Fish oil decreases hepatic cholesteryl ester secretion but not apoB secretion in African green monkeys

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Abstract Two groups of African green monkeys were fed diets containing 40% of calories as fat with half of the fat calories as either fish oil or lard. The fish oil-fed animals had lower cholesterol concentrations in blood plasma (33%) and low density lipoproteins (LDL) (34%) than did animals fed lard. Size and cholesteryl ester (CE) content of LDL, strong predictors of coronary artery atherosclerosis in monkeys, were significantly less for the fish oil-fed animals although the apoB and LDL particle concentrations in plasma were similar for both diet groups. We hypothesized that decreased hepatic CE secretion led to the smaller size and reduced CE content of LDL in the fish oil-fed animals. Hepatic CE secretion was studied using recirculating perfusion of monkey livers that were infused during perfusion with fatty acids (85% 18:1 and 15% n-3) at a rate of 0.1 μmol/min per g liver. The rate of cholesterol secretion was less (P = 0.055) for the livers of fish oil versus lard-fed animals (3.3 ± 0.5 vs. 6.0 ± 1.2 mg/h per 100 g, mean ± SEM) but the rate of apoB secretion was similar for both groups (0.92 ± 0.15 vs. 1.01 ± 0.13 mg/h per 100 g, respectively). The hepatic triglyceride secretion rate was also less ($P<0.05$) for the fish oil-fed animals (8.3 ± 2.5 vs. 18.3 ± 4.4 mg/h per 100 g). Liver CE content was lower ($P<0.006$) in fish oil-fed animals (4.1 ± 0.8 vs. 7.4 ± 0.7 mg/g) and this was reflected in a lower ($P<0.04$) esterified to total cholesterol ratio of perfusate VLDL (0.21 ± 0.045 vs. 0.41 ± 0.06). The hepatic VLDL of animals fed fish oil had 40–50% lower ratios of triglyceride to protein and total cholesterol to protein. From these data we conclude that livers from monkeys fed fish oil secreted similar numbers of VLDL particles as those of lard-fed animals although the hepatic VLDL of fish oil-fed animals were smaller in size and relatively enriched in surface material and depleted of core constituents. Positive correlations between plasma LDL size and both hepatic CE content (r = 0.87) and hepatic VLDL cholesterol secretion rate (r = 0.84) were also found. Therefore, the reduced size of plasma LDL in fish oil-fed compared to lard-fed African green monkeys results, at least in part, from reduced hepatic CE accumulation and the secretion of less CE in the hepatic precursor particles destined to become plasma LDL. **—Parks, J. S., M. D. Wilson, F. L. Johnson, and L. L. Rudel.** Fish oil decreases hepatic cholesteryl ester secretion but not apoB secretion in African green monkeys. *J. Lipid Res.* 1989. **30:** 1535–1544.

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Nonhuman primates consuming diets containing 0.8 mg cholesterol/kcal and 40% of calories as saturated fat become hypercholesterolemic and have increased coronary artery atherosclerosis compared to those animals fed saturated fat with 0.02 mg cholesterol/kcal (1). When monkeys develop hypercholesterolemia, plasma LDL concentrations and LDL size increase (2–4). LDL size is the most powerful predictor of coronary artery atherosclerosis in monkeys explaining from 50–80% of the individual animal variability in extent and severity of disease (1). When LDL size increases the number of cholesteryl ester (CE) molecules per particle increases out of proportion to other chemical constituents of LDL (3). Most of the change in CE number is due to an increase in saturated and monounsaturated CE with little or no change in the number of polyunsaturated species (2, 4–6). Most saturated and monounsaturated cholesteryl esters are thought to be synthesized by the intracellular enzyme, acyl coenzyme A:cholesterol acyltransferase (ACAT) (4). Since plasma LDL arise predominantly from the intravascular catabolism of hepatic VLDL (4), the increased content of saturated and monounsaturated CE in plasma LDL is likely to be derived from the liver via CE secretion in hepatic VLDL.

Isocaloric substitution of dietary fish oil for lard in African green monkeys was associated with decreased plasma LDL size and cholesteryl ester content; however, plasma apoB and LDL particle concentrations remained unchanged (7). The relative CE deficiency of LDL from animals fed fish oil could result from decreased hepatic CE synthesis by ACAT or from decreased plasma CE syn-

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); CE, cholesteryl ester(s); ACAT, acyl CoA:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); IDL, intermediate density lipoprotein(s).

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thesis by lecithin:cholesterol acyl transferase (LCAT). We have recently reported that LCAT utilization of n-3 fatty acids for CE synthesis is poor relative to that for other fatty acids and this may contribute to the formation of the smaller, relatively CE-deficient LDL particles found in plasma of animals fed fish oil (8). On the other hand, little is known about the role of hepatic CE metabolism in determination of the size and CE content of plasma LDL in fish oil-fed animals. In a previous study we have found a strong correlation between plasma LDL size and the rate of accumulation of total cholesterol during perfusions of livers from African green monkeys fed either saturated or n-6 polyunsaturated dietary fat (9). These data suggested an important role for the liver in determining the size and CE content of nonhuman primate plasma LDL. Although it previously has been demonstrated that diets enriched in fish oil reduce hepatic triglyceride secretion (10–16), little is known about the effect of fish oil diets on hepatic cholesterol secretion.

The purpose of this study was to establish interrelationships between plasma LDL size and hepatic CE content and secretion after long-term feeding of diets containing lard or fish oil to African green monkeys. We hypothesized that the decreased LDL size associated with fish oil feeding of monkeys was due to decreased hepatic CE secretion. Given the close similarities in lipoprotein metabolism of the African green monkey model to that in human beings, we have used this species previously to document changes in plasma lipoproteins when dietary fish oil was fed (7, 17) and have also used this animal model to study the effects of saturated and n-6 polyunsaturated fat diets on hepatic lipoprotein secretion (9). Our data suggest that hepatic cholesteryl ester content and secretion are important in determination of plasma LDL size, which is known to be related to the atherogenic properties of these particles in the primate model (1, 2, 4–6).

METHODS

Animals

Twenty four adult male African green monkeys of the grivet subspecies (*Cercopithecus aethiops aethiops*), purchased from Primate Imports (Port Washington, NY), were fed atherogenic diets containing 40% of calories as fat and 0.76 mg chol/kcal for 2.5–3 years before initiation of this study. Detailed compositions of the diets have been published (17). Half of the fat calories of the diets were derived from lard or menhaden oil and half were from egg yolk and egg yolk replacement, a low cholesterol mixture made to resemble the composition of egg yolk (**Table 1**). The percentage distribution of saturated, monounsaturated, and polyunsaturated fatty acids in the diets for all identified fatty acids (95% of total) is also shown in Table 1. Note that the saturated fatty acid percentage was nearly equal for both diets. The animals were fed 30 g diet per kg body weight per day. On average this was equivalent to a daily intake of 545 mg cholesterol for both diet groups and an intake of 3.8 mg of n-3 fatty acids per day for the fish oil group. A subset of (n = 14) of the animals was used for liver perfusion studies.

TABLE 1. Diet compositions

Composition	Lard Diet	Fish Oil Diet
	g/100 g diet	
Protein	21	21
Carbohydrate	45	45
Fat		
Lard	11	
Menhaden oil		11
Dried egg yolk	15	11.3
Egg yolk replacement		3.7
Alphacel and minerals	8	8
	100	100
Fatty acid composition		
% Saturated	38.3	39.7
% Monounsaturated	44.0	34.3
% Polyunsaturated n-6	12.5	9.7
% Polyunsaturated n-3		11.6
Cholesterol[a]	0.340	0.339

[a]Cholesterol concentration of diet constituents: lard (0.95 mg/g), menhaden oil (8.1 mg/g), egg yolk (22 mg/g), and egg yolk replacement (0.37 mg/g). Cholesterol in diet was equivalent to 0.76 mg/Kcal.

Plasma lipoprotein characterization

Blood samples were taken from animals after an overnight (18 h) fast. After administration of ketamine (10 mg/kg) to each animal, blood was taken from the femoral vein and was put into chilled tubes (4°C) containing 0.1% EDTA, 0.02% NaN_3, 0.04% DTNB (final concentrations) at pH 7.4. LDL molecular weight was measured using the agarose column method after isolation of the $d < 1.225$ g/ml lipoproteins from plasma (3). Plasma cholesterol and triglyceride determinations were performed with enzymatic methods using the Technicon RA-500 Analyzer according to method SM4-0139A85 for cholesterol and SM4-0189K87, GPO blank method, for triglyceride (Technicon Instruments Corp., Tarrytown, NY). For cholesterol determinations, Boehringer Mannheim reagents (BMD 236691) were used in place of Technicon reagents. All cholesterol and triglyceride determinations were standardized according to the CDC-NHLBI Lipid Standardization Program (18). HDL cholesterol concentrations were quantitated after precipitation of apoB containing lipoproteins from plasma with heparin-manganese chloride (19).

Liver perfusions

Perfusion of the isolated livers was performed as described previously (9, 20). Donor animals were fed their last meal 18 h prior to liver perfusion. The perfusion medium consisted of Krebs-Henseleit original Ringer bicarbonate buffer, containing D-glucose, amino acids, insulin, hydrocortisone, streptomycin, penicillin, and washed human erythrocytes at 22% hematocrit, pH 7.4. Recirculating liver perfusion was performed with 280–320 ml of medium for 90 min, after which the liver was flushed free of the original perfusate by nonrecirculating perfusion. Recirculating perfusion was then resumed with 280–320 ml of fresh medium for 4 h. All medium changes were performed without interruption of perfusion. Liver color, rate of bile production, rate of oxygen consumption, and rate of perfusate cholesterol accumulation were monitored throughout perfusion to assure the livers remained viable (20).

Oleic acid and a mixture of n-3 fatty acids (EPA mixture 50%) were purchased from the Nu-Chek Prep (Elysian, MN); the latter mixture contained 54% 20:5 and 18% 22:6. To prepare the fatty acid mixture used for liver perfusions, 16.8 g of 18:1 and 5.4 g of the n-3 fatty acid mixture were weighed and placed in an Erlenmeyer flask. Then 730 ml of Krebs-Henseleit original Ringer bicarbonate (KRB) buffer without Ca^{2+} was added to the flask followed by 1.1 equivalents of NaOH. After the solution turned clear the final volume was adjusted to 771 ml to give a 100 mM solution of fatty acid soaps. Fifty-ml aliquots of this mixture were gassed with N_2 and frozen at −20°C until use. All aliquots of this mixture were used within 4 months. Just prior to liver perfusion the fatty acid mixture was thawed and diluted with Ca^{2+}-free KRB buffer that had been adjusted to pH 10.3, to give a final concentration of 10–20 mM. The fatty acid mixture was infused during the 90-min wash-out period as well as during the 4-h second period perfusion. The fatty acid composition of the fatty acid infusate, determined by gas-liquid chromatography (7), was as follows (mean ± SEM): 18:1, 82.9 ± 1.5%; 20:5, 7.8 ± 0.4%; 22:5, 1.9 ± 0.1%; 22:6, 1.5 ± 0.1%; and other, 5.9% (n = 12). The final fatty acid concentration for each perfusion was adjusted to give an estimated perfusion rate of 0.1 μmol/min per g liver using a pump rate of 0.475 ml/min, assuming liver weight was 2.2% of the body weight of the animal. Fatty acid infusion rates, normalized for actual liver weight determined at the completion of perfusion, were 0.109 ± 0.004 and 0.098 ± 0.005 μmol fatty acid/min per g liver for lard and fish oil-fed animals, respectively, and were not statistically different from one another.

Aliquots of liver perfusate were taken every 30 min during the 4 h of perfusion for total cholesterol, triglyceride, and apoprotein analyses. Total cholesterol was assayed by the method of Rudel and Morris (21), triglycerides were assayed enzymatically using the RA 500 autoanalyzer, and apoB concentrations were measured by an enzyme-linked immunosorbent assay (see below). After 4 h of perfusion, the perfusate was collected on ice and adjusted to 0.1% EDTA, 0.1% NaN_3, 0.04% DTNB, pH 7.4. Erythrocytes were removed by low-speed centrifugation and perfusate VLDL were isolated by ultracentrifugation at d 1.006 g/ml as previously described (9, 20).

Immunoassays

Plasma and perfusate apoB concentration was measured with a two-site sandwich enzyme-linked immunosorbent assay, performed essentially as described by us for apoA-I (22). Briefly, apoB isolated from plasma LDL of cynomolgus monkeys (23) was used to immunize goats. IgG were isolated from the antisera by ammonium sulfate precipitation and apoB antibodies were isolated by affinity chromatography (22). An aliquot of the apoB antibodies was taken for conjugation with horseradish peroxidase (24), and other aliquots were diluted for coating the microtiter plates with 500 ng per well. Standards and samples were then added to the coated plates in appropriate dilutions, made in 0.01 M phosphate buffer, pH 7.0, 0.1% Tween 20, 0.1% bovine serum albumin. Each dilution was heated at 37°C for 3 h before plating. Purified African green monkey LDL, of known protein concentration (25) and containing essentially only apoB (>98% as determined by ELISA for other apoproteins) was used as a primary reference standard, and was used to standardize a plasma pool. The coefficient of variation of the apoB assay among 48 separate assays was 8.2%, for an African green monkey plasma pool with an apoB concentration of 87 mg/dl. All perfusion samples were diluted several times and at least three of these dilutions were shown to fall on a line parallel to the LDL standard and the reference plasma. The linear range of the assay was 16 to 128 ng of apoB.

Analytical procedures

Lipids were extracted from livers and lipoproteins by the method of Folch, Lees, and Sloane Stanley (26). Cholesterol content was quantitated by the method of Rudel and Morris (21). Lipid phosphorus was measured by the method of Fiske and SubbaRow (27). Protein content was quantitated by the method of Lowry et al. (25), using extraction with hexane to remove turbidity after color development. Triglyceride assay of liver lipids was done by the procedure of Sardesai and Manning (28). Thin-layer chromatography and fatty acid analyses (7) were performed as described previously.

Statistical analyses

Values are given as the mean ± standard error of the mean (SEM). Statistical comparisons between the two

diet groups were made using the Student's *t*-test. Logarithmic transformation was used for variables that were not normally distributed before using the *t*-test (29). For comparison of rates of accumulation of constituents in the liver perfusate, repeated measures analysis of variance was used (29).

RESULTS

Detailed studies of the plasma lipoproteins of African green monkeys fed the fish oil or lard diets for 8 months have been published previously (7, 17). The studies reported here utilized a subset of the original study group. Some of the lipoprotein characteristics of this subset of animals are given in **Table 2**. The total plasma, IDL + LDL, HDL cholesterol, and plasma triglyceride concentrations for each animal are the mean of the last five to six bimonthly determinations before liver perfusion studies were initiated. The apoprotein concentrations and LDL molecular weight values were determined on one plasma sample taken from each animal within 2 months of the date of necropsy and liver perfusion. Total plasma and VLDL + LDL cholesterol concentrations were 45–50% lower ($P < 0.01$) for the fish oil group compared to the lard group. We have previously shown that <5% of the total plasma cholesterol of both groups of animals was in lipoprotein fractions less dense than LDL (i.e., VLDL, IDL; ref. 17). HDL cholesterol concentrations were 25% lower on average for the fish oil group but this difference was not statistically significant ($P = 0.11$). ApoA-I concentrations in plasma were 37% lower ($P < 0.01$) for the fish oil group but apoB concentrations were not significantly different between diet groups. LDL size was also smaller for fish oil-fed animals (3.25 ± 0.14 vs. 2.57 ± 0.09 g/μmol; $P < 0.01$).

Rates of accumulation of apoB, total cholesterol, and triglyceride in the perfusate were determined during the recirculating perfusion of African green monkey livers. These data are shown in **Fig. 1**. The data were normalized to 100 g liver weight to correct for variations in liver weight among animals. Accumulation rates of apoB, triglyceride, and total cholesterol in the perfusate were calculated from the slope of the line of best fit for the accumulation versus perfusion time plots from 30 min to 240 min. ApoB accumulation in the perfusate was similar for animals fed the lard or fish oil diets (1.01 ± 0.13 vs. 0.92 ± 0.15 mg/h per 100 g liver, respectively). The distribution of apoB among perfusate lipoproteins was also similar for both diet groups with the percentage perfusate apoB in the VLDL fraction averaging 82.5 ± 3.0% for the lard group and 81.6 ± 3.2% for the fish oil group. Assuming that each hepatic VLDL particle had only one apoB molecule, the data suggest that hepatic VLDL particle secretion was the same for both diet groups.

Triglyceride accumulation in the liver perfusate was significantly less ($P < 0.05$) for the livers of fish oil-fed animals compared to their lard-fed counterparts (Fig. 1). The rate of triglyceride accumulation was 18.3 ± 4.4 mg/h per 100 g liver for the lard group and 8.3 ± 2.5 mg/h per 100 g liver for the fish oil group. Total cholesterol accumulation in the perfusate was also less ($P = 0.055$) for the fish oil group (3.3 ± 0.5 mg/h per 100 g liver) compared to the lard group (6.0 ± 1.2 mg/h per 100 g liver).

Hepatic lipid and protein concentrations were quantitated for each of the animals (**Table 3**). The data in Table 3 include seven additional animals (five lard and two fish oil) that were not part of the liver perfusion studies. Hepatic lipid compositions for this subset were similar to those subjected to liver perfusion (n = 14), so the values have been combined in the Table 3. There were no significant differences in hepatic phospholipid, triglyceride, or protein concentrations between the two dietary groups. However, hepatic free and esterified cholesterol concentrations were 30% and 45% lower, respectively, in the fish oil group compared to their lard counterparts.

Fig. 2 shows the interrelationships between hepatic CE concentration with hepatic VLDL cholesterol secretion rate and with plasma LDL size, measured as LDL molecular weight. There was a statistically significant relation-

TABLE 2. Lipoprotein characteristics of African green monkeys fed lard or fish oil diets

	Plasma Cholesterol			Whole Plasma			
Diet	Total	VLDL + LDL	HDL	ApoB	ApoA-I	TG	LDL Mol Wt
			mg/dl				*g/μmol*
Lard (n = 7)	410 ± 56	327 ± 58	83 ± 10	128 ± 19	305 ± 35	13 ± 2	3.25 ± 0.14
Fish oil (n = 7)	225 ± 40	162 ± 44	63 ± 7	100 ± 14	192 ± 13	26 ± 2	2.57 ± 0.09
P Value[a]	<0.01	<0.01	NS	NS	<0.01	<0.01	<0.01

Values are mean ± SEM. Total plasma, VLDL + LDL, and HDL cholesterol values for each animal are the mean of the last five to six bimonthly samples before liver perfusion. Apoprotein concentrations and LDL molecular weight were obtained on a single blood sample for each animal within 2 months of liver perfusion studies.

[a] *P* value from Student's *t*-test after logarithmic transformation of the data; NS, not significant at $P = 0.05$.

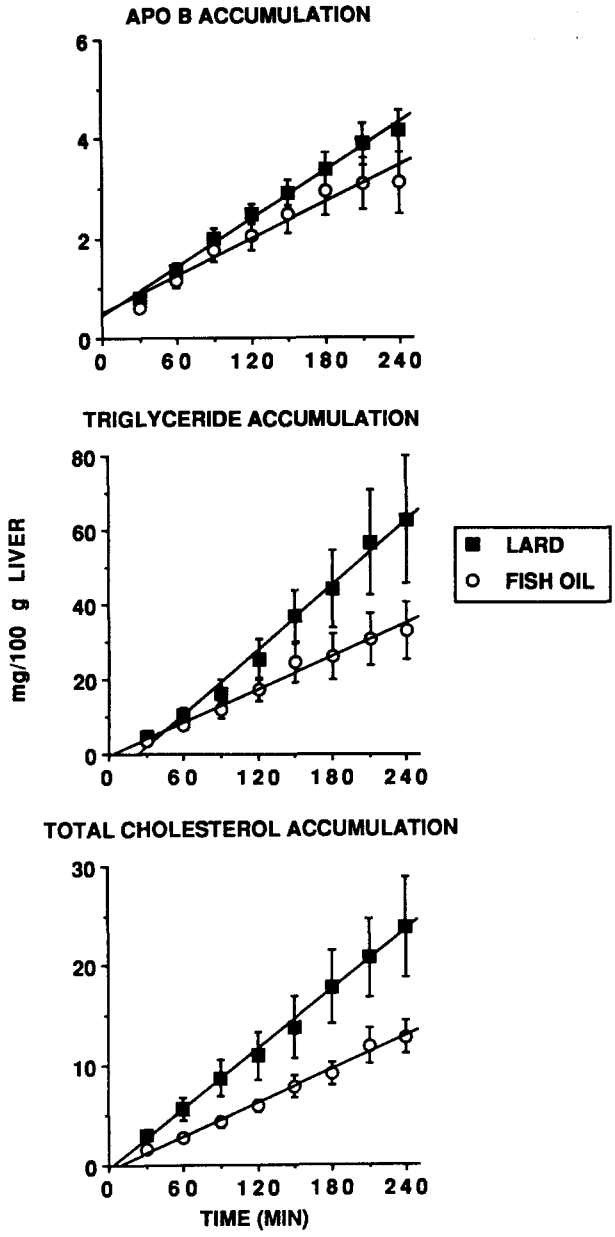

Fig. 1. Accumulation of apoB, triglyceride, and total cholesterol in recirculating liver perfusate of African green monkeys. Aliquots of perfusate were taken every 30 min for analysis during the second period of the perfusion. Details of the liver perfusion and assays are given in the Methods section. Each point represents the mean ± SEM for 5-7 animals in each diet group. The best fit line was determined for each group by linear regression.

ship (r = 0.85; $P<0.01$) between liver CE concentration and VLDL cholesterol secretion (top panel). On average, the VLDL cholesterol secretion rate for the animals fed fish oil (2.42 ± 0.44 mg/h per 100 g liver) was half the rate for the animals fed lard (4.42 ± 0.87 mg/h per 100 g liver; P = 0.063). The percentage of total perfusate cholesterol distributed in VLDL was similar for both diet groups [76 ± 3% (lard) vs. 70 ± 4% (fish oil)]. There was also a statistically significant relationship between hepatic CE concentration and LDL size (Fig. 2 middle; r = 0.90; $P<0.01$). There was also a strong correlation between hepatic VLDL cholesterol secretion and LDL molecular weight (r = 0.81) which can be seen in the bottom panel of Fig. 2. Note that LDL molecular weight was an in vivo plasma measurement determined for each animal prior to liver perfusion while hepatic VLDL cholesterol secretion is an in vitro measurement acquired during liver perfusion. Such good agreement between in vivo and in vitro measurements documents the reliability of the perfusion data.

The chemical compositions of the hepatic VLDL for the two diet groups are given in **Table 4**. In general, the fish oil group had hepatic VLDL that contained a greater percentage of total protein and phospholipid with less cholesteryl ester and triglyceride compared to the lard group. Thus, the hepatic VLDL derived from the fish oil group were relatively enriched in surface constituents and poorer in core constituents compared to their lard counterparts. Based on these data, we would predict that the hepatic VLDL of the fish oil group were smaller in size than the lard-derived particles.

The ratios of hepatic VLDL constituents are presented in **Table 5**. There was a 40–50% reduction in the triglyceride to protein ratio, the esterified cholesterol to total cholesterol ratio, and the total cholesterol to protein ratio for the particles derived from animals fed fish oil compared to those fed lard. The phospholipid to protein ratio of hepatic VLDL was also lower (P = 0.07) for the fish oil group. No significant difference was found for the phospholipid to total cholesterol ratio between the two groups but the free cholesterol to total cholesterol ratio was significantly higher for the fish oil group.

The fatty acid compositions for liver CE and hepatic VLDL CE are shown in **Table 6**. The group fed the fish

TABLE 3. Effect of dietary fat on concentrations of liver lipids and protein

Diet	PL	FC	TG	CE	Protein
			mg/g wet wt		
Lard (n = 12)	30.9 ± 2.0	3.9 ± 0.2	10.0 ± 1.0	7.4 ± 0.7	166.7 ± 7.1
Fish oil (n = 9)	26.1 ± 2.7	2.7 ± 0.2	10.6 ± 2.0	4.1 ± 0.8	170.1 ± 5.7
P Value	NS	<0.01	NS	0.006	NS

Values are given as mean ± SEM; NS, not significant at P = 0.05.

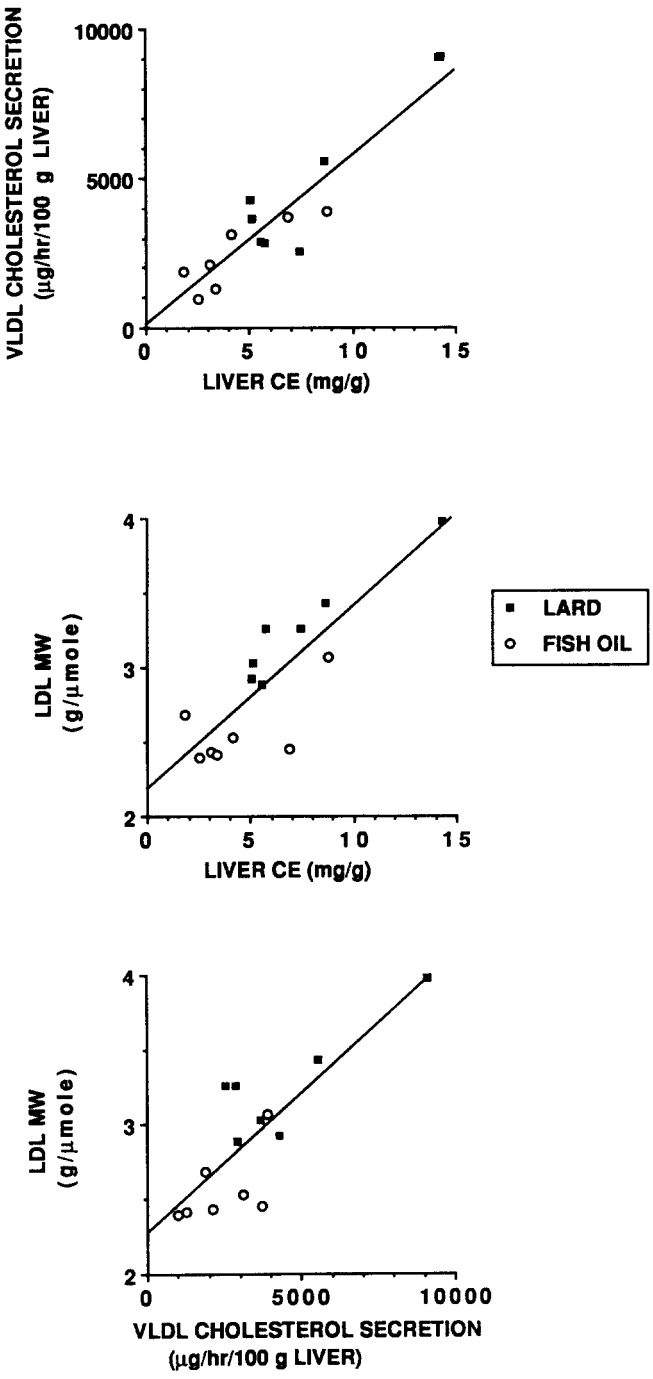

Fig. 2. Relationship of hepatic CE concentration to hepatic VLDL cholesterol accumulation (top); hepatic CE concentration to plasma LDL molecular weight (middle); and hepatic VLDL cholesterol accumulation to LDL molecular weight (bottom). Hepatic VLDL cholesterol accumulation rate was determined between the 30-min and 240-min time periods of the perfusion. LDL molecular weight was determined from a plasma sample within 2 months prior to the perfusion. Each point represents a single animal and the line of best fit for each graph is shown. Statistically significant correlations ($P<0.01$) were found for all three graphs (VLDL cholesterol secretion vs. liver CE, $r = 0.85$; LDL molecular weight vs. liver CE, $r = 0.90$; VLDL cholesterol secretion vs. LDL molecular weight, $r = 0.81$).

oil diet had relatively more 16:1 and n-3 fatty acids (20:5, 22:5, 22:6) and less 18:1 and 20:4 in hepatic CE compared to the lard group. There was no difference in the relative amount of saturated CE species between the two diet groups. In general, similar trends were seen for the CE fatty acid composition of hepatic VLDL from each diet group. Within each diet group, the fatty acid profile between liver CE and hepatic VLDL CE was similar indicating that the cholesteryl esters secreted by the perfused liver were similar in composition to those of the storage pool of CE in the liver. We have previously demonstrated that a low level of LCAT activity accumulates in the perfusates of African green monkey livers (30); therefore, the similarity in composition between the cholesteryl esters of liver and of secreted VLDL is probably because both pools are derived from synthesis by ACAT.

DISCUSSION

The purpose of this study was to establish the interrelationships between hepatic CE metabolism and the smaller size of plasma LDL in African green monkeys fed a diet containing fish oil substituted for lard. Our previous studies in animals fed saturated fat have suggested that the liver is a primary source of the additional CE molecules of enlarged plasma LDL given that they are enriched with cholesteryl oleate, the predominant product of liver ACAT and the predominant cholesteryl ester secreted by the perfused monkey liver (9, 20). Therefore, in the present study we hypothesized that the smaller size of LDL in animals fed dietary fish oil was due to decreased hepatic CE secretion. We found that livers of animals chronically fed fish oil-containing diets secreted the same number of VLDL particles as their counterparts fed lard, but that these VLDL were relatively enriched in surface material and poor in core constituents (i.e., CE and TG) and therefore, were smaller in size than hepatic VLDL of animals fed the lard diet. Since there was a strong correlation between hepatic CE content and hepatic VLDL cholesterol secretion (Fig. 2), the CE depletion of hepatic VLDL of the fish oil group appeared to result from the depletion of the liver CE pool. The smaller size of plasma LDL appears to be a consequence of these events. The reduced rate of CE secretion in hepatic precursor particles destined to become plasma LDL is likely to result in a reduced CE content of mature plasma LDL. The poor utilization of n-3 fatty acids by LCAT for the generation of plasma CE is also likely to result in fewer CE molecules per LDL particle (8). The common denominator for both of these outcomes may be the inability of the enzymes that esterify cholesterol (ACAT and LCAT) to efficiently utilize long chain n-3 fatty acids. In support of this hypothe-

TABLE 4. Effect of dietary fat on hepatic VLDL composition

Diet	Protein	PL	FC	CE	TG
			%		
Lard (n = 7)	10.2 ± 0.6[a]	25.4 ± 1.4	6.4 ± 0.3	9.4 ± 2.8	48.6 ± 2.7
Fish oil (n = 6)	15.3 ± 1.4	31.5 ± 2.1	6.8 ± 0.4	3.8 ± 1.3	42.7 ± 3.7
P Value[b]	0.005	0.03	NS	0.07	NS

[a]Mean ± SEM.
[b]*P* value from Student's *t*-test after logarithmic transformation of the data; NS, not significant at $P = 0.05$.

sis, Rustan et al. (31) have recently demonstrated that, relative to other fatty acids, n-3 fatty acids were not good substrates for hepatic ACAT. Since LDL size has been found to be positively correlated with the extent of coronary artery atherosclerosis in nonhuman primates (1, 2, 4-6), dietary fish oil would appear to prevent atherosclerosis development by decreasing CE enrichment of LDL. In this context, it is important to note that this observation in African green monkeys occurs in a primate species that responds to an atherogenic diet with only a modest degree of hepatic CE accumulation, a situation similar to that in human beings (32).

It is unlikely that our experimental findings resulted from decreased hepatic cholesterol synthesis or changes in apoB particle metabolism. Although we did not measure it, hepatic cholesterol synthesis should have been very low in these animals because of the cholesterol in the diets. In what may be an analogous situation, Spady and Dietschy (33) have shown that diets containing n-6 polyunsaturated fat resulted in more hepatic cholesterol synthesis when compared to diets with saturated fat (coconut oil), even when hepatic cholesterol concentrations were equivalent for diet groups. It also seems unlikely that alterations in intravascular apoB metabolism can explain the smaller LDL particles of the fish oil group. Huff and Telford (34) recently showed that LDL apoB and cholesterol concentrations were reduced in pigs fed fish oil compared to corn oil. This reduction was associated with an increased intravascular conversion of VLDL to LDL and increased rate of catabolism of LDL but less direct hepatic production of LDL in the fish oil group. However, in our study there was no difference in whole plasma apoB concentration (>90% of which was in LDL), in hepatic apoB secretion rate, or in distribution of apoB among liver perfusate lipoproteins between the two diet groups. Although we cannot exclude the possibility that alterations in intravascular apoB metabolism occurred in the animals fed fish oil that resulted in LDL apoB concentrations that were similar to those of the lard group, we have no evidence for such a mechanism. Rather, we favor the interpretation that LDL particle metabolism, including intravascular conversion of hepatic VLDL to LDL, direct hepatic secretion of LDL, and LDL catabolism were similar for both groups, but that the plasma LDL of animals fed fish oil were smaller and the LDL cholesterol concentrations lower because of decreased hepatic secretion of CE and less intravascular synthesis of CE by LCAT (8).

Although the decreased secretion of hepatic triglyceride that accompanies fish oil feeding has been a consistent finding in studies of the effects of fish oil ingestion, the effect on apoB secretion is more controversial. Based on VLDL apoB turnover studies, Nestel and co-workers (12) concluded that plasma VLDL apoB production was decreased in human subjects fed fish oil for 2-5 weeks. However, four of the six subjects in that study had an increased rate of VLDL apoB turnover as well. Wong and Nestel (16) demonstrated that in short term incubations of HepG-2 cells with eicosapentaenoic acid, compared to oleic acid or linoleic acid, a decreased incorporation of [^{3}H]leucine into VLDL apoB occurred. On the other hand, Nossen et al. (13) could find no difference in the extent of incorporation of [^{3}H]valine into immunoprecipitated apoB in cultured rat hepatocytes incubated with eicosapentaenoic acid or oleic acid. In the present study we found no difference in the rate of apoB mass accumulation in total perfusate or in VLDL between livers of the animals fed lard or fish oil even though hepatic cho-

TABLE 5. Ratios of chemical constituents of hepatic VLDL

Diet	PL/Protein	TG/Protein	EC/TC	FC/TC	PL/TC	TC/Protein
Lard (n = 7)	2.52 ± 0.09	4.94 ± 0.52	0.41 ± 0.06	0.59 ± 0.06	2.42 ± 0.35	1.22 ± 0.24
Fish oil (n = 6)	2.12 ± 0.19	2.98 ± 0.43	0.21 ± 0.05	0.79 ± 0.05	3.70 ± 0.49	0.58 ± 0.07
P Value	NS	0.014	0.04	0.03	NS	0.026

Values are given as mean ± SEM; NS, not significant at $P = 0.05$.

TABLE 6. Percentage fatty acid compositions of hepatic and hepatic VLDL CE

	% Composition			
	Hepatic CE		Hepatic VLDL CE	
Fatty Acid	Lard	Fish Oil	Lard	Fish Oil
14:0	1.7 ± 0.3	3.1 ± 0.7	3.0 ± 0.4	2.4 ± 0.4
16:0	19.8 ± 1.7	23.7 ± 1.9	16.2 ± 1.4	17.8 ± 2.0
16:1	2.6 ± 0.1	4.5 ± 0.2[a]	4.5 ± 0.6	5.7 ± 0.4
18:0	8.7 ± 0.3	9.2 ± 0.8	8.6 ± 1.2	8.2 ± 1.2
18:1	46.0 ± 2.5	32.1 ± 2.3[a]	44.8 ± 4.1	38.8 ± 5.2
18:2	4.8 ± 0.3	4.8 ± 0.4	10.5 ± 0.6	8.2 ± 1.0
20:4	1.9 ± 0.2	1.2 ± 0.1[a]	1.4 ± 0.1	1.7 ± 0.1[b]
22:4	1.6 ± 0.2	0.6 ± 0.1[a]		
20:5 (n-3)	0.5 ± 0.1	3.1 ± 0.2[a]	1.0 ± 0.2	3.5 ± 0.4[a]
22:5 (n-3)	2.1 ± 0.5	5.2 ± 0.5[a]	2.1 ± 0.6	2.1 ± 0.8
22:6 (n-3)	0.3 ± 0.03	2.0 ± 0.1[a]	0.5 ± 0.2	1.8 ± 0.3[a]
Other	10.0 ± 0.8	11.3 ± 2.3	7.4 ± 1.5	9.7 ± 2.9

Values are given as mean ± SEM.
[a] $P < 0.01$ by t-test (lard vs. fish oil).
[b] $P < 0.05$ by t-test (lard vs. fish oil).

lesterol and triglyceride secretion were reduced for the fish oil group. In addition, we found no difference in the plasma concentrations of apoB (Table 1) or in the hepatic concentration of mRNA for apoB (J. S. Parks et al., unpublished data). Taken together these data suggest that an extended time of fish oil feeding to African green monkeys did not change the various aspects of apoB metabolism. Instead, the hepatic and intravascular metabolism of neutral lipids appeared to be the major site of action of fish oil on lipoprotein metabolism. One distinct difference in our study compared to those of others was the long-term feeding of the experimental diets (~2.5 yr). Numerous other experimental variables among studies may also explain some the differences observed in hepatic apoB production, but our data are the first to be internally consistent for the outcome both in vivo and in vitro.

African green monkeys fed experimental diets enriched in n-6 polyunsaturated fatty acids had a greater accumulation of hepatic CE than animals fed a more saturated dietary fat (9), even though the plasma cholesterol concentration was lower. Therefore, in the present study, we were surprised to see that CE accumulation was less in the livers of the monkeys fed the fish oil diet compared to their lard-fed counterparts. One potential explanation for this outcome may be that a less efficient utilization of n-3 fatty acids by hepatic ACAT occurred, as suggested by Rustan et al. (31). Alternatively, a decreased efficiency of absorption of dietary cholesterol might have occurred when fish oil was fed, with the result being a decrease in the amount of cholesterol in chylomicron remnants reaching the liver for subsequent metabolism or storage. Still another possibility is that the fish oil-fed animals more efficiently secreted hepatic cholesterol in the bile. However, during liver perfusion with a constant rate of bile acid infusion, we found no difference in the rate of biliary cholesterol secretion between the lard versus fish oil-fed animals of this study (35). In any case, a positive and opposite effect of n-3 versus n-6 fatty acids on hepatic cholesterol accumulation appears to occur that would apparently benefit the liver in maintaining a more normal cholesterol content.

In our earlier study of the effects of n-6 polyunsaturated fat (safflower oil) on hepatic and plasma CE metabolism, the n-6 polyunsaturated fat-fed group had a reduced size and concentration of plasma LDL compared to that of the saturated (butter) fat group (9). There was also a strong correlation between the rate of hepatic cholesterol secretion and LDL size. However, hepatic VLDL of the animals fed safflower oil contained more CE molecules per particle even though, on the mean, the plasma LDL were of smaller size than in the saturated fat group. This outcome is markedly different from that in animals fed fish oil versus lard where the plasma LDL size and the hepatic CE concentration both were lower in the polyunsaturated fat-fed group. Hepatic VLDL of animals fed safflower oil had an average of 3000 CE molecules per particle and would have had to lose CE in the circulation to become a typical plasma LDL with 2000–2500 molecules of CE (6, 7). Hepatic VLDL of animals fed fish oil, however, have an average of 800 molecules of CE per particle and must gain CE in the circulation to have the CE content typical of plasma LDL. These particles can gain CE in plasma through the generation of CE by the LCAT reaction and subsequent transfer of these CE into LDL. However, as mentioned previously, the reactivity of LCAT towards phospholipid substrates containing n-3 fatty acids is relatively low (8). Thus, both secretion and intravascular generation of CE appear to be contributing

to the smaller LDL size in the n-3 fatty acid group. These data point out that several interesting and fundamental aspects of CE metabolism are different between animals fed n-3 versus n-6 fatty acids.

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