*Kidney International, Vol. 64 (2003), pp. 192–198*



# HMG-CoA reductase, cholesterol 7α-hydroxylase, LCAT, ACAT, LDL receptor, and SRB-1 in hereditary analbuminemia

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**HMG-CoA reductase, cholesterol 7α-hydroxylase, LCAT, ACAT, LDL receptor, and SRB-1 in hereditary analbuminemia.**

*Background.* Hereditary analbuminemia is associated with hypercholesterolemia, which has been shown to be primarily caused by increased extrahepatic production of cholesterol. Nagase rats with hereditary analbuminemia (NAR) have been used as a model to dissect the effect of primary hypoalbuminemia from that caused by proteinuria in nephrotic syndrome. The present study was undertaken to explore the effect of hereditary analbuminemia on protein expression of the key factors involved in cholesterol metabolism.

*Methods.* Hepatic tissue protein abundance of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase (a rate-limiting enzyme in cholesterol catabolism), low density lipoprotein (LDL) receptor, high density lipoprotein (HDL) receptor (SRB-1), acyl-coA cholesterol acyltransferase-2 (ACAT-2), and plasma concentration of lecithin cholesterol acyltransferase (LCAT), as well as HMG-CoA reductase, ACAT, and LCAT activities were determined in fasting male NAR and Sprague-Dawley control rats.

*Results.* The NAR group exhibited significant up-regulation of HMG-CoA reductase protein abundance but normal HMG-CoA reductase enzymatic activity. This was coupled with a significant up-regulation of cholesterol 7α-hydroxylase and a mild up-regulation of ACAT protein abundance and activity. However, hepatic LDL receptor and HDL receptor and plasma LCAT protein concentration and activity were normal in NAR.

*Conclusion.* Hypercholesterolemia in NAR is associated with elevated hepatic HMG-CoA reductase protein abundance, but normal HMG-CoA reductase activity. These findings point to post-translational regulation of this enzyme and favor an extrahepatic origin of hypercholesterolemia in NAR. The observed up-regulation of cholesterol 7α-hydroxylase represents a compensatory response to the associated hypercholesterolemia. Unlike nephrotic syndrome, which causes severe LDL receptor, HDL receptor, and LCAT deficiencies, hereditary analbuminemia does not affect these proteins.

**Key words:** Nagase rats, nephrotic syndrome, hypoalbuminemia, hyperlipidemia, hypercholesterolemia, HMG-CoA reductase, LCAT, ACAT, LDL receptor, HDL, bile acid.

Received for publication October 4, 2002
and in revised form December 28, 2002, and January 28, 2003
Accepted for publication February 18, 2003



Nagase analbuminemic rats (NAR) were derived from Sprague Dawley strain by Sumi Nagase in 1979 [1]. Analbuminemia in the Nagase rats is caused by a defective albumin mRNA processing, resulting in exon skipping and a virtual lack of albumin gene expression [2, 3].

Hereditary analbuminemia is associated with hypercholesterolemia and hypertriglyceridemia in both rats [4, 5] and humans [6]. This is accompanied by significant elevations of plasma Apo A1, Apo B, and Apo E concentrations [7]. While less severe, alterations of plasma lipids and apoprotein profile in hereditary analbuminemia generally resemble those seen in nephrotic syndrome, in which hypoalbuminemia is caused by albuminuria. The fact that the common denominator in both conditions is hypoalbuminemia and depressed plasma oncotic pressure suggests that increased lipid and apoprotein synthesis may be, in part, mediated by hypoalbuminemia, as proposed by Marsh and Drabkin [8].

The liver plays a central role in regulation of cholesterol metabolism. The contribution of the liver to cholesterol metabolism is multifaceted and includes its role in biosynthesis of cholesterol and cholesterol-carrying apoproteins; catabolism of cholesterol to bile acids for disposal in the intestinal tract; receptor-mediated clearance of cholesterol-containing lipoproteins [low density lipoprotein (LDL), high density lipoprotein (HDL), and intermediate density lipoprotein (IDL)]; and intracellular and extracellular esterification of cholesterol. Several major proteins are involved in the above mentioned processes, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is the rate-limiting step in cholesterol synthesis; cholesterol 7α-hydroxylase, the rate-controlling enzyme in cholesterol catabolism to bile acids; LDL receptor; HDL receptor, otherwise known as scavenger receptor class B type 1 (SRB-1); acyl-CoA cholesterol acyltransferase (ACAT); and lecithin cholesterol acyltransferase (LCAT).

While hepatic expression of various apoproteins in hereditary analbuminemia has been previously investi-

gated [5, 7], hepatic production of the majority of the enzymes and receptors listed above has not been explored. The present study was therefore undertaken to determine immunodetectable abundance of HMG-CoA reductase, cholesterol 7α-hydroxylase, LDL receptor, HDL receptor, ACAT, and LCAT in the NAR and in normal Sprague-Dawley controls. In addition, enzymatic activities of HMG-CoA reductase, ACAT, and LCAT were determined.

## METHODS

### Animals

Male Nagase rats (SLC, Inc., Hamamatsu, Japan) and age-matched male Sprague Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA) with an average body weight of 180 g were employed in the study. The animals were housed in a climate-controlled, light-regulated space with 12-hour dark-light cycles. They were provided access to regular rat chow (Purina Mills, Inc., Brentwood, MO, USA) and water ad libitum. The animals were maintained for 4 weeks, at which point they were placed in individual metabolic cages for a 24-hour urine collection. Animals were pair-fed during the last week of the observation period. No food was provided after 5 P.M. the day before sacrifice. However, water was provided at all times. The animals were subsequently anesthetized with pentobarbitol 50 mg intraperitoneally and euthanized by exsanguination using cardiac puncture between the hours of 9 and 11 A.M. The liver was promptly removed and immediately frozen in liquid nitrogen and stored at −70°C until processing. In addition, plasma was separated and stored at −70°C. Six animals were included in each group. The protocol employed in the study was approved by the institutional animal care and use committee of the University of California, Irvine.

### Miscellaneous assays

Urine protein concentration was determined by a quantitative colorimetric assay using a kit purchased from Sigma Chemical Company (St. Louis, MO, USA). Serum albumin concentration was quantified by bromocresol green method employing a kit purchased from Wako Chemicals USA, Inc. (Richmond, VA, USA). A colorimetric assay (Sigma Chemical Company) was used to measure serum and urine creatinine concentrations. Plasma concentrations of total cholesterol and hepatic tissue free cholesterol were measured by enzymatic colorimetric assays using kits supplied by Wako Chemicals USA, Inc. Plasma HDL and LDL cholesterol concentrations and triglyceride levels were determined by kits purchased from Sigma Chemical Company.

### HMG-CoA reductase protein determination

Frozen rat liver tissue was homogenized in 1 mL of 20 mmol Tris-HCl (pH 7.5) buffer containing 2 μm $MgCl_2$, 0.2 mol/L sucrose, 5 μm phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin, 10 μg/mL aprotinin, and 3 μg/mL pepstatin A. The crude extract was centrifuged at 12,000*g* at 4°C for 10 minutes to remove tissue debris. The supernatant was processed for determination of HMG-CoA reductase. Protein concentration of the supernatant was determined with a bicinchoninic acid (BCA) protein assay kit (Pierce Inc., Rockford, IL, USA). HMG-CoA reductase protein abundance in the liver extract was quantified by Western blot analysis as described by Ness et al [9]. Briefly, aliquots containing 100 μg proteins were fractionated on 4-20% Tris-glycine gel (Novex, Inc., San Diego, CA, USA) at 120 V for 2 hours. After electrophoresis, proteins were transferred to Hybond-ECL membrane (Amersham Life Science, Inc., Arlington Heights, IL, USA). The membrane was incubated for 1 hour in blocking buffer [1 × tris-buffered saline (TBS), 0.1% Tween 20, and 7% nonfat milk] and then overnight in the same buffer containing 1:7500 polyclonal anti HMG-CoA reductase antibody (generously provided by Professor Ness GC, Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, FL, USA). The membrane was washed four times for 10 minutes in 1 × TBS, 0.1% Tween 20 prior to a 2-hour incubation in blocking buffer plus diluted (1:10,000) horseradish peroxidase–linked anti-rabbit immunoglobulin (Ig)G (Amersham Life Science, Inc., Arlington Heights, IL, USA). The washes were repeated before the membranes were developed with chemiluminescent agents (ECL; Amersham Life Science, Inc.) and subjected to autoluminography for 3 minutes.

### Determination of cholesterol 7α-hydroxylase protein

Cholesterol 7α-hydroxylase protein abundance in the liver tissue preparation was determined by Western blot analysis using a rabbit anti-rat cholesterol 7α-hydroxylase antibody as described in our earlier studies [10]. The antibody employed in this assay was generously provided by Professor John Y.L. Chiang (Northeastern Ohio University College of Medicine).

### Determination of LDL receptor protein

Hepatic LDL receptor protein abundance was determined in the plasma membrane preparation by Western blot analysis using a mouse antibovine LDL receptor antibody (Cortex Biochem, Inc., Davis, CA, USA) as described in our previous study [11].

### Determination of ACAT-2 protein

Hepatic acyl-coA cholesterol acyltransferase-2 (ACAT-2) protein abundance was quantified by Western analysis using a polyclonal antibody against ACAT-2 as described in our recent study [12]. The antibody employed in these experiments was a generous gift from Professor Lawrence L. Rudel, Department of Biochemistry and Com-

parative Medicine, Wake Forest University, Winston-Salem, NC.

### Determination of plasma LCAT protein

Plasma lecithin cholesterol acyltransferase (LCAT) protein concentration was measured in triplicate by enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human LCAT antibody as described by Wang et al [13]. This antibody was generously supplied by Professor John S. Parks, Wake Forest University, Winston-Salem, NC. Briefly, 10 μL of plasma were diluted in binding buffer (1 × TBS, 0.05% NaN3, 200 μL final volume) and incubated at 4°C overnight in microtiter plates. The wells were washed 3 times with washing buffer (1 × TBS and 0.05% Tween 20), blocked for 3 hours at room temperature with blocking buffer [0.5% bovine serum albumin (BSA) and 0.05% Tween 20 in 1 × TBS), and washed. A 1:2000 dilution of rabbit anti-human LCAT antibody in blocking buffer were added to the well and incubated at room temperature for 3 hours. The plates were washed and incubated with 1:2000 anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (Sigma Chemical Co.), developed with 1-Step™ Turbo-TMB-ELISA kit (Pierce, Inc.), and read at 450 nm after the color developed. Purified human plasma LCAT was used as standard for the assay.

### Determination of HDL receptor (SRB-1)

SRB-1 protein abundance in the plasma membrane preparation of the liver was quantified by Western analysis using a polyclonal antibody against SRB-1 (Novus Biological, Inc., Littleton, CO, USA) as described in our previous study [14].

### Determination of enzymatic activities

Enzymatic activities of hepatic HMG-CoA reductase and ACAT, as well as plasma LCAT were determined by use of methods that were described in our earlier studies [12, 15, 16].

### Data analysis

Student *t* test and regression analysis were used in statistical analysis of the data, which are presented as mean ± SE. *P* values less than 0.05 were considered significant.

## RESULTS

### General data

Data are shown in Table 1. As expected, serum albumin concentration in the NAR group was severely reduced compared with the normal control group. Urinary protein excretion in the NAR group was minimal and virtually identical to that in the control group. Likewise, creatinine clearance was similar in the two groups. The NAR group showed significantly higher serum total cholesterol and triglyceride levels compared to the corresponding values in the control group. Likewise, LDL-cholesterol and very low density lipoprotein (VLDL) cholesterol levels were higher in the NAR group than in the control animals. However, HDL/total cholesterol ratios were similar in the two groups. Liver tissue free cholesterol concentration in the NAR group was two-fold greater than that in the normal control group.

### HMG-CoA reductase data

As illustrated in Figure 1, hepatic tissue HMG-CoA reductase protein abundance in the NAR group was two-fold greater than that found in the control group. In contrast, hepatic HMG-CoA reductase activity in the NAR group was similar to that found in the control group. These findings point to posttranslational regulation of this enzyme.

### Cholesterol 7α-hydroxylase data

Results are shown in Figure 2. The NAR group showed a significant up-regulation of hepatic cholesterol 7α-hydroxylase when compared with that found in the control group. A significant correlation was found between liver tissue cholesterol 7α-hydroxylase abundance and liver-free cholesterol concentration ($r = 0.74$, $P < 0.05$).

### LDL-receptor and SRB-1 data

Data depicted in Figures 3 and 4 illustrate that no significant difference was found in hepatic tissue LDL receptor protein abundance between the NAR and the control groups. Likewise, liver tissue abundance of SRB-1, otherwise known as HDL receptor, was virtually identical in the two groups.

### ACAT data

As presented in Figure 5, liver tissue ACAT-2 protein abundance and ACAT enzymatic activity were mildly elevated in the NAR rats as compared to that found in the Sprague Dawley control rats.

### Plasma LCAT data

Data are shown in Figure 6. No significant difference was found in plasma LCAT protein abundance between the NAR and Sprague Dawley control rats. Likewise, plasma LCAT enzymatic activity was similar in the two groups.

## DISCUSSION

The male NAR rats employed in the present study exhibited a significant increase in hepatic tissue HMG-CoA reductase protein abundance. However, HMG-CoA reductase enzymatic activity was normal in the NAR group. The observed discordance between hepatic

**Table 1.** Serum concentrations of total cholesterol, triglycerides, VLDL-cholesterol and albumin, serum HDL-chol/total cholesterol ratio, creatinine clearance, urinary protein excretion, and liver tissue free cholesterol content in male Sprague-Dawley control and Nagase analbuminemic rats (NAR)

| Groups | Control ($N = 6$) | NAR ($N = 6$) | $P$ value |
|---|---|---|---|
| Serum cholesterol *mg/dL* | 67.4 ± 3.9 | 112.7 ± 3.5 | <0.01 |
| Serum LDL *mg/dL* | 36.9 ± 4.0 | 52.5 ± 3.8 | <0.01 |
| Serum VLDL *mg/dL* | 10.4 ± 1.5 | 23.6 ± 2.7 | <0.01 |
| HDL/cholesterol (ratio) | 0.3 ± 0.05 | 0.3 ± 0.06 | NS |
| Serum triglycerides *mg/mL* | 50.8 ± 6.5 | 81.4 ± 7.9 | <0.01 |
| Liver free cholesterol *mg/g protein* | 16.1 ± 0.6 | 31.3 ± 1.2 | <0.01 |
| Serum albumin *g/dL* | 3.9 ± 0.05 | 0.13 ± 0.02 | <0.001 |
| Creatinine clearance *mL/min* | 1.2 ± 0.1 | 1.3 ± 0.1 | NS |
| Urine protein *mg/24 hr* | 7.7 ± 1.0 | 8.2 ± 1.0 | NS |

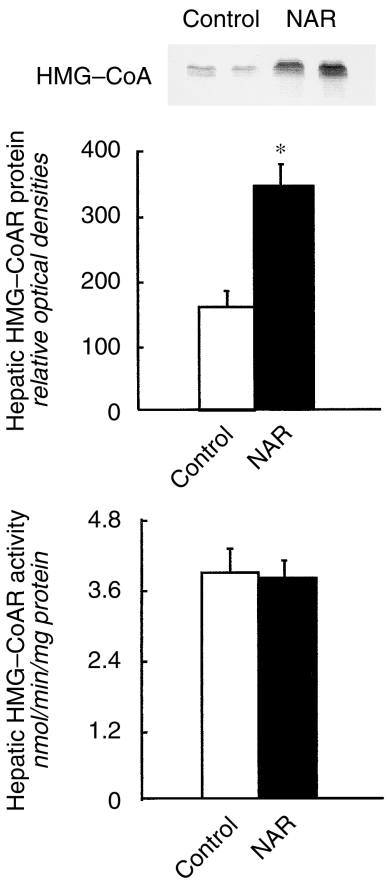


**Fig. 1. Representative Western blot and group data depicting HMG-CoA reductase protein abundance and enzymatic activity in the liver of Nagase rats with inherited analbuminemia (NAR) and Sprague Dawley control (CTL) rats.** $N = 6$ in each group; $^*P < 0.01$.

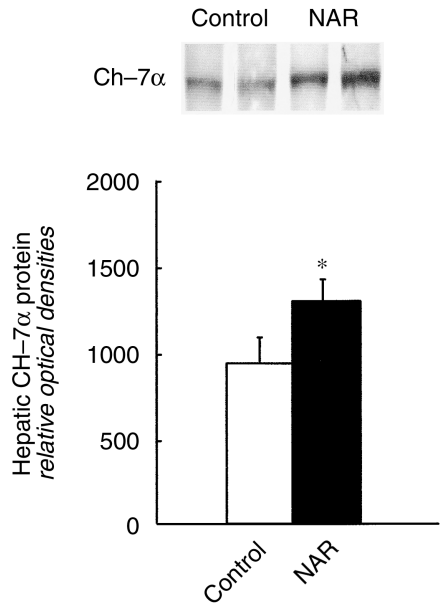


**Fig. 2. Representative Western blot and group data depicting cholesterol 7α-hydroxylase (CH-7α) protein abundance in the liver of Nagase rats with inherited analbuminemia (NAR) and Sprague Dawley control (CTL) rats.** $N = 6$ in each group; $^*P < 0.05$.

HMG-CoA reductase protein abundance and its enzymatic activity points to posttranslational regulation of this enzyme. HMG-CoA reductase is an endoplasmic reticulum–bound enzyme that is the rate-controlling step in cholesterol biosynthesis. While this enzyme is present in all tissues, it is most abundantly expressed in the liver, which plays a central part in regulation of plasma cholesterol concentration [17].

Earlier studies by our group demonstrated up-regulation of HMG-CoA reductase mRNA, enzymatic activity, and immunoreactive protein in the liver of rats with puromycin-induced nephrotic syndrome [15] and in the Imai rats with spontaneous focal glomerulosclerosis [18]. However, up-regulation of hepatic HMG-CoA reductase protein expression in our rats with hereditary analbuminemia was accompanied by normal HMG-CoA reductase activity. Using tritiated water incorporation, Joles et al [19] demonstrated a fourfold increase in $^3H$ incorporation into plasma cholesterol in female Nagase rats, which exhibit a more severe hyperlipidemia than their male counterpart. This was associated with a much greater increase in the rate of biosynthesis of cholesterol and its main precursors in the carcass than in the liver of their female NAR. These observations clearly indicate

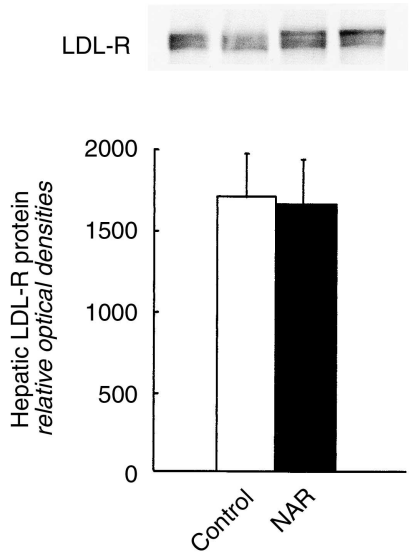


**Fig. 3. Representative Western blot and group data depicting low-density lipoprotein (LDL) receptor protein abundance in the liver of Nagase rats with inherited analbuminemia (NAR) and Sprague Dawley control (CTL) rats.** $N = 6$ in each group.

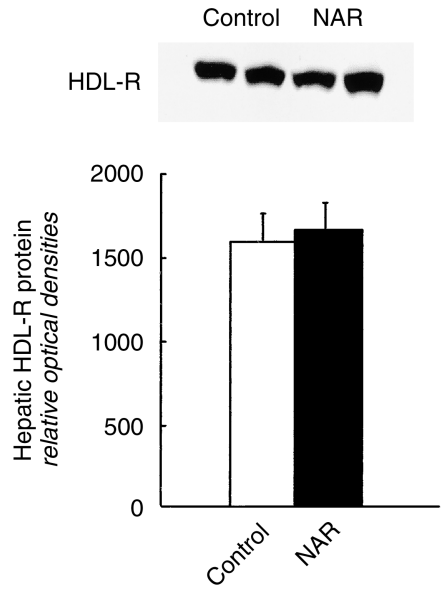


**Fig. 4. Representative Western blot and group data depicting high-density lipoprotein (HDL) receptor (SRB-1) protein abundance in the liver of Nagase rats with inherited analbuminemia (NAR) and Sprague Dawley control (CTL) rats.** $N = 6$ in each group.

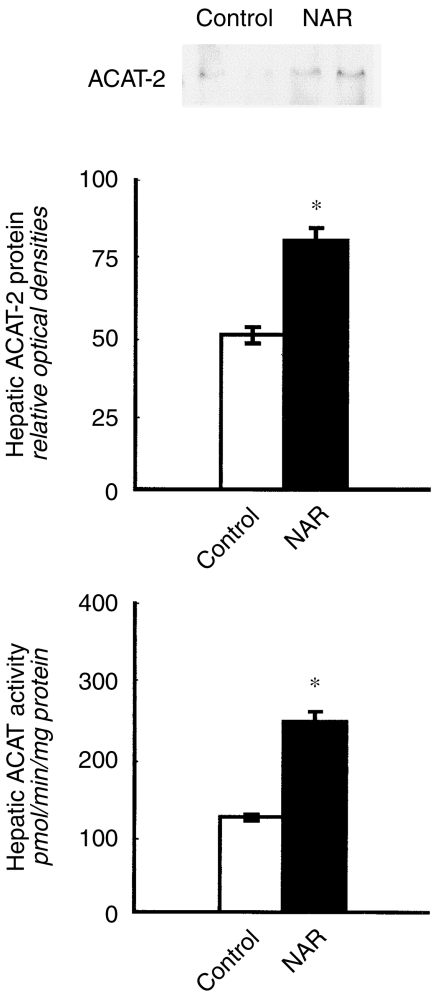


**Fig. 5. Representative Western blot and group data depicting acyl-coA cholesterol acyltransferase-2 (ACAT-2) protein abundance and acyl-CoA cholesterol acyltransferase (ACAT) enzymatic activity in the liver of Nagase rats with inherited analbuminemia (NAR) and Sprague Dawley control (CTL) rats.** $N = 6$ in each group; $^*P < 0.05$.

the role of increased total body cholesterol production in the pathogenesis of hypercholesterolemia in female NAR rats. As with the present study, whole liver microsomal HMG-CoA reductase enzymatic activity in their female NAR was nearly identical to that found in their Sprague Dawley control rats. Based on these observations, the authors concluded that increased cholesterol biosynthesis in extrahepatic tissues is largely responsible for the associated hypercholesterolemia in their female NAR.

The NAR group exhibited a significant up-regulation of hepatic cholesterol 7α-hydroxylase, which is the rate-limiting step in cholesterol conversion to bile acids for disposal in the gut. Up-regulation of hepatic cholesterol 7α-hydroxylase in NAR shown here provides the molecular basis of the reported increased rate of fecal bile acid excretion in this model [20]. Expression of cholesterol 7α-hydroxylase is augmented by increased free cholesterol concentration in the hepatocyte [21, 22]. It is of note that liver tissue free cholesterol concentration in our NAR rats was nearly twice that of the Sprague Dawley controls. This phenomenon can account for the observed up-regulation of cholesterol 7α-hydroxylase in our NAR. The compensatory up-regulation of cholesterol 7α-hydroxylase, which is the main pathway of cholesterol catabolism, can

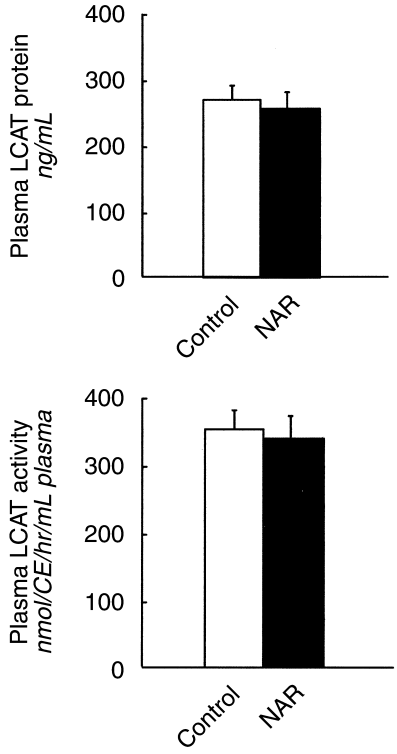


**Fig. 6. Plasma concentration of the immunodetectable lecithin cholesterol acyltransferase (LCAT) protein and enzymatic activity in the Nagase rats with hereditary analbuminemia (NAR) and Sprague Dawley control (CTL) rats.** $N = 6$ in each group.

mitigate the impact of increased cholesterol biosynthesis in the NAR. In contrast to rats with hereditary analbuminemia, rats with nephrotic syndrome exhibit no rise in hepatic cholesterol 7α-hydroxylase [10, 18]. Thus, the presence of up-regulation of cholesterol 7α-hydroxylase in the NAR and its absence in nephrotic syndrome can, in part, account for the greater severity of hypercholesterolemia in nephrotic syndrome as compared to that in hereditary analbuminemia.

Hypercholesterolemia in hereditary analbuminemia is primarily due to increased LDL and HDL levels [4]. Receptor-mediated uptake of LDL and HDL by the liver represents the primary pathway of plasma cholesterol clearance. We therefore sought to determine the abundance of LDL receptor and HDL receptor, SRB-1, in the NAR. Hepatic LDL receptor and HDL receptor proteins in the male NAR were virtually identical to those in Sprague-Dawley control rats. Thus, elevation of plasma LDL and HDL cholesterol concentrations cannot be attributed to either LDL receptor or HDL receptor deficiency. In contrast to the NAR, nephrotic animals exhibit severe acquired LDL receptor and HDL receptor deficiencies [11, 14, 18]. Once again, the presence of LDL receptor and HDL receptor deficiencies in nephrotic syndrome and their absence in hereditary analbuminemia can, in part, account for the greater severity of hypercholesterolemia in the former compared to the latter condition.

Hepatic ACAT-2 protein abundance and ACAT activity in NAR was mildly elevated compared to that found in the Sprague Dawley control rats. ACAT catalyzes the intracellular esterification of cholesterol-to-cholesterol ester in nearly all mammalian cells [23]. Two isoforms of this enzyme have been identified thus far, including ACAT-1, which is expressed in most tissues [24], and ACAT-2, which is primarily expressed in the liver and intestine [25–27]. Esterification of cholesterol in the liver by ACAT is involved in the packaging and secretion of apoprotein B–containing lipoproteins [28–30]. Mild up-regulation of hepatic ACAT-2 in our NAR was associated with an increase in the intracellular free cholesterol concentration. This observation suggests that up-regulation of ACAT-2 may be a compensatory response to increased hepatic tissue free cholesterol concentration. In our recent studies, we found a severe fourfold increase in hepatic ACAT-2 abundance that was accompanied by a significant reduction of hepatic tissue free cholesterol concentration in rats with nephrotic syndrome [12, 18}. Thus, severe up-regulation of hepatic ACAT-2 expression in nephrotic syndrome appears to represent a primary maladaptive phenomenon as opposed to an appropriate physiologic response seen in our rats with hereditary analbuminemia.

Plasma LCAT protein concentration and enzymatic activity in the NAR were virtually identical to those of the Sprague Dawley controls. LCAT is a glycoprotein enzyme that is produced in the liver and secreted in the plasma. LCAT catalyzes hydrolysis of a free fatty acid from phospholipid (phospholipase-2 activity) and transesterification of the fatty acid to the 3-hydroxyl group of cholesterol (acyltransferase activity) to produce cholesterol ester [31]. HDL is the preferred vehicle of LCAT in the plasma and the primary platform for its reactions. By converting free cholesterol-to-cholesterol ester, LCAT helps to maintain a favorable chemical concentration gradient for maximal efflux of cholesterol from the peripheral cells onto the surface of HDL particle. Thus, LCAT plays a central role in HDL-mediated transport of surplus cholesterol from peripheral tissues for disposal in the liver. In fact, hereditary LCAT deficiency is associated with significant reduction of plasma HDL cholesterol concentration, formation of cholesterol-laden cells in various tissues, corneal opacification, premature atherosclerotic cardiovascular disease, and progressive renal insufficiency [32]. In a recent study, we found heavy urinary losses of LCAT leading to severe LCAT deficiency in rats with nephrotic syndrome [18, 33]. This phenomenon could account for impaired HDL maturation and depressed HDL/total cholesterol ratio in nephrotic syndrome. In contrast to the nephrotic rats, NAR

have normal plasma immunodetectable LCAT and activity, which accounts for their normal plasma HDL/total cholesterol ratio. In an earlier study, van Tol et al [5] reported a significant increase in plasma LCAT enzymatic activity in female NAR compared to the female Sprague-Dawley control rats. In contrast, we found no significant difference in either immunodetectable or LCAT enzymatic activity between the male NAR and Sprague-Dawley control rats. The reason for the difference in LCAT enzymatic activity between male and female NAR is uncertain and requires further investigation. It is of note that hyperlipidemia in female NAR rats is far more severe than in the male NAR rats [4]. Thus, gender differences can, in part, account for the different LCAT activity measurements in this model.

## CONCLUSION

Male NAR exhibit normal hepatic HMG-CoA reductase activity, despite a significant increase in HMG-CoA reductase protein abundance. This is coupled with marked up-regulation of cholesterol 7α-hydroxylase and a mild rise in ACAT-2 abundance in the liver. However, hepatic LDL receptor and SRB-1 abundance and plasma LCAT concentration and activity, which are severely deficient in nephrotic syndrome, are normal in hereditary analbuminemia.

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