

Processing of vitamin A and E in the human gastrointestinal tract

PATRICK BOREL,¹ BERENGERE PASQUIER,² MARTINE ARMAND,² VIVIANE TYSSANDIER,¹
PASCAL GROLIER,¹ MARIE-CECILE ALEXANDRE-GOUABAU,¹ MARC ANDRE,²
MICHELE SENFT,² JACQUES PEYROT,³ VERONIQUE JAUSSAN,⁴ DENIS LAIRON,²
AND VERONIQUE AZAIS-BRAESCO¹

¹Unité des Maladies Métaboliques et Micronutriments, Institut National de la Recherche Agronomique, Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle; ²Unité 476, Institut National de la Santé et de la Recherche Médicale, 13009 Marseille; ³Service d'Hepato-Gastro-Enterologie, Centre Hospitalier Universitaire Nord, 13015 Marseille; and ⁴Clintec Technologies, Velizy, France

Received 4 May 2000; accepted in final form 4 August 2000

Borel, Patrick, Berengere Pasquier, Martine Armand, Viviane Tyssandier, Pascal Grolier, Marie-Cecile Alexandre-Gouabau, Marc Andre, Michele Senft, Jacques Peyrot, Veronique Jaussan, Denis Lairon, and Veronique Azais-Braesco. Processing of vitamin A and E in the human gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* 280: G95–G103, 2001.—We aimed to provide basic data on the processing of vitamin A and E in the human gastrointestinal tract and to assess whether the size of emulsion fat globules affects the bioavailability of these vitamins. Eight healthy men received intragastrically two lipid formulas differing in their fat-globule median diameter (0.7 vs. 10.1 μm). Formulas provided 28 mg vitamin A as retinyl palmitate and 440 mg vitamin E as all-*rac* α -tocopherol. Vitamins were measured in gastric and duodenal aspirates, as well as in chylomicrons, during the postprandial period. The gastric emptying rate of lipids and vitamin A and E was similar. The free retinol/total vitamin A ratio was not significantly modified in the stomach, whereas it was dramatically increased in the duodenum. The proportion of ingested lipid and vitamins was very similar in the duodenal content. The chylomicron response of lipids and vitamins was not significantly different between the two emulsions. Our main conclusions are as follows: 1) there is no significant metabolism of vitamin A and E in the human stomach, 2) the enzyme(s) present in the duodenal lumen is significantly involved in the hydrolysis of retinyl esters, and 3) the size of emulsion fat globules has no major effect on the overall absorption of vitamin A and E.

retinol; α -tocopherol; bioavailability; emulsification; digestion

VITAMIN A IS ESSENTIAL for the growth and general health of higher animals. It is needed for vision, reproduction, and development and maintenance of differentiated tissues. It is present in the diet mainly as esterified retinol (preformed vitamin A). Vitamin E is the main fat-soluble micronutrient involved in the antioxidant defenses in humans. It is present in the diet mainly as

free α -tocopherol. Surprisingly, the processing of these essential fat-soluble micronutrients in the human gastrointestinal tract is poorly documented. It has been shown that the absorption efficiency of these fat-soluble vitamins is very different; absorption is apparently very efficient, i.e., $\sim 99\%$, for preformed vitamin A (34) and much less efficient for vitamin E, i.e., between 15 and 65% (13, 36), but the reason for such a difference is not well understood.

The digestion of vitamin A and E consists of several sequential steps including physicochemical and enzymatic events (24–26). It begins in the stomach, where foods are subjected to acidity and gastric enzymes. In humans, it is assumed that this organ does not play a role in the hydrolysis of vitamin A esters (21), but data on this topic are very old and have been obtained only in animal models (18). Since gastric lipase can hydrolyze up to 30% of ingested triacylglycerols (1, 2, 12, 20), it cannot be excluded that this enzyme plays a role in the digestion of vitamin A by hydrolyzing a fraction of retinyl esters. The role of the stomach in vitamin E digestion is suspected to be limited as this vitamin is naturally present in its free form, but no study has assessed whether there is any degradation of this essential antioxidant in the stomach.

Digestion continues in the duodenum where pancreatic and biliary secretions occur. Previous works suggest that retinyl esters are hydrolyzed by pancreatic enzymes secreted in the intestinal lumen (15, 37, 38) and/or by retinyl ester hydrolases associated with intestinal brush border membrane (32, 33). However, the relative importance of the pancreatic and the brush border enzymes in the digestion of retinyl esters remains to be determined (21).

During digestion it is assumed that vitamin A and E are transferred from the emulsion fat globules to water-soluble structures made of phospholipids and bile salts forming multi- or unilamellar vesicles and mixed

Address for reprint requests and other correspondence: P. Borel, INRA, UMMM-Equipe vitamines, Centre INRA de Clermont-Fd/Theix, 63122 Saint-Genès-Champanelle, France (E-mail: Patrick.Borel@clermont.inra.fr).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

micelles (11, 22, 23, 30). However, it is not known whether there is a different efficiency of transfer between the vitamins.

Vitamin A and E are solubilized in the fat phase of the meal. This phase is emulsified in the gastrointestinal tract so that lipases can hydrolyze triacylglycerols. The characteristic feature of lipases is their specificity to act at the surface of fat globules (5). The higher the surface available for lipase is, the higher the enzyme velocity is (3). For a constant lipid volume, the smaller the fat globules are, the higher the oil/water interface is. Thus lipid emulsification has to be considered as a fundamental step in fat digestion (1, 2, 11). In vitro experiments have shown that the extent of lipid emulsification affects the activity of gastrointestinal lipases (3, 7). Moreover, a study conducted in the rat has shown that emulsions with different droplet sizes behave differently in the digestive tract and are digested differently (8). Since fat-soluble vitamins are trapped in the emulsion fat globules and have to be released from these droplets to be absorbed, it is possible that lipid emulsification can affect their bioavailability. The answer to this question is of major importance for the preparation of enteral emulsions.

The aims of this study are to enhance our knowledge on the processing of vitamin A and E in the human gastrointestinal tract and to test the hypothesis that differences in the size of emulsion fat globules could affect their bioavailability.

METHODS

Subjects. Eight healthy male volunteers (24.0 ± 1.4 yr, 174 ± 3 cm and 66 ± 5 kg) were enrolled in the study after giving written, informed consent. The study protocol was approved by the local Medical Ethics Committee (CCPPRB, Marseille, France). No volunteer had history of gastrointestinal or lipid metabolic disorders, as checked by medical history and fasting blood lipid measures [triacylglycerols (0.92 ± 0.09 mmol/l) and total cholesterol (4.19 ± 0.29 mmol/l) were in the normal range]. No volunteer was taking drugs that might affect gastrointestinal function or lipid metabolism.

A 3-day diet record was used to determine the dietary habits of the subjects. Diets were analyzed using the GENI software package (Micro 6, Nancy, France). The subjects consumed a typical Western diet with $10,810 \pm 707$ kJ/day, $16.1 \pm 1.1\%$ of energy as proteins, $43.6 \pm 2.7\%$ as fat, and $40.3 \pm 3.4\%$ as carbohydrates. Vitamin A [653 ± 146 retinol equivalents (RE)/day] and vitamin E (8.9 ± 1.6 mg/day) intakes were in keeping both with the population reference intakes (PRI) of the European Community and of the recommended dietary allowances (RDAs) in the United States. Furthermore, all subjects showed fasting plasma concentrations of retinol and α -tocopherol in the normal range. The subjects were asked to have a light dinner the day before each experiment.

Formulas. Each subject was given two formulas (Clintec Technologies, Velizy, France) differing only in the size of the fat globules, 1 wk apart and in a random order. The formula composition is given in Table 1. One formula contained small-sized fat globules with a median droplet diameter of 0.7 ± 0.2 μ m (fine emulsion), and the other contained fat globules sized 10.1 ± 0.9 μ m (coarse emulsion). The median

Table 1. *Composition of the formulas*

Component	Amount
Fish oil	24 g
Olive oil	24 g
Soybean lecithin	2.3 g
Glycerol stearate	3.5 g
Milk and lactoserum proteins	33.8 g
Carbohydrates	50 g
Vitamin A	28.4 mg (15,500 RE)
Vitamin E	440 mg (326 TE)
PEG-4000	0.25 g
Water	500 ml

The fatty acid blend of the triacylglycerol mixture (fish oil and olive oil) 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 -linolenic acid (0.8%), arachidic acid (1.2%), arachidonic acid (0.5%), eicosapentaenoic acid (8.2%), docosapentaenoic acid (0.9%), and docosahexaenoic acid (3.3%). There were only trace amounts (<0.1 mg/g triacylglycerol) of free fatty acids, mono-glycerides, and diglycerides in the emulsions. For vitamin A, composition was 99.65 mole % as all-*trans* retinyl palmitate and 0.35 mole % as all-*trans* retinol, as measured by HPLC; RE, retinol equivalents. For vitamin E, composition was all-*rac* α -tocopherol; TE (α -tocopherol equivalents). Polyethylene glycol (PEG-4000; Merck, Darmstadt, Germany) was used to measure the gastric volumes (see METHODS). Water was added to bring the volume to 500 ml.

diameter of the fat globules was determined as previously reported (1, 2) by using a particle-size analyzer (model Capa-700; Horiba, Kyoto, Japan).

The formulas provided 787 kcal (3,290 kJ), 57% as fat, 26% as carbohydrates, and 17% as proteins. The amount of triacylglycerols given (48 g/meal) is the usual fat intake for most people who live in industrialized countries. The amounts of vitamin A (28 mg/bolus, 99.7% as retinyl palmitate and 0.3% as free retinol, 15,500 RE) and vitamin E (440 mg as all-*rac* α -tocopherol, i.e., 326 α -tocopherol equivalents) were high compared with the dietary intakes (around 35 times the RDAs), but they were chosen to follow accurately the processing of these vitamins in the gastrointestinal tract and in the chylomicron fraction.

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

Study design. Each procedure started at 9:00 AM after subjects had fasted overnight. Each subject was intubated with a single-lumen nasogastric tube (outer diameter 0.50 cm; Mallinckrodt Laboratories, 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. The position of the tubes was checked under X-ray before emulsion intake and 2 h thereafter. After the tubes had been placed, the volunteer assumed a sitting position that was maintained until the end of the study (27). An antecubital vein was catheterized with an intravenous cannula (Jelco-Critikon, Chatenay-Malabry, France).

Fasting gastric juice (37 ± 7 ml) and duodenal fluid (6 ± 1 ml) were removed by manual aspiration just before ingestion of the formula to determine fasting values. The 500-ml formula was intubated intragastrically with a 60-ml syringe over a 10-min period. A 2-ml sample of the initial formula

was retained for measuring lipid droplet size and for chemical analysis. Large samples (100–200 ml) of the stomach contents were aspirated 0.5 h after feeding and at 1, 2, 3, and 4 h by gentle aspiration with a 60-ml syringe to obtain representative samples (1, 2). Then, a 20-ml aliquot was taken from each gastric sample for analytical determinations. The respective remaining contents were promptly re-injected into the subject's stomach via the tubes. Duodenal fluid was aspirated 0.5 h after formula infusion and every hour for 4 h. Note that the duodenal sample that could be aspirated was remarkably constant during digestion (~7.5 ml).

To prevent any bacterial growth, 5 μ l 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 sample (1, 22).

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 analysis. A protease inhibitor from soybeans (Sigma Chemical, La Verpilliere, France) was added (0.5 g/l aspirate) to a 1- to 5-ml duodenal sample immediately after collections. The samples were immediately placed on ice, rapidly frozen, and stored at -20°C for determining pancreatic lipase activity.

Another fraction of the gastric and duodenal samples was placed into glass vials containing a methanolic solution of lipase inhibitors (5% vol/vol) (1, 22) to measure lipids.

The remaining fraction of the samples was placed into opaque glass vials and stored at -20°C until vitamin measurements.

A baseline fasting blood sample (0 h) was collected. Blood samples (10 ml) were drawn every hour for 7 h after formula intake. Serum was separated from whole blood by centrifugation (910 g, 15 min). Chylomicron plus large chylomicron remnants ($S_f > 1,000$) were isolated by ultracentrifugation as previously described (14).

Estimating the gastric content volume. We have used the double-sampling marker-dilution method of George (19), with PEG-4000 as the nonabsorbable marker, to estimate the gastric content volume. The meals used were fully homogenized liquid test meals that have been shown (28) to lead to simultaneous emptying of the aqueous and lipid phases. Thus, under these conditions PEG-4000 is a marker of the aqueous phase and can be used to monitor the emptying of the total meal components. The method was as follows: at each collection time a large sample (100–200 ml) of stomach content was aspirated. Then, after taking an aliquot (2 ml) for the first PEG measurement, a new dose of PEG marker (1 ml containing 150 mg PEG) was added to the remaining stomach sample. After reinjection of the sample in the stomach, the stomach content was gently homogenized with a syringe connected to the nasogastric tube. Then a 50-ml sample of the stomach content was aspirated, another 2-ml aliquot was collected for the second PEG measurement, and the remaining sample was reinjected in the stomach through the nasogastric tube. Volumes present in the stomach were calculated using the equation of George (19). PEG-4000 concentration in gastric contents (0.1–1 ml samples) was determined according to the method of Hyden (28).

The gastric content volumes were used to calculate the amount of lipids and vitamins in the stomach at each collection time. Fat and vitamin emptying rates were calculated from the initial quantity of fat and vitamins ingested (see Table 1) and the total amount of fat and vitamins that were present in the stomach at each collection time.

Determining the size of emulsion fat droplets. The median size of fat globules was determined in the initial formulas and in the gastric and duodenal aspirates as previously reported (1, 2) by using a particle size analyzer (Capa-700; Horiba, Kyoto, Japan).

Isolating and analyzing vesicular and micellar structures. Vesicles and micelles present in the duodenal content were separated from emulsion fat globules by ultracentrifugation (9, 35). Briefly, 500 μ l of duodenal content was put in a polyallomer tube containing 9.5 ml distilled water. The tube was centrifuged for 3 h at 170,000 g at 10°C in a Kontron swinging bucket rotor (31). Two milliliters of the aqueous infranatant were taken with a syringe. The pelleted fraction was obtained first by removing all the infranatant, then by washing the bottom of the tube with 500 μ l chloroform/methanol 2:1 (vol/vol). Vitamins and lipids recovered in the infranatant and in the pellet were extracted, separated, and measured as described above. Results of infranatant and pellet were pooled.

Lipid analysis. Lipids from initial emulsions and gastric and duodenal samples were extracted in chloroform/methanol (2:1 vol/vol) (17). The chloroformic lower phases were evaporated to dryness under nitrogen. Total lipids were determined gravimetrically.

Total bile salt concentration in duodenal contents was measured by an enzymatic method as previously described (1). Chylomicron triacylglycerols were determined by enzymatic procedures (10) with commercial kits (BioMerieux, Marcy l'Etoile, France).

Vitamin analysis. Vitamin A (retinyl palmitate and retinol) and vitamin E (α -tocopherol) were quantified by reverse-phase HPLC on a Kontron (Zurich, Switzerland) apparatus with ultraviolet detection at 325 nm and 292 nm for vitamin A and E, respectively. The column was a C_{18} Nucleosil (250 \times 4.6 mm, 5 μ m) and the mobile phase was 100% methanol. Retinyl laurate (4) and all-*rac* α -tocopherol acetate (Hoffmann-La Roche, Basel, Switzerland) were used as internal standards. Quantifications were carried out using Kontron MT2 software.

Lipase activity measurements. Gastric lipase activity was determined using a pH-stat titrator (Metrohm, Herisau, Switzerland) at pH 5.40 and 37°C and with tributyrin as the substrate (1, 2). Pancreatic lipase activity was measured in the same conditions except that this was done at pH 8.0 and with an excess of colipase (Boehringer, Mannheim, Germany) (2).

Statistical analysis. Two-factor repeated-measures ANOVA (meal by time) was used to analyze curves showing patterns of change in lipid and vitamin concentrations over time after ingestion of the two meals (39). Student's *t*-test for paired values was used to compare data obtained after ingestion of the two formulas. $P < 0.05$ was considered significant. The StatView 4.5 software (Abacus, Berkeley, CA) was used to perform these calculations.

RESULTS

Gastric parameters. The gastric pH was very low in basal conditions (1.9 ± 0.1). It markedly increased up to 6.1 at 30 min after formula intake and returned to values comparable to baseline after 3 h. The gastric lipase activity dropped markedly after 30 min (from 56 U/ml at baseline to 10 U/ml) and then returned to levels close to fasting value 2 h after feeding the formulas (data not shown). There was no effect of emulsion droplet size on these parameters.

Duodenal Parameters. The pH of the duodenal content did not significantly vary throughout the 4-h digestion period, ranging from 5.2 to 6.7 (data not shown). Pancreatic lipase activity increased ~15-fold over fasting values after infusion of formulas and remained relatively constant throughout the 4-h digestion (2,500–3,700 U/ml) (data not shown). Bile salt concentrations ranged from 2.5 to 4.7 mmol/l in fasting samples and significantly increased during the post-prandial period, reaching 6.4 to 10.7 mmol/l (data not shown). Again, there was no effect of emulsion droplet size on these parameters.

Changes in emulsion droplet size. Table 2 shows that the coarse emulsion kept a comparable median diameter throughout 3-h digestion in the stomach and in the duodenum. Conversely, the median diameter of the fine emulsion significantly increased in the stomach to reach around half the median diameter of the coarse emulsion after 30-min digestion. Then it kept comparable values (ranging from 2.75 ± 0.72 to 6.97 ± 2.51 μm). Note that, although there was an increase in the median diameter of the small emulsion, its median diameter remained significantly lower than that of the coarse emulsion in the stomach.

Changes in gastric content of lipids and vitamins during digestion. The total amount of lipids (triacylglycerols + triacylglycerol lipolysis products), vitamin A (retinyl palmitate + retinol), and vitamin E (α -tocopherol) in the stomach content at each collection time is presented in Fig. 1. The gastric content in lipids and vitamins exhibited a biphasic curve: in the first 30 min, most lipids (60–71%) and vitamins (76–77% for vitamin A and 75–87% for vitamin E) disappeared from the stomach, then the disappearance rate (percent emptied per hour) was slower and relatively constant. Note that there was no significant effect of emulsion droplet size on the stomach content of lipids and vitamins.

Table 2. Median diameter of emulsion fat globules before and during digestion in digestive tract

	Fine Emulsion	Coarse Emulsion
Intubated emulsion	0.7 \pm 0.2	10.1 \pm 0.9*
Stomach		
30 min	4.97 \pm 1.59	10.93 \pm 1.34*
1 h	2.75 \pm 0.72	9.55 \pm 1.87*
2 h	6.20 \pm 1.77†	15.40 \pm 3.20*†
3 h	5.37 \pm 0.78†	10.27 \pm 1.32*
Duodenum		
30 min	6.51 \pm 2.76†	8.17 \pm 1.45
1 h	2.14 \pm 0.67	9.77 \pm 1.86*
2 h	6.97 \pm 2.51†	11.20 \pm 2.20
3 h	3.27 \pm 0.23	8.96 \pm 2.75*

Values are means \pm SE of 8 determinations. Measurements were done with a particle size analyzer (Capa 700, Horiba). For more details see METHODS. *Significant difference ($P < 0.05$) between the fine and the coarse emulsion at a given time point (Bonferroni t -test; SAS/STAT software, Cary, NC). †Significant difference between initial emulsion and the corresponding emulsion found in the gastrointestinal tract at a given time point.

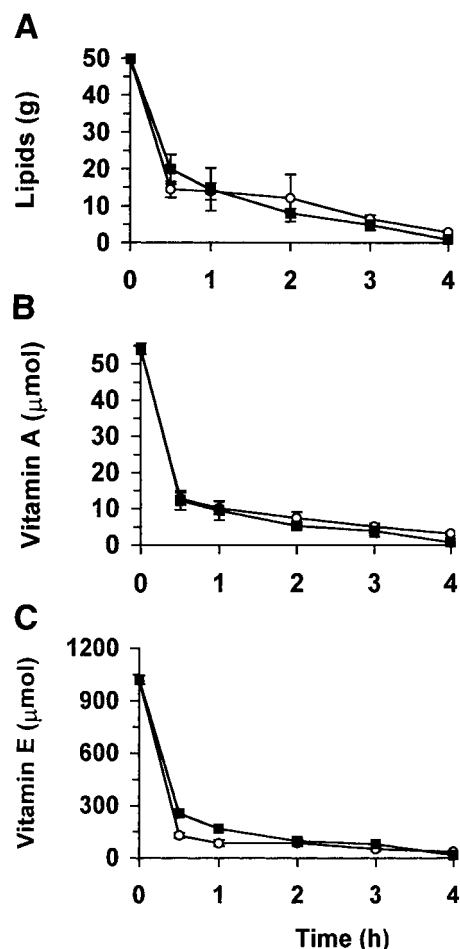


Fig. 1. Lipids (triacylglycerols + triacylglycerol lipolysis products) (A), vitamin A (retinyl palmitate + retinol) (B), and vitamin E (α -tocopherol) (C) content of the stomach as a function of digestive time. The total amount of lipids and vitamins was calculated by multiplying the concentration of each molecule by the gastric content volume. Results were obtained after ingestion of the fine emulsion (\circ) and after ingestion of the coarse emulsion (\blacksquare). Values are means \pm SE of 8 determinations. For lipids, 2-factor repeated-measures ANOVA showed no meal effect ($P = 0.828$), a time effect ($P = 0.0054$), and no meal \times time interaction ($P = 0.457$). For vitamin A, there was no meal effect ($P = 0.36$), a time effect ($P = 0.0001$), and no meal \times time interaction ($P = 0.96$). For vitamin E, there was no meal effect ($P = 0.101$), a time effect ($P = 0.0002$), and no meal \times time interaction ($P = 0.194$).

Concentration of lipids and vitamins in the duodenal content during digestion. The concentration of lipids and vitamins in the duodenal content as a function of time is shown in Fig. 2. There was a continuous decrease in the concentration of all the nutrients measured. As observed in the stomach, there was no significant effect of the emulsion droplet size on the concentration of lipids or vitamins in this part of the gastrointestinal tract.

Comparison between lipid and vitamin processing in the gastrointestinal tract. To compare the processing of triacylglycerols with that of vitamins in the gastrointestinal tract, we have represented the percentage of each ingested nutrient recovered in the stomach and the duodenum as a function of digestive time (Fig. 3).

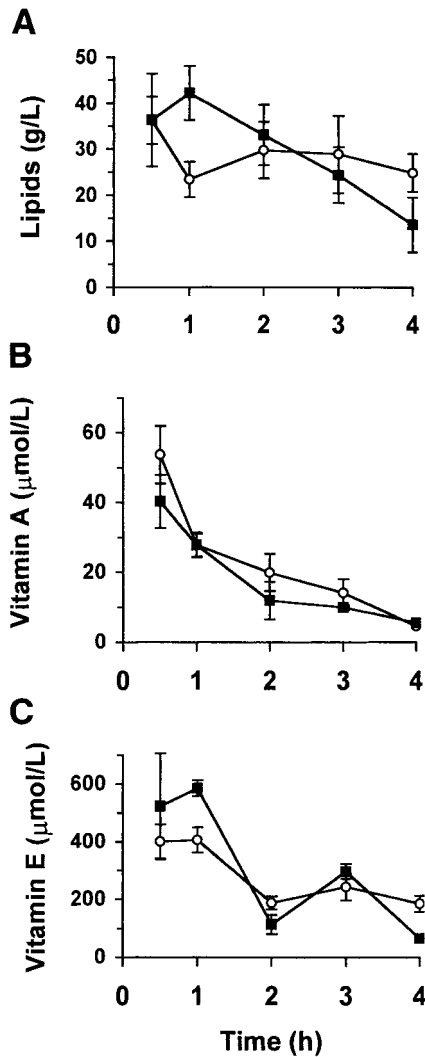


Fig. 2. Lipids (triacylglycerols + triacylglycerol lipolysis products) (A), vitamin A (retinyl palmitate + retinol) (B), and vitamin E (α -tocopherol) (C) concentrations in the duodenum as a function of digestive time. Results were obtained after ingestion of the fine emulsion (○) and after ingestion of the coarse emulsion (■). Values are means \pm SE of 8 determinations. For lipids, 2-factor repeated-measures ANOVA showed no meal effect ($P = 0.78$), a time effect ($P = 0.018$), and no meal \times time interaction ($P = 0.063$). For vitamin A, the statistical test showed no meal effect ($P = 0.199$), a time effect ($P = 0.0001$), and no meal \times time interaction ($P = 0.294$). For vitamin E, there was no meal effect ($P = 0.909$), a time effect ($P = 0.0001$), and no meal \times time interaction ($P = 0.32$).

Note that, in Fig. 3, we have pooled the data obtained with the two formulas, since there was no significant difference between the two emulsions with regard to the lipid and vitamin concentrations measured in the gastrointestinal tract. Figure 3A shows that, globally, the percentage of ingested lipids and vitamins remaining in the stomach at each digestive time was similar. Figure 3B shows that the percentage of the three ingested nutrients present in the duodenum was also very similar.

Hydrolysis of vitamin A in the stomach and duodenum. Figure 4 shows the proportion of vitamin A recovered as free retinol in the stomach (Fig. 4A) and the

duodenum (Fig. 4B) as a function of digestive time. Note that the proportion of free retinol in the test meals was only 0.3% and that there was no significant difference in the free retinol/total vitamin A ratio of the two emulsions. The ratio was significantly higher than the initial ratio after 30 min in the stomach. However, there was no further increase of the ratio in the stomach during digestion (there was no effect of time on the proportion of free retinol in the stomach) (Fig. 4A). The meal had an effect on the percentage of free retinol in the stomach. More precisely, this percentage was ~ 2.6 times higher in the case of the fine emulsion than in the case of the coarse one. Conversely, in the duodenum there was no effect of the meal but an effect of time on the retinol/retinyl palmitate ratio. Finally, note that the ratio was much higher in the duodenum than in the stomach and that it could increase up to 42% after 4-h digestion.

Distribution of vitamins between fat globules and micellar phase. Figure 5 shows the proportion of the vitamins recovered in the micelle + vesicle fraction as

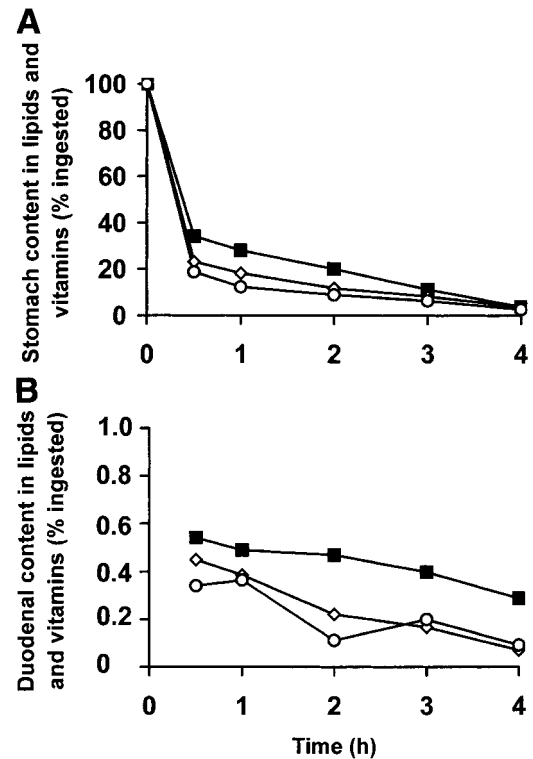


Fig. 3. Percentage of ingested triacylglycerols (■), vitamin A (◇), and vitamin E (○) recovered in the stomach (A) and the duodenum (B) at different digestive times. Percentages remaining in the stomach were calculated by multiplying the concentration of each molecule in the gastric content (mg/ml) by the volume of the gastric content (ml) and by dividing it by the amount of each molecule in the test meal (mg). Percentages in the duodenum were estimated by multiplying the concentration of each molecule in the duodenal content by 7.5 ml, which is the mean volume of the duodenal content that can be recovered (see METHODS), and by dividing it by the amount of each molecule in the test meal. For greater clarity and because there was no significant effect of the meal on the concentration of the nutrients in the stomach and the duodenum, the data obtained after the two test meals were pooled. Values therefore represent the means of 16 measurements.

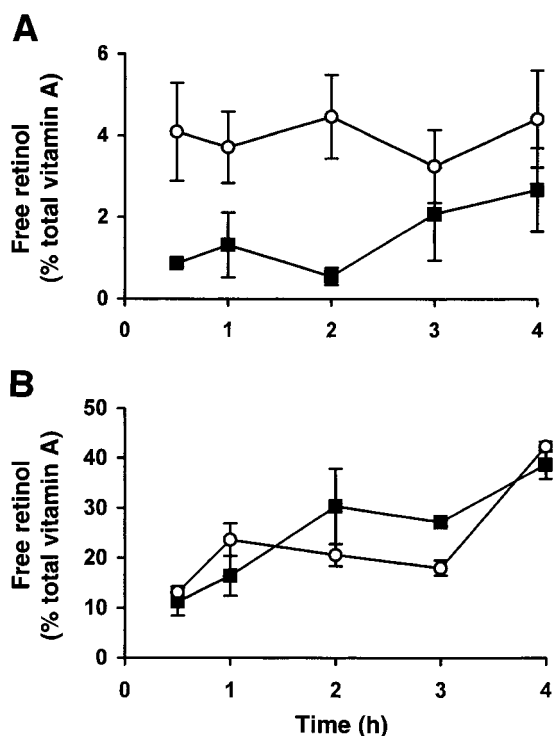


Fig. 4. Extent of intragastric (A) and intraduodenal (B) hydrolysis of vitamin A. Hydrolysis is expressed as the percentage of vitamin A recovered as free retinol. Note that in the test meals 99.7% of vitamin A was retinyl palmitate and 0.3% was free retinol. Results were obtained after ingestion of the fine emulsion (\circ) and after ingestion of the coarse emulsion (\blacksquare). Values are mean \pm SE of 8 measurements. In the stomach, 2-factor repeated-measures ANOVA showed a meal effect ($P = 0.034$), no time effect ($P = 0.182$), and no meal \times time interaction ($P = 0.73$). In the duodenum, there was no meal effect ($P = 0.395$), a time effect ($P = 0.0001$), and a meal \times time interaction ($P = 0.0058$).

a function of digestive time. It is reasonable to assume that the remaining fractions of the vitamins were located in the fat globules. Note that because there was great variability in the results, and because there was no significant difference between the results obtained with the two meals (data not shown), we pooled the value of the two test meals to present more accurate data. Only 2–12% retinyl palmitate was recovered in the micellar phase (Fig. 5A), whereas most free retinol was recovered in this fraction (Fig. 5B). α -Tocopherol exhibited an intermediate pattern: 12–41% of this vitamin was recovered in the micelle + vesicle fraction (Fig. 5C).

Note that we did not find any vitamin A or E in the aqueous phase of the stomach content (data not shown).

Postprandial chylomicron responses. Postprandial triacylglycerol and vitamin A and E responses were determined to compare the time course of fat and vitamin absorption.

Chylomicron triacylglycerols increased over baseline after 1 h and peaked at 3 h after intake of the coarse emulsion and at 4 h after intake of the fine one (Fig. 6A).

The response of chylomicron retinyl palmitate (Fig. 6B) was very close to that of chylomicron triacylglycerol with a maximal rise at 3 h for the coarse emulsion and at 4 h for the fine one.

The chylomicron α -tocopherol responses (Fig. 6C) were slightly delayed compared with those observed for triacylglycerols and retinyl palmitate. More precisely, the increase over baseline began at 2 h, instead of 1 h for the other two nutrients, and the peaks were delayed by half an hour compared with those of the other nutrients.

For all the nutrients measured, there was no significant effect of the meal on the whole chylomicron response as measured by area under the curve (AUC) (Table 3). Conversely, there was a significant effect of the meal on the time at which the maximal concentration of each nutrient appeared in the chylomicron fraction.

DISCUSSION

The gastric emptying of vitamin A and E follows that of lipids. The fact that the percentage of ingested lipid and vitamins that remains in the stomach at each

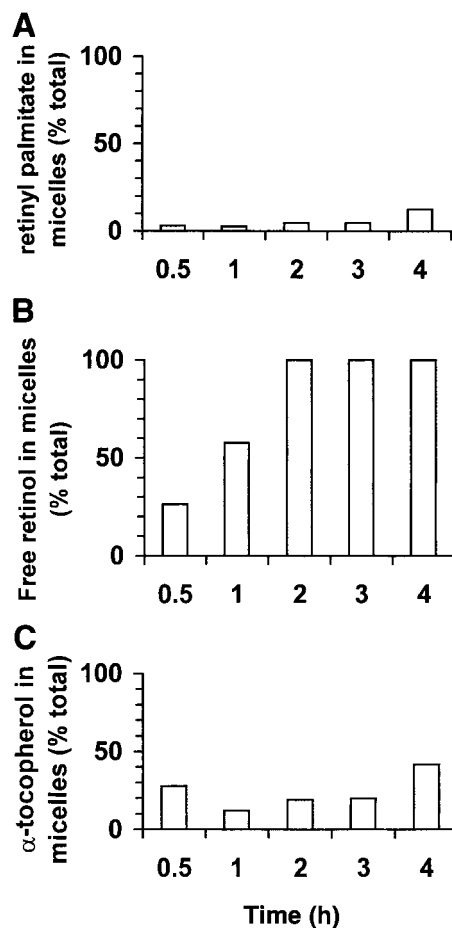


Fig. 5. Percentage of duodenal content retinyl palmitate (A), free retinol (B), and α -tocopherol (C) recovered in the micellar phase. Because there was great variability in the results and because there was no significant difference between the results obtained with the two emulsions, the data obtained after ingestion of the two emulsions were pooled.

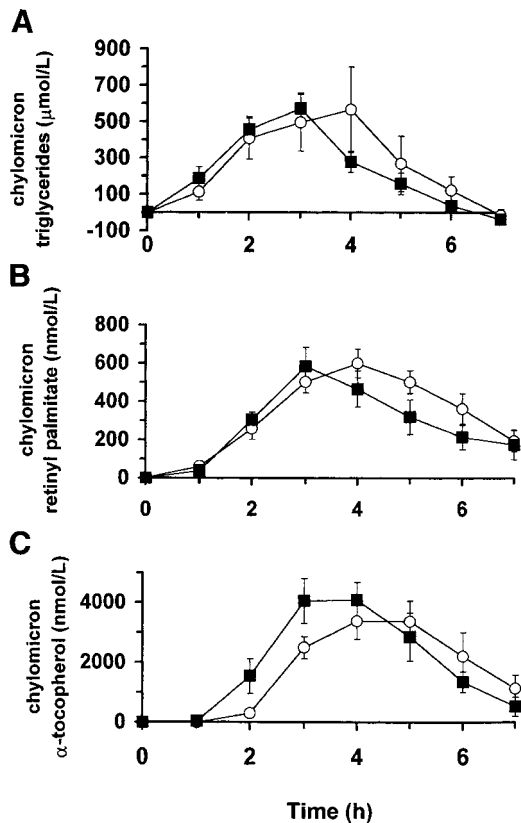


Fig. 6. Chylomicron triacylglycerols (A), retinyl palmitate (B), and α -tocopherol (C) responses to vitamin A- and E-rich meals. Results were obtained after ingestion of the fine emulsion (○) and after ingestion of the coarse emulsion (■). Values are concentration changes from fasting (0 h) values and are means \pm SE of 8 determinations. For triacylglycerols, 2-factor repeated-measures ANOVA gives no meal effect ($P = 0.704$), a time effect ($P < 0.0001$), and no meal \times time interaction ($P = 0.1809$). For vitamin A, there was no meal effect ($P = 0.37$), a time effect ($P = 0.0001$), and no meal \times time interaction ($P = 0.183$). For vitamin E, there was no meal effect ($P = 0.648$), a time effect ($P = 0.0001$), and a meal \times time interaction ($P = 0.049$). Student's t -test for paired values showed that the emulsion droplet size significantly ($P = 0.041$) affects the time at which the maximal concentration of chylomicron triacylglycerols was observed. This was also observed for chylomicron retinyl palmitate ($P = 0.0066$) and for chylomicron vitamin E ($P = 0.0479$).

digestive time was similar confirms what is generally assumed but has never been demonstrated, i.e., that the gastric emptying of vitamin A and E follows that of lipids. This is not surprising since these vitamins are fat soluble and since they remained in the fat phase of the meal in the stomach (there was no detectable vitamin A and E in the aqueous phase of the stomach content whatever the digestive time; data not shown).

There is no significant hydrolysis of retinyl palmitate in the human stomach. Because the processing of vitamin A in the human stomach is poorly understood (18, 21), we aimed to assess whether retinyl esters are hydrolyzed in this digestive organ. To do so, we measured the retinol/total vitamin A ratio in the stomach content during digestion. It is reasonable to assume that a modification of this ratio, compared with the one measured in the meal, indicates an hydrolysis of retinyl esters. The results clearly show that there is no

major hydrolysis of retinyl palmitate in the human stomach (>95% vitamin A present in the stomach remained as retinyl palmitate whatever the digestive time and the test meal). Nevertheless, the fact that there was a slight, but significant, increase in the ratio (as compared with the ratio measured in the test meals) after 30-min digestion was noteworthy. This increase can be due either to a small secretion of free retinol by the stomach, to a contamination by free retinol coming from the duodenum, or to a slight hydrolysis of retinyl palmitate in the stomach. The fact that the level of hydrolysis was droplet size-dependent (the ratio was significantly higher in the case of the fine emulsion than in the case of the coarse one) supports the last hypothesis and suggests that this increase was due to a hydrolysis by a lipase (the activity of these enzymes depends on fat emulsification). However, data obtained in this experiment are not conclusive on the exact nature of the lipase involved, i.e., gastric lipase or contaminating pancreatic lipase coming from the duodenum (in this experiment the pylorus was continuously open by the nasoduodenal tube during digestion).

There is no significant metabolism of vitamin E in the human stomach. There is virtually no information on the processing of vitamin E in the human stomach (13, 36). Data obtained in this experiment suggest that free α -tocopherol is not significantly altered or absorbed in the human stomach. Indeed, as the percentage of ingested α -tocopherol recovered in the stomach decreased similarly to the percentage of lipids and vitamin A, this suggests that the α -tocopherol decrease was only due to gastric emptying and that this vitamin was not significantly degraded or absorbed in the human stomach.

An enzyme present in the duodenal lumen, probably pancreatic lipase, hydrolyzes a significant proportion of retinyl esters. Because several enzymes are potentially able to hydrolyze retinyl esters in the human duodenum (15, 32, 33, 37, 38), our aim was to assess the relative role of luminal and brush border membrane enzymes in the hydrolysis of retinyl esters.

Because 11–42% vitamin A was present as free retinol in the duodenal content (Fig. 4B), we concluded that a large proportion of retinyl palmitate was hydro-

Table 3. Effect of the particle size of emulsion fat globules on the bioavailability of triacylglycerols and vitamin A and E

	Fine Emulsion	Coarse Emulsion
AUC, $\mu\text{mol}\cdot\text{h}\cdot\text{l}^{-1}$		
Triacylglycerols	3,530 \pm 1,180	3,330 \pm 790
Retinyl palmitate	2,378 \pm 148	2,014 \pm 361
α -Tocopherol	12,328 \pm 1,844	14,193 \pm 2,564

Values are means \pm SE. Bioavailability was estimated with chylomicron 0–7 h areas under the curve (AUC), which were calculated by using the trapezoidal method. The fine emulsion had a fat globule median diameter of 0.7 μm , and the coarse emulsion had a median diameter of 10 μm . No significant difference was found between the AUCs obtained after ingestion of the two emulsions (Student's t -test for paired values, $P < 0.05$).

lyzed in the duodenal lumen. Theoretically, retinyl palmitate can be hydrolyzed either at the fat globule interface by pancreatic lipase or in the mixed micelles by cholesterol esterase (15). However, recent data have established that cholesterol esterase is not significantly involved in the hydrolysis of retinyl esters (37, 38). The fact that at 0.5 and 1 h a significant proportion of free retinol was associated with the fat globules (Fig. 5B) adds further evidence for a role of pancreatic lipase in the hydrolysis of retinyl esters.

The fact that enzymes present in the duodenal lumen play a major role in the hydrolysis of retinyl palmitate is in agreement with observations showing that subjects with pancreatic insufficiency have a lower absorption of vitamin A, given as retinyl palmitate, than healthy subjects (29) and that tetrahydrolipstatin, a gut lipase inhibitor, inhibits the absorption of retinyl palmitate but not that of retinol (16).

The transport of vitamin E in the enterocyte is apparently less efficient than that of triacylglycerols and vitamin A. The 0.5-h delay in the appearance of vitamin E in the chylomicron fraction, compared with that of vitamin A and triacylglycerols, suggests that the absorption kinetic of vitamin E is different from that of the two other nutrients. However, because the gastric emptying rate of vitamin E was not markedly different from that of lipids and vitamin A, and because the proportion of vitamin E in the duodenum was not markedly different from that of lipids and vitamin A, this delay was not apparently due to a major difference in the processing of this vitamin in the gastrointestinal tract. To explain this delay, we suggest that the transport of α -tocopherol in the enterocyte is less efficient than that of lipids and vitamin A.

There is no significant effect of lipid emulsification on the bioavailability of vitamin A and E. Data obtained provide evidence that the particle size of emulsion fat globules (between 0.7 and 10 μm) has no major effect on the overall absorption of these vitamins (as assessed by their overall chylomicron response, i.e., AUC). This can be explained either by the fact that the median diameter of the fine emulsion was increased to half the size of the coarse emulsion in the stomach before the emulsion entered the duodenum or by the fact that the very efficient digestion of lipids (>95% in healthy subjects) has overridden a potential effect of fat droplet size on vitamin absorption. Perhaps the results would have been different in subjects suffering from fat malabsorption.

In summary, this study provides new fundamental data on the processing of vitamin A and E in the human gastrointestinal tract. It definitively establishes that the human stomach has no significant role in the hydrolysis of retinyl esters. It also suggests that there is no significant metabolism, i.e., absorption or degradation, of dietary vitamin E (free α -tocopherol) in the stomach. It provides evidence that a large proportion of retinyl esters is hydrolyzed in the duodenal lumen, suggesting that a pancreatic enzyme, probably pancreatic lipase, plays a major role in the digestion of preformed vitamin A in healthy adults. Finally, it demon-

strates that lipid emulsification (between 0.7 and 10 μm) has no major effect on the efficiency of intestinal absorption of vitamin A and E in healthy adults.

We thank J. L. Trouilly and F. Audry for valuable help during the conception of emulsions, and we thank Y. Rochette, J. Léonardi, M. Sacco-Cosson, and M. Bonneil for technical assistance.

This research was conducted with financial support from Clintec Technologies and from the Institut Français de Nutrition (to M. Armand).

REFERENCES

1. **Armand M, Borel P, Dubois C, Senft M, Peyrot J, Salducci J, Lafont H, and Lairon D.** Characterization of emulsions and lipolysis of dietary lipids in human stomach. *Am J Physiol Gastrointest Liver Physiol* 266: G372–G381, 1994.
2. **Armand M, Borel P, Pasquier B, Dubois C, Senft M, André M, Peyrot J, Salducci J, and Lairon D.** Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. *Am J Physiol Gastrointest Liver Physiol* 271: G172–G183, 1996.
3. **Armand M, Borel P, Ythier P, Dutot G, Melin C, Senft M, Lafont H, and Lairon D.** Effects of droplet size, triacylglycerol composition, and calcium on the hydrolysis of complex emulsions by pancreatic lipase: an in vitro study. *J Nutr Biochem* 3: 333–341, 1992.
4. **Azais-Braesco V, Forget A, Mercier M, and Grolier P.** Rapid synthesis and purification of vitamin A esters. *J Am Oil Chem Soc* 69: 1272–1273, 1992.
5. **Benzonana G and Desnuelle P.** Etude cinétique de l'action de la lipase pancréatique sur des triglycérides en émulsion. Essai d'une enzymologie en milieu hétérogène. *Biochim Biophys Acta* 105: 121–136, 1965.
6. **Bitman J and Wood DL.** Quantitative densitometry in situ of lipids separated by thin layer chromatography. *J Liq Chromatogr* 4: 1023–1034, 1981.
7. **Borel P, Armand M, Ythier P, Dutot G, Melin C, Senft M, Lafont H, and Lairon D.** Hydrolysis of emulsions with different triglycerides and droplet sizes by gastric lipase in vitro. Effect on pancreatic lipase activity. *J Nutr Biochem* 5: 124–133, 1994.
8. **Borel P, Armand M, Ythier P, Dutot G, Melin C, Senft M, Lafont L, and Lairon D.** Digestion and absorption of tube-feeding emulsions with different droplet sizes and compositions in the rat. *JPEN J Parenter Enteral Nutr* 18: 534–543, 1994.
9. **Borel P, Grolier P, Armand M, Partier A, Lafont H, Lairon D, and Azais-Braesco V.** Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets. *J Lipid Res* 37: 250–261, 1996.
10. **Buccolo G and David H.** Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 19: 476–482, 1973.
11. **Carey MC, Small DM, and Bliss CM.** Lipid digestion and absorption. *Annu Rev Physiol* 45: 651–677, 1983.
12. **Carrière F, Barrowman JA, Verger R, and Laugier R.** Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterology* 105: 876–888, 1993.
13. **Drevon CA.** Absorption, transport and metabolism of vitamin E. *Free Radic Res* 14: 229–246, 1991.
14. **Dubois C, Armand M, Azais-Braesco V, Portugal H, Pauli AM, Bernard PM, Latgé C, Lafont H, Borel P, and Lairon D.** Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipemic adults. *Am J Clin Nutr* 60: 374–382, 1994.
15. **Erlanson C and Borgstrom B.** The identity of vitamin A esterase activity of rat pancreatic juice. *Biochim Biophys Acta* 167: 629–631, 1968.
16. **Fernandez E and Borgström B.** Intestinal absorption of retinol and retinyl palmitate in the rat. Effects of tetrahydrolipstatin. *Lipids* 25: 549–552, 1990.

17. **Foleh J, Lees M, and Stanley JHG.** A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 226: 498–509, 1957.
18. **Ganguly J.** Absorption, transport, and storage of vitamin A. *Vitam Horm* 18: 387–402, 1960.
19. **George JD.** New clinical method for measuring the rate of gastric emptying: the double sampling test meal. *Gut* 9: 237–242, 1968.
20. **Hamosh M.** *Lingual and Gastric Lipases: Their Role in Fat Digestion.* Boca Raton, FL: CRC, 1990, p. 1–239.
21. **Harrison EH.** Lipases and carboxylesterases: possible roles in the hepatic metabolism of retinol. *Annu Rev Nutr* 18: 259–276, 1998.
22. **Hernell O, Stagers JE, and Carey MC.** Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry* 29: 2041–2056, 1990.
23. **Hoffman AF and Borgström B.** Physicochemical state of lipids in intestinal content during their digestion and absorption. *J Clin Invest* 43: 43–49, 1964.
24. **Hollander D and Muralidhara KS.** Vitamin A-1 intestinal absorption in vivo. Influence of luminal factors in transport. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 232: E471–E477, 1977.
25. **Hollander D, Rim E, and Muralidhara KS.** Mechanism and site of small intestinal absorption of α -tocopherol in the rat. *Gastroenterology* 68: 1492–1499, 1975.
26. **Hollander D.** Intestinal absorption of vitamins A, E, D and K. *J Lab Clin Med* 97: 449–462, 1981.
27. **Horowitz M, Jones K, Edelbroek MAL, Smout AJPM, and Read NW.** The effect of posture on gastric emptying and intra-gastric distribution of oil and aqueous meal components and appetite. *Gastroenterology* 105: 382–390, 1993.
28. **Hyden S.** A turbidimetric method for the determination of higher polyethylene glycols in biological materials. *Kungl Lantbrukshögskolans Annaler* 22: 139–145, 1955.
29. **Johnson EJ, Krasinski SD, Howard LJ, Alger SA, Dutta SK, and Russell RM.** Evaluation of vitamin A absorption by using oil-soluble and water-miscible vitamin A preparations in normal adults and in patients with gastrointestinal disease. *Am J Clin Nutr* 55: 857–864, 1992.
30. **Mansbach CM, Cohen RS, and Leff PB.** Isolation and properties of the mixed lipid micelles present in intestinal content during fat digestion in man. *J Clin Invest* 56: 781–791, 1975.
31. **Potter HP and Saunders DR.** Isolation of the aqueous phase of human intestinal contents during the digestion of a fatty meal. *Gastroenterology* 60: 997–1007, 1971.
32. **Rigtrup KM, Kakkad B, and Ong DE.** Purification and partial characterization of retinyl ester hydrolase from brush border of rat small intestinal mucosa: probable identity with brush border phospholipase B. *Biochemistry* 33: 2661–2666, 1994.
33. **Rigtrup KM, McEwen LR, Said HM, and Ong DE.** Retinyl ester hydrolytic activity associated with human intestinal brush border membranes. *Am J Clin Nutr* 60: 111–116, 1994.
34. **Sivakumar B and Reddy B.** Absorption of labelled vitamin A in children during infection. *Br J Nutr* 27: 299–304, 1972.
35. **Stagers JE, Hernell O, Stafford J, and Carey MC.** Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption 1 Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. *Biochemistry* 29: 2028–2040, 1990.
36. **Traber MG and Sies H.** Vitamin E in humans: demand and delivery. *Annu Rev Nutr* 16: 321–347, 1996.
37. **Van Bennekum AM, Fisher EA, Blaner WS, and Harrison EH.** Hydrolysis of retinyl esters by pancreatic triglyceride lipase. *Biochemistry* 39: 4900–4906, 2000.
38. **Weng W, van Bennekum AM, Potter SH, Harrison EH, Blaner WS, Breslow JL, and Fisher EA.** Intestinal absorption of dietary cholesteryl ester is decreased but retinyl ester absorption is normal in carboxyl ester lipase knockout mice. *Biochemistry* 38: 4143–4149, 1999.
39. **Winer BJ.** *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.