Dietary saturated fatty acid content affects lymph lipoproteins: studies in the rat

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Abstract We examined effects on intestinal absorption of cholesterol and triglycerides and intestinal lipoprotein formation by feeding rats diets in which saturated fatty acids (palmitic plus stearic) comprised 78%, 68%, 48%, or 38% of triglyceride fatty acids. Absorption into lymph of radiolabeled cholesterol was proportional to triglyceride absorption. The rates of absorption of these lipids were related inversely to the % saturated fatty acids fed. The distribution of newly absorbed cholesterol and triglyceride into intestinal lipoproteins differed. With increasing cholesterol absorption more was recovered in very low density lipoproteins in contrast to the appearance preferentially in chylomicrons of larger quantities of fatty acid. Lymph lipid content did not reflect a consistent pattern in relation to the experimental diet fed. The fatty acid composition of triglyceride-rich lymph lipoproteins resembled the diet closely. One-quarter of the intestinal lymph particles from rats fed the highly saturated diets was flattened and polygonal as judged by electron microscopy if cooled to room temperature; whereas with the same diets, particles collected and isolated at 37°C were round. Proportions of A-I and C apolipoproteins in triglyceride-rich intestinal particles varied inversely; apoA-I increased as fat/cholesterol absorption was greater. Diet-induced alterations in plasma lipoproteins and increased circulating triglycerides in this study in rats were unrelated to the variations in intestinal absorption or lymph lipoprotein formation.—**Feldman, E.B., B. S. Russell, R. Chen, J. Johnson, T. Forte, and S. Bennett Clark.** Dietary saturated fatty acid content affects lymph lipoproteins: studies in the rat. *J. Lipid Res.* 1983. **24:** 967–976.

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The fatty acid composition of dietary fat influences the concentration and composition of circulating lipoproteins. In previous studies we fed rats diets of 10–15% triglyceride by weight with 100% or 78% of triglyceride fatty acids as palmitate (16:0), stearate (18:0), oleate (18:1), or linoleate (18:2); we demonstrated specific effects of each fatty acid on levels of plasma lipids and lipoproteins, cholesterol absorption and turnover (1, 2), and lymph lipoprotein (LP) lipids and apolipoproteins (3). Of interest was the observation in electron micrographs that lymph chylomicrons and very low density lipoproteins (VLDL) of rats fed the 16:0 and 18:0 diets were flattened and polygonal in contrast to the round particles obtained in rats fed the unsaturated fats. Additional experiments demonstrated that the chylomicrons and VLDL enriched in saturated fats were secreted as metastable undercooled liquids; with isolation of LP at the usual temperature range of 4–15°C, triglyceride crystallized and lipoprotein structure was altered (4).

The present study was undertaken to determine in rats the level of dietary saturated fatty acids at which these EM physical alterations were observed. We also characterized the effects on intestinal LP formation of defined mixtures of 68%, 48%, or 38% saturated fatty acids compared to the 78% saturated fatty acid diets fed previously. With less than 50% saturated fatty acids in the diet, cooled lymph lipoproteins appeared spherical (3); with 68% saturated fatty acids, we observed phase transitions by differential scanning calorimetry on cooling from >37°C to between 24–20°C, similar to those reported earlier by us (4).

METHODS

Diets

Male Sprague-Dawley rats were obtained as weanlings. They were fed a standard stock diet (Wayne Lab Blox) until they were between 6 and 7 weeks of age. The mean body weights of the four groups of rats studied, 10–16 animals per group, ranged from 169 ± 11 g to 202 ± 15 g. The rats were switched to purified diets (1) with 15% (weight/weight) triglyceride added

Abbreviations: LP, lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; apo, apolipoprotein; EM, electron microscopy.

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to provide 78% saturated fatty acids (tripalmitin, palm oil), 68% saturated fatty acids (palm oil, tripalmitin, high oleic safflower oil), 48% saturated fatty acids (palm oil, tripalmitin, safflower oil), or 38% saturated fatty acids (high oleic safflower oil, tristearin, palm oil). The 78%, 68%, and 38% saturated fatty acid diets also contained 0.7% safflower oil as a source of essential fatty acids. The diet specified was fed for 4 weeks. Food intake, body weight, and levels of plasma cholesterol (5), high density lipoprotein (HDL) cholesterol (6), and triglycerides (7) were monitored. Food was introduced in the late afternoon and withdrawn about 8 AM. Blood samples were obtained at about 11 AM.

Lymph cannulation

On day 31 the major mesenteric lymphatic trunk was cannulated (8) in four to six rats per dietary group; ketamine anesthesia was used. Following surgery the rats were kept in restraint cages. Lymph from rats fed the 78% and 38% saturated fatty acid diets was collected in test tubes kept in an ice bath. The rats fed the 68% and 48% saturated fatty acid diets were kept in a constant temperature room at 37°C after surgery and lymph was collected at that temperature. Collection tubes contained EDTA, NaN_3, and DTNB to achieve final concentrations of 1 g/l, 1 g/l, and 4 mM, respectively. In the immediate postoperative 24 hr, the rats were offered a solution of 10% dextrose and 0.5 N NaCl ad libitum. The next morning the rats were given by gavage 2 ml of an emulsion prepared in Krebs-Ringer phosphate buffer containing 20 mM Na taurodeoxycholate. Per ml the gavages contained: 20 μmol of triglyceride (100% 16:0, 69.1% 16:0, 44.5% 16:0, or 36% 18:0 for the respective four diet groups); 0.25 mg cholesterol; 20 μCi[^{3}H]tripalmitin to trace the predominant fatty acid in the gavages in groups 1–3, [^{3}H]triolein to trace the predominant fatty acid in the gavage for group 4; and 4 μCi[^{14}C]cholesterol, dispersed by sonication. Lymph was collected for 8 hr after the gavage. The specific diet was then reintroduced and lymph was collected for an additional 16 hr while rats consumed about 10 g of food, approximately half their usual daily intake. Data are reported only for rats with lymph flow exceeding 0.5 ml/hr.

Lipoprotein studies

The LP of 10-ml aliquots of the lymph samples was separated by serial preparative ultracentrifugation (4, 9) begun the day of collection. Where samples had been collected on ice, ultracentrifugation was performed at 15°C. Where the samples were collected at 37°C, ultracentrifugation was carried out at that temperature. Aliquots of lymph (9–10 ml) were overlayed with 2–3 ml of a d 1.006 g/ml solution and the chylomicrons were floated in a SW 40 rotor at 2.5×10^6 $g \cdot min$ (41,000 g, 60 min). The top (chylomicron) fraction was harvested and the infranatant was spun in a 50.3 rotor for 1.3×10^8 $g \cdot min$ (120,000 g, 18 hr), using two tubes per lymph sample to yield small intestinal particles (VLDL). The infranatant was spun for 1.3×10^8 $g \cdot min$ (120,000 g, 18 hr) at d 1.063 g/ml to yield low density lipoprotein (LDL), and that infranatant was spun at 3.2×10^8 $g \cdot min$ (120,000 g, 44 hr) d 1.21 to yield HDL. Aliquots of lymph and LP samples were analyzed for total and free cholesterol (5), and triglyceride (7), and LP samples were also analyzed for protein (10). Lymph and LP samples were extracted with chloroform–methanol 2:1 v/v (11). Radioactivity was quantified (12). Triglycerides were separated from other lipid classes by ascending thin-layer chromatography (TLC) on silica gel G (12). Fatty acid composition was determined by gas–liquid chromatography (GLC) of eluted methyl esters using 15% High Eff 2BP (13). Radioactivity was measured by counting the silica gel scrapings in scintillation fluid (12). Apolipoproteins (apoproteins) of delipidated LP were determined by electrophoresis in polyacrylamide disc gels containing 10% sodium dodecyl sulfate (SDS-PAGE, 14). Apoprotein patterns were stained with Coomassie blue and the density of staining was measured by a Beckman densitometer (15). LP and lymph samples were shipped to the Donner Laboratories to arrive within 5 days of lymph collection, and EM was performed (16). Samples that had been collected on ice were shipped on ice; samples collected at 37°C were shipped at that temperature. Temperatures of samples were maintained at 4°C and >27°C, respectively, and measured on receipt of samples. Differential scanning calorimetry of lymph chylomicron and VLDL samples from rats fed the 78% and 68% saturated fatty diets was carried out as described previously (4). These samples were collected and isolated at 37°C and maintained above 29°C during shipment (validated on receipt). The fatty acid composition of aliquots of the diets and gavages was determined in duplicate extracts. The radioactivity and mass administered by gavage were quantitated in duplicate aliquots to determine specific activities.

RESULTS

The fatty acid composition of triglycerides of whole lymph, chylomicrons, and VLDL resembled closely that of the diet fed in samples from all four test diet groups (**Table 1**). The lymph triglyceride fatty acids of rats fed the 48% saturated fatty acid diet resembled the diet most closely. The percent of saturated fatty acids was similar in chylomicrons and VLDL with the exception

TABLE 1. Fatty acid compositions of diets fed and of intestinal lymph and lymph lipoprotein triglycerides

Sample	Diet Group % Saturates	Fatty Acid Percent by Weight[a] 16:0	18:0	18:1	18:2
Diet	78%	77.6[b]	1.2	14.7	5.3
	68%	65.5	2.4	15.8	16.3
	48%	44.8	2.7	22.2	30.2
	38%	13.1	25.3	43.1	16.7
Lymph	78%	56.0 ± 2.0[c]	2.7 ± 0.5	31.2 ± 1.1	7.8 ± 1.4
	68%	45.6 ± 5.0[d]	8.9 ± 0.6	22.3 ± 0.7	14.0 ± 2.5
	48%	36.1 ± 1.0[d]	5.4 ± 0.3	26.7 ± 0.6	26.5
	38%	17.4 ± 2.0[e]	15.9 ± 0.6	50.0 ± 4.0	5.7 ± 1.0
Chylomicrons	78%	62.2 ± 3.4	2.3 ± 0.6	26.8 ± 2.6	6.6 ± 0.6
	68%	46.6 ± 4.0	7.9 ± 2.0	18.4 ± 1.0	16.4 ± 2.0
	48%	39.0 ± 1.0	5.7 ± 1.0	27.8 ± 1.0	19.8 ± 1.0
	38%	14.6 ± 1.0	17.0 ± 3.0	51.5 ± 7.0	5.9 ± 1.0
VLDL	78%	51.9 ± 3.2	3.2 ± 0.5	32.4 ± 3.2	7.9 ± 0.7
	68%	44.2 ± 4.0	10.0 ± 1.0	21.0 ± 1.0	15.0 ± 3.0
	48%	41.0 ± 1.0	9.1 ± 1.0	28.2 ± 0.5	15.5 ± 0.5
	38%	18.3	16.1	48.4	8.6

[a] Triglycerides were separated by TLC and eluted and methyl esters were analyzed by GLC.
[b] Average of two determinations in duplicate for all diets.
[c] Mean ± SE, n = 6 for all samples of 78% diet.
[d] Mean ± SE, n = 3 for all samples of 68% diet
[e] Mean ± SE, n = 3 for all samples of 38% diet, except VLDL which was an average of two samples.

of the 78% diet in which VLDL was less saturated than chylomicrons. The fatty acid composition of lymph collected for 8 hr following the four gavages also resembled that of the diet.

Radiolabeled cholesterol and triglyceride absorptions were correlated significantly with all diets (**Fig. 1**). Radioactivity data were incomplete with the 48% diet be-

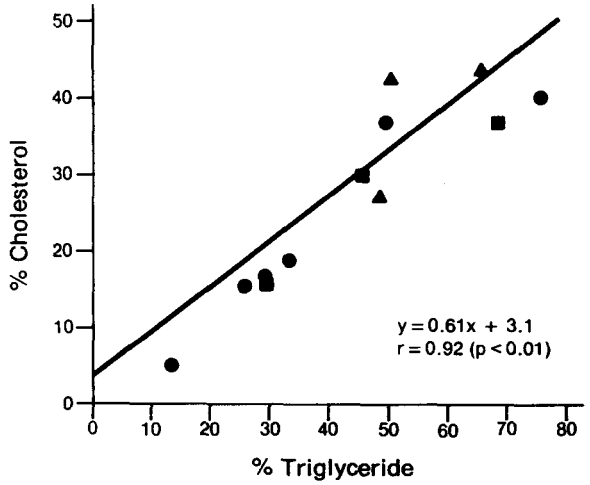


Fig. 1. Lymphatic absorption of cholesterol vs triglycerides: % radiolabeled cholesterol and triglyceride recovered in lymph collected for 24 hr after rats were given gavages of radiolabeled cholesterol and triglyceride, with the diet fed during hours 8–24. The symbols represent the % saturated fatty acids fed: ●, 78%; ■, 68%; ▲, 38%. Each point represents data from one rat. Mean % radiolabeled cholesterol and triglyceride absorptions with each diet are given in the text.

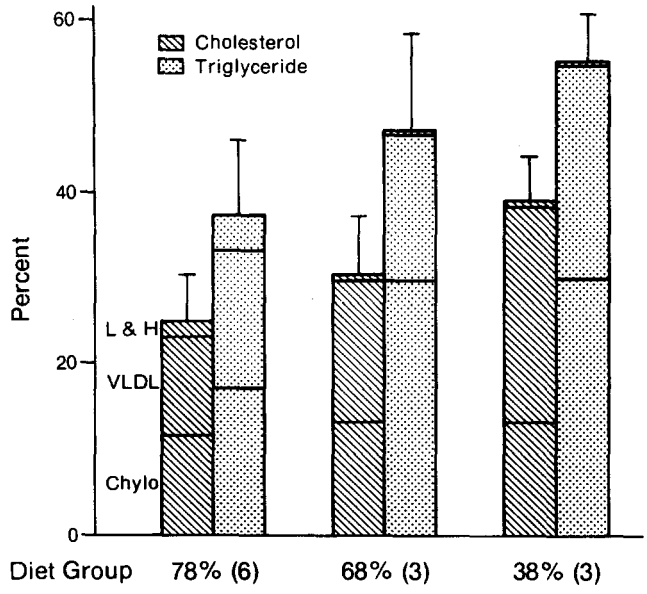


Fig. 2. Absorption of radiolabeled cholesterol and triglyceride into lymph LP in rats fed diets with different percentages of saturated fatty acids. Lymph was collected for 24 hr after gavage of radiolabeled cholesterol and triglyceride, with the specific diet fed from hours 8–24. The height of the left bar of each pair represents the mean % of radiolabeled cholesterol recovered in lymph. The height of the right bar of each pair represents the mean % of radiolabeled triglyceride recovered in lymph. The vertical line represents 1 SE. 78%, 68%, 38% represent the saturation of the dietary triglycerides fed to groups of rats. The numbers in parentheses are the numbers of rats with lymph collections. Chylo, chylomicrons; L, LDL; H, HDL. LP were separated by serial ultracentrifugation. Radioactivity was quantitated in lipid extracts.

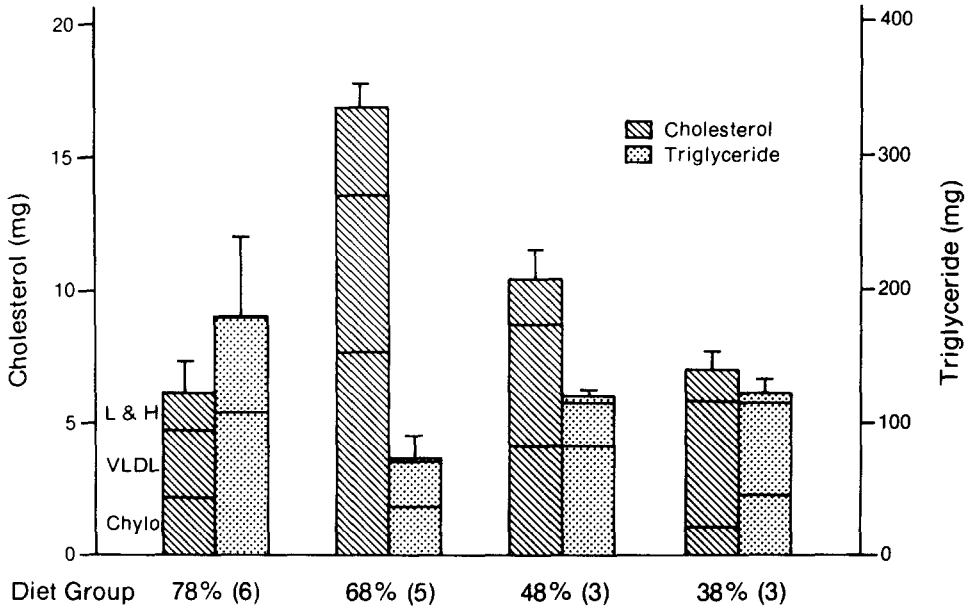


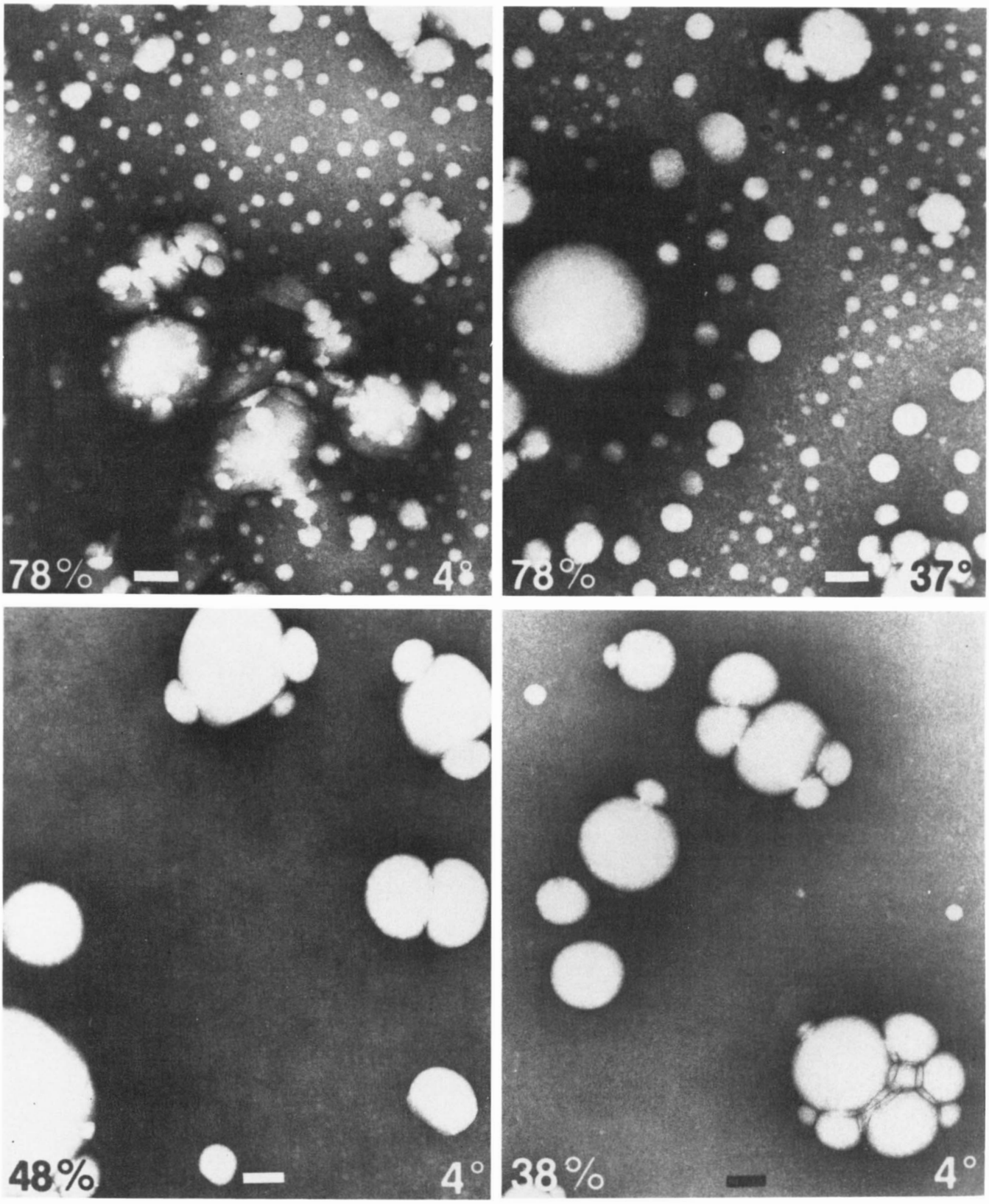
Fig. 3. Distribution of cholesterol and triglyceride in lymph LP of rats fed diets with different concentrations of saturated fatty acids. Lymph was collected for 16 hr from rats fed each diet. The height of the left bar of each pair represents the mean content of cholesterol. The height of the right bar of each pair represents the mean triglyceride content. The vertical line represents 1 SE. See legend to Fig. 2 for abbreviations. The numbers in parentheses are the numbers of rats with lymph collections analyzed. Volumes of lymph collected were (mean ± 1 SE): 78%, 27.1 ± 6.6 ml; 68%, 40.3 ± 7.5 ml; 48%, 23.5 ± 2.8 ml; 38%, 33.7 ± 4.9 ml.

cause of technical problems and are not shown. Cholesterol absorption was 61% of triglyceride, $r = 0.92$ ($P < 0.01$). The percent of radiolabeled cholesterol and triglyceride absorbed into lymph was least with the 78% saturated fatty acid diet (25.4 ± 5.5; 37.5 ± 8.9%, respectively), intermediate with the 68% saturated fat diet (30.4 ± 6.6; 47.3 ± 11.6%), and greatest with the 38% saturated fat diet (40.7 ± 5.2; 54.6 ± 5.4%). As cholesterol radioactivity increased, more radiolabeled cholesterol was found in lymph VLDL vs chylomicrons (**Fig. 2**). As triglyceride radioactivity increased, more label was found proportionally in chylomicrons.

Lymph lipid mass content was variable (**Fig. 3**). Triglyceride levels in lymph were greater with the 78% saturated fat diet compared to each of the other diets ($P < 0.001$, <0.01, <0.01). Cholesterol levels in lymph did not show any pattern of correlation with the percent of saturated fats fed. Lymph cholesterol levels were significantly greater in samples from rats fed the 68% saturated fat diet compared to the 78% saturated fat diet ($P < 0.05$). The distribution of triglyceride into lymph LP varied and showed no dietary pattern. Lymph chylomicron triglyceride levels were greatest with the 78% and 48% saturated fat diets. VLDL triglycerides were greatest in the 78% saturated fat diet, intermediate with the 38% saturated fat diet, and least with the 48% and 68% saturated fat diets. Cholesterol levels in lymph chylomicrons paralleled those in whole lymph; highest levels occurred with the 68% saturated fat diet, intermediate levels with the 48% saturated fat diet, and lowest levels with the 38% and 78% saturated fat diets. VLDL cholesterol levels were correlated less well with total cholesterol in lymph. With the exception of the 68% saturated fat diet, the major portion of lymph LP cholesterol was carried in VLDL.

Chylomicron size increased progressively as the chylomicron lipids became less saturated (**Fig. 4**). VLDL particles were largest with the 78% saturated fatty acid

Fig. 4. Chylomicron morphology as a function of the saturated fatty acid composition of the diet and temperature. Chylomicrons were isolated at 37°C from lymph of rats fed a diet of 78% saturated fatty acids and were negatively stained. Additional samples were then cooled to 4°C. Lymph from rats fed diets of 48% or 38% saturated fatty acids was collected at 4°C; chylomicrons were separated at 15°C and stored at 4°C. The numbers represent the % saturated fatty acids and temperatures. EM size data represent mean ± SD of 100–300 particles. Chylomicrons from the 78% saturated fatty acid diet are smaller in diameter (89.2 ± 39.0, 82.6 ± 40.1, 75.5 ± 34.5 nm, 4°C; 112 ± 57.7, 71.1 ± 40.6 nm, 37°C) compared to the 48% and 38% saturated fatty acid diets (169 ± 69, 128 ± 74 nm; 155 ± 57.6, 151 ± 93.4 nm). Chylomicrons (not shown) from the 68% diet, isolated at 37°C measured 131 ± 51 nm and at 4°C measured 106 ± 53 nm and 104 ± 36 nm. Chylomicrons of the 78% saturated fatty acid diet are round at 37°C but after cooling to 4°C many particles are flattened and polygonal in shape. Chylomicrons of the 48% and 38% saturated fatty acid diets are round at 4°C. Photographs and measurements were made at a magnification of ×60,000. The bar marker represents 100 nm. (Slightly reduced in reproduction.)



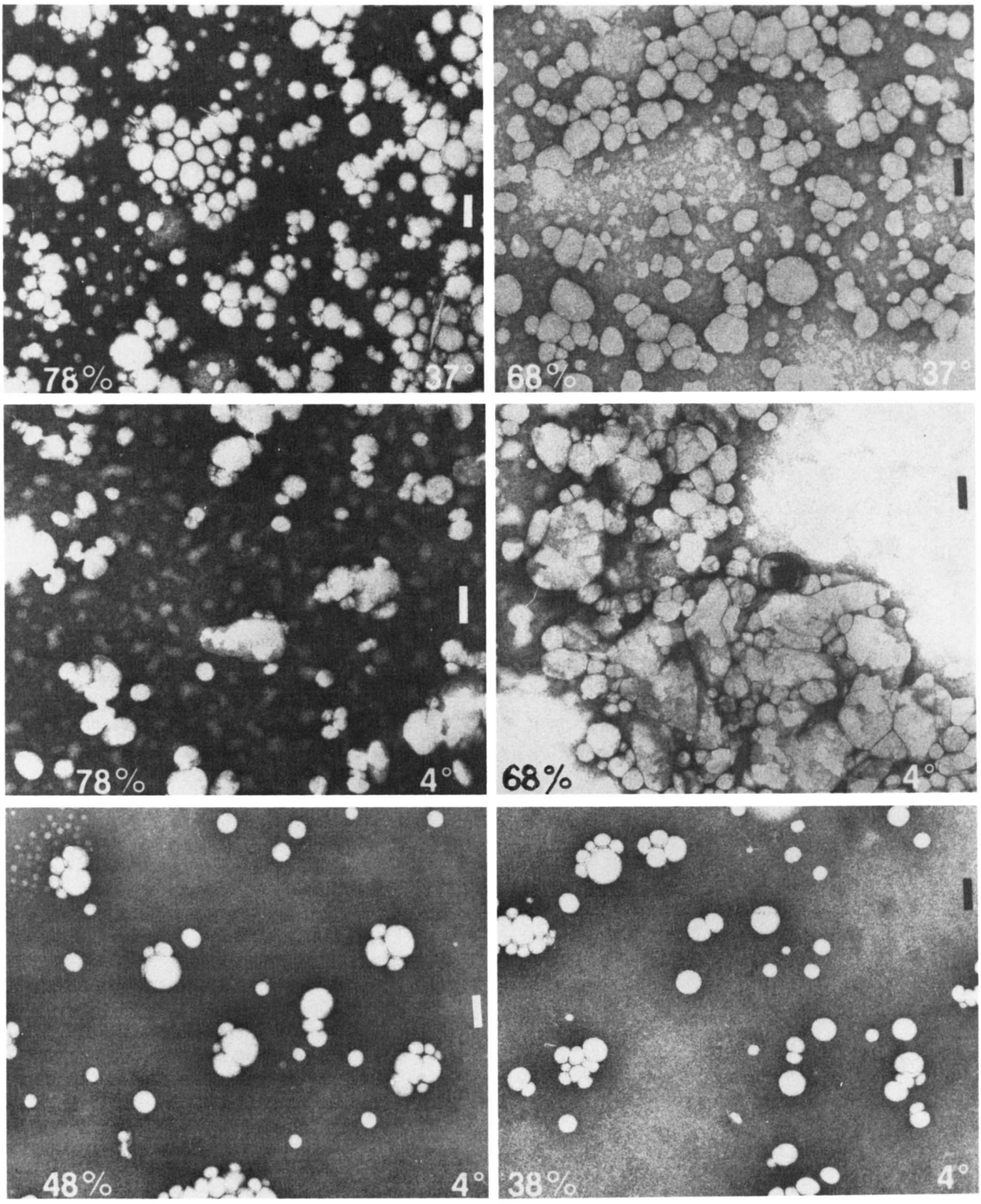


Fig. 5. Morphology of lymph VLDL as a function of the saturated fatty acid composition of the diet and temperature. VLDL were isolated at 37°C from rats fed diets of 78% and 68% saturated fatty acids and were negatively stained. Additional samples were then cooled to 4°C. Lymph from rats fed diets of 48% or 38% saturated fatty acids was collected at 4°C; VLDL were separated at 15°C and stored at 4°C. The numbers

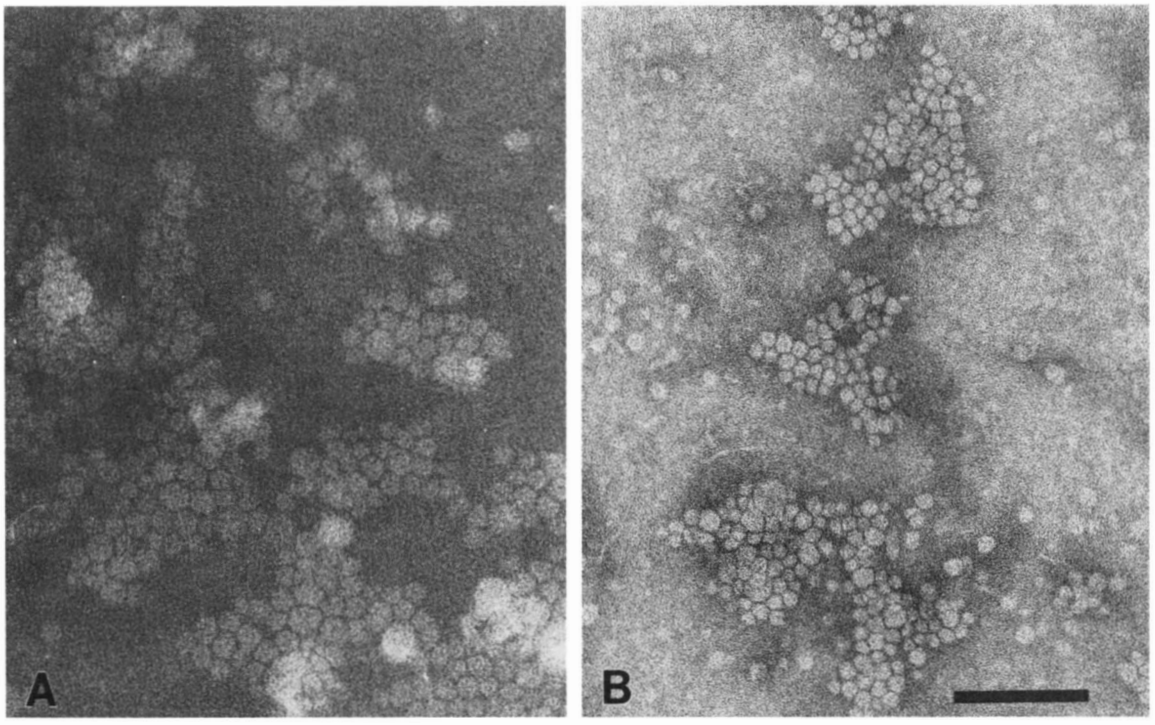


Fig. 6. Electron micrographs of lymph HDL of rats fed diets differing in saturated fatty acid concentration. Lymph was collected at 4°C; HDL were separated at 15°C and stored at 4°C. LP were negatively stained. Round particles were the rule. EM size data represent the mean ± SD of 100–300 particles. A, with the 78% saturated fatty acid diet, particle diameter averaged 12.0 ± 2.1, 11.8 ± 2.3, 11.3 ± 2.2, 11.3 ± 2.1 nm. B, with the 38% saturated fatty acid diet particle diameter averaged 9.6 ± 1.8, 9.5 ± 1.7 nm. Photographs and measurements were made at a magnification of ×160,000. The bar marker on B represents 100 nm and applies to both micrographs.

diet, were intermediate with the 68% diet, and were smaller and of similar size with the two less saturated diets (**Fig. 5**). HDL particles were larger with the 78% and 68% saturated fatty acid diets in comparison to the 38% saturated fatty acid diet (**Fig. 6**). In electron micrographs, chylomicrons and VLDL from the 78% and 68% saturated fatty acid diets that were cooled to 4°C showed flattened polygonal particles in contrast to their round shape at 37°C. These irregular shapes were not observed in cooled triglyceride-rich LP of the 48% or 38% diets (Figs. 4 and 5). Varying proportions of discoidal HDL (17) were seen following all gavages, with the exception of the 48% diet where there was insufficient HDL for studies by EM; round particles, however, were the rule. Lymph chylomicrons obtained from rats fed diets with 78% or 68% saturated fatty acids examined by differential scanning calorimetry (4) were liquid when secreted and crystallized at 20–24°C when cooled from 37°C. The crystallized chylomicron lipids began to melt at 15–23°C on reheating from 2°C, with melting complete at about 60°C.

Apoprotein distributions were examined in lymph VLDL and HDL samples in rats ingesting the four diets (**Table 2**). These data were compared in samples collected and/or stored at 4°C prior to ultracentrifugation. Within each group the distribution of apoproteins was similar in the two collections, after the gavage or while ingesting the diet. The major apoprotein for VLDL samples was apoA-I from rats fed all diets except the 78% saturated fatty acid diet. In the 78% saturated fatty acid diet, C apoproteins predominated in VLDL. In another group of three rats fed the 78% saturated fatty acid diet, lymph was collected over the two time periods and lipoproteins were isolated in samples kept throughout at 37°C. The mean apoprotein composition of those VLDL samples was: A-I, 40%; A-IV, 25%; C's, 18%; and E, 16%. The percent of C apoprotein in VLDL decreased progressively as percent saturated

represent the % saturated fatty acids fed and the temperature. EM size data represent mean ± SD of 100–300 particles. VLDL from the 78% saturated fatty acid diet are larger (79 ± 40.9, 74.8 ± 30.2 nm, 4°C; 59.4 ± 19.3 nm, 37°C) compared to small intestinal particles from the other three diets (70.5 ± 25.1 nm, 4°C; 51.7 ± 17.4 nm, 37°C; 63.7 ± 18.8, 59.6 ± 16.0 nm and 61.9 ± 23.7, 61.3 ± 20.8 nm, respectively). VLDL of the 78% and 68% saturated fatty acid diets are round at 37°C but after cooling to 4°C many particles are large, flattened, and irregular in shape. VLDL of the 48% and 38% saturated fatty acid diets are round at 4°C. Photographs and measurements were made at a magnification of ×60,000 for all samples except 38% saturated fatty acids, ×62,000. The bar marker represents 100 nm. (Slightly reduced in reproduction.)

TABLE 2. Apoproteins of intestinal lymph lipoproteins

Lipoprotein	Diet Group % Saturates	Apoprotein[a] A-I	A-IV	C's	E
		% [b]			
VLDL	78	24	12	52	12
	68	43	10	26	22
	48	59	4	22	15
	38	48	24	20	8
HDL	78	50	12	29	3
	68	66		20	14
	48	66	34		
	38	76	6	12	6

[a] Apoproteins were separated by SDS-PAGE and scanned by densitometry of stained gels.
[b] Average of determinations on two collections each of two or three rats. Lymph samples were collected and/or stored at 4°C prior to ultracentrifugation.

fatty acids in diet and lymph triglycerides declined. VLDL apoA-IV was greatest in samples from rats fed the 38% saturated fat diet. VLDL apoE was greatest in the 68% saturated fat diet. HDL apoprotein patterns showed a progressive increase in apoA-I as the percent of saturated fatty acids declined. This was associated with a decline in apoC's of HDL. HDL apoE was relatively increased in the 68% saturated fatty acid diet. Significant amounts of apoA-IV were observed in HDL from the 48% saturated fatty acid diet.

In comparison with samples from rats that continued to be fed the stock diet (<5% fat), plasma cholesterol was increased only with the 68% saturated fatty acid diet (**Table 3**). This increase in plasma cholesterol was associated with a significant increase in HDL cholesterol. With the other diets, plasma cholesterol either was unchanged or decreased compared to the stock diet, with a decrease in absolute and relative amounts of HDL cholesterol. In rats fed all the high fat diets, plasma triglyceride levels increased from two- to fivefold compared to the stock diet. The highest triglyceride levels were observed with the 68% saturated fatty acid diet. As a result of the plasma triglyceride elevation, the LDL:HDL ratio decreased even though HDL cholesterol declined in general.

DISCUSSION

The present study confirms previous reports from our laboratory (1–3) and by others (18, 19) that cholesterol absorption in rats is greater in the presence of dietary triglycerides with large concentrations of unsaturated fatty acids. We have also demonstrated, in agreement with others, that the increase in exogenous cholesterol absorption is recovered in VLDL (20), whereas more triglyceride is recovered in the chylomicron fraction of lymph LP (21). The fatty acid composition of lymph triglycerides reflected that of the test diet more closely with the more unsaturated fat diets in association with their increased absorption. These differences suggest that the mechanism controlling the "packaging" of cholesterol differs from that for triglyceride, and that chylomicron and VLDL are formed independently in the intestine in response to the amount of triglyceride and cholesterol absorbed.

Only a small portion of the cholesterol transported in lymph was of exogenous origin. Redgrave and Dunne (21) suggested that cholesterol newly synthesized in the ileum may provide the source for the endogenous cholesterol, whereas Vahouny, Fawal, and Treadwell (22) suggested that the increase in lymph cholesterol was derived from bile. Our experiments did not permit identifying the source of lymph cholesterol but indicated a responsiveness of the mechanism to the quantity and nature of dietary fat.

TABLE 3. Plasma lipids

Diet Group % Saturates	Total Cholesterol mg/dl	HDL Cholesterol mg/dl	% Total	LDL:HDL	Triglyceride mg/dl
78%	43 ± 2[a] (13)[b]	22 ± 1[c] (17)	50	0.18	83 ± 9[c] (12)
68%	71 ± 3[cf] (16)	52 ± 7[ef] (5)	73	0/52	190 ± 12[cf] (16)
48%	49 ± 1[cg] (21)	21 ± 1[c] (20)	43	0/21	108 ± 11[c] (21)
38%	57 ± 1[f] (13)	25 ± 1[dh] (13)	43	0.56	88 ± 11[c] (27)
Stock	58 ± 2 (36)	34 ± 3 (9)	59	0.47	39 ± 5 (35)

[a] Mean ± SE.
[b] Number of samples.
[c] $P < 0.001$ compared to the stock diet.
[d] $0.01 > P > 0.001$ compared to the stock diet.
[e] $0.05 > P > 0.02$ compared to the stock diet.
[f] $P < 0.001$ compared to the 78% saturate diet.
[g] $0.02 > P > 0.01$ compared to the 78% saturate diet.
[h] $0.05 > P > 0.02$ compared to the 78% saturate diet.

We had reported the presence of flattened and polygonal-shaped particles in triglyceride-rich lymph LP (chylomicrons and VLDL) collected at 4°C from rats fed diets with 78% or more of saturated fatty acids (16:0 or 18:0) (3). The high content of saturated fatty acids resulted in secretion in vivo of undercooled particles that crystallize at room temperature (below 22°C) (4). In the present study these distorted particles were obtained with cooling when the saturated fatty acid concentration of triglyceride-rich particles exceeded 54%. Florén and Nilsson (23) had probably observed a similar phenomenon in their study of lymph LP in cream-fed rats; they suggested that the saturated triacylglycerols of these chylomicrons had crystallized partially during cooling. Zilversmit (24) also had suggested earlier that chylomicrons from cream-fed dogs are semisolid at body temperature. The fatty acid composition of cream (62% saturated fatty acids) is intermediate between the 68% and 48% saturated fatty acid diets of our experiments. Similarly, Puppione et al. (25) have observed flattened and asymmetric triglyceride-rich lipoproteins in refrigerated samples of lymph and blood of ruminating cattle, where the sum of 16:0 + 18:0 fatty acids represented 78% of the fatty acid mass. In this study and in our previous report (4), we avoided the phase transitions when we collected lymph and isolated LP at 37°C. At that temperature lymph chylomicrons obtained from rats fed the 68%+ saturated fatty acids are undercooled liquids, crystallize when cooled to 24°C, and do not remelt until heated to 60°C. A similar study was reported by Parks et al. (26) with triglyceride-rich LP collected at 39°C in one vervet monkey fed butter fat (61% 14:0 + 16:0 + 18:0). These data suggest that the percentage of saturated fatty acids in the diet required to produce undercooled triglyceride-rich LP particles is intermediate between 48 and 61%, with a ratio of saturated: unsaturated fatty acids between 1 and 2. The exact level is undetermined as yet. We suggest that triglyceride-rich LP for metabolic studies obtained from experimental animals or human subjects fed diets with saturated fatty acids exceeding 50% of total fatty acids should be collected and isolated at temperatures exceeding 24°C. In human subjects ingesting quantities of butter fat, cream, or other highly saturated fats (coconut oil, palm kernel oil, cocoa butter), cooling of LP circulating in blood vessels exposed to environmental cold, such as at the tip of the nose or fingers with resultant crystallization, might have adverse effects. Dr. Puppione has speculated[2] that manatees along the coast of Georgia are prone to hypothermia and death in cold weather is perhaps related to solidifying of highly saturated depot fat making this usual source of energy unavailable for metabolism.

The observation of larger chylomicron particles where triglyceride absorption was greater is in accord with previous studies by ourselves (3) and others (21, 27). The inverse relation of VLDL and HDL to chylomicron size observed in this study also occurred in our previous study on feeding diets of 16:0, 18:0, 18:1, and 18:2 fatty acids at 78%+ of total triglyceride fatty acids.

The apoprotein composition of lymph VLDL and HDL observed by us resembles that reported by others in the rat (28–30). The variation in apoprotein composition of VLDL and HDL with diet suggested that the increase in apoA-I is related to increased fat absorption since apoA-I levels were greater with the diets high in unsaturated fatty acids which were absorbed better. In the rat, the intestine contributes more A-apoproteins to plasma than apoB or C apoproteins (31), and increased apoA-I levels in the proximal intestine have been related to rapid absorption of fat. The cooling of LP fractions with high concentrations of saturated fatty acids may also influence their apoprotein composition. We have collected lymph samples at 37°C from a group of rats fed the 68% saturated fatty acid diet. The samples were separated into two aliquots for LP fractionation, one chilled to 4°C and the other maintained at 37°C. The apoprotein composition of VLDL and HDL differed in the aliquots with reciprocal changes in apoA-I and C apoproteins. Exchange of apoproteins may have occurred at 37°C. The specifics of the lipid and apoprotein content of these samples are detailed elsewhere (32).

Feeding diets differing in fatty acid composition has variable effects on plasma lipids and LP. The results of the present study do not indicate that mechanisms of intestinal absorption or lymph LP formation are responsible for the alterations in circulating LP observed in these groups of rats. Lymph apoprotein formation, partition of cholesterol and triglycerides into lymph LP, and the biophysical (EM) properties of lymph LP were, however, affected by the absorbability of dietary fat and cholesterol into lymph via the intestine.

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[2] Puppione, D.L. Personal communication.

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