HMG-CoA reductase inhibition reverses LCAT and LDL receptor deficiencies and improves HDL in rats with chronic renal failure

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Liang, K., C. H. Kim, and N. D. Vaziri. HMG-CoA reductase inhibition reverses LCAT and LDL receptor deficiencies and improves HDL in rats with chronic renal failure. Am J Physiol Renal Physiol 288: F539–F544, 2005. First published October 26, 2004; doi:10.1152/ajprenal.00074.2004.—Dyslipidemia is a prominent feature of chronic renal failure (CRF) and a major risk factor for atherosclerosis and the progression of renal disease. CRF-induced dyslipidemia is marked by hypertriglyceridemia and a shift in plasma cholesterol from HDL to the ApoB-containing lipoproteins. Several studies have demonstrated a favorable response to administration of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors (statins) in CRF. This study was intended to explore the effect of statin therapy on key enzymes and receptors involved in cholesterol metabolism. Accordingly, CRF (% nephrectomized) and sham-operated rats were randomized to untreated and statin-treated (rosuvastatin 20 mg·kg⁻¹·day⁻¹) groups and observed for 6 wk. The untreated CRF rats exhibited increased total cholesterol-to-HDL cholesterol ratio, diminished plasma lecithin:cholesterol acyltransferase (LCAT) and LDL receptor, significant reductions of total cholesterol-to-HDL cholesterol, hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, or HDL receptor (SRB-1). Statin administration lowered HMG-CoA reductase activity, normalized plasma LCAT, total cholesterol-to-HDL cholesterol ratio, and hepatic LDL receptor but did not significantly change either plasma total cholesterol, hepatic cholesterol 7α-hydroxylase, total ACAT activity, or SRB-1 in the CRF animals. Statin administration to the normal control rats led to significant increases in plasma LCAT and hepatic LDL receptor, significant reductions of total cholesterol-to-HDL cholesterol ratio, hepatic HMG-CoA reductase activity, and cholesterol 7α-hydroxylase abundance with virtually no change in plasma cholesterol concentration. Thus administration of rosuvastatin reversed LCAT and LDL receptor deficiencies and promoted a shift in plasma cholesterol from ApoB-containing lipoproteins to HDL in CRF rats.

3-hydroxy-3-methylglutaryl; atherosclerosis; lipid disorders; renal disease; SRB-1; bile acid; cholesterol; triglyceride

CHRONIC RENAL FAILURE (CRF) results in profound abnormalities of lipid metabolism and marked alteration of plasma lipid profile. The associated dyslipidemia contributes to the atherogenic diathesis and progression of renal insufficiency in patients with chronic kidney disease (3, 7, 12, 28). The typical features of CRF-induced dyslipidemia include hypertriglyceridemia; elevated level and impaired clearance of VLDL, IDL, and LDL; inappropriately reduced HDL cholesterol; and impaired maturation of cholesterol-poor HDL-3 to cardioprotective cholesterol ester-rich HDL-2 (3, 7, 12, 28). The primary underlying mechanism of hypertriglyceridemia and elevation of plasma triglyceride-rich lipoproteins (VLDL, IDL, and chy-
Cheshire, UK) mixed in powdered rat chow. The treatment was continued for 6 wk. The untreated CRF and sham-operated control groups received drug-free powdered food. Six animals were included in each group.

At the conclusion of the treatment period, the animals were placed in metabolic cages for a 24-h urine collection. They were then killed by exsanguination via cardiac puncture between the hours of 9 and 11 AM. The liver was removed, immediately frozen in liquid nitrogen, and stored at −70°C until processed. Serum cholesterol, triglyceride, LDL, VLDL, and creatinine concentrations and urinary protein and creatinine were determined as described previously (22). Plasma HDL cholesterol was measured in the supernatant of plasma after precipitation of LDL and VLDL using dextran sulfate (0.91 mg/ml), and HDL-3 cholesterol was measured after precipitation of HDL-2, LDL, and VLDL using dextran sulfate (1.74 mg/ml) as described by Warnick and Cheung (38). After centrifugation at 1,500 g for 30 min, supernatants were collected and analyzed for total HDL and HDL-3 cholesterol using a cholesterol reagent kit (Pointe Scientific). The liver was removed, immediately frozen in liquid nitrogen, and stored at −70°C until used.

Measurement of LDL receptor protein. LDL receptor protein abundance in the liver tissue was measured by Western blot analysis using a mouse antibovine LDL receptor antibody (Cortex Biochem, Davis, CA) as described in our previous study (29). Hepatic tissue HDL receptor protein abundance was determined by enzyme-linked immunosorbent assay (ELISA) using a rabbit anti-human HDL receptor antibody (generously provided by Dr. John S. Parks, Wake Forest University, Winston-Salem, NC) as described in our earlier studies (34).

Acyl-CoA:cholesterol acyltransferase measurements. Acyl-CoA:cholesterol acyltransferase (ACAT)-2 protein abundance in the liver was determined by Western blot analysis using a polyclonal ACAT-2 antibody (generously provided by Dr. Lawrence Rudel, Wake Forest University) as described previously (33). Hepatic microsomal ACAT activity was quantified in a manner identical to that described in our earlier studies (33).

Data analysis. ANOVA and a multiple range test were used in the statistical evaluation of the data, which are shown as means ± SE. P values <0.05 were considered significant.

RESULTS

General data. Data are summarized in Table 1. The untreated CRF animals exhibited a significant reduction in creatinine clearance, mild hypoalbuminemia, severe hypertriglyceridemia, marked elevation of plasma VLDL, moderate elevation of plasma total cholesterol and LDL cholesterol concentrations, and a significant increase in plasma total cholesterol-to-HDL cholesterol ratio. Statin administration resulted in significant improvements in plasma VLDL, LDL, and triglyceride concentrations, marked elevation of plasma HDL cholesterol, normalization of plasma total cholesterol-to-HDL cholesterol ratio, with virtually no change in plasma total cholesterol concentration in the treated compared with the untreated CRF rats. In the sham-operated rats, statin administration resulted in no significant change in serum lipid levels except for a mild rise in HDL cholesterol and a modest decline in plasma total cholesterol-to-HDL cholesterol ratio. Statin administration resulted in a significant rise in both HDL-2 and HDL-3 cholesterol in the CRF group. However, the magnitude of the rise in HDL-2 cholesterol was greater than that of HDL-3 cholesterol concentration in the CRF animals. In contrast, the impact of statin therapy on cholesterol contents of HDL-2 and HDL-3 in the normal controls was less conspicuous than that seen in the CRF animals.

Body weight in the CRF groups was lower than that in the sham-operated controls. No significant difference was found in

### Table 1. General data for the sham-operated control group, untreated rats with chronic renal failure, and 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor-treated control and chronic renal failure rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol, mg/dl</th>
<th>LDL cholesterol, mg/dl</th>
<th>VLDL cholesterol, mg/dl</th>
<th>HDL cholesterol, mg/dl</th>
<th>HDL-2 cholesterol, mg/dl</th>
<th>HDL-3 cholesterol, mg/dl</th>
<th>Total/HDL cholesterol ratio</th>
<th>Triglycerides, mg/dl</th>
<th>Albumin, g/dl</th>
<th>Creatinine, m/l/d</th>
<th>Ccr, ml/24 h</th>
<th>Urine protein, mg/24 h</th>
<th>Body wt, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>59 ± 2.2</td>
<td>18.7 ± 2.7</td>
<td>9.3 ± 0.9</td>
<td>23 ± 2.4</td>
<td>10 ± 2.6</td>
<td>13 ± 0.8</td>
<td>2.7 ± 0.3</td>
<td>46.6 ± 4.5</td>
<td>3.8 ± 0.1</td>
<td>0.38 ± 0.01</td>
<td>2.6 ± 0.2</td>
<td>10.6 ± 1.3</td>
<td>365 ± 25</td>
</tr>
<tr>
<td>CRF</td>
<td>71 ± 3.2</td>
<td>21.1 ± 1.6</td>
<td>9.4 ± 0.8</td>
<td>26 ± 2.3</td>
<td>11 ± 0.9</td>
<td>15 ± 1.2</td>
<td>2.1 ± 0.2*</td>
<td>47.0 ± 4.1</td>
<td>3.7 ± 0.2</td>
<td>0.40 ± 0.03</td>
<td>2.5 ± 0.2</td>
<td>10.7 ± 2.3</td>
<td>348 ± 28</td>
</tr>
<tr>
<td>CRF-HI</td>
<td>124 ± 11*</td>
<td>69 ± 10*</td>
<td>33 ± 3.4*</td>
<td>36 ± 4.2*</td>
<td>19 ± 3.7*</td>
<td>17 ± 3.3</td>
<td>3.7 ± 0.5*</td>
<td>166 ± 17*</td>
<td>3.1 ± 0.06</td>
<td>1.3 ± 0.2*</td>
<td>0.56 ± 0.09*</td>
<td>72 ± 7.1*</td>
<td>339 ± 18*</td>
</tr>
<tr>
<td>CRF-HI</td>
<td>124 ± 2.3*</td>
<td>43 ± 8*†</td>
<td>19 ± 1.9†</td>
<td>63 ± 6.2†</td>
<td>36 ± 1.5†</td>
<td>27 ± 0.7†</td>
<td>2.1 ± 0.2*</td>
<td>94 ± 9.3*</td>
<td>3.1 ± 0.2</td>
<td>0.9 ± 0.08*</td>
<td>0.98 ± 0.07†</td>
<td>63 ± 6.7*</td>
<td>283 ± 21†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. CTL, sham-operated control; CRF, chronic renal failure; CTL-HI, HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA reductase-treated control; CRF-HI, HMG-CoA-treated CRF rate; Ccr, creatinine clearance. *P < 0.05 vs. CTL. †P < 0.05 vs. CRF.
body weight among the statin-treated and untreated control groups. However, body weight in the statin-treated CRF group was significantly lower than that in the untreated CRF group.

**Hepatic HMG-CoA reductase.** Data are depicted in Fig. 1. Hepatic HMG-CoA reductase protein abundance and microsomal HMG-CoA reductase activity in the untreated CRF rats were unchanged compared with the corresponding values found in the sham-operated control animals. Statin administration resulted in significant reduction of hepatic HMG-CoA reductase activity without significant change in the abundance of the immundetectable enzyme. Similarly, statin therapy led to a significant fall in HMG-CoA reductase activity with no significant change in its protein abundance in the treated controls.

**LDL and HDL receptors.** Data are given in Fig. 2. Hepatic tissue LDL receptor protein abundance in the untreated CRF animals was significantly lower than that found in the sham-operated control rats. Statin administration led to normalization of liver tissue LDL receptor in the treated CRF group. No significant difference was found in SRB-1 abundance among the three groups. Statin therapy raised hepatic LDL receptor but did not affect HDL receptor abundance in the liver of sham-operated controls.

**Plasma LCAT concentration.** Data are shown in Fig. 3. In confirmation of our previous studies (34), plasma LCAT concentration was significantly reduced in the untreated CRF rats compared with that in the control group. Plasma LCAT concentration was fully normalized in the statin-treated CRF group. A mild but significant rise was observed in plasma LCAT level in the statin-treated controls.

**ACAT.** Data are illustrated in Fig. 4. In confirmation of our recent studies (23), hepatic ACAT-2 protein abundance and microsomal ACAT enzymatic activity were significantly elevated in the untreated CRF group compared with the corresponding values in the control animals. Both ACAT-2 protein abundance and enzymatic activity remained significantly elevated in the statin-treated CRF group. In the treated subgroup of sham-operated control rats, statin administration resulted in a mild reduction in hepatic ACAT activity.

**Cholesterol 7α-hydroxylase.** Data are shown in Fig. 5. No significant difference was found in hepatic tissue cholesterol 7α-hydroxylase abundance between the untreated CRF rats and the sham-operated control animals. Although the mean value of the enzyme abundance was lower in the statin-treated CRF animals, the difference did not reach statistical significance. However, statin administration led to a significant fall in the enzyme abundance among the treated controls.

**DISCUSSION**

Cholesterol homeostasis in the peripheral tissues is primarily dependent on the balance between receptor-mediated cholesterol influx via ApoB-containing lipoproteins and HDL-mediated efflux of surplus cholesterol (10). Accordingly, the ratio of cholesterol in the ApoB-containing lipoproteins to that in HDL serves as a useful indicator of cholesterol homeostasis in the artery wall, glomerular mesangium, and a number of other tissues. The untreated CRF animals employed in the present study exhibited a significant increase in plasma total cholesterol-to-HDL cholesterol ratio, representing an atherogenic profile. This was associated with a severe reduction in plasma LCAT concentration, confirming the results of earlier studies.
in rats and humans with CRF (28, 34). LCAT deficiency can account for impaired HDL maturation, defective reverse cholesterol transport, and abnormal total cholesterol-to-HDL cholesterol ratio in patients and animals with CRF. Statin administration for 6 wk reversed CRF-induced LCAT deficiency. Correction of LCAT deficiency was associated with, and most likely responsible for, the marked rise in plasma HDL cholesterol concentration observed in the statin-treated CRF animals.

Long-term statin administration in the CRF rats resulted in a significant decline in plasma VLDL and LDL cholesterol concentrations. This phenomenon can be attributed to diminished cholesterol biosynthesis occasioned by the observed inhibition of hepatic HMG-CoA reductase activity and enhanced LDL clearance occasioned by the reversal of hepatic LDL receptor deficiency. The decline in plasma VLDL and LDL cholesterol concentrations in the statin-treated CRF rats was matched by a marked rise in plasma HDL cholesterol concentration. Consequently, plasma total cholesterol concentration remained virtually unchanged and total cholesterol-to-HDL cholesterol concentration ratio was normalized. It should be noted that, unlike humans, rats lack CETP (27). This protein mediates the exchange of cholesterol ester contained in HDL-2 for triglyceride contained in IDL within the circulation. The absence of CETP in the rat and its presence in humans are responsible for the relatively higher level of HDL cholesterol in the rat plasma than that in the human plasma. Accordingly, due to the CETP-mediated transport of cholesterol from HDL to ApoB-containing lipoproteins, the magnitude of the rise in HDL cholesterol and the corresponding fall in total cholesterol-to-HDL cholesterol ratio in response to inhibition of HMG-CoA reductase may be less striking in humans with CRF than in the rats with CRF.

Despite the inhibition of HMG-CoA reductase activity, the expression of HMG-CoA reductase and cholesterol 7α-hydroxylase (the rate-limiting enzymes in cholesterol biosynthesis and catabolism, respectively) remained unchanged in the CRF animals. This phenomenon is most likely due to enhanced hepatic cholesterol uptake from the circulation, occasioned by reversal of LDL receptor and LCAT deficiencies. Consequently, the resultant improvements in hepatic LDL uptake and HDL-mediated reverse cholesterol transport must have precluded the compensatory upregulation of HMG-CoA reductase.

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Fig. 3. Plasma lecithin:cholesterol acyltransferase (LCAT) concentration in CRF, CRF-HI, CTL, and CTL-HI groups; n = 6/group. *P < 0.01 vs. other group(s).

Fig. 4. Representative Western blot and group data depicting hepatic acyl-CoA:cholesterol acyltransferase (ACAT)-2 protein abundance and microsomal ACAT activity in CRF, CRF-HI, CTL, and CTL-HI groups; n = 6/group. *P < 0.01 vs. CTL group. P < 0.05 vs. CRF group.

Fig. 5. Representative Western blot and group data depicting hepatic cholesterol 7α-hydroxylase (CH-7α) protein abundance in CRF, CRF-HI, CTL, and CTL-HI groups; n = 6/group. *P < 0.05.
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and downregulation of cholesterol 7α-hydroxylase in response to the statin-induced reduction of cholesterol biosynthesis in the hepatocytes of these animals.

ACAT catalyzes intracellular esterification of cholesterol and as such is involved in intestinal absorption of cholesterol, hepatic assembly of ApoB-containing lipoproteins, foam cell formation, and atherogenesis (2, 5, 6, 8, 9, 16, 26). In confirmation of our earlier studies (23), the CRF rats employed here showed a marked upregulation of ACAT activity and liver-specific ACAT-2 protein, which remained elevated despite statin administration.

SRB-1 serves as a docking platform for unloading the HDL lipid cargo in the hepatocyte (1) and as such represents a critical link in reverse cholesterol transport. Hepatic SRB-1 protein abundance was unchanged in the liver of CRF animals and was not affected by statin administration.

In the sham-operated control rats, statin administration led to significant rises in plasma LCAT and the hepatic LDL receptor. This was coupled with a significant fall in cholesterol and was not affected by statin administration.

The body weight of the statin-treated CRF rats was significantly below that found in the untreated CRF animals. In contrast, the drug did not significantly affect body weight in the treated control rats. The reason for the observed difference in weight between the statin-treated CRF and control rats is not clear and warrants further studies of the effect of CRF on metabolism of the statin used here and other statins and the possible need for dosage adjustment. The lower body weight and food intake in the statin-treated CRF rats may have contributed, in part, to the observed changes in lipoprotein metabolism in these animals. It should be noted, however, that we have observed significant improvements in the lipoprotein profile, as well as hepatic expression, of the LDL receptor, HDL receptor, and plasma LCAT in rats with nephrotic syndrome (and normal creatinine clearance) with administration of the same statin preparation, despite there being no significant change in body weight (unpublished data).

In conclusion, long-term inhibition of HMG-CoA reductase reversed LCAT and LDL receptor deficiencies and improved the plasma lipid profile in rats with renal mass reduction.

GRANTS

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REFERENCES