Impaired function of lecithin:cholesterol acyltransferase in atherosclerosis-susceptible White Carneau pigeons: possible effects on metabolism of oxidized phospholipids

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Abstract Although White Carneau (WC) pigeons are known to be more susceptible to atherosclerosis than Show Racer (SR) pigeons, the reasons for this difference are not fully understood. While no major differences are known in the lipoprotein composition, a difference in the cholesteryl ester (CE) composition was reported. However, there is little information on the activity or specificity of lecithin:cholesterol acyltransferase (LCAT), the major source of plasma CE. In order to determine whether the esterification of cholesterol or other functions of LCAT are compromised in WC pigeons, we studied the various reactions catalyzed by LCAT in the two groups. The cholesterol esterification was found to be significantly lower in WC pigeons, whether assayed with exogenous or endogenous substrates. Furthermore, lyso phosphatidylcholine (PC) esterification and oxidized PC hydrolysis, two other reactions carried out by LCAT, were also lower in WC. We found evidence for the presence of an active lysophospholipase in pigeon plasma, and this activity was also lower in WC compared to SR. A significant increase in the FC/PC ratio, another reported atherogenic risk factor, was found in WC plasma. Because of the absence of other hydrolytic enzymes in pigeon plasma, LCAT may play an important role in the metabolism of oxidized PC generated during lipoprotein oxidation, and therefore a decrease in its activity in White Carneau pigeons may contribute to increased risk of atherosclerosis.—**Liu, M., R. W. St. Clair, and P. V. Subbaiah.** Impaired function of lecithin:cholesterol acyltransferase in atherosclerosis-susceptible White Carneau pigeons: possible effects on metabolism of oxidized phospholipids. *J. Lipid Res.* 1998. **39:** 245–254.

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The importance of lecithin:cholesterol acyltransferase (LCAT) in the esterification of plasma cholesterol and in the reverse cholesterol transport is well established (1, 2). Our previous studies showed that LCAT also carries out several ancillary reactions not involving cholesterol. These reactions include the esterification of lyso PC to PC (3), hydrolysis and transesterification of platelet activating factor (PAF) (4), and hydrolysis and transesterification of truncated phospholipids generated during lipoprotein oxidation (5). Although PAF and the oxidized phospholipids are believed to be hydrolyzed mainly by the PAF-acetylhydrolase (PAF-AH) in human plasma (6), this enzyme activity is absent in avian plasma (7). Moreover, paraoxonase, a calcium-dependent phosphodiesterase which is thought to be involved in the metabolism of oxidized phospholipids (8), is also absent in avian plasma (9). Therefore, the possible role of LCAT in the metabolism of these bioactive phospholipids may be more important in avian species. It is known that White Carneau (WC) pigeons are more susceptible to spontaneous atherosclerosis than Show Racer (SR) pigeons (10), although the mechanisms underlying this difference in susceptibility are not well understood. Most previous studies found no major differences in the lipoprotein composition between the two breeds of pigeons (11), although a difference in cholesteryl ester (CE) fatty acid composition has been reported (12). A defect in the efflux of cholesterol from the macrophages, primarily caused by impaired hydrolysis of CE in the cells, may account for at least some of the increased susceptibility of WC pigeons (13, 14). Other differences reported between the two types of pigeons include increased synthesis of prostaglandins in the aorta (15), and decreased post-heparin lipase activity (16) in WC pigeons, compared to SR.

Abbreviations: AAPH, 2,2′-azo-*bis*-(2-amidinopropane) dihydrochloride; ACAT, acyl-CoA:cholesterol acyltransferase; CE, cholesteryl ester; FC, free (unesterified) cholesterol; LAT, lysolecithin acyltransferase; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; PAF, platelet activating factor; PAF-AH, PAF-acetylhydrolase; PC, phosphatidylcholine; SR, Show Racer; WC, White Carneau.

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However, no information is available on the activity or specificity of LCAT in the two groups of pigeons. Epidemiological studies in humans show that a decrease in the LCAT activity is associated with increased risk of coronary disease (17, 18), whereas over-expression of the enzyme activity in transgenic rabbits has been shown to prevent diet-induced atherosclerosis (19). The study of Young and Middleton (12) showed an increase in 16:0 CE and a decrease in 20:4 CE in WC pigeons, although the possible reasons for these changes were not explored. Our recent results show that the 16:0/20:4 ratio in plasma CE is significantly higher in atherosclerosis-susceptible species such as rabbit, guinea pig, and human, compared to the atherosclerosis-resistant species such as rat and mouse (20). We further showed that this difference in CE composition is due to the substrate and positional specificities of the respective LCATs and not due to alterations in the diet. It is therefore of interest to find out whether the activity or specificity of LCAT is different between the two breeds of pigeons, and whether such a difference could account for the reported differences in CE composition and atherosclerosis-susceptibility.

The results presented here show that there is a significant reduction in LCAT activity in WC pigeons compared to SR pigeons. WC pigeons also showed an increase in the free cholesterol/PC ratio in their plasma, a little-known but powerful risk factor for atherosclerosis (21, 22). The decrease in cholesterol esterification by LCAT is accompanied by a decrease in its other functions, namely lyso PC esterification and hydrolysis of oxidized PC. In addition, we found evidence for the presence of an active lyso PC hydrolase in pigeon plasma, and this activity was also significantly lower in WC pigeons compared to SR. As the hydrolysis of oxidized PC and lyso PC is important in the detoxification of oxidized phospholipids, the decrease in LCAT activity may contribute to increased risk of atherosclerosis in WC pigeons, especially as the PAF-AH is absent in birds.

MATERIALS AND METHODS

Materials

1-[1-^{14}C-palmitoyl]lyso PC (56.8 mCi/mmol), [4-^{14}C] cholesterol (33 mCi/mmol), 1-palmitoyl 2-[1-^{14}C-arachidonoyl] PC, and 2[^{3}H-acetyl] PAF (10 Ci/mmol) were all purchased from DuPont NEN, Egg PC, unlabeled lyso PC (1-palmitoyl), and free cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). AAPH was purchased from Wako Chemical Co. (Richmond, VA).

Plasma samples

Blood was drawn in EDTA (1 mg/ml) from WC or SR pigeons maintained at Bowman Gray School of Medicine. Approximately equal number of males and females (ages ranging from 5 months to 2 years) were used from each group. The birds were fed a normal diet of commercial pigeon pellets. Blood was collected from the non-fasted pigeons without anesthesia from the alar vein. Plasma was separated by centrifugation at 1000 *g*, and the samples were shipped the same day on wet ice to Chicago. The enzyme assays and lipid analyses were performed within 4 days of blood collection. Samples from female pigeons in their egg-laying cycle that were grossly hyperlipemic were excluded from the study.

Enzyme assays

LCAT activity was measured by two different procedures. In the procedure of Stokke and Norum (23), labeled cholesterol was first equilibrated with endogenous cholesterol in the presence of DTNB, a reversible inhibitor of LCAT, and the esterification of labeled cholesterol was measured after the reversal of the inhibition with mercaptoethanol as described earlier (24). This method measures the esterification of endogenous cholesterol and reflects not only enzyme concentration but also the effect of substrate and inhibitor composition. In the proteoliposome assay (25), an external substrate, containing labeled cholesterol, PC, and human apoA-I, was added to 20 μl of plasma, and the esterification of labeled cholesterol was determined (24). This method reflects the concentration of active enzyme in the plasma and is believed to be unaffected by endogenous substrates, activators, or inhibitors (25).

LAT I activity of LCAT was assayed by the conversion of labeled lyso PC to PC, as described earlier (24, 26). Briefly, the incubation mixture contained 60 nmol of 1-[1-C^{14}-palmitoyl] lyso PC, 100 μl of plasma, 10 mm of plasma, 10 mm mercaptoethanol, and 1 mm EDTA–10 mm Tris-NaCl buffer, pH 7.4, in 0.4 ml total volume. The reactions were carried out for 1–2 h at 37°C and stopped by the addition of 1 ml methanol. The lipids were extracted (27) and the radioactivity in lyso PC and PC was determined.

The PAF-AH activity was estimated from the release of labeled acetate from [^{3}H]acetyl PAF (5). The reaction mixture contained 32 nmol of labeled PAF and 10 μl of plasma in 10 mm Tris and 0.15 m NaCl, pH 7.4, in a final volume of 0.4 ml. The incubation was carried out for 30 min at 37°C, and reaction mixture was extracted by the procedure of Bligh and Dyer (27). Ali-

quots of the aqueous and chloroform layers were taken for the determination of radioactivity. The counts in the aqueous layer (free acetate) represent the extent of hydrolysis of PAF. A control tube containing no plasma was included in all experiments in order to correct for any nonenzymatic hydrolysis or incomplete extraction.

The LAT II activity (the transfer of truncated acyl group from the oxidized PC to lyso PC) was determined essentially by the same procedure as LAT I, but the radioactivity in the sphingomyelin region of the TLC plate was determined, because the polar PC moves with sphingomyelin in the solvent system used (28).

Lyso PC hydrolase was determined by the release of labeled free fatty acid from acyl-labeled 1-palmitoyl lyso PC. The reaction mixture contained 100 μl of plasma and 60 nmol of labeled lyso PC, and 10 mm Tris-HCl, pH 7.4, in a final volume of 0.4 ml. Incubation was carried out for 1 h at 37°C, and the sample was extracted by the procedure of Bligh and Dyer (27). The lipid extract was separated on TLC plate with the solvent system of chloroform–methanol–water 65:25:4 (v/v) and the spots corresponding to lyso PC and free fatty acids were scraped and counted for radioactivity.

Lipid analyses

Free and esterified cholesterol were determined with the commercial enzymatic kits (Boehringer Mannheim) (26). The phospholipids were estimated by lipid phosphorus, after their separation on silica gel TLC plates with the solvent system of chloroform–methanol–water 65:25:4 (v/v). The spots corresponding to standard lyso PC, sphingomyelin, PC, PI + PS, and PE were scraped from the plate and the lipid phosphorus was determined by the modified Bartlett procedure (29).

The CE composition was determined by the gas chromatographic analysis of the methyl esters. CE was first separated from the rest of the lipids by TLC on silica gel with the solvent system of hexane–ethyl ether–acetic acid 70:30:1 (v/v). The CE spot was scraped from the plate and treated directly with BF_3-methanol as described earlier (30) to prepare the fatty acid methyl esters, which were then analyzed on a capillary column in a Shimadzu gas chromatographic apparatus equipped with a flame ionization detector (30).

The in vitro synthesis of CE species in whole plasma was estimated by studying the formation of labeled CE from [4-^{14}C]cholesterol which was equilibrated with endogenous cholesterol (30). The labeled plasma was incubated for 16 h at 37°C and the synthesized CE were separated by reverse phase HPLC into various molecular species using the solvent system of acetonitrile–tetrahydrofuran–water 65:35:2 (v/v) at a flow rate of 2 ml/min. The radioactivity in the effluent was measured with the help of a flow-through radioactivity detector (Radiomatic FLO-ONE Beta, Packard Company), and the peaks were quantitated using a computer program (EZChrom, Scientific Software Inc., San Ramon, CA).

Statistics

The statistical significance of differences between the two breeds of pigeons was calculated by Student's *t* test (SPSS for Windows software, SPSS Inc., Chicago).

RESULTS

Lipid composition

The cholesterol and phospholipid composition of plasma from WC and SR pigeons is shown in **Table 1**. Both free and esterified cholesterol were significantly higher in WC pigeons compared to SR, while the FC/EC ratio was not different between the two groups. There was also no difference in the phospholipid composition between the two groups. However, the FC/PC ratio, reported to be an independent risk factor for atherosclerosis (21), was significantly higher in WC group.

Enzyme activities

As shown in **Fig. 1**, the cholesterol esterification activity of LCAT, measured either with an exogenous substrate (LCATp) or endogenous substrates (LCATsn) was significantly higher in SR pigeons compared to WC. The molar esterification activity (nmol of cholesterol esterified/h/ml) was not significantly different between the two groups, because of increased FC concentration in WC plasma (results not shown). The LAT I activity of LCAT was also significantly higher in SR group, and the difference was statistically significant when expressed as molar rate also (not shown). In ad-

TABLE 1. Lipid composition in White Carneau and Show Racer pigeons

Lipid	WC	SR	P Value
		μmol/ml	
CE	4.82 ± 0.19	3.9 ± 0.10	<0.001
FC	2.01 ± 0.09	1.72 ± 0.02	<0.005
FC/CE ratio	0.43 ± 0.03	0.44 ± 0.01	NS
Lyso PC	0.19 ± 0.01	0.19 ± 0.01	NS
Sphingomyelin	0.45 ± 0.01	0.44 ± 0.02	NS
PC	4.48 ± 0.10	4.77 ± 0.15	NS
PI + PS	0.23 ± 0.01	0.22 ± 0.01	NS
PE	0.60 ± 0.02	0.57 ± 0.03	NS
FC/PC ratio	0.44 ± 0.04	0.36 ± 0.04	<0.001

All values shown are mean ± SEM of 12 samples. The *P* value was calculated with Student's *t* test. Abbreviations: FC, free cholesterol; CE, cholesteryl ester; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

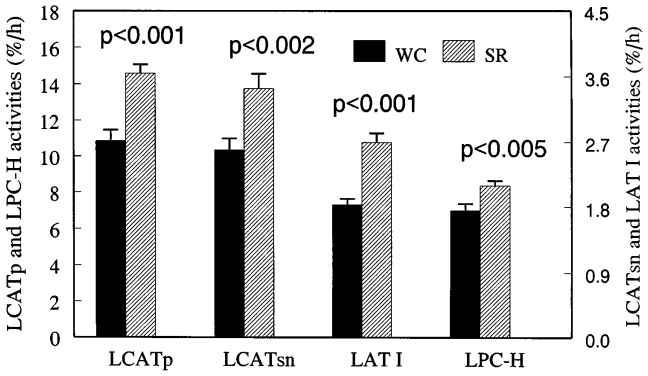

Fig. 1. Plasma enzyme activities (% substrate converted/h) in WC and SR pigeons. LCATp: cholesterol esterification assayed with proteoliposome (exogenous) substrate; LCATsn: cholesterol esterification assayed by Stokke and Norum procedure (endogenous substrates); LAT I: Lyso PC acyltransferase activity (long chain acyltransfer); LPC-H: LysoPC hydrolase. All values shown are mean ± SEM of 17 samples for each group, except for LPC-H values which are for 24 samples from each group.

dition to the various esterification reactions carried out by LCAT, the lyso PC hydrolase activity, which may play an important role in the catabolism of lyso PC, was also significantly higher in SR pigeons. To our knowledge, this activity has not been previously reported in pigeon plasma. It may also be pointed out that the lyso PC hydrolase activity was 5- to 10-fold higher in pigeon plasma, compared to human plasma (results not shown).

Cholesteryl ester (CE) composition

We have previously reported that the specificity of LCAT, and hence the CE composition of plasma, is an indicator of the atherogenic risk among various animal species (20). It was therefore of interest to determine whether the difference in the atherogenic risk between the two groups of pigeons is also reflected in their CE composition. Furthermore, the lower LCAT activity in WC pigeons should result in lower concentration of 18:2 CE and higher concentration of 18:1 CE, based on the known specificity of LCAT and ACAT (1, 31). As shown in **Table 2**, there was indeed somewhat less 18:2 CE and more 18:1 CE and 16:0 CE in WC pigeons, as expected from the lowered LCAT activity, and increased contribution of tissue ACAT. However, these differences between the two groups of pigeons did not reach statistical significance, possibly because of small sample size. There was also less 20:4 CE, and a higher ratio of saturated CE/20:4 CE in the WC pigeons, but these changes also were not statistically significant.

In vitro synthesis of CE species

In order to determine whether the specificity of LCAT is different in the WC and SR pigeons, the plasma samples (6 each from WC and SR) were prelabeled with [^{14}C]cholesterol and incubated at 37°C for 16 h. The labeled CE formed were then separated on reverse phase HPLC, and their radioactivity was quantitated as described in Methods. As shown in **Table 3**, there were no significant differences between WC and SR with regard to the percentage composition of the CE synthesized in vitro, indicating that the specificity of LCAT is not different in the two groups. The formation of 20:4 CE was actually higher in WC pigeons, with the difference being close to significance level ($P = 0.057$). Therefore the decrease in 20:4 CE in these birds as reported by Young and Middleton (12), and as suggested in Table 1, is unlikely due to LCAT specificity, but probably due to changes in PC molecular species composition.

TABLE 2. Fatty acid composition of cholesteryl esters in whole plasma

CE Species	WC (n = 14)	SR (n = 13)
	% of total	
14:0	0.12 ± 0.05	0.06 ± 0.03
16:0	10.12 ± 0.56	9.19 ± 0.28
16:1 (n–7)	1.65 ± 0.25	2.19 ± 0.27
18:0	3.32 ± 0.29	2.69 ± 0.13
18:1 (n–9)	19.06 ± 1.46	17.82 ± 0.97
18:1 (n–7)	1.79 ± 0.15	1.56 ± 0.09
18:2 (n–6)	60.45 ± 2.19	63.06 ± 1.09
18:3 (n–6)	0.32 ± 0.06	0.13 ± 0.07
18:3 (n–3)	0.49 ± 0.11	0.38 ± 0.13
20:3 (n–6)	0.18 ± 0.08	0.30 ± 0.12
20:4 (n–6)	2.12 ± 0.10	2.28 ± 0.15
20:5 (n–3)	0.16 ± 0.07	0.20 ± 0.10
22:6 (n–3)	0.21 ± 0.09	0.14 ± 0.08
Saturated[a]/20:4	6.58 ± 0.45	5.46 ± 0.32

All values shown are mean ± SEM.
[a] 14:0 + 16:0 + 18:0.

TABLE 3. Synthesis of cholesteryl esters in vitro

CE Species	WC (n = 10)	SR (n = 9)
	% of total CE	
16:0	9.75 ± 0.65	9.01 ± 0.41
18:0	1.60 ± 0.21	1.77 ± 0.23
18:1 (n–9)	14.63 ± 2.40	16.77 ± 2.11
18:2 (n–6)	67.89 ± 2.16	68.04 ± 2.18
20:4 (n–6)	5.09 ± 0.44	4.07 ±0.17[a]
20:5 (n–3) + 22:6 (n–3)	0.75 ± 0.21	0.70 ± 0.19
16:0/20:4 ratio	1.93 ± 0.19	2.32 ± 0.24
18:1/18:2 ratio	0.23 ± 0.05	0.26 ± 0.04

[a] $P = 0.057$.

are detoxified, primarily through their hydrolysis to lyso PC (32, 34). Because PAF-AH, the major enzyme that carries out the hydrolysis of the oxidized phospholipids in human plasma, is absent in avian plasma (7), the oxidized lipids may accumulate more in pigeon plasma, compared to human plasma. Furthermore, if LCAT plays a role in the metabolism of oxidized phospholipids, the accumulation of oxidized products may differ between the two groups, because of differences in LCAT activity. To test these hypotheses, plasma was oxidized with 50 mm AAPH for 16 h and the phospholipid composition was determined after separation on TLC. As shown in **Fig. 2**, there was a decrease in PC, phosphatidylethanolamine (PE), and phosphatidylinositol + serine (PI+PS) after oxidation in both WC and SR plasmas, but there was no significant difference between the two breeds. There was an increase in the amounts of lyso PC and sphingomyelin, as found in human plasma (28). The increase in lyso PC was significantly higher in WC plasma, while the increase in sphingomyelin was similar in the two groups. We showed previously that the increase in sphingomyelin in human plasma is due to the accumulation of oxidized PC with truncated acyl groups (28). Compared to human plasma, the accumulation of polar PC is higher in both groups of pigeons, indicating the importance of PAF-AH in its degradation. However, the increase in sphingomyelin and lyso PC could account only for about one-third of the decrease in PC, showing that there is a considerable degradation of polar PCs to water-soluble products in pigeons, although PAF-AH is absent. The significantly higher accumulation of lyso PC in WC pigeons may be due to either the decreased lyso PC hydrolase activity (Fig. 1) or the increased accumulation of polar PCs that migrate with lyso PC (28, 35). Preliminary results with the oxidation of *sn*-2-acyl labeled 16:0–18:2 PC in presence of plasma show that a significant percent (16–25%) of the label appears in the lyso PC region on TLC, indicating the formation of polar PCs that move with lyso PC. There was more of this label in lyso PC in the WC group compared to the SR group (results not shown).

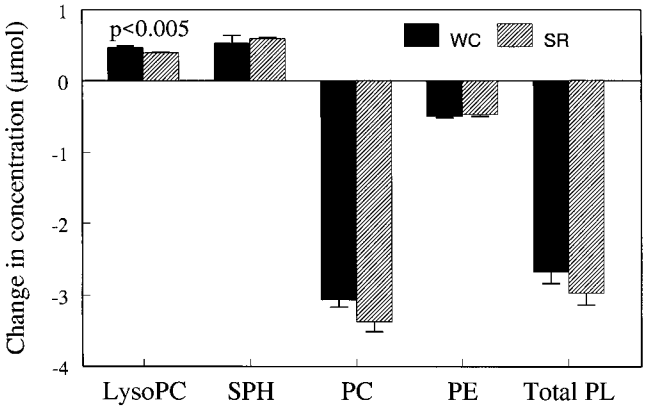

Fig. 2. Changes in plasma phospholipid concentrations after oxidation. Plasma samples (100 μl) from WC or SR pigeons were oxidized with 50 mm AAPH for 16 h in a final volume of 0.4 ml. Samples were analyzed for phospholipid composition before and after oxidation, and the changes (post-pre, μmol/ml plasma) are plotted. All values shown are mean ± SEM of 10 samples in each group. The statistical significance of the differences was calculated by Student's *t* test.

Effect of oxidation on enzyme activities

As shown in **Fig. 3**, oxidation of plasma resulted in inactivation of LCAT (cholesterol esterification) and LAT I

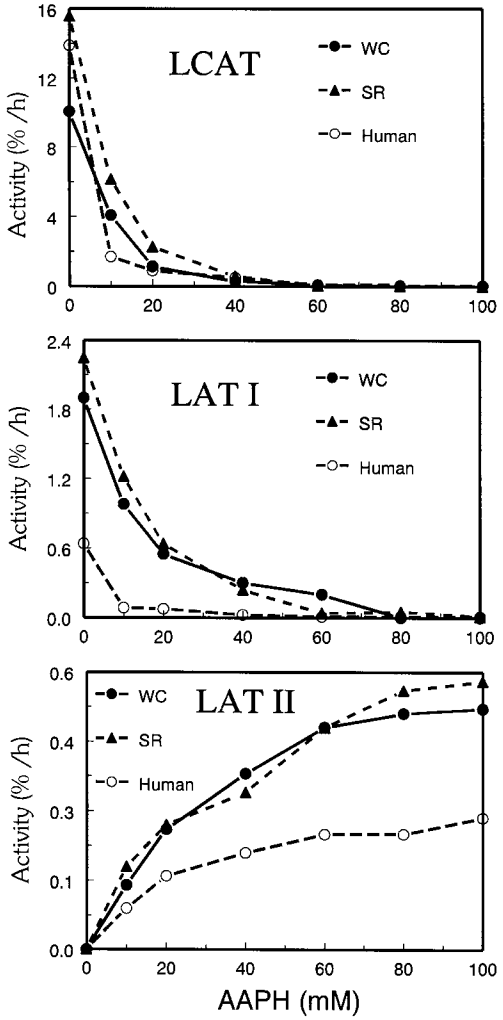

Fig. 3. Effect of oxidation on the various reactions catalyzed by LCAT. Pigeon or human plasma was oxidized with the indicated concentration of AAPH for 16 h at 37°C. Aliquots (100 μl) were taken for the assay of LCAT activity by proteoliposome assay and for the LAT I and LAT II activities, as described in the Methods. The activities (expressed as % substrate esterified/h) are averages of duplicate samples.

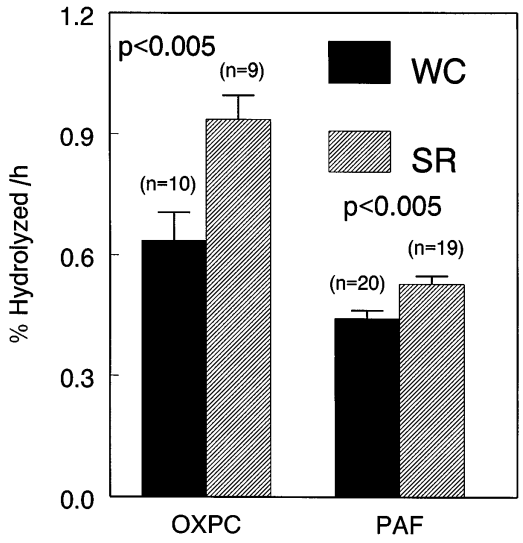

Fig. 4. Hydrolysis of oxidized PC and PAF by WC and SR plasma. Labeled oxidized PC (OXPC) was prepared by the oxidation of 1 mg 1-16:0-2-[1-^{14}C]-20:4 PC with 50 mm AAPH for 2 h at 37°C. The oxidized PC products were separated on silica gel TLC plate with the solvent system of chloroform–methanol–water 65:25:4 (v/v), and the spot corresponding to sphingomyelin was scraped and eluted (27). After estimation of the lipid phosphorus, 60 nmol of the labeled OXPC (containing 25,00 dpm) was dispersed in 10 mm Tris-HCl buffer, pH 7.4, and incubated with 20 μl of whole plasma in a final volume of 0.4 ml for 1 h. The reaction mixture was extracted (27), and aliquots of the aqueous and chloroform layers were taken for radioactivity measurement. The percent of total counts appearing in the aqueous layer measure the extent of hydrolysis of OXPC. PAF hydrolysis was determined similarly by the release of labeled acetate from [^{3}H]acetate-labeled PAF, after incubation with 10 μl plasma. For both assays, control reactions without plasma were included to correct for any nonenzymatic hydrolysis or incomplete extraction.

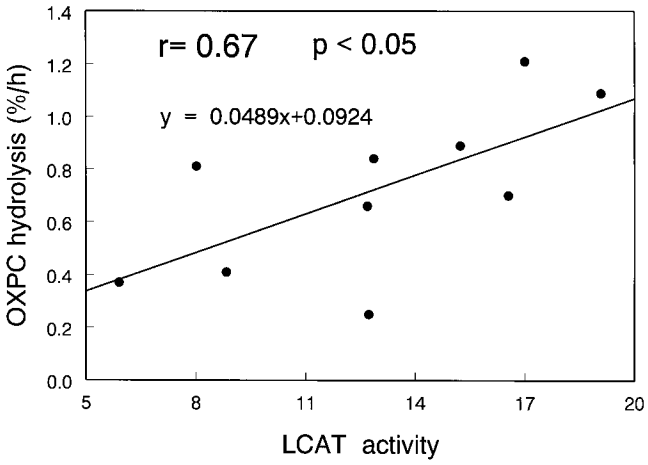

Fig. 5. Correlation (Pearson) of hydrolysis of oxidized PC and LCAT reaction. LCAT activities shown are with proteoliposome assay (% esterification/30 min). Only the samples in which both assays were performed at the same time were included. The values shown include 5 from SR and 5 from WC group.

(lyso PC esterification) activities in the pigeon plasma, as it does in human plasma (28). However, the enzyme activities in pigeon plasma appear to be relatively more resistant to oxidation than in human plasma, because at 10 mm AAPH concentration, where the human plasma lost most of the activity, the pigeon plasma retained up to 50% of LAT I and up to 37% of LCAT activity. A similar resistance to oxidation was noticed in chicken plasma LCAT (5), and this may be due to the lack of the sulfhydryl groups at residues 31 and 184 in avian LCAT (36). The LAT II activity (transfer of truncated acyl group from the oxidized PC to lyso PC) increased over 10-fold in the pigeon plasma after oxidation, as observed in human plasma (28). These results show that oxidation specifically affects the transfer of long chain acyl group by LCAT. They also show that the LAT II activity is carried out by LCAT, and not by PAF-AH because the latter enzyme is absent in pigeon plasma. There was no difference in the effect of oxidation on the various enzyme activities carried out by LCAT between the two types of pigeons. The lyso PC hydrolase activities also were decreased by 80% after oxidation with AAPH in both groups of birds (results not shown).

Hydrolysis of oxidized PC by plasma

In order to study the possible effect of reduced LCAT activity in WC pigeons on their ability to hydrolyze oxidized PC, we isolated oxidized PC after the oxidation of 1-16:0-2[1-^{14}C-20:4] PC by AAPH, and studied its hydrolysis by both types of plasma. Labeled PC first was oxidized with 50 mm AAPH for 2 h at 37°C, and the total lipid extract was then separated on silica gel TLC plate with the solvent system of chloroform–methanol–water 65:25:4 (v/v). The polar PC, corresponding to the sphingomyelin spot was scraped and eluted (27). The labeled polar PC, containing 60 nmol lipid phosphorus, was then incubated with 20 μl of whole plasma in a final volume of 0.4 ml in Tris-Cl buffer, pH 7.4, for 1 h at 37°C. After extraction of the lipids by the procedure of Bligh and Dyer (27), aliquots from the chloroform and aqueous layers were taken for radioactivity determination. The counts in the aqueous layers represent the hydrolysis of the polar PC. As seen in **Fig. 4** the degradation of oxidized PC was significantly higher in SR plasma. The SR plasma also showed higher hydrolysis of PAF, although the overall rate of hydrolysis of PAF is very low compared to human plasma. As the PAF-AH is absent in avian plasma, the activity towards polar PC and PAF is probably due to LCAT itself. Supporting this possibility is the positive correlation between the hydrolysis of polar PC and the LCAT activity ($r = 0.67$; $P < 0.05$) (**Fig. 5**).

DISCUSSION

Although it is well established that WC pigeons are more susceptible to spontaneous atherosclerosis than SR pigeons, the underlying mechanisms for this are not completely understood. While the majority of the studies show no significant difference in the lipoprotein composition between the two groups of pigeons, some of the earlier studies reported significant differences in the plasma CE composition (12). There is evidence that the plasma CE composition is correlated with the risk of atherosclerosis (20, 37). The source for the majority of CE in pigeon plasma is the LCAT reaction, as in humans. However, surprisingly little information is available on the activity or the specificity of LCAT in the two groups of pigeons. The results presented here clearly show a significantly lower LCAT activity in WC pigeons compared to SR, although there was no difference in the specificity of the enzyme. As the lower activity was evident with the exogenous substrate also, it appears that there is a decrease in the concentration of active enzyme in the plasma, rather than a change in the substrate or activator concentrations. Because it is known that the LCAT activity is decreased dramatically in egg-laying chickens (38), we considered the possibility that the lower activity in WC pigeons is due to the inclusion of some birds that were in their egg-laying phase. However this appears unlikely because we have excluded the plasma samples that were grossly hyperlipidemic (an indication of the egg-laying phase), and most other birds have not yet reached sexual maturity. Furthermore, we selected birds randomly and thus would expect approximately equal numbers of males and females in both groups. In addition, the differences between the WC and SR pigeons were evident even when only male birds were compared (results not shown).

The decrease in LCAT activity in WC pigeons in vivo would be expected to result in an increased FC/CE ratio and altered CE composition in plasma. However, we did not find a significant change in the FC/CE ratios, possibly because the contribution of ACAT to plasma CE may have compensated for the decreased activity of LCAT. It is also possible that the individual lipoprotein fractions differ in the FC/CE ratio, but we did not separate the lipoproteins in this study. It is of interest to note that Langelier, Connelly, and Subbiah (39) reported a significantly lower percent of esterified cholesterol in the LDL of WC pigeons. Although we found a trend towards increased 18:1 CE and decreased 18:2 CE in WC pigeons, as would be expected from the decreased contribution of LCAT (and increased contribution of ACAT), the differences were not statistically significant. The increase in the saturated CE/ 20:4 CE ratio in WC pigeons also was not statistically significant possibly because of the small sample size. Using a larger sample size (60 pigeons in each group), Young and Middleton (12) reported a significant decrease in 20:4 CE and an increase in 16:0 CE in WC pigeons. It is therefore possible that there is a small but real difference in the CE composition between the two groups. It is unlikely, however, that the difference is due to a change in the specificity of LCAT, because our results show that the 20:4 CE synthesis in vitro is slightly higher in WC plasma compared to SR plasma.

The CE composition of plasma lipoproteins may affect reverse cholesterol transport because the hydrolysis of CE by the aortic CE hydrolase shows specificity in the order of 18:2 CE > 18:1 CE > 16:0 CE (40). Therefore, the increased saturation in plasma CE can contribute to decreased mobilization from the aorta, as previously reported in WC macrophages (13). This assumes that some of the CE are taken up and stored intact (without hydrolysis and reesterification) in the arterial cells. The decrease in 20:4 CE in WC pigeons may also be related to increased risk of atherosclerosis, because previous studies showed that the atherosclerosis-susceptible animal species have increased 16:0 CE and decreased 20:4 CE (20, 37). Furthermore, cholesterol feeding has been shown to result in a 90% reduction in plasma 20:4 CE in atherosclerosis-susceptible mice and a 50% reduction in resistant mice (22).

The lower LCAT activity in WC pigeons may be relevant to their increased risk of atherosclerosis for the following reasons: *i*) Epidemiological studies in humans showed a negative correlation between LCAT activity and risk of atherosclerosis (17, 18). Furthermore, over-expressing LCAT activity in transgenic rabbits was shown to increase their HDL levels dramatically, and protect them from the diet-induced atherosclerosis (41). *ii*) In addition to its HDL-raising effects, LCAT may directly affect cholesterol efflux from the arterial cells. For example, it has been shown that an inhibition of LCAT in the surrounding serum results in the abolition of net cholesterol efflux from cells, although the unidirectional flux is not affected (42). The enzyme therefore appears to inhibit the influx of cholesterol into the cells by reducing the free cholesterol pool size in plasma. *iii*). The increase we observed in the FC/PC ratio in WC pigeons, and which has been reported to be an independent risk factor for atherosclerosis (21, 22), may also be the consequence of decreased LCAT activity. Although FC and PC are consumed in equimolar amounts by the LCAT reaction, the accumulation of FC in plasma exceeds that of PC in LCAT deficiency, leading to increased FC/PC ratios (43). This increase in FC/PC is found even in heterozygous subjects who have an apparently normal rate of endogenous cholesterol esterification, but contain only a half normal concentration of

LCAT (44). The higher ratio of FC/PC in lipoproteins has been shown to be inhibitory for cholesterol efflux (45), and thus may be considered atherogenic. It is possible that this ratio is also increased in cell membranes because both FC and PC exchange readily between lipoproteins and cell membranes (45), and this could compromise membrane structure and function. Although previous studies in cultured macrophages from WC and SR pigeons did not show a difference in cholesterol desorption from membranes (14), it is possible that any differences in membrane composition were lost when the cells were cultured for over 72 h in a common serum-containing medium prior to the efflux experiments. *iv*). LCAT may play an important role in the hydrolysis of oxidized phospholipids formed during lipoprotein oxidation. As both PAF-AH and paraoxonase activities appear to be absent in avian plasma (7, 9), the potential role of LCAT in the detoxification of oxidized PC may be more important in birds than in humans. It has been shown that the oxidized phospholipids have several potentially atherogenic effects such as induction of chemotactic and adhesion molecules in arterial cells (46, 47). Our in vitro studies with labeled oxidized PC show that the hydrolysis of these compounds is indeed lower in WC pigeons compared to SR (Fig. 4).

The present study also showed, for the first time, the presence of a lyso PC hydrolase activity in pigeon plasma. Although it is generally assumed that the lyso PC generated by the LCAT and phospholipase activities in plasma is bound to albumin and transported to liver and other tissues for further metabolism, significant amounts of it are also bound to lipoproteins, especially LDL (48). Lyso PC also has been shown to be a powerful chemotactic factor for monocytes (49), an inducer of adhesion molecules in endothelial cells (50), and an inhibitor of endothelium-dependent relaxation (51). The hydrolysis of lyso PC by the hydrolase activity is therefore potentially beneficial. It is of interest to note that this activity also is significantly lower in WC pigeons, and therefore may contribute to increased risk of atherosclerosis.

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