Gastric Digestion Modifies Absorption of Butterfat into Lymph Chylomicrons in Rats\textsuperscript{1,2}

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ABSTRACT Our objective was to characterize the time course of mesenteric lymph output, lipid composition and size of lymph chylomicrons in rats given gastric infusion of lipid emulsions containing defined fractions of butterfat, palm oil or corn oil. The concentrations of cholesterol, triacylglycerol (TAG) and phospholipid in lymph obtained before lipid infusion were 1.4–2.5-fold greater in rats chronically fed palm oil or solid butterfat compared with corn oil or liquid butterfat ($P = 0.02$). Total lymph chylomicron TAG output (mg/24 h) stimulated by gastric lipid infusion was 21% greater with corn oil compared with all saturated fats ($P = 0.02$). Total lymph chylomicron cholesterol output was 1.3–8.6-fold greater than the amount infused in all groups ($P = 0.03$) and was independent of the amount of cholesterol infused. The size distribution as well as the mean, median and modal diameters of lymph chylomicrons isolated during peak lymphatic TAG output were not significantly different among treatments. The fatty acid and TAG profiles of lymph chylomicrons obtained from rats infused with corn or palm oil did not differ significantly from that of the emulsion infused. In contrast, gastric lipolysis of butterfat significantly modified the lipid composition of lymph chylomicrons. We observed progressive disappearance of short- and medium-chain fatty acids in gastric contents and an absence of detectable short-chain fatty acids with concurrent proportionate increases in long-chain fatty acids and large TAG molecules in lymph chylomicrons compared with butterfat emulsions. These studies demonstrate that gastric digestion is an important modifier of lipid absorption. J. Nutr. 128: 2403–2410, 1998.

KEY WORDS: chylomicron • lymph • saturated fat • butterfat • triacylglycerol • rats

Chylomicrons are the transport vehicles for intestinally absorbed lipids, and subsequent metabolism of chylomicrons in the systemic circulation impacts the metabolism of plasma lipoproteins and cholesterol. A variety of studies have examined the effects of dietary fat saturation on the composition and size of mesenteric lymph chylomicrons with somewhat differing results due to differences in methodology (Renner et al. 1986). Relatively few studies have addressed how natural dietary sources of saturated and unsaturated fats affect chylomicron composition (Degrace et al. 1996, Kalogeris et al. 1992a and 1992b). Digestion of dietary fat begins in the stomach and is completed in the small intestine where final absorption occurs. Recent studies in humans indicate that gastric lipolysis can help to increase emulsification of fat in the stomach (Armand et al. 1994a) and that a high-fat diet increases activity of gastric lipase (Armand et al. 1994b). In humans, a preduodenal lipase of gastric origin cleaves ester bonds at the sn-1 and sn-3 positions of the triacylglycerol (TAG)\textsuperscript{4} molecule. In the rat, gastric lipolysis is catalyzed by an enzyme with properties similar to that of human gastric lipase but the enzyme originates in the tongue and is called lingual lipase (Liao et al. 1983).

Butterfat has a unique profile of short (4–6 C)- and medium-chain (8–10 C) fatty acids (25 mol/100 mol) (Lai et al. 1995a) which are located predominantly at the sn-3 position (Gresti et al. 1993) and thus are presumably subject to gastric lipolysis (Paltauf et al. 1974, Staggers et al. 1981). To determine how ingestion of butterfat affects chylomicron composition, it is important to use gastric rather than intestinal infusion of lipid prior to collection of lymph. However, few studies have used gastric infusion of butterfat.

Our objective was to characterize the time course of lymph output and the composition and size of chylomicrons isolated from mesenteric lymph in rats given gastric infusion of lipid emulsions containing defined fractions of butterfat, palm oil or corn oil. There are several unique and physiologically relevant features of our experimental approach. First, rats were prefed the experimental fats for 3–4 wk prior to collection of lymph. Thus, our results reflect chronic ingestion of the dietary fats rather than an acute response to a bolus of fat. Second, we used gastric infusion of lipid emulsions prepared from dietary fats.

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\textsuperscript{4} Abbreviations used: LCFA, long chain fatty acids; TAG, triacylglycerol; 2-MAG, 2-monoacylglycerols.
Third, we examined dynamic changes in lymph flow and chylomicron composition by characterizing lymph output throughout the entire postprandial phase rather than just at steady state of fat absorption.

**MATERIALS AND METHODS**

**Animals and diets.** The animal facilities and protocols were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Harlan Sprague Dawley, Madison, WI) initially weighing 140–170 g were used in all experiments. The rats were housed in individual stainless steel cages and maintained at 25°C with a 12 h light-dark cycle (lights on, 1100–2300 h). Rats were adapted to eat a daily 3-h meal (800–1100 h) containing 16% corn oil or 2% corn oil plus 14% palm oil, liquid butterfat or solid butterfat as previously described (Lai et al. 1995b) and were then meal-fed the experimental diets for 3–4 wk prior to tissue collection or lymph cannulation. The compositions of fatty acid and TAG (based on total acyl carbon number) profiles of the diets were previously reported (Lai et al. 1995a, Ney et al. 1991). The diets contained (per kilogram diet) 160 g of corn oil or 20 g of corn oil (to provide essential fatty acids) plus 140 g of palm oil, solid butterfat or liquid butterfat. Crystalline cholesterol (catalog no. C8503, Sigma Chemical, St. Louis, MO) was added to the corn oil and palm oil diets based on the cholesterol concentration of the intact anhydrous butterfat from which the liquid and solid butterfat were fractionated. The liquid butterfat contained 0.48 g cholesterol/kg diet, the solid butterfat diet contained 0.17 g cholesterol/kg diet and the palm oil and corn oil diets contained 0.35 g cholesterol-ol/kg diet (Lai et al. 1995a). The major differences between liquid and solid butterfat fractions were in the contents of short (4–6 C) and medium-chain (8–10 C) fatty acids (27 mol/100 mol in liquid and 19 mol/100 mol in solid), palmitate (20 mol/100 mol in liquid and 35 mol/100 mol in solid), stearate (7 mol/100 mol in liquid and 13 mol/100 mol in solid), and oleate (24 mol/100 mol in liquid and 16 mol/100 mol in solid).

**Experimental Design**

**Experiment 1: time course of lipid digestion.** The time course of lipid digestion was examined indirectly by measuring the TAG content remaining in the gastrointestinal lumen at 2, 5 and 9 h after ingestion of a 4-g meal. All rats ate the meal within 30 min. At the defined time points, subgroups (of the four treatment groups were randomly assigned to one of four diets) were collected for 2 h and then the glucose infusion was replaced by gastric infusion of a lipid emulsion at a constant rate of 3 mL/h for 3 h followed by infusion of sterile saline (0.15 mol/L NaCl) for 21 h. Lymph samples were collected hourly for 13 h after lipid infusion; afterward they were collected at 20 (containing lymph secreted from 13–20 h inclusive), 22 and 24 h. Lymph was collected at 30°C in 15 mL Nalgene clear centrifuge tubes. The following were added to the lymph samples (final concentrations): disodium EDTA (2.5 mol/mL), reduced glutathione (1.5 mol/mL) and gentamicin sulfate (1.2 mg/gentamicin/L). Lymph was centrifuged for 5 min at 2000 × g at 37°C. Clotted fibrins were wound out using wooden applicator sticks and the supernant cell-free lymph was transferred to a clean tube.

**Lipid emulsions.** Lipid emulsions were formulated to contain the amounts of fat and cholesterol equivalent to those contained in 5 g of the experimental diet. The corn oil and palm oil emulsions contained 800 mg/L fat, 1.75 mg added cholesterol and 90 mg sodium taurocholate in 9 mL of PBS (6.75 mmol/L Na2HPO4, 16.5 mmol/L NaH2PO4, 115 mmol/L NaCl and 5 mmol/L KCl). The compositions of liquid and solid butterfat emulsions were the same as those of the corn oil and palm oil except for cholesterol: the liquid butterfat emulsion contained 2.73 mg naturally occurring cholesterol per mL emulsion and the solid butterfat emulsion contained 0.94 mg/mL respectively (Lai et al. 1995a). Cholesterol, dissolved in chloroform, was added to the corn and palm oil emulsions (1.75 mg cholesterol in 9 mL) and then the solvent was evaporated. Immediately before administration, 9 mL of PBS containing 18.6 mmol/L sodium taurocholate was added to the lipid, and the solution was sonicated until no floating lipid droplets were visible. Corn oil and liquid butterfat emulsions were prepared at 23°C. Palm oil and solid butterfat were heated to 40–50°C before mixing and sonication. In a preliminary experiment, the stability of palm oil and solid butterfat emulsions was verified by demonstrating that TAG concentration in the emulsions was relatively constant over a 3-h period at 30°C.

**Isolation of lymph chylomicrons.** Chylomicrons were isolated from lymph samples by ultracentrifugation. One to two milliliters of lymph was carefully overlaid by 3–4 mL of a d = 1.0063 kg/L NaCl solution containing 0.15 mg of phenylmethylsulfonyl fluoride per milliliter of lymph. The chylomicrons were centrifuged at 85,200 × g for 20 h at 4°C in a Sorvall (Sorvall, Inc., Newton, MA) SS-34 rotor at 25°C. The chylomicrons were then collected by sequential ultracentrifugation of the infranatant at 100,000 × g for 15 h (x 2) at 4°C in a Sorvall SS-34 rotor. The chylomicrons were washed once by re-centrifugation at 100,000 × g for 15 h at 4°C in a Sorvall SS-34 rotor. The precipitated chylomicrons were resuspended in 1 mL of PBS and the TAG content was determined by capillary gas chromatography with flame ionization detection, using a Varian 3400 gas chromatograph (Varian, Walnut Creek, CA) as previously described (Monsma et al. 1993). TAG concentrations of the stomach and intestinal lipid extracts were determined according to the method described by Fletcher (1968), multiplicated by the total volume of the lipid extracts, and expressed as milligrams.

Lymph chylomicrons were analyzed for concentrations of TAG (Sigma triglyceride kit, No. 337, Sigma Chemical Company, St. Louis, MO), total cholesterol (Allain et al. 1974) and phospholipids within 1–2 h following isolation. Phospholipid concentration was determined using an enzymatic method based on the measurement of the choline moiety of the phospholipids (Warnick 1986).

**Determination of fatty acid and TAG profiles.** TAG from lipid extracts of the diets, emulsions, luminal contents from the stomach and intestines, and lymph chylomicrons were isolated on 3 mL aminopropyl (NH2) Bond Eluts (catalog no. 1212–4038, Varian) as previously described (Monsma et al. 1993). The fatty acid composition of the TAG fraction from various lipid extracts was determined by capillary gas chromatography with flame ionization detection, using a Varian 3400 gas chromatograph (Varian, Sugarland, TX) equipped with an on-column temperature pro-
grammary injector and a DB2330 capillary column (J&W Scientific, Folsom, CA) as previously described (Lai et al. 1995a, Ney et al. 1991). TAG profiles were determined based on total acyl carbon number, i.e., total number of carbon atoms from all fatty acids esterified to the glycerol backbone, using a Varian gas chromatograph equipped with a TAP CB (75% phenyl, 25% methylpolysiloxane) on a WCOT Ultimetal column (25 M × .25 mm i.d., .80 mm o.d., .10 mm film; Chrompack, Raritan, NY) as described previously (Lai et al. 1995a, Ney et al. 1991). Values are expressed as the molar proportion of total fatty acids or TAG molecular species identified.

**Chylomicron size determination.** Lymph chylomicron samples from individual rats were pooled according to stages of TAG absorption as indicated by the rates of lymph secretion (mL/h) and chylomicron TAG secretion (mg/h). Chylomicron samples collected from each rat with the five highest TAG secretions (usually 3- to 7-h samples) were pooled for determination of particle size by electron microscopy.

Electron microscopy was performed on chylomicron samples collected within 1–2 d during which they were kept at 37°C to prevent crystallization of saturated fatty acids from the TAG molecules and distortion of chylomicron particles (Renner et al. 1986). Chylomicron samples were fixed in 4% O,O4 (1:1, v/v), stained with 1% ammonium molybdate and then sprayed onto formvar/carbon-coated grids. Grids were air-dried and then viewed in a Jeol CX100 electron microscope (Japan).

Electron microscope negatives of negatively stained lipid droplets were scanned using a Leafscan 45 film scanner connected to a Power Macintosh running Adobe Photoshop 3.0.4. The images were imported into IPLab Spectrum 2.5.7, and the measurement parameters were set in nm as follows: area and perimeter, 20 – 20,000 minimum-maximum; eccentricity, 0 – 0.7 minimum-maximum. Approximately 200–1000 particles were counted for each sample.

**Statistical analyses.** For experiment 1, differences among dietary treatment groups were assessed by two-way ANOVA using generalized linear models in SAS (Version 6.12, SAS Institute, Inc., Cary, NC 1996). For experiment 2, differences among dietary treatment groups were assessed by two-factor repeated measures analysis using one-way ANOVA at each time point. When repeated measures analysis or F tests from ANOVA indicated significant differences (P < 0.05), a multiple comparison was performed to compare group means using the least significant difference technique. Values are expressed as means ± SD or as means with pooled SD. Finally, frequency distributions of lymph chylomicron particle sizes were assessed by chi-square analysis, and differences in the mean, median (the 50th percentile value) and modal (the most commonly occurring value) diameters for chylomicron sizes among treatment groups were assessed by one-way ANOVA.

**RESULTS**

**Time course of lipid digestion.** The time course of lipid digestion was examined indirectly by measuring the TAG contents remaining in the gastrointestinal lumen at 2, 5 and 9 h after meal ingestion. As expected, the amount of TAG present in the stomach increased dramatically in response to meal ingestion (P < 0.0001, effect due to time, two-way ANOVA, Fig. 1). No significant differences in stomach TAG contents were noted among dietary groups, indicating similar rates of gastric emptying of TAG. However, rats fed liquid or solid butterfat retained 18–23% more TAG in the stomach (P = 0.06, effect due to fat, two-way ANOVA) than rats fed palm or corn oil. The amount of TAG remaining at 2 h after meal ingestion ranged from 240 to 321 mg (275 ± 69 mg), which corresponded to 38%–50% of the total fat intake. This suggests that >50% of the fat ingested was digested by the stomach and released to the small intestines 2 h after meal ingestion (ANOVA). Only 12–17% and 2–4% of the ingested fat still remained in the stomach at 5 and 9 h, respectively, after meal ingestion.

The amount of TAG remaining in small intestinal lumen (from pylorus to 60 cm distal to pylorus) ranged from 1.2 to 4.1 mg at all time points measured after meal ingestion. This suggests that gastric release of dietary fat into the proximal small intestinal lumen was relatively comparable over time. Overall, TAG contents in the intestinal lumen peaked at 5 h, which reflected the period of active fat digestion and absorption in all dietary groups.

Rats fed liquid (341 ± 15 g) or solid (340 ± 14 g) butterfat showed significantly greater final body weights than rats fed corn oil (326 ± 20 g) or palm oil (329 ± 19 g) with no significant difference in food intake. Both the type of fat and the feeding state affected liver weight. Liver weight was the highest at 2 h after meal ingestion. Rats fed the liquid butterfat had significantly greater liver weights (3.85 ± 0.23 g/100 g body) than the other groups (3.58 ± 0.36 g/100 g body weight [P = 0.01]).

**Mesenteric lymph flow after gastric infusion.** Figure 2 shows the volume of mesenteric lymph collected after gastric infusion at 3 mL/h of a lipid emulsion containing 0.94–2.73 mg cholesterol and 800 mg fat, an amount of lipids equivalent to that present in 5 g of the diet fed to the animals for 3–4 wk prior to lymph experiment. Baseline lymph output ranged from 1.5–2 mL/h as measured for 2 h before lipid infusion. Initially, lymph output decreased for the first hour in response to lipid infusion. This may reflect a decrease in the rate of gastric emptying due to gastric infusion of dietary fat. Thereafter, lymph output increased steadily, peaked at ~4–6 h, then...
decreased to 1.8–2.4 mL/h at 24 h. The rate of lymph flow did not differ significantly among treatment groups.

**Baseline lymph lipid concentration.** Lymph lipid composition before initiation of lipid infusion was measured to determine the chronic effect of ingesting the experimental fats. The mass lipid outputs (milligrams per hour of lymph output) of cholesterol, TAG and phospholipid in baseline lymph samples are shown in Figure 3. Overall, rats chronically fed palm oil or solid butterfat showed 1.4–2.5-fold greater mass output of cholesterol, TAG and phospholipid in baseline lymph samples than rats chronically fed corn oil or liquid butterfat (P = 0.02). The current observation of similar lymph lipid contents in the corn oil and liquid butterfat groups as compared to the palm oil and solid butterfat groups is consistent with our earlier report assessing postprandial lipemia using these dietary fats (Lai et al. 1995b).

**Time course of lymph chylomicron lipid output after gastric lipid infusion.** The time course of lymph chylomicron TAG, cholesterol and phospholipid outputs (lymph flow multiplied by lipid concentration) in response to lipid infusion are shown in Figure 4. Lymph chylomicron TAG output increased after lipid infusion and peaked at ~5–6 h. Peak TAG output were 9–27-fold higher than baseline TAG output. Repeated measures analysis showed a significant fat by time interaction (P = 0.01), indicating that the time course of chylomicron TAG output was affected by the type of fat infused. This effect was primarily due to the corn oil group, which showed relatively higher rates of chylomicron TAG secretion between 5 and 9 h compared to other groups.

Integration of the area under the TAG output curves (corrected for baseline contribution) reflected the total amount of TAG, cholesterol and phospholipid outputs (lymph flow multiplied by lipid concentration) in response to lipid infusion are shown in Figure 4. Lymph chylomicron TAG output increased after lipid infusion and peaked at ~5–6 h. Peak TAG output were 9–27-fold higher than baseline TAG output. Repeated measures analysis showed a significant fat by time interaction (P = 0.01), indicating that the time course of chylomicron TAG output was affected by the type of fat infused. This effect was primarily due to the corn oil group, which showed relatively higher rates of chylomicron TAG secretion between 5 and 9 h compared to other groups.

Integration of the area under the TAG output curves (corrected for baseline contribution) reflected the total amount of
TAG mass output stimulated by intragastric lipid infusion. Chylomicron TAG output during peak lymph secretion, which occurred between 3 and 7 h for most animals, accounted for 41–47% of total TAG output over the 24-h period. Overall, corn oil infusion resulted in 31% greater amount of TAG output during peak lymph flow period and 21% greater amount during the 24-h period compared to infusion of saturated fats (P = 0.02).

Similar to chylomicron TAG output, lymph chylomicron cholesterol output increased after lipid infusion and peaked at 4–6 h. Peak cholesterol output increased 2–5-fold compared to baseline cholesterol output, reflecting the need for cholesterol in packaging the chylomicron particles for transport of dietary TAG absorbed by the gut. Repeated measures analysis indicated that type of fat did not affect the time course of chylomicron cholesterol output.

Total chylomicron cholesterol outputs calculated by integration of the area under the cholesterol output curves (corrected for baseline contribution) ranged from 2.3 to 7.7 mg, which were 1.3–8.6-fold greater than the amount infused (0.94–2.73 mg), and varied greatly among treatment groups (Fig. 4). Total chylomicron cholesterol output was the highest with corn oil infusion, slightly lower with infusion of the two butterfat fractions and significantly lower with palm oil compared with corn oil infusion (P = 0.03). Chylomicron cholesterol output during peak lymph secretion, i.e., 3–7 h, accounted for 62% of the total output over 24 h for the palm oil group but only 31–41% for the other three groups.

The time course of lymph chylomicron phospholipid output in response to lipid infusion showed significant differences among dietary fats at 7, 11, 12 and 13 h (P < 0.05, one-way ANOVA). Overall, palm oil infusion resulted in the highest chylomicron phospholipid output and liquid butterfat infusion resulted in the lowest chylomicron phospholipid output. At 11, 12 and 13 h chylomicron phospholipid secretion was significantly higher in rats infused with palm oil or solid butterfat compared to rats infused with corn oil or liquid butterfat. Over the 24-h period, palm oil infusion resulted in the greatest total amount of chylomicron phospholipid output (80 mg) which was twofold greater than infusion of liquid butterfat (P = 0.16). However, these differences were not statistically significant. Chylomicron phospholipid output during peak lymph output, i.e., 3–7 h, accounted for 35–36% of total output over 24 h in all groups.

**Lymph chylomicron size distribution.** Lymph chylomicrons produced during peak lymphatic TAG output (usually 3–7 h postprandially) were examined under electron microscope for determination of particle size. Approximately 75% of the lymph chylomicron particles produced during peak lymphatic TAG output were between 20 to 120 nm in diameter in all treatment groups and this result is consistent with an earlier study (Kalogeris and Story 1992). However, mean chylomicron diameter varied substantially among rats within the same treatment group. For example, mean chylomicron diameters for the three animals in the corn oil group were 121, 106 and 82 nm, respectively, and those for the palm oil group were 115, 80 and 44 nm, respectively. Overall, mean diameters (mean ± SD, n = 3) were as follows: corn oil, 103 ± 20 nm; palm oil, 80 ± 35 nm; liquid butterfat, 112 ± 52 nm; and solid butterfat, 132 ± 20 nm. Median diameters (mean ± SD, n = 3) were as follows: corn oil, 71 ± 2 nm; palm oil, 62 ± 32 nm; liquid butterfat, 74 ± 24 nm; and solid butterfat, 90 ± 7 nm. Modal diameters (mean ± SD, n = 3) were as follows: corn oil, 41 ± 27 nm; palm oil, 44 ± 6 nm; liquid butterfat, 46 ± 17 nm; and solid butterfat, 59 ± 1 nm. No significant differences in lymph chylomicron size were noted among treatment groups in the mean, median and modal diameters (P > 0.05, one-way ANOVA) as well as in the frequency distributions of particle sizes (chi-square, P > 0.05).

**Fatty acid composition and triacylglycerol molecular species in digested and absorbed TAG.** Fatty acid and TAG compositions of the experimental diets, stomach lipid extracts and lymph chylomicron lipid extracts were analyzed to examine if the fatty acid and TAG species contained in the corn oil, palm oil, liquid butterfat and solid butterfat were altered by lipid digestion and absorption. As reported in a previous study (Lai et al. 1995a), corn oil and palm oil diets contained >99 mol/100 mol of the total fatty acids as 16–18 carbon fatty acids (palmitic, stearic, oleic and linoleic acids). In contrast, the solid and liquid butterfat diets contained 16 mol/100 mol and 24 mol/100 mol, respectively, of short- and medium-chain fatty acids (4–10 C) fatty acids. Additional differences in the fatty acid composition between the solid and liquid butterfat diets were observed in palmitate (32 mol/100 mol in solid and 20 mol/100 mol in liquid), stearate (14 mol/100 mol in solid and 6 mol/100 mol in liquid) and oleate (15 mol/100 mol in solid and 24 mol/100 mol in liquid). In the present study, fatty acid composition of TAG remaining in the stomach at 2, 5 and 9 h postprandially in rats fed corn oil and palm oil were found to reflect that of the diets (data not shown) with <2% changes noted in the relative proportions of the four major fatty acids after meal ingestion. Similarly, the fatty acid composition of lymph chylomicron TAG collected from rats infused with corn or palm oil emulsion did not differ significantly from that of emulsion fatty acids.

Unlike the corn oil and palm oil groups, we observed substantial modifications of the fatty acid composition in postprandial TAG remaining in the stomach and in TAG isolated from lymph chylomicrons compared to that of the butterfat diets or emulsions (Fig. 5). Progressive decreases in short- and medium-chain fatty acids (4–10 C) with concurrent increases in long-chain fatty acids (16–18 C) were noted in TAG remaining in the stomach as digestion and absorption of the liquid or solid butterfat occurred (P < 0.05 of effect due to time, two-way ANOVA). Fatty acid composition of the TAG remaining in the stomach at 9 h after ingestion of the liquid or solid butterfat meal showed a 4–5-fold reduction in short- and medium-chain fatty acids, suggesting that these fatty acids were preferentially digested by gastric lipolysis. Furthermore, lymph chylomicron TAG isolated from rats infused with liquid or solid butterfat contained no detectable short-chain fatty acids (4–6 C).

In addition to changes in the fatty acid composition, modifications in the TAG molecular species of TAG remaining in the stomach and TAG isolated from lymph chylomicrons in response to ingestion/infusion of liquid or solid butterfat were also observed. As shown in **Figure 6**, the relative proportions of 30–34 C TAG molecular species decreased in postprandial TAG remaining in the stomach and these TAG disappeared in lymph chylomicrons. Since these TAG species contained at least one short- or medium-chain fatty acids (4–10 C), the disappearance of these TAG in lymph chylomicron suggest that the majority of the 4–10 C fatty acids were not secreted into lymph. Similarly, the relative proportions of 36–40 C TAG molecules also decreased substantially.

**DISCUSSION**

Evidence suggests that gastric lipolysis and stimulation of gastric hormones impacts fat digestion and absorption (Bernbach et al. 1989, Roy et al. 1979). However, intestinal infusion of lipid emulsions has been utilized in most reports assessing
the composition of lymph chylomicrons (Bergsted et al. 1990, Green et al. 1984, Kalogeris et al. 1992a and 1992b, Renner et al. 1986). In this study, we describe the time course of lymph output and the chemical composition and size of lymph chylomicrons isolated from rats given gastric infusion of liquid or solid butterfat, palm oil or corn oil. Our approach is particularly relevant to digestion of butterfat due to its content of short- and medium-chain fatty acids (25 mol/100 mol) (Lai et al. 1995a), which are located predominantly at the sn-3 position (Gresti et al. 1993) and are presumably subject to gastric lipolysis (Paltauf et al. 1974, Staggers et al. 1981). In contrast to palm oil or corn oil, gastric infusion of butterfat resulted in dramatic changes in the fatty acid and TAG profiles of lymph chylomicrons compared to that of the lipid emulsions infused.

A variety of studies using different types of feeding models have reported a lower apparent efficiency of absorption for saturated relative to unsaturated fatty acids (McDonald et al. 1980, Ney et al. 1991, Ockner et al. 1972, Sheehe et al. 1980). We noted that total lymph chylomicron TAG production (mg/24 h) stimulated by gastric infusion was 21% greater with infusion of corn oil compared to the saturated butterfat and palm oil emulsions. Degrace et al. (1996) also demonstrated a
lower recovery of lymphatic TAG with gastric administration of butter in comparison with corn oil. Our observation that rats fed liquid or solid butterfat showed significantly greater final body weight with no significant differences in food intake compared to rats fed corn oil or palm oil suggests that low recovery of lymph TAG with butterfat feeding does not affect growth as would be expected with decreased efficiency of lymphatic absorption. In addition, we show that the majority of short- and medium-chain fatty acids found in butterfat are preferentially digested in the stomach and do not appear in TAG isolated from lymph chylomicrons. These data indicate that overall digestion and absorption of saturated fatty acids from butterfat may be underestimated due to the artifact introduced by intestinal infusion in previous studies.

The effects of dietary fat saturation on chylomicron size are inconsistent. Several investigators have reported that absorption of unsaturated fats produced larger chylomicrons compared to absorption of saturated fats (Boquillon et al. 1977, Feldman et al. 1983b, Hayashi et al. 1992, Kalogeris et al. 1992a and 1992b). Feldman et al. (1983b) demonstrated that chylomicron diameter was largest in rats fed diet rich in oleic acid, intermediate in rats fed diet rich in linoleic acid and smallest in rats fed diet rich in palmitic acid. Consistently, Kalogeris et al. (1992a and 1992b) also reported smaller chylomicron size in rats infused duodenally with butter oil compared to corn oil or olive oil. In contrast, Fraser et al. (1968) and Renner et al. (1986) reported that fat saturation had no effect on particle size.

Differences in methodology have been cited as a likely reason to explain the discrepancy. More specifically, collection and isolation of chylomicrons using temperature below 25°C would cause crystallization of saturated fatty acids and distortion of chylomicron particles (Bennet Clark et al. 1982, Feldman et al. 1983a and 1983b, Renner et al. 1986). In the present study, lymph was collected at 30°C and the chylomicron fractions were isolated at 37°C. Under these conditions, electron microscopy of lymph chylomicrons showed no significant difference in the size distribution of lymph chylomicron particles produced with gastric infusion of corn oil, palm oil, solid or liquid butterfat.

Analytical differences in the determination of chylomicron size may also help to explain why previous results (Feldman et al. 1983b, Kalogeris et al. 1992a and 1992b) showed smaller chylomicrons with absorption of saturated relative to unsaturated fats while our results showed no significant differences. In the studies of Feldman et al. (1983b) and Kalogeris (1992a and 1992b) lymph chylomicrons collected from individual rats in the same treatment group were pooled, and sizes of 200–1000 particles were determined by electron microscopy for each treatment. This approach gives one representative sample per treatment group. However, it eliminates the opportunity to obtain “between-animal variation on the mean diameter,” which is defined as the SD of the means from n divided by the square root of n, where n is the number of rats per treatment group. Such between-animal variation on the mean diameter is essential to determine statistical significance of mean diameter differences among treatment groups. In earlier studies (Feldman et al. 1983b, Kalogeris et al. 1992a and 1992b) statistical significance in mean diameter differences among treatment groups was assessed using SEM computed from the SD of all particles divided by the number of particles from the single representative sample per treatment group. Such SEM represented the variation between-animal variation on the mean diameter as defined above, nor did it represent the “within-animal variation on all particles” (defined as the SD of all particles divided by the number of particles) because the latter can only be computed based on data from a single animal.

In our study, statistical significance of the mean diameter differences among treatment groups was assessed based on the between-animal variation on the mean diameter. Because we observed a relatively large between-animal variation on the mean diameter (e.g., coefficient of variation of 15–46%), insignificant treatment effects were noted. If data from the three rats within the same treatment group had been pooled with variation computed based on the number of particles in a manner similar to earlier reports, significantly greater mean particle diameter with corn oil compared with palm oil would be observed.

Taken together, chylomicron sizes determined in our study appeared to be comparable to those reported previously. However, unlike previous reports, chylomicron sizes were not smaller with absorption of palm oil, solid butterfat or liquid butterfat compared with corn oil. This conclusion was based on comparisons of the mean, median and modal diameters, as well as the entire size distribution profiles among treatment groups.

We observed a greater lymph chylomicron cholesterol output (mg/24 h) compared to the amount infused with all four fats. This indicated that supply of endogenous cholesterol, potentially from biliary secretion or intestinal de novo synthesis (Stange et al. 1985), is necessary for packaging chylomicron particles during fat absorption. In addition, the degree of endogenous cholesterol supply appears to be independent of the amount of dietary cholesterol. For example, corn oil infusion resulted in threefold greater chylomicron cholesterol output (7.7 mg/24 h) compared to palm oil infusion (2.3 mg/24 h) despite infusion of the same amount of cholesterol (1.75 mg). In contrast, infusion of corn oil, liquid and solid butterfat resulted in similar total cholesterol output despite differing amounts of cholesterol infused. Therefore, in addition to dietary cholesterol, composition of dietary fat may influence lymphatic cholesterol secretion (Kalogeris et al. 1992a).

Our results of diminishing proportions of the short- and medium-chain fatty acids (4–10 C) in the stomach from early to late postprandial phases provide further evidence of gastric lipolysis of butterfat (Aw et al. 1980, Fernando-Warnakulasuriya et al. 1981). In rats, the major lipase functioning in the stomach is lingual lipase (Liao et al. 1983) which hydrolyzes fatty acids at the sn-3 position in preference to the sn-1 position (Faltauf et al. 1974, Staggers et al. 1981). The majority of short- and medium-chain fatty acids in butterfat are esterified to the sn-3 position (Gresti et al. 1993). Thus, the reduction in 4–10 C fatty acids in stomach and lymphatic TAG after gastric infusion of butterfat can be explained by lingual lipase action in the stomach and absorption of fatty acids independent of lymph.

There was a pronounced redistribution of lymph chylomicron TAG profiles toward larger TAG molecules (46–52 C) with infusion of liquid or solid butterfat. This raises a question regarding how the long-chain (12–18 C) fatty acids (LCFA) hydrolyzed in the intestine with infusion of liquid or solid butterfat were resynthesized into TAG for transport in lymph chylomicrons. If these LCFA were resynthesized into TAG by esterification to the 2-monocglycerols (2-MAG) produced during fat digestion, substantial supply of endogenous fatty acids would be needed to replace the lost short- and medium-chain fatty acids in order for all the free fatty acids and 2-MAG to be reassembled into TAG. If supply of endogenous fatty acids was limited, there would be an excess of 2-MAG which would need to be metabolized by the enterocyte.

Comparison of the fatty acid profile of the solid butterfat
emulsion with the fatty acid profile of the lymph chylomicron allowed us to estimate the contribution of endogenous LCFA to resynthesis of absorbed LCFA from solid butterfat in the lymph. As shown in Figure 5, all of the 4–10 C fatty acids in the solid butterfat emulsion (16 mol/100 mol) were not detected in the lymph; these fatty acids most likely did not enter lymph. Therefore, the remaining LCFA in solid butterfat emulsion would have a percent composition of 15 mol/100 mol (12–14 C), 38 mol/100 mol (16:0), 16 mol/100 mol (18:0), 18 mol/100 mol (18:1) and 8 mol/100 mol (18:2) entering into lymph. The above LCFA molar composition is very similar to what was detected in peak lymph (14 mol/100 mol for 12–14 C, 38 mol/100 mol for 16:0, 14 mol/100 mol (18:0), 20 mol/100 mol (18:1) and 9 mol/100 mol (18:2)). Together with our observation that total lymph TAG mass output (0–24 h) for solid butterfat group was 82% that of the corn oil group (Fig. 4), these findings lead us to speculate that the original 12–18 C fatty acids present in solid butterfat emulsion were resynthesized into lymph TAG with minimal contribution of endogenous LCFA. It is more difficult to perform the aforementioned estimation for the liquid butterfat group, because the 8–10 C fatty acids detected in the lymph could not be quantified in relation to emulsion 8–10 C fatty acids without data on absolute mass.

In conclusion, results from the present study demonstrated complex profiles of gastrointestinal digestion and absorption of liquid and solid butterfat, presumably due to their different physical properties and unique composition of saturated fatty acids and TAG molecular species. In contrast to earlier reports utilizing intestinal infusion, ingestion of butterfat does not show smaller lymph chylomicron particles compared to unsaturated corn oil. This finding may reflect methodological differences in determining chylomicron size as well as modifications in lymph chylomicron lipid composition due to gastric lipolysis. These studies demonstrate that gastric digestion is an important modifier of lipid absorption.

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LITERATURE CITED