Specificity of lecithin:cholesterol acyltransferase and atherogenic risk: comparative studies on the plasma composition and in vitro synthesis of cholesteryl esters in 14 vertebrate species

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Abstract To determine whether the specificity of lecithin: cholesterol acyltransferase (LCAT) influences the susceptibility to atherosclerosis, we compared the composition and in vitro synthesis of cholesteryl ester (CE) in the plasmas of 14 vertebrate species with varying predisposition to atherosclerosis. The susceptible species (Group I) had significantly higher ratios of 16:0 CE/20:4 CE in their plasma than the resistant species (Group II). The in vitro formation of labeled CE species in native plasma from labeled cholesterol correlated highly with the mass composition, showing that the LCAT reaction is the predominant source of plasma CE in all the animal species examined. Isolated LCATs from Group I species also synthesized CE with higher ratios of 16:0/20:4 than LCATs from Group II when egg phosphatidylcholine (PC) was used as the acyl donor. In addition, the Group I LCATs exhibited lower specificity towards *sn*-2-20:4 and *sn*-2-22:6 PCs, and higher specificity towards *sn*-2-18:2 PC species than Group II LCATs. With 16:0–20:4 PC as the substrate, all Group I LCATs synthesized more 16:0 CE than 20:4 CE, whereas all Group II LCATs, with the exception of dog enzyme, synthesized predominantly 20:4 CE, showing that the two types of LCAT have different positional specificities towards this PC. These results suggest that there are two classes of LCAT in nature that differ from each other in their substrate and positional specificities, possibly because of differences in their active-site architectures. We propose that the presence of one type of LCAT, which cannot efficiently transfer certain long chain polyunsaturated acyl groups and which consequently synthesizes more saturated CE, may increase the risk of atherosclerosis.—**Liu, M., J. D. Bagdade, and P. V. Subbaiah.** Specificity of lecithin:cholesterol acyltransferase and atherogenic risk: comparative studies on the plasma composition and in vitro synthesis of cholesteryl esters in 14 vertebrate species. *J. Lipid Res.* 1995. **36:** 1813–1824.

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About 70% of cholesterol in human plasma is present as cholesteryl ester (CE). The fatty acid composition of plasma CE varies widely from species to species (1) and is affected not only by the dietary fatty acid composition, but also by endogenous factors. Several studies have suggested that CE fatty acid composition influences the atherogenic susceptibility (2–7). For example, Swell, Field, and Treadwell (2) reported that the animal species resistant to atherosclerosis have a higher concentration of 20:4 CE in their plasma than susceptible species. Similarly Chen and Li (8) have recently shown a close correlation between the polyunsaturated/saturated fatty acid ratio in plasma CE and atherogenic risk in different animal species. In addition, Schrade, Biegler, and Bohle (5) found that the plasma concentrations of 16:0 CE were higher and those of 18:2 CE and 20:4 CE significantly lower in patients with atherosclerosis than in healthy controls. The study of Young and Middleton (6) showed that White Carneau pigeons, which develop spontaneous atherosclerosis, have lower concentration of 20:4 CE than the atherosclerosis-resistant Show Racer pigeons fed the same diet. Recent investigations (9, 10) also indicate a possible relationship between plasma CE composition and insulin sensitivity, another known car-

Abbreviations: ACAT, acyl CoA:cholesterol acyltransferase; CE, cholesteryl ester; GLC, gas–liquid chromatography; HPLC, high performance liquid chromatography; LCAT, lecithin:cholesterol acyltransferase; PC, phosphatidylcholine; TLC, thin-layer chromatography.

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diovascular risk factor. Despite these studies demonstrating the relevance of CE fatty acid composition to atherogenic risk, none has addressed the causes that underlie the differences in CE composition of various animal species or the mechanisms by which this composition might influence atherogenic risk.

There are two possible sources for the CE present in plasma: *1)* the lecithin:cholesterol acyltransferase (LCAT) reaction in the plasma, which transfers a fatty acid from the phosphatidylcholine (PC) to free cholesterol to form CE on the lipoprotein surface, and *2),* the acyl CoA:cholesterol acyltransferase (ACAT) reaction in the tissues that esterifies cholesterol with an acyl CoA in the endoplasmic reticulum, after which the CE is incorporated into lipoproteins and secreted into circulation. As these two enzymes differ in their fatty acid specificity (11, 12), the relative contribution of the two reactions determines the overall CE composition of plasma. Previous studies have suggested that most CE present in human plasma is derived from the LCAT reaction, whereas in rats a significant fraction of plasma CE may be derived from the intestinal and hepatic ACAT reactions (13, 14). The plasma CE composition is also affected by the substrate specificity of LCAT and the molecular species composition of PC present in a given plasma. For example, rat LCAT is known to prefer 16:0–20:4 PC, and produce predominantly 20:4 CE, whereas human LCAT prefers 16:0–18:2 PC and synthesizes predominantly 18:2 CE, from the same mixture of PC species (15–17). These differences are further exaggerated by the presence of higher concentrations of *sn*-2 20:4 PCs in rat plasma compared to human plasma (13, 18). We have recently shown that human and rat LCATs differ from each other not only in their specificity towards PC species, but also in their positional specificity in the presence of 16:0–20:4 PC (19–21). Thus human LCAT, which is normally specific for the *sn*-2 position of most natural PCs, alters its positional specificity in the presence of 16:0–20:4 PC and produces more 16:0 CE than 20:4 CE. Rat LCAT, on the other hand, synthesizes only 20:4 CE under the same conditions (19). Thus several factors affect the CE composition in the plasma, and presumably the atherogenic risk. The aim of the present study was to determine to what extent the specificity of LCAT influences plasma CE composition in various animal species and whether LCAT specificity correlates with the susceptibility to atherosclerosis. First, we studied the plasma CE composition from 14 different vertebrate species, with a broad range of susceptibility to atherosclerosis. We next examined the synthesis of CE species in vitro by LCAT in native plasma to correlate the CE composition with LCAT specificity in the presence of endogenous PC substrates. Finally, we have determined the substrate and positional specificities of isolated LCATs from various animal species in the presence of synthetic PC substrates. The results obtained show that LCATs from atherosclerosis-resistant animals have significantly different substrate and positional specificities than the enzymes from the susceptible animals, and suggest that LCAT specificity may be a factor that influences the atherogenic risk.

MATERIALS AND METHODS

Plasma samples

All plasma or serum samples were obtained from animals or humans who consumed their respective normal' diets. The sources of plasma (or serum) for the purification of the various LCATs were as follows. Pooled frozen sera (non-sterile, trace-hemolyzed) from dog, rat, mouse, rabbit, guinea pig, chicken, horse, and sheep were purchased from Pel-Freez Biologicals (Rogers, AR). Pig plasma was obtained from Dr. Jan Rapacz of University of Wisconsin. Cow plasma was obtained from Miles Inc. (Kankakee, IL), whereas the pooled baboon plasma was obtained from Dr. Ram Kushwaha, Southwest Foundation for Biomedical Research (San Antonio, TX). Outdated human plasma was purchased from the local blood bank (United Blood Services, Chicago). Pooled plasma samples from cat and hamster were obtained from the Biological Resource Laboratories, University of Illinois at Chicago.

Fresh plasma samples were obtained for the analyses of CE fatty acid composition and in vitro synthesis of labeled CE. Most of these samples were obtained from the Biological Resource Laboratories of University of Illinois at Chicago. Samples from pigs and baboons were supplied by Drs. Rapacz and Kushwaha, respectively. Fasting blood samples from healthy human volunteers (both sexes, ages 25–50 years) were drawn in EDTA after obtaining informed consent. All samples were analyzed within 48 h for the study of labeled CE formation and CE fatty acid composition.

Enzyme purification

Human LCAT was purified to homogeneity from the outdated plasma by the procedure previously described (19, 22). All other LCATs were purified by the same procedure, excepting that the purification was carried out only up to phenyl-Sepharose step. The enzymes from cow and rat plasmas were purified further by DEAE-Sepharose and hydroxyapatite columns, similar to those used for human LCAT purification (19, 22). The substrate and positional specificities of these highly purified LCATs were similar to the corresponding partially pure (phenyl-Sepharose) LCATs (results not shown). Therefore, all LCAT purifications were rou-

tinely carried out only up to the phenyl-Sepharose step. The concentration of PC or apolipoprotein A-I present in the LCAT preparations was negligible.

Lipid analyses

Total cholesterol, free cholesterol, and triacylglycerol were estimated by the enzymatic procedures as described previously (23). Total lipids were extracted by the procedure of Bligh and Dyer (24), and separated on silica gel TLC plates with the solvent system of hexane–diethyl ether–acetic acid 70:30:1 (v/v). The spot corresponding to CE was scraped from the plate, eluted twice with diethyl ether (2 ml each), and the fatty acids were transmethylated with BF_3 in methanol for 90 min at 85°C (25). The fatty acid methyl esters were analyzed on a Shimadzu GLC equipment, as described previously (25). The fatty acid composition is expressed as weight% of the total.

In vitro synthesis of CE species in native plasma

Fresh plasma was labeled with [4-^{14}C]cholesterol, by incubating with human serum albumin-labeled cholesterol complex (26) at 4°C for 18 h. The sample was then incubated at 37°C for 6 h to allow the formation of CE by the action of endogenous LCAT, and the reaction was stopped by the addition of methanol. The lipids were extracted by the Bligh and Dyer procedure (24), and the total lipid extract was subjected to reverse phase HPLC on C-18 column with the solvent system of acetonitrile–tetrahydrofuran–water 65:35:1.5 (v/v) at a flow rate of 2 ml/min. The temperature of the column was maintained at 30°C with the help of a column heater. The identification of various CE species was done by running authentic synthetic CE species under identical conditions and monitoring their absorbance at 214 nm. Peaks from the whole plasma were also subjected to GLC analysis of the CE fatty acids, to confirm the identification. Fractions of 1 ml each (0.5 min) were collected in scintillation vials, and the solvent was evaporated off by placing them in a fume hood overnight at room temperature. Scintillation fluid (5 ml CytoScint, ICN Biomedical) was added to each vial and the radioactivity was determined in a Beckman liquid scintillation counter. The radioactivity in each CE peak was calculated and expressed as % of the total CE formed. Values for 22:6 CE and 20:5 CE peaks were combined because these two peaks were not always well separated.

Activity and specificity of purified LCATs

Egg PC and synthetic PCs were purchased from either Sigma Chemical Co. or Avanti Polar Lipids. The positional purity of various synthetic PCs was determined from the fatty acid composition of the free fatty acids and lyso PC generated by the action of snake venom phospholipase A_2. Proteoliposomes, which contained PC, [4-^{14}C]cholesterol, and human apolipoprotein A-I at molar ratios of 250:12.5:0.8, respectively, were prepared by the cholate-dialysis procedure of Chen and Albers (27). Purified LCAT was incubated with the labeled substrate in the presence of 5 mM mercaptoethanol and 0.625% (w/v) human serum albumin in 10 mM Tris-HCl buffer, pH 7.4, for 30 min at 37°C in a final volume of 0.4 ml. The reaction was stopped by the addition of 2.5 ml ethanol, and the lipids were extracted twice with 2 ml hexane containing 0.2 mg each of free cholesterol and cholesteryl oleate. The combined lipid extract was subjected to silica gel TLC with the solvent system of petroleum ether–ethyl acetate 85:15 and the spots were visualized by exposure to iodine vapors. The spots corresponding to free cholesterol and CE were scraped and their radioactivity was determined in a liquid scintillation counter. The percent of cholesterol esterified was calculated, and the enzyme activity is expressed as nmol of cholesterol esterified/h. Because of the wide variation in the activities of the various enzymes with different substrates, all enzyme activities were calculated as fraction of the activity in the presence of 16:0–18:2 PC that was assayed with the same enzyme at the same time. It is not possible to compare the absolute activities of LCATs from different species with each other because of the variation in the extent of purification and stability of various the enzymes.

The positional specificities of various LCATs were determined from the composition of labeled CE species formed from each PC. For these purposes the PCs tested were assumed to be 100% positionally pure. However, because in practice all synthetic PCs have a certain percentage of contamination from the positional isomers, the values obtained for the positional specificities may have to be corrected (20).

Statistical analyses

All statistical analyses were carried out using SPSS-PC software for Windows. Differences between various animal species were tested by one-way analysis of variance (ANOVA), with Tukey's honestly significant difference (HSD) post hoc test, and with the significance level set at 0.05. To differentiate the atherosclerosis-susceptible animals from the resistant ones, we performed discriminant function analysis, grouping rat, mouse, cat, and dog in one group (resistant), and rabbit, guinea pig, pig, and chicken in the other (susceptible), and using a step-wise method of variable entry for the plasma concentration of all CE species. The variable that minimized the overall Wilke's lambda was entered at each step. The discriminant score was calculated for individual cases to classify each of the "unknown" cases into susceptible or resistant categories. Pearson correlation coefficients

were used to measure the correlation between CE composition and in vitro synthesis of CE species.

RESULTS

Plasma CE composition

Table 1 shows the fatty acid composition of plasma CE (weight %) from 13 animal species and humans. As all plasma samples were obtained from the animals or human volunteers consuming 'normal' diets, the CE composition of each species presented here should reflect the normal pattern. As we were especially interested in 16:0 CE and 20:4 CE concentrations, we presented the results in the order of decreasing 16:0 CE/20:4 CE ratios, which are shown in **Fig. 1.** This order is maintained in all tables and figures for ease of comparison. The most abundant CE was 18:2 CE in all species except in rat where 20:4 CE was the major species. 16:0 CE was the predominant saturated CE in all species, although its concentration varied widely from 5.6% (cow) to 23.1% (rabbit). The concentration of 20:4 CE varied even more, from 0.5% (horse) to 46.3% (rat). Consequently the 16:0/20:4 ratio in plasma CE showed a wide variation among species (0.22 to 18.1). Where comparisons could be made, the CE composition reported here closely agreed with the literature values (8, 28–30). If one arbitrarily divides the animal species into two groups, Group I species with the 16:0/20:4 ratio equal to or higher than in humans and Group II with the ratio lower than in humans, the latter group contained mostly atherosclerosis-resistant species. Therefore, the high 16:0/20:4 ratio in plasma CE may be a better indicator of atherogenic susceptibility than either high 16:0 CE or low 20:4 CE concentration alone. By ANOVA, significant differences were also found between various animal species in the concentrations of individual CE species, but there was considerable overlap between the two groups (Table 1).

To investigate the possible utility of CE fatty acid composition as a "predictor" of atherogenic risk, we calculated the discriminant function coefficients for the known susceptible species and the known resistant species, and then used them to "predict" the susceptibility of other species. Based on the published data, we designated rat, mouse, cat, and dog as the resistant group, and rabbit, guinea pig, pig, and chicken as the susceptible group. Applying the step-wise discriminant function

TABLE 1. Cholesteryl ester composition in plasma (weight% of CE fatty acids, mean ± SD)

Species (n)	14:0	16:0	16:1(n-7)	1;8:0	18:1(n-9)	18:1(n-7)	18:2(n-6)
Horse (4)	0.46 ± 0.15^{a,b,c,d}	9.22 ± 0.51^{a,b}	1.14 ± 0.26^{a,b}	1.64 ± 0.69^{a,b}	8.45 ± 1.68^{b}	0.52 ± 0.09^{a}	76.88 ± 2.32^{g}
Rabbit (5)	0.79 ± 0.18^{c,d}	23.10 ± 2.35^{d}	1.34 ± 0.06^{b,c,d}	4.72 ± 1.44^{b,c}	26.34 ± 1.19^{e}	1.15 ± 0.03^{a,b,c}	38.12 ± 4.16^{b,c}
Guinea pig (6)	0.81 ± 0.51^{c,d}	16.73 ± 4.81^{c,d}	1.47 ± 0.29^{a,b,c,d}	6.99 ± 5.40^{b,c}	17.74 ± 5.03^{c,d}	1.60 ± 0.45^{b,c,d}	49.64 ± 16.55^{b,c,d,e}
Sheep (4)	0.61 ± 0.42^{b,c,d}	13.26 ± 2.15^{b,c,d}	2.24 ± 0.73^{b,c,d}	6.17 ± 2.31^{b,c}	21.25 ± 3.76^{c,d,e}	2.62 ± 2.27^{c,d}	47.73 ± 3.35^{b,c,d,e}
Chicken (4)	0.21 ± 0.17^{a}	17.70 ± 3.25^{c,d}	2.94 ± 0.26^{c,d}	13.30 ± 7.48^{c}	19.02 ± 4.74^{c,d,e}	2.11 ± 0.46^{c,d}	34.76 ± 5.68^{a,b}
Hamster (7)	0.23 ± 0.07^{a,b}	9.94 ± 1.67^{a,b}	1.44 ± 0.20^{a,b,c}	1.99 ± 0.77^{a,b}	17.25 ± 2.49^{c,d,e}	1.25 ± 0.17^{a,b,c,d}	61.29 ± 4.28^{e,f,g}
Pig (7)	0.94 ± 0.23^{d}	12.80 ± 1.70^{b,c,d}	0.88 ± 0.21^{a}	2.43 ± 0.26^{a,b}	25.82 ± 1.50^{d,e}	2.20 ± 0.21^{c,d,e}	47.31 ± 4.62^{b,c,d,e}
Baboon (7)	0.49 ± 0.13^{b,c,d}	11.80 ± 3.02^{b,c}	1.64 ± 0.55^{b,c,d}	5.73 ± 6.46^{b,c}	16.69 ± 6.51^{c}	1.50 ± 0.33^{b,c,d}	53.12 ± 8.21^{d,e}
Man (6)	0.49 ± 0.26^{b,c,d}	10.98 ± 1.44^{b,c}	3.35 ± 2.07^{d}	1.13 ± 0.43^{a}	19.02 ± 2.25^{c,d,e}	1.33 ± 0.17^{a,b,c,d}	53.73 ± 5.39^{d,e,f}
Cow (4)	0.70 ± 0.04^{c,d}	5.83 ± 0.54^{a}	1.38 ± 0.42^{a,b,c}	2.23 ± 1.56^{a,b}	3.95 ± 1.02^{a}	0.65 ± 0.03^{a,b}	74.34 ± 5.39^{f,g}
Cat (4)	0.48 ± 0.19^{a,b,c,d}	9.28 ± 0.96^{a,b}	1.82 ± 0.27^{b,c,d}	3.03 ± 0.50^{a,b}	22.42 ± 1.60^{c,d,e}	1.66 ± 0.21^{b,c,d}	49.86 ± 0.80^{c,d,e}
Dog (7)	0.41 ± 0.17^{a,b,c}	10.22 ± 2.02^{a,b}	1.45 ± 0.27^{a,b,c}	2.38 ± 0.66^{a,b}	15.36 ± 1.14^{c}	2.86 ± 0.68^{d}	44.43 ± 4.66^{b,c,d}
Mouse (6)	0.43 ± 0.29^{a,b,c,d}	7.00 ± 3.50^{a}	2.20 ± 1.07^{b,c,d,e}	1.16 ± 0.64^{a}	7.43 ± 2.29^{b}	3.38 ± 3.17^{b,c,d}	42.32 ± 2.76^{b,c,d}
Rat (5)	0.49 ± 0.21^{b,c,d}	10.28 ± 2.45^{a,b,c}	2.22 ± 0.42^{b,c,d}	2.74 ± 1.84^{a,b}	6.42 ± 2.35^{a,b}	1.06 ± 0.24^{a,b,c}	24.77 ± 2.08^{a}

Species (n)	18:3(n-6)	18:3(n-3)	20:3(n-6)	20:4(n-6)	20:5(n-3)	22:6(n-3)
Horse (4)	0.14 ± 0.04^{a,b}	0.28 ± 0.09^{a,b}	N.D.	0.52 ± 0.07^{a}	N.D.	N.D.
Rabbit (5)	0.21 ± 0.12^{a,b}	2.31 ± 1.27^{e}	0.53 ± 0.13^{c,d}	1.40 ± 0.37^{b}	N.D.	N.D.
Guinea pig	2.07 ± 2.48^{c,d,e}	1.36 ± 1.28^{a,b,c,d}	0.10 ± 0.07^{a}	1.33 ± 0.42^{b}	N.D.	0.12 ± 0.16^{a,b}
Sheep (4)	1.17 ± 0.76^{d,e}	2.45 ± 1.01^{d,e}	0.09 ± 0.09^{a,b}	2.34 ± 0.40^{b,c}	0.06 ± 0.12^{a,b}	N.D.
Chicken (4)	0.96 ± 0.17^{d,e}	0.72 ± 0.32^{b,c,d,e}	1.89 ± 0.70^{e}	3.80 ± 0.73^{c,d}	N.D.	1.30 ± 0.96^{e}
Hamster (7)	0.08 ± 0.04^{a}	0.60 ± 0.20^{b,c,d}	0.19 ± 0.06^{a,b}	3.62 ± 1.10^{c,d}	1.16 ± 0.40^{b,d,e}	0.96 ± 0.34^{c,e}
Pig (7)	0.48 ± 0.17^{b,c,d,e}	0.47 ± 0.09^{a,b,c,d}	0.54 ± 0.26^{c,d}	5.85 ± 2.38^{d,e,f}	N.D.	N.D.
Baboon (7)	0.34 ± 0.16^{b,c,d}	0.59 ± 0.29^{b,c,d}	0.46 ± 0.09^{c,d}	6.02 ± 1.98^{d,e,f}	0.77 ± 0.20^{b,c,d}	0.84 ± 0.18^{c,d}
Man (6)	0.98 ± 0.28^{d,e}	0.41 ± 0.13^{a,b,c}	0.60 ± 0.25^{c,d}	7.27 ± 1.47^{e,f}	0.30 ± 0.26^{a,b,c}	0.41 ± 0.12^{b,c,d}
Cow (4)	1.36 ± 0.11^{e}	1.25 ± 0.11^{c,d,e}	1.00 ± 0.18^{d,e}	4.13 ± 1.01^{c,d,e}	0.88 ± 0.60^{b,c,d,e}	0.16 ± 0.20^{a,b,c}
Cat (4)	0.23 ± 0.03^{a,b,c}	0.38 ± 0.17^{a,b,c}	0.18 ± 0.21^{b,c,d}	9.53 ± 2.01^{f}	0.36 ± 0.05^{a,b,c}	0.76 ± 0.30^{c,d}
Dog (7)	0.54 ± 0.33^{c,d,e}	0.13 ± 0.09^{a}	0.48 ± 0.15^{c,d}	21.35 ± 6.94^{g}	0.14 ± 0.11^{a}	0.16 ± 0.05^{a}
Mouse (6)	0.45 ± 0.09^{b,c,d,e}	0.44 ± 0.17^{a,b,c}	0.82 ± 0.28^{d,e}	23.70 ± 9.59^{g}	4.17 ± 4.08^{d,e}	6.06 ± 2.19^{f}
Rat (5)	0.53 ± 0.14^{b,c,d,e}	0.41 ± 0.05^{a,b,c}	0.31 ± 0.03^{c}	46.30 ± 2.01^{h}	2.68 ± 3.67^{e}	1.72 ± 1.15^{e}

Values with identical superscripts in the same column are not significantly different from each other by one way ANOVA, which was performed on $\log_{10}$ transformed values, with Tukey-HSD post-hoc test, significance level, 0.05; N.D., not detected.

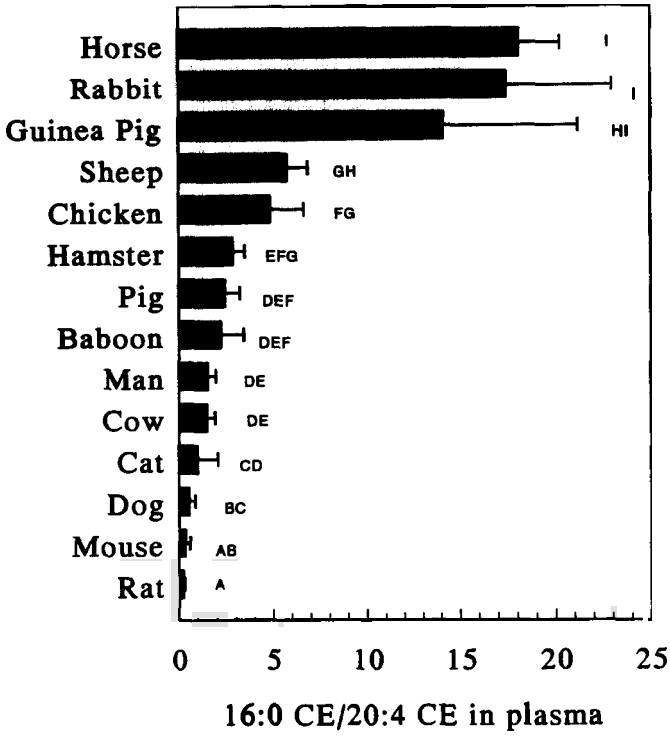

Fig. 1. Ratios of 16:0/20:4 in plasma CE of 14 vertebrate species (mean ± SD). The values are arranged in the decreasing order of the ratio, and the same order is maintained for all the results in the study paper, for ease of comparison. Bars with identical letters at top are not significantly different from each other by ANOVA, at the significance level of 0.05.

analysis to the CE values from these species, we obtained the canonical discriminant functions, which were then used to predict the susceptibility of individual animals (cases). By these criteria, all cases from horse, baboon, hamster, man, sheep, and cow were classified as susceptible. Although the 16:0/20:4 ratio in cow plasma is lower than in human plasma (Fig. 1) and the 16:0 CE concentration is the lowest among all species, the statistical test used above predicts that this species is also susceptible to atherosclerosis on the basis of total CE composition. It remains to be seen whether this prediction can be verified experimentally. It may be pointed out that the "pooled within-group correlations" between the discriminant function and various CE concentrations showed that 20:4 CE had the highest contribution to the function and 16:0 had the next highest contribution, although these two CEs affected the function oppositely.

In vitro formation of CE species by LCAT in intact plasma

Although the fatty acid analysis of CE was performed on plasma obtained from animals on their normal diets, the effect of dietary fatty acids on plasma CE composition cannot be excluded because "normal" diets of various animals can differ considerably in their fatty acid composition. It is also important to determine whether the differences in plasma CE composition are due to differences in LCAT specificity or due to varying contributions of tissue ACAT. To examine these possibilities, we obtained freshly drawn plasma from various species and studied the formation of labeled CE from labeled cholesterol that has been equilibrated with endogenous cholesterol. Prelabeled plasma was incubated for 6 h at 37°C and the labeled CE species formed were separated from each other on reverse phase HPLC as described in Methods. As all the labeled CE formed are due to LCAT reaction alone, the distribution of label among various CE species represents the specificity of LCAT in native plasma. The number of labeled CE species detected by this procedure is lower than that indicated by chemical composition, but it is clear that all the major CE species in plasma are formed by the LCAT reaction (**Table 2**). The major labeled CE formed was 18:2 CE in all species

TABLE 2. Synthesis of cholesteryl esters by LCAT in native plasma (% of total labeled CE)

Species	22:6 + 20:5 CE	20:4 CE	18:2 CE	18:1 CE	16:0 CE	18:0 CE	16:0/20:4 Ratio
Horse (4)	$3.45 \pm 1.28^{a,b,c,d}$	3.90 ± 0.58^{a}	$75.81 \pm 4.25^{f,g}$	$8.48 \pm 1.81^{b,d}$	$8.26 \pm 1.57^{c,d}$	$0.11 \pm 0.15^{a,b}$	2.21 ± 0.29^{i}
Rabbit (5)	6.93 ± 3.03^{d}	7.84 ± 1.87^{b}	$58.03 \pm 6.29^{d,e}$	$11.56 \pm 1.89^{d,e}$	15.40 ± 1.99^{e}	$0.14 \pm 0.15^{a,b}$	$1.98 \pm 0.26^{h,i}$
Guinea pig (6)	1.72 ± 0.65^{a}	7.18 ± 1.61^{b}	$64.82 \pm 3.63^{e,f}$	$11.34 \pm 1.68^{d,e}$	14.80 ± 1.58^{e}	$0.14 \pm 0.09^{a,b}$	2.13 ± 0.40^{i}
Sheep (4)	$1.93 \pm 0.94^{a,b}$	$9.42 \pm 1.06^{b,c}$	$61.32 \pm 3.95^{d,e}$	$16.30 \pm 3.14^{e,f}$	$10.87 \pm 2.10^{d,e}$	$0.16 \pm 0.13^{a,b}$	$1.15 \pm 0.14^{g,h}$
Chicken (4)	$5.42 \pm 0.88^{c,d}$	$12.63 \pm 1.24^{c,d,e}$	45.06 ± 2.06^{c}	$21.34 \pm 2.13^{f,g}$	$13.40 \pm 1.95^{d,e}$	2.15 ± 2.29^{b}	1.08 ± 0.24^{g}
Hamster (6)	$3.44 \pm 1.32^{a,b,c,d}$	$12.25 \pm 2.68^{c,d,e}$	$59.13 \pm 4.15^{d,e}$	$15.81 \pm 2.07^{e,f}$	$9.10 \pm 2.17^{c,d}$	$0.28 \pm 0.23^{a,b}$	$0.75 \pm 0.13^{e,f,g}$
Pig (6)	$2.68 \pm 0.49^{a,b,c,d}$	$15.03 \pm 2.34^{e,f}$	46.10 ± 3.05^{c}	24.18 ± 2.15^{g}	$11.83 \pm 2.56^{d,e}$	$0.20 \pm 0.09^{a,b}$	$0.81 \pm 0.22^{e,f,g}$
Baboon (6)	$2.37 \pm 0.37^{a,b,c}$	19.80 ± 1.59^{f}	54.50 ± 2.88^{d}	$11.11 \pm 1.78^{d,e}$	$12.10 \pm 1.11^{d,e}$	$0.13 \pm 0.07^{a,b}$	$0.61 \pm 0.06^{d,e,f}$
Man (6)	$3.45 \pm 2.03^{a,b,c,d}$	$13.58 \pm 2.07^{d,e}$	$59.21 \pm 4.27^{d,e}$	$11.94 \pm 2.87^{d,e}$	$11.55 \pm 1.46^{d,e}$	$0.28 \pm 0.11^{a,b}$	$0.88 \pm 0.24^{f,g}$
Cow (4)	$1.85 \pm 0.73^{a,b}$	$9.26 \pm 1.36^{b,c,d}$	78.84 ± 1.74^{g}	$4.62 \pm 0.99^{a,b}$	4.41 ± 1.73^{b}	0.84 ± 0.37^{b}	$0.48 \pm 0.16^{c,d}$
Cat (4)	$4.80 \pm 1.37^{b,c,d}$	$13.06 \pm 1.40^{c,d,e}$	$57.00 \pm 3.68^{d,e}$	$18.78 \pm 1.85^{f,g}$	$6.20 \pm 1.36^{b,c}$	$0.17 \pm 0.13^{a,b}$	$0.48 \pm 0.12^{c,d,e}$
Dog (7)	$3.82 \pm 0.50^{a,b,c,d}$	30.56 ± 3.27^{g}	43.83 ± 5.23^{c}	$11.59 \pm 2.38^{d,e}$	$10.05 \pm 1.96^{c,d}$	$0.16 \pm 0.13^{a,b}$	$0.33 \pm 0.09^{b,c}$
Mouse (6)	5.90 ± 1.07^{d}	48.30 ± 3.56^{h}	37.79 ± 2.61^{b}	$5.48 \pm 1.04^{a,b,c}$	2.42 ± 0.77^{a}	0.11 ± 0.15^{a}	0.05 ± 0.02^{a}
Rat (5)	$4.27 \pm 0.66^{b,c,d}$	49.07 ± 1.60^{h}	31.43 ± 1.95^{a}	5.08 ± 0.83^{a}	$10.08 \pm 1.55^{d,e}$	$0.07 \pm 0.08^{a,b}$	0.21 ± 0.03^{b}

Values with identical superscripts vertically are not significantly different from each other by one way ANOVA, which was performed on $\log_{10}$ transformed values, with Tukey-HSD post-hoc test, significance level, 0.05.

except in mouse and rat plasmas which synthesized more 20:4 CE than 18:2 CE. In general there was very high correlation between the percentage of labeled CE formed and the corresponding concentration of the CE in plasma, except for 18:0 CE (correlation coefficients were: 20:4, $r = 0.91$; 18:2, $r = 0.70$; 18:1(n–9), $r = 0.75$; 16:0, $r = 0.66$; 18:0, $r = 0.22$). The ratios of labeled 16:0 CE/ labeled 20:4 CE are also shown in Table 2. ANOVA analysis showed that there was virtually no overlap in the ratio between the two groups of animals. The correlation between this ratio in labeled CE and in plasma CE was excellent, as shown in **Fig. 2** ($r = 0.86$). A notable deviation from linearity was observed for the values from mouse plasma, in which the ratio in labeled CE was consistently lower than that expected from the plasma CE concentrations. This may be due to a significant contribution of 16:0 CE by ACAT reaction in mouse, although other possibilities, such as differential turnover of various CE species, cannot be excluded.

In vitro synthesis of CE by partially purified LCATs in the presence of egg PC

Although the synthesis of labeled CE in intact plasma most probably represents the specificity of LCAT in vivo, it is possible that the composition of endogenous PC substrates, activators, and inhibitors influences the composition of CE synthesized by LCAT. In order to study the substrate specificity of LCATs from various species under identical conditions, we partially purified the enzyme from each of the 14 species, and determined the CE species formed in the presence of a common substrate (egg PC), which contains a mixture of PC species, and a common apoprotein activator (human apolipoprotein A-I). The molecular species composition of egg PC, as well as the fatty acid composition at *sn*-1 and *sn*-2 positions are given in **Table 3.** Proteoliposome substrates containing egg PC, human apolipoprotein A-I, and labeled cholesterol were prepared by established procedures (27), reacted with partially purified LCATs, and the composition of labeled CE species synthesized was determined. Our studies with human, bovine, and rat LCATs showed that there is no difference in the specificities of highly purified and partially purified LCATs. Similar results were reported by Grove and Pownall (16). The percentages of labeled CE species formed in the presence of egg PC substrate and various LCATs are shown in **Table 4.** The major CE formed was 18:1 CE with most LCATs, except for cow and mouse

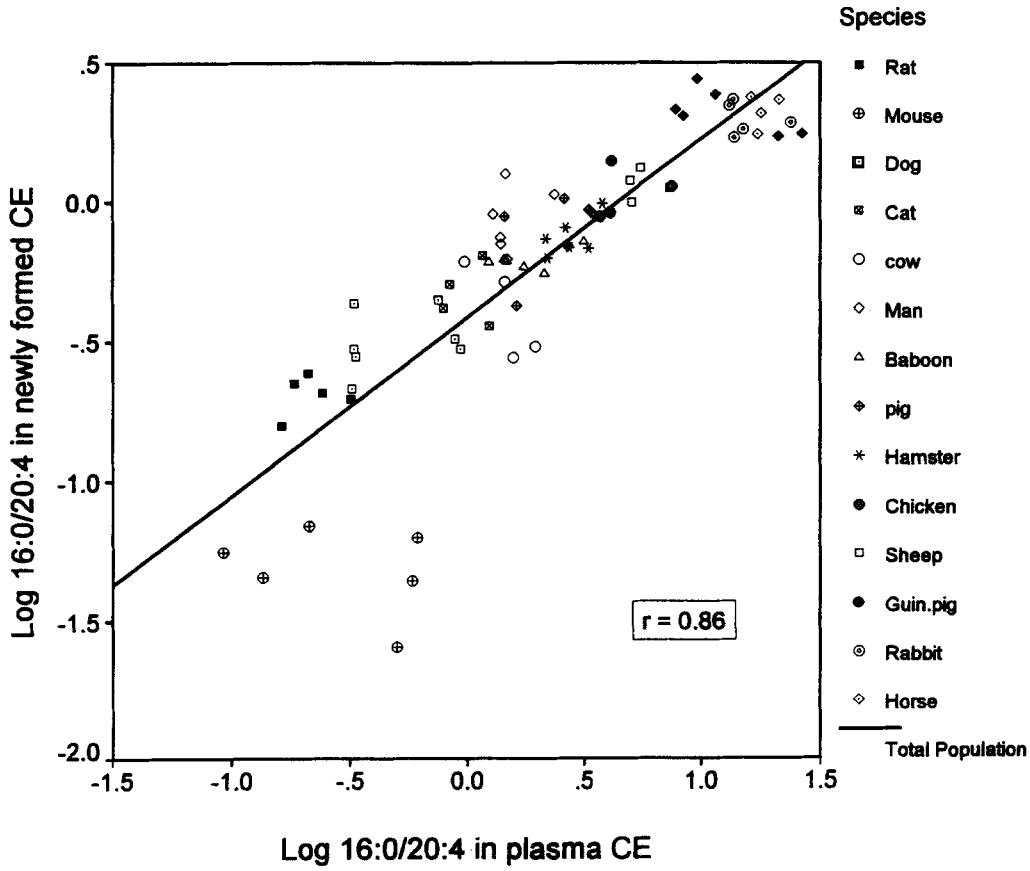

Fig. 2. Correlation of 16:0 CE/20:4 CE (mass) ratio in the plasma with the same ratio in the newly formed (labeled) CE by LCAT in native plasma.

TABLE 3. Molecular species composition and positional distribution of fatty acids in egg PC

Molecular Species of PC		Positional Distribution of Fatty Acids		
PC Species	% of Total PC	Fatty Acid	*sn*-1	*sn*-2
			%	
16:0–22:6	0.99	14:0	0.26	0.06
18:1–20:4	0.10	16:0	68.98	1.46
16:0–20:4	1.94	16:1(n–7)	0.84	0.95
18:1–18:2	1.02	18:0	25.46	0.46
16:0–18:2	18.24	18:1(n–9)	3.07	59.80
18:0–20:4	2.89	18:1(n–7)	0.72	1.65
18:1–18:1	2.18	18:2(n–6)	0.41	27.67
16:0–18:1	49.37	18:3(n–6)	0.08	0.02
18:0–18:2	11.27	20:1(n–9)	0.08	0.11
18:0–18:1	12.01	20:2(n–6)	0.06	0.31
		20:3(n–6)	0.00	0.55
		20:4(n–6)	0.03	4.62
		22:4(n–6)	0.00	1.27
		22:6(n–3)	0.00	0.86

Molecular species composition of PC was analyzed by HPLC, after derivatization to diacylglycerol benzoates. To determine the positional distribution of fatty acids, egg PC was first treated with snake venom phospholipase A_2, and the fatty acid composition of lyso PC (*sn*-1) and free fatty acids (*sn*-2) was analyzed by GLC, after methylation.

LCATs which formed more 18:2 CE. More importantly, the 16:0/20:4 ratio in newly formed CE correlated positively with the ratio in plasma CE mass ($r = 0.74$) (results not shown). Similarly, a high correlation was found between the ratio of labeled CE formed with endogenous substrates and with egg PC ($r = 0.87$) (**Fig. 3**), indicating that LCAT specificity, and not the PC substrate composition, is the primary determinant of this ratio.

If one assumes that all the acyl groups for CE synthesis are derived from the *sn*-2 position of PC, the selectivity for each fatty acid can be calculated by dividing the amount of labeled CE formed by the concentration of the same fatty acid at *sn*-2 position of PC. A selectivity value of > 1.0 indicates the preferential utilization of the acyl group, whereas a selectivity value of < 1.0 shows that the acyl group is a poor substrate compared to the 'average' *sn*-2 acyl group. The results presented in **Table 5** show that the greatest variation in selectivity was found for 20:4, with the LCATs from cow, cat, mouse, and rat showing high selectivity for this fatty acid, while the rest of the LCATs showed low preference for it. On the other hand, LCATs from all the species examined exhibited high selectivity values for 16:0. This may result from either a preferential utilization of *sn*-2-16:0 over other *sn*-2 acyl groups or from the utilization of 16:0 from the

TABLE 4. CE species formed by isolated LCATs in the presence of egg PC

	% of Total Labeled CE Formed					
LCAT	20:4	18:2	18:1	16:0	18:0	16:0/20:4
Horse	1.5	40.4	47.2	9.0	0.9	6.0
Rabbit	3.1	31.2	43.9	17.1	0.2	5.5
Guinea pig	2.9	34.4	45.5	11.6	0.0	4.0
Sheep	3.1	36.7	49.5	8.2	0.3	2.6
Chicken	1.9	39.5	44.6	12.5	0.0	6.6
Hamster	3.5	35.7	51.3	7.1	0.0	2.0
Pig	2.4	35.7	47.4	14.5	0.0	6.0
Baboon	2.5	34.6	49.7	11.0	0.0	4.4
Man	3.8	29.9	46.1	11.5	0.7	3.0
Cow	4.9	56.9	19.1	12.1	0.5	2.5
Cat	10.0	33.7	38.2	11.2	0.0	1.1
Dog	3.1	35.2	51.2	8.5	0.0	2.7
Mouse	15.0	36.5	24.5	3.9	0.0	0.3
Rat	11.8	31.3	36.9	12.2	1.1	1.0

Isolated LCATs were reacted with a proteoliposome substrate containing egg PC–labeled free cholesterol–human apolipoprotein A-I at the molar ratios of 250:12.5:0.8 for 2 h at 37°C, and the labeled CE species formed were analyzed by HPLC as described in the text.

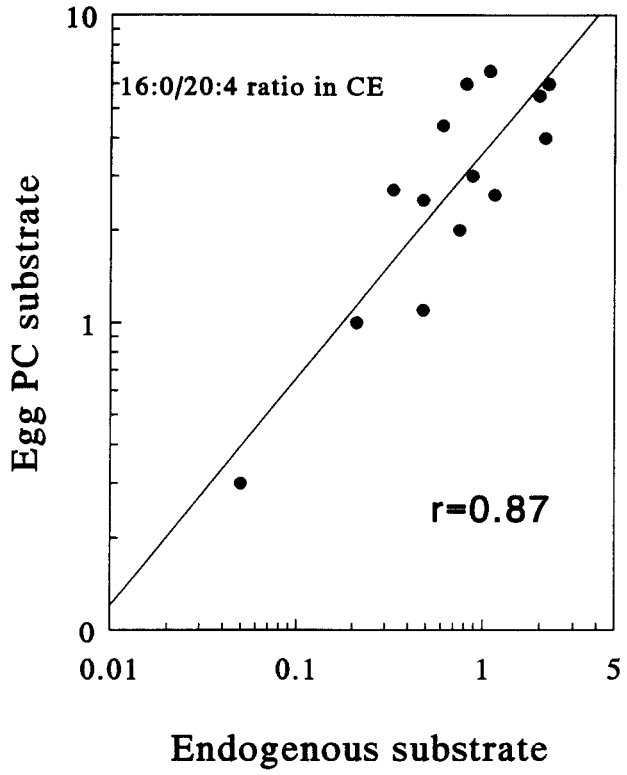

Fig. 3. Correlation of 16:0 CE/20:4 CE in plasma with the same ratio in the newly synthesized CE by the isolated LCATs in the presence of egg PC as acyl donor, and human apolipoprotein A-I as the activator.

sn-1 position of certain PC species, as we demonstrated for human plasma (19). All the LCATs showed low selectivity for 18:1, the major fatty acid at the *sn*-2 position of egg PC.

Substrate and positional specificities of isolated LCATs in the presence of synthetic PCs

We next determined the substrate specificities of various purified LCATs using proteoliposome substrates containing single species of synthetic PCs. In addition to showing the specificity towards various naturally occurring PCs under defined conditions, these experiments allowed us to determine the positional specificities of different LCATs from the composition of labeled CE formed. To normalize for the variations in LCAT potency among animal species, we calculated the relative activities of the enzymes by taking the activity in the presence of 16:0–18:2 PC as 1.0, as this PC was a good substrate for all LCATs. The results presented in **Table 6** show wide variations in the relative activities of various LCATs, especially between Group I and Group II LCATs. All LCATs showed lower activity with 16:0–18:1 PC (compared to 16:0–18:2 PC) and all enzymes, except cat, mouse, and rat LCATs also showed lower relative reactivity with 16:0–20:4 PC. In general, Group II LCATs exhibited higher activity with *sn*-2-20:4 PCs compared to Group I LCATs. One exception was guinea pig LCAT, which showed high activity with 14:0–20:4 PC.

Group I and Group II LCATs showed marked differences in their positional specificities. While all LCATs were specific for the *sn*-2 position of 16:0–18:2, 16:0–18:1, and 16:0–18:3 PCs, Group I LCATs derived less than 30% of the acyl groups from the *sn*-2 position of 16:0–20:4 PC. On the other hand, all Group II enzymes, with the exception of dog LCAT, derived the majority of the acyl groups from the *sn*-2 position of this PC. It should be pointed out that the synthetic PCs used contained a small percentage of contamination with positional isomers, and the percentage contribution

TABLE 5. Selectivity of various LCATs for the *sn*-2 acyl groups of egg PC

	Selectivity for the Acyl Group[a]				
LCAT	20:4	18:2	18:1	16:0	18:0
Horse	0.32	1.46	0.77	6.16	1.96
Rabbit	0.67	1.13	0.71	11.71	0.43
Guinea pig	0.63	1.24	0.74	7.95	0.00
Sheep	0.67	1.33	0.81	5.62	0.65
Chicken	0.41	1.43	0.73	8.56	0.00
Hamster	0.76	1.29	0.83	4.86	0.00
Pig	0.52	1.29	0.77	9.93	0.00
Baboon	0.54	1.25	0.81	7.53	0.00
Man	0.82	1.08	0.75	7.88	1.52
Cow	1.06	2.06	0.31	8.29	1.09
Cat	2.16	1.22	0.62	4.17	0.00
Dog	0.67	1.27	0.83	5.82	0.00
Mouse	3.25	1.32	0.40	2.67	0.00
Rat	2.55	1.13	0.60	8.36	2.39

[a]Ratio of % of labeled CE formed/% of corresponding acyl group at *sn*-2 position of egg PC.

TABLE 6. Activities and positional specificities of various LCATs in the presence of synthetic PC substrates[a]

LCAT	16:0–18:2 (93.2%)[a]		16:0–18:1 (97.6%)[a]		16:0–18:3 (75.3%)[a]		14:0–20:4 (85.7%)[a]		16:0–20:4 (85.8%)[a]		18:0–20:4 (84.6%)[a]		16:0–22:6 (94.5%)[a]	
	Activity[b]	% from *sn*-2	Relative Activity[c]	% from *sn*-2	Relative Activity[c]	% from *sn*-2	Relative Activity[c]	% from *sn*-2	Relative Activity[c]	% from *sn*-2	Relative Activity[c]	% from *sn*-2	Relative Activity[c]	% from *sn*-2
Horse	3.29	98.2	0.55	89.6	0.48	73.2	0.23	26.1	0.28	23.9	0.12	86.5	0.14	23.3
Rabbit	3.08	91.0	0.72	88.6	0.86	59.3	0.41	11.1	0.65	21.6	0.24	83.9	0.37	29.0
Guinea pig	2.20	92.3	0.64	89.7	1.05	58.6	0.32	84.7	0.63	24.2	0.14	91.7	0.50	6.0
Sheep	2.52	92.5	0.89	87.1	1.01	63.2	0.21	25.1	0.49	26.2	0.13	75.5	0.31	13.9
Chicken	2.87	89.4	0.83	81.7	0.98	86.6	0.23	15.8	0.51	26.9	0.13	75.3	0.33	27.7
Hamster	4.17	94.0	0.74	91.7	0.68	63.2	0.22	30.0	0.41	28.0	0.11	82.1	0.15	16.2
Pig	1.91	95.8	0.69	91.8	0.71	66.2	0.35	16.2	0.44	21.0	0.22	92.5	0.29	15.9
Baboon	3.38	93.8	0.57	91.0	0.62	86.2	0.19	27.9	0.53	30.0	0.14	82.0	0.23	11.4
Man	2.87	92.9	0.74	94.0	0.94	67.5	0.47	24.5	0.60	20.9	0.14	78.9	0.32	20.7
Cow	4.50	92.5	0.59	97.6	0.74	93.7	0.37	18.5	0.40	87.4	0.18	97.6	0.07	48.1
Cat	1.75	92.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.08	62.2	N.D.	N.D.	0.62	34.5
Dog	3.93	94.9	0.55	93.9	0.66	65.4	0.25	30.3	0.54	33.9	0.15	83.9	0.20	25.4
Mouse	2.00	92.5	0.79	90.8	1.05	77.7	1.48	29.9	1.50	84.8	0.99	98.5	1.13	68.0
Rat	1.90	90.3	0.77	88.6	1.13	76.4	1.07	50.8	1.45	75.1	0.97	100.0	0.90	34.1

[a]Values in parentheses denote positional purity as determined by snake venom phospholipase A_2 treatment: N.D., not determined.
[b]Activity given as nmol cholesterol esterified/h.
[c]Activity relative to 16:0–18:2 PC.

from *sn*-2 position may therefore be slightly higher than that shown in Table 5 for most PCs (20). Nevertheless, the positional specificity in the presence of 16:0–20:4 PC appears to differentiate the two groups of LCAT from each other. In contrast to the results with 16:0–20:4 PC, the positional specificity was not altered in the presence of 18:0–20:4 PC, because all LCATs transferred predominantly the 20:4 acyl group from this PC. The positional specificities in the presence of 14:0–20:4 PC and 16:0–22:6 PC, on the other hand, showed differences between the two groups of LCAT, although the distinctions were not as clear cut as with 16:0–20:4 PC. These differences in positional specificity may be partly responsible for the observed variations in the 16:0/20:4 ratios in plasma CE.

DISCUSSION

Although several previous studies have shown that low concentration of 20:4 or high concentration of 16:0 in plasma CE is correlated with atherogenic risk (2, 5, 6, 8), the factors contributing to the differences in CE composition have not been investigated. The CE composition of plasma is affected by several factors that include dietary fatty acid composition, molecular species composition of plasma PC, specificity of LCAT towards various PC species, and the relative contributions of tissue ACAT. The results presented here clearly show that the majority of differences in plasma CE composition among various animal species can be attributed to the substrate and positional specificities of LCAT, rather than to the diet or ACAT contribution. This is especially true for the ratio of 16:0 CE/20:4 CE, which we used here as a potential marker for atherogenic susceptibility, rather than the individual concentrations of the CEs. This ratio is significantly higher in those animal species known to be susceptible to atherosclerosis, compared to those that are resistant.

Although rabbit, guinea pig, chicken, pig, and baboon are known to be susceptible, we are not aware of any careful study that shows atherosclerotic risk in horse, the species with the highest 16:0 CE/20:4 CE ratio. Similarly, rat, mouse, cat, and dog are known to be relatively resistant to atherosclerosis, but the susceptibility of cow, which also has a relatively low 16:0/20:4 ratio, is unknown. There was a close correlation between the 16:0/20:4 ratio in plasma CE and the same ratio in LCAT-synthesized CE in freshly prepared plasma. In fact, this ratio in the newly synthesized CE distinguishes the two groups better than the plasma CE (mass) ratio. Even when egg PC was used as the substrate for the partially purified LCATs, the 16:0/20:4 ratio in the newly synthesized CE correlated highly with the ratio in plasma CE, indicating that the differences in PC substrate composition do not significantly affect the ratio in most cases. On the other hand, the percentages of 18:1 CE and 18:2 CE formed by LCAT may be more reflective of the fatty acid composition of the PC substrate. Thus, LCATs from most species synthesized high percentage of 18:1 CE in the presence of egg PC, which has a high concentration of *sn*-2-18:1 PC (Table 3), whereas in native plasma the synthesis of this CE is much lower than that of 18:2 CE in all species (Table 2).

Furthermore, the correlation between the synthesis of 18:1 CE or 18:2 CE in the presence of egg PC with the synthesis of the same CE species in native plasma was weak (r = 0.54 and 0.50, respectively, for 18:1 CE and 18:2 CE). In contrast, there was a strong correlation between the 16:0/20:4 ratios of newly formed CE in the presence of both egg PC and in native plasma (r = 0.87) (Fig. 3).

Our studies with partially purified enzymes and synthetic PC substrates also showed wide variations in the substrate specificities of various LCATs. Although Grove and Pownall (16) previously studied the substrate specificity of several of the LCATs studied here, they used diacyl PCs containing the same fatty acid at both positions. For this reason, their results cannot be extrapolated to PC substrates in native plasma, which mostly contain a saturated fatty acid at *sn*-1 position and an unsaturated fatty acid at *sn*-2 position. It is not surprising, therefore, that our results with naturally occurring asymmetric PCs differ significantly from those reported by Grove and Pownall (16). For example, several of the LCATs (from man, guinea pig, rabbit, chicken, horse) showed no activity in the presence of 20:4–20:4 PC or 22:6–22:6 PC, whereas the present studies show that all enzymes can utilize 16:0–20:4 PC and 16:0–22:6 PC, albeit at widely varying rates. The specificity results of various LCATs with respect to the PC substrates are also different for the symmetric and asymmetric PCs. For example, cow LCAT was shown to be relatively nonspecific with respect to various diacyl symmetric PCs whereas our studies showed that this enzyme preferred 16:0–18:2 PC over 16:0–20:4 PC and 16:0–22:6 PC by 2.5- and 14-fold, respectively. This shows that the substrate specificity is influenced strongly by the *sn*-1 acyl group of PC, although this acyl group is not transferred to cholesterol significantly in most cases.

Another advantage of using the naturally occurring asymmetric PCs is that one can determine the positional specificity of LCAT at the same time. A major finding of the present work is that there are two distinct classes of LCAT in nature with differing positional specificity toward 16:0–20:4 PC. Furthermore, the two classes of LCAT appear to be distributed according to the known atherogenic risks of various animal species. Thus LCATs from Group I animals are unable to efficiently transfer 20:4 acyl group from the *sn*-2 position of 16:0–20:4 PC, whereas the LCATs from Group II animals can. An exception in the latter group is dog LCAT, which transfers more 16:0 than 20:4 to cholesterol (Table 6). However, our recent studies show that dog plasma contains an unusually high concentration of 18:0–20:4 PC (34% of total PC, compared to only 6% in human plasma, and 14% in rat plasma; P. V. Subbaiah and M. Liu, manuscript in preparation). As all LCATs synthesize only 20:4 CE in the presence of this PC (Table 6), the low 16:0/20:4 ratio in dog plasma CE may be the result of unusual substrate composition rather than LCAT specificity. Group I animals are mostly susceptible to diet-induced atherosclerosis, whereas the Group II animals are more resistant. Thus, in general, the LCAT specificity appears to correlate with atherogenic susceptibility, although a causal relationship obviously cannot be established from the present studies.

The molecular basis for the altered positional specificity of LCATs from Group I animals is under study in our laboratory. Our recent studies show that the size of the *sn*-2 acyl group is one critical determinant of LCAT positional specificity (20). Thus we found a positive correlation between the effective chain length of the *sn*-2 acyl group and the utilization of *sn*-1 acyl group by human LCAT. Using chimeric constructs of mouse and human recombinant enzymes, we have also demonstrated that the difference in the positional specificities of the two enzymes is attributable to the central 1/3 sequence of the enzyme protein, corresponding to amino acid residues 130–306 in human LCAT (21). This segment of the enzyme contains not only the active site serine involved in the catalytic step, but also one of the two critical free cysteine residues, the N-glycosylation site previously postulated to be involved in modulating the acceptor specificity of the enzyme (31), and Ser_{216}, which affects the overall activity of the enzyme without participating in the catalytic process (32). We postulate that the Group I and Group II enzymes differ from each other in one or more critical residues in this region, resulting in differences in the architecture of the respective active sites, which in turn affect the substrate and positional specificities of the enzymes. The amino acid sequences of at least four LCATs studied here (human, mouse, rat, and baboon) have been deduced from their cDNA sequences (33–36). The sequences for pig and rabbit LCATs have also been determined recently (P. H. Pritchard, personal communication). A preliminary comparison of mouse and rat LCATs on the one hand, and human, rabbit, pig, and baboon LCATs on the other, shows differences at several residues in the central 1/3 sequence of the enzyme (21). However, site-directed mutagenesis of the various suspected residues needs to be carried out in order to identify the putative sites responsible for the specificity.

The possible mechanism by which the LCAT specificity and plasma CE composition influence the atherogenic susceptibility is unknown. There is some evidence that the saturated CE are cleared from cultured cells (37) and tissues (7) more sluggishly than the polyunsaturated CE. Other in vitro studies have demonstrated that the CE hydrolases of macrophages (38) and smooth muscle cells (39) preferentially hydrolyze the polyunsaturated

CEs, and consequently 18:1 CE and 16:0 CE accumulate in these cells even in the absence of ACAT activity. These studies show the possible role of lipoprotein CE composition in the formation of foam cells. It also appears possible that the 20:4 CE in the lipoproteins is the source of arachidonate for eicosanoid synthesis in several tissues (40), and that a decrease in its concentration may lead to impaired eicosanoid production. Previous studies have suggested that certain eicosanoids stimulate the clearance of CE from the cultured cells (41), and that the eicosanoid synthesis in the cells is directly correlated the 20:4 CE content of the lipoproteins in the medium (42). It is therefore conceivable that the synthesis of 20:4 CE by LCAT indirectly promotes the clearance of all CE species from arterial cells.

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