Effect of saturated and unsaturated lipid on the composition of mesenteric triglyceride-rich lipoproteins in the rat

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Abstract The effect of saturated and unsaturated lipids on the composition of mesenteric lymph triglyceride-rich lipoproteins was studied in rats. A short-term steady-state infusion model was developed in mesenteric lymph fistula rats. Micellar solutions of linoleate, oleate, or palmitate were infused intraduodenally. Steady-state conditions of lymph flow, triglyceride, and apoA-I and apoB secretion rates were achieved in hours 3–5 after the start of the infusion. During this steady-state period, triglyceride-rich lipoproteins were prepared and characterized. With lipid infusion there were the expected increases in secretion rates of triglyceride, apoB, and apoA-I both in whole lymph and in the d < 1.006 g/ml lipoproteins. Compositional analysis of d < 1.006 g/ml lipoproteins revealed no difference in the ratios of phospholipid or apoA-I (surface) to triglyceride (core) constituents between saturated and unsaturated lipids, suggesting a similar particle size. This was directly verified by agarose gel filtration and electron microscopy carried out at 27°C, which showed no difference in particle size between linoleate and palmitate chylomicrons. When these lipoproteins were prepared at 4°C, palmitate lipoproteins exhibited dramatically changed gel filtration elution profiles, suggesting a shift to smaller or at least distorted particles and questioning earlier results suggesting a smaller size for saturated fat d < 1.006 g/ml lipoproteins. Despite the similarity of size between saturated and unsaturated chylomicrons, the apoB content of unsaturated linoleate chylomicrons was significantly lower than that of palmitate chylomicrons. This difference was present whether chylomicrons were prepared by centrifugation or by gel filtration. The clearance of palmitate chylomicrons from the circulation of recipient rats was slightly more rapid than that of linoleate chylomicrons. The mechanism for this apparently selective increase in the apoB content of saturated fat chylomicrons is unknown but the present studies suggest that these changes may be of physiologic significance, perhaps relating to the potential atherogenicity of saturated lipids. —**Renner, F., A. Samuelson, M. Rogers, and R. M. Glickman.** Effect of saturated and unsaturated lipid on the composition of mesenteric triglyceride-rich lipoproteins in the rat. *J. Lipid Res.* 1986. **27:** 72–81.

Supplementary key words mesenteric lymph lipoproteins • chylomicrons • apoA-I apoB • triglycerides

Dietary fat saturation and cholesterol have been strongly implicated in human and experimental atherogenesis (1). These lipids are absorbed by the intestine and must be incorporated into lipoproteins before they are secreted into the systemic circulation (2, 3). A variety of studies have examined the effects of saturated and unsaturated lipids on the secretion and composition of mesenteric lymph lipoproteins with somewhat differing results. In some studies, saturated lipid feeding resulted in the secretion of smaller triglyceride-rich lipoproteins when compared to unsaturated fat. This was shown by electron microscopy of mesenteric lymph lipoproteins (4, 5) as well as by the demonstration of increased triglyceride transport in smaller VLDL-size particles during saturated fat infusion in the rat (6). Other studies, however, failed to show any particle size differences between these two kinds of lipids (7, 8). It should be noted that, with the exception of one recent study (5), all of these studies have utilized lipoproteins prepared below 27°C. It has been shown recently that low temperatures result in partial crystallization of saturated core lipids with resultant changes in the size, shape, and density of these lipoproteins (9, 10). We therefore felt it was important to reexamine the effect of dietary fat saturation on the size and composition of mesenteric lymph lipoproteins. In addition, the finding of Ockner, Pittman, and Yager (11) that saturated and unsaturated fatty acids may be absorbed over different lengths of intestine complicates the interpretation of size changes resulting from saturated and unsaturated lipid feeding. Such measurements should be performed under conditions of constant infusion and comparable steady-state lymph triglyceride secretion rates. This would minimize intestinal absorptive differences between saturated and unsaturated fat. The present study develops and validates such a model. In addition, these studies have

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; SDS, sodium dodecyl sulfate; apo, apolipoprotein; RIA, radioimmunoassay.

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examined the secretion rates of intestinal apoproteins (apoA-I, apoB) under constant conditions of either saturated or unsaturated fat infusion. This is of interest inasmuch as the metabolic fate of lipoproteins can be influenced by specific apoproteins (12, 13) and might differ as a result of the degree of fatty acid saturation.

This present study describes a model for short term steady-state lipid absorption with comparable mesenteric triglyceride secretion rates for saturated and unsaturated fat absorption. Using this model we have studied whether the degree of fat saturation results in differences in the size and lipid and apoprotein composition of triglyceride-rich particles ($d < 1.006$ g/ml) in rat mesenteric lymph. Our studies show that there is a selective difference in the content of apoB between palmitate and linoleate chylomicrons, despite the constancy of other surface constituents and despite an almost identical particle size. The consequences of these findings are discussed.

METHODS

Animals and operative procedures

Male Sprague-Dawley rats (250–300 g) were fed a conventional rat chow diet (Agway, Inc., Syracuse, NY) containing about 6% fat until the day of operation. With the rats under pentobarbital anesthesia, mesenteric lymph duct cannulation was performed by a modification of the technique described by Bollman, Corin, and Grindlay (14) using a polyethylene catheter (PE 40, Clay-Adams, Inc., Parsippany, NJ). Another polyethylene catheter (PE 90, Clay-Adams, Inc.) was inserted into the duodenum and secured by a purse-string suture. Operated animals were placed in restraining cages (15) with free access to drinking water but no food. Animals were usually operated upon in the afternoon and infused intraduodenally with 0.9% (w/v) saline and 5% (w/v) dextrose at an infusion rate of 7 ml/kg per hr. Animals recovered overnight and lipid infusions were started the next morning.

Mixed micellar solutions

Mixes micelles were prepared according to the method of Ockner, Hughes, and Isselbacher (6). Fatty acids (palmitic, oleic, or linoleic acid; Nu-Chek-Prep, Inc., Elysian, MN) and monoolein (Nu-Chek-Prep, Inc., Elysian, MN) were dissolved to a final concentration of 19.2 mM fatty acid, 19.2 mM monoolein, and 20 mM taurocholate (Calbiochem-Behring, La Jolla, CA). The pH of all micellar solutions was raised to 7.4 and well sonicated to ensure solubilization of fatty acids. Each micellar solution was marked with 10 to 50 μCi of the appropriate fatty acid: either [^{3}H]palmitic acid or [^{3}H]oleic acid (New England Nuclear, Boston, MA) or [^{14}C]linoleic acid (Amersham, Arlington Heights, IL).

Intraduodenal infusion and lymph collection

Basal lymph was collected for 1 to 2 hr immediately before micellar lipid infusion was begun. During this period the dextrose saline solution was infused but drinking water was removed. Thereafter, 5 ml of the mixed micelles was infused over 30 min. Thirty minutes later a constant micellar lipid infusion was begun at an infusion rate of 5 ml/hr of micelles diluted 1:1 with phosphate-buffered saline. This infusion was continued for the next 5–7 hr. One hr before lipid infusion, hourly lymph samples were collected in polyethylene tubes and kept at 30°C until further analysis. In most cases two animals were infused in parellel, one with saturated and one with unsaturated micelles. These paired experiments were carried out to minimize possible day to day variations in experimental conditions. At the conclusion of an experiment, aliquots of lymph were stored overnight at 4°C in order to determine the effect of low temperatures on lipoprotein size (see below). The remaining lymph samples were kept at temperatures above 27°C and analyzed by gel filtration or subjected to ultracentrifugation for further analysis.

Isolation of lipoproteins

After lymph was defibrinated with wooden applicator sticks, aliquots were subjected to a 1×10^8 *g*-min spin in a Beckman L5-75 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) using an SW 50.1 swinging-bucket rotor to obtain the $d < 1.006$ g/ml lipoproteins. This fraction was taken off by means of a tube slicer. In some experiments a 3×10^6 *g*-min chylomicron spin preceded the 1×10^8 *g*-min spin. All centrifugations were carried out at 27–29°C.

Compositional analysis

Triglycerides were determined enzymatically (Worthington Diagnostic Systems, Inc., Freehold, NJ), and phospholipids were determined according to Bartlett (16) after lipid extraction with chloroform–methanol 2:1 (v/v) as described by Folch, Lees, and Sloane Stanley (17).

Apoproteins A-I and B were measured by radioimmunoassay (RIA). For the apoA-I RIA, 10 μg of apoA-I was iodinated by the chloramine T method using 1 mCi of ^{125}I (> 350 mCi/ml; New England Nuclear, Boston, MA) as described previously (18). The RIA was performed by adding 100 μl of sample to 20 μl of a 5% Triton–phosphate-buffered saline albumin solution (PBSA). After a 30-min incubation at 37°C, 300 μl of a solution containing a 1:500 dilution of nonimmune rabbit serum, and 20–30,000 cpm of ^{125}I-labeled apoA-I was added following which 100 μl of antisera to apoA-I was added. After a 48-hr incubation at 4°C, goat anti-rabbit IgG was added. The immune complex was harvested 16 hr later by centrifugation at 3000 rpm in a Sorvall centrifuge, resuspended in PBSA, and recentrifuged. Radioactivity was

measured in a gamma counter (LKB-1270). All samples were assayed in triplicate. The working range of the assay was 1–500 ng of apoA-I. The within-assay coefficient of variation was 3.2% and the between-assay coefficient of variation was 1.2%. Rat plasma LDL (d 1.030–1.050 g/ml) was used as a standard for the apoB RIA, which was similar to the above procedure with the following modifications. Rat plasma LDL was examined on 5.6% SDS polyacrylamide gel electrophoresis and found to contain apoB as the sole apoprotein. The iodination procedure used 20 μg of apoB and was stopped after 10 sec with sodium metabisulfite. A second column of Sephadex G-200 was used in addition to the G-25 column to purify the iodinated LDL. The anti-apoB antibody employed in the assay was raised in rabbits and has been shown to recognize both forms of apoB (apoB-48 and apoB-100). Silicone-treated tubes were used to minimize nonspecific binding of the iodinated apoB. About 7000 cpm was added to each tube in the assay. The working range of the assay was 15–3500 ng of apoB. The within-assay coefficient of variation was 5% and the between-assay coefficient of variation was 10%. Fasting plasma levels of apoB using this assay ranged from 30 to 40 mg/dl.

Electron microscopy

Chylomicron fractions from saturated fatty acid- (palmitate) or unsaturated fatty acid (linoleate)-infused animals were examined by negative stain electron microscopy. All chylomicron preparations were kept at temperatures above 27°C during the staining procedure. Chylomicrons were placed in dialysis bags and dialyzed against 1% osmium tetroxide in phosphate-buffered saline, pH 7.4, for 60 min. Fixed chylomicrons were either examined directly or after pelleting by low speed centrifugation on Formvar-coated grids by negative stain electron microscopy in a Phillips 200 electron microscope. Particles were measured with a micrometer eyepiece in two dimensions and an average diameter was determined.

Density gradient centrifugation

Aliquots of [^{14}C]linoleate and [^{3}H]palmitate chylomicrons were mixed and subjected to density gradient centrifugation on a 30–37% sucrose gradient by a modification of the method of Pinter and Zilversmit (19) as described in detail previously (20). Aliquots of each fraction were analyzed for both ^{3}H and ^{14}C radioactivity in a Beckman LS-350 liquid scintillation counter after appropriate window settings.

Gel filtration studies

Gel filtration was used to obtain chylomicrons without ultracentrifugation and to estimate the size spectrum of triglyceride-rich mesenteric lipoproteins after saturated and unsaturated lipid infusions. Furthermore, the effects of temperature on the gel filtration profile of these lipoproteins was studied. Agarose column chromatography was performed on 4% Sepharose 4B CL (Pharmacia Fine Chemicals, Uppsala, Sweden) on 1.0 × 120 cm columns (Bio-Rad, Richmond, CA). One column was mantled with insulation material and aluminum foil and kept at room temperature (ca. 24°C). Before use the column temperature was raised with prewarmed buffer (35°C containing 0.9% NaCl and 0.01% EDTA, pH 7.0). The effluent temperature always exceeded 27°C. An identical column was kept at 4°C in the cold and buffer of this temperature was used. Flow rates were 10.0 ml/hr for the prewarmed column and 9.5 ml/hr for the chilled column. Fraction volumes were 4 ml. Columns were calibrated with blue dextran as a void volume indicator. Rat plasma and lymph VLDL obtained by sequential ultracentrifugation and checked for homogeneity by agarose lipoprotein electrophoresis served for identification of the gel chromatography elution profiles. Equal aliquots of lymph from [^{3}H]palmitate and [^{14}C]linoleate experiments were pooled and gel-filtered at 27° or 4°C. Column effluents were collected and each fraction was analyzed for both ^{3}H and ^{14}C radioactivity as indicated above.

Chylomicron infusion studies

The clearance of linoleate and palmitate chylomicrons was studied after intravenous bolus injection into recipient rats. Chylomicrons were harvested as described above by ultracentrifugation during periods of steady state infusion of either palmitate or linoleate micelles. The palmitate micellar solution contained 275 μCi of [7-^{3}H(N)]cholesterol (New England Nuclear, Boston, MA) and the linolate micellar solution contained 30 μCi of [4-^{14}C]cholesterol (New England Nuclear, Boston, MA) to label core cholesteryl esters. Chylomicrons were harvested at 3 × 10^{6} *g*-min at 27°C and the creamy layer was aspirated with a drawn pipette. After determining triglyceride enzymatically (see above) an equal triglyceride mass of each chylomicron sample was mixed to give a total of 1.5 to 2.25 mg of triglyceride in a volume of 60–200 μl. This volume was made up to 1 ml with sterile 5% dextrose in normal saline. The chylomicron solution was injected as a bolus via the femoral vein at time zero. Samples of blood (0.6 ml) were removed 5, 10, 15, and 30 min later from the opposite femoral vein. The blood was placed in a microfuge tube containing 9 μl of 5% Na_2 EDTA as an anticoagulant. The blood was centrifuged in a Beckman microfuge for 4 min and a 225-μl aliquot of plasma was counted in 12 ml of Ultraflor (National Diagnostics, Somerville, NJ) as described above.

Statistical analysis

Secretion rates of baseline and steady-state lipid absorption values (hours 3–5) were compared using a two-factor repeated measures analysis of variance. Ratios of d < 1.006 g/ml, VLDL, and chylomicrons were analyzed

by a one-factor analysis of variance. When comparison between only two groups was performed, the two-tailed Student's *t*-test was applied. The size histograms of linoleate and palmitate chylomicron size distributions were analyzed using the *t*-test for large sample sizes (z-test). The plasma half-time of linoleate and palmitate chylomicrons in the recipient rats was calculated with the semi-log linear regression function on a Ti-55II calculator. The slopes of the regression lines were compared using the two-tailed Student's *t*-test.

RESULTS

Initial studies were conducted to validate that steady-state conditions were achieved by the infusion conditions employed. The time course of lymph flow, triglyceride, apoA-I, and apoB secretion was determined after infusions of linoleate, oleate, and palmitate micellar solutions (**Fig. 1**). Constant parameters were achieved 3–5 hr after the start of micellar infusion. During hours 3 to 5 all parameters increased when compared to the basal period. There were no significant differences in lymph flow, triglycerides, apoA-I, or B secretion rates among all groups during hours 3–5, indicating that comparable steady-state conditions had been achieved during this time period. Therefore, further analyses were performed on pooled lymph samples from hours 3 to 5 in a given animal.

Effects of lipid absorption on mesenteric lymph secretion

As shown in **Table 1,** all monitored parameters revealed highly significant increases over basal values during lipid absorption. Of note are the increases in mesenteric transport rates of apoB and, to a lesser extent, of apoA-I. No significant differences in the net secretion rates of apoB and apoA-I during steady state absorption period (hours 3–5) or in the increases over time could be documented among the three groups. There was, however, a tendency toward lower apoB secretion rates in the linoleate group as compared to palmitate despite almost identical triglyceride secretion rates. Since apoA-I is found in mesenteric lymph in HDL particles as well as in triglyceride-rich particles, changes in apoA-I transport rates in whole lymph may not reflect changes that might be present in triglyceride-rich particles of lymph. As also shown in Table 1, there was a highly significant increase in apoA-I transport rates in the d < 1.006 g/ml lymph lipoproteins in all lipid groups over baseline values. No significant differences were found among the three groups during steady-state secretion periods (hours 3–5). It was also of interest to determine the distribution of lymph apoB during the above conditions. During basal conditions approximately 50% of apoB was carried in the d < 1.006 g/ml lipoproteins (d < 1.006 g/ml/whole lymph, 0.49 ± 0.06). During lipid absorption this rose to 67% of apoB carried in the d < 1.006 g/ml lipoproteins with no differences seen among the three infusion groups.

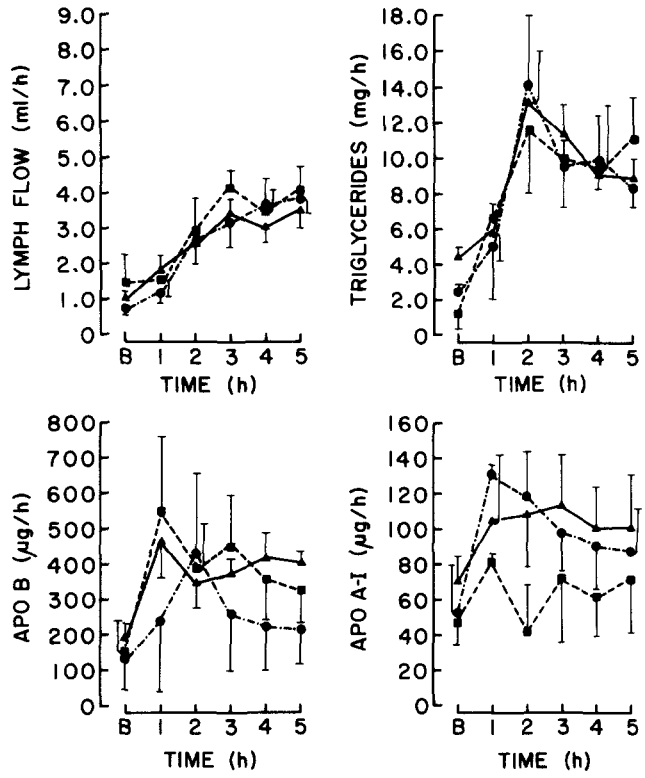

Fig. 1. Mesenteric lymph flow and transport rates of triglycerides, apoA-I, and apoB in rats immediately before and during intraduodenal infusions of micellar lipid solutions. (▲) Palmitate, monoolein; (■) oleate, monoolein; (●) linoleate, monoolein. Data points are mean ± standard error (bars) of four or five animals in each group. B, basal collection period; hours 1–5, intraduodenal lipid infusion. During hours 3 to 5 there was no difference in lymph flow, triglycerides, apoA-I, and apoB among the three lipids studied as calculated by analysis of variance.

Relationship between apoA-I, apoB, and phospholipids (surface constituents) and triglyceride (core) in d < 1.006 g/ml lymph lipoproteins

A major focus of this study was to determine whether significant differences in d < 1.006 g/ml lipoprotein composition exist after saturated and unsaturated fat feeding. D < 1.006 g/ml lipoproteins in lymph from linoleate-, oleate-, and palmitate-treated animals were analyzed for triglyceride, phospholipids, apoA-I, and apoB content. **Table 2** shows ratios of surface to core chylomicron constituents. As can be seen, significantly higher apoB/triglyceride ratios were found in the palmitate group whereas the other surface to core ratios were not significantly different, suggesting selective changes in the apoB content of saturated versus unsaturated chylomicrons.

Since d < 1.006 g/ml lipoproteins of mesenteric lymph

TABLE 1. Mesenteric lymph flow and transport rates of triglyceride, apoA-I, and apoB under basal conditions and during steady state lipid absorption (hours 3-5)

	Whole Lymph				d < 1.006 g/ml
	Lymph Flow	Triglyceride	ApoB	ApoA-I	ApoA-I
	ml/hr	*mg/hr*	*μg/hr*	*μg/hr*	*μg/hr*
Linoleate	(7)	(7)	(7)	(5)	(4)
Basal	0.9 ± 0.2	2.5 ± 0.9	133 ± 9	50 ± 13	23 ± 5
Hr 3-5	3.5 ± 0.4	9.3 ± 2.0	236 ± 133	83 ± 24	41 ± 10
Oleate	(4)	(4)	(4)	(4)	(3)
Basal	1.4 ± 0.8	1.2 ± 0.8	144 ± 89	49 ± 26	14 ± 7
Hr 3-5	3.9 ± 0.7	10.2 ± 2.0	391 ± 138	65 ± 29	34 ± 11
Palmitate	(7)	(7)	(7)	(5)	(3)
Basal	1.3 ± 0.4	4.3 ± 0.4	199 ± 25	72 ± 12	22 ± 4
Hr 3-5	3.0 ± 0.3	9.3 ± 1.1	396 ± 42	105 ± 27	48 ± 12
Total	(18)	(18)	(18)	(14)	
Basal	1.2 ± 0.2	2.9 ± 0.5	157 ± 42	57 ± 9	
Hr 3-5	3.4 ± 0.3	9.6 ± 0.9	342 ± 66	85 ± 14	
Analysis of variance					
Basal vs. hr 3-5	$P < 0.0001$	$P < 0.001$	$P < 0.002$	$P < 0.01$	$P < 0.005$
Linoleate vs. oleate vs. palmitate	NS	NS	NS	NS	NS

Lymph was collected for 1 hr (basal) before the intraduodenal infusion of the indicated micellar solution. Lymph from 3-5 hr (steady-state period) was pooled and the secretion rates of each component were measured (mean ± SEM). Numbers in parentheses indicate the available experimental data for analysis. Differences as calculated by analysis of variance are as indicated. NS, no significant difference.

contain triglyceride-rich particles of varying sizes, this fraction was divided into chylomicron and VLDL fractions by ultracentrifugation and these ratios were re-examined in additional linoleate- and palmitate-infused animals (**Table 3**). Linoleate chylomicrons exhibited a strikingly lower apoB and total surface to core triglyceride ratio as compared to palmitate. A similar apoB/triglyceride relationship was observed when chylomicrons were prepared by gel filtration. VLDL prepared by centrifugation (Table 3) did not show this relationship, indicating that this effect was restricted to large triglyceride-rich particles (i.e., chylomicrons). These data suggest that the relative content of apoB differs between saturated and unsaturated chylomicrons. This appears to be a selective effect since the other compositional data are similar and suggest no differences in particle size. The apoprotein pattern of saturated and unsaturated chylomicrons did not differ. Specifically, both types of chylomicrons contained apoB-48 as the sole apoB species present.

Effect of fatty acid saturation on chylomicron size: electron microscopic examinations

Since the above compositional data suggested that there were no major size differences between saturated and unsaturated chylomicrons, direct electron microscopic measurements were performed to verify these conclusions. Chylomicrons were obtained as stated in Methods and processed above 27°C to avoid temperature-induced artifacts. The size distribution of linoleate and plamitate chylomicrons is shown in **Fig. 2.** Neither the size distribution nor the mean diameters (linoleate: 1058 ± 22 Å, median diameter 925Å, n = 443; palmitate: 992 ± 23 Å, median diameter 850 Å, n = 205) differed significantly

TABLE 2. Relationship between surface (apoA-I, apoB, phospholipids) and core (triglycerides) components of mesenteric d < 1.006 g/ml lipoproteins during steady-state lipid absorption

	N	ApoA-I/TG	ApoB/TG	PL/TG	ApoA-I + ApoB + PL/TG
Linoleate	6	0.013 ± 0.008	0.016 ± 0.003[a]	0.095 ± 0.013	0.116 ± 0.013
Oleate	4	0.005 ± 0.002	0.018 ± 0.003	0.147 ± 0.022	0.170 ± 0.026
Palmitate	8	0.008 ± 0.002	0.039 ± 0.009[a]	0.121 ± 0.009	0.163 ± 0.006

The d < 1.006 g/ml fraction of mesenteric lymph collected during steady state absorption of unsaturated and saturated lipid micelles was analyzed for its major components and surface-to-core ratios were calculated (means ± SEM). N, number of experiments; TG, triglycerides; PL, phospholipids.

[a] $P < 0.05$, by analysis of variance.

TABLE 3. Relationship between surface (apoA-I, apoB, phospholipids) and core (triglycerides) components of mesenteric chylomicrons and VLDL during steady-state lipid absorption

	N	ApoA-I/TG	ApoB/TG	PL/TG	ApoA-I + ApoB + PL/TG
Chylomicrons					
Linoleate	7	0.004 ± 0.001	0.008 ± 0.001[a]	0.088 ± 0.009	0.099 ± 0.008[a]
Palmitate	7	0.005 ± 0.002	0.016 ± 0.003[a]	0.137 ± 0.021	0.160 ± 0.022[a]
VLDL					
Linoleate	4	0.018 ± 0.004	0.044 ± 0.015	0.388 ± 0.111	0.450 ± 0.125
Palmitate	4	0.022 ± 0.006	0.056 ± 0.024	0.291 ± 0.004	0.369 ± 0.019

Whole lymph collected during steady-state lipid absorption was separated into chylomicron and VLDL fractions by ultracentrifugation at 27°C. Ratios of the major surface (apoA-I, apoB, phospholipids) and core (triglycerides) components were calculated. N, number of experiments (mean ± SEM).

[a]Statistical significant differences between groups calculated by analysis of variance, $P < 0.05$.

between the two groups ($P < 0.01$ that particle sizes differ).

Effect of fatty acid saturation on chylomicron density as assessed by density gradient centrifugation

Since the above results indicate relative enrichment of apoB in saturated chylomicrons despite similar particle sizes, it would be predicted that palmitate chylomicrons should exhibit a greater density than linoleate chylomicrons. Compatible with this assumption are the results of sucrose gradient centrifugation of mixtures of [^{3}H]palmitate- and [^{14}C]linoleate-labeled chylomicrons (**Fig. 3**). As can be seen, more palmitate radioactivity remained at the bottom of the gradient when compared to linoleate chylomicrons, suggesting a slightly increased density for palmitate chylomicrons. This difference, while modest, was uniformly observed in each of five separate experiments.

Effect of fatty acid saturation and temperature on gel filtration elution profiles of mesenteric lymph lipoproteins

Since earlier reports of chylomicron size differences utilized low temperatures in lipoprotein preparation, we wished to determine the effects of fatty acid saturation and temperature on the size distribution of mesenteric triglyceride-rich lipoproteins. The elution profiles of whole lymph collected during steady-state absorption of [^{14}C]linoleate or [^{3}H]palmitate micelles (see Methods) were studied by agarose gel filtration. As assessed by thin-layer chromatography, more the 70% of lipid-extractable counts were recovered in triglyceride, validating that the column profiles of radioactivity reflect triglyceride distribution.

Representative elution profiles are shown in **Fig. 4.** A consistent peak of both isotopes eluted with the void volume representing the bulk of transported triglycerides as chylomicrons. When processed at 27°C, chylomicron peaks from saturated and unsaturated fat-fed animals consistently coeluted in the same fractions. The further elution profile formed a shoulder representing mainly VLDL. In contrast, after overnight storage at 4°C, profound changes in the elution profile of saturated fat-containing lymph occurred. The [^{3}H]palmitate peak reproducibly shifted one fraction to the right and a greater percentage of the palmitate label eluted in later fractions. These findings indicate a marked influence of temperature on the gel filtration properties of mesenteric saturated lipid-rich lipoproteins.

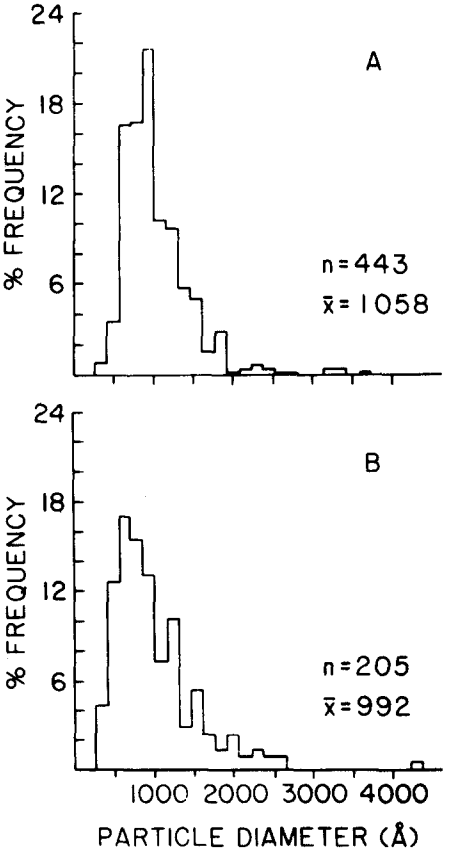

Fig. 2. Size distribution of mesenteric lymph chylomicrons harvested by a 3 × 10^6 *g*-min spin, isolation temperature above 27°C, during steady-state absorption of linoleate monoolein (A) or palmitate monoolein (B) micellar solutions. No statistically significant difference in the size distribution was found.

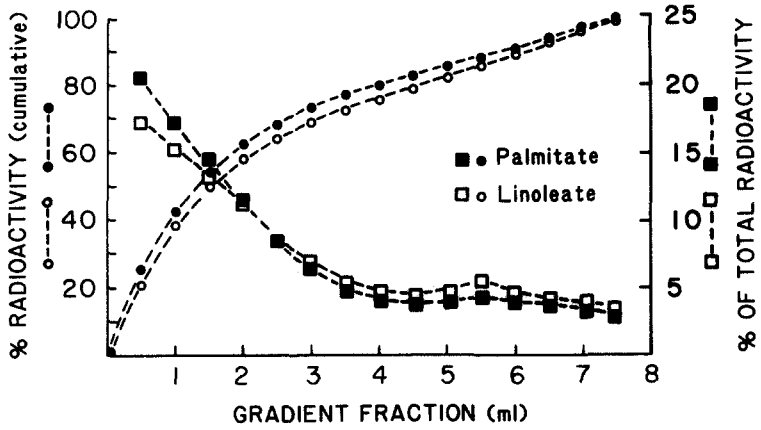

Fig. 3. Sucrose density gradient distribution of [^{3}H]palmitate and [^{14}C]linoleate chylomicrons harvested at 3×10^6 *g*-min and subjected to 30–37% density gradient centrifugation (see Methods). At all stages temperature was kept above 27°C. Fraction 1 is the bottom of the gradient.

Chylomicron infusion studies

Having determined that, when prepared above 27°C, palmitate chylomicrons were of a similar size but enriched in apoB when compared to linoleate chylomicrons, we wished to determine whether these changed altered the metabolism of these particles. We therefore compared the plasma clearance in recipient rats of palmitate and linoleate chylomicrons whose core was marked with [^{3}H]- or [^{14}C]cholesteryl ester (see Methods). As shown in **Fig. 5**, the clearance of palmitate chylomicrons was slightly but significantly more rapid than that of linoleate chylomicrons in four separate experiments.

DISCUSSION

This report extends earlier studies on the effects of saturated and unsaturated fat absorption on intestinal lipoprotein formation. This study differs from many previous studies in that a constant steady-state model of fat absorption was used in all experiments and lipoproteins were handled above 27°C to avoid the recently described effects of low temperatures on saturated lipid crystallization (9, 10). It was important to establish a short-term steady-state model of absorption of different fatty acids since it is known that palmitate absorption requires a greater length of intestine, involving more distal parts of the small bowel (11). Since it is thought that one determinant of particle size relates to the amount of lipid fluxing across a given segment of intestinal mucosa, non-steady-state conditions of triglyceride secretion might affect particle size. The present model achieved comparable lymph flows and triglyceride secretion rates with all the fatty acids tested and minimized effects of variable absorption (Fig. 1, Table 1). In addition, a short-term steady-state infusion model avoids the effects of unphysiological intraduodenal bolus fat loads and decreases the inevitable protein depletion during long-term lymph drainage experiments. Reaching a steady state of triglyceride absorption after 2–3 hr of constant intraduodenal lipid infusion is compatible with other reports in the literature (21, 22) although different amounts of lipid have been used.

The fatty acid composition of the d < 1.006 g/ml triglycerides was not analyzed in detail in this study because

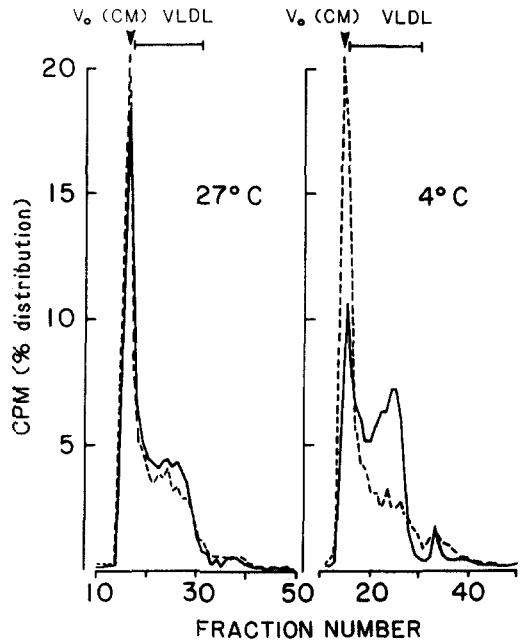

Fig. 4. The effect of temperature on gel filtration of mesenteric lymph lipoproteins. Animals were infused with either palmitate-monoolein (^{3}H-labeled) or linoleate-monoolein (^{14}C-labeled) micelles. Equal volumes of lymph from each animal were mixed and gel-filtered on 4% agarose columns at either 27°C or 4°C (see Methods). The elution positions of lymph chylomicrons and VLDL are indicated. (—) Palmitate; (---) linoleate.

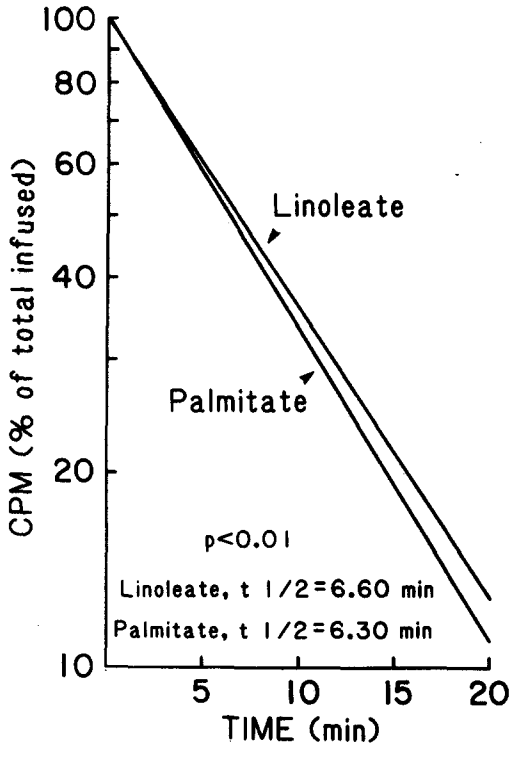

Fig. 5. The clearance of aplmitate and linoleate chylomicrons. Palmitate and linoleate chylomicrons labeled with either [^{3}H]- or [^{14}C]cholesterol were prepared from donor rats (Methods) and infused into recipient rats. The disappearance of radioactivity was followed by tail-vein bleedings at the indicated times.

detailed data using an almost identical infusion schedule have been reported by Ockner et al. (6) showing that chylomicron and, to a lesser extent, VLDL largely reflect dietary fatty acid composition.

The apoA-I secretion rates, both basal and during fat absorption, in the present study are somewhat lower than in other reports (23–25). However, differing lipid infusions and differing methods of apoprotein quantitation make strict comparisons difficult. The relative increases in apoA-I secretion during lipid absorption are in good agreement with other reports (25, 26).

ApoB secretion rates as determined by radioimmunoassay were in close agreement to those reported by Krause et al. (25) using the tetramethyl urea precipitation method. With both saturated and unsaturated lipid, apoB secretion rates markedly increased with lipid absorption (Table 1, Fig. 1). An additional finding of the present study was that even during lipid absorption approximately one-third of lymph apoB was contained in particles of d > 1.006 g/ml. The nature and source of such particles and their metabolic fate require further study. Preliminary studies performed recently have shown that these apoB-bearing particles are mainly IDL and LDL and, at least in part, originate from the intestine (F. Renner, unpublished observations).

With the parameters of this model established, we examined possible change produced in lipoprotein size and composition by fatty acids of different saturation. Several lines of evidence point to the conclusion that saturated lipids such as palmitate do not result in major size changes in the d < 1.006 g/ml lymph lipoproteins. At comparable rates of lymph triglyceride secretion, there was no difference in the relationship of several surface components (i.e., apoA-I, phospholipids) to core triglyceride between saturated and unsaturated fat infusion either in the total d < 1.006 g/ml lymph lipoprotein fraction or in chylomicrons or VLDL when analyzed separately. Direct electron microscopic measurements of diameters of linoleate and palmitate chylomicrons processed above 27°C confirm a similar size distribution. This finding is in contrast with previous reports (4, 5) describing larger particles in mesenteric lymph during unsaturated lipid absorption; however, these studies either were not performed under steady state absorptive conditions (5) or lipoproteins were prepared at low temperatures (4). In addition, gel filtration studies of mixtures of palmitate and linoleate particles showed similar elution profiles when processed above 27°C. Although gel filtration does not allow sizing of chylomicrons eluting with the void volume, the size distribution of smaller particles (i.e., VLDL) carrying exogenous labeled fatty acids can be estimated. As seen in Fig. 4, there was a slight shift towards smaller particles in the VLDL region with palmitate lipoproteins above 27°C; however, this difference was slight and represented only a small percentage of triglyceride mass as judged by radioactivity elution profiles. When lipoproteins were prepared and stored at low temperatures (4°C) marked changes in gel filtration profiles were seen in saturated (palmitate) lipoproteins. Less triglyceride eluted in the void volume with proportionally more material eluting in the VLDL range. These studies are in good agreement with data of Feldman et al. (5), Bennett Clark et al. (10), and Parks et al. (9), showing crystallization of up to 75% of core lipid (palmitate) at low temperatures leading to particle deformation and smaller particles.

A major finding in this study was the difference in the apoB content of mesenteric chylomicrons during steady-state palmitate and linoleate absorption. The fact that particle size of saturated and unsaturated chylomicrons was similar and that other surface constituents were unchanged suggests a specific effect of fatty acid saturation on chylomicron apoB content. It is hard to conclude from our experimental design whether linoleate absorption leads to an apoB depletion or palmitate to an apoB enrichment of chylomicrons. Smaller particles (VLDL) did not show this effect (Table 3). Secretion data (Table 1) suggest that a suppressive effect of linoleate absorption on apoB secretion may be more likely since apoB secretion rates with

palmitate and oleate were similar, while apoB secretion rates with linoleate were lower.

Several explanations for this finding are possible but unproven. It is possible that there are different effects of saturated and unsaturated fat feeding on the intestinal synthesis of apoB. This is the subject of current experiments in our laboratory. Studies in nonhuman primates maintained on chronic unsaturated fat diets suggest a suppressive effect of unsaturated fatty acids on apoA-I synthesis (27); however, no data on apoB are available. In man, a suppression of apoA-I and apoB synthetic rates in a type IIb hyperlipoproteinemic patient on a chronic unsaturated diet has been observed (28). Although it is thought that hepatic apoB synthesis is mainly responsible for such changes, the intestine may have a role in modulation of apoB synthesis as a function of dietary fat saturation. An alternative possibility is that saturated lipid by virtue of its physical properties may associate with different amounts of apoB within the enterocyte. Triglyceride formation from saturated fat is less rapid than from unsaturated lipids and shows less affinity for fatty acid binding protein (29).

Although the precise mechanisms of apoB association with triglyceride are unknown, apolipoprotein-lipid interactions within secretory organelles (i.e., endoplasmic reticulum, Golgi) are undoubtedly important. Changes in membrane fluidity have been described with chronic saturated fat feeding (30) and could alter the association of apoproteins and lipids; however, it is highly unlikely that these changes occur over the short duration of the present study.

The finding that differences in fatty acid saturation influence chylomicron apoprotein content (apoB) is of interest since these changes could result in an altered catabolism of these particles. Chylomicron remnant clearance is believed to be via specific hepatic receptors recognizing mainly apoE on the particle (12, 31). No quantitative data about the apoE content of saturated and unsaturated fat chylomicrons were determined in the present study. It is therefore not clear whether altered fatty acid or apoprotein composition is responsible for the accelerated palmitate chylomicron remnant clearance observed in the present study. The observation, however, agrees with the data of Nestel and Scow (32) describing a more rapid systemic removal of cream-derived chylomicrons as compared to corn oil-derived chylomicrons in dogs and rats. Whether different sites of catabolism are present for saturated versus unsaturated fat chylomicrons was not determined in the present study but is of potential interest. Remnants produced from apoB-enriched particles could result in catabolism by extrahepatic sites and could be a factor in the development of lipid deposition at sites of atherosclerosis. The metabolic fate of these particles requires further study.

In summary, the present studies support the concept that saturated and unsaturated fat have differential effects on the composition and catabolism of intestinal triglyceride-rich lipoproteins.

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