Influence of dietary fatty acid composition on the relationship between CETP activity and plasma lipoproteins in monkeys

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Abstract **CETP activity, measured as transfer of cholesteryl ester from exogenous HDL to exogenous VLDL and LDL, reflecting CETP mass as determined by ELISA, was documented in three groups of St. Kitts vervet monkeys fed diets enriched in saturated (Sat), monounsaturated (Mono), or n-6 polyunsaturated (Poly) fatty acids. CETP activity was not different when comparing the three dietary fats. However, CETP activity was significantly higher when cholesterol was added to each of the diets. Significant positive associations between CETP activity and VLDL and LDL cholesterol concentrations were found whereas significant negative associations were seen between CETP activity and HDL cholesterol in each of the diet groups. The strength of these associations was highest in the Sat group. Cholesteryl ester (CE) fatty acid composition of lipoproteins varied widely among diet groups, with the more polyunsaturated CE of the Poly group being associated with a higher rate of CE transfer to endogenous acceptor apolipoprotein B-containing lipoproteins. Finally, only the Sat diet group showed significant positive correlations of CETP activity with LDL particle diameter ($r = 0.76$), cholesteryl ester percentage ($r = 0.67$), and a strong negative correlation ($r = -0.86$) with LDL receptor function, estimated as the difference between native and methylated LDL turnover rates. We speculate that strong associations between CETP and LDL metabolism may explain, at least in part, the increased atherogenicity of dietary saturated fat.**—Fusegawa, Y., K. L. Kelley, J. K. Sawyer, R. N. Shah, and L. L. Rudel. **Influence of dietary fatty acid composition on the relationship between CETP activity and plasma lipoproteins in monkeys.** ***J. Lipid Res.*** **2001.** 42: **1849–1857.**

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Elevated low density lipoprotein cholesterol and decreased high density lipoprotein cholesterol concentrations are associated with increased coronary heart disease in humans and coronary artery atherosclerosis in nonhuman primate models (1–3). The types of fatty acids in diets are also known to modify the development of coronary artery atherosclerosis (4–6). The effects of dietary fatty acids often seem paradoxical, as when polyunsaturated fat lowers the concentration of HDL cholesterol and decreases the extent of atherosclerosis (5, 6) or when monounsaturated fat lowers LDL cholesterol concentrations without decreasing atherosclerosis (6). One possible explanation for these outcomes is that in addition to effects on concentrations, dietary fatty acids alter lipoprotein particle compositions, with some compositions being more atherogenic than others (5, 7). Studies of animal models of atherosclerosis can help clarify the contribution(s) of the factors that alter both concentrations and compositions of plasma lipoproteins in atherogenesis.

One of the factors in plasma that modifies lipid composition in lipoprotein particles and may play a role in atherogenicity is CETP (8). This protein transfers cholesteryl esters (CE) among lipoprotein particles and exchanges CE for triacylglycerols. Exchange of CE for triacylglycerol is thought to be important in reverse cholesterol transport when CE moves from HDL to VLDL and IDL for rapid removal of CE from plasma (9, 10). The presence of apolipoprotein C-I (apoC-I) on HDL may serve as an inhibitor of CE transfer from HDL to other lipoproteins by CETP (11). In addition, the presence of a lipid transfer inhibitor protein (apoF) on LDL particles may function to assure directional transfer of CE from HDL to VLDL (10). Dietary fatty acids alter the composition of phospholipids and influence the types of CE made by LCAT (12), and may alter the types of CE made by hepatic ACAT2 for secretion by the liver in lipoproteins (7, 13). Such modifications can increase the proportion of higher melting temperature CE in the core of the LDL particle, which in turn may slow transfer via CETP (14). In addition, many studies have shown that different dietary fatty acids directly affect CETP levels, although general agreement on

Abbreviations: CE, cholesteryl ester; FCR, fractional clearance rate; LDLr, LDL receptor; Mono, monounsaturated; Poly, polyunsaturated; Sat, saturated; TPC, total plasma cholesterol.

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the specific effects of individual fatty acids on CETP expression has not been achieved (15–17).

In primates, a positive correlation between CETP concentration and LDL cholesterol and an inverse correlation with HDL cholesterol were seen (8); CETP concentration was positively correlated with the extent of coronary artery atherosclerosis. Plasma CETP levels also were highly correlated with CETP production during liver perfusion. This outcome indicates that regulation of CETP production by the liver will affect CETP availability in plasma and modulate any influence on the relative degree of atherogenicity. The extent to which individual fatty acids from the diet can modulate the influence of CETP and lipoprotein on atherosclerosis is the topic of this presentation.

Accordingly, we examined the effects of three different dietary fatty acid enrichments, with and without added dietary cholesterol, on CETP activity in St. Kitts vervet monkeys, in which individual animals exhibit a highly variable response to diet. Correlations between CETP activity and several plasma lipoprotein end points were evaluated as a means to detect whole animal responses of CETP and lipoproteins that may not be otherwise apparent. To the extent that individual fatty acids differently modify CETP and its interaction with lipoproteins, these studies may help further our understanding of fatty acid influences on the pathogenesis of atherosclerosis by capitalizing on the extensive variability among these animals to diet induction.

MATERIALS AND METHODS

Animal colony and blood sampling

The monkeys used in these studies were feral adult male St. Kitts vervets (*Cercopithecus aethiops sabeus*). The groups of animals fed each of the three dietary fats were preselected to have equivalent average ± SD plasma cholesterol responses to a challenge diet containing cholesterol. Each group was fed their particular fat without added cholesterol for 20 weeks, and after that, a diet containing the same fat with added cholesterol (0.4 mg/kcal) was fed for a period of about 36 months. The makeup of the three different diets has been described (18).

Periodic blood samples were taken from each of the animals. After an overnight fast, sedation with ketamine hydrochloride (10 mg/kg) was performed and blood was withdrawn from the femoral vein into tubes containing 0.1% EDTA and a protease inhibitor cocktail (19). Plasma was isolated by centrifugation and fractionated immediately or stored at −80°C until NMR analyses of lipoproteins were performed.

Lipoprotein separation and analysis

Lipoproteins, at a density of 1.225 g/ml, were initially isolated from plasma by ultracentrifugation and individual lipoprotein classes were separated by gel-filtration chromatography, using a Superose 6 HPLC column. Fractions containing VLDL plus IDL, LDL, and HDL were pooled for further analysis and LDL particle size was measured (20). Cholesterol distribution among lipoprotein classes was measured enzymatically after chromatographic separation (21) and in whole plasma by NMR (22–24). For lipoprotein compositions, protein and phospholipid phosphorus were measured directly in isolated fractions whereas free cholesterol, CE mass and fatty acids, and triacylglycerol were all quantified after extraction and separation of lipid classes by thin-layer chromatography (25). In some cases, the LDL receptor-mediated clearance rate of LDL apoB was estimated in vivo by measuring the difference in turnover rate of homologous ^{125}I-labeled LDL and methylated heterologous ^{131}I-labeled LDL (26). HDL subfraction distribution of protein was measured by NMR (22–24) and in some cases after nondenaturing PAGE, using 4–30% polyacrylamide gradient gels (27). After fixation of the gels and Coomassie blue staining, HDL protein percentage distribution was determined by densitometric scanning and deconvolution analysis by the method of Verdery et al. (28).

CETP assay

HDL labeled with [^{3}H]CE was prepared after whole plasma incubation with [^{3}H]cholesterol as described by Tall et al. (29). After overnight incubation, more than 97% of the [^{3}H]cholesterol radioactivity was isolated in the CE band produced by thin-layer chromatography. CETP activity was determined as the percentage of labeled CE transferred in 1 h at 37°C from isolated human HDL to a mixture of human VLDL and LDL added in excess (30). Each assay tube contained [^{3}H]CE-HDL$_3$ (30 μg of cholesterol) as the donor, unlabeled VLDL and LDL (200 μg of cholesterol) as the acceptor, and 20 μl of whole monkey plasma as the source of CETP in a final volume of 200 μl. The tubes were incubated for 30 min at 37°C in a shaking water bath. This time point was within the linear first-order portion of the rate curve, and represented an incubation time at which less that 30% of the CE was transferred. After incubation, the tubes were immediately chilled on ice for at least 30 min to stop the reaction. The donor and acceptor lipoproteins were separated by heparin-manganese precipitation, and radioactivity was determined in an aliquot of supernatant. The percent transfer of radioactivity from HDL$_3$ to VLDL and LDL was used as the detection parameter. All assays were conducted in duplicate.

CETP mass was measured by a two-site sandwich enzyme-linked immunosorbent assay (31) using monoclonal human CETP antibody (TP20) for coating the microtiter plate as the capture antibody. A second antibody (TP2), conjugated with horseradish peroxidase, was added to decorate the CETP antigen. Both antibodies were purchased from the University of Ottawa Heart Institute (Ottawa, Canada). Plasma dilutions in the range of 1:5 to 1:50 were made and 200 μl was then added to the plate for detection of CETP. Color detection was as described earlier using H_2O_2 as substrate and *o*-dianisidine as the chromogen (31).

A CE transfer ratio was also calculated as the proportion of CE transferred from exogenous labeled HDL into endogenous apoB-containing lipoproteins in whole plasma. A 300-μl aliquot of whole plasma was incubated with 30 μg of cholesterol in [^{3}H]CE-HDL at 37°C for 30 min. After chilling on ice, HDL was separated from other lipoproteins by the heparin-manganese precipitation procedure (32), and the ratio (converted to percent) of [^{3}H]CE in apoB-containing lipoproteins compared with the amount added in HDL was measured.

Statistical analyses were done as indicated. ANOVA and repeated measures ANOVA were done to compare for dietary fat effects, and Fisher's preferred least significant difference (PLSD) post hoc analysis was used to differentiate individual fatty acid effects. A paired *t*-test was also used to determine dietary cholesterol effects within a diet fat group. Correlation analyses were used to determine statistically significant relationships among various parameters to capitalize on the large individual animal variability. A difference of $P < 0.05$ was considered statistically significant.

RESULTS

At the end of an initial cholesterol-free diet period of 16 weeks and then at regular intervals after feeding a cholesterol-enriched diet in the atherosclerosis progression phase of the study, total plasma cholesterol (TPC), HDL cholesterol, and LDL cholesterol were measured (**Fig. 1**). During the period before cholesterol was added to the diet, TPC was near 150 mg/dl in the polyunsaturated (Poly), monounsaturated (Mono), and saturated (Sat) fatty acid groups. Plasma cholesterol rose quickly and significantly in all groups after cholesterol was added to the diet. The apparently higher average plasma cholesterol concentrations in the Mono group were not significantly different from the other groups as determined by repeated measures ANOVA. LDL cholesterol rose quickly and significantly when cholesterol was added to the diet, but remained equivalent among the three diet fat groups. The pattern was for TPC and LDL cholesterol to overshoot initially between 3 to 6 months, and then fall back only to rise again between 12 and 18 months on diet before settling onto a plateau after about 20 months on diet. HDL cholesterol was significantly lower in the Poly group than in either of the other two dietary fat groups when the diet contained no cholesterol. HDL cholesterol levels fell significantly for all three fat groups when cholesterol was added to the diet. HDL cholesterol was consistently 20 mg/dl lower in the Poly group than in the other groups throughout the study, and this difference was statistically significant. In all dietary fat groups, TPC and LDL cholesterol were increased and HDL cholesterol was decreased by adding cholesterol to the diet.

CETP activity and CETP mass concentration as measured by ELISA were compared in a subset of the animals

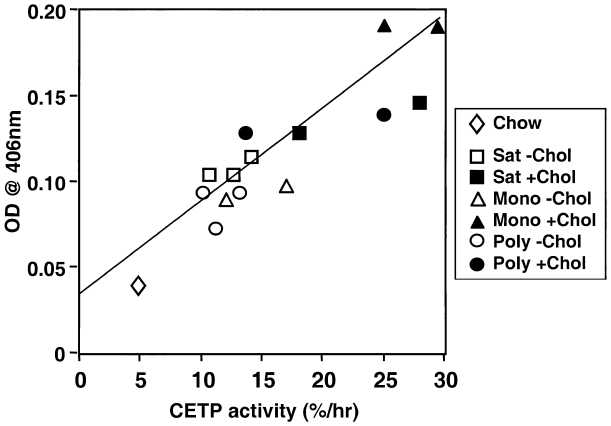

Fig. 2. Comparison of CETP activity with CETP mass estimated by ELISA. Whole plasma samples from each diet group were evaluated. All values were plotted and the linear best fit regression line is shown along with the correlation coefficient, $r = 0.89$, $P < 0.0001$.

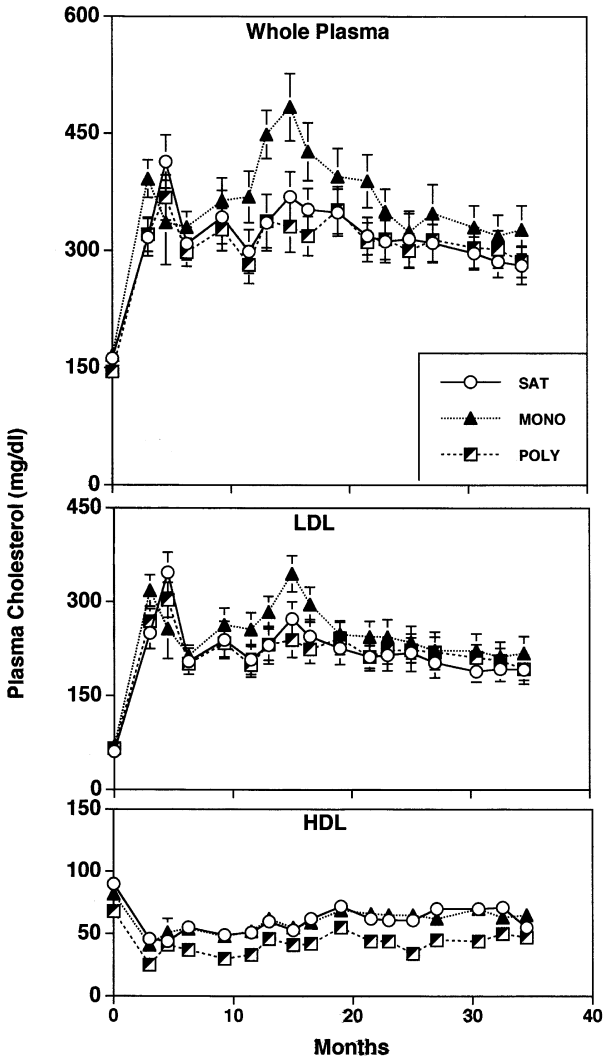

Fig. 1. Plasma cholesterol, LDL cholesterol, and HDL cholesterol measured for all animals of the saturated (Sat), monounsaturated (Mono), and polyunsaturated (Poly) fat groups when fed a zero cholesterol diet (0 months), and at various times (months) after cholesterol was added to the diet. All values represent means ± SEM for 10 to 13 measurements at each time. The lower HDL cholesterol in the Poly group was statistically significant by repeated measures ANOVA, $P < 0.01$.

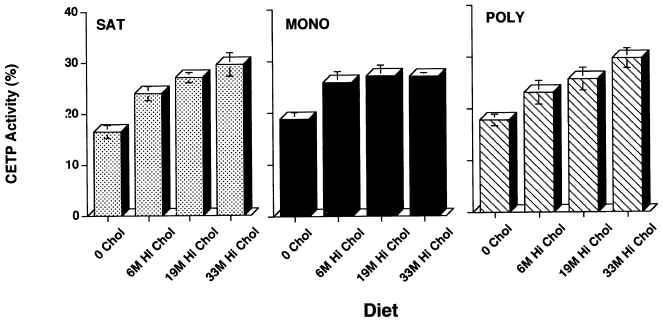

Fig. 3. CETP activity measured in plasma samples taken when the animals were fed the diets indicated. Each column represents the mean ± SEM for 10 to 13 animals. CETP activity is presented as the percentage of radiolabeled CE in HDL transferred in 1 h when exogenous LDL, and VLDL, are added for the assay. The dietary cholesterol effect was significant in each diet fat group, Sat, $P < 0.001$; Mono, $P < 0.02$; and Poly, $P < 0.01$. In the Poly group, the 6-month value was significantly lower than the 33-month value, $P < 0.02$.

TABLE 1. Correlation coefficients between CETP activity and cholesterol concentration in lipoproteins

Lipoprotein	Saturated	Monounsaturated	Polyunsaturated
Whole plasma	0.63 n = 40 $P < 0.0001$	0.41 n = 39 $P < 0.01$	0.48 n = 0.47 $P < 0.001$
VLDL + IDL-C	0.77 n = 29 $P < 0.0001$	0.28 n = 28 NS	0.56 n = 34 $P < 0.001$
LDL Cholesterol	0.76 n = 29 $P < 0.0001$	0.53 n = 28 $P < 0.01$	0.58 n = 34 $P < 0.001$
HDL Cholesterol	−0.73 n = 29 $P < 0.0001$	−0.48 n = 28 $P < 0.01$	−0.56 n = 34 $P < 0.001$

Correlation coefficients were determined in each plasma sample for which both CETP activity and lipoprotein cholesterol distribution was measured.

used in these experiments (**Fig. 2**). The agreement between the two methods was excellent ($r = 0.89$, $P < 0.0001$), indicating that the assay of CETP activity, as done here, is an accurate reflection of CETP mass concentration. For CETP activity shown in **Fig. 3**, activity increased when the diets enriched with cholesterol were compared with the zero cholesterol diet by paired *t*-test in Sat ($P < 0.001$), Mono ($P < 0.02$), and Poly ($P < 0.01$) diet groups. No significant dietary fat-related differences were found at any time point selected for study by ANOVA ($P = 0.45$–0.77). When the high cholesterol diet was fed to the Poly group, the 33-month time point showed higher CETP activity than at 6 months by paired *t*-test ($P < 0.02$). In **Table 1**, the correlation coefficients between CETP activity and each measure of lipoprotein cholesterol concentration at all four time points are shown. In all dietary fat groups, CETP activity was significantly and positively correlated with cholesterol concentrations in whole plasma, VLDL plus IDL (except in the Mono group), and LDL. Comparisons of the correlation between CETP activity and LDL cholesterol concentrations in each diet fat group are shown in **Fig. 4**. The correlation in the Sat group was higher ($r = 0.76$, $P < 0.0001$) than for the Mono group ($r = 0.53$, $P < 0.004$) and Poly group ($r = 0.58$, $P < 0.001$) although the range of CETP activity values and the slopes of the regression lines were similar for each diet group. CETP activity was negatively correlated with HDL cholesterol concentration in each dietary fat group, with the best correlation again seen in the Sat group.

In **Fig. 5**, the CE transfer ratio from exogenous CE-labeled HDL to endogenous apoB-containing lipoproteins in whole plasma (in vivo) was compared for a monkey fed a commercial chow diet and one to which the Poly diet with cholesterol was fed. The CE transfer ratio in the Poly diet animal was markedly higher at each time point. On the basis of these data, the CE transfer ratio after 30 min of incubation was determined for each animal during the cholesterol-enriched diet period. The data in **Fig. 6** show that a wide range of CE transfer ratios was obtained for each diet group, with significantly higher values being seen in the Poly group compared with the Sat and Mono groups (ANOVA, $P = 0.0037$). The data in **Fig. 7** show that the CE transfer ratio in whole plasma was significantly correlated with the CETP activity when both values were measured in the same plasma samples from animals in each diet group ($r = 0.60$, $P < 0.0003$). This outcome indicates that the transfer efficiency of CE in plasma is apparently higher in the Poly group, even though the amount of transfer protein is essentially equivalent among diet groups.

During the zero cholesterol and cholesterol-supplemented diet periods, the relationship between LDL particle concentration and CETP was compared. Subsets of animals in each dietary fat group were evaluated by comparing CETP activity with LDL receptor (LDLr) function, measured as the proportion of the fractional clearance rate (FCR, pools per day) of iodinated LDL protein due to the LDLr (**Fig. 8**). In the Sat group, a strong negative correlation ($r = -0.86$, $P < 0.0001$) was seen between CETP and LDLr FCR, but no significant association with CETP activity was identified for either of the other two diet groups. This outcome suggests that much of the difference measured in LDL cholesterol concentration may be due to factors that simultaneously control CETP production and LDLr activity in the liver when saturated fat-enriched diets are fed. Dietary monounsaturated and polyunsaturated fatty acids altered this relationship.

The particle sizes and percentage compositions were measured for LDL collected during the cholesterol-

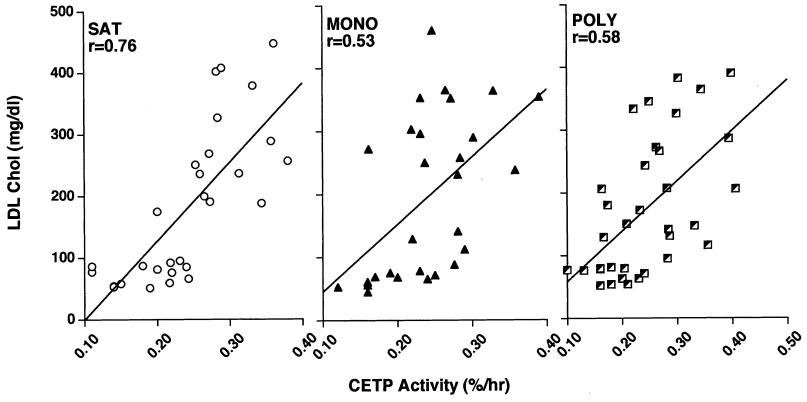

Fig. 4. Correlation between CETP activity and LDL cholesterol concentration in all of the plasma samples for which CETP assays were done. The lines drawn represent the linear best fit regression lines. Each animal in the study may be represented three or four times. The levels of significance were as follows: Sat, $P < 0.0001$; Mono, $P < 0.004$; and Poly, $P < 0.001$.

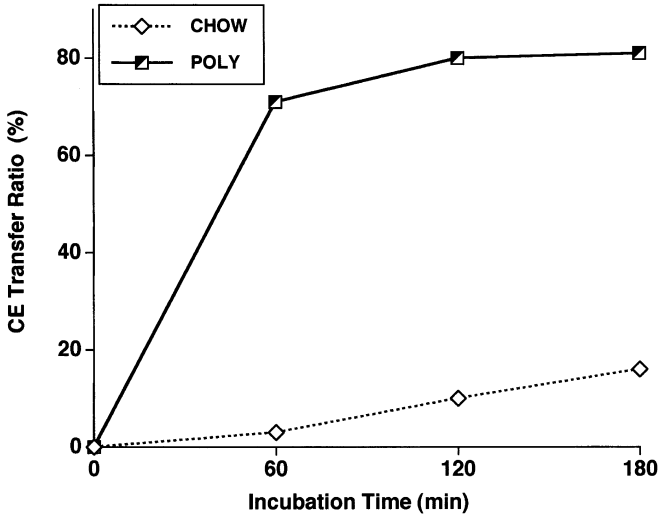

Fig. 5. Time course of transfer of radiolabeled CE from HDL added in tracer amounts to plasma taken from a monkey fed monkey chow and from another monkey fed a saturated fat diet. Acceptor lipoproteins were the endogenous lipoproteins in the plasma sample.

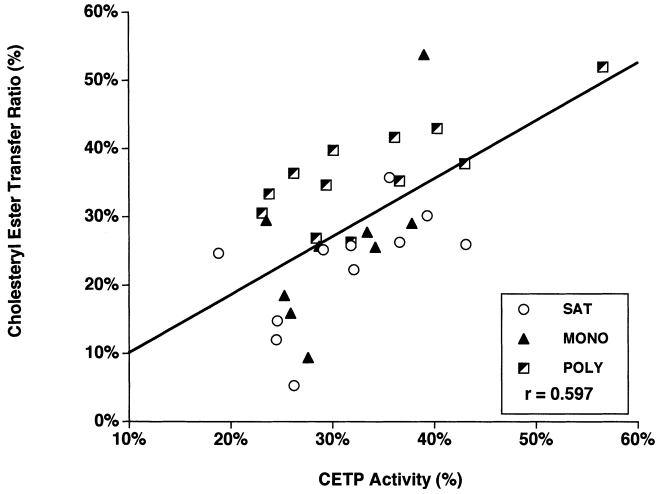

Fig. 7. Comparison between CETP activity and the CE transfer ratio in the same plasma sample taken from animals in each diet group. All plasma samples were taken during the moderate cholesterol diet period. The line drawn represents the linear best fit regression line, and the level of significance for this correlation was $P < 0.0003$.

enriched diet period, and the data are shown in **Table 2**. The values for LDL particle diameter show that the average size for LDL particles in the Mono group was significantly larger than for the particles in the Sat and Poly groups ($P = 0.0015$ by ANOVA). LDL percentage compositions also were different among diet groups. In the Mono group, the phospholipid and free cholesterol percentages were lower and the CE percentage was higher than in the Sat group. This outcome is consistent with the larger average LDL particle size in the Mono group compared with the Sat group. The percentage values for surface versus core in the Poly group suggest that these LDL particles may be intermediate in size between Sat and Mono LDL, although particle diameter measurements showed a similar size for Poly LDL and Sat LDL, both being smaller than LDL in the Mono animals. This may reflect differences in the degree of fatty acid unsaturation and altered molecular associations within the particles, as has been suggested earlier (33).

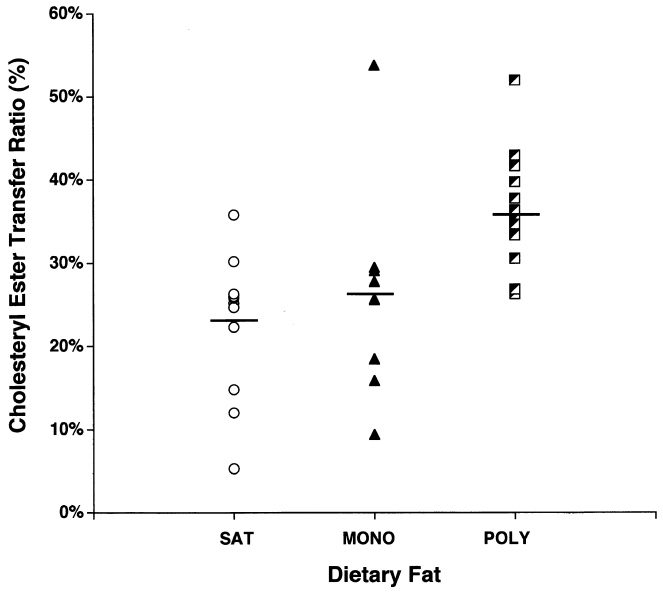

Fig. 6. Comparison of fatty acid effects on the CE transfer ratio measured in plasma samples taken from monkeys in each dietary fat group during the moderate cholesterol diet period. The horizontal lines represent means for all the observations within a group. The Poly group values were significantly ($P < 0.004$) higher than the values for the other two groups.

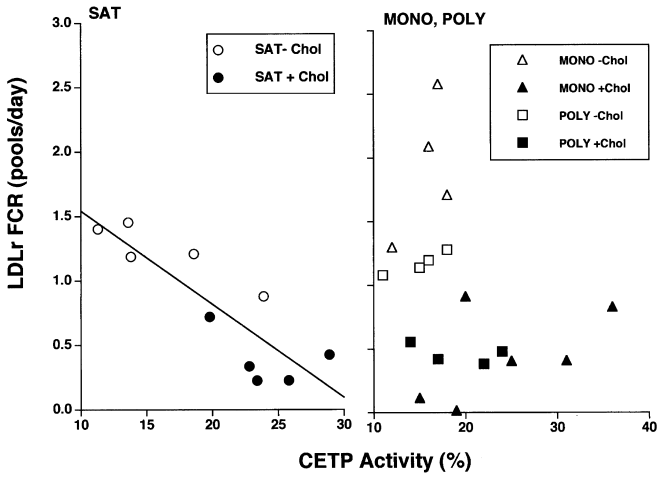

Fig. 8. Comparison between CETP activity and LDL receptor (LDLr)-mediated fractional clearance rate (FRC) measured in each animal by using ^{131}I-labeled methylated LDL and ^{125}I-labeled native LDL, during the low cholesterol diet period and again during the moderate cholesterol diet period. The linear best fit regression line is shown where a significant correlation existed ($r = -0.86$, $P < 0.0001$).

TABLE 2. Percentage compositions and average particle sizes of LDL

Component	Diet Fat		
	Saturated	Monounsaturated	Polyunsaturated
	n = 12	*n = 13*	*n = 12*
Protein	19.73 ± 0.46	19.08 ± 0.34	20.26 ± 0.23
Phospholipid	20.80 ± 0.37^{m,p}	18.80 ± 0.40^{s}	18.59 ± 0.35^{s}
Free cholesterol	10.35 ± 0.34^{m,p}	9.43 ± 0.15^{s}	9.67 ± 0.15^{s}
Cholesteryl ester	48.04 ± 0.83^{m,p}	51.96 ± 0.62^{s}	50.68 ± 0.44^{s}
Triacylglycerol	1.08 ± 0.22	0.72 ± 0.12	0.80 ± 0.09
Particle diameter (Å)	256.8 ± 5.3^{m}	278.2 ± 3.7^{s,p}	247.4 ± 2.0^{m}

All values represent means ± SEM for assays done on LDL isolated from each animal at two different times; a superscript letter indicates a statistically significant difference from saturated (s), monounsaturated (m), or polyunsaturated (p) fat group based on ANOVA with Fisher's protected least significant difference test.

A statistically significant positive correlation ($r = 0.76$, $P < 0.01$) between CETP activity and LDL particle diameter was seen but only in the Sat diet group. Significant correlations between LDL composition and CETP activity were found, again only in the Sat group, where a significant positive correlation coefficient ($r = 0.67$, $P < 0.02$) was seen with CE percentage and a significant negative correlation ($r = -0.69$, $P < 0.02$) was seen with triacylglycerol percentage.

The CE percentage compositions were also measured for LDL and for HDL collected during the period when dietary cholesterol was fed (**Table 3**). The data show significant differences among diet fat groups in each of the four major CE for both LDL and HDL. The most remarkable difference is the shift in cholesteryl oleate from 73.4% to 18.7% in the LDL from Mono and Poly diet group animals, respectively. Cholesteryl linoleate shifts inversely from 16.8% to 69.0% for LDL of the same groups. HDL show similar major shifts in CE percentages although the actual percentage values between LDL and HDL for cholesterol oleate were significantly different in the Sat and Mono groups whereas the percentages of cholesteryl linoleate were significantly different between LDL and HDL in the Poly group. The fact that percentages of CE in LDL and HDL are close within each dietary fat group, but are widely different among fat groups, suggests that CETP is relatively efficient in the exchange of many different CE.

TABLE 3. HDL and LDL cholesteryl ester percentages

CE Lipoprotein	Diet Fat		
	Saturated	Monounsaturated	Polyunsaturated
16:0 HDL	14.2 ± 0.27^{m,p,L}	7.64 ± 0.46^{s,L}	7.06 ± 0.52^{s}
16:0 LDL	16.5 ± 0.81^{m,p,H}	6.32 ± 0.4^{s,p,H}	8.59 ± 1.02^{s,m}
18:0 HDL	2.84 ± 0.20^{m,p,L}	0.97 ± 0.11^{s,L}	0.85 ± 0.07^{s,L}
18:0 LDL	5.59 ± 0.33^{m,p,H}	1.83 ± 0.18^{s,H}	1.78 ± 0.24^{s,H}
18:1 HDL	47.00 ± 1.62^{m,p,L}	62.99 ± 2.48^{s,p,L}	18.94 ± 0.63^{s,m}
18:1 LDL	48.85 ± 1.66^{m,p,H}	73.40 ± 1.17^{s,p,H}	18.68 ± 0.55^{s,m}
18:2 HDL	26.27 ± 1.68^{m,p}	20.85 ± 1.83^{s,p}	65.35 ± 1.04^{s,m,L}
18:2 LDL	25.67 ± 1.88^{m,p}	16.77 ± 0.77^{s,p}	69.03 ± 1.07^{s,m,H}

All values represent means ± SEM; percentages were done on LDL and HDL prepared from the plasma of each of the animals in each diet fat group. In each case, the percentages for each of the cholesteryl esters (CE: 16:0, cholesteryl palmitate; 18:0, cholesteryl stearate; 18:1, cholesteryl oleate; 18:2, cholesteryl linoleate) were measured. Superscript letters s, m, and p indicate statistically significant dietary fat-related differences, and superscript letters L and H indicate statistically significant differences between LDL and HDL.

TABLE 4. HDL and HDL subfraction cholesterol concentrations (mg/dl)

	Diet Fat		
	Saturated	Monounsaturated	Polyunsaturated
Total HDL	66.7 ± 6.4^{p}	65.3 ± 5.9^{p}	45.9 ± 4.1^{m,s}
HDL fraction			
HDL_5	13.3 ± 2.3	13.9 ± 3.3	7.4 ± 1.0
HDL_4	10.8 ± 3.6	8.6 ± 2.0	2.8 ± 1.0
HDL_3	21.2 ± 3.4^{p}	18.9 ± 2.2^{p}	8.8 ± 1.9^{m,s}
HDL_2	6.7 ± 1.2	9.7 ± 2.2	7.6 ± 1.8
HDL_1	14.8 ± 2.5	14.2 ± 2.8	19.3 ± 2.8

All values represent means ± SEM for four separate measurements on blood drawn between months 26 and 34 of the cholesterol-enriched diet period for each animal (n = 12, 10, and 13 for Sat, Mono, and Poly, respectively). HDL_5, HDL_4, HDL_3, HDL_2, and HDL_1 cholesterol concentrations were measured by NMR, and these fractions approximate, from larger to smaller, HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c}, respectively. Superscript letters s, m, and p indicate statistically significant dietary fat-related differences.

HDL particle distribution during the high cholesterol diet period was measured as the concentration of cholesterol in five different-sized HDL subfractions whose concentrations were measured by the NMR technique (22–24) (**Table 4**). A similar outcome was obtained when HDL subfraction distribution of protein was measured by nondenaturing PAGE on 4–30% gels (data not shown). NMR subfractions were labeled (from largest to smallest) HDL_5, HDL_4, HDL_3, HDL_2, and HDL_1, and these fractions are similar to HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c}, respectively, as seen on nondenaturing PAGE. Total HDL cholesterol concentration was significantly lower in the Poly group than in the other two groups in these samples. This difference was mainly due to significantly lower cholesterol concentrations in the HDL_3 subfraction of the Poly animals. Lower average values for cholesterol in the larger HDL_5 and HDL_4 in the Poly group were not statistically significant. The smaller HDL_2 showed no difference and the smallest HDL_1 appeared to show a higher average cholesterol concentration in the Poly group, although this was not a statistically significant difference. HDL_1 was the major cholesterol-carrying class in the Poly group, whereas HDL_3 was the subpopulation with the highest cholesterol concentration in the Sat and Mono groups. Correlations of HDL subfraction cholesterol concentrations with CETP activity were generally weak ($r < 0.5$) although a significant negative correlation ($r = -0.65$) with the concentration of HDL_3 cholesterol was found in the Sat group.

DISCUSSION

An original finding in this study was the higher strength of the association between CETP and plasma lipoprotein

end points among the animals fed the Sat diet compared with those fed Mono and Poly diets. This occurred in the face of the observation that no differences in average CETP activity were seen among dietary fat groups. The strong positive associations of CETP activity with LDL cholesterol concentration, LDL particle size and chemical composition, and, inversely, with LDLr FCR, were highly significant in the Sat diet group. Except for LDL cholesterol concentration, these correlations with CETP were statistically significant only in the Sat group. These end points have been significantly correlated with coronary artery atherosclerosis in primates, and thus it is of interest to find a dietary fatty acid effect on associations between the lipoprotein end points and CETP. Whether these associations indicate that CETP contributes to the increased atherogenicity of dietary saturated fat in primates (6) cannot be ascertained with certainty. However, these observations are made here for the first time, and suggest that regulation of CETP and its effects on lipoprotein metabolism are dietary saturated fat specific. To the extent that other dietary fats modify these associations suggests that the influence of CETP on atherogenicity could be less in these situations.

In reviews by Morton (10) and by Inazu, Koizumi, and Mabuch (34), the possible pro- versus antiatherogenic effects of CETP were considered. In the cholesterol-fed primate, a positive association between CETP mass and atherosclerosis has been reported (8). CETP transgenic/LDLr knockout mice showed 1.8-fold more atherosclerosis than LDLr knockout mice (35). A more indirect indication that CETP may promote atherosclerosis is the finding that a CETP inhibitor attenuated atherosclerosis in rabbits fed an atherogenic diet (36), and this diet would have suppressed LDLr function (37). These studies indicate that high CETP activity is proatherogenic when LDLr function is strongly suppressed. The strong negative correlation between CETP activity and LDLr function in the Sat diet group (Fig. 8) indicates that Sat diet monkeys with lowest LDLr function have the highest CETP activity. This would apparently have the effect of increasing the efficiency of moving CE into VLDL and LDL while simultaneously limiting the clearance of LDL from plasma, and thus would appear proatherogenic. The reason(s) that the Sat fat diet potentiates this effect is as yet unknown. Downregulation of mRNA for LDLr in CETP transgenic mice has been reported (38), and these authors indicated that CETP-mediated downregulation of LDLr might be due to enhanced return of cholesterol to liver. Spady, Woollett, and Dietschy (39) have suggested that saturated fatty acids uniquely redistribute cholesterol in hamster liver into a regulatory pool for the LDLr. The CETP gene has been activated by sterol regulatory element-binding protein (SREBP) 1 (40), and an isoform of this transcriptional factor (SREBP2) regulates gene expression of the LDLr (41). Suppression of SREBP1 by dietary polyunsaturated fatty acids but not saturated fatty acids has been reported (42, 43). In any case, our data suggest that CETP could be more atherogenic among populations that eat saturated fat-enriched diets. Effects of increased CETP activity on atherosclerosis in each diet group will be directly estimated when these animals are terminated.

It has been suggested that a CETP polymorphism is related to LDL particle size (44), and humans with CETP deficiency or alcohol drinkers with low CETP activity have small LDL particles (45, 46). However, CETP activity did not show a direct relation with LDL size and LDL composition in healthy young humans (47). Typically, individuals with higher plasma triacylglycerol concentrations show an inverse association with LDL particle size and this is likely due to the transfer of triacylglycerol molecules for CE into LDL, with the subsequent removal of the triacylglycerols from the particle (48). In combination, the previous studies indicate that an effect of CETP, per se, on LDL particle size and composition may not be consistent. In the present study, only the Sat group showed statistically significant relationship between LDL particle diameter, CE percentage, and CETP activity. The relative dietary fatty acid status in the human studies is unknown in most cases, but perhaps could be a contributing factor to the different outcomes among studies.

When cholesterol was added to the diet in these studies, LDL cholesterol concentrations went up and HDL cholesterol concentrations went down (Fig. 3). At the same time, CETP activity went up and a significant correlation of CETP activity with LDL and HDL cholesterol was found. The findings suggest that at least part of the reason for the shift in cholesterol distribution from HDL to LDL was due to higher CETP activity. The significant positive correlation between LDL cholesterol and CETP activity, and the significant negative correlation between HDL cholesterol and CETP activity, agree with a previous study in which CETP mass concentration was measured (8), and support this possibility for CETP-mediated CE redistribution. The fact that CETP associations were highest in the Sat group may indicate that CE transfer is somewhat less efficient when the lipoprotein core CE phase contains more saturated fatty acids and a higher melting temperature (14). The data in Fig. 6 showing an increased CE transfer ratio in the Poly group appear to support this possibility.

The fact that the HDL cholesterol concentration is lower in the Poly group than in the other groups while CETP activity is not different suggests that the lower HDL in Poly animals is not due to CETP mass. A positive correlation between the CE transfer ratio and CETP activity reflecting CETP mass (Fig. 7) suggests that the CE transfer in vivo is determined at least in part by the concentration of CETP. However, a contribution of other factors to CE transfer is indicated, because the correlation between CE transfer ratio and CETP activity was not as strong as compared with the correlation between CETP activity and CETP mass (Fig. 2). In whole plasma, lipid transfer inhibitor protein (10), apoC-I (11), and nonesterified fatty acids (49) all should affect CE transfer. Moreover, the lower HDL concentration in the Poly diet group could have affected the CE transfer ratio, because exogenous labeled HDL would have been diluted somewhat less by endogenous HDL in this method (added HDL cholesterol in the assay is about 11% in the Sat group vs. 16% of the HDL

cholesterol in the Poly group). For these reasons, we cannot be sure about the contribution of enhanced CE transfer to the lower HDL cholesterol concentrations in the Poly diet group.

It is curious that the percentage of cholesteryl linoleate in the Poly group was higher in LDL than in HDL. The anticipated difference would be for cholesteryl linoleate to be higher in HDL than LDL as a result of synthesis of this CE in HDL by LCAT. To the extent that LDL CE composition reflects that of the CE secreted from the liver in apoB-containing lipoproteins (7), LDL might be expected to have a higher percentage of ACAT2-derived cholesteryl oleate. The data were consistent with this possibility in the Sat and Mono groups, although the CETP activity in plasma appears to have been sufficient to randomize most of the CE among lipoprotein classes in all dietary fat groups regardless of metabolic source (50).

The finding in this study that during cholesterol feeding, higher CETP activity is associated with higher LDL and lower HDL, strongly supports this possibility. The increased strength of association between CETP and plasma lipoprotein end points in the Sat group may also point to the fact that higher CETP levels represent a more atherogenic situation. One difference in the present study of St. Kitts vervets is that no dietary fat-related differences in LDL cholesterol concentrations were found, whereas this was not the case in an earlier study of African green monkeys (6). It is not certain how CETP activities would compare between these two studies, but the fact that we saw the fat-related differences in lipoprotein-CETP associations in the present study in the absence of differences in LDL cholesterol concentrations was considered important.

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