Trans unsaturated fatty acids inhibit lecithin: cholesterol acyltransferase and alter its positional specificity[1]

Papasani V. Subbaiah,[2] Veedamali S. Subramanian, and Ming Liu

Departments of Medicine and Biochemistry, Rush Medical College, 1653 West Congress Parkway, Chicago, IL 60612

Abstract **Although dietary *trans* unsaturated fatty acids (TUFA) are known to decrease plasma HDL, the underlying mechanisms for this effect are unclear. We tested the hypothesis that the decreased HDL is due to an inhibition of lecithin:cholesterol acyltransferase (LCAT), the enzyme essential for the formation of HDL, by determining the activity of purified LCAT in the presence of synthetic phosphatidylcholine (PC) substrates containing TUFA. Both human and rat LCATs exhibited significantly lower activity (−37% to −50%) with PCs containing 18:1*t* or 18:2*t*, when compared with the PCs containing corresponding *cis* isomers. TUFA-containing PCs also inhibited the enzyme activity competitively, when added to egg PC substrate. The inhibition of LCAT activity was not due to changes in the fluidity of the substrate particle. However, the inhibition depended on the position occupied by TUFA in the PC, as well as on the paired fatty acid. Thus, for human LCAT, 18:1*t* was more inhibitory when present at *sn*-2 position of PC, than at *sn*-1, when paired with 16:0. In contrast, when paired with 20:4, 18:1*t* was more inhibitory at *sn*-1 position of PC. Both human and rat LCATs, which are normally specific for the *sn*-2 acyl group of PC, exhibited an alteration in their positional specificity when 16:0–18:1*t* PC or 16:1*t*–20:4 PC was used as substrate, deriving 26–86% of the total acyl groups for cholesterol esterification from the *sn*-1 position. These results show that the *trans* fatty acids decrease high density lipoprotein through their inhibition of lecithin: cholesterol acyltransferase (LCAT) activity, and also alter LCAT's positional specificity, inducing the formation of more saturated cholesteryl esters, which are more atherogenic.—**Subbaiah, P. V., V. S. Subramanian, and M. Liu. ***Trans* **unsaturated fatty acids inhibit lecithin:cholesterol acyltransferase and alter its positional specificity. *J. Lipid Res.* 1998.** 39: **1438–1447.**

Supplementary key words high density lipoproteins • LCAT • cholesterol esterification • fluidity • acyl chain length

Trans unsaturated fatty acids (TUFA) constitute a significant fraction of the fat in Western diets because they are present in hydrogenated vegetable fats such as margarines, as well as in dairy products such as butter and milk. It has been estimated that, on average, up to 10% of fatty acids consumed by U.S. population is TUFA, with some sections of population consuming up to 25% of total dietary fatty acids as TUFA (1, 2). Several reports have shown that TUFA adversely affect the cardiovascular risk by increasing total cholesterol, low density lipoprotein (LDL), and lipoprotein[a] (Lp[a]) levels, and by decreasing high density lipoprotein (HDL) levels (1, 3–6). In fact, TUFA have more adverse effects on these risk factors than equal amounts of saturated fatty acids (4, 6, 7). However, the mechanisms by which TUFA affect the lipoprotein levels are not clearly understood. The increase in LDL concentration has been attributed in part to the down-regulation of LDL receptor (8), whereas the decrease in HDL has been postulated to be due to the stimulation of cholesteryl ester transfer protein (CETP) activity, which transfers cholesteryl ester (CE) from HDL to very low density lipoprotein (VLDL) and LDL (4, 9, 10). However, TUFA feeding is also known to decrease HDL in animal species such as pigs (11, 12) and rats (13, 14), which do not have CETP activity, suggesting that additional mechanisms may be operative. The major source of HDL CE in plasma of all vertebrates is the action of lecithin:cholesterol acyltransferase (LCAT), which esterifies free cholesterol in plasma by transferring an acyl group from phosphatidylcholine (PC). Patients who have congenital deficiency of LCAT (15), or those with decreased levels of enzyme because of liver disease (16) have very low levels of HDL, showing the critical importance of the plasma LCAT activity in maintaining HDL concentration. However, the possible effects of TUFA on LCAT activity have not attracted much attention. It is known that the incorporation of 18:1*t* into plasma CE is much lower than that of 18:1*c* (2, 17). A study in essential fatty acid-deficient rats showed that dietary 18:1*t* did not affect LCAT activity in whole plasma, whereas 18:2*t* significantly decreased it (18). However it is not known whether the decrease in LCAT activity was due to changes in the lipoprotein structure or a direct effect of TUFA on the enzyme. The study of Moore, Alfin-Slater,

Abbreviations: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; PC, phosphatidylcholine; POPC diether, 1-O-hexadecyl 2-octadecenyl *sn*-glycerophosphorylcholine; TUFA, *trans* unsaturated fatty acid(s).

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[2]To whom correspondence should be addressed.

and Aftergood (19) in normal rats also showed a decrease in cholesterol esterification after TUFA feeding, but the decrease was found to be due to a reduction in the free cholesterol concentration rather than a decrease in the esterification rate. Because a significant percentage of dietary TUFA is incorporated into plasma PC (17) which is the acyl donor for LCAT reaction, it would be important to determine whether the PC species that contain TUFA are good substrates for LCAT, and whether the reduction in plasma HDL after treatment with TUFA containing diets is due to impaired LCAT activity.

Although human LCAT normally transfers the *sn*-2 acyl group from most natural PCs, our earlier studies showed that when the *sn*-2 position of PC is occupied by a long chain fatty acid such as 20:4 or 18:0, the positional specificity of the enzyme is altered, and that a substantial percentage of the acyl groups for CE formation is derived from *sn*-1 position (20, 21). As the *sn*-1 position in plasma PC is usually occupied by a saturated fatty acid, this alteration results in the formation of saturated CE species, which appear to be more atherogenic than the unsaturated CE species (22–24). Based on our studies with various synthetic PC species, we proposed that the chain length of the *sn*-2 acyl group is a major determinant of the positional specificity of LCAT, and that when the *sn*-2 acyl group exceeds certain length, the utilization of the *sn*-1 acyl group for CE formation is increased (21). Because the presence of a *trans* double bond does not shorten the chain length as much as the *cis* double bond, the chain length of 18:1*t* is greater than that of 18:1*c*, and therefore it may behave more like 18:0 and alter the positional specificity of LCAT, when present at *sn*-2 position of PC. In the present study we tested this hypothesis, in addition to studying the effects of TUFA on LCAT activity, by using purified enzyme and synthetic PC substrates containing the three most common TUFA present in human diet (18:1*t*, 18:2*t*, and 16:1*t*). We found that LCAT activity is inhibited by the presence of TUFA in PC, and that the positional specificity of the enzyme is altered when 18:1*t* or 18:2*t* is present at *sn*-2 position. These results thus provide a novel mechanism for the decrease in HDL levels and the increased risk of atherosclerosis caused by TUFA-containing diets.

MATERIALS AND METHODS

PC substrates

PC species containing the various TUFA at specific positions were synthesized chemically from the corresponding lyso PCs and free fatty acids, following the procedure described by Paltauf and Hermetter (25). The lyso PCs used were prepared by the hydrolysis of the corresponding diacyl PCs (Avanti Polar Lipids) with snake venom phospholipase A_2. The lyso PCs were purified by silica gel thin-layer chromatography (TLC), and used immediately for the synthesis of PCs. The synthesis was carried out for 17 h at 37°C in the dark and under N_2 to minimize the lipid oxidation. The position of the double bond in the fatty acids used was as follows: 16:1*t* and 16:1*c* ($\Delta 7$), 18:1*t* and 18:1*c* ($\Delta 9$), 18:2*t* and 18:2*c* ($\Delta^{9,12}$) The PCs were purified by TLC on silica gel with the solvent system of chloroform–methanol–water 65:25:4 (v/v). The positional purity of all PCs was tested by treatment of an aliquot with snake venom phospholipase A_2, and analyzing the fatty acid composition of lysoPC and free fatty acids (20). Egg PC was purchased from Sigma Chemical Co, and POPC diether was a gift from Dr. F. Paltauf (Technicsche Universitat, Graz, Austria).

Enzyme and apo A-I preparations

LCAT was purified from human plasma, obtained from a local blood bank, or rat plasma purchased from Pel-Freez Biologics (Rogers, AR). The purification procedures were as described earlier (20, 26), using a combination of ultracentrifugation and column chromatography procedures. In many experiments the phenyl Sepharose eluates were used as the enzyme source, rather than the highly pure preparations, because of the greater stability of the enzyme in phenyl Sepharose eluates. There is no difference in the specificity of the partially purified and highly purified enzyme preparations (21).

ApoA-I was purified from human plasma HDL as described earlier (20). The preparation gave a single protein band on SDS gels.

Enzyme assays

LCAT activity was assayed by using proteoliposome substrates, prepared according to the method of Chen and Albers (27). The substrate contained PC:[4-^{14}C] cholesterol:apoA-I at molar ratios of 250:12.5:0.8. Where indicated, 90% of PC was replaced by POPC diether, in order to provide a uniform matrix, for the comparison of activities with different PC substrates (28). The incubation mixture for the enzyme assay included 100 μl of proteoliposomes, 10–25 μl of enzyme preparation, 5 mm mercaptoethanol, 0.5% human serum albumin, and 10 mm Tris-Cl buffer, pH 7.4, containing 0.15 m NaCl and 1 mm EDTA, in a final volume of 0.4 ml. The reactions were carried out for 30 min at 37°C in shaking water bath, and terminated by the addition of 1 ml methanol containing 10 μg each of unlabeled free cholesterol and cholesteryl oleate. The lipids were extracted by the Bligh and Dyer procedure (29), and free cholesterol and cholesteryl ester were separated on silica gel TLC with the solvent system of petroleum ether–ethyl acetate 85:15 (v/v). The lipids were visualized by brief exposure to iodine vapors, the radioactivity in cholesterol and cholesteryl ester was determined by liquid scintillation counting, and the percent of cholesterol esterified was calculated.

Positional specificity of LCAT

The specificity of LCAT for each position of PC was determined by the HPLC separation of the labeled CE formed after a 2-h incubation, and quantitating their radioactivity with a flow-through detector (Radiomatic Flo-one, Packard Instruments Co, Meriden, CT). The mobile phase was acetonitrile–tetrahydrofuran–water 65:35:1.5 (v/v), and the flow rate of the solvent was 2 ml/min. The temperature of the column was maintained at 27°C with the help of a column heater. The ratio of scintillation fluid to solvent flow was set at 2:1, and the detector update time was 10 sec. Background subtraction was set at 25 dpm. The peaks of radioactivity were quantitated using EZchrom software (Scientific Software Ins, San Ramon, CA). As all synthetic PCs have a certain amount of contamination with the positional isomers, and the contribution of the contaminant for the enzyme reaction can distort the positional specificity results significantly, we used mixtures of positional isomers of known positional purity, and obtained best fit curves for the contribution of each position of PC. Extrapolating the curves to 100% purity gives the true positional specificity of the enzyme for a given PC species (21).

Chain lengths of acyl groups

The determination of the acyl chain lengths was performed by computer modeling of the structures with Chem3D program

(Cambridge Software), using the coordinates published by Applegate and Glomset (30), and MM2 energy minimization. The length of the acyl group was measured from the O18 of the carboxyl function to the terminal hydrogen of the methyl group, and adding the vanderWaal's radii for the terminal atoms (30).

RESULTS

Effects of TUFA-containing PCs on LCAT activity

Three major TUFA present in human diets were tested in the present study: 18:1*t* and 18:2*t*, which are the predominant TUFA present in hydrogenated vegetable oils, and 16:1*t*, which is the major TUFA in dairy fat. In order to determine the effect of TUFA on LCAT activity, we synthesized PCs containing 16:1*t*, 18:1*t* or 18:2*t* at *sn*-1 or *sn*-2 positions (pairing with 20:4 and 16:0, respectively). The synthetic PCs were incorporated into proteoliposome substrates (27) containing labeled free cholesterol and apolipoprotein A-I, and the conversion of labeled cholesterol to CE was estimated in presence of human or rat LCAT. The enzyme activity in presence of TUFA-containing PC was compared with the activity in presence of corresponding *cis* isomer. The LCAT activity is known to be affected not only by the specificity of the enzyme for a given substrate, but also by the physicochemical properties of the substrate particle (28). As the *trans* and *cis* double bonds affect the physical properties of PC differently, it is necessary to distinguish the bulk phase effects of the *trans* double bond from its effects on the interaction of the substrate with the active site of the enzyme. For this purpose, we prepared all the substrates in this experiment with 90% POPC diether, which provides a uniform "inert" matrix (28), and 10% test PC. The enzyme activities obtained in presence of such substrates should be independent of the bulk phase effects of the PC substrates.

As shown in **Fig. 1**, human LCAT showed 41% lower activity with 16:0–18:1*t* PC and 50% lower activity with 16:0–18:2*t* PC, compared with the corresponding *cis* isomers. Rat LCAT showed 27% lower activity with 16:0–18:1*t* PC and 37% lower activity with 16:0–18:2*t* PC, compared to the corresponding *cis* isomers. In contrast to the effect of TUFA at the *sn*-2 position, their incorporation into *sn*-1 position resulted in lesser inhibition of LCAT activity (**Fig. 2**). With both 16:1*t*–20:4 PC and 18:1*t*–20:4 PC, human LCAT showed about 30% lower activity, compared to the respective *cis* isomers. The activity of rat LCAT, however, was not affected by the either 16:1*t*–20:4 PC or 18:1*t*–20:4 PC. These results thus show that the presence of TUFA in PC substrate results in decreased LCAT activity, the decrease being more pronounced when the TUFA occupies the *sn*-2 position. The impaired enzyme activity is not due to changes in the bulk phase properties of the substrate because all substrates were incorporated into a common POPC diether matrix.

Effect of positional isomers of TUFA-containing PCs

TUFA are known to be incorporated into both *sn*-1 and *sn*-2 positions of plasma PC (17). Although the above experiments showed differences in the inhibitory effects of *sn*-1 TUFA versus *sn*-2 TUFA, it is necessary to determine whether the effects of TUFA depend upon the position they occupy in PC or the 'normal' acyl group with which they were paired, because in one experiment they were paired with 20:4 and in another with 16:0. Therefore we determined the effects of positional isomers of PCs that contained a TUFA either at *sn*-1 or at *sn*-2 position. Because in practice it is difficult to synthesize 100% (positionally) pure PCs in the laboratory, we determined the activities using a series of mixtures of isomers with known

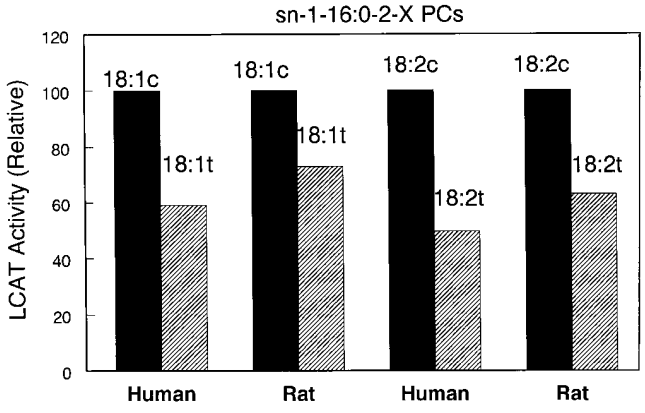

Fig. 1. Effect of the presence of *trans*-fatty acid at *sn*-2 position of substrate PC on LCAT activity. Synthetic PCs, containing 16:0 at *sn*-1 position and the indicated fatty acid at *sn*-2, were incorporated into proteoliposome substrates (27), which contained a common 'inert' matrix (90% POPC diether and 10% test PC), in addition to labeled cholesterol and apoA-I. Cholesterol esterification was determined as described in Methods, and the esterification rate obtained in presence of the *trans*-PC was expressed as the % of the activity obtained with the corresponding *cis* isomer under identical conditions. The results shown are averages of two separate experiments. The double bond was at carbon 9 for all 18:1 acyl groups, and at carbons 9 and 12 for all 18:2 acyl groups.

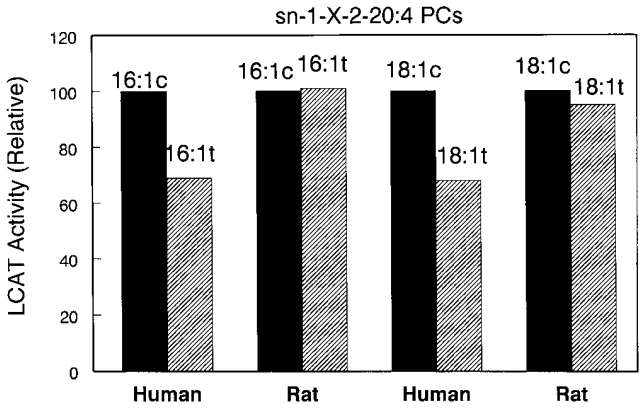

Fig. 2. Effect of the presence of *trans*-fatty acid at *sn*-1 position of substrate PC on LCAT activity. Synthetic PCs that contained the indicated fatty acid at *sn*-1 and 20:4 at *sn*-2 were incorporated into proteoliposomes (27) with 90% POPC diether matrix, as described under Fig. 1. The enzyme activity obtained with *trans*-PC is expressed as % of the activity obtained in presence of the corresponding *cis*- isomer. The results presented are averages of two separate experiments. The position of double bond for 16:1 was at carbon 7 and for 18:1 at carbon 9.

positional purity. Extrapolating the best fit curves obtained from these results to 100% purity gave the theoretical activity with each pure isomer. The results from these experiments were also used to determine the positional specificity (see below). As the aim of the experiments was to compare the activities of the two positional isomers with each other, and as they should have the same physicochemical properties (31), POPC diether matrix was not used in these experiments.

Figure 3 shows the effect of pairing 18:1*t* with 16:0, the predominant saturated fatty acid in plasma PC. When 18:1*t* was present exclusively at *sn*-1 position (18:1*t*–16:0, the left Y intercept), human LCAT activity showed about 60% lower activity when compared with the activity in presence of 18:1*c*–16:0 PC. On the other hand, the enzyme was inhibited almost completely when 18:1*t* was present at *sn*-2 position (16:0–18:1*t*, right Y intercept). This loss of activity in presence of 16:0–18:1*t* is more pronounced than that found in presence of a POPC ether matrix (Fig. 1), suggesting additional effects of TUFA on the bulk phase properties of the substrate, or the direct effects of POPC diether on the activities (32). Essentially similar results were obtained with rat LCAT, although the inhibition was less pronounced. These results show that, when paired with 16:0 acyl group, 18:1*t* was more inhibitory when it was present at *sn*-2 position of PC than at *sn*-1 position. They also show that 18:1*t* was a poorer substrate than 18:1*c* at either position.

The effects of pairing 18:1*t* with 20:4, the major 20 carbon polyunsaturated fatty acid in plasma PC, are shown in **Fig. 4**. In contrast to the results seen above with the 16:0–18:1 series, 18:1*t* was inhibitory to human LCAT, when present at *sn*-1 position, rather than at *sn*-2 position. Thus, the activity in presence of pure 18:1*t*–20:4 PC was only 30% of that obtained in presence of pure 18:1*c*–20:4 PC (right Y intercepts, Fig. 4 top), while the activities in presence of pure 20:4–18:1*t* and 20:4–18:1*c* were comparable (left Y intercepts, Fig. 4 top). Therefore the inhibitory effect of 18:1*t* on human LCAT was dependent not only on

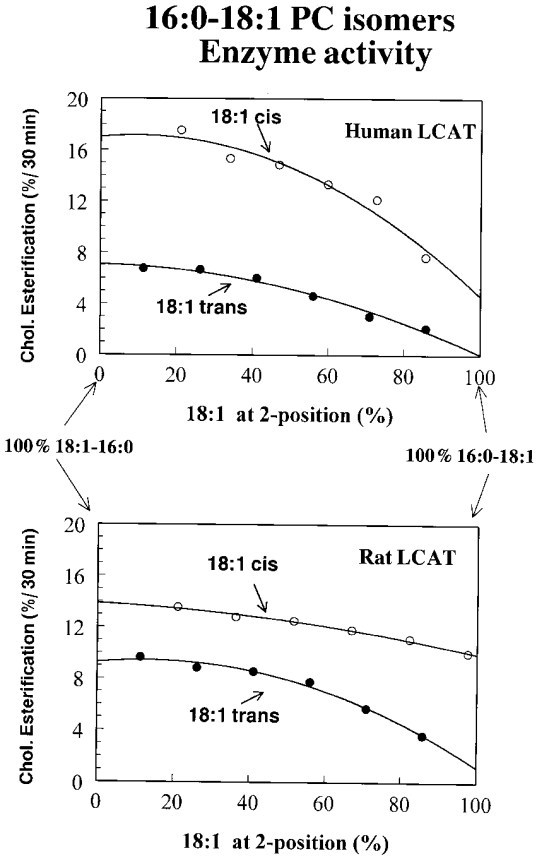

Fig. 3. Effect of positional isomers of 16:0–18:1 PC on LCAT activity. 16:0–18:1*t* PC of known positional purity was incorporated into proteoliposome substrate in the absence of POPC diether. The cholesterol esterification rates were determined as described in Methods, and a best-fit curve was plotted (SlideWrite Plus program) between positional purity of the substrate and enzyme activity (human LCAT on top, rat LCAT on bottom). Extrapolating the curve to 100% pure 16:0–18:1 PC isomer (right Y intercept) gives the theoretical activity with the pure isomer. Similarly, the left Y intercept gives the activity with pure 18:1–16:0 PC isomer. For comparison, similar curves were obtained for 16:0–18:1*c* PC isomers under identical conditions.

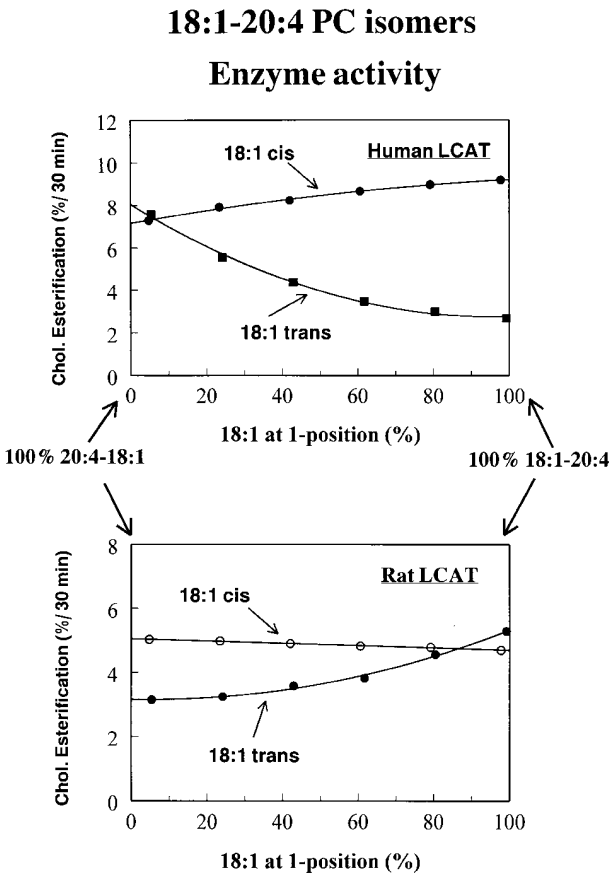

Fig. 4. Effect of positional isomers of 18:1–20:4 PC on LCAT activity. 18:1*c*–20:4 PC or 18:1*t*–20:4 PC of known positional purity was incorporated into proteoliposome substrates and the activity for human LCAT (top) or rat LCAT (bottom) was determined. Best-fit curves were obtained between the activity and positional purity of the PC substrate. The right Y intercepts give the activities in presence of pure 18:1–20:4 PCs and the left Y intercepts give the activities in presence of pure 20:4–18:1 PCs.

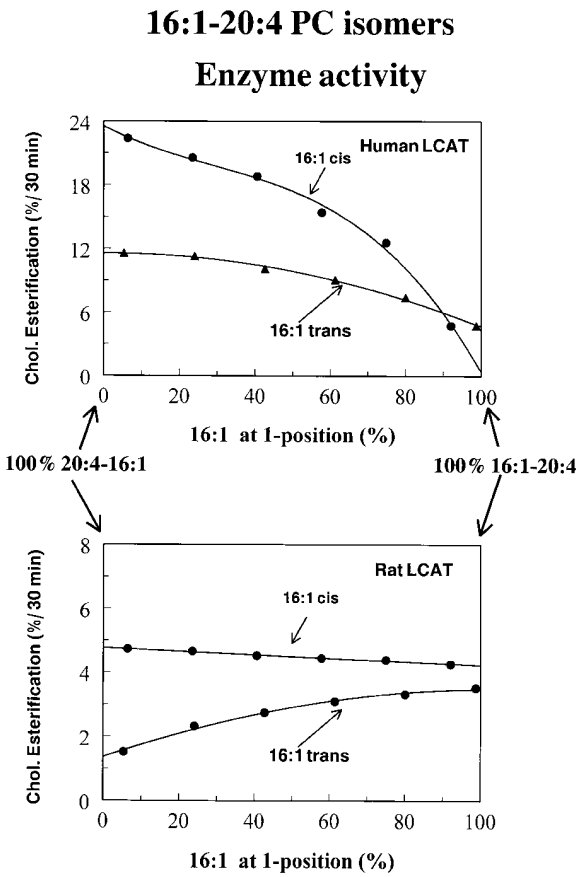

Fig. 5. Effect of positional isomers of 16:1–20:4 PC on LCAT activity. 16:1*c*–20:4 PC or 16:1*t*–20:4 PC of varying positional purities were incorporated into proteoliposome substrates, and the activities of human LCAT (top) and rat LCAT (bottom) were determined. The activities with 100% pure PCs were obtained from the best-fit curves, as described under Figs. 3 and 4.

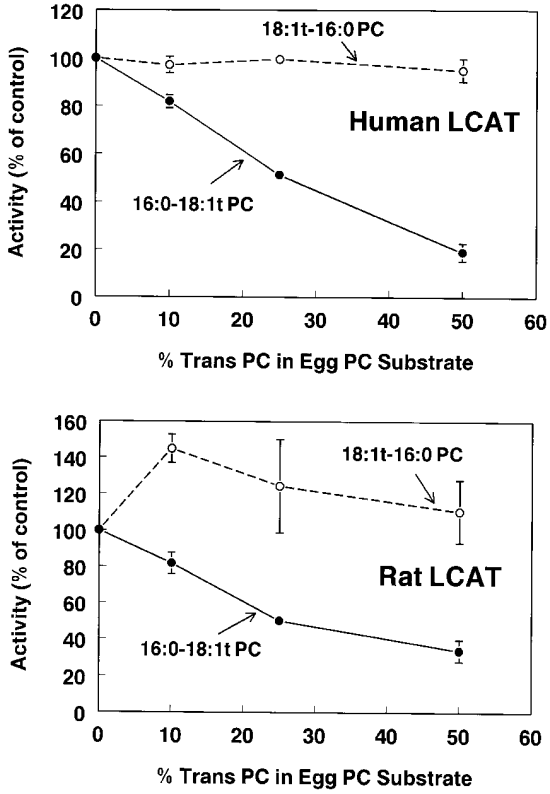

Fig. 6. Inhibition of LCAT activity, assayed with egg PC substrate, by TUFA-containing PC. Varying amounts of 16:0–18:1*t* PC or 18:1*t*–16:0 PC were substituted for egg PC in the standard proteoliposome substrate (27) (keeping the total PC concentration constant) and activities of purified human LCAT (top) and rat LCAT (bottom) were assayed in presence of the mixed substrates. All activities are expressed as % of the activity obtained in presence of 100% egg PC. The values presented are mean ± SEM of 4 experiments. As the two positional isomers of the *trans*- PC should have similar effects on the physicochemical properties of the substrate, the inhibition of activity by 16:0–18:1*t* isomer is not due to its effects on the fluidity of the substrate particle.

the position it occupied but also the acyl group it was paired with. When paired with 16:0, it was more inhibitory at *sn*-2, whereas when paired with 20:4, it was more inhibitory at *sn*-1. The effects of 18:1*t*–20:4 PCs on rat LCAT, however, were opposite to those seen with human LCAT because the presence of 18:1*t* at *sn*-2 was more inhibitory than at *sn*-1 (Fig. 4 bottom).

The results of pairing 16:1*t*, another common TUFA, with 20:4 are shown in **Fig. 5**. When 16:1*t* was present at *sn*-2 position (left Y intercept), human LCAT showed 2-fold higher activity than when it was present at *sn*-1 position (right Y intercepts). However, when compared to the *cis* isomer at the same position, 16:1*t* was inhibitory at *sn*-2, but stimulatory at *sn*-1. Rat LCAT showed lower activity with 16:1*t*, compared to 16:1*c*, whether the *trans* acid was present at *sn*-1 (18% decrease) or at *sn*-2 (72% decrease) (Fig. 5 bottom).

Competition of TUFA-containing PC with egg PC substrate

In order to determine whether the TUFA-containing PCs are simply poor substrates for LCAT or also inhibitors of LCAT activity assayed in presence of 'normal' PC, we prepared egg PC proteoliposome substrates in which increasing percentage of 16:0–18:1*t* PC was substituted for egg PC, keeping the total PC concentration in the substrate constant. Egg PC is a mixture of several PC species, the most predominant being 16:0–18:1*c* (49% of total) (23). As shown in **Fig. 6**, substitution of egg PC with 16:0–18:1*t* PC resulted in a linear inhibition of enzyme activity of both human and rat LCATs. However, when the positional isomer 18:1*t*–16:0 PC replaced the egg PC, there was either no effect (human LCAT) or even stimulation (rat LCAT) of activity. As 16:0–18:1*t* PC and 18:1*t*–16:0 PC should have similar effects on the matrix properties of the substrate particles (31), it is unlikely that the binding of the enzyme to the two particles is different. These results therefore indicate that the inhibition by *sn*-2–18:1*t* PC is not due to its effect on the fluidity or a simple dilution of the substrate, but most probably due to a competition for binding at the active site.

Effect of TUFA on the positional specificity of LCAT

The positional specificity of LCAT was determined by estimating the percentage of the labeled CE species formed

after incubation of the enzyme with each PC species and labeled cholesterol. As most synthetic PCs have varying amounts of positional isomers as contaminants, and as only a small percentage of total PC is consumed during the incubation, it is necessary to correct for the possible contribution of the contaminating isomer. For this purpose we used the series of mixtures of positional isomers as described above for enzyme activity, and obtained best fit curves for the synthesis of each CE species plotted against the positional purity of PC. Extrapolating the curve to 100% purity gives the true specificity of the enzyme for the given isomer (21).

Figure 7 shows the positional specificity curves for mixtures of 16:0–18:1 PC isomers. When 18:1*t* was present exclusively at *sn*-1 (left Y intercept) it was not utilized by either human or rat LCAT for the formation of CE, indicating absolute specificity of both enzymes for the *sn*-2 position of 18:1*t*–16:0 PC. However, when 18:1*t* was present exclusively at *sn*- 2 (right Y intercept) only 19% of the total CE formed in presence of human LCAT, and 14% of total CE formed in presence of rat LCAT was 18:1*t* CE, showing that both enzymes predominantly transferred the *sn*-1 acyl group (16:0) to cholesterol. When 18:1*c* was present at *sn*-2, both enzymes derived >95% of acyl groups from *sn*-2. These results show that both human and rat LCATs, which are normally specific for the *sn*-2 position of PC, alter their positional specificity when 18:1*t* is present at *sn*-2.

We have also determined the CE species formed in presence of 16:0–18:2*t* PC, using only a single substrate whose positional purity was 89% (89% 18:2*t* and 11% 16:0 at *sn*-2). The composition of labeled CE formed was 54% 16:0 CE and 46% 18:2*t* CE for human LCAT, and 59% 16:0 CE and 41% 18:2*t* CE for rat LCAT. Although these values may be slightly different for the 100% positionally pure 16;0–18:2*t* PC, these results nevertheless show that 18:2*t* also significantly alters positional specificity of LCAT when present at *sn*-2 position of PC.

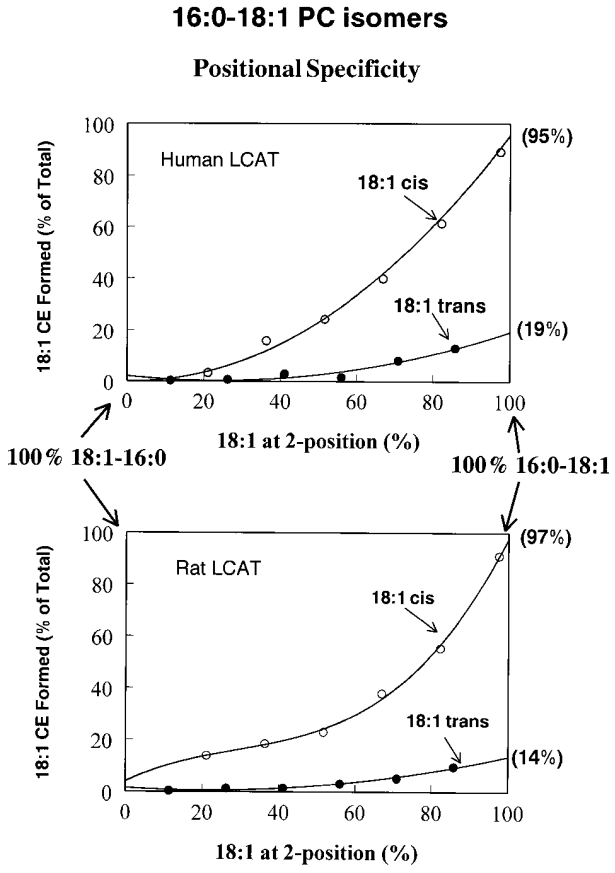

Fig. 7. Effects of pairing 18:1 (*cis*- or *trans*-) with 16:0 on the positional specificity of LCAT. 16:0–18:1*t* PC or 16:0–18:1*c* PC of known positional purity was incorporated into standard proteoliposomes (no POPC diether) and reacted with purified human (top) or rat (bottom) LCAT for 2 h at 37°C. The ^{14}C-labeled cholesteryl esters (CE) formed during the incubation were then separated on reverse-phase HPLC columns with the solvent system of acetonitrile–tetrahydrofuran–water 65:35:1.5 (v/v), and the radioactivity was quantitated with the help of a flow-through detector. Best-fit curves were plotted for the formation of labeled 18:1(t or c) CE (% of total labeled CE) against percentage of 18:1 at *sn*-2 position of PC. Extrapolating the curves to right Y axis gives the specificity of the enzyme for 100% pure 16:0–18:1 PC (21).

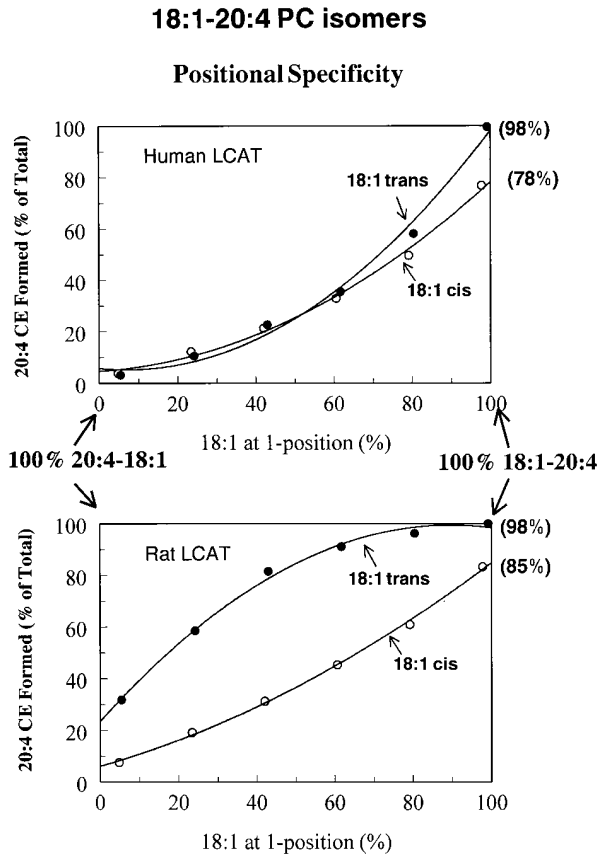

Fig. 8. Effect of pairing 18:1 (*cis*- or *trans*-) with 20:4 on the positional specificities of human (top) and rat (bottom) LCATs. The enzymes were incubated with proteoliposomes containing 18:1*c*–20:4 PC or 18:1*t*–20:4 PC of known positional purity for 2 h, and the formation of labeled CE species was determined by HPLC. The synthesis of labeled 20:4 CE (as % of total labeled CE) was plotted against the concentration of 18:1 at *sn*-1 (i.e, concentration of 20:4 at *sn*-2). Extrapolation of the best-fit curves to the right Y axis gives the positional specificity of the enzyme for 100% pure 18:1–20:4 PC. The left Y intercept gives positional specificity in presence of 100% pure 20:4–18:1 PC.

The effect of pairing 18:1*t* with 20:4 on the positional specificities of the two enzymes is shown in **Fig. 8**. When 18:1*t* was present at *sn*-1 position (right Y intercepts), virtually no 18:1*t* CE was formed in presence of either human or rat LCAT. However, when it was present at *sn*-2 (left Y intercepts), it provided 94% and 77% of the acyl groups for CE synthesis by human and rat LCATs, respectively. These results show that when 18:1*t* is paired with 20:4, the alteration in positional specificity of LCAT is less significant than when it is paired with 16:0. An interesting observation in these experiments is the utilization of significant percentage of *sn*-1 acyl group (15–22%) from 18:1*c*–20:4 PC (right Y intercepts) by both enzymes. While the utilization of 18:1*c* from *sn*-1 by human LCAT is expected based on our earlier data with 16:0–20:4 PC (21), its utilization by rat enzyme is unexpected because of its known strong preference for *sn*-2–20:4 (21, 33).

The effect of pairing of 16:1*t* with 20:4 on the positional specificities is shown in **Fig. 9**. In contrast to the results with 18:1*t*–20:4 PC, human LCAT showed dramatic change in positional specificity in the presence of 16:1*t*–20:4 PC, with only 25% of the acyl groups for CE synthesis being derived from *sn*-2 position (Fig. 9, right Y intercept). Rat LCAT also showed alteration in positional specificity, although the effect was less marked (74% from *sn*-2). Neither enzyme showed an alteration in positional specificity when 16:1*t* was present at *sn*-2 position of PC, i.e., they formed 16:1*t* CE almost exclusively.

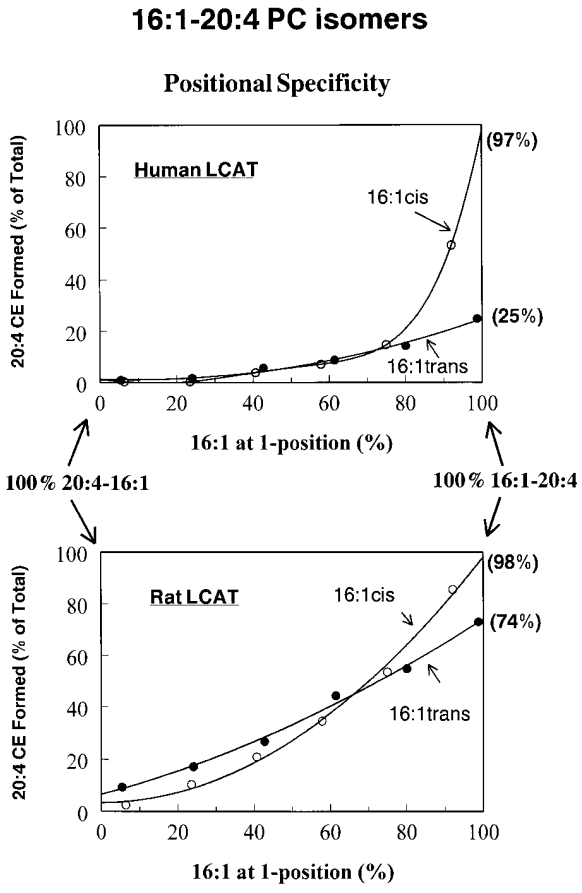

Fig. 9. Effect of pairing 16:1*c* or 16:1*t* with 20:4 on the positional specificity. 16:1*c*–20:4 PC or 16:1*t*–20:4 PC of known positional purity was reacted with either human or rat LCAT and the radioactive CE species formed were quantitated by HPLC as described in Methods. Best-fit curves were plotted for the 20:4 CE formation (% of total labeled CE) against the % of 16:1 at *sn*-1 (i.e., % of 20:4 at *sn*-2). The percentage of 20:4 CE formed in presence of pure 16:1–20:4 PC is obtained from right Y intercepts of the curve. The percentage of 20:4 CE formed in presence of pure 20:4–16:1 PC is obtained from the left Y intercepts.

Sn-2 chain length and positional specificity

As our previous studies showed a correlation between the chain length at *sn*-2 position of PC and the positional specificity of LCAT (21), we determined whether such relationship existed with the various 18 carbon acyl groups tested here. We plotted the utilization of 16:0 from the various 16:0–18:X PC species, against the chain length of 18:X, as determined by computer modeling. As shown in **Fig. 10**, a sigmoidal relationship was found between the *sn*-2 acyl chain length and the alteration in positional specificity (16:0 CE formation) by both human and rat LCATs. The values for 16:0–20:4 PC are also shown as reference, because this PC distinguishes the two classes of LCAT in vertebrates (23). The positional specificity of the enzyme is altered sharply above a certain *sn*-2 acyl chain length, this limiting length being higher for rat LCAT.

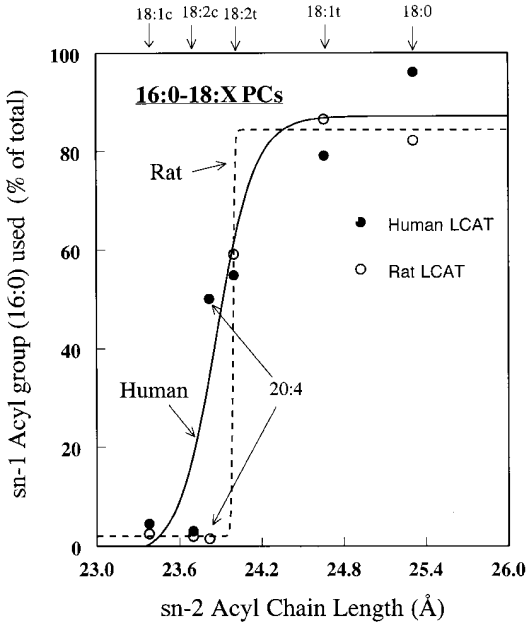

Fig. 10. *Sn*-2-acyl chain length of 16:0–18:× PC and the positional specificity. The chain lengths of the *sn*-2 acyl groups, as determined by computer modeling, were plotted against the utilization of the *sn*-1 acyl group (16:0) for CE synthesis by human and rat LCATs. The values for *sn*-2–20:4 are included for reference. All values are obtained from the extrapolation of the best-fit curves to 100% purity, except for the 16:0–18:2(t) values which were obtained with a single substrate, whose purity was 89%. A sigmoidal relationship was obtained for both LCATs, although the inflection point for rat LCAT is shifted to the right of that of human enzyme, suggesting a greater tolerance of rat enzyme for the *sn*-2 chain length.

DISCUSSION

The present study provides evidence for a novel mechanism by which TUFA may decrease HDL levels, namely the inhibition of LCAT activity. Although the increase in CETP activity by TUFA, as reported by several laboratories may play a significant role, it cannot explain the decrease of HDL in animal species such as rats and pigs which do not have CETP activity (11–14). Even in human studies, the decrease in HDL cholesterol is not always accompanied by an increase in CETP activity (7). Thus an inhibition of CE synthesis by LCAT may contribute, at least in part, to the reported decrease in HDL after ingestion of TUFA-containing fats. Although our results provide only in vitro evidence for the LCAT inhibition, the following lines of evidence indicate that this inhibition occurs in vivo also. *a*) In swine fed hydrogenated *trans* fat, the increase in plasma free cholesterol was higher than that of esterified cholesterol, indicating a decreased rate of plasma cholesterol esterification in vivo. *b*) Feeding TUFA decreases HDL_2 levels much more than HDL_3 (3, 7). The formation of HDL_2 takes place mainly in plasma compartment through the action of LCAT on HDL_3 (15, 34). Therefore, an inhibition of LCAT activity would be expected to decrease HDL_2 levels. CETP, on the other hand, is known to favor transfer of CE from HDL_3 (35), and therefore an increase in CETP activity would be expected to result in a preferential depletion of HDL_3 cholesterol. *c*) The incorporation of 18:1*t* (relative to 18:1*c*) into human plasma CE is much lower than its incorporation into the *sn*-2 position of PC (17). Since most of plasma CE in humans is formed by the LCAT reaction, this impaired transfer of 18:1*t* from PC to CE shows that 18:1*t*-containing PCs are poor substrates for LCAT, in accordance with the results presented here. *d*) A study by Privett et al. (18) in the essential fatty acid-deficient rats showed that serum LCAT activity, as measured with endogenous substrates, was decreased by >85% after feeding 18:2*t* (although not 18:1*t*). The synthesis of 20:3 CE in plasma was also inhibited significantly, showing an inhibition of LCAT activity in vivo. The different effects of 18:1*t* and 18:2*t* in this in vivo study may be related to the positional distribution of these two fatty acids in plasma PC. It is likely that 18:2*t* is incorporated into *sn*-2 position and therefore inhibited LCAT activity. On the other hand, 18:1*t* was probably incorporated more into *sn*-1 position (17), which leads to little or no inhibition of rat LCAT (see Figs. 3 and 4). Such a possibility is supported by the studies in rat brain by Karney and Dhopeshwarkar (36), who showed that >90% of 18:2*t* was incorporated into the *sn*-2 position of PC, whereas about 66% of 18:1*t* was incorporated into the *sn*-1 position. In the case of human plasma, the incorporation of 18:1*t* even into *sn*-1 position of PC would be inhibitory to LCAT reaction (Figs. 3 and 4).

The results presented here demonstrate that the decrease in LCAT activity after TUFA feeding occurs not only because the TUFA-containing PCs are poor substrates for LCAT, but also because they inhibit LCAT activity against other PC species, probably by competing with them at the active site. The decreased activity of the enzyme is not due to changes in the fluidity of the substrate particle because the inhibition was observed in presence of a uniform matrix. It may also be noted that under physiological conditions, the incorporation of dietary TUFA into lipoproteins does not alter the gross physical properties of lipoproteins significantly (11). Furthermore, the results in Fig. 6 show that while 16:0–18:1*t* PC was inhibitory, its positional isomer (18:1*t*–16:0 PC) was not. As the fluidity characteristics of these two isomers should be the same (31), the specific inhibition by the sn-2–18:1*t* isomer cannot be due to fluidity effects or a simple dilution of the 'natural' substrate.

Another important finding of the present study is that the position occupied by the TUFA in the substrate PC, as well as the fatty acid it is paired with, are critical in determining the inhibitory effects. Thus *sn*-2–18:1*t* was inhibitory when it was paired with 16:0 (at *sn*-1), but not when it was paired with 20:4. Conversely, *sn*-1–18:1*t* was inhibitory when paired with 20:4, but not with 16:0 (at *sn*-2). As the TUFA are incorporated into either position of PC (17), the fatty acids paired with TUFA are also important in determining the LCAT activity. Similarly, the alteration in the positional specificity of the enzyme is dependent upon the position occupied by the TUFA, as well as the structure of the paired fatty acid. For example, *sn*-2–18:1*t* alters positional specificity when paired with *sn*-1–16:0, but not when paired with *sn*-1–20:4.

The poor incorporation of 18:1*t* and 18:2*t* into plasma CE as reported by previous studies (2, 17) appears to be not only because of decreased LCAT activity, but also as a result of an alteration in the positional specificity of the enzyme when the TUFA occupy the *sn*-2 position. This results in the formation of more saturated CE rather than TUFA-CE. This is consistent with our hypothesis that the chain length of *sn*-2 acyl group is a critical determinant of its utilization by LCAT (21). The introduction of one *trans* double bond at C9 of 18:0 reduces the chain length by about 0.65 Å, and decreases its transition temperature by 26°C, whereas the introduction of a *cis* double bond at the same position results in shortening of the acyl chain by 2 Å, and a decrease in transition temperature by 57°C (2, 17). Even when two *trans* double bonds are present (18:2 9*t*, 12*t*), the chain length is not decreased by as much as with a single *cis* double bond (18:1 9*c*). When the positional specificity of LCAT is plotted against the chain length of *sn*-2 acyl group of PC, the alteration in specificity (utilization of *sn*-1 acyl) occurred only when the *sn*-2 chain length exceeded 23.7 Å for human LCAT and 24.0 Å for rat LCAT (Fig. 10). The chain lengths of both 18:1*t* and 18:2*t* are above these limits, and therefore their utilization for CE synthesis is low.

The physicochemical properties of TUFA are intermediate between the corresponding saturated and *cis* unsaturated fatty acids (2). However, many studies showed that the adverse effects of TUFA on the coronary risk factors (increase in LDL, decrease in HDL) are more severe than those of saturated fatty acids (4, 6, 7). One reason for this is that while the saturated fatty acids increase the HDL

cholesterol, TUFA decrease it significantly (5, 6). Another possible reason for this is that unlike the saturated fatty acids which are almost exclusively incorporated into *sn*-1 position of PC, TUFA are incorporated into either position of PC (17). Because of the alteration in positional specificity of LCAT when a TUFA occupies *sn*-2, the synthesis of saturated CE may be paradoxically increased after feeding TUFA than after feeding saturated fat diets. As the saturated CE are relatively better substrates for CETP than polyunsaturated CE (37), this may increase the CETP activity (as is observed) and thereby lead to elevated LDL and decreased HDL levels. Saturated CE are also more atherogenic because of their increased deposition and reduced clearance from arterial tissue (38, 39). The ratio of saturated CE/20:4 CE in plasma is strongly correlated with atherogenic risk (22, 23). The 18:1/18:2 ratio in plasma CE may also be increased because the relative contribution of acyl-CoA:cholesterol acyltransferase (which synthesizes predominantly 18:1 CE) would be increased due to the decrease in LCAT contribution (which produces predominantly 18:2 CE). Taken together, these results suggest that the TUFA feeding not only decreases HDL cholesterol, but may also result in the formation of functionally impaired HDL.

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