmstadt, Germany) was added to the bolus (0.05%) to measure gastric volumes and to enable monitoring of the gastric-emptying rate for the fat emulsions. Before administration, the 500-mL emulsion was brought to 37 °C in a water bath.

Study design

Each procedure started at 0900 after subjects fasted overnight. Each subject was intubated with a single-lumen nasogastric

tube (outer diameter 0.50 cm; Mallinckrodt Laboratories Ltd, Athlone, Ireland) and a single-lumen nasoduodenal tube (outer diameter 0.30 cm). The gastric tube was located 45–50 cm from the nose in the stomach at the corpus-antrum junction. The duodenal tube was located at the junction of the second and the third portion of the duodenum after the papilla of Vater. Ten aspirating ports were located in the 7 cm at the ends of the tubes. The tubes were firmly secured to the nose with adhesive tape. The position of the tubes was checked by X-ray before infusion of the emulsions and at the second hour of the procedure. After the tubes had been placed, the subject assumed a sitting position in bed, which he maintained until the end of the study. An antecubital vein was then catheterized with an intravenous cannula equipped with disposable obturators (Jelco-Critikon, Chatenay-Malabry, France).

Fasting gastric juice (36.5 ± 7.7–36.7 ± 5.9 mL) and duodenal fluid (5.6 ± 1.1–6.6 ± 1.0 mL) were removed by manual aspiration just before ingestion of the emulsion to determine basal fasting values. The 500-mL emulsion was intubated intragastrically with a 60-mL syringe over a 10-min period. A 2-mL sample of the initial emulsion was retained for measurement of lipid droplet size and chemical analysis. Large samples (100–200 mL) of the stomach contents were aspirated 0.5 h after feeding and every hour for 4–5 h after administration of the emulsion by gentle aspiration with a 60-mL syringe to obtain representative samples (14, 15). Then, a 20-mL aliquot was taken from each gastric sample for analytic determinations. The last sample obtained after 4 or 5 h was the entire volume (29 ± 7 mL with the fine emulsion and 11.9 ± 7.8 mL with the coarse emulsion) of the remaining contents. Duodenal fluid was aspirated and a 5–10-mL sample was collected 0.5 h after emulsion infusion and every hour for 4–5 h. The respective remaining contents were promptly reinjected into the subject's stomach or duodenum via the tubes.

A fraction (5 mL) of the retained gastric sample was immediately placed on ice, neutralized with NaOH when necessary to reach pH 5.0–5.4 to prevent inactivation of the gastric lipase, rapidly frozen, and stored at −20°C until gastric lipase activity was assayed. To prevent proteolytic inactivation of pancreatic lipase, a protease inhibitor from soybeans (Sigma Chemical Co, La Verpilliere, France) was added (final concentration: 0.5 g/L aspirate) to a 1–5-mL duodenal specimen immediately after collection (14, 15). These samples were immediately placed on ice, rapidly frozen, and stored at −20°C for determination of pancreatic lipase activity. The remainder of each gastric or duodenal sample was placed into glass vials containing a methanolic solution of lipase inhibitors (5% by vol), as described in detail previously (8, 14). To prevent any bacterial growth, 5 μL each of an aqueous solution of 4% NaN_3 (wt:vol) (Prolabo, Vitry-sur-Seine, France) and 5% chloramphenicol (wt:vol; Sigma) per milliliter of aspirate were added to each test tube (8, 14). These concentrations of additives were shown in preliminary experiments to have no effect on the droplet size distribution of the emulsions. The pH was measured in each sample to the nearest 0.1 pH unit. A baseline fasting blood sample (0 h) was collected. Blood samples (10 mL) were drawn at 0.5 h and every hour for 7 h after emulsion infusion.

Gastric volume, fat emptying, and PEG-4000 determinations

We used the double-sampling marker-dilution method of George (25), with PEG 4000 as the nonabsorbable marker, to measure total gastric volume and to compare the gastric-emptying rate of lipids for the 2 emulsions. The meals used were fully homogenized liquid test meals that have been shown (26, 27) to lead to simultaneous emptying of the aqueous and lipid phases (26, 27). Thus, under these conditions, PEG 4000, like phenol red (28), is a marker of the aqueous phase and can be used to monitor the emptying of the total meal components. A small amount of concentrated PEG 4000 was added (1 mL containing 150 mg PEG 4000) at each collection time after a sample was collected and just before addition of a new dose of marker. Volumes present in the stomach were calculated by using the following equation of George (25):

$$V_1 = V_2 (C_2 - C_3)/(C_3 - C_1) \quad (1)$$

where V_1 is the volume to be determined, C_1 is the initial concentration of marker in V_1, V_2 is the volume of concentrated marker added to V_1, C_2 is the concentration of added marker, and C_3 is the final concentration of the marker; the mass of marker in V_1 is given by V_1C_1 and the mass of marker in V_2 is given by V_2C_2. PEG 4000 concentration in gastric contents (0.1–1-mL samples) was determined according to the method of Hyden (29).

The amount of fat present in the stomach contents was calculated by multiplying the gastric volume by the fat concentration at each time point. Rates of fat emptying were thus calculated by knowing the initial quantity of fat ingested (48 g) and the total amount of fat that was present in the stomach at each time point.

Measurement of emulsion droplet size

The distribution of the emulsion droplet sizes was determined in the initial emulsions and the gastric and duodenal aspirates as described previously (14, 15) by using a particle-size analyzer (Capa-700; Horiba, Kyoto, Japan). Before measurements, a calibration was done by using microparticles in the size range of 0.2–100 μm (polystyrene size standard kit; Polysciences, Warrington, PA) to check the accuracy of the data given by the apparatus. The lower threshold of the apparatus used is 0.01 μm. Because no measurable proportion of very small droplets (0.1–0.02 μm) had been found in gut contents during previous experiments, we selected a lower limit of 0.09 μm. Measurements were done at 560 nm by using gradient mode analysis at a constant centrifuge acceleration rate (1 × *g*/min), which allowed accurate measurement of both large (100 μm) and small (0.09 μm) particles. Results are given as droplet size distributions calculated by the particle-sizer software (Horiba) as a fraction of the total volume occupied by lipid droplets in a given sample. The median size values were calculated by the particle-sizer software from the droplet size distribution. From the distribution of particle sizes obtained for a given emulsion, the particle-sizer software calculated the specific surface area (Sw, expressed as m^2/g emulsified fat) as described previously (14, 15).

Analysis of triacylglycerols and lipolysis products

Lipids in the initial emulsions and the gastric and duodenal samples were extracted in chloroform:methanol (2:1, by vol) (30). To ensure complete protonation of fatty acids, the organic solvent phases were partitioned with 20% (by vol) 150 mmol aqueous NaCl/L containing 2% glacial acetic acid (by vol, pH 3.0), as described previously (8, 14, 15). The chloroformic lower phases were evaporated to dryness under nitrogen. Total lipids were determined gravimetrically. Total phospholipids were measured as phosphorus after being reduced to ash as described previously (14). Neutral lipid classes (triacylglyc-

erols, diacylglycerols, monoacylglycerols, fatty acids, and cholesterol) were separated by 2-stage, 1-dimensional thin-layer chromatography according to Bitman and Wood (31) and lipids were quantified densitometrically by using a video densitometry system and standard calibration curves, as was described in detail (14, 15). Values were converted into moles by using average molecular masses calculated according to the fatty acid composition of the emulsions (triacylglycerol: 870.34; diacylglycerol: 592.89; monoacylglycerol: 315.44; fatty acids: 277.45). The extent of apparent triacylglycerol lipolysis was calculated as the relative disappearence (%) of triacylglycerols from total glycerides present (triacylglycerols + diacylglycerols + monoacylglycerols) and from triacylglycerols originally present in the emulsions (97%), as described previously (14, 15, 19). Total bile salt concentration in either duodenal or gastric contents was measured by an enzymatic method described previously (14) to check for contamination of gastric contents by duodenal fluid.

Isolation and analysis of vesicular and micellar structures

Small vesicles plus mixed micelles were isolated from duodenal contents collected after 2 h of digestion. Most large fat droplets were removed by prior centrifugation (6560 × *g* for 10 min) and the micelles + vesicles were subsequently isolated by gel filtration (Sepharose CL 4B, 30 × 1.5 cm column; Pharmacia, Uppsala, Sweden) following a procedure described previously (5). Eluted fractions containing both vesicular and micellar lipids were extracted by using the method of Folch et al (30). Lipid classes were separated by 2-stage, 1-dimensional thin-layer chromatography, as was described previously (15, 31). Quantification was done by using video densitometry, neutral lipids standard curves, and a lysophosphatidylcholine and phosphatidylcholine standard mixture (2–25 μg), as already described (14, 15).

Lipase activity measurements

Gastric lipase activity was determined in aliquots (200 μL) of gastric aspirates free of lipase inhibitors with a pH-Stat titrator (Metrohm, Herisau, Switzerland) at pH 5.40 and 37 °C by using tributyrin as substrate, 150 mmol NaCl/L, 6 mmol $CaCL_2$/L, 2 mmol taurodeoxycholate/L, and 1.5 μmol bovine serum albumin/L (Sigma), as described previously (14, 15).

As described previously (15), the activity of pancreatic lipase in either duodenal samples or gastric specimens (to check for duodenal contamination in the stomach) was measured by using a pH-Stat titrator at pH 8.0 and 37 °C with tributyrin as substrate, 2 mmol tris buffer/L (Sigma), 150 mmol NaCl/L, 10 mmol $CaCl_2$/L, 8 mmol taurodeoxycholate/L (Sigma), and excess colipase (Boehringer Mannheim, Mannheim, Germany). One lipase unit was defined as 1 μmol fatty acid titrated per minute.

Serum preparation and analytic determinations

Serum was separated from whole blood by centrifugation (910 × *g* for 15 min at 4 °C). The chylomicron fraction (S_f >1000) was isolated from 2 mL serum layered under 3 mL 0.9% NaCl by ultracentrifugation at 10 °C (25 000 × *g*, 1 h) in a Beckman centrifuge (40.3 rotor; Beckman Instruments, Palo Alto, CA) as described previously (32).

Serum and chylomicron triacylglycerols were determined by using enzymatic procedures with commercial kits (BioMerieux, Marcy l'Etoile, France) (33). Retinyl palmitate was determined in chylomicrons by using reversed-phase HPLC on a Kontron apparatus (Zurich, Switzerland), as described previously (34). Chylomicron droplet size was determined by using a quasielastic light-scattering detector (SEMAtech, Nice, France).

Statistics

Analytic determinations were made in duplicate, except for droplet size measurements because of the measurement duration (≈1 h). The values reported are means ± SEs from 8 subjects. The 0–7-h area under the curve (AUC) was calculated by using the trapezoidal method (32). Statistical significance of the data was analyzed by Bonferroni *t* test and two-way analysis of variance for repeated measures with contrast analysis when interaction terms were significant, ie, $P < 0.05$ (version 6.12; SAS/STAT software, SAS Institute Inc, Cary, NC; SuperANOVA programs for the Macintosh computer, Abacus Concepts, Berkeley, CA).

RESULTS

Gastric measures

Similar patterns were observed for postprandial variations of pH and gastric lipase activity, independent of the emulsion used. The gastric pH was very low in basal conditions (from 1.84 ± 0.08 to 1.98 ± 0.14), increased markedly up to 6.1 at 0.5 h after emulsion intubation ($P < 0.05$), and then returned to values comparable with baseline after 3 h (data not shown). Gastric lipase activity, expressed as U/L gastric content, dropped markedly by 83.9% and 72.5% ($P < 0.05$) by 0.5 h for the fine and coarse emulsions, respectively, and then returned to higher values close to the fasting value 2 h after feeding (**Figure 2**).

As shown in Figure 2, gastric volumes (meal + secretion) were not significantly different for the 2 emulsions, except at 2 and 4 h, when the volumes were significantly higher with the fine emulsion. The data show that 58.8% and 55.2% of the fat emulsion was emptied 0.5 h after infusion of the fine and coarse emulsions, respectively. Two hours after infusion, gastric emptying of lipids tended to be slower with the fine emulsion, with a significant difference after 4 h only (Figure 2).

Duodenal measures

The pH of the duodenal contents was constant throughout the 5-h digestion period, ranging from 5.2 to 6.7, independently of the type of emulsion used (data not shown). Pancreatic lipase activity (U/L) increased significantly from 14.6 to 16.2 times over fasting values after infusion of both emulsions. Lipase concentration was relatively constant throughout 4 h of digestion and decreased after 5 h to reach a value comparable with baseline for the coarse emulsion or an intermediate value for the fine emulsion (5670 ± 3000 and 17 360 ± 9600 U/L, respectively) (Figure 2). In the duodenal contents, bile salt concentrations ranged from 2.5 to 4.7 mmol/L in fasting samples and increased significantly postprandially, reaching 6.4–10.7 mmol/L, and then plateaued for the 5 h of digestion, independently of the emulsion intubated (data not shown).

Changes in emulsion droplet size

Median diameters of the fat droplets in the 2 emulsions in stomach and duodenal contents throughout digestion are shown in **Figure 3**. Throughout 4 h of digestion, the diam-

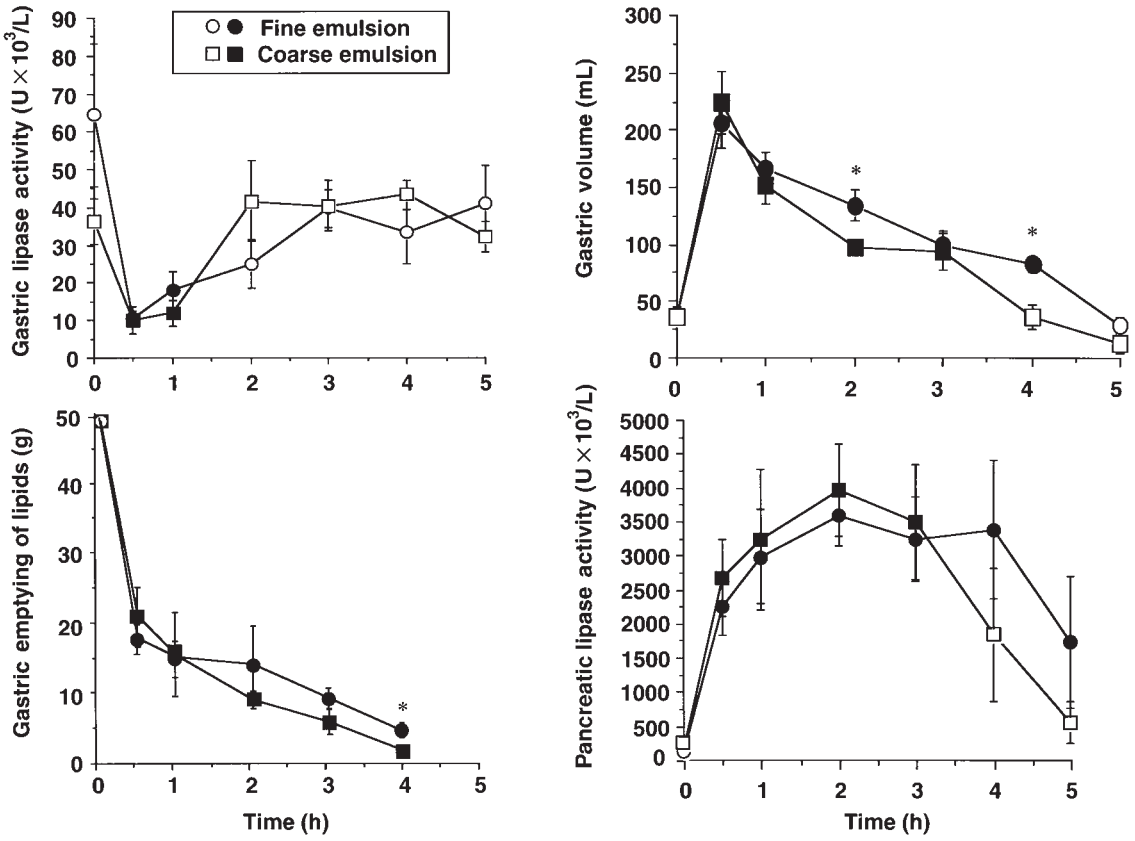

FIGURE 2. Mean (±SE) gastric lipase activity, gastric volume, gastric emptying rate of fat, and pancreatic lipase activity after infusion of fine and coarse emulsions. Filled symbols indicate a significant difference from the baseline value. *Significantly different from the coarse emulsion, $P < 0.05$ (two-way analysis of variance for repeated measures with contrast analysis). $n = 8$ subjects.

eter of the droplets in the coarse emulsion did not change significantly in the stomach or duodenum, and thus, droplet size distributions (**Table 1**), emulsion median diameters (Figure 3), and Sw values (1.28–3.21 compared with 1.4 m^2/g emulsified fat) were close to the initial values. The median diameter of the fat droplets in the fine emulsion increased in the stomach (2.75–6.20 compared with 0.7 μm initially) but was not significantly different from that in the duodenum (2.14–6.51 μm) (Figure 3). Consequently, the Sw decreased to 2.5–8.2 compared with 20.3 m^2/g initially. On the whole (Table 1), the <1-μm fraction decreased (10.0–39.1% compared with 79.5%) and droplets sized between 1 and 10 μm increased significantly at each time point (37.7–80.3% compared with 18.9%). The 10–50-μm droplets increased significantly after 0.5, 2, and 3 h of digestion in the stomach (26.2–29.6% compared with 1.4%) and after 0.5 and 2 h digestion in the duodenum (26.6% and 26.5% compared with 1.4%). Nevertheless, the median diameter of the fat droplets in the fine emulsion was significantly lower than that of the coarse emulsion in the stomach (0.5–3 h) and in the duodenum (1, 3, and 4 h). Consequently, the Sw of the fine emulsion remained significantly higher than that of the coarse emulsion in the stomach (2.7–7.0 compared with 1.3–1.8 m^2/g) and tended to be higher in the duodenum (2.5–5.2 compared with 2.3–3.2 m^2/g) at the same sampling time during digestion.

Lipid composition of gastric and duodenal contents throughout digestion

The concentrations of lipid species in the stomach and duodenal contents at each collection time are presented in **Table 2**. In the stomach, fatty acids, monoacylglycerols, and diacylglycerols were generated as a result of triacylglycerol hydrolysis by gastric lipase. With the fine emulsion, fatty acid concentrations (7.5–29.5 mmol/L) were significantly higher than with the coarse emulsion (2.2–12.3 mmol/L) at each time during digestion. Diacylglycerol concentrations (7.1–20.2 compared with 5.1–12.7 mmol/L) were generally higher with the fine emulsion. More monoacylglycerols were generated from the fine emulsion throughout digestion, with significantly higher concentrations at 3 and 4 h (2.8–9.8 compared with 0.7–4.1 mmol/L).

In the duodenum, fatty acid concentrations were 0.5–4- and 1.5–10-fold higher than in the stomach with the fine and the coarse emulsions, respectively, as a result of the lipolysis of triacylglycerols and diacylglycerols by both gastric and pancreatic lipases. With the fine emulsion, fatty acid concentrations were on the whole moderately higher (significantly higher at 4 h only). In the same way, monoacylglycerol concentrations were moderately higher at each time during digestion with the fine emulsion ($P < 0.05$ at 1 h). Moderately higher or nonsignificantly different concentrations of diacylglycerols were observed with the fine emulsion. The triacylglycerol concentrations were higher with the coarse emulsion, especially after 1 h.

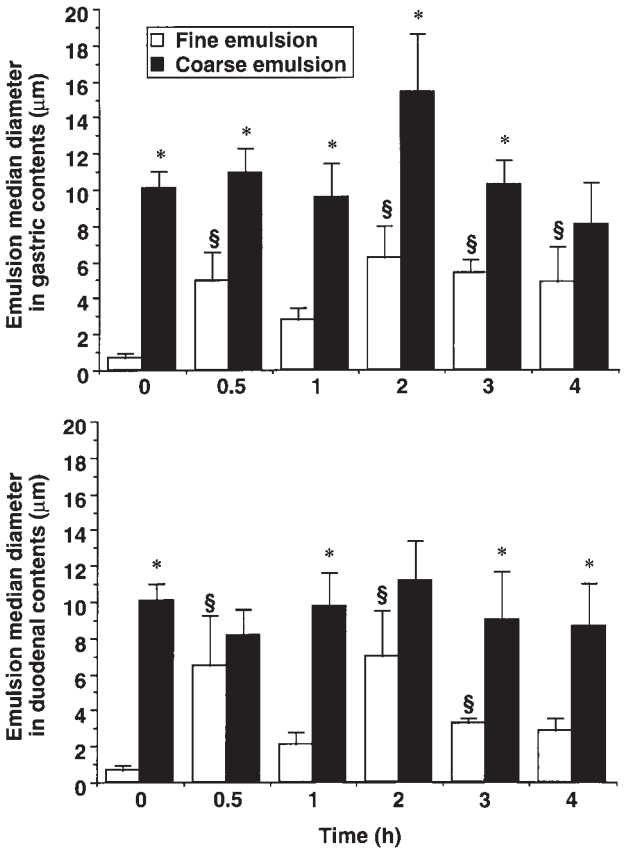

FIGURE 3. Median diameter of droplets in the fine and coarse emulsions in gastric and duodenal contents after 0.5, 1, 2, 3, and 4 h of digestion. Measurements were made by using a particle-size analyzer (Capa-700; Horiba, Kyoto, Japan). For more details, *see* Methods. Results are means ± SEs of 8 determinations. §Significantly different from the initial emulsion, $P < 0.05$ (Bonferroni *t* test). *Significantly different from the fine emulsion, $P < 0.05$ (Bonferroni *t* test).

Gastric and duodenal lipolysis

As compared with the initial emulsions intubated, the relative amount of triacylglycerols present in the gastric aspirates decreased by 12.7–35.6% with the fine emulsion and by 4.2–14.8% with the coarse emulsion during 4 h of digestion (**Figure 4**). These data indicate that the extent of gastric lipolysis was significantly higher at each sampling time when the fine emulsion was given than when the coarse emulsion was given. In the duodenal contents (Figure 4), the decrease in the relative amount of triacylglycerol was significantly more extensive ($P < 0.05$) than in the stomach and was significantly higher with the fine emulsion after 1, 3, and 4 h of digestion (56.8–73.3%) than with the coarse emulsion (34.2–46.3%).

Micellar and vesicular structures

In the micelles + vesicles fraction, the major lipid species were fatty acids, lysophospholipids, and phospholipids. Fatty acid concentrations were significantly different, ie, 1.12 ± 0.09 and 0.53 ± 0.11 mmol/L duodenal content, after intubation of the fine and coarse emulsions, respectively. No significant differences were observed between emulsions concerning concentrations of phospholipids (0.17 ± 0.04 compared with 0.12 ± 0.04 mmol/L duodenal content) and lysophospholipids (2.74 ± 0.74 compared with 1.84 ± 0.84 mmol/L duodenal content).

Postprandial responses

The serum triacylglycerol responses had bell-shaped curves from 0.5 to 7 h after intubation of emulsions, as shown in **Figure 5**. The triacylglycerol concentration increased significantly over baseline from 1–2 to 4–5 h in both cases but peaked at significantly different times, ie, at 3 h, 56 ± 0.41 min with the fine emulsion and at 2 h, 50 ± 0.24 min with the coarse emulsion. The maximal increases averaged 0.890 ± 0.162 and 0.889 ± 0.289 mmol/L over basal values with the coarse and the fine emulsions, respectively. After 7 h, plasma triacylglycerol concentrations returned to values close to fasting after both emulsions. The 0–7-h AUCs for serum triacylglycerol showed overall comparable values after ingestion of the 2 emulsions (Figure 5 insert).

The occurrence of chylomicrons (chylomicrons and large chylomicron remnants) in the serum is shown in Figure 5. As for triacylglycerols, chylomicron triacylglycerols increased significantly over baseline after 1 or 2 h and peaked 3 (0.503 ± 0.054 mmol/L) or 4 (0.510 ± 0.194 mmol/L) h after administration of the coarse and fine emulsions, respectively. Nevertheless, the amplitude of the responses was comparable for the 2 emulsions as shown by the 0–7-h AUCs.

The chylomicron particle diameters were not significantly different between emulsions and ranged between 122.5 and 138.5 nm with the fine emulsion and between 120.9 and 138.5 nm with the coarse emulsion (data not shown). Chylomicron retinyl palmitate followed a bell-shaped curve with a maximal rise after 3 h with the coarse emulsion and after 4 h with the fine emulsion, as did triacylglycerols (data not shown). The 0–7-h AUCs were comparable with the 2 emulsions (2.29 ± 0.82 and 2.25 ± 0.50 nmol · h/L with the fine and coarse emulsions, respectively).

DISCUSSION

It was observed previously that lipase activities are sensitive to the size of fat droplets in vitro (20, 22, 23). A first in vivo study conducted in rats given emulsions with droplets of different sizes (0.8–22 μm) suggested that the initial size of the emulsion particles governs the digestive and metabolic behavior of dietary lipids (24). Further studies were necessary to show the full effect of fat droplet size on fat digestion and absorption in humans. Thus, the purpose of this study was to determine the physicochemical properties of 2 emulsions with particles of different sizes in the digestive tract of healthy humans and the consequences on fat digestion and assimilation of these differences. As discussed below, relevant aspects were the differential evolution in droplet size of the 2 emulsions, the relation between fat droplet size and the extent of lipolysis, and the resultant postprandial appearance of triacylglycerols and fat-soluble vitamins in the plasma.

A 10-μm coarse emulsion kept a constant particle size throughout digestion, presumably because the size of the droplets is close to that resulting from the new steady state reached in the stomach that ranges from 17 to 37 μm in rats (24) and humans (14). On the contrary, a fine emulsion (median diameter <1 μm) underwent a marked alteration in the gastric environment, leading to a 4- to 9-fold increase in median droplet size, without any measurable breakage of the emulsion, as occurs

TABLE 1
Droplet size distribution pattern in initial emulsions and gastric and duodenal contents during digestion[1]

Emulsion and time	Fine emulsion				Coarse emulsion			
	50–200 μm	10–50 μm	1–10 μm	<1 μm	50–200 μm	10–50 μm	1–10 μm	<1 μm
	%				%			
Initial emulsion	0.3 ± 0.3	1.4 ± 0.9[a]	18.9 ± 4.9[a]	79.5 ± 5.4[c]	0.03 ± 0.03	42.4 ± 5.3	51.5 ± 5.1	3.6 ± 0.6
Gastric contents								
0.5 h	3.7 ± 2.7	29.6 ± 9.2[b]	37.7 ± 6.0[b]	29.0 ± 5.2[a,b]	4.8 ± 2.8	43.4 ± 7.9	46.0 ± 8.0	5.8 ± 1.1
1 h	3.3 ± 2.2	14.2 ± 5.3[a,b]	45.7 ± 5.7[b]	36.8 ± 7.3[b]	2.4 ± 2.1	42.6 ± 4.4	49.8 ± 5.3	5.2 ± 0.7
2 h	0.1 ± 0.1	28.9 ± 6.8[b]	60.3 ± 6.1[b,c]	10.8 ± 3.4[a]	7.6 ± 5.0	48.2 ± 4.8	38.0 ± 4.5	6.1 ± 2.0
3 h	2.1 ± 1.4	26.2 ± 5.5[b]	63.2 ± 4.5[c]	8.5 ± 2.0[a]	1.4 ± 1.0	39.1 ± 5.1	52.8 ± 5.6	6.8 ± 1.5
4 h	2.6 ± 2.4	14.2 ± 7.3[a,b]	68.3 ± 5.7[c]	14.3 ± 4.0[a]	0.0 ± 0.0	34.1 ± 17.1	58.0 ± 15.5	7.9 ± 2.3
Duodenal contents								
0.5 h	0.1 ± 0.1	26.6 ± 22.1[b]	63.4 ± 19.2[c]	10.0 ± 2.9[a]	0.6 ± 0.6	35.3 ± 9.3	56.6 ± 9.5	7.5 ± 0.5
1 h	1.0 ± 0.6	9.7 ± 4.7[a,c]	50.3 ± 10.6[b]	39.1 ± 12.0[b]	1.8 ± 1.7	34.6 ± 7.4	52.7 ± 8.3	11.0 ± 2.3
2 h	6.7 ± 4.7	26.5 ± 2.0[b,c]	55.8 ± 5.8[b,c]	11.0 ± 2.3[a]	3.3 ± 2.1	43.8 ± 8.5	45.3 ± 6.8	7.5 ± 2.9
3 h	0.1 ± 0.1	6.4 ± 3.7[a]	80.3 ± 4.3[d]	13.4 ± 1.6[a]	5.8 ± 3.4	28.3 ± 11.1	53.9 ± 3.6	11.9 ± 4.1
4 h	2.2 ± 2.2	5.8 ± 5.7[a]	70.6 ± 3.0[c]	21.4 ± 5.0[a,b]	0.1 ± 0.1	43.5 ± 3.4	48.3 ± 3.8	8.2 ± 0.6

[1] $\bar{x}$ ± SE; n = 8 determinations. Different letter superscripts indicate significant differences for a given droplet class and emulsion between initial values and values obtained after gastric and duodenal digestion, $P < 0.05$ (two-way analysis of variance for repeated measures with contrast analysis).

in the digestive tract of rats (24). Several factors could lead to this alteration: *1*) the high-to-moderate acidity of stomach contents during digestion might destabilize the emulsified droplets and induce rearrangements leading to a new steady state situation (35), and *2*) the lack of conditions required to improve emulsification or to reverse or prevent destabilization of the emulsion such as sufficient mechanical energy supply or the availability of emulsifiers.

In fact, alteration of the fine emulsion was not likely due to gastric pH, because an increase in fat droplet size occurred as early as 30 min after intubation when intragastric pH was ≈6.0. No correlation was found between droplet size of the emulsions

TABLE 2
Concentrations of lipid species in gastric and duodenal contents after emulsion intubation[1]

Location and time	Fatty acids	Monoacylglycerols	Diacylglycerols	Triacylglycerols	Phospholipids
			mmol/L		
Gastric contents					
Fine emulsion					
0.5 h	7.5 ± 1.9[2]	2.8 ± 1.2	7.1 ± 1.4	68.1 ± 4.7	2.2 ± 0.3
1 h	10.5 ± 2.4[2]	5.4 ± 2.5	11.0 ± 3.0	71.6 ± 6.4	2.9 ± 0.5
2 h	17.4 ± 2.7[2]	5.9 ± 1.8	12.9 ± 2.7	82.8 ± 4.2	1.5 ± 0.2[2]
3 h	29.5 ± 2.6[2]	9.8 ± 0.9[2]	20.2 ± 2.9[2]	59.1 ± 8.4	3.1 ± 0.7[2]
4 h	17.7 ± 4.8[2]	6.2 ± 2.0[2]	12.2 ± 2.7[2]	23.9 ± 8.4	2.0 ± 1.0
Coarse emulsion					
0.5 h	2.2 ± 1.1	1.6 ± 1.0	5.1 ± 1.0	94.8 ± 15.0	3.2 ± 0.5
1 h	3.5 ± 0.9	1.8 ± 0.8	6.7 ± 1.3	86.3 ± 23.9	3.2 ± 0.6
2 h	12.8 ± 2.5	4.1 ± 1.0	12.7 ± 2.4	77.0 ± 10.7	2.5 ± 0.3
3 h	6.1 ± 1.4	1.9 ± 0.7	5.5 ± 0.8	45.5 ± 9.7	1.4 ± 0.2
4 h	4.7 ± 2.8	0.7 ± 0.6	3.5 ± 2.2	12.3 ± 4.7	0.8 ± 0.2
Duodenal contents					
Fine emulsion					
0.5 h	29.8 ± 5.6	16.5 ± 3.0	9.2 ± 3.3	14.8 ± 7.6	4.8 ± 1.1
1 h	24.1 ± 5.9	18.0 ± 3.1[2]	5.7 ± 1.7	8.2 ± 2.2[2]	3.2 ± 1.0
2 h	23.1 ± 5.3	9.8 ± 3.1	7.6 ± 1.8	17.0 ± 4.9	2.7 ± 0.6
3 h	15.1 ± 3.8	14.9 ± 4.7	5.3 ± 1.8	16.2 ± 7.7	3.3 ± 0.8
4 h	18.0 ± 5.0[2]	7.6 ± 2.8	7.4 ± 1.4[2]	10.0 ± 3.8	2.0 ± 0.4
Coarse emulsion					
0.5 h	22.2 ± 5.6	10.5 ± 3.0	9.0 ± 3.1	26.8 ± 4.7	3.9 ± 0.9
1 h	23.1 ± 5.0	9.4 ± 2.3	10.7 ± 2.2	30.4 ± 4.9	3.5 ± 0.9
2 h	18.1 ± 3.3	8.5 ± 1.5	9.7 ± 2.5	21.4 ± 4.6	2.5 ± 0.5
3 h	15.1 ± 4.2	5.2 ± 1.9	4.0 ± 1.3	16.9 ± 4.8	2.2 ± 0.4
4 h	7.0 ± 2.1	2.2 ± 1.0	1.9 ± 1.0	8.4 ± 4.3	1.5 ± 0.2

[1] $\bar{x}$ ± SE; n = 8 determinations.
[2] Significantly different from the coarse emulsion, $P < 0.05$ (two-way analysis of variance for repeated measures with contrast analysis).

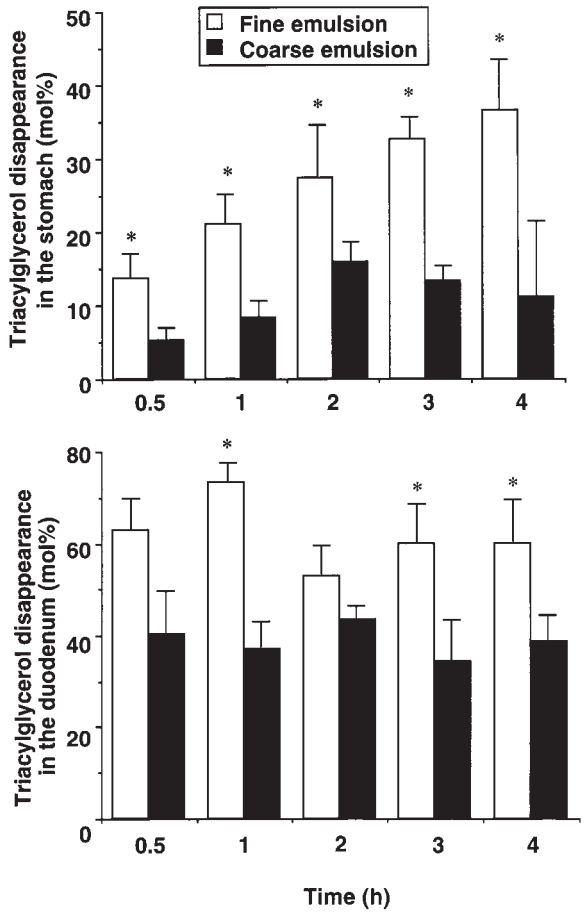

FIGURE 4. Mean (±SE) extent of intragastric and intraduodenal triacylglycerol lipolysis expressed as a percentage of initial triacylglycerols hydrolyzed as a function of time for the fine and coarse emulsions; n = 8 determinations. Details of the calculation are given in Methods. *Significantly different from the coarse emulsion, $P < 0.05$ (two-way analysis of variance for repeated measures with contrast analysis).

and gastric pH values (r^2 = 0.275, P = 0.03). Availability of emulsifying agents was not expected to be a limiting factor because phospholipids are initially present in excess amounts in the emulsions and because lipolysis generates new amphipaths, such as fatty acids, at the interface surface (1, 8). We suggest that a partial coalescence or formation of droplet aggregates could have resulted from production and accumulation of long-chain fatty acids at the droplet surface in the stomach contents (1, 8). Despite the observed change in droplet size, the fine emulsion resulted in smaller fat droplet sizes in the stomach (from 1.7- to 3.5-fold) than did the coarse emulsion.

Because triacylglycerols are the main components (97%) of the emulsified droplets and are absorbed in the upper half of the small intestine, and because some bile lipids are considered to be emulsifying agents (1), we expected a marked shift in the pattern of the droplet size distribution toward smaller droplets in the duodenum, as suggested earlier in rat studies by Senior (36) and Frazer et al (37). This was not observed in our study with either emulsion because the droplet-distribution patterns obtained in the duodenum were generally no different from those observed in the stomach, as was shown previously in rats (24) and in healthy humans (14, 15) given a very coarse emulsion. The method used to measure fat droplet size allowed us to measure emulsion fat droplets without interference from multi- or unilamellar vesicles (13–25 nm) or micelles and mixed micelles (3–6 nm) coexisting with fat droplets in the duodenal contents (1, 8).

Several explanations for our results can be proposed. First, emulsifiers from bile do not significantly contribute to further emulsification of dietary fat. Indeed, bile lipids distribute at the surface of fat droplets (38, 39) creating a crystalline monolayer preventing coalescence of globules and thus have a stabilizing rather than an emulsifying effect (38). Furthermore, phospholipids are massively distributed in the aqueous phase to form micellar structures when the bile salt concentration is >4 mmol/L (1, 39, 40), which was the case in our study. We showed previously in vitro that mixing emulsions with a micellar bile salt solution did not change their droplet size (22). Second, there is not enough mechanical energy in the duodenum to allow further fat emulsification (41). In the study by Frazer et al (37), fat emulsification was observed in the duodenum probably because oil being infused directly into the duodenum had been mixed with bile lipids. In fact, in physiologic conditions, fat emulsification occurs in the stomach rather than in the duodenum (14, 15). The last possible explanation is that very small droplets are more rapidly hydrolyzed and absorbed and thus do not accumulate in the duodenum (1, 8). In fact, if an emulsification process does not take place in the duodenum, other essential physicochemical events occur that involve absorption of lipid nutrients (4, 6–8). For example, in the duodenum, several kinds of dispersed structures are formed and coexist, such as microemulsion particles and lamellar liquid crystals remaining at the oil-subphase interface, unilamellar vesicles, and micelles in the aqueous phase (6–8, 15). In our study, vesicle and micelle compositions were significantly different depending on the emulsion intubated. Two times more fatty acids were incorporated into these structures after digestion of the fine emulsion than after the coarse one.

Gastric and duodenal variables such as pH, gastric and pancreatic lipase-colipase–dependent concentrations, and bile salt concentrations were not affected significantly by the type of emulsion intubated and the values obtained confirm data from previous studies (1, 2, 13–15, 42, 43). At the same time, a marked difference was observed in the extent of lipolysis of the emulsions. Intragastric lipolysis was 1.7–3.3 times higher with the fine emulsion than with the coarse one, which suggests a direct relation with fat droplet size. This phenomenon can be explained by the fact that a lipid emulsion composed of small-sized fat globules offers, for a given lipid mass, a larger lipid surface area than does an emulsion with bigger droplets. The measured total surface area of the fine emulsion ranged from 138 to 672 m^2/L gastric content whereas it ranged from 30 to 126 m^2/L gastric content with the coarse emulsion, offering ≈5 times more interface area accessible to lipase. In the digestive tract, lipases are present in excess amount and thus, the larger the available lipid surface the higher the number of lipase molecules able to bind at the oil-water interface. For a given period of time, higher amounts of fatty acids should thus be generated; this was the case in this study with 7.5–29.5 mmol fatty acids/L found in the stomach contents with the fine emulsion compared with 2.2–12.8 mmol/L with the coarse emulsion.

Another explanation is that gastric lipase activity is inhibited by the long-chain fatty acids generated (14, 15, 21, 23) by a surface-dependent phenomenon, as shown in vitro (23). With the 10-μm emulsion, gastric lipolysis was maximal until fatty acid concentration reached 121 μmol/m^2 emulsion surface, ie, after 2 h digestion. This concentration is close to that obtained after 4 h of digestion with the fine emulsion (128 μmol/m^2). Thus, in the human stomach, the larger the emulsion surface area, the higher the rate of lipolysis before surface area concentrations of fatty acids are generated that inhibit lipolysis. Because fatty acid and monoacylglycerol absorption is assumed to begin in the duodenum, the lipolysis pattern of both emulsions is more difficult to analyze. Nevertheless, the fact that triacylglycerol concentrations in duodenal contents were always higher with the coarse emulsion suggests that the fine emulsion was more hydrolyzed in the duodenum also. The delay in lipolysis of the coarse emulsion observed in the stomach seems to be compensated for because the ratio of lipolysis of the fine to the coarse emulsion decreased from 1.7–3.3 in the stomach to 1.04–1.99 in the duodenum, probably because of the high activity of pancreatic lipase.

Given that this study was conducted in healthy humans and that fat absorption is very efficient in such subjects (1), we did not foresee marked differences in overall fat absorption between the 2 emulsions. In fact, we found postprandial plasma triacylglycerol responses with similar amplitudes and that are in good agreement with responses that have been already observed with test meals with comparable amounts of fat (32, 44). However, the kinetics of changes in postprandial plasma lipid variables were different, showing a measurable and significant delay in the appearence of the triacylglycerol peak in the case of the fine emulsion, as was shown in a previous study in rats (24). This delay was also found for changes in retinyl palmitate concentration. This apparently conflicting observation could be explained by slightly different rates of gastric emptying of the 2 emulsions—that of the fine

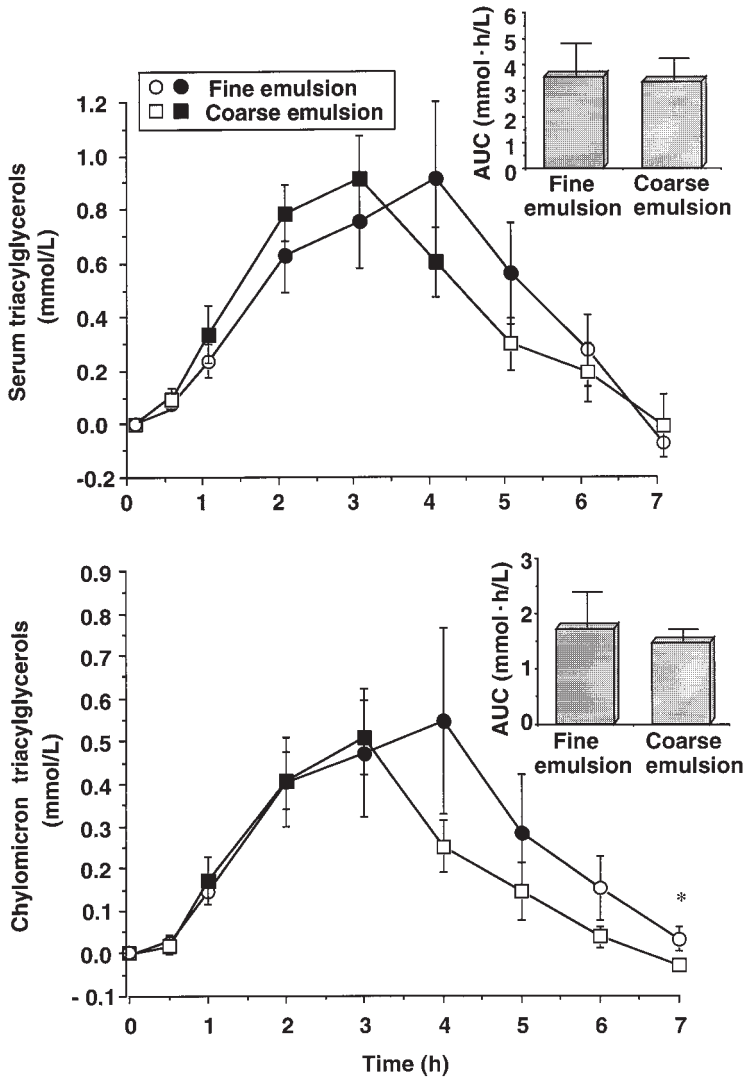

FIGURE 5. Mean (±SE) change in serum and chylomicron triacylglycerols from 0 to 7 h after intubation of the fine and coarse emulsion; $n = 8$ determinations. Filled symbols indicate a significant difference from the fasting value, $P < 0.05$ (two-way analysis of variance for repeated measures with contrast analysis). *Significantly different from the coarse emulsion, $P < 0.05$ (two-way analysis of variance for repeated measures with contrast analysis). Inserts: amplitude of the response is given as the 0–7-h area under the curve (AUC).

emulsion being slower. This agrees with the notion that a slow gastric-emptying rate can be a limiting factor in fat assimilation (45). Nevertheless, we observed that the gastric-emptying rate was significantly lower with the fine emulsion but only after >70% of the fat had left the stomach. Another possibility is a different absorption rate or handling of lipid nutrients by the small intestinal mucosa, depending on the different physicochemical behaviors of the 2 emulsions. Several of the steps involved in pre- and postabsorptive processes might be involved, such as the formation of mixed micelles or liquid-crystalline vesicles of which the specific role in the uptake of fatty acids and monoacylglycerols is unresolved, the diffusion of lipid nutrients across the unstirred water layer through a carrier-dependent or passive process, and the formation and secretion of intestinal chylomicrons, which depend on the luminal supply of phospholipids (46). These processes, and their regulation, are not well understood (46). For instance, triacylglycerols have been reported to peak earlier postprandially, ie, at 2 h, when subjects received a solid complex meal than when they received the meal in liquid form (32, 44). Although the mechanisms involved need further investigation, it appears that ingestion by healthy human subjects of emulsions with particles of different sizes leads to different time courses of appearance of lipid in the plasma postprandially.

This study, performed in healthy humans, was dedicated to increasing our knowledge in the field of nutrition and of the gastrointestinal tract, and could improve enteral nutrition therapy. Indeed, >100 enteral formulas are currently available, and new emphasis is now being placed on disease-specific formulas (47, 48). These formulas are for those patients with damaged fat digestion and absorption processes (eg, extensive stomach and small bowel resection, severe exocrine pancreas insufficiency such as chronic pancreatitis, cystic fibrosis, and premature infants), which lead to fat malabsorption, reduced energy and fat-soluble vitamin supplies, steatorrhea, and severe health complications. The present study points out that the physicochemical properties of lipid emulsions, such as fat droplet size, should be taken into account in the formulation of enteral nutrition, given their consequences on fat digestion. Further in vivo studies are necessary to test the hypothesis that the use of fine-particle emulsions in the enteral nutrition of patients with pancreatic insufficiency improves their intragastric fat digestion and thus could facilitate further absorption of lipid nutrients.

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