Upregulation of acyl-CoA:cholesterol acyltransferase in chronic renal failure

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Liang, Kaihui, and N. D. Vaziri. Upregulation of acyl-CoA:cholesterol acyltransferase in chronic renal failure. Am J Physiol Endocrinol Metab 283: E676–E681, 2002.—Chronic renal failure (CRF) is associated with profound abnormalities of lipid metabolism and accelerated atherosclerotic cardiovascular disease. In a recent study, we found marked downregulation of hepatic lecithin-cholesterol acyltransferase, or LCAT, expression, which can account for impaired HDL maturation and depressed HDL cholesterol concentration in CRF. Here, we report on the effect of CRF on acyl-CoA:cholesterol acyltransferase (ACAT) expression. ACAT is an intracellular enzyme that catalyzes esterification of free cholesterol to cholesterol ester for storage or secretion. ACAT plays a major role in hepatic production and release of VLDL, intestinal absorption of cholesterol, foam cell formation, and atherogenesis. We examined hepatic expression of ACAT-1 and ACAT-2 mRNA (Northern blot) and protein (Western blot) abundance and total ACAT activity in male CRF rats (6 wk after 5/6 nephrectomy) and sham-operated controls. The CRF animals showed a significant reduction in creatinine clearance, marked hypertriglyceridemia, modest hypercholesterolemia, and significant upregulation of hepatic tissue ACAT-2 protein and mRNA abundance. In contrast, hepatic ACAT-1 mRNA and protein abundance were unaffected by CRF. Upregulation of ACAT-2 expression was accompanied by a significant increase in hepatic ACAT activity and a significant decrease in hepatic microsomal and whole liver free cholesterol concentration. Thus CRF results in significant upregulation of hepatic ACAT-2 (but not ACAT-1) expression and ACAT activity, which may, in part, contribute to the associated lipid disorders.

uremia; renal insufficiency; hyperlipidemia; cholesterol; atherosclerosis; cardiovascular disease

CHRONIC RENAL FAILURE (CRF) is associated with a highly atherogenic diathesis, which is, in part, due to the associated dyslipidemia. CRF-induced lipid abnormalities include elevated plasma concentrations of triglyceride, VLDL, lipoprotein a, and apolipoprotein B; depressed plasma HDL cholesterol concentration; and normal, reduced, or increased plasma total cholesterol concentration (2, 8, 12, 13, 20, 25, 26). In a series of earlier studies undertaken to explore the molecular basis of CRF-associated dyslipidemia, we found marked downregulations of skeletal muscle and adipose tissue lipoprotein lipase and VLDL receptor expressions (17, 29, 30, 34) in rats with CRF produced by 5/6 nephrectomy. In addition, Klin et al. (16) found marked downregulation of hepatic triglyceride lipase in rats with experimental CRF (16). The observed deficiencies of lipoprotein lipase and VLDL receptor can, in part, account for the associated hypertriglyceridemia, elevation of plasma VLDL, and impaired clearance of triglyceride-rich lipoproteins in CRF. In addition, we have found severe downregulation of apoprotein-A1 and lecithin-cholesterol acyltransferase, or LCAT, in CRF rats (28, 32). The latter abnormalities can account for the reduction in plasma HDL concentration and impaired maturation of lipid-poor HDL-3 to cardioprotective lipid-rich HDL-2 in patients and animals with CRF (26).

Acyl-CoA:cholesterol acyltransferase (ACAT) catalyzes intracellular esterification of cholesterol and formation of cholesterol ester in nearly all mammalian tissues (3). ACAT-mediated esterification of cholesterol serves several important functions. 1) Esterification of cholesterol by ACAT limits its solubility in the cell membrane lipids and promotes accumulation of cholesterol ester in the fat droplets within cytoplasm. This process prevents toxic accumulation of free cholesterol in various cell membrane fractions. 2) ACAT plays a major role in intestinal transport of cholesterol, since most of the cholesterol absorbed undergoes esterification by ACAT (24) before incorporation in chylomicrons (15). 3) ACAT-mediated esterification of cholesterol is involved in production and release of apoB-containing lipoproteins (e.g., VLDL) by the liver (6, 11, 14). 4) ACAT appears to be involved in regulation of hepatic cholesterol 7α-hydroxylase, the rate-controlling enzyme in cholesterol catabolism (23). 5) ACAT has a role in accumulation of cholesterol ester in macrophages and vascular tissue, an event that is central to foam cell formation and atherosclerosis (4).

Given the critical role of ACAT in lipid metabolism and atherogenesis, we hypothesized that CRF may result in upregulation of ACAT expression. The present study was designed to test this hypothesis.
METHODS

Animal models. Male Sprague-Dawley rats weighing 225–250 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). They were housed in a climate-controlled, light-regulated facility with 12:12-h day-night cycles. The animals were fed regular rat chow (Purina Mills, Brentwood, MO) and water ad libitum and randomly assigned to the CRF and control groups of six animals each. The animals assigned to the CRF group were subjected to 5/6 nephrectomy by surgical resection using a dorsal incision, as described previously (34). The animals assigned to the control group were subjected to sham operation and were provided free access to feed and water.

The animals were observed for 6 wk, at which point they were placed in metabolic cages for a 24-h urine collection. They were then killed by exsanguination by cardiac puncture between the hours of 9 and 11 AM. 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 strict hemostasis and aseptic techniques were observed. Serum cholesterol, triglyceride, and creatinine concentrations and urinary protein and creatinine contents were determined as described previously (31). Arterial blood pressure was determined by a tail sphygmomanometer (Harvard Apparatus, South Natick, MA) as described in our earlier studies (33).

Western blot analysis. Frozen rat liver tissue was homogenized in 1 ml of 20 mM Tris-HCl (pH 7.5) buffer containing 2 mM MgCl₂, 0.2 M sucrose, 5 mM phenylmethylsulfonyl fluoride (PMSF), 5 μg/ml leupeptin, 10 μg/ml aprotonin, and 3 μg/ml pepstatin A. The crude extract was centrifuged at 12,000 g at 4°C for 10 min to remove tissue debris. The supernatant was processed for determination of ACAT-2 and ACAT-1 abundance by Western blot and protein concentration by a bicinechonic acid (BCA) protein assay kit (Pierce, Rockford, IL). Aliquots containing 100-μg proteins were fractionated on 4–20% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 2 h. After electrophoresis, proteins were transferred to Hybond enhanced chemiluminescence (ECL) membrane (Amersham Life Science, Arlington Heights, IL). The membrane was incubated for 1 h in blocking buffer (1× Tris-buffered saline (TBS), 0.1% Tween 20, and 7% nonfat milk) and then overnight in the same buffer containing either 1.5000 polyonal ACAT-2 antibody or 1.3000 polyonal ACAT-1 antibody (generously provided by Professor Lawrence L. Rudel, Department of Biochemistry and Comparative Medicine, Wake Forest University, Winston Salem, NC). Membrane was washed four times for 10 min in 1× TBS, 0.1% Tween 20 before a 2-h incubation in blocking buffer plus diluted (1:6,0000) horseradish peroxidase-linked anti-rabbit IgG (Amersham Life Science, Arlington Heights, IL). The washes were repeated before the membranes were developed with chemiluminescent agents (ECL, Amersham Life Science) and subjected to autoluminography for 1 min.

ACAT activity assay. ACAT activity in the microsomal preparation was determined from the rate of [14C]oleoyl-CoA incorporation into stearyl ester with a modification of the method described by Chang et al. (9). Briefly, 200 mg of the frozen liver were homogenized in 3 ml of ice-cold buffer A (in mM: 50 Tris, pH 7.8, 1 EDTA, and 1 PMSF), and the homogenate was centrifuged at 10,000 g for 15 min at 4°C. The microsome-enriched supernatant was harvested and centrifuged at 100,000 g for 60 min. The pellet was resuspended in 2 ml of buffer A and then centrifuged at 100,000 g for 45 min at 4°C, and the final pellet was suspended in the same buffer.

Protein concentration in this microsomal preparation was determined by means of the BCA protein assay kit (Pierce).

Two hundred microliters of the microsomal protein were incubated with 20 μl of the substrate mixture (0.25 nml fatty acid-free BSA and 0.25 nml [14C]oleoyl-CoA in 104 mM Tris, pH 7.8) in a total volume of 80 μl at 37°C for 5 min. At the conclusion of the incubation period, the reaction was stopped by addition of 1.5 ml of chloroform-methanol (2:1, vol/vol). Phase separation was accomplished by addition of 500 μl of water and 80 μg of cholesterol oleate, which served as internal standard for the thin-layer chromatography procedure. The chloroform layer containing the lipid fraction was dried under nitrogen, resuspended in 60 μl of ethyl acetate, and separated on silica gel (Sigma Chemical, St. Louis, MO) by use of a mixture of petroleum ether, anhydrous ether, and acetic acid (90:10:1) as the mobile phase. Thereafter, the cholesterol ester bands were visualized by iodine vapor staining. The bands were then removed and transferred to scintillation vials before counting. The specific activity of ACAT was expressed as picomoles of oleoyl-CoA converted to stearyl ester per minute per milligram protein.

Northern blot analysis. Total RNA was prepared from 0.2 g of frozen liver tissue with RNAzol by the manufacturer’s recommended procedure (Tel-Test, Friendswood, TX). RNA concentration was determined from the absorbance at 260 nm with a spectrophotometer (Gene-Quat, Bio-Rad, Hercules, CA). Twenty-microgram aliquots of total RNA were denatured in 2.2 M formaldehyde at 65°C for 15 min and run on 1% agarose-2.2 M formaldehyde gels at 40 V for 5 h. The separated RNA was transferred to the nylon membrane (Zeta probe, Bio-Rad) by capillary blotting in 6× SSC buffer (0.9 M NaCl and 0.09 M Na citrate, pH 7.0) overnight and immobilized by UV irradiation (Ultraviolette Crosslinker, Fisher Scientific, Pittsburgh, PA). The membrane was incubated at 65°C in a solution containing 5× sodium chloride-sodium phosphate-EDTA (SSPE; in mM: 0.75 NaCl, 0.05 NaH₂PO₄, 0.005 EDTA, pH 7.4), 5× Denhardt’s solution [Ficoll (type 400), polyvinylpyrrolidone and BSA, 1 g/l each], 1% SDS, and 100 μg/ml salmon sperm DNA for 2 h. The cDNA probes for monkey ACAT-2 (a 950-bp BamHI fragment) and ACAT-1 (a 1.7-Kb HindIII fragment) were generously supplied by Professor Lawrence L. Rudel, and rat GAPDH (1.3-Kb PstI fragment) was obtained from American Type Culture Collection (Rockville, MD). All probes were labeled with [32P]dCTP (3,000 Ci/mmol; New England Nuclear, Boston, MA) by the random primer method (Promega, Madison, WI). Hybridization was carried out at 65°C in a prehybridization solution with [32P]-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 min each. The washed blots were exposed to X-ray film (New England Nuclear) at −80°C for 8 h for GAPDH and 2 days for ACAT-1 and ACAT-2. 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.

Cholesterol measurements. Whole liver tissue extract was obtained by centrifugation of the homogenate at 2,000 g for 15 min at 4°C. Hepatic microsomes were isolated by centrifugation of the homogenate at 10,000 g for 15 min at 4°C, followed by centrifugation of the supernatant at 100,000 g for 60 min at 4°C. The resultant pellet was washed and centrifuged as described in ACAT activity assay. Total cholesterol concentration in the whole liver extract and microsomal preparations was determined by use of the Wako cholesterol CII kit. The corresponding free cholesterol concentrations.
were quantified by means of a Wako free cholesterol C kit, and esterified cholesterol was determined by subtracting free cholesterol from total cholesterol concentrations.

Data analysis. Student’s t-test and regression analysis were used in statistical evaluation of the data, which are presented as means ± SE. P values <0.05 were considered significant.

RESULTS

General data. Data are shown in Table 1. The CRF group exhibited a significant increase in plasma creatinine concentration and a significant reduction in creatinine clearance compared with the sham-operated control group. Compared with the control group, the CRF animals exhibited a significant rise in arterial blood pressure. The CRF group showed a significant increase in plasma triglyceride, total cholesterol, LDL cholesterol, and VLDL cholesterol concentrations. However, the plasma HDL cholesterol-to-total cholesterol concentration ratio was markedly reduced in the CRF group compared with the corresponding value found in the control group. Hepatic tissue-free cholesterol concentration and microsomal free cholesterol concentration values in the CRF group were significantly lower than those found in the control group. The whole liver and microsomal esterified cholesterol concentrations in the CRF group were comparable with those of the control group. No significant difference was found in either total microsomal cholesterol concentration or whole liver cholesterol concentration between the two groups.

ACAT data. Data are depicted in Figs. 1-5. The CRF group exhibited a 5.7-fold increase in liver ACAT-2 protein abundance compared with the normal control group. The observed upregulation of ACAT-2 protein abundance in the CRF group was associated with a 1.7-fold increase in hepatic ACAT-2 mRNA abundance. In contrast, liver ACAT-1 protein and ACAT-1 mRNA abundance values in the CRF group were similar to those found in the control group. Measurement of ACAT enzymatic activity revealed a 1.6-fold increase in the CRF group compared with that found in the control rats.

**DISCUSSION**

ACAT is a membrane-associated enzyme that is primarily localized in the endoplasmic reticulum. Its localization in the endoplasmic reticulum is consistent with its demonstrated role in cholesterol transport within the liver and intestinal mucosa. cDNA for ACAT was initially isolated from human genomic DNA by Chang et al. (10) and expressed in mutant Chinese hamster ovary cells lacking ACAT activity. An ACAT with a nearly identical cDNA sequence was subsequently found in other animals, namely, mice, hamsters, and rabbits (5, 22, 27). mRNA for this ACAT is frequently found in other animals, namely, mice, hamsters, and rabbits (5, 22, 27). mRNA for this ACAT gene in mice resulted in loss of ACAT activity in embryonic fibroblasts and marked reduction in cholesterol ester in peritoneal macrophages and adrenal cortex (19). However, cholesterol ester accumulation in

![Image](https://example.com/image.png)

**Table 1. Serum concentrations and kinetic measurement data of CRF and CTL groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>CTL</th>
<th>CRF</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol, mg/dl</td>
<td>72 ± 2</td>
<td>162.7 ± 10</td>
<td>≤0.003</td>
</tr>
<tr>
<td>HDL/cholesterol ratio</td>
<td>0.4 ± 0.02</td>
<td>0.2 ± 0.01</td>
<td>≤0.003</td>
</tr>
<tr>
<td>Serum triglycerides, mg/ml</td>
<td>50.1 ± 4.8</td>
<td>152.4 ± 24</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Ccr, ml/min</td>
<td>1.9 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>8.8 ± 1.0</td>
<td>35.2 ± 4.4</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>115 ± 5.6</td>
<td>153 ± 8.1</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>445 ± 7.6</td>
<td>407 ± 5.8</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Liver microsomal total choles¬terol, mg/g protein</td>
<td>27.1 ± 3.6</td>
<td>26.9 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Liver microsomal free choles¬terol, mg/g protein</td>
<td>14.1 ± 1.9</td>
<td>10.9 ± 1.1</td>
<td>≤0.006</td>
</tr>
<tr>
<td>Liver microsomal esterified cholesterol, mg/g protein</td>
<td>13.2 ± 2.7</td>
<td>16.8 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Liver total cholesterol, mg/g protein</td>
<td>32.3 ± 1.9</td>
<td>29.2 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Liver free cholesterol, mg/g protein</td>
<td>14.8 ± 0.9</td>
<td>11.9 ± 0.4</td>
<td>≤0.04</td>
</tr>
<tr>
<td>Liver esterified cholesterol, mg/g protein</td>
<td>17.6 ± 1.1</td>
<td>17.3 ± 0.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. CRF, chronic renal failure; CTL, control; Ccr, creatinine clearance; NS, not significant.

**Fig. 1.** Representative Western blot and group data depicting hepatic tissue acyl-CoA cholesterol transferase (ACAT)-2 protein abundance in rats with chronic renal failure (CRF) and sham-operated control animals (CTL). Values are means ± SE; n = 6 in each group. *P < 0.001.
hepatocytes and intestinal cholesterol absorption were normal in these animals (19), pointing to the presence of additional ACAT isotype(s) with different tissue distributions. In fact, recently, three separate groups simultaneously identified a second ACAT isotype, termed ACAT-2 (1, 7, 21). Oelkers et al. (21) isolated the full-length cDNA clone of ACAT-2 from the human hepatoma cell (HepG2) cDNA library. Cultured HepG2 cells and human enterocytes (CaCo2) expressed ACAT-2, but cultured endothelial cells (HeLa), monocytes (undifferentiated THP1), and macrophages (differentiated THP1) did not. Simultaneously, Cases et al. (7) identified the second mammalian ACAT enzyme, designated as ACAT-2, in the mouse liver. As expected, ACAT-2 was primarily expressed in the mouse liver and intestine and exhibited different IC50 values in response to different ACAT inhibitors compared with the original ACAT (7). As with the study of Oelkers et al., Cases et al. found abundant ACAT-2 expression in human hepatoma HepG2 cells and human intestinal cells, but not in human fibroblasts. Likewise, they found the highest ACAT-2 mRNA expression in mouse liver and small intestine, as well as in human liver (7). At the same time, Anderson et al. (1) identified two distinct forms of ACAT in the hepatocytes of the African green monkey. One of the two enzymes was similar to that originally cloned from human genomic DNA, which they termed ACAT-1 (10). The second ACAT

Fig. 2. Representative Northern blot and group data depicting hepatic ACAT-2 and GAPDH mRNA in animals of the CRF and CTL groups. Values are means ± SE; n = 6 animals in each group. *P < 0.01.

Fig. 4. Representative Northern blot and group data depicting hepatic ACAT-1 and GAPDH mRNA abundance in animals with CRF and CTL animals. No significant difference was found between the 2 groups. Values are means ± SE; n = 6 in each group.

ACAT-2, but cultured endothelial cells (HeLa), monocytes (undifferentiated THP1), and macrophages (differentiated THP1) did not. Simultaneously, Cases et al. (7) identified the second mammalian ACAT enzyme, designated as ACAT-2, in the mouse liver. As expected, ACAT-2 was primarily expressed in the mouse liver and intestine and exhibited different IC50 values in response to different ACAT inhibitors compared with the original ACAT (7). As with the study of Oelkers et al., Cases et al. found abundant ACAT-2 expression in human hepatoma HepG2 cells and human intestinal cells, but not in human fibroblasts. Likewise, they found the highest ACAT-2 mRNA expression in mouse liver and small intestine, as well as in human liver (7). At the same time, Anderson et al. (1) identified two distinct forms of ACAT in the hepatocytes of the African green monkey. One of the two enzymes was similar to that originally cloned from human genomic DNA, which they termed ACAT-1 (10). The second ACAT

Fig. 3. Representative Western blots and group data depicting hepatic ACAT-1 protein abundance in CRF and CTL animal groups. No significant difference was found between CRF and CTL groups. Values are means ± SE; n = 6 in each group.

Fig. 5. Hepatic microsomal ACAT activity in rats of CRF and CTL groups. Values are means ± SE; n = 6 in each group. *P < 0.001.
isoform, termed ACAT-2, was primarily expressed in the liver and intestine. Sequence analysis of ACAT-2 DNA identified a 562-amino acid protein (1).

The CRF animals employed in the present study exhibited a significant upregulation of ACAT-2 protein abundance and a significant increase in ACAT-2 mRNA abundance in the liver tissue. This observation suggests that elevation of ACAT-2 protein abundance in the CRF animals is primarily due to upregulation of hepatic ACAT-2 gene expression. To our knowledge, ours is the first study to demonstrate upregulation of ACAT in chronic renal failure. In a recent study (30a), we showed marked upregulation of hepatic ACAT-2 in rats with puromycin-induced nephrotic syndrome. The observed upregulation of ACAT-2 in the CRF animals found in the present study is not due to proteinuria, because, unlike nephrotic animals, heavy proteinuria was absent in the CRF groups.

In contrast to ACAT-2, hepatic tissue ACAT-1 protein and mRNA abundance in the CRF animals were virtually identical to those found in the sham-operated control rats. Thus CRF appears to specifically affect ACAT-2 but not ACAT-1 abundance in the liver. This was accompanied by a significant increase in hepatic tissue ACAT enzymatic activity.

Elevation of plasma VLDL is one of the most common features of CRF-associated dyslipidemia (2, 8, 16–18, 29, 30, 34). Esterification of cholesterol by ACAT isosforms has been shown to play an important role in production and secretion of apoB-containing lipoproteins, i.e., VLDL by the liver (6, 11, 14). Upregulation of hepatic ACAT-2 expression and ACAT enzymatic activity shown here can, therefore, contribute to elevation of plasma VLDL by compounding the effect of CRF-induced deficiencies of lipoprotein lipase, hepatic triglyceride lipase, and VLDL receptor, which impair VLDL clearance (16, 17, 28–30, 32, 34).

In conclusion, CRF results in significant upregulation of hepatic ACAT-2 (but not ACAT-1) abundance and ACAT enzymatic activity. Upregulation of hepatic ACAT-2 expression and ACAT activity, shown for the first time here, can potentially contribute to dysregulation of lipid metabolism and the atherogenic diathesis in chronic renal insufficiency.

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REFERENCES


