Dietary fatty acid modification of HDL phospholipid molecular species alters lecithin:cholesterol acyltransferase reactivity in cynomolgus monkeys

J. Todd Thornburg, John S. Parks, and Lawrence L. Rudel[1]

Department of Comparative Medicine, Bowman Gray School of Medicine of Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157-1040

Abstract In the following report, cynomolgous monkeys, fed atherogenic diets containing either saturated, monounsaturated, polyunsaturated (n-6 Poly) or fish oil (n-3 Poly) fat as 35% of total calories, provide a model for the study of dietary fat effects on plasma lipoproteins and atherosclerosis. We have previously described the ability of polyunsaturated fat diets to lower plasma high density lipoprotein (HDL) cholesterol levels and alter HDL subpopulation distribution in the primate model. These experiments investigate possible mechanisms responsible for such modifications. Animals fed polyunsaturated fat had significantly lower plasma concentrations of HDL cholesterol, total plasma cholesterol, and apolipoprotein A-I. Such changes were reflected in the distribution of protein among HDL subfractions, with the most remarkable modification in subclass distribution being the preponderance of small HDL particles in the n-3 Poly-fed animals. Striking alterations were also observed in the distribution of phosphatidylcholine (PC) molecular species (diet effect $P < 0.0001$ for all major molecular species). Phosphatidylcholine isolated from lipoproteins were used to make recombinant HDL (rHDL) particles. The reaction rate of purified lecithin:cholesterol acyltransferase (LCAT) with particles made from n-3 Poly-derived PC was 50% of that determined using rHDL formed with PC from other dietary groups ($P < 0.0001$). When the distribution of LCAT-derived rHDL cholesteryl esters was analyzed, LCAT demonstrated little selectivity for certain PC molecular species except in n-3 Poly-derived rHDL where 18:2-containing PC was selectively utilized. These data demonstrate that differences in dietary fat intake can significantly alter HDL PC concentration and molecular species distribution. We suggest that diet-induced alterations in HDL PC molecular species modify the type of cholesteryl esters produced during the LCAT reaction thereby affecting the plasma cholesteryl ester pool. We also propose that dietary n-3 Poly affects cholesteryl ester metabolism in part via LCAT by lowering PC (LCAT substrate) availability, altering the rate of the LCAT reaction, and decreasing HDL cholesterol concentrations; however, n-6 Poly dietary fat effects on HDL concentration appear to be through some mechanism other than LCAT.—**Thornburg, J. T., J. S. Parks, and L. L. Rudel.** Dietary fatty acid modification of HDL phospholipid molecular species alters lecithin:cholesterol acyltransferase reactivity in cynomolgus monkeys. *J. Lipid Res.* 1995. **36:** 277–289.

Supplementary key words high density lipoproteins • fatty acid metabolism • cholesteryl esters

This laboratory has developed the use of monkeys fed different types of dietary fat as a model to examine lipoprotein metabolism in atherosclerosis. Several studies have demonstrated that when dietary fat intake is altered, the distribution of plasma cholesteryl ester molecular species changes and that these changes are an important indicator of atherogenic risk (1–4). In particular, an increased ratio of cholesteryl oleate to cholesteryl linoleate appears to be indicative of increased risk of atherosclerosis in African green monkeys (1, 2). We have also reported that when polyunsaturated fats are fed, plasma HDL cholesterol levels are reduced and the subpopulation distribution of HDL-associated protein is altered (5, 6). While the mechanisms underlying such changes are as yet unknown, we have previously suggested that cholesteryl ester (CE) metabolism is central in determining HDL composition.

An important factor in the production of plasma CE is the enzyme lecithin:cholesterol acyltransferase (LCAT). This enzyme utilizes a fatty acyl moiety of phosphatidylcholine (PC) to convert cholesterol to cholesteryl ester (7). HDL particles (8–10) are the best source of substrate lipids for this enzyme. The most likely substrates for the LCAT reaction appear to be newly formed HDL particles. Such nascent particles appear in plasma as small, dense spheres or disks which are secreted by the liver and intestine or are released as redundant surface material from chylomicrons after lipolysis. These nascent particles are thought to act as acceptors of free cholesterol from peripheral tissues (11). Nascent particles are short-lived in plasma and discoidal HDL are rarely identified in whole

Abbreviations: Abbreviations: HDL, high density lipoprotein; PC, phosphatidylcholine; rHDL, recombinant HDL; LCAT, lecithin:cholesterol acyltransferase; CE, cholesteryl ester; apo, apolipoprotein; TLC, thin-layer chromatography.

[1]To whom correspondence should be addressed.

plasma. This is likely because once associated with the HDL particle, cholesterol is converted to cholesteryl ester by LCAT, increasing the lipid content of the particle core, lowering particle density, and converting the disk-like particle into a more spherical HDL. LCAT, therefore, is capable of directly influencing the HDL subfraction distribution, plasma cholesteryl ester molecular species, and may play a key role in dietary modifications of HDL cholesterol levels.

One possible way that dietary fat alters CE availability and HDL particle biochemistry is by altering the composition of the phospholipid substrate pool for LCAT in the HDL precursor particles. In situations using simple phospholipid mixtures, several laboratories have shown that selected PC molecules are variously better substrates for LCAT (12–15). However, little information is available describing the effects of long-term ingestion of different dietary fats on lipoprotein phospholipid composition and how changes in phospholipid composition would alter production of cholesteryl esters via the LCAT reaction. Such studies generally have short-term dietary challenge (16–18) and may not reflect the longer term steady state changes in whole body fatty acid flux as it affects cholesteryl ester molecular species and other plasma lipids. Other studies have utilized radiolabeled cholesterol added to total plasma as a substrate for the LCAT reaction, a situation in which the real LCAT substrate is ill-defined as to particle size, substrate configuration, and localization of radiolabeled cholesterol (16, 17, 19).

The goal of this study was to document the effect of dietary fatty acids of different types on HDL-associated PC and to determine how these effects may alter CE availability for HDL formation by modifying the rate of the LCAT reaction and the products it forms. For these studies we have used discoidal, recombinant HDL particles as the LCAT substrate as this most closely resembles the physiologic substrate of the enzyme. We have chosen to use an experimental primate model of atherosclerosis, the cynomolgus monkey, for these studies because the animals can be maintained for long periods under tight dietary control and the effects of dietary fatty acids on HDL particle biochemistry in this model appear to mimic those in human beings. In this situation, the information derived should have maximal extrapolation to humans.

Results indicate that polyunsaturated fats (n–6 and n–3) decrease plasma levels of apolipoprotein (apo) A-I, apoA-II, HDL cholesterol and HDL-associated PC with respect to animals fed the saturated fat (Sat) diet. Phosphatidylcholine molecular species distribution was significantly altered by diet and a relationship was determined between changes in dietary fat, PC molecular species distribution, and LCAT-derived CE molecular species. The n–3 Poly diet, rich in n–3 polyunsaturated fatty acids, was unique in its capacity to decrease the rate of cholesteryl ester production by the LCAT enzyme. Such changes in the rate of reaction (enzyme reactivity) with only minimal alterations in plasma enzyme activity (a reflection of enzyme mass) were presumably due to the diet's ability to increase the amount of HDL-associated PC containing n–3 fatty acids in the *sn*-2 position. The n–6 poly diet appeared to alter plasma cholesteryl ester abundance and distribution through a separate mechanism.

EXPERIMENTAL PROCEDURES

Animals and diets

Forty two Indonesian cynomolgus monkeys (*Macaca fascicularis*) were purchased and placed in quarantine for 14 weeks during which they received a monkey chow diet. The animals were then fed a challenge diet containing 0.45 mg/kg cholesterol and 35% of calories as fat (lard) for 12 weeks. Four experimental groups each with 10 or 11 animals were then established so that each group demonstrated equivalent means for several reference parameters during the challenge period. These parameters included cholesterol concentrations in whole plasma, low density lipoproteins, and high density lipoproteins, and concentrations of apoB, apoA-I, and apoE. The groups were also equivalent with respect to mean body weight as well as diastolic and systolic blood pressure. Subsequently, all groups were then fed a monkey chow diet for 14 weeks as a wash-out phase during which total plasma cholesterol values returned to within 3% of pre-challenge levels. Each group was then fed one of four experimental diets (Sat, Mono, n–6 Poly, or n–3 Poly). All diets contained 0.4 mg/kcal cholesterol and 35% of calories as fat. The compositions of the different diets are presented in **Table 1** and the fatty acid analysis of the diets is listed in **Table 2.** Blood samples were taken from the animals for lipoprotein analysis after the experimental diets had been fed for at least 18 months. The amount of cholesterol in these diets was targeted to produce average total plasma cholesterol levels between 300 and 400 mg/dl, the range of high risk for premature coronary heart disease in the North American population.

High density lipoprotein isolation

Immediately prior to drawing blood, monkeys were fasted overnight (about 16 h). Beginning at 8:00 AM, ketamine hydrochloride (10 mg/kg) was administered. Ten minutes later, blood was drawn by venipuncture from the femoral vein into chilled tubes containing EDTA and NaN_3 to give final concentrations of 0.1% and 0.02%, respectively. Red blood cells were then removed from plasma by centrifugation at 4°C (1,000 *g*, 30 min). Plasma density was increased to 1.225 g/ml using KBr and the lipoproteins were isolated by centrifugation at 15°C (50,000 rpm, 24 h) in a Beckman Ti 70.1 rotor. Lipoprotein classes were then separated by gel filtration

TABLE 1. Diet composition

Ingredient	All Diets[a]	Experimental Group			
		Sat	Mono	n-6 Poly	n-3 Poly
		g/100 g			
Wheat flour	35.00				
Dextrin	9.60				
Sucrose	10.00				
Casein	9.00				
Lactalbumin	5.00				
Alphacel	7.1				
Hegsted salts	5.00				
Vitamin mixture	2.60				
Vitamin D_3 in corn oil	0.0625				
Fat					
Lard		16.40			8.20
Oleinate-rich safflower oil			16.40		
Safflower oil				16.40	
Fish oil[b]					8.20
Crystalline cholesterol		0.16	0.17	0.17	0.149
β-Sitosterol		0.0746			0.0746
α-Tocopherol		0.0080	0.0026	0.0026	
γ-Tocopherol		0.0082	0.0075	0.0075	
Tenox 20A[c]		0.0082	0.0082	0.0082	

[a] All diets contain a calorie distribution of 35% fat, 48% carbohydrate, and 17% protein.
[b] Fish oil contains 2.6 mg cholesterol, 1.0 mg α-tocopherol, 1.0 mg γ-tocopherol, and 1 mg Tenox 20A per g of oil.
[c] Tenox 20A is an antioxidant.

chromatography using 4% agarose (Bio-Gel A15m, 200–400 mesh) with the elution buffer 0.15 M NaCl containing 0.01% EDTA and 0.01% NaN_3 as previously described (20). The column elution profile generated by monitoring the absorbance at 280 nm indicated the HDL-containing fractions, which were then collected.

Gradient Gel Electrophoresis

Isolated plasma HDL, recombinant HDL samples, and high molecular weight standards were subjected to electrophoresis under non-denaturing conditions in 4–30% polyacrylamide gradient gels (Pharmacia, Piscataway, NJ) as described by Babiak et al. (21). The gels were stained using Coomassie G-250 and scanned using a Zeineh laser densitometric scanner (Biomed Instruments, Fullerton, CA). The densitometric tracings were analyzed according to Verdery et al. (22).

TABLE 2. Fatty acid composition of experimental diets[a]

Fatty Acid	Sat	Mono	n-6 Poly	n-3 Poly
14:0	1.8	0.4	0.4	4.9
16:0	25.0	6.5	7.5	22.2
16:1 n-7	2.3	0.1	N.D.	6.3
18:0	15.1	2.7	2.1	9.9
18:1 n-7 + 9	41.6	72.1	12.2	29.8
18:2 n-6	12.5	17.5	77.5	8.3
20:5 n-3	N.D.	N.D.	N.D.	7.3
22:6 n-3	N.D.	N.D.	N.D.	5.0
Unidentified	1.7	0.7	0.3	6.3

[a] Moles % of total fatty acid; N.D., not detected.

Chemical analysis

Cholesterol concentration in whole plasma was measured by procedure of Rudel and Morris (23) while the cholesterol concentrations in the lipoprotein fractions were determined using cholesterol oxidase (Guilford Diagnostics, Cleveland, OH) (24). Phospholipid phosphorous was determined according to Rouser, Fleischer, and Yamamoto (25). Whole plasma apoA-I and apoA-II concentrations were measured by enzyme-linked immunosorbent assay (ELISA) by the procedure described previously (26).

Preparation of recombinant HDL particles

Monkey lipoprotein PC for use in the formation of the rHDL substrates was isolated by extraction of d < 1.225 g/ml lipoproteins pooled from animals within a diet group and subsequent separation by HPLC using a Phenomenex (Phenomenex, Torrance, CA) Partisil 5 silica column (250 × 4.6 mm). Two solvent mixtures were used to create the mobile phase (A: isopropanol–hexane, 8:6 w/1.5% H_2O; B: isopropanol–hexane, 8:6 w/9% H_2O) with the following gradient system used for elution (45 min total elution time): 0–10 min 85%A:15%B, gradient to 27%A:73%B at 18 min, gradient to 100%B at 25 min and

isocratic system at 100%B to 45 min. For assays designed to determine the amount of LCAT enzyme in plasma, egg PC (Serdary Research, Port Huron, MI) was used as the phospholipid component. ApoA-I was purified from human plasma HDL using AcA 34 size exclusion column chromatography (Spectrum, Los Angeles, CA) (27). The cholate dialysis procedure of Chen and Albers (28) as modified by Parks, Bullock, and Rudel (15) was used to form the rHDL substrates. The [4-^{14}C]cholesterol used for the assays (New England Nuclear, Boston, MA) had a specific activity of 50.3 mCi/mmol. The phospholipid/cholesterol/apoA-I molar ratio for all disks was 106:2.7:1 ± 5% after dialysis. Each rHDL preparation contained approximately 1.6 mCi [4-^{14}C]cholesterol/ml and 50 μl of this preparation was used in each assay.

Lecithin:cholesterol acyltransferase assays

In assays designed to assess LCAT enzyme concentration in plasma, 10 μl plasma and 50 μl rHDL preparation was added to 10 mM β-mercaptoethanol, 2% bovine serum albumin (final concentrations) and buffer (140 mM NaCl, 10 mM Tris, 0.01% EDTA, 0.01% NaN_3, pH 7.4) to give a final volume of 500 μl. When determining the effect of different PC molecular species on LCAT's rate of reaction, incubation conditions were similar except purified LCAT was used. The method of Chen and Albers (29) as modified by Babiak et al. (21) was used to obtain purified LCAT. The LCAT used in these experiments had a specific activity of 2.1 nmol/mg-h of cholesteryl ester formed and was devoid of cholesteryl ester transfer protein activity.

Phospholipid analysis

Phospholipids were extracted according to Bligh and Dyer (30) and class separation was achieved using thin-layer chromatography (TLC) on silica gel H plates (Analtech, Newark, DE) using the solvent system chloroform–methanol–glacial acetic acid–water 65:45:12:6. The phospholipid classes were visualized using primulin spray reagent (1 mg/100 ml in acetone–water 4:1) and eluted from the silica as described by Christianson (31). Recoveries of phospholipid phosphorus from the TLC plate varied between 85% to 90%. Isolated PC was then hydrolyzed to the corresponding mixture of diradylglycerols using phospholipase C from *Bacillus cereus* (Grade 1, Boehringer Mannheim, Indianapolis, IN) followed by conversion to the diradylbenzoylglycerols as described by Blank et al. (32). This mixture was separated into subclasses according to Chabot et al. (33) and the isolated diacylbenzoylglycerols were further separated into molecular species (32). Quantification of the diacylbenzoylglycerols was achieved using a Hewlett-Packard 3390 A integrator. Recoveries from isolated PC through molecular species analysis were routinely > 95%. The HPLC elution profile for the diacylbenzoylglycerols was compared to that described by Blank et al. (32) and the identity of each molecular species was verified by separation of the methyl ester derivatives of fatty acids obtained from each molecular species using the Hewlett-Packard (Kennett Square, PA) 5890 Series 2 gas chromatograph equipped with a 7673 automatic sample injector and a DB 225 fused silica column (J&W Scientific, Folsom, CA) as described elsewhere (34). In peaks containing two molecular species, the contribution of each was determined by calculation after quantitative analysis of the fatty acids. In all procedures described above, lipids and reagents remained immersed in an argon atmosphere throughout. To assure that the values obtained for molecular species were accurate, isolated PC was subjected to saponification and the fatty acid methyl esters were quantitatively measured using gas chromatography. The relative proportions of each fatty acid determined in this manner never differed more than 10% from the value summed from molecular species distributions (data not shown).

Determination of apoA-I α-helical content

The α-helical content of apoA-I associated with rHDL particles was determined by a Jasco 720 spectropolarimeter. The rHDL were in disk buffer containing 140 mM NaCl, 10 mM Tris, 0.01% NaN_3, 0.01% EDTA, pH 7.4, at a concentration of 30 μg apoA-I/ml in an 0.05-cm cell. Scans were from 260 nm to 200 nm.

Statistical analysis

Results are given as mean ± standard error of the mean. Statistical analysis was done using the Statview™ SE+ program for the Macintosh computer. Where appropriate, one-way analysis of variance (ANOVA) with the Fisher's one-way post hoc least significant difference test or repeated measures analysis of variance was done. (The data in Fig. 4 were subjected to linear regression analysis to determine the line of best fit.)

RESULTS

HDL particles

Table 3 presents the dietary impact on the plasma concentrations of HDL particle components. This allows a comparison of the dietary fat effects on each class of molecules making up the HDL. As predicted, diets containing polyunsaturated fatty acids (n-6 Poly and n-3 Poly) were found to significantly lower plasma HDL cholesterol levels when compared to the saturated fat diet. The phospholipid content and amounts of apolipoproteins A-I (apoA-I) and A-II (apoA-II) in plasma (detected by ELISA assay) followed a similar trend. The apoA-I/cholesterol ratio is an estimate of protein-to-neutral lipid ratio and may reflect both particle size and density. In this case the Sat and n-3 Poly HDL would be predicted to be

TABLE 3. HDL particle characteristics

	Sat	Mono	n-6 Poly	n-3 Poly
TPC, mg/dl	423 (32)^{a,c}	318 (50)^{a,b}	293 (38)^{b}	443 (29)^{c}
HDL C, mg/dl	26.9 (1.4)^{a}	23.5 (1.7)^{a,b}	19.1 (2.7)^{b,c}	17.5 (1.1)^{c}
HDL PL, mg/dl	38.0 (3.8)^{a,c}	39.0 (6.4)^{a}	23.0 (5.0)^{b}	26.0 (1.8)^{b,c}
ApoA-I, mg/dl	99.6 (14.4)^{a}	64.4 (8.4)^{b}	52.4 (6.8)^{b}	59.9 (4.9)^{b}
ApoA-II, mg/dl	8.41 (1.31)^{a}	6.22 (0.56)^{a,b}	5.31 (0.78)^{b}	5.38 (0.22)^{b}
A-I/Chol.	3.7	2.7	2.7	3.4
A-I/A-II	11.8	10.3	9.9	11.1
PL/Chol	1.4	1.7	1.2	1.5

All values except ratios, mean (SEM). Values with different superscript are significantly different ($P < 0.05$).

somewhat small denser particles as they have a higher apoA-I/cholesterol ratio. The apoA-I/apoA-II ratio varied little between diet groups although it was slightly higher in the Sat- and n-3 Poly-fed animals, indicating that these particles may have slightly less apoA-II than HDL isolated from animals on the Mono and n-6 Poly diets. The phospholipid to cholesterol ratio showed no clear trend, perhaps due to changes in particle number as well as size distribution.

Upon analysis using non-denaturing 4% to 30% gradient gel electrophoresis, the HDL subfraction distribution was indeed found to vary between diet groups (**Fig. 1**) (P value for diet effect given below each subfraction). The Coomassie-stained gels were analyzed by Gaussian summation analysis as described by Verdery et al. (22). The Mono- and n-6 Poly-fed animals had, respectively, 51% and 49% of HDL protein associated with the HDL 2a and 2b fractions, indicating an increased percentage of larger HDL when compared to the Sat- and n-3 Poly-fed animals which had 39% and 32%, respectively, of HDL protein in the same size range. The greatest difference in size distribution was detected in the HDL from n-3 Poly-fed animals in which over 40% of the HDL protein was associated with small, dense HDL 3b and 3c particles. In general, the concentration of n-3 Poly HDL protein was significantly decreased (see letters above bars) in the larger subfractions (2b, 2a, and 3a) with increasing protein concentrations in the smaller (3b and 3c) particles.

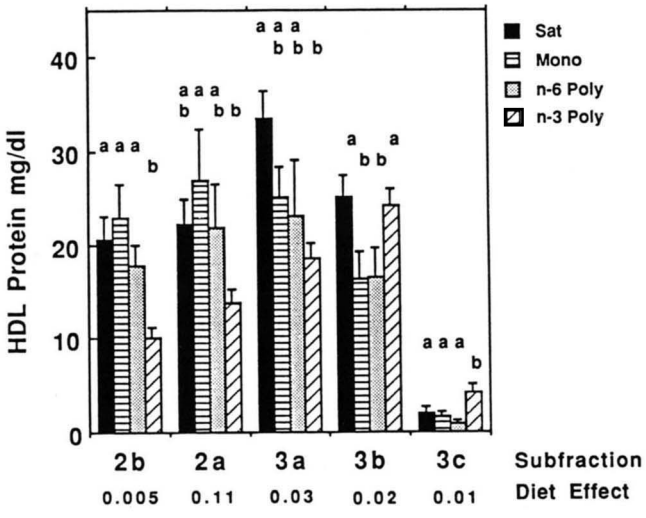

Fig. 1. Relative amounts of protein in different HDL subfractions. HDL were isolated from all monkeys as described in Experimental Procedures. Total HDL protein was measured directly and the concentration of protein in each subfraction was calculated using the percentage area determined by densiometric scanning after separation of HDL subfractions with 4–30% polyacrylamide gradient gels (22). Bars indicate mean ± SEM HDL protein concentrations of each of the five HDL subfractions. The significance of the dietary fat effects on particular HDL subfractions is indicated by P values (ANOVA) given below the subfraction data. Different letters above the bars indicate statistically significant differences ($P \leq 0.05$) in mean protein concentrations between diet groups within a particular subfraction.

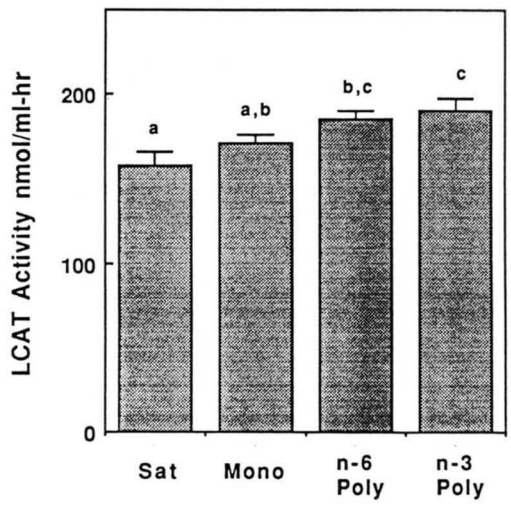

Fig. 2. Plasma LCAT activity indicating circulating levels of enzyme. Ten microliters of plasma from each animal was incubated with rHDL particles (phospholipid:[^{14}C]cholesterol (1600 pmol):apoA-I molar ratio = 106:2.7:1) for 15 min and the amount of esterified cholesterol produced (10–15%) was quantitatively determined by separation of lipids using TLC and the amounts of radioactivity associated with free and esterified cholesterol determined by liquid scintillation spectrometry. Bars indicate mean ± SEM LCAT activity for each diet group. Columns with different letters above them indicate statistically significant differences between means at $P \leq 0.05$ level (ANOVA).

TABLE 4. HDL phospholipid class composition[a]

Class	Sat	Mono	n-6 Poly	n-3 Poly
Lyso PC	1.67 (0.24)[a]	1.85 (0.42)[a]	1.48 (0.27)[a]	1.54 (0.16)[a]
Sph.	5.15 (0.33)[a]	5.87 (0.57)[a]	5.02 (0.43)[a]	6.09 (0.47)[a]
PC	85.7 (0.8)[a]	84.9 (0.9)[a]	86.9 (0.9)[a]	84.6 (0.7)[a]
PS/PI	3.70 (0.28)[a]	3.08 (0.24)[a]	2.99 (0.47)[a]	3.17 (0.38)[a]
PE	3.81 (0.28)[a,b]	4.26 (0.31)[a,b]	3.56 (0.34)[a]	4.64 (0.19)[b]

All values, mean (SEM). Values for each class with different superscript are significantly different ($P < 0.05$).
[a]Moles % of total phospholipid.

LCAT activity in plasma

Effects of dietary fat on plasma levels of the LCAT enzyme were also tested (**Fig. 2**). Plasma LCAT activity was measured using a recombinant HDL (rHDL) substrate made with purified human apoA-I, egg lecithin PC, and radiolabeled cholesterol. Recombinant particles contributed 76% of the free cholesterol and 93% of the phospholipid in the incubation mixture (the balance coming from plasma added as a source of enzyme) so that the measure of activity represented a direct estimate of enzyme mass. Plasma LCAT concentrations were found to be significantly altered by diet (see letters above bars), with measured activity in Sat-fed animals significantly (15%) lower than that measured in the animals fed polyunsaturated fats. LCAT plasma concentrations were significantly higher in the n-3 Poly-fed animals than in the Mono-fed animals while the difference in plasma LCAT concentrations between animals fed the n-6 Poly and Mono diets was not statistically significant.

HDL phospholipids

Dietary fat had little effect on the distribution of the major phospholipid classes as shown in **Table 4.** The predominant phospholipid class of HDL was PC which comprised approximately 85% of all phospholipid regardless of diet. Levels of lysophosphatidylcholine (LPC) were very low in all particles and sphingomyelin levels were constant between diet groups at about 5% of total phospholipid. The only statistically significant change in phospholipid distribution was the increased proportion of phosphatidylethanolamine in the n-3 Poly HDL when compared to the n-6 Poly particles.

Phosphatidylcholine from the HDL was isolated by TLC and hydrolyzed by phospholipase-C to produce the corresponding diradylglycerols. After derivitization, the benzoyldiradylglycerols were then separated into diacyl, 1-*O*-alkyl acyl and alk-1-enyl acyl subclasses by normal phase HPLC. The results are presented in **Table 5.** Although statistically significant differences in certain subclasses were detected among diet groups, the absolute extent of differences were small. It may be of interest, however, to note that 5-6% of the PC found in HDL, regardless of diet, contains a 1-*O*-alkyl linkage.

HDL phosphatidylcholine molecular species

After subclass separation, the benzoylated diacylglycerol subclass was further separated into molecular species using reverse phase HPLC. Changes in dietary fat intake significantly altered the distribution of HDL-associated PC molecular species among each of 18 molecular species shown in **Fig. 3** (diet effect $P < 0.0001$ for each species except 16:0-16:0 where $P = 0.04$). While six other molecular species were identified and quantitatively measured, they comprised less than 1% of the molecular species in each diet group (data not shown). The combined percentage of molecular species with 16:0 in the *sn*-1 position varied significantly (diet effect $P < 0.001$) among diet groups (46.3%, Sat; 45.0%, Mono; 41.3%, n-6 Poly; 52.4%, n-3 Poly) as did the percentage of molecular species with 18:0 in the *sn*-1 position (36.7%, Sat; 26.9%, Mono; 36.8%, n-6 Poly; 44.0%, n-3 Poly). In the Sat, Mono, and n-6 Poly groups, the majority of the isolated PC (81%, 86%, and 87%, respectively) had 18:1 or 18:2 in the *sn*-2 position. This was significantly different from n-3 Poly-derived HDL phospholipid in which only 32% of the PC contains 18:1 or 18:2 in the *sn*-2 position.

In the Sat diet group, the major molecular species in-

TABLE 5. HDL Phosphatidylcholine Subclass Composition[a]

Subclass	Sat	Mono	n-6 Poly	n-3 Poly
Diacyl	93.5 (0.3)[a,b]	93.7 (0.6)[a,b]	94.6 (0.5)[b]	92.4 (0.6)[a]
1-*O*-Alkyl-acyl	5.86 (0.34)[a]	6.05 (0.62)[a]	5.13 (0.55)[a]	6.88 (0.65)[a]
Alk-1-enyl-acyl	0.62 (0.10)[a,d]	0.29 (0.06)[b,c]	0.27 (0.10)[c]	0.76 (0.09)[d]

All values, mean (SEM). Values with different superscripts are significantly different ($P < 0.05$).
[a]Moles % of total phosphatidylcholine.

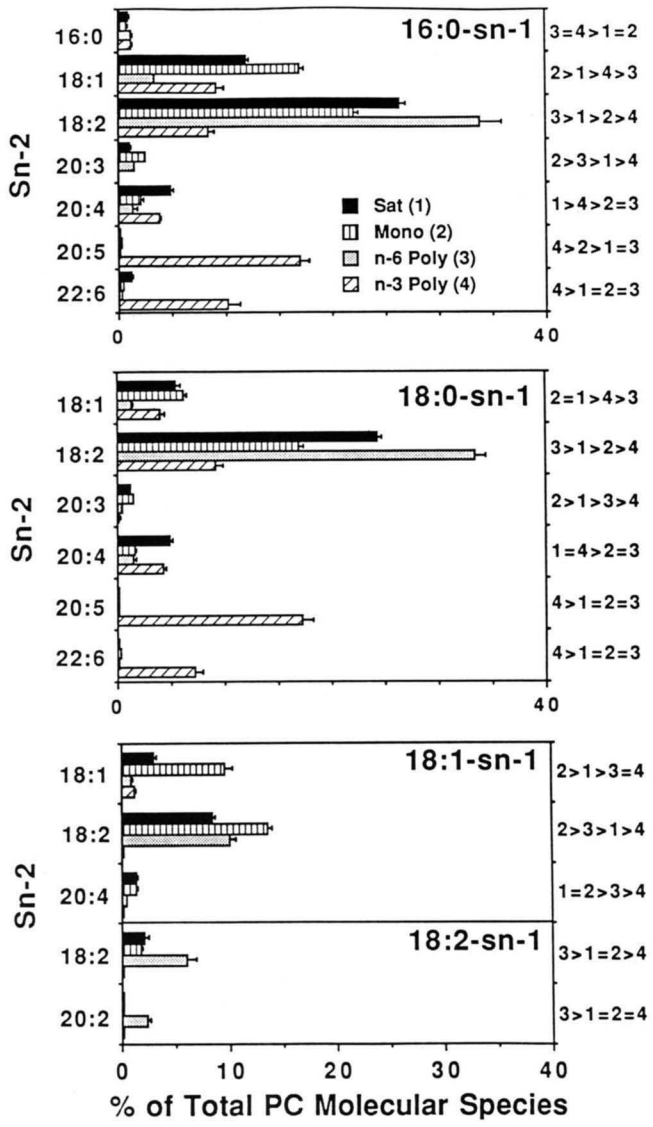

Fig. 3. HDL phosphatidylcholine molecular species. Phosphatidylcholine was purified from isolated HDL and converted to diradylbenzoylglycerols. The diacylbenzoylglycerols were then isolated and further separated into molecular species as described in Experimental Procedures. Bars represent the mean (± SEM) per cent of each molecular species as a proportion of total PC molecular species isolated. Mass of each molecular species was determined using integration of detector response during HPLC separation monitored at 230 nm. Numbers at the right of the graph indicate different diet groups as indicated in figure legend. The > between numbers indicates statistically significant differences between means at $P \leq 0.05$ level (ANOVA). The = symbol indicates $P > 0.05$ between diet groups. Numbers in the top right corner of each panel indicate the fatty acid in the *sn*-1 (terminal glycerol carbon) position of the phospholipid while the numbers at the left of the figure indicate the fatty acid at the *sn*-2 (middle glycerol carbon) position of a particular molecular species of PC. A large subset of animals are represented in the data (Sat, n = 9; Mono, n = 10; n-6 Poly, n = 7; n-3 Poly, n = 11).

cluded 16:0 and 18:0 in the *sn*-1 position, with less 18:1 and 18:2 in this position than for Mono and n-6 Poly HDL PC. Interestingly, however, the reduction of PC containing unsaturated fatty acids in the *sn*-1 position was even more pronounced in the n-3 Poly animals. In Sat-fed animals the predominant *sn*-2 fatty acid was 18:2 (26%, 16:0–18:2; 24%, 18:0–18:2) with about one third as much 18:1, the next most abundant fatty acid in the *sn*-2 position (12.0%, 16:0–18:1; 5.5%, 18:0–18:1). Surprisingly, nearly 15% of the molecular species of the Sat-fed HDL PC had unsaturated fatty acids in the *sn*-1 position.

In HDL PC from Mono-fed animals, 16:0–18:2 and 18:0–18:2 were again the most common molecular species, but the percentage of these was lower compared to the HDL PC of the Sat group. The Mono diet, rich in 18:1-containing triglyceride, increased the proportion of 16:0–18:1 and 18:1–18:1 molecular species relative to the Sat diet ($P < 0.05$) while the amount of 18:0–18:1 was not significantly increased. The amount of PC with 18:1 as the *sn*-1 fatty acid was increased considerably with the Mono diet with over 25% of all molecular species having an unsaturated fatty acid in the *sn*-1 position, significantly higher than all other diet groups. The proportion of molecular species containing 20:3 was also highest in the Mono HDL PC. Interestingly, the relative amounts of 16:0–20:4 and 18:0–20:4 were low in the Mono- as well as n-6 Poly-fed animals.

The n-6 Poly diet, rich in 18:2-containing triglycerides, produced the highest percentage of 16:0–18:2 and 18:0–18:2 PC. The largest decreases resulting from the 18:2 enrichment were in the 18:1-containing molecules. Interestingly, the relative amount of 18:1–18:2 PC was not elevated in the n-6 Poly HDL. The n-3 polyunsaturated fatty acids of the n-3 Poly diet had the most profound effect on the amounts of 18:2-containing molecular species. The appearance of n-3 polyunsaturated fatty acids in the phospholipids of n-3 Poly animals was substantial, with 51% (one of every two molecules) of the PC containing either 20:5 or 22:6 esterified to the *sn*-2 position. Due to the decrease in 18:2-containing molecular species in the n-3 Poly group, the total proportion of PC containing polyunsaturated fatty acids was lower in this group than the n-6 Poly group (82% and 91%, respectively, $P < 0.05$). The reduction of 18:2 molecular species was consistently of greater magnitude than the reduction of 18:1 molecular species. The molecular species distribution of the 1-*O*-alkyl-2-acyl PC was also measured with similar diet effects being observed (data not shown).

LCAT reactivity

Isolated lipoproteins from each diet group ($d < 1.225$ g/ml) were extracted, and the purified PC was used to form recombinant HDL (rHDL) particles. To assure that any apparent difference in the rate of reaction would be due to changes in the composition of phospholipid molecular species and not to particle characteristics such as size or protein conformation, rHDL particles were subjected to 4% to 30% gradient gel electrophoresis. Although the particles formed were heterogeneous in size,

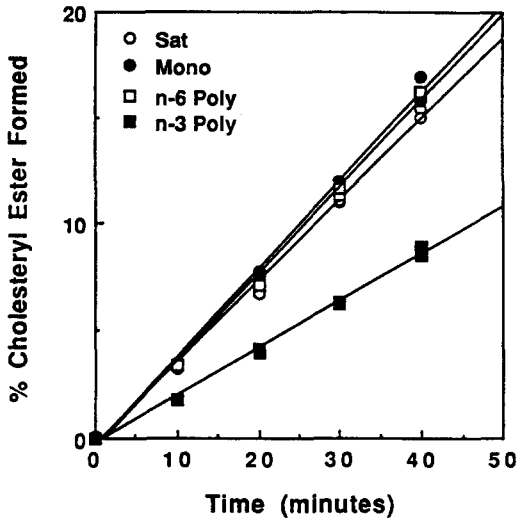

Fig. 4. The rate for LCAT-catalyzed cholesteryl ester production using rHDL made with PC purified from HDL isolated from the plasma of animals fed different dietary fats. HDL phosphatidylcholine was isolated from four animals in each diet group and used to make rHDL particles as described in Experimental Procedures. The rHDL particles (phospholipid:[^{14}C]cholesterol (1600 pmol):apoA-I molar ratio = 106:2.7:1) were incubated with purified human LCAT for the indicated times and the amount of esterified cholesterol produced was determined as indicated in Fig. 2. Points indicate the values of duplicate experiments. Data were subjected to linear regression analysis to determine the line of best fit and repeated measures analysis of variance was used to detect statistically significant differences between rates (see text).

this examination revealed that the particle size distribution was essentially the same for disks made from PC isolated from each diet group. Most of the particles (87.5% ± 0.9 SD) had Stokes radii between 4.6 nm and 6.3 nm. The rest of the particles were smaller falling in the range of 3.7 nm to 4.6 nm particles. Further study showed little difference (75% ± 1.8 SD) in the α-helical content of apoA-I associated with the particles originating from PC isolated from different diet groups. The free cholesterol concentration, determined by the measuring radioactive cholesterol associated with the disks, was also identical.

Figure 4 shows the rate of cholesteryl ester formation by semi-purified LCAT when rHDL, differing only in their PC molecular species distribution, were used as substrates. The results indicate that rHDL made with PC from n-3 Poly-fed animals were a relatively poor substrate for LCAT and demonstrated a rate of cholesteryl ester formation (2.14 nmol/mg-h, Sat; 2.31 nmol/mg-h, Mono; 2.27 nmol/mg-h, n-6 Poly; 1.23 nmol/mg-h, n-3 Poly) nearly half the other observed rates ($P < 0.0001$). Repeated measures analysis of variance also showed that the rate observed with Sat rHDL as significantly lower than the rate observed using Mono rHDL ($P = 0.044$) although this difference was small.

Selectivity of LCAT

After performing experiments similar to those described above, the molecular species distribution of LCAT products was determined. The rHDL were incubated for 40 and 60 min with purified LCAT. The cholesteryl esters produced during the reaction were extracted and separated into discrete molecular species using reverse phase HPLC. Relative amounts of radioactivity in peaks comigrating with known standards were used to determine the distribution of cholesteryl esters (see product column, **Table 6**). This distribution was found to be identical at the two different time points studied. Regardless of diet, the major molecular species

TABLE 6. LCAT selectivity

CE or *sn*-2 FA	Diet	% of rHDL PC (Substrate)[a]	% of rHDL CE (Product)[b]	Ratio %CE/%PC
18:1	Sat	20.4 ± 0.6	24.2 ± 0.3	1.2
	Mono	32.7 ± 0.6	37.8 ± 0.9	1.1
	n-6 Poly	5.3 ± 0.3	7.9 ± 0.2	1.5
	n-3 Poly	14.6 ± 1.4	24.1 ± 1.2	1.6
18:2	Sat	61.1 ± 1.0	60.0 ± 0.8	1.0
	Mono	53.6 ± 0.7	45.4 ± 1.5	0.8
	n-6 Poly	81.7 ± 2.1	78.9 ± 0.3	1.0
	n-3 Poly	17.4 ± 1.4	44.6 ± 0.9	2.6
20:4	Sat	11.0 ± 0.3	1.9 ± 0.3	0.2
	Mono	5.1 ± 0.3	1.8 ± 0.4	0.3
	n-6 Poly	3.3 ± 0.3	1.3 ± 0.1	0.4
	n-3 Poly	8.1 ± 0.3	3.8 ± 0.5	0.5
20:5 + 22:6	Sat	1.5 ± 0.2	3.8 ± 0.1	2.5
	Mono	1.7 ± 0.1	0.2 ± 0.1	0.1
	n-6 Poly	0.4 ± 0.04	1.6 ± 0.4	4.0
	n-3 Poly	51.4 ± 2.6	10.1 ± 1.5	0.2

All values, mean ± SEM.
[a]Moles % of total phosphatidylcholine molecular species.
[b]Percent of total cholesteryl ester radioactivity.

produced were cholesteryl linoleate and cholesteryl oleate; the relative proportion of these two molecular species, however, varied over 8-fold (cholesteryl linoleate/cholesteryl oleate: 2.5, Sat; 1.2, Mono; 10.0, n-6 Poly; 1.8, n-3 Poly). The Mono rHDL substrates yielded the highest percentage of cholesteryl oleate; n-6 Poly rHDL led to the highest proportion of cholesteryl linoleate, while n-3 Poly and Mono rHDL substrates demonstrated identical percentages of cholesteryl oleate but different amounts of cholesteryl linoleate (Table 6). Phospholipids containing 20:4 in the *sn*-2 position were poor substrates for the LCAT reaction regardless of other molecular species present. Although LCAT's major source of fatty acid is considered to be the *sn*-2 position of PC, the enzyme apparently can also utilize palmitate at the *sn*-1 position at a lower rate. In these assays cholesteryl palmitate was generated (6.8%, Sat; 9.6%, Mono; 5.2%, n-6 Poly; 13.5%, n-3 Poly) and is considered to be derived from such a reaction as the amount of PC molecular species containing palmitate in the *sn*-2 position is no more than 1% regardless of diet group. Subbaiah and coworkers (35) have reported combined levels of 18:2-16:0 and 18:1-16:0 as high as 1.5% of total LDL PC and this may contribute to the production of cholesteryl palmitate, though there is no precedent to suggest that levels of such molecular species can be altered significantly. While small amounts of these molecular species would not be detected using the methods used in the current study, such low amounts of PC containing 16:0 in the *sn*-2 position (2% or less) cannot account for the significant levels of cholesteryl palmitate generated by LCAT. No measurable cholesteryl stearate was produced in these reactions.

To determine whether differences in product formation were due to selectivity of the LCAT enzyme, the proportion of individual CE molecular species (LCAT product) was divided by the proportion of rHDL PC molecular species containing the same fatty acid in the *sn*-2 position (LCAT substrate column of Table 6) and the ratio of percentage product over substrate percentage was calculated (last column Table 6). This can be considered a selectivity factor where a ratio greater than 1.0 indicates selective utilization of a certain molecular species and a ratio less than 1.0 signifies a less reactive substrate. For the 18:1-containing molecular species, each of the three diet groups, Sat, Mono, and n-6 Poly, show a slight selective utilization of rHDL PC containing 18:1 in the *sn*-2 position. In the same diet groups, however, there appears to be no selectivity for the utilization of 18:2-containing molecular species (ratio $\leq$ 1). A selectivity ratio for arachidonate esters was also calculated; it was less than one and indicated that arachidonate-containing PC was underutilized in the Sat, Mono, and n-6 Poly substrate particles (Table 6).

When rHDL made from n-3 Poly-derived PC were used as substrates for the LCAT reaction, the enzyme selectively utilized PC with 18:2 in the *sn*-2 position (ratio = 2.6). Selectivity for PC molecular species with 18:1 in the *sn*-2 position was also observed, (ratio = 1.6) although less pronounced. These increases in selectivity for 18:1- and 18:2-containing PC substrates were offset by decreased utilization of PC molecules containing 20:5 or 22:6 as the *sn*-2 fatty acid (ratio = 0.2). While just over half of the available PC substrate contained 20:5 or 22:6, only 10% of the cholesteryl esters produced were 20:5 or 22:6 CE molecular species. The data would suggest the selective utilization of 20:5 and/or 22:6 in the Sat and n-6 Poly particles; however, the substrate and products are in very small proportions and contribute very little to the total cholesteryl ester pool. The product/substrate ratio for the mono particle was the lowest observed, an outcome for which the significance is not clear.

DISCUSSION

This study demonstrates that dietary fat intake significantly alters the composition of PC molecular species in circulating HDL. In addition to direct effects on composition of the phospholipid monolayer surrounding the lipoprotein particle, changes in the HDL PC molecular species are reflected in LCAT-derived cholesteryl esters and therefore would affect the core composition of circulating lipoproteins. The results also support the hypothesis that effects of dietary fat on HDL cholesterol concentration and subpopulation distribution are mechanistically linked to the HDL metabolizing enzyme LCAT. While dietary fat has only small effects on the absolute amount of circulating enzyme (Fig. 2), the ability of diet to affect changes in HDL PC concentration (Table 3) and in the molecular structure of the substrate (Fig. 3) appears capable of altering the rate of reaction of this enzyme (Fig. 4). Changes in HDL PC molecular species due to ingestion of an n-3 Poly diet appear to lead to a lower concentration of HDL PC and a decreased rate of reaction of substrate particles with LCAT. As a result, the HDL subpopulation distribution tends toward smaller, denser, cholesteryl ester-depleted lipoproteins due to limited cholesteryl ester availability (Fig. 1).

As LCAT is known to utilize predominantly the *sn*-2 fatty acid of HDL PC for the acyltransferase reaction catalyzed by this enzyme, the distribution of fatty acids at this position were of particular interest. Oleic and linoleic acid were the two most common fatty acids in the *sn*-2 position for the Sat, Mono and n-6 Poly while eicosapetaenoic acid and docosahexaenoic acid are the most prominent *sn*-2 fatty acids in the n-3 Poly HDL PC. Such effects on molecular species distribution may have a significant influence on HDL composition as it has been proposed that plasma enzymes essential for proper maturation of these particles selectively utilize certain molecu-

lar species. LCAT's rate of reaction, for example, has been shown to be lower when 20:5- or 22:6-containing PC is the major substrate available (15) while 16:0-18:2 PC has been proposed as a preferred substrate for the LCAT reaction (14). It is in this context that dramatic, diet-induced changes in HDL phospholipid composition such as those described here become significant in relation to plasma cholesteryl ester metabolism. Indeed, the percentage of 16:0-18:2 and 18:0-18:2 in n-6 Poly HDL was significantly higher than any other diet group (Fig. 3) and therefore might be expected to produce populations of larger, more cholesteryl ester-rich particles although this was not the case (Fig. 1, Table 3). The monounsaturated fat diet also made a significant impact on the available substrate containing linoleic acid. There was a 5-fold greater proportion of 16:0-18:1 and 18:0-18:1 in the Mono HDL when compared to the n-6 Poly HDL. This was balanced by proportionately lower amounts of 16:0-18:2 and 18:0-18:2. The Mono HDL, however, were not significantly different in size or cholesteryl ester content from the Sat HDL (Fig. 1, Table 3).

The most striking decreases in 18:2-containing molecular species were in the n-3 Poly HDL. The portion of 16:0-18:2 in the n-3 Poly HDL was 3-fold lower than was found in any of the other diet groups and the percentage of 18:0-18:2 was decreased 2- to 3-fold. Earlier studies (15) predict that such a significant replacement of 18:2 fatty acid with 20:5 and 22:6 fatty acids would significantly lower LCAT's rate of reaction with these HDL particles and smaller more cholesteryl ester-depleted particles were observed in the plasma of n-3 Poly-fed animals (Fig. 1, Table 3). The effects of fish oil were not as pronounced in 18:1-containing molecular species although the percentage was low. In n-3 Poly-fed animals, the proportion of 16:0-18:2 in HDL is only 30% of that found in Sat HDL, while n-3 Poly HDL have 75% of the 16:0-18:1 found in HDL from Sat-fed animals (Fig. 3). Such observations offer further support for a possible selective effect of dietary n-3 fatty acids on fatty acid metabolism or phospholipid synthesis in the liver (36-39).

The source of fatty acyl moiety (PC) and positional specificity (> 90% *sn*-2) of LCAT has been well documented. Others have also looked at the fatty acyl selectivity of LCAT by using a single molecular species as substrate or reconstituting certain phospholipid molecular species in an inert matrix, such as dialkyl phospholipids (12, 13), and comparing relative rates of reaction. Although studies seem to suggest that 16:0-18:2 is a preferred substrate for LCAT (14, 15), results have not been consistent (13, 14). Purified PC from the different diet groups was used to make rHDL to test LCAT reactivity using well-defined substrates. In these experiments changes in reactivity were directly attributable to altered molecular species distribution as composition, size distribution, and protein conformation appeared nearly identical among the different rHDL substrates tested. The results in Fig. 4 demonstrate that rHDL made from n-3 Poly PC are a poor substrate for LCAT. Also demonstrated here is that the difference in reactivity reported earlier by Parks et al. (15) using total plasma lipid was most likely due to differences in PC molecular species in particular and not differences in the distribution of phospholipid classes or subclasses in general. Perhaps surprising was LCAT's similar rate of reaction against rHDL made from Sat-, Mono-, or n-6 Poly-derived PC. If LCAT prefers the 16:0-18:2 molecular species as a phospholipid substrate, rHDL made with PC from n-6 Poly-fed animals should be considerably better substrates than Mono rHDL. This was not the case, however, and suggests that LCAT's selectivity for substrate, as determined using in vivo derived PC mixtures, may not be as pronounced as previously thought.

We have also examined the specificity of LCAT by defining the cholesteryl esters produced by the enzyme during its reaction with the rHDL particles described above and comparing that to the fatty acids available as substrate in the *sn*-2 position of the rHDL phospholipid. Such a comparison reveals that PC containing 18:1 in the *sn*-2 position is utilized as readily as 18:2 in rHDL particles derived from Sat, Mono, or n-6 Poly lipoproteins (Table 6). The Mono diet appears to produce the highest proportion of cholesteryl oleate while the n-6 Poly diet produces the lowest. Indeed, in the same circumstances where the amount of available 16:0-18:2 PC was decreased 40% (n-6 Poly vs. Mono), and alterations in the cholesteryl esters produced by LCAT were as dramatic (decrease in 18:2 CE, 42%), the rate of the LCAT reaction remained unchanged.

The ratio of %CE to %PC is used in this study as a measure of selectivity and differs from the values found in an earlier study (15). One reason for this is that the earlier study used a ratio generated from the weight per cent of total fatty acid as opposed to the current study which uses moles per cent cholesteryl ester divided by moles per cent of phospholipids having the fatty acid of interest in the *sn*-2 position. This would automatically make the numbers about 2-fold lower in the present manuscript which should provide greater accuracy. This does not explain the selective use of 18:1 and 18:2 found in the earlier study using phospholipids from lard-fed animals. Such differences may be due to the fact that the earlier paper used total serum phospholipids which would include significant amounts of fatty acids esterified to sphingomyelin and phosphatidylethanolamine (mainly from apoB-containing lipoproteins) which are not substrates for the LCAT reaction and may alter the apparent selectivity.

The apparent selectivity by LCAT for 18:1- and 18:2-containing PC is demonstrated when the n-3 Poly diet is fed, in which case PC species containing either fatty acid in the *sn*-2 position act as the preferred substrate

(Table 6) and is consistent with earlier findings in African green monkeys (15). We would propose that this is a reflection of LCAT's inability to easily utilize phospholipid substrates containing 20:5 or 22:6 in the *sn*-2 position and therefore an affinity of LCAT for *sn*-2 fatty acids of shorter chain length and/or decreased polyunsaturation seems apparent. This hypothesis is supported by the substantially lower rate of reaction displayed in Fig. 4 and by the fact that phospholipids containing arachidonate in the *sn*-2 position appear to be poor substrates for LCAT (Table 6). As the n-3 Poly rHDL have 51% of the PC molecular species with 20:5 or 22:6 in the *sn*-2 position, the interaction of LCAT with these particles seems significantly altered. The data presented cannot distinguish whether this is due to a decreased binding of the particles, a compromised binding of the substrate molecule, or normal binding with a decrease in the rate of acyl hydrolysis and/or acyl transfer by LCAT. It is, however, intriguing that selectivity for the 18:2-containing molecular species is most apparent when substantial quantities of 20:5 and 22:6 PCs are present in the substrate. This situation occurs in monkey plasma lipoproteins after long-term feeding of the oil from cold-water fish. These circumstances may, in effect, be similar to placing 16:0-18:2 in an inert matrix such as dialkyl PC, a method used to identify preferred substrates for the LCAT reaction. These data indicate that the phospholipid environment in which LCAT contacts and binds its substrate may be important in determining the apparent affinity of the enzyme for any one substrate molecular species. The apparent selectivity of LCAT for 20:5 and 22:6 fatty acids in Sat and n-6 Poly experiments (Table 6) is likely a statistical artifact due to the low percentage of both substrate and product measured.

It appears that LCAT is essential in the maturation of smaller HDL to larger less dense particles through its conversion of accumulated HDL free cholesterol to cholesteryl ester. One line of evidence for this relationship comes from the fact that the plasma of LCAT-deficient patients is enriched in small, dense spherical or discoidal particles similar to nascent HDL (40). The predominant proteins of these nascent particles are apoA-I and apoA-II. The similar ratio of apoA-I to apoA-II between diet groups indicates that dietary fat does not have a selective effect on the metabolism of these two proteins. As, on average, 80% of HDL cholesterol is esterified, the apoA-I/cholesterol ratio is an indicator of the average surface-to-core ratio for the HDL. The data would predict that HDL from the Mono- and n-6 Poly-fed animals might contain a larger core and therefore show a particle size distribution slightly larger than HDL from the Sat- and n-3 Poly-fed animals. Figure 1 identifies n-3 Poly HDL as consisting predominantly of smaller, denser HDL particles, which is consistent with the finding that LCAT reactivity is lower against n-3 Poly rHDL. Therefore, inhibition of LCAT's rate of reaction by n-3 polyunsaturated fatty acids found in the n-3 Poly diet may cause a decrease in the HDL cholesteryl ester pool size to the extent that the concentration of larger (2b and 2a) HDL is significantly decreased. This inhibition may also contribute to an overall lower concentration of HDL cholesterol in these animals.

While such effects on LCAT selectivity and reactivity may explain alterations in HDL metabolism during n-3 Poly intake, such a hypothesis does not explain the effects of the n-6 Poly diet. The data suggest different mechanisms are at work with respect to these two diets. The availability of HDL PC is one factor that may provide a clue to the effects of the n-6 Poly diet. While the lower mean plasma HDL phospholipid level in n-3 Poly-fed animals does not reach significance when compared to plasma from Sat-fed animals, the n-6 Poly diet significantly decreases HDL phospholipid levels by 40% when compared to the Sat and Mono diets. The different effects of Poly diets could, therefore, be due to the ability of the n-6 Poly diet to lower HDL phospholipid concentration and consequently LCAT substrate availability and the n-3 Poly diet's additional capacity to affect LCAT's rate of reaction. Both diets, high in polyunsaturated fatty acids, appear equal in their ability to slightly raise the concentration of LCAT enzyme in circulation when compared to a saturated fat diet.

Clearly, however, LCAT is not solely responsible for overall dietary effects on plasma cholesteryl ester levels. For example, the n-6 Poly animals were found to have levels of total plasma cholesterol and HDL cholesterol 30% lower than the Sat animals. In contrast, n-3 Poly animals showed no reduction in total plasma cholesterol levels but did have 35% lower HDL cholesterol levels when compared to Sat animals. These data would indicate that under some conditions, such as n-3 Poly feeding, decreased LCAT reactivity may play a dominant role in determining HDL size, density, and cholesterol concentration without significantly affecting total plasma cholesterol levels. Under other dietary conditions, such as in the n-6 Poly feeding, the impact of other factors such as phospholipid secretion, VLDL catabolism, or hepatic acyl CoA:cholesterol acyltransferase activity may be the predominant influence on plasma and HDL cholesterol levels.

This work was supported by NIH grant numbers HL-24736, HL-49373, and HL-07115.

Manuscript received 22 October 1993, in revised form 1 April 1994, and in revised form 8 September 1994.

REFERENCES

1. Rudel, L. L., M. G. Bond, and B. C. Bullock. 1985. LDL heterogeneity and atherosclerosis in nonhuman primates. *Ann. N.Y. Acad. Sci.* **454:** 248-253.

2. Rudel, L. L., J. S. Parks, F. L. Johnson, and J. Babiak. 1986. Low density lipoproteins in atherosclerosis. *J. Lipid Res.* **27:** 465–474.
3. Rudel, L. L., J. S. Parks, and M. G. Bond. 1986. Dietary polyunsaturated fat effects on atherosclerosis and plasma lipoproteins in African green monkeys. *In* Nutritional Diseases: Research Directions in Comparative Pathology. D. G. Scarpelli, and G. Migaki, editors. Alan R. Liss, Inc., New York. 501–523.
4. Parks, J. S., and L. L. Rudel. 1990. Effect of fish oil on atherosclerosis and lipoprotein metabolism. *Atherosclerosis.* **84:** 83–94.
5. Babiak, J., F. T. Lindgren, and L. L. Rudel. 1988. Effects of saturated and polyunsaturated dietary fat on the concentrations of HDL subpopulations in African green monkeys. *Arteriosclerosis.* **8:** 22–32.
6. Parks, J. S., J. A. Martin, B. L. Sonbert, and B. C. Bullock. 1987. Alteration of high density lipoprotein subfractions of nonhuman primates fed fish-oil diets. *Arteriosclerosis.* **7:** 71–79.
7. Glomset, J. A. 1962. The mechanism of the plasma cholesterol esterification reaction: plasma fatty acid transferase. *Biochim. Biophys. Acta.* **65:** 128–135.
8. Sparks, D. L., and P. H. Pritchard. 1989. The neutral lipid composition and size of recombinant high density lipoproteins regulates lecithin:cholesterol acyltransferase activity. *Biochem. Cell Biol.* **67:** 358–364.
9. Karpe, F., J. Johansson, and L. A. Carlson. 1990. Studies on the lecithin:cholesterol acyltransferase substrate properties of HDL as determined by its subclass distribution analysed by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **1042:** 310–314.
10. Jonas, A. 1991. Lecithin-cholesterol acyltransferase in the metabolism of high-density lipoproteins. *Biochim. Biophys. Acta.* **1084:** 205–220.
11. Johnson, W. J., F. H. Mahlberg, G. H. Rothblat, and M. C. Phillips. 1991. Cholesterol transport between cells and high-density lipoproteins. *Biochim. Biophys. Acta.* **1085:** 273–298.
12. Pownall, H. J., Q. Pao, and J. B. Massey. 1985. Acyl chain and headgroup specificity of human plasma lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **260:** 2146–2152.
13. Jonas, A., N. L. Zorich, K. E. Kézdy, and W. E. Trick. 1987. Reaction of discoidal complexes of apolipoprotein A-I and various phosphatidylcholines with lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **262:** 3969–3974.
14. Subbaiah, P. V., and H. Monshizadegan. 1988. Substrate specificity of human plasma lecithin-cholesterol acyltransferase towards molecular species of phosphatidylcholine in native plasma. *Biochim. Biophys. Acta.* **963:** 445–455.
15. Parks, J. S., B. C. Bullock, and L. L. Rudel. 1989. The reactivity of plasma phospholipids with lecithin:cholesterol acyltransferase is decreased in fish oil-fed monkeys. *J. Biol. Chem.* **264:** 2545–2551.
16. Subbaiah, P. V., D. Kaufman, and J. D. Bagdade. 1993. Incorporation of dietary n-3 fatty acids into molecular species of phosphatidylcholine and cholesteryl ester in normal human plasma. *Am. J. Clin. Nutr.* **58:** 360–368.
17. Holub, B. J., D. J. Bakker, and C. M. Skeaff. 1987. Alterations in molecular species of cholesterol esters formed via plasma lecithin-cholesterol acyltransferase in human subjects consuming fish oil. *Atherosclerosis.* **66:** 11–18.
18. Bronsgeest-Schoute, H. C., C. M. van Gent, J. B. Luten, and A. Ruiter. 1981. The effect of various intakes of ω-3 fatty acids on the blood lipid composition in healthy human subjects. *Am. J. Clin. Nutr.* **34:** 1752–1757.
19. Lichtenstein, A. H., R. J. Nicolosi, and K. C. Hayes. 1980. Dietary fat and cholesterol effects on plasma lecithin:cholesterol acyltransferase activity in cebus and squirrel monkeys. *Atherosclerosis.* **37:** 603–616.
20. Rudel, L. L., J. A. Lee, M. D. Morris, and J. M. Felts. 1974. Characterization of plasma lipoproteins separated and purified by agarose-column chromatography. *Biochem. J.* **139:** 89–95.
21. Babiak, J., H. Tamachi, F. L. Johnson, J. S. Parks, and L. L. Rudel. 1986. Lecithin:cholesterol acyltransferase-induced modifications of liver perfusate discoidal high density lipoproteins from African green monkeys. *J. Lipid Res.* **27:** 1304–1317.
22. Verdery, R. B., D. F. Benham, H. L. Baldwin, A. P. Goldberg, and A. V. Nichols. 1989. Measurement of normative HDL subfraction cholesterol levels by Gaussian summation analysis of gradient gels. *J. Lipid Res.* **30:** 1085–1095.
23. Rudel, L. L., and M. D. Morris. 1973. Determination of cholesterol using *o*-phthalaldehyde. *J. Lipid Res.* **14:** 364–366.
24. Carr, T. P., C. J. Andreson, and L. L. Rudel. 1993. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clin. Biochem.* **26:** 39–42.
25. Rouser, G., S. Fleischer, and A. Yamamoto. 1970. Two-dimensional thin-layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids.* **5:** 494–496.
26. Koritnik, D. L., and L. L. Rudel. 1983. Measurement of apolipoprotein A-I concentration in nonhuman primate serum by enzyme-linked immunosorbent assay (ELISA). *J. Lipid Res.* **24:** 1639–1645.
27. Parks, J. S., and L. L. Rudel. 1979. Isolation and characterization of high density lipoprotein apoproteins in the non-human primate (vervet). *J. Biol. Chem.* **254:** 6716–6723.
28. Chen, C-H., and J. J. Albers. 1982. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
29. Chen, C-H., and J. J. Albers. 1985. A rapid large-scale procedure for purification of lecithin-cholesterol acyltransferase from human and animal plasma. *Biochim. Biophys. Acta.* **834:** 188–195.
30. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
31. Christiansen, K. 1975. Lipid extraction procedure for in vitro studies of glyceride synthesis with labeled fatty acids. *Anal. Biochem.* **66:** 93–99.
32. Blank, M. L., M. Robinson, V. Fitzgerald, and F. Snyder. 1984. Novel quantitative method for determination of molecular species of phospholipids and diglycerides. *J. Chromatogr.* **298:** 473–482.
33. Chabot, M. C., D. G. Greene, J. K. Brockschmidt, R. L. Capizzi, and R. L. Wykle. 1990. Ether-linked phosphoglyceride content of human leukemia cells. *Cancer Res.* **50:** 7174–7178.
34. Parks, J. S., and B. C. Bullock. 1987. Effect of fish oil versus lard diets on the chemical and physical properties of low density lipoproteins of nonhuman primates. *J. Lipid Res.* **28:** 173–182.
35. Subbaiah, P. V., C-H. Chen, J. D. Bagdade, and J. J. Albers. 1985. Substrate specificity of plasma lysolecithin acyltransferase and the molecular species of lecithin formed by the reaction. *J. Biol. Chem.* **260:** 5308–5314.
36. Parks, J. S., and A. K. Gebre. 1991. Studies on the effect of

dietary fish oil on the physical and chemical properties of low density lipoproteins in cynomolgus monkeys. *J. Lipid Res.* **32:** 305–315.
37. Edwards, I. J., A. K. Gebre, W. D. Wagner, and J. S. Parks. 1991. Reduced proteoglycan binding of low density lipoproteins from monkeys (*Macaca fascicularis*) fed a fish oil versus lard diet. *Arterioscler. Thromb.* **11:** 1778–1785.
38. Scobey, M. W., F. L. Johnson, J. S. Parks, and L. L. Rudel. 1991. Dietary fish oil effects on biliary lipid secretion and cholesterol gallstone formation in the African green monkey. *Hepatology.* **14:** 679–684.
39. Parks, J. S., J. Kaduck-Sawyer, B. C. Bullock, and L. L. Rudel. 1990. Effect of dietary fish oil on coronary artery and aortic atherosclerosis in African green monkeys. *Arteriosclerosis.* **10:** 1102–1112.
40. Glomset, J. A., and K. R. Norum. 1970. The metabolic role of lecithin:cholesterol acyltransferase: perspectives from pathology. *Annu. Rev. Biochem.* **9:** 1–65.