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Down-regulation of hepatic lecithin:cholesterol acyltransferase gene expression in chronic renal failure

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Down-regulation of hepatic lecithin:cholesterol acyltransferase gene expression in chronic renal failure.

Background. Chronic renal failure (CRF) is associated with premature arteriosclerosis, impaired high-density lipoprotein (HDL) maturation, increased pre-β HDL (a lipid-poor HDL species), reduced HDL/total cholesterol ratio, hypertriglyceridemia, and depressed lipolytic activity. The latter has been, in part, attributed to elevated pre-β HDL, which is a potent inhibitor of lipoprotein lipase (LPL). Accumulation of cholesterol in the arterial wall is a critical step in atherogenesis, and HDL-mediated cholesterol removal from peripheral tissues mitigates atherosclerosis. Lecithin:cholesterol acyltransferase (LCAT) is essential for maturation of HDL and cholesterol removal by HDL from peripheral tissues. Earlier studies have revealed depressed plasma LCAT enzymatic activity in patients with CRF. This study was conducted to determine whether impaired LCAT activity can be confirmed in CRF animals and if so whether it is due to down-regulation of hepatic LCAT expression.

Methods. Hepatic tissue LCAT mRNA and plasma LCAT enzymatic activity were measured in male Sprague-Dawley rats six weeks after excisional 5/6 nephrectomy or sham operation.

Results. Compared with the controls, the CRF group exhibited a significant reduction of hepatic tissue LCAT mRNA abundance. The reduction in hepatic LCAT mRNA was accompanied by a marked reduction of plasma LCAT activity and elevation of serum-free cholesterol in the CRF animals. LCAT activity correlated positively with the HDL/total cholesterol ratio and inversely with free cholesterol and triglyceride concentrations.

Conclusions. CRF leads to a marked down-regulation of hepatic LCAT mRNA expression and plasma LCAT activity. This abnormality can impair HDL-mediated cholesterol uptake from the vascular tissue and contribute to cardiovascular disease. In addition, LCAT deficiency can, in part, account for elevated serum-free cholesterol, reduced HDL/total cholesterol, and elevated pre-β HDL in CRF. The latter can, in turn, depress lipolytic activity and hinder triglyceride-rich lipoprotein clearance in CRF.

Key words: high-density lipoprotein, LCAT, lipid disorder, arteriosclerosis, cardiovascular disease, hypertriglyceridemia.

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Chronic renal failure (CRF) is associated with increased risk of arteriosclerotic cardiovascular disease and profound abnormalities of lipid metabolism [1]. The reported CRF-induced lipid abnormalities include hypertriglyceridemia, elevated plasma very low-density lipoprotein (VLDL), apolipoprotein B (apoB), pre-β high-density lipoprotein (HDL), and lipoprotein (a), coupled with diminished plasma apoA-1 and HDL cholesterol concentrations [2–5]. This is associated with triglyceride enrichment and impaired clearance of VLDL and chylomicrons and elevated plasma concentrations of VLDL and chylomicron remnants [2, 6, 7]. The latter abnormalities are largely due to CRF-induced down-regulation of lipoprotein lipase (LPL), hepatic triglyceride lipase, and VLDL receptor expression [8–11] coupled with elevations of plasma, apoC-III, and pre-β HDL, which are potent inhibitors of LPL [12].

Plasma total cholesterol is frequently low to normal and only occasionally elevated in CRF patients. In addition, HMG CoA reductase, the rate-limiting step in cholesterol biosynthesis, and cholesterol 7α-hydroxylase, the rate-limiting step in cholesterol catabolism, are unaffected by CRF [13]. Moreover, LDL receptor and scavenger receptor BI, the primary pathways of hepatic cholesterol uptake, are normal in CRF [14]. However, HDL maturation is impaired. The plasma HDL cholesterol-to-total cholesterol ratio is markedly reduced [2], and pre-β HDL, which is a lipid-poor HDL species, is elevated in CRF [12].

Lecithin:cholesterol acyltransferase (LCAT) is a 63 to 65 kD plasma glycoprotein that is responsible for the synthesis of most cholesteryl esters (CEs) found in plasma [15]. The enzyme catalyzes the hydrolysis of a sn-2 fatty acid from phospholipid and the transesterification of the fatty acid to the 3-hydroxyl group of cholesterol to form CE and lysophospholipid [15]. ApoA-I serves as a cofactor for the reaction and is the major structural apolipoprotein of HDLs, which are the preferred macromolecular substrate particle for the LCAT reaction [16–18]. LCAT is essential for maturation of

HDLs that are secreted from the liver and intestine or formed during lipolysis of triglyceride-rich lipoproteins [19]. The generation of CE by LCAT converts nascent discoidal HDL to spherical particles, which are the predominant form of HDL in plasma. The importance of LCAT in the HDL maturation process is best illustrated by genetic [20] or induced [21] LCAT deficiency states in which nascent discoidal HDL accumulate in plasma and CE concentrations are very low. LCAT is also thought to play an important role in reverse cholesterol transport, the process by which excess free cholesterol is removed from peripheral tissues by HDL and transported to the liver for excretion [15]. The role of LCAT in this process appears to be the generation of a chemical gradient for the flux of free cholesterol from cells into HDL by decreasing the concentration of free cholesterol in HDL through its conversion to CE.

Earlier studies have shown a reduction in plasma LCAT enzymatic activity in dialysis-dependent patients with end-stage renal disease [22]. Given the central role of LCAT in reverse cholesterol transport by HDL, its deficiency can account for low HDL cholesterol concentration and increased pre-β HDL in CRF. The present study was designed to determine whether the reported impairment of plasma LCAT activity in patients can be confirmed in CRF animals and, if so, whether it is due to depressed LCAT gene expression.

METHODS

Animal models

Male Sprague-Dawley rats weighing 225 to 250 g were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN, USA). They were housed in a climate-controlled, light-regulated facility with 12-hour day (~500 lux) and 12-hour night (<5 lux) cycles. The animals were fed regular rat chow (Purina Mills, Brentwood, MO, USA) and water ad libitum. They were then randomly assigned to the following groups. A minimum of six animals was used in each group.

(1) The CRF group. Animals assigned to the CRF group were subjected to 5/6 nephrectomy by surgical resection using a dorsal incision as described previously [9].

(2) The control group. The animals assigned to the control group were subjected to sham operation and were provided free access to food and water.

The study groups were observed for five weeks, at which point they were placed in metabolic cages for a 24-hour urine collection. They were then sacrificed between the hours of 9 and 11 a.m. by exsanguination using cardiac puncture. The liver was removed immediately, snap frozen in liquid nitrogen, and stored at −70°C until processed. All surgical procedures were carried out under general anesthesia (Nembutal, 50 mg/kg, IP) while observing strict hemostasis and aseptic techniques. Serum cholesterol, triglyceride, and creatinine concentrations and urinary protein and creatinine contents were determined as described previously [13]. Arterial blood pressure was determined by a tail sphygmomanometer (Harvard Apparatus, South Natick, MA, USA).

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 Inc., Friendswood, TX, USA). RNA concentration was determined from the absorbance at 260 nm using a spectrophotometer (GeneQuat; Bio-Rad, Hercules, CA, USA). Twenty-five microgram aliquots of total RNA were denatured in 2.2 mol/L formaldehyde at 65°C for 15 minutes and run on 1% agarose/2.2 mol/L formaldehyde gel at 40 V for five hours. The separated RNA was transferred to the nylon membrane (Zeta probe; Bio-Rad) by capillary blotting in 6 × standard saline citrate (SSC) buffer (0.9 mol/L NaCl, 0.09 mol/L Na citrate, pH 7.0) overnight and immobilized by ultraviolet irradiation (Ultraviolet Crosslinker; Fisher Scientific, Pittsburgh, PA, USA). The membrane was incubated at 65°C in a solution containing 5 × SSPE [0.75 mol/L NaCl, 0.05 mol/L NaH_2PO_4, 0.005 mol/L ethylenediaminetetraacetic acid (EDTA), pH 7.4], 5 × Denhard's [Ficoll (type 400), polyvinylpyrrolidone, bovine serum albumin (fraction 5) 1 g/L each], 1% sodium dodecyl sulfate (SDS), and 100 μg/mL salmon sperm DNA for two hours. The cDNA probes for rat LCAT (1.35 kb EcoR I and *Hin*dII fragment of R_1P_1) were supplied by Professor John S. Parks (Wake Forest University School of Medicine, Winston-Salem, NC, USA), and rat glyceraldehyde phosphate dehydrogenase (GAPDH; 1.3 kb Pst I fragment) was obtained from American Type Culture Collection (Rockville, MD, USA). Both probes were labeled with [^{32}P] dCTP (3000 Ci/mmol; New England Nuclear Inc., Boston, MA, USA) by the random primer method (Promega Inc., Madison, WI, USA). Hybridization was carried out at 65°C in a prehybridization solution with ^{32}P-labeled cDNA. The blots were washed twice in 2 × SSPE/0.5% SDS solution at room temperature, twice in 1 × SSPE/0.5% SDS solution at 37°C, and twice in 0.1 × SSPE/0.5% SDS solution at 65°C for 15 minutes each. The washed blots were exposed to x-ray film (New England Nuclear Inc.) at −80°C for six hours for GAPDH and two days for LCAT. The autoradiographs were scanned with a laser densitometer (Molecular Dynamics, Sunnyvale, CA, USA) to determine relative mRNA levels. The values obtained for GAPDH were used as the internal control.

LCAT activity assay

Lecithin:cholesterol acyltransferase incubations were performed in duplicate using an exogenous substrate

Table 1. Creatinine clearance (C_{Cr}), serum concentrations of creatinine, triglycerides, total cholesterol, free cholesterol, LDL, HDL, and VLDL, HDL cholesterol-to-total cholesterol (HDL-C/TC) ratio, urinary protein excretion, systolic arterial pressure and body weight in chronic renal failure (CRF) and sham-operated control (CTL) groups

	Control ($N=6$)	CRF ($N=6$)	*P* values
Creatinine clearance *mL/min*	1.82 ± 0.15	0.48 ± 0.05	< 0.001
Serum creatinine *mg/mL*	0.6 ± 0.06	1.62 ± 0.12	< 0.001
Serum triglycerides *mg/mL*	43.5 ± 1.6	108.6 ± 9.3	< 0.001
Serum cholesterol *mg/dL*	73.5 ± 2.4	163.8 ± 9.6	< 0.001
Serum LDL-chol *mg/dL*	37 ± 1.7	91 ± 8.8	< 0.001
Serum HDL-chol *mg/dL*	28.5 ± 1.2	33.2 ± 53	NS
Serum free cholesterol	23.7 ± 1.4	50.5 ± 6.0	< 0.001
Serum VLDL-chol *mg/mL*	7.7 ± 1.2	30.2 ± 5.2	< 0.001
HDL-C/TC (ratio)	0.38 ± 0.01	0.26 ± 0.01	< 0.005
Urine protein *mg/24 h*	8.3 ± 0.7	33.6 ± 4.0	< 0.001
Blood pressure *mm Hg*	108 ± 6	147 ± 11	< 0.005
Body weight *g*	442 ± 7.8	409 ± 5.9	<0.05

Abbreviations are: LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein.

assay as described previously [23, 24]. The exogenous substrate consisted of recombinant HDL (rHDL) particles containing sn-1 16:0, sn-2 20:4 phosphatidylcholine, ^{3}H-cholesterol, and human apoA-I, made by cholate dialysis [23]. Assays of initial reaction velocity were performed in 0.5 mL buffer (10 mmol/L Tris, 140 mmol/L NaCl, 0.01% EDTA, 0.01% NaN_3, pH 7.4) containing rHDL (1.2 μg cholesterol; saturating substrate concentration), 0.6% bovine serum albumin (fatty acid-free; Sigma Chemical Co., St. Louis, MO, USA), 2 mmol/L β-mercaptoethanol, and 5 μL rat plasma as a source of LCAT. Incubations were performed for 15 minutes, after which the free and esterified cholesterol radiolabel was extracted and quantitated [25].

Miscellaneous assays

A colorimetric assay was used to measure serum and urine creatinine concentrations using a kit obtained from Sigma Chemical Co. Plasma concentrations of total cholesterol and free cholesterol were measured by enzymatic colorimetric assays using kits supplied by Wako Chemical Company (Richmond, VA, USA). Plasma HDL and LDL cholesterol concentrations and triglyceride level were determined by kits purchased from Sigma Chemical Co.

Data analysis

Student *t* test and regression analysis were performed in statistical analysis of the data that are presented as mean ± SEM. *P* values less than 0.05 were considered significant.

RESULTS

General data

As shown in Table 1, the CRF group exhibited a significant increase in plasma creatinine concentration and a significant reduction in creatinine clearance as compared with the sham-operated control group. The CRF group showed a significant increase in plasma triglyceride, total cholesterol, free cholesterol, LDL cholesterol, and VLDL cholesterol concentrations. However, despite significant elevations of total cholesterol concentrations, plasma HDL cholesterol was unchanged, and the plasma HDL cholesterol-to-total cholesterol concentration ratio was markedly reduced in the CRF group as compared with the corresponding values found in the control group. Compared with the control group, the CRF animals exhibited a significant rise in arterial blood pressure.

LCAT data

Hepatic tissue LCAT mRNA abundance was significantly lower in the CRF group as compared with that found in the sham-operated controls (Fig. 1). The reduction in hepatic tissue LCAT mRNA was accompanied by a parallel fall in plasma LCAT activity in the CRF group (Fig. 2). These observations point to acquired down-regulation of LCAT in rats with CRF. Plasma LCAT activity was directly related to hepatic LCAT mRNA abundance ($r = 0.87$, $P < 0.001$) pointing to transcriptional regulation of LCAT in the study animals. Plasma LCAT activity was directly related to the HDL/total cholesterol ratio ($r = 0.84$, $P < 0.005$) and inversely to the plasma triglyceride concentration ($r = -0.81$, $P < 0.005$) and plasma-free cholesterol concentration ($r = -0.78$, $P < 0.05$).

DISCUSSION

The CRF animals employed in the present study exhibited a marked reduction of plasma LCAT enzymatic activity. The reduction in plasma LCAT activity in our CRF rats is consistent with an earlier report by Bories, Subbaiah, and Bagdade, who showed depressed plasma LCAT enzymatic activity in patients with end-stage renal disease [22]. The reduction in plasma LCAT enzymatic activity using an exogenous substrate assay in our CRF

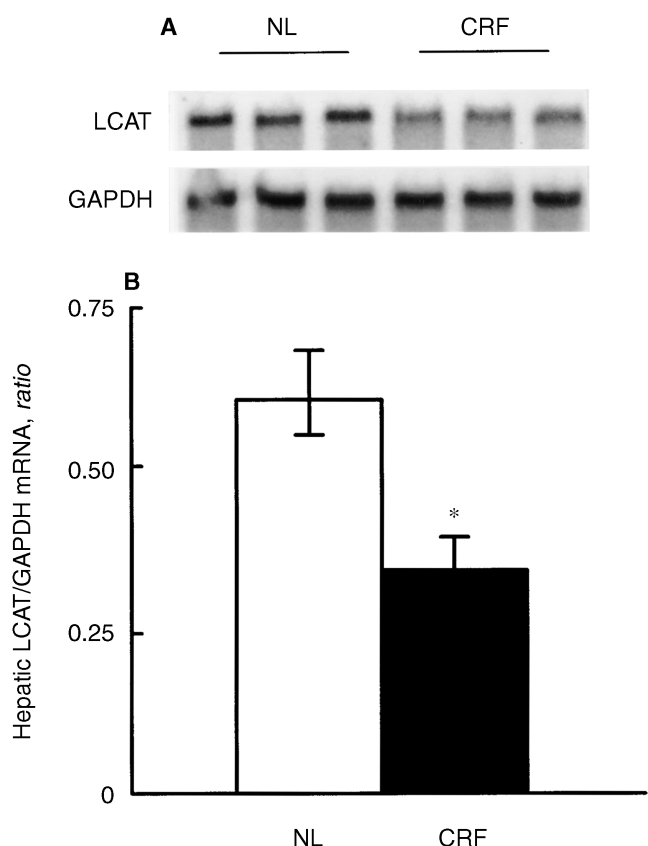

Fig. 1. (*A*) Representative Northern blot of hepatic lecithin:cholesterol acyltransferase (LCAT) and glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA in three animals with chronic renal failure (CRF) and three sham-operated normal control (NL) rats. (*B*) Group data depicting the hepatic LCAT/GAPDH mRNA ratio in the study groups. $N = 6$ animals in each group. $*P < 0.01$.

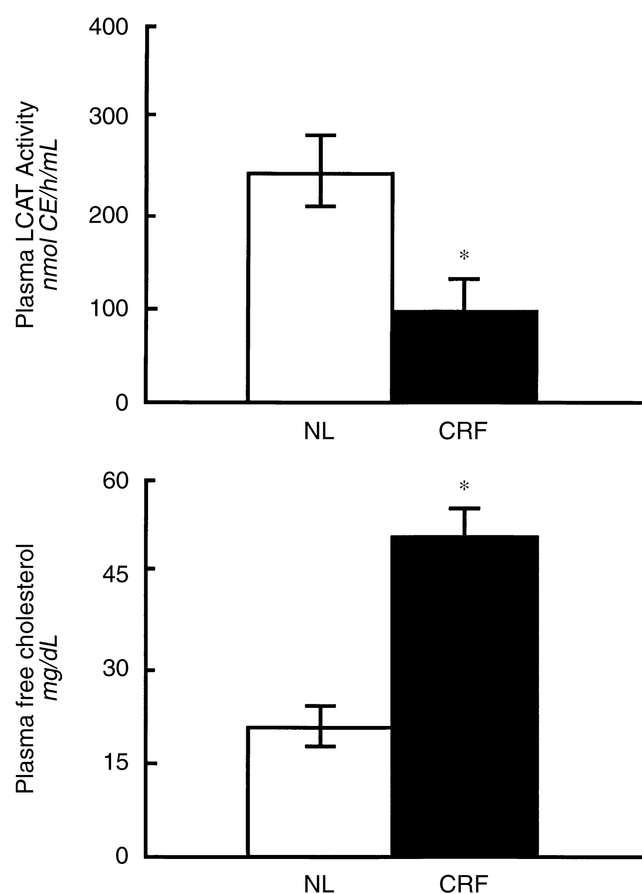

Fig. 2. Plasma LCAT enzymatic activity (*A*) and plasma-free cholesterol concentration (*B*) in six rats with chronic renal failure (CRF) and six normal control (NL) animals. $*P < 0.001$.

animals was accompanied by a significant reduction in hepatic tissue LCAT mRNA abundance when compared with the control group. This observation suggests that impaired plasma LCAT activity reported in CRF patients previously [22] and shown in CRF rats in our current study must be due to quantitative deficiency of the enzyme as opposed to its inhibition by the uremic milieu. Accordingly, these data suggest that experimental CRF leads to an acquired down-regulation of hepatic LCAT gene expression in the rat.

The available data do not allow definitive conclusions as to the mechanism(s) by which CRF leads to down-regulation of hepatic LCAT gene expression. Little is known about LCAT metabolism and regulation in health and disease. LCAT appears to be constitutively secreted by the liver, and very few perturbations are known to affect LCAT biosynthesis except for inflammatory mediators which depress LCAT production [21, 26]. Interestingly, inflammation has been shown to be a common feature of CRF [27]. Therefore, the associated inflammatory state can potentially contribute to down-regulation of hepatic LCAT production in CRF. In fact, the CRF-associated inflammation has been implicated in the pathogenesis of impaired biosynthesis of albumin and transferrin, which like LCAT, are produced and secreted by the liver [26].

As noted in the introduction, LCAT plays a critical role in the HDL-mediated cholesterol uptake from the peripheral tissues. Thus, LCAT deficiency can, at least in part, account for the reduction of plasma HDL cholesterol-to-total cholesterol ratio known to occur in CRF [2] and exemplified by CRF animals employed in this study. Similarly, LCAT deficiency can contribute to the reported elevation of pre-β HDL, which is a lipid-free HDL species, in CRF [12]. It is of interest that pre-β HDL has been recently shown to be a potent inhibitor of LPL. Elevation of pre-β HDL is thought to contribute to impaired lipolytic activity and depressed clearance of triglyceride-rich lipoproteins in CRF [12]. Accordingly, the CRF-induced LCAT deficiency may indirectly impact triglyceride metabolism via elevation of pre-β HDL and the resultant inhibition of lipolytic pathway. Moreover, in humans who unlike rats possess cholesterol ester

transfer protein (CETP), the reduction in cholesterol ester content of HDL can possibly limit the triglyceride for CE exchange between VLDL and HDL in the circulation. This can contribute further to triglyceride enrichment of VLDL particles and their remnants in CRF [2].

In conclusion, CRF results in marked down-regulation of LCAT expression and activity. Acquired LCAT deficiency can depress cholesterol uptake from peripheral tissues by HDL and contribute to accumulation of cholesterol in the vascular tissue and atherosclerosis in CRF. Moreover, reduced cholesterol uptake can, in part, account for CRF-induced abnormalities of plasma lipid profile.

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