Acquired lecithin-cholesterol acyltransferase deficiency in nephrotic syndrome

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Vaziri, N. D., K. Liang, and J. S. Parks. Acquired lecithin-cholesterol acyltransferase deficiency in nephrotic syndrome. Am J Physiol Renal Physiol 49: F823–F828, 2001.—Lecithin-cholesterol acyltransferase (LCAT) is involved in the synthesis of plasma cholesteryl esters and is pivotal in the maturation of plasma high-density lipoprotein (HDL) and conversion of HDL3 to HDL2. In nephrotic syndrome (NS), the ratio of HDL2 to HDL3 is low even though the total concentration of HDL is generally normal. We hypothesize that the reduced HDL2/HDL3 ratio in NS is due to urinary losses of LCAT, leading to plasma LCAT deficiency. To test this hypothesis, Sprague-Dawley rats were randomized to NS (given 130 mg puromycin aminonucleoside on day 1 and 60 mg ip on day 14) or control groups and were studied on day 30. To dissect the effect of proteinuria from hypoalbuminemia, a group of Nagase rats with inherited hypoalbuminemia was included. Hepatic LCAT and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA abundance and plasma and urine LCAT activity were measured. The NS group showed a fourfold rise in serum cholesterol and triglycerides, a fivefold rise in free cholesterol, and a fourfold fall in the HDL-to-total cholesterol ratio. Despite severe hypoalbuminemia, the Nagase rats showed only a mild elevation of serum cholesterol and triglycerides with a normal serum free cholesterol and HDL-to-total cholesterol ratio. The NS group exhibited a normal hepatic LCAT-to-GAPDH mRNA ratio, a marked reduction in plasma LCAT activity, and a significant increase in urinary LCAT excretion. LCAT/GAPDH mRNA and plasma and urine LCAT were normal in Nagase rats. Thus NS led to heavy urinary losses and reduced plasma concentration of LCAT, despite normal hepatic LCAT mRNA abundance. However, hypoalbuminemia, per se, without proteinuria as seen in the Nagase rats had no effect on plasma LCAT or the HDL-to-total cholesterol ratio. Therefore, proteinuria, not hypoalbuminemia, causes LCAT deficiency and a depressed HDL-to-total cholesterol ratio in NS.

proteinuria; hyperlipidemia; hypercholesterolemia; hypoalbuminemia; hypertriglyceridemia; arteriosclerosis; high-density lipoprotein

LECITHIN-CHOLESTEROL ACYLTRANSFERASE (LCAT) is a 63-kDa glycoprotein enzyme that is synthesized in the liver and secreted in plasma (10, 43) where it catalyzes the removal of the fatty acyl group from the sn-2 position of lecithin and its transfer to free cholesterol to form cholesteryl ester (13). Thus the enzyme possesses a phospholipase A2-like activity and an acyltransferase activity. The enzymatic activity of LCAT depends on the presence of apolipoprotein a-1, which serves as its cofactor (3, 9). LCAT plays a central role in cholesterol uptake by high-density lipoprotein (HDL) particles from the peripheral tissues and maturation of HDL to cholesterol ester-rich HDL2 particles (12). Inherited LCAT deficiency is associated with a marked reduction in HDL-mediated reverse cholesterol transport, a depressed ratio of cholesterol-rich HDL2 to cholesterol-poor HDL3, the presence of cholesterol-laden foam cells in various tissues, accelerated atherosclerotic cardiovascular disease, corneal opacification, and progressive renal disease (20).

Heavy glomerular proteinuria, otherwise known as nephrotic syndrome (NS), is associated with profound hyperlipidemia. Nephrotic hyperlipidemia is characterized by hypercholesterolemia, hypertriglyceridemia, and elevations of low-density (LDL) and very-low-density (VLDL) lipoproteins as well as lipoprotein (a) (5, 44). Although HDL levels are generally normal, the maturation of cholesterol-poor HDL3 to cholesterol-rich cardiovascular protective HDL2 is impaired in NS (18, 32). Because LCAT is necessary for maturation of HDL and generation of HDL2, we hypothesized that this phenomenon may be indicative of urinary losses of LCAT and acquired LCAT deficiency in NS. The present study was designed to test this hypothesis.

METHODS

Study groups. Male Sprague-Dawley rats weighing 180–200 g were housed in a temperature- and light-controlled space with 12:12-h light (500 lux)-dark (<5 lux) cycles. To dissect the effect of proteinuria from hypoalbuminemia, a group of Nagase rats with inherited hypoalbuminemia was included. Hepatic LCAT and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA abundance and plasma and urine LCAT activity were measured. The NS group showed a fourfold rise in serum cholesterol and triglycerides, a fivefold rise in free cholesterol, and a fourfold fall in the HDL-to-total cholesterol ratio. Despite severe hypoalbuminemia, the Nagase rats showed only a mild elevation of serum cholesterol and triglycerides with a normal serum free cholesterol and HDL-to-total cholesterol ratio. Thus NS led to heavy urinary losses and reduced plasma concentration of LCAT, despite normal hepatic LCAT mRNA abundance. However, hypoalbuminemia, per se, without proteinuria as seen in the Nagase rats had no effect on plasma LCAT or the HDL-to-total cholesterol ratio. Therefore, proteinuria, not hypoalbuminemia, causes LCAT deficiency and a depressed HDL-to-total cholesterol ratio in NS.

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5% dextrose in water. In an attempt to dissect the possible effect of proteinuria from that of hypoalbuminemia, a group of male age-matched Nagase hypoalbuminemic rats (Japan SLC, Hamamatsu, Japan) was included as well. Thirty days after the initial piroxicam or placebo injections, animals \( (n = 6/\text{group}) \) were placed in individual metabolic cages for a 24-h urine collection. The next day, under general anesthesia (50 mg/kg ip Nembutal), the animals were killed between the hours of 9 and 11 AM, and the liver was removed immediately, frozen in liquid nitrogen, and stored at \(-70^\circ\)C for subsequent processing. In addition, blood was collected using cardiac puncture.

**RNA preparation and Northern blot analysis.** Total RNA was prepared from 0.2 g of frozen liver tissue with RNazol using the manufacturer’s recommended procedure (Tel-Test, Friendswood, TX). RNA concentration was determined from the absorbance at 260 nm using a spectrophotometer (GeneCare, Friendswood, TX). The membrane was immobilized by ultraviolet irradiation (UltravioletCrosslinker; Fisher Scientific, Pittsburgh, PA). The separated RNA was transferred to the nylon membrane (Zeta probe; Bio-Rad) by capillary blotting in 6× 0.9 M NaCl and 0.09 sodium citrate (pH = 7.0) overnight and immobilized by ultraviolet irradiation (Ultraviolet Crosslinker; Fisher Scientific, Pittsburgh, PA). The membrane was incubated at 65°C in a solution containing 5× 0.75 M NaCl, 0.05 M NaH₂PO₄, and 0.005 M EDTA (pH 7.4; SSPE), 5× Denhardt’s [Ficoll (type 400), polyvinylpyrrolidone, BSA (fraction 5) 1 g/l each], 1% SDS, and 100 μg/ml salmon sperm DNA for 2 h. The cDNA probe for rat LCAT (1.35 kb EcoR I and Hind II fragment of R₈₂₄) was supplied by Parks, and rat glyceroldehyde phosphate dehydrogenase (GAPDH; 1.3 kb Pst I fragment) was obtained from American Type Culture Collection (Rockville, MD). Both probes were labeled with \[^{32}P\]dCTP (3,000 Ci/mmoll; NEN, Boston, MA) by the random primer method (Promega, Madison, WI). Hybridization was carried out at 65°C in a prehybridization solution with \[^{32}P\]-labeled cDNA. The blots were washed two times in 2× SSPE-0.5% SDS solution at room temperature, two times in 1× SSPE-0.5% SDS solution at 37°C, and two times in 0.1× SSPE-0.5% SDS solution at 65°C for 15 min each. The washed blots were exposed to X-ray film (NEN) at \(-80^\circ\)C for 6 h for GAPDH and 2 days for LCAT. The autoradiographs were scanned with a laser densitometer (Molecular Dynamics, Sunnyvale, CA) to determine relative mRNA levels. The values obtained for GAPDH were used as the internal control.

**LCAT activity assay.** LCAT incubations were performed in duplicate using an exogenous substrate assay as described previously (29). The exogenous substrate consisted of recombinant HDL (rHDL) particles containing sn-1(16:0), sn-2(20:4) phosphatidycholine, \[^{3}H\]cholesterol, and human apolipoprotein A-I made by cholate dialysis (14). Assays of initial reaction velocity were performed in 0.5 ml buffer (10 mM Tris, 140 mM NaCl, 0.01% EDTA, and 0.01% Na₂SO₄, pH 7.4) containing rHDL (1.2 μg cholesterol; saturating substrate concentration), 0.6% BSA (fatty acid-free; Sigma, St. Louis, MO), 2 mM β-mercaptoethanol, and 5 μl rat plasma as a source of LCAT. Incubations were performed for 15 min, after which the free and esterified cholesterol radiolabel was extracted and quantified (27).

**Miscellaneous assays.** Urine protein concentration was determined by a quantitative colorimetric assay using a kit purchased from Sigma. Serum albumin concentration was quantified by the bromocresol green method employing a kit purchased from Wako Diagnostics and Chemicals (Richmond, VA). A colorimetric assay was used to measure serum and urine creatinine concentrations using a kit obtained from Sigma Chemical. Plasma concentrations of total cholesterol and free cholesterol were measured by enzymatic colorimetric assays using kits supplied by Wako Diagnostics and Chemicals. Plasma HDL and LDL cholesterol concentrations and triglyceride level were determined by kits purchased from Sigma.

**Data analysis.** ANOVA, Duncan’s multiple range test, and regression analysis were performed for statistical analysis of the data, which are presented as means \pm SE. \( P \) values <0.05 were considered statistically significant.

**RESULTS**

**General findings.** As expected, both nephrotic and hypoalbuminemic Nagase rats exhibited marked hypoalbuminemia compared with the control group (Table 1). Hypoalbuminemia was associated with severe proteinuria in the nephrotic group. However, urinary protein excretion was normal in the Nagase rats. No significant difference was found in either serum creatinine or creatinine clearance among the three groups. The NS group showed a 4-fold elevation of serum total cholesterol, a 5-fold rise in plasma free cholesterol, a nearly 4.5-fold increase in serum triglyceride level, and a 50% reduction in the serum

| Table 1. Serum concentrations of total cholesterol, LDL-cholesterol, triglycerides, VLDL-cholesterol, albumin and creatinine, serum HDL-cholesterol-to-total cholesterol ratio, 24-h urinary protein excretion, and creatinine clearance in control, nephrotic, and analbuminemic Nagase rats |
|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Control                | Nephrotic              | Nagase                 | \( P \) Values (ANOVA) |
|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Serum cholesterol, mg/dl| 68.1 ± 2.8             | 290 ± 19♀*              | 112 ± 2.2♂              | ≤0.001                |
| Serum LDL-cholesterol, mg/dl| 35.7 ± 5.6              | 188 ± 14♀*              | 54 ± 4♂                | ≤0.001                |
| Serum VLDL-cholesterol, mg/ml| 9.2 ± 1.3               | 48.4 ± 11.7♀*           | 21.7 ± 3.7♂           | ≤0.001                |
| HDL-cholesterol-to-total cholesterol ratio| 0.34 ± 0.03            | 0.18 ± 0.01♀*           | 0.32 ± 0.02♂           | ≤0.003                |
| Serum free cholesterol, mg/dl| 19 ± 1.8                | 97 ± 7♀*                | 30 ± 3♂                | ≤0.001                |
| Serum triglycerides, mg/ml| 51.5 ± 5.6               | 224 ± 24.9♀*            | 81.4 ± 7.5♀           | ≤0.001                |
| Serum creatinine, mg/ml| 0.6 ± 0.02              | 0.6 ± 0.01              | 0.5 ± 0.02             | NS                    |
| Creatinine clearance, ml/min| 1.1 ± 0.1               | 1.3 ± 0.1               | 1.2 ± 0.2              | NS                    |
| Urine protein, mg/24 h| 7.4 ± 0.6               | 256 ± 18.2♀*            | 7.2 ± 0.4              | ≤0.003                |
| Serum albumin, g/dl| 3.8 ± 0.03              | 2.1 ± 0.08♀*            | 0.14 ± 0.02♀          | ≤0.0001               |

Values are means ± SE; \( n = 6 \) rats in each group. LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein. NS, not significant. *\( P < 0.05 \) vs. control group. †\( P < 0.05 \) vs. Nagase rats.
HDL cholesterol-to-total cholesterol concentration ratio compared with the control group. However, serum cholesterol and triglyceride concentrations were only mildly elevated, and the HDL cholesterol-to-total cholesterol ratio and plasma free cholesterol were normal in the Nagase rats despite extreme hypoalbuminemia.

**Plasma and urine LCAT activities.** The nephrotic animals exhibited a marked fourfold reduction in plasma LCAT activity coupled with a fourfold increase in urine LCAT excretion when compared with the normal control group. In contrast, despite severe hypoalbuminemia, the Nagase rats showed normal plasma and urinary LCAT values (Fig. 1). Plasma LCAT activity was inversely related to urine LCAT activity \( (r = -0.95, P < 0.001) \) and urine protein excretion \( (r = -0.86, P < 0.005) \). No correlation was found between plasma LCAT activity and serum albumin concentration among the study animals (Fig. 2).

**Hepatic LCAT mRNA.** Despite severe reduction in plasma LCAT activity, hepatic tissue LCAT mRNA was normal in the NS group. Likewise, hepatic LCAT mRNA abundance was unchanged in the Nagase rats (Fig. 3). Thus the reduction in plasma LCAT activity in the NS group was not due to its diminished transcript abundance in the liver.

**DISCUSSION**

NS is associated with marked hyperlipidemia and profound abnormalities of cholesterol and triglyceride metabolisms. In an earlier study, we found a marked upregulation of hepatic 3-hydroxy-3-glutaryl-CoA (HMG-CoA) reductase (the rate-limiting step in cholesterol biosynthesis) during the induction phase of NS and its inappropriate elevation during the chronic phase of NS in rats (36). We subsequently found an inappropriately low expression of hepatic cholesterol 7α-hydroxylase (the rate-limiting enzyme in cholesterol catabolism to bile acids) in the nephrotic rats (26). Despite severe hypercholesterolemia, the nephrotic rats employed in the latter studies showed normal hepatocyte cholesterol concentrations, which contrasted the marked elevation of hepatocyte cholesterol content in the control rats with diet-induced hypercholesterolemia of equal magnitude (26, 36). Because intracellular, not extracellular, cholesterol is involved in regulation of HMG-CoA reductase and cholesterol 7α-hydroxylase expression (30, 42), we supposed that the lack of a rise in intracellular cholesterol in the face of severe hypercholesterolemia may account for the failure of the liver to suppress biosynthetic and stimulate catabolic pathways of cholesterol metabolism in the nephrotic animals. Moreover, we hypothesized that the apparent discordance between the intracellular and extracellular compartments in the liver with respect to chol-
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In a series of subsequent experiments, expressions of LDL receptor and scavenger receptor class B type I (SR-BI), which represent the principal pathways of cholesterol uptake by the liver, were studied. These studies revealed marked downregulations of hepatic LDL receptor and SR-BI proteins with normal mRNA abundance in both instances (25, 37). The above studies explored the effect of NS on hepatic metabolism of cholesterol but did not address cholesterol esterification and uptake from the peripheral tissues. In a series of separate studies designed to explore the molecular basis of the NS-induced abnormalities of triglyceride-rich lipoproteins (4, 5, 18, 21), we found marked downregulation of lipoprotein lipase, hepatic triglyceride lipase, and VLDL receptor expression in nephrotic rats (22–24).

The present study revealed a marked reduction in plasma LCAT activity in NS rats, denoting a severe acquired deficiency state. The reduction in plasma LCAT activity in the NS group was accompanied by heavy urinary losses of the enzyme. However, LCAT mRNA abundance in the liver tissue was not altered by NS. Plasma LCAT activity was inversely related to its urinary excretion. Taken together these findings suggest that the urinary losses must have contributed to the observed LCAT deficiency in NS, a condition characterized by urinary losses of proteins of intermediate molecular weight. The molecular weight of LCAT (63 kDa) is very close to that of albumin, whose urinary losses and depressed plasma concentration constitute the defining features of NS. Thus it is not surprising that, as with albumin, urinary losses can lead to a low plasma concentration of LCAT. In fact, urinary losses have been shown to result in the reduction of plasma concentrations of numerous other proteins and proteinbound agents in this condition. These include but are not limited to hormone and hormone-binding proteins (1, 6, 19, 35, 45), coagulation and fibrinolytic factors (33, 34, 39–41), IgG, metal-binding proteins (8), etc.

It should be noted that the effect of NS on metabolism of plasma proteins is usually not limited to their losses in the urine. In fact, NS frequently affects biosynthesis and catabolism of many proteins. (17) Available data on LCAT metabolism are extremely limited, and information on the tissue uptake and catabolism of LCAT is lacking. LCAT appears to be constitutively secreted by the liver, and very few perturbations are known to affect LCAT biosynthesis, except inflammatory mediators (7) and chronic renal failure (38). While demonstrating urinary losses of LCAT, we have not explored its biosynthesis and catabolism in the present study. Similarly, we have not examined the possible effect of the nephrotic milieu on LCAT activity. Further studies are required to elucidate LCAT metabolism in health and disease in general and the specific issues relevant to NS, noted above.

In an attempt to dissect the possible role of proteinuria from that of hypoalbuminemia, we included a group of Nagase rats with inherited hypoalbuminemia and no proteinuria. In contrast to the nephrotic animals, the Nagase rats showed normal plasma and urine LCAT activity. These observations suggested that LCAT deficiency in the NS group was not due to hypoalbuminemia, which was common to both NS and Nagase rats, but rather was related to proteinuria, which was present in the former but not the latter group.

Cholesterol content of the peripheral cells is a function of the combined cholesterol influx and de novo synthesis on the one hand and cholesterol efflux on the other. The primary pathway of cholesterol influx in the peripheral tissues is the LDL receptor pathway. The principal pathway for removal of surplus cholesterol from the peripheral cells is cholesterol uptake and its disposal in the liver by HDL. Optimal cholesterol uptake by HDL depends on the presence of LCAT, which catalyzes the removal of a fatty acyl group from the sn-2 position of lecithin and its transfer to free cholesterol to form cholesterol ester. Because of its increased hydrophobicity, cholesterol ester formed on the surface of HDL (12). This process helps to maintain a favorable gradient for continued diffusion of free cholesterol from the cell to the surface of HDL (11, 13). However, in the presence of LCAT deficiency, accumulation of unprocessed free cholesterol on the surface of HDL limits the gradient-driven cholesterol uptake by HDL. Consequently, maturation of cholesterol-poor HDL₃ to cholesterol ester-rich HDL₂ diminishes, the HDL₂-to-HDL₃ ratio falls, and cellular cholesterol rises (20). The functional significance of the observed LCAT deficiency is evidenced by marked elevation of plasma free cholesterol and the reduction in the HDL-to-total cholesterol ratio in the NS animals.
On the basis of the above observations, severe LCAT deficiency shown here can contribute to the previously known abnormalities of HDL maturation and metabolism (18, 32) in NS. This is further supported by the recent demonstration in NS of aquired deficiency of SR-BI, which facilitates hepatic uptake of lipids from HDL particles (25). Together these findings point to a profound dysregulation of HDL metabolism and hence impaired reverse cholesterol transport in NS.

The observed reduction in plasma LCAT enzymatic activity found here in rats with puromycin-induced NS is consistent with the earlier reports in rats with Hay-mann nephritis (31) and humans with NS (28) and is consistent with the earlier reports in rats with Hay-mann nephritis (31) and humans with NS (28) and contrasts the results reported by other investigators who have shown either normal or elevated LCAT activity (15, 32). The reason for the observed disparity is uncertain and may be due to methodological differences.

In conclusion, NS results in profound deficiency and urinary losses of LCAT. The observed LCAT deficiency is related to proteinuria but not hypoalbuminemia. This phenomenon can contribute to the reported abnormalities of HDL metabolism in NS.

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REFERENCES


