Targeted disruption of the murine lecithin:cholesterol acyltransferase gene is associated with reductions in plasma paraoxonase and platelet-activating factor acetylhydrolase activities but not in apolipoprotein J concentration[1]

Trudy M. Forte,[2,*] Michael N. Oda,[*] Laura Knoff,[*] Balz Frei,[†] Jung Suh,[†] Judith A. K. Harmony,[§] William D. Stuart,[§] Edward M. Rubin,[*] and Dominic S. Ng[3,*]

Life Sciences Division, Lawrence Berkeley National Laboratory,* University of California, Berkeley, CA 94729; Linus Pauling Institute,† Oregon State University, 571 Weniger Hall, Corvallis, OR 97331; and Department of Pharmacology and Cell Biophysics, College of Medicine,§ University of Cincinnati, 231 Bethesda Avenue, Cincinnati, OH 45267

Abstract **Lecithin:cholesteryl acyltransferase (LCAT) deficiency resulting from targeted disruption of the *Lcat* gene in the mouse is associated with dramatic decreases in HDL concentration and the accumulation of nascent HDL in the plasma. We examined whether LCAT deficiency in mice is associated with a concomitant decrease in two antioxidative enzymes, paraoxonase (PON) and platelet-activating factor acetylhydrolase (PAF-AH). In control *Lcat* (+/+) mice both these enzymes are transported on HDL. Compared to *Lcat* (+/+) mice, HDL-cholesterol is reduced 94% and apoA-I, 90%, in *Lcat* (−/−) mice; this reduction in HDL is paralleled by a 71% decrease in PAF-AH activity and in a 58% decrease in PON activity. Apolipoprotein J (apoJ) levels, rather than being decreased, were significantly ($P = 0.01$) higher (36%) in *Lcat* (−/−) than in *Lcat* (+/+) mice, and the apo J/PON ratio was 3-fold greater in *Lcat* (−/−) than in *Lcat* (+/+) animals. Even though apolipoprotein A-I (apoA-I) concentration and PON activity were drastically reduced, there was no reduction in apoA-I and PON liver mRNA levels suggesting that post-transcriptional events are responsible for the reduction of plasma PON and apoA-I levels. Fast protein liquid chromatography (FPLC) revealed that in *Lcat* (+/+) mice both PON and PAF-AH activity is associated with large, apoA-I-containing HDL particles (9.7 nm by non-denaturing gradient gel electrophoresis) while in *Lcat* (−/−) mice both enzymes are associated with small 8.2 nm particles. We conclude that the concomitant reduction in HDL and apoA-I concentrations and PON and PAF-AH activities is best explained by rapid clearance of the small HDL particles found in LCAT deficiency.**—Forte, T. M., M. N. Oda, L. Knoff, B. Frei, J. Suh, J. A. K. Harmony, W. D. Stuart, E. M. Rubin, and D. S. Ng. **Targeted disruption of the murine lecithin:cholesterol acyltransferase gene is associated with reductions in plasma paraoxonase and platelet-activating factor acetylhydrolase activities but not in apolipoprotein J concentration. *J. Lipid Res.* 1999.** 40: **1276–1283.**

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Numerous epidemiologic studies have shown that elevated high density lipoprotein (HDL) concentrations are protective against coronary heart disease (CHD). However, the mechanism(s) whereby HDL protects against CHD is still not fully understood. In part, this protection may be explained by several enzyme functions associated with HDL which might be considered anti-atherogenic, including lecithin:cholesterol acyltransferase (LCAT), paraoxonase (PON), and platelet-activating factor acetylhydrolase (PAF-AH). The HDL-associated enzyme, LCAT, mediates "reverse cholesterol transport," the process whereby excess cholesterol removed from cells, after esterification on HDL, is returned to the liver for catabolism (1). A deficiency of LCAT activity has been associated with severely reduced concentrations of HDL (1), early atherosclerosis in some patients (1, 2), and accumulation of lipids in renal arteries and arterioles (1). LCAT deficiency also prevents the normal maturation of plasma HDL and re-

Abbreviations: CHD, coronary heart disease; LCAT, lecithin:cholesterol acyltransferase; PON, paraoxonase; PAF-AH, platelet-activating acetylhydrolase; HDL, high density lipoproteins; LDL, low density lipoproteins; apoA-I, apolipoprotein A-I; apoJ, apolipoprotein J; VHDL, very high density lipoproteins; FPLC, fast protein liquid chromatography

[1] Dr. Arthur A. Spector served as editor of this article.

[2] To whom correspondence should be addressed.

[3] Present address: St. Michael's Hospital, Annex 2-013, 38 Shuter Street, Toronto, Ontario, M5B 1A6, Canada.

sults in the accumulation of discoidal, i.e., nascent, HDL in the plasma albeit these aberrant particles are present in low concentration (3).

Paraoxonase, a 44 kDa glycoprotein, is a calcium-dependent enzyme synthesized by the liver and transported almost exclusively on HDL. As in humans, PON activity in the mouse is also transported on HDL particles (4). The association of PON with HDL was first described by Uriel (5) and later studies from several laboratories have confirmed this association (6–8). Because PON and apoA-I co-isolate during purification of the enzyme, it has also been suggested that apoA-I and PON may be closely associated. Several reports suggest that reduced HDL concentrations are correlated with reduced PON activity (7, 9); in fish-eye disease and Tangier disease, both syndromes with severely depleted HDL, PON activity is concomitantly severely reduced (10). The mechanism whereby PON protects against atherosclerosis is not completely understood. However, several in vitro studies utilizing HDL–PON complexes in conjunction with LDL exposed to oxidative compounds have suggested that this enzyme prevents the oxidation of LDL (11, 12). The latter is important because oxidized LDLs initiate the early events in atherosclerosis and the formation of arterial foam cells. The protective role of PON may be related to its ability to prevent the accumulation of lipid hydroperoxides on LDL. The in vivo relationship between PON activity and atherosclerosis has recently been illustrated unequivocally in PON knockout mice (13). In these studies, a deficiency of PON was associated with increased lipid hydroperoxides on HDL; in addition, when mice were placed on a high fat/cholesterol diet, PON-deficient animals had a 2-fold increase in aortic lesion size compared to wild-type littermates, suggesting that PON has a protective role in atherosclerosis.

Platelet-activating factor acetylhydrolase is a 45 kDa protein secreted primarily by macrophages (14, 15). This enzyme hydrolyzes platelet-activating factor which is a proinflammatory molecule and is considered a prime suspect in the early events of atherosclerosis (16). PAF-AH also has the ability to hydrolyze oxidized phospholipids possessing short-chain acyl moieties in the *sn*-2 position (17). In normal human subjects, a relatively small fraction (15–30%) of total PAF-AH activity is transported on HDL while the majority of activity resides on LDL (18, 19). PAF-AH has been demonstrated to protect LDL from the accumulation of oxidized lipids (20, 21) and therefore functions as an antioxidative enzyme. Unlike humans, in the mouse, PAF-AH is transported almost exclusively by HDL (4, 22) which may, in part, result from the near absence of LDL in this species.

In human subjects, a subset of HDL-PON particles is associated with a unique protein termed "clusterin" or apoJ (8, 23, 24). The latter protein is highly glycosylated with a molecular mass of approximately 70 kDa (25, 26). ApoJ is multifunctional and is important in tissue remodeling, cell death, and membrane stabilization (24). Because of its induction at fluid–tissue interfaces, it has been hypothesized that apoJ has a role in protecting cell interfaces from potentially toxic substances (27). Recent investigations with cholesterol-loaded macrophages indicate that apoJ induces cholesterol efflux from cells, thus suggesting that this apoprotein may have an anti-atherogenic function (28). Interestingly, in a series of studies with atherosclerosis-prone mice, including cholesterol-fed C57Bl/6 mice, apoE knockout mice, and LDL receptor-negative mice, Navab et al. (9) demonstrated that plasma PON activity is greatly reduced but that apoJ concentrations are unaffected or somewhat elevated thus resulting in an elevated apoJ/PON ratio. These authors suggested that the apoJ/PON ratio may be a better predictor of atherosclerosis than plasma HDL concentrations. Although the physiology of apoJ is not completely understood, the overall inference from the numerous studies with apoJ is that this HDL accessory protein may have a role in cell protection and/or be a marker for adverse changes occurring in tissues.

We have recently developed a mouse in which the endogenous LCAT gene was disrupted leading to the complete absence of plasma LCAT activity (29). In addition to the absence of LCAT, these mice had severely reduced HDL-cholesterol and apoA-I concentrations; moreover, because of impairment of cholesteryl ester formation, HDL possessed numerous discoidal, i.e., nascent, particles. Because of the severe reduction in HDL and apoA-I in the LCAT-deficient mice, we hypothesized that there should be a concomitant reduction in the antioxidative enzymes, PON and PAF-AH, and potentially a reduction in apoJ concentration. The present study demonstrates that LCAT deficiency increases, rather than decreases, apoJ concentration, suggesting that apoA-I-containing HDL are not a strict requirement for apoJ transport. Moreover, we demonstrate that the activities of both PON and PAF-AH are decreased in parallel with reduction of HDL concentrations. In addition, the distributions of enzyme activities are altered, suggesting that HDL particle composition and/or morphology may regulate the transport of these enzymes.

EXPERIMENTAL PROCEDURES

Transgenic mice

LCAT-deficient mice created by LCAT gene disruption (chimeric mice were bred with DBA × C57BL/6 F1 hybrids) as previously described by Ng et al. (29) were used in these studies. PON and PAF-AH activities of *Lcat* (−/−) and (+/−) mice were compared to control littermates, *Lcat* (+/+).

Analysis of PON and PAF-AH activity

Blood from fasting mice approximately four months of age was collected into 0.3 ml heparinized tubes (Sarstedt); heparin was used as the anticoagulant because EDTA irreversibly inhibits PON activity. Plasma was obtained after centrifuging blood samples for 10 min at 5,000 rpm. Paraoxonase activity in plasma was measured as arylesterase activity using phenylacetate as substrate and including 1 mm calcium (30). The arylesterase reaction was initiated by adding 20 μl heparinized plasma to a cuvette containing 1.8 ml 50 mm Tris/HCl (pH 8.0), 1 mm $CaCl_2$, and 10 mm phenylacetate; enzyme activity was determined by recording absorbance at 270 nm for 30 sec. One unit of arylesterase activity = 1 μmole phenylacetate hydrolyzed per min.

PAF-AH activity was determined by the release of (^{3}H)acetate from 2-(acetyl-^{3}H)PAF essentially as described by Stafforini, Prescott, and McIntyre (31). Mouse plasma samples were diluted 1:150 before use. Results are expressed as nmoles acetate released per hour per ml.

Cholesterol and apoA-I analyses

Fasting plasma cholesterol was analyzed using enzyme end point kits for total cholesterol (Boehringer Mannheim); cholesteryl ester was calculated from the difference between total and free cholesterol. HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with polyethylene glycol. ApoA-I was determined by radial immunodiffusion using mouse-specific anti-apoA-I essentially as previously described (32).

Distribution of enzyme activity

Ultracentrifugation was found to displace a high percentage of both PON and PAF-AH activity from HDL particles; therefore, lipoproteins were fractionated by fast protein liquid column chromatography (FPLC) using two Superose 6 columns in series. For FPLC, heparinized plasma from a pool of 5–6 mice was used; both *Lcat* (−/−) and *Lcat* (+/+) animals were examined. The FPLC was calibrated with isolated VLDL/LDL (d < 1.063 g/ml) and HDL (d 1.063–1.21 g/ml) isolated from pooled control mouse plasma.

Isolation and analysis of PON and apoA-I mRNA by Northern blot hybridization

Liver RNA was determined for four *Lcat* (−/−) and three *Lcat* (+/+) mice. The RNA was isolated from approximately 200 mg of liver tissue which had been flash frozen and pulverized in liquid nitrogen. The total RNA fraction was extracted into Tripure reagent (Boehringer Mannheim) from the pulverized liver tissue. After centrifugation, the aqueous phase was precipitated with 70% ethanol and stored at −70°C. Equivalent loads of total liver RNA (20 μg) were resolved in 1.1% agarose formaldehyde MOPS gel; analyses were carried out in duplicate. The RNA was transferred to Hybond nylon membranes and probed with PCR-generated digoxigenin–UTP-incorporated cDNA fragments. The cDNA fragments, ~300 bp in length, were specific for murine apoA-I, paraoxonase, or β-actin cDNA. The blots were probed with antidioxigenin alkaline phosphatase-conjugated antibodies and the immunoreactive bands were visualized on Kodak XO-Mat film by CDP-Star chemiluminescence; exposures were within the linear range of the film. Quantitation of the mRNA species was performed by densitometric analysis of the film using the levels of β-actin mRNA as an internal standard for normalizing the total RNA load per sample.

Lipid hydroperoxide detection

Pooled EDTA plasma was obtained from *Lcat* (+/+) and *Lcat* (−/−) mice that were fasted overnight; pooled plasma consisting of a single pool for *Lcat* (+/+) mice (7 months of age) and two separate pools for *Lcat* (−/−) mice (one pool from mice 7 months of age and the other 13 months of age) were immediately frozen at −80°C, placed on dry ice, and shipped to Dr. Balz Frei (Oregon State University) where analysis of lipid hydroperoxides was carried out. The presence of cholesteryl ester and phospholipid hydroperoxides was assessed by high performance liquid chromatography with isoluminol chemiluminescence detection as previously described (33, 34).

ApoJ analysis

Mouse plasma was diluted 1/40 with PBS and gel loading buffer without reductant and the equivalent of 0.5 μl plasma was loaded onto 10% SDS-PAGE minigels. The equivalent of 0.1, 0.3, 0.5, 0.7, and 1.0 μl plasma from two different mice was also loaded to determine the linearity of the conditions. After electrophoresis, the protein was blotted onto nitrocellulose paper; Ponceau S protein staining confirmed even loading and transfer. Blocking and all incubations were with 5% non-fat milk and 0.05% Tween 20 (Sigma). Blots were incubated with 1/3,000 dilution of rabbit anti-rat SGP2 antibody (courtesy of Dr. Griswold) which also recognized murine apoJ followed by goat anti-rabbit IgG HRP conjugate (Boehringer Mannheim) at a 1/10,000 dilution. After thorough washing, blots were developed with the enhanced chemiluminescence kit (Amersham) and Kodak BioMax film. Exposure times were 30, 45, and 70 sec. The film was scanned into a computer and bands were quantified using NIH-image. The standards were plotted and the linearity of a 0.5 μl plasma load was confirmed at all three exposures. The data is expressed as integrated density units × 100.

Statistical analysis

Values are reported as mean ± SD. Differences between groups of mice were determined by either the paired *t*-test or unpaired *t*-test and values of $P < 0.05$ were considered significant.

RESULTS

Plasma lipid and apoA-I concentrations and PON and PAF-AH activity

Plasma total cholesterol, HDL-cholesterol, and apoA-I concentrations for the various LCAT genotypes are shown in **Table 1**. The *Lcat* (−/−) genotype, which is associated with a complete absence of LCAT activity (29), has a 62.5% reduction in total cholesterol compared to *Lcat* (+/+) mice; however, the reduction in HDL-cholesterol is even more remarkable where there is a 94% reduction in *Lcat* (−/−) mice compared to *Lcat* (+/+) mice. Although there is a trend toward reduction of total and

TABLE 1. Total cholesterol, HDL-cholesterol, and apoA-I concentrations in plasma of mice with different LCAT genotypes

Genotype	n	TC	HDL-C	ApoA-I	Non-HDL-C	Non-HDL-C
			mg/dl			*%*
+/+	10	128.1 ± 41.7	105.9 ± 39.1	205 ± 51	22.2 ± 5.1	18.3
+/−	10	102.6 ± 17.4	82.7 ± 17.7	166 ± 40	20.0 ± 4.3	19.9
−/−	10	48.4 ± 18.1[a]	6.8 ± 2.5[b]	21 ± 9[b]	41.5 ± 16.1[c]	85.1

[a] $P < 0.0002$ compared with *Lcat* (+/+).
[b] $P < 0.0001$ compared with *Lcat* (+/+).
[c] $P < 0.004$ compared with *Lcat* (+/+).

TABLE 2. PON (arylesterase) and PAF-AH activity in plasma of mice with different LCAT genotypes

Genotype	n	PON	PAF-AH
		U/ml	*nmol acetate/h/ml*
+/+	10	92.5 ± 18.1	84,968 ± 29,656
+/−	10	86.1 ± 10.4	66,487 ± 15,025
−/−	10	38.6 ± 6.9[a]	24,262 ± 5,654[a]

[a] $P < 0.0001$ compared with *Lcat* (+/+).

HDL-cholesterol in *Lcat* (+/−) mice, this difference was not significant ($P = 0.08$) compared to *Lcat* (+/+) mice. The data also indicate that the distribution of cholesterol is greatly altered in *Lcat* (−/−) mice where 85% is in the non-HDL, i.e., VLDL/LDL, fraction in these mice compared to either control or heterozygous mice (18% and 20%, respectively). Consistent with the severely reduced concentration of HDL-cholesterol, apoA-I is drastically reduced (90%) in *Lcat* (−/−) mice compared to controls.

Table 2 indicates that PON and PAF-AH activity are both drastically reduced in *Lcat* (−/−) mice compared to controls (58% and 71%, respectively). Although there is a slight reduction in PON and PAF-AH activities in heterozygote mice, this reduction was not significantly different from controls. Because HDL is the primary transporter of PON and PAF-AH in mice, the substantial reduction in activity of both enzymes in *Lcat* (−/−) mice suggests a strict requirement of plasma HDL for transport of these enzymes.

Distribution of PON and PAF-AH activity in control and LCAT deficient mice

We previously demonstrated by non-denaturing gradient gel electrophoresis that the lipoprotein profile of LCAT-deficient mice is altered, particularly the HDL fraction which exhibits a skewing to small, dense particles (29). Because of this observation, we asked the question whether the distribution of PON and PAF-AH activity is also altered. To address this question we used FPLC for isolation of lipoproteins. **Figure 1A** and **B** shows representative profiles of PON and PAF-AH activity, respectively, across the various elution fractions; cholesterol and apoA-

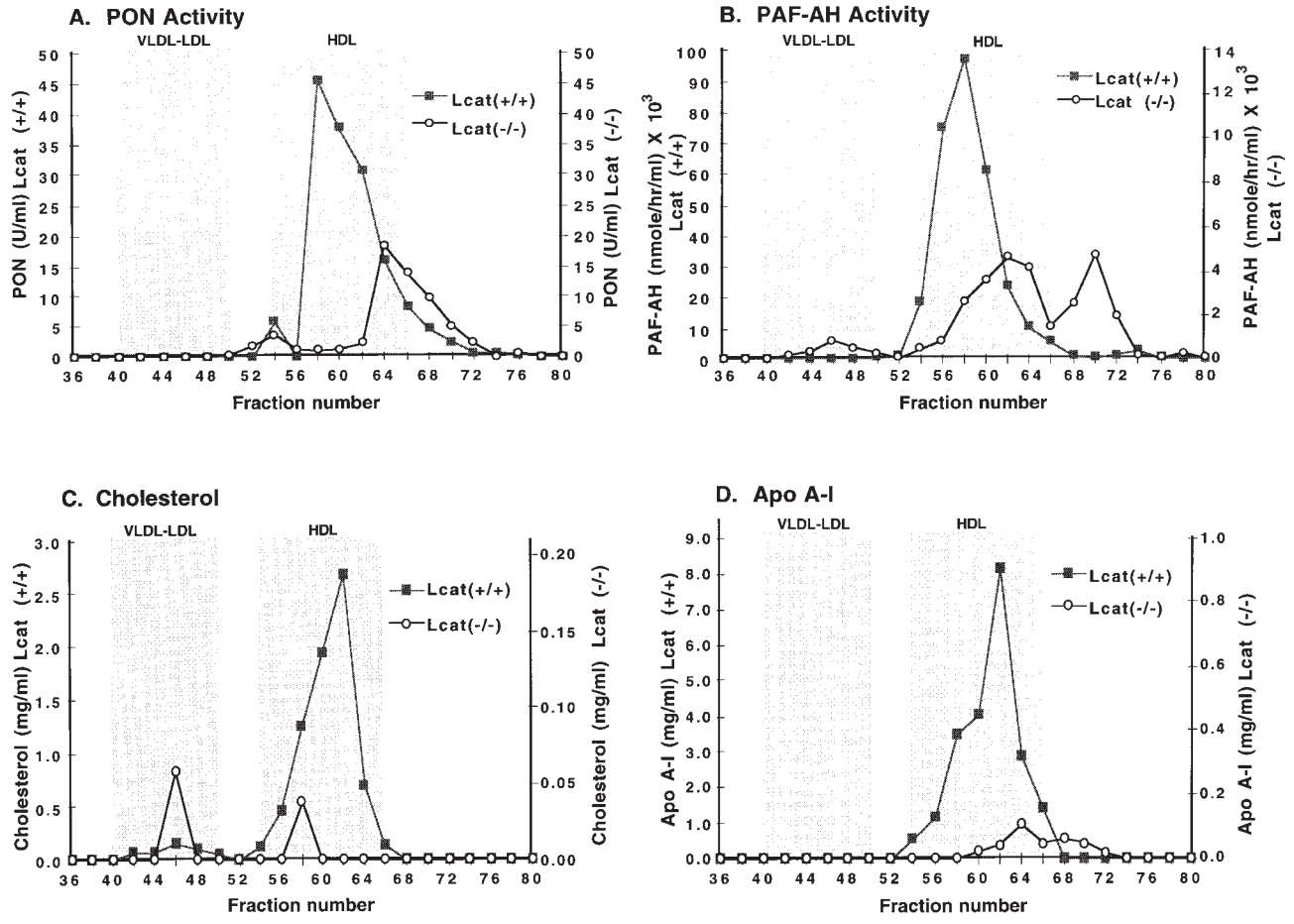

Fig. 1. Fast protein liquid chromatography fractionation of plasma from *Lcat* (+/+) (●—●) and *Lcat* (−/−) (○—○) mice showing the distribution of PON, PAF-AH, cholesterol and apoA-I. Half milliliter samples were collected; each point represents two consecutive 0.5-ml fractions which were pooled. Samples were concentrated to 0.2 ml. (A) The profile for PON activity, (B) profile for PAF-AH activity, (C) cholesterol distribution, and (D) apoA-I distribution. The gray bar between fractions 40–50 delineates the VLDL/LDL fraction while the gray bar between fractions 54–66 delineates the HDL region. The column was calibrated with lipoprotein fractions isolated from plasma of control mice.

I were also quantified and are shown in Fig. 1C and D, respectively. In control mice (*Lcat* (+/+)) both PON and PAF-AH activities have a major peak at fraction 58 in the HDL region. The latter coincides with a shoulder in the apoA-I distribution seen in Fig. 1D and the cholesterol peak in Fig. 1C. Together, these data suggest that in control mice PON and PAF-AH may be co-transported on the same particle and that the major HDL transporter is a large-sized apoA-I-containing particle.

The distribution of PON activity is significantly altered in *Lcat* (−/−) mice compared to controls (Fig. 1A). Whereas PON activity in control mice peaks with large HDL particles, PON activity in *Lcat* (−/−) mice peaks at fraction 66 which represents small HDL particles. Unlike *Lcat* (+/+) mice, where the majority of the enzyme activity is associated with lipoprotein particles, 51.4% of the total PON activity in *Lcat* (−/−) mice is in the lipid-poor region.

Compared to controls, PAF-AH activity in *Lcat* (−/−) mice has a trimodal distribution consisting of two major and one minor peak (Fig. 1B); one major peak is in the region of fractions 62–64 which corresponds to small-sized HDL particles and the other is in the lipid-poor region. The latter represents 37.8% of the total activity. Unlike LCAT-deficient mice, control mice do not exhibit PAF-AH activity in the lipid-poor region and the preponderance of activity is associated with large HDL. In *Lcat* (−/−) mice there is also a minor peak of PAF-AH activity in the VLDL-LDL region (fraction 46) which coincides with a cholesterol peak in the same region suggesting that, under the appropriate conditions, PAF-AH activity can associate with lipoproteins of lower density.

Taken together, these data suggest that both PON and PAF-AH activities are redistributed in LCAT-deficient mice where, rather than associating with large HDL particles, both enzymes associate with small HDL or remain unassociated with lipid. We have previously reported that in LCAT-deficient mice the predominant HDL particle isolated by ultracentrifugation has a diameter of 7.6 nm compared to 9.5 nm for controls; moreover, the morphology of HDL in LCAT deficiency was discoidal rather than spherical (29). Because both PON and PAF-AH activities in *Lcat* (−/−) mice are associated with the smallest lipid-containing particles in the FPLC profile, it is likely that they correspond to the small, nascent HDL previously noted in non-denaturing gradient gel profiles. To confirm this, we pooled FPLC fractions 54–64 from both control and LCAT-deficient mice and subjected this material to ultracentrifugation at d 1.21 g/ml. The floating lipid product of d < 1.21 g/ml contained particles 8.2 nm in the case of the LCAT deficiency and 9.7 nm for controls (data not shown) confirming that enzyme activity is associated with small HDL in *Lcat* (−/−) mice.

Liver mRNA levels for apoA-I and PON

PON is expressed primarily in the liver (35) which is also a major site of apoA-I synthesis. Because both these moieties are exceedingly reduced in LCAT-deficient mice, we tested whether disruption of the *Lcat* gene reduced the level of mRNA transcription of apoA-I and/or PON. The

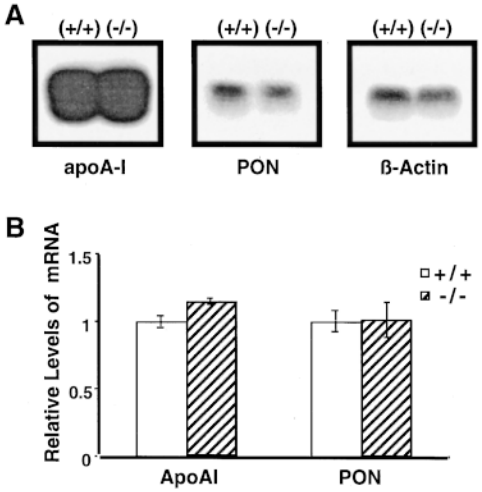

Fig. 2. Liver mRNA levels for PON and apoA-I. Liver mRNA for apoA-I and PON was quantified by densitometric analysis as described in Methods to determine whether message for one or both these proteins was altered in LCAT-deficient mice. The relative mRNA levels for PON and apoA-I from four *Lcat* (−/−) and three *Lcat* (+/+) mice are shown (open bars represent *Lcat* (+/+) mice and hatched bars, *Lcat* (−/−) mice). *$P = 0.053$ compared to *Lcat* (+/+).

relative levels of PON and apoA-I mRNA from liver extracts of *Lcat* (+/+) and *Lcat* (−/−) mice are exhibited in **Fig. 2**. There was no reduction in PON mRNA levels in *Lcat* (−/−) mice compared to *Lcat* (+/+) mice (1.017 ± 0.26 and 1.0 ± 0.16, respectively). There is, however, a slight increase in apoA-I mRNA in *Lcat* (−/−) compared to *Lcat* (+/+) mice (1.15 ± 0.05 and 1.0 ± 0.10, respectively) which is within the margins of statistical significance ($P = 0.053$). The absence of any significant reduction in mRNA transcription for either PON or apoA-I suggests that post-transcriptional events and not reduced transcription, per se, are responsible for the reduction of plasma enzyme activity and apoA-I concentration.

ApoJ levels

Because apoJ in human plasma is found in association with apoA-I- and PON-containing lipoproteins, we investigated whether reduction of HDL in LCAT deficiency is associated with a reduction of apoJ. The data presented in **Fig. 3** indicate that levels of apoJ are not decreased in *Lcat* (−/−) mice, but rather exhibit a modest (36%) but significant increase ($P = 0.01$). In contrast, PON activity is significantly decreased ($P < 0.0001$). The net result is that the apoJ/PON ratio is increased 3-fold in *Lcat* (−/−) mice compared to controls (1.4 and 4.1 for *Lcat* (+/+) and *Lcat* (−/−), respectively).

Plasma lipid hydroperoxides

Several reports using in vitro models have suggested that PON and PAF-AH protect against lipid peroxidation, therefore we investigated whether lipid hydroperoxides are present at increased levels in the plasma of *Lcat* (−/−) mice compared to controls. A sensitive chemiluminescent

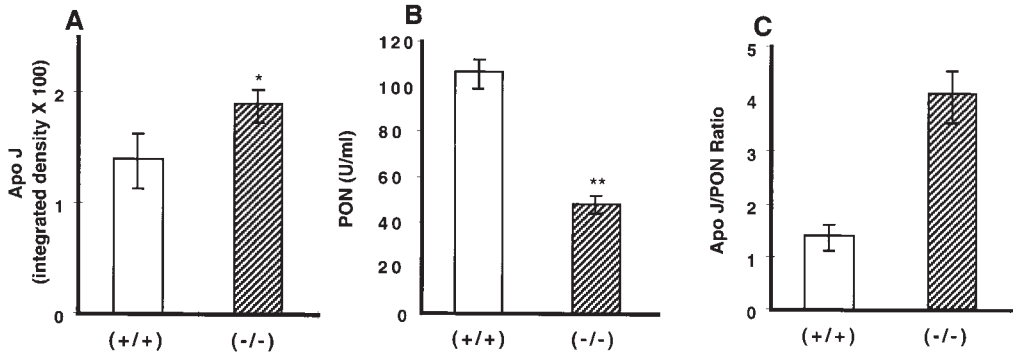

Fig. 3. Changes in (A) apoJ and (B) PON levels and (C) apoJ/PON ratio in *Lcat* (+/+) and *Lcat* (−/−) mice. The relative amounts of apoJ were calculated from densitometric scans of plasma samples probed with antibody to mouse apoJ and visualized by chemiluminescence; these arbitrary units are multiplied by 100. Arylesterase activity was used to determine PON activity. The data represent a total of 10 mice in each group. * $P = 0.01$ and ** $P < 0.0001$ comparing *Lcat* (−/−) to *Lcat* (+/+).

detection method (33) was used to assess the accumulation of cholesteryl ester and phospholipid hydroperoxides; however, no lipid hydroperoxides could be detected (<5 nm) in the plasma samples of either *Lcat* (−/−) or *Lcat* (+/+) mice (data not shown).

DISCUSSION

In vitro studies with both PON and PAF-AH have demonstrated that these enzymes have the ability to hydrolyze oxidized lipids on LDL thus providing a potential in vivo mechanism for the putative antiatherogenic properties of these enzymes. Although there are no clinical studies linking a decrease in PAF-AH with increased risk for atherosclerosis, several studies have suggested an inverse relationship between serum PON activity and cardiovascular disease risk (9, 36–39). Serum PON activity is significantly lower in insulin-dependent (7, 36) and non-insulin-dependent diabetes (37), in familial hypercholesterolemia (38) and in subjects with myocardial infarcts (9, 39) compared to controls. In mice, PON activity is significantly decreased in the C57Bl/6 atherosclerosis-susceptible strain maintained on an atherogenic diet demonstrating that, in this strain of mice, low PON activity is associated with aortic lesion formation (4). Because of their putative role in inhibiting the oxidation of LDL and HDL, an extreme reduction in both PON and PAF-AH activities in LCAT-deficient mice might be expected to result in the accumulation of plasma lipid hydroperoxides. However, in the present study we found no lipid hydroperoxides in *Lcat* (−/−) mouse plasma, suggesting that hydroperoxide accumulation may be irrelevant unless the animals are stressed. Alternatively, lack of plasma lipid hydroperoxides in LCAT-deficient mice could indicate that other compensatory mechanisms may be involved.

Several laboratories have demonstrated that, in humans, apoJ appears in an HDL complex containing PON and apoA-I (8, 23). The majority of the PON/apoJ/apoA-I complexes appear in the density range of HDL_3 and VHDL (8, 23, 40); in addition, apoJ is also found complexed to the SC5b-9 complement fraction (24, 41). The physiology of apoJ is not fully understood but it has been proposed that the protein may help cells contend with unfavorable conditions during normal function. Recent studies by Navab et al. (9) demonstrated that apoJ in atherosclerosis-prone mice, including LDL receptor negative and apoE-deficient mice, have high apoJ levels and low PON activity. When mice were injected with minimally oxidized LDL, apoJ was increased along with a concomitant decrease in PON activity (9). Furthermore, these authors showed that patients with angiographically documented coronary atherosclerosis have elevated apoJ compared to controls and decreased PON activity, suggesting that an elevated apoJ/PON ratio may be a useful marker for increased risk for atherosclerosis. In our LCAT-deficient mice, the apoJ/PON ratio was elevated but there was no indication of oxidative stress in the plasma that might contribute to increased risk. ApoJ in our LCAT-deficient mice remained elevated while PON and PAF-AH activity and apoA-I concentration were drastically reduced suggesting that apoJ is not associated with nascent HDL particles. Although speculative, the lack of association of apoJ with HDL may help explain the somewhat higher concentration of this protein in *Lcat* (−/−) mice; in the lipid-poor form the turnover of apoJ may be reduced. The lack of association of apoJ with nascent HDL particles is consistent with the observation of Burkey, Stuart, and Harmony (42) that the majority of apoJ secreted by HepG2 cells is in the lipid-poor, $d > 1.25$ g/ml fraction. Like our LCAT-deficient mice, HepG2 cell medium is deficient in LCAT and contains nascent HDL (43). Our data are also consistent with the observation in Tangier patients, that apoJ concentrations are normal even though HDL and apoA-I concentrations are extremely low (41). In the latter case, apoJ was observed in the lipid-poor fraction associated with the SC5b-9 complement complex, implying that lack of reduction of apoJ concentrations in HDL deficiency may in part be accounted for by apoJ's ability to associate with other proteins.

Shih et al. (4) showed that a reduction in PON activity in lesion-prone C57Bl/6 mice on an atherogenic diet is associated with decreased PON mRNA levels in the liver thus accounting for the reduction of serum PON activity in this murine model. Because of the close association between apoA-I and PON, we examined liver mRNA levels of both proteins to determine whether synthesis of one or both of these proteins is reduced in LCAT deficiency. The data clearly demonstrated that there was no decrease in PON or apoA-I mRNA in LCAT-deficient mice which contrasts sharply from the studies with lesion-prone mice. It is likely that in LCAT deficiency both proteins are secreted normally and associate with HDL particles, but that small, nascent HDL particles in *Lcat* (−/−) mice are rapidly cleared from the circulation. Furthermore, our FPLC data suggest that in LCAT-deficient mice, apoA-I and PAF-AH, as well as PON, co-localize on particles of the same size; thus it is possible that all three proteins are removed simultaneously from the plasma. The hypothesis that rapid clearance of HDL can account for the low PON and PAF-AH activity in *Lcat* (−/−) mice is supported by the HDL turnover studies in human familial LCAT-deficient subjects which demonstrated that low plasma HDL concentration is associated with rapid clearance of HDL (44).

Like LCAT-deficient mice, apoA-I knockout mice that express no apoA-I and have low HDL-cholesterol have low PON activity; however, activity is reduced only 33% (T. M. Forte, L. Knoff, and M. R. McCall, unpublished observation) as compared with the 60% reduction found in LCAT deficiency, providing indirect evidence that apoA-I is not an absolute requirement for PON activity. Moreover, preliminary FPLC analysis of apoA-I-deficient plasma showed that PON activity was associated with large HDL, suggesting that association of the enzyme with large HDL may be important in preventing the rapid clearance of the enzyme.

The distribution of both PON and PAF-AH activity within the HDL density range is altered in LCAT-deficient mice. In both instances, enzyme activity in LCAT deficiency is almost equally distributed between small dense HDL particles and the lipid-poor region. Although speculative, the appearance of both enzymes in the latter fraction may be due to the altered structure and composition of HDL in LCAT deficiency. These small discoidal particles that are rich in free cholesterol may have a limited capacity to bind both PON and PAF-AH.

In human subjects, it has been reported that PAF-AH activity is associated primarily with LDL (18, 19) and with small, very dense HDL as judged by density gradient ultracentrifugation (19). Unlike in humans, PAF-AH activity in our control mice was associated with a population of large buoyant HDL rather than small dense HDL after isolation by FPLC. These differences may be explained by the different methods used to isolate the HDL fractions and/or species differences. There was no evidence of PAF-AH in the lower density fractions (VLDL/LDL) of control mice which is consistent with previous reports that, in the mouse, PAF-AH is associated with HDL (22). However, FPLC of *Lcat* (−/−) plasma showed a small peak of PAF-AH activity in the VLDL/LDL fraction that was not present in controls. This peak coincided with an increase in cholesterol in this fraction and suggests that PAF-AH in the mouse can associate with lower density particles under the appropriate conditions. Clearly, further studies are required to determine the parameters necessary for binding of PAF-AH to low density particles in the mouse.

From the present studies we conclude that, in the mouse, LCAT deficiency, which is associated with a pronounced decrease in HDL and apoA-I, is also associated with a pronounced decrease in PON and PAF-AH activity which is likely to be the result of rapid clearance of small HDL particles found in LCAT deficiency. The dramatic change in distribution of both enzyme activities is probably a combination of altered morphology and composition of the particles which is likely to be less favorable for binding of the enzymes to the apoA-I–lipid complex.

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