View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 63 (2003), pp. 1756–1763

# Molecular mechanisms of altered cholesterol metabolism in rats with spontaneous focal glomerulosclerosis

NOSRATOLA D. VAZIRI, TADASHI SATO, and KAIHUI LIANG

*Division of Nephrology and Hypertension, University of California, Irvine, California; and Department of Pediatrics, Saga Medical School Saga City, Saga, Japan*

**Molecular mechanisms of altered cholesterol metabolism in rats with spontaneous focal glomerulosclerosis.**

*Background.* Imai rats exhibit spontaneous focal glomerulosclerosis (FGS), which is marked by heavy proteinuria, severe hyperlipidemia, and progressive renal insufficiency beginning at 8 to 10 weeks of age. In an earlier study, we reported severe skeletal muscle and adipose tissue lipoprotein lipase, and very low-density lipoprotein (VLDL) receptor deficiencies, which account for elevated plasma VLDL and triglycerides in Imai rats at 34 weeks of age. In this study, we investigated key factors involved in cholesterol metabolism.

*Methods.* Male Imai and Sprague-Dawley control rats were fed a regular rat chow and observed from age 8 through 34 weeks. Hepatic 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, low-density lipoprotein (LDL) receptor and acyl Co A:cholesterol acyltransferase (ACAT) were measured by Western blot and plasma lecithin:cholesterol acyltransferase (LCAT) protein was measured by enzyme-linked immunosorbent assay (ELISA).

*Results.* At 34 weeks of age, the Imai rats showed severe proteinuria, hypoalbuminemia, 60% reduction in glomerular filtration rate (GFR), elevated plasma total and LDL cholesterol and LDL/high-density lipoprotein (HDL) ratio. Imai rats showed a twofold elevation of hepatic HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis, but no significant change in cholesterol 7α-hydroxylase, the rate-limiting enzyme in cholesterol catabolism to bile acids. This was accompanied by and largely due to a threefold down-regulation of hepatic LDL receptor, which limits hepatic uptake of LDL; and a threefold up-regulation of hepatic ACAT ($P < 0.01$), which augments esterification of hepatocyte free cholesterol, thus, limiting cholesterol-mediated feedback regulation of cholesterol synthesis and catabolism. Moreover, plasma LCAT concentration was severely depressed (by fourfold) in Imai rats. This abnormality can impair HDL-mediated cholesterol transport from extrahepatic tissues to the liver.

*Conclusion.* The study revealed marked abnormalities of the key proteins involved in regulation of hepatic cholesterol metabolism. These abnormalities can account for severe dysregulation of cholesterol metabolism in Imai rats with spontaneous FGS, which closely resembles FGS in humans.

**Key words:** nephrotic syndrome, hyperlipidemia, hypercholesterolemia, HMG-CoA reductase, cholesterol-7α-hydroxylase, LDL receptor, LCAT, ACAT, bile acids.

Received for publication August 7, 2002
and in revised form September 23, 2002
Accepted for publication December 17, 2002

© 2003 by the International Society of Nephrology

Imai rats were originally derived from the mating of a male Sprague-Dawley rat that exhibited spontaneous renal disease and hyperlipidemia while consuming a low-fat basal diet [1]. Male Imai rats develop a progressive focal glomerulosclerosis (FGS), which presents with mild proteinuria at 5 to 6 weeks of age progressing to frank proteinuria and hyperlipidemia by 8 to 10 weeks of age, and significant renal insufficiency by 30 to 40 weeks of age [2]. The severity of the disease is considerably less and the onset of the disease is significantly delayed in female animals [1]. The histologic and biochemical features of FGS in the Imai rats closely resemble those of progressive FGS in humans [2, 3].

Severe nephrotic proteinuria in the Imai rats is accompanied by marked elevation of plasma cholesterol, triglycerides, low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) and phospholipid concentrations [1, 2]. In a recent study, we found marked down-regulations of lipoprotein lipase and VLDL receptor in adipose tissue and skeletal muscle of 34-week-old Imai rats with severe nephrotic proteinuria and moderate renal insufficiency [4]. These findings helped to elucidate the molecular mechanism of hypertriglyceridemia and elevation of plasma VLDL in this model. The observed down-regulations of lipoprotein lipase and VLDL receptor in 34-week-old Imai rats exhibiting severe nephrotic syndrome and moderate renal insufficiency paralleled the findings of our earlier studies in rats with puromycin-induced nephrotic syndrome [5, 6] and those with chronic renal insufficiency induced by 5/6 nephrectomy [7–9].

The present study was designed to discern the molecular basis of hypercholesterolemia, elevation of plasma LDL and LDL to high-density lipoprotein (HDL) cholesterol ratio in Imai rats with spontaneous FGS. Accordingly, we examined hepatic expressions of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (the

rate-limiting step in cholesterol synthesis), cholesterol 7α-hydroxylase (the rate-limiting step in cholesterol catabolism to bile acids), LDL receptor (the principal pathway of cholesterol clearance) and acyl CoA:cholesterol acyltransferase (ACAT) (the main enzyme responsible for intracellular esterification of cholesterol) in male Imai rats with FGS and age-matched male Sprague-Dawley control rats. In addition, plasma immunodetectable lecithin:cholesterol acyltransferase (LCAT), which is the main enzyme responsible for esterification of cholesterol in the plasma and maturation of HDL was measured. We studied the Imai rats at 34 weeks of age at which point severe nephrotic syndrome and moderate renal insufficiency were present. It is of note that earlier studies from our group and other investigators had explored the effects of nephrotic syndrome and chronic renal insufficiency separately. However, little is known about the effects of the combination of the two conditions, which frequently occurs in advanced stages of FGS and many other glomerulopathies in animals and humans. Investigation of the Imai rats at 34 weeks of age afforded the opportunity to explore this issue.

## METHODS

### Animals

Imai hyperlipidemic rats (Biological Research Laboratories, Central Research Division of Takeda Chemical Industries, Osaka, Japan) were bred in the animal facility of Saga Medical School (Saga, Japan). Six male Imai rats and six male Sprague-Dawley control rats were monitored for 34 weeks. They were fed a regular rat chow (containing 24.8% protein, and 0.08% cholesterol) and housed in a climate-controlled space with 12-hour light, 12-hour dark cycles. All animals were allowed access to food and water ad libitum. Arterial pressure was measured by a tail plethysmograph (Harvard Apparatus, Cambridge, MA, USA). A 24-hour urine collection was obtained for determinations of urinary protein and creatinine excretion using metabolic cages. At the conclusion of each observation period, animals were anesthetized with intraperitoneal injections of pentobarbital (50 mg/kg) and euthanized by exsanguination using cardiac puncture. Liver was harvested and frozen in liquid nitrogen immediately and stored at −70°C until processed. In addition, plasma was separated and used for measurements of lipids, creatinine and albumin concentrations, as well as LCAT protein abundance.

### Routine biochemical determinations

Serum total cholesterol, triglyceride, creatinine, and albumin concentrations were measured by a synchro CX3 autoanalyzer (Beckman Instruments, Inc., Fullerton, CA, USA). Urinary protein concentration was determined by a kit purchased from Wako Pure Chemical Industries, Tokyo, Japan.

Creatinine clearance was calculated from urinary and plasma creatinine values using the standard formula. Plasma HDL, LDL, and VLDL levels were determined by ultracentrifugation method as described by Lasser et al [10].

### HMG-CoA reductase protein determination

Frozen rat liver tissue was homogenized in 1 mL of 20 mmol/L Tris-HCl (pH 7.5) buffer containing 2 mmol/L $MgCl_2$, 0.2 mol/L sucrose, 5 mmol/L 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 by 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, Chambers, and Lopez [11]. Briefly, aliquots containing 100 μg proteins were fractionated on 4% to 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 G.C. Ness, Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, Florida). 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 antirabbit immunoglobulin G (IgG) (Amersham Life Science, Inc.). 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 antirat cholesterol 7α-hydroxylase antibody as described in our earlier studies [12]. The antibody employed in this assay was generously provided by Professor John Y.L. Chiang (Northeastern Ohio University College of Medicine, Rootstown, OH).

### 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 Biochemi, Inc., Davis, CA, USA) as described in our previous study [13].

### Determination of ACAT-2 protein

Hepatic ACAT-2 protein abundance was quantified by Western analysis using a polyclonal antibody against ACAT-2 as described in our recent study [14]. The antibody employed in these experiments was a generous gift from Professor Lawrence L. Rudel, Department of Biochemistry and Comparative Medicine, Wake Forest University, Winston-Salem, North Carolina.

### Determination of plasma LCAT protein

Plasma LCAT protein concentration was measured in triplicate by enzyme-linked immunosorbent assay (ELISA) method using rabbit antihuman LCAT antibody as described by Wang et al [15]. This antibody was generously supplied by Professor John S. Parks, Wake Forest University, Winston-Salem, North Carolina. Briefly, 10 uL of plasma were diluted in binding buffer (1 × TBS, 0.05% $NaN_3$, 200 uL 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) and blocked for 3 hours at room temperature with blocking buffer [0.5% bovine serum albumin (BSA) and 0.05% Tween-20 in 1 × TBA] and washed. A 1:2000 dilution of rabbit antihuman LCAT antibody in blocking buffer were added into the well and incubated at room temperature for 3 hours. The plates were washed and incubated with 1:2000 antirabbit IgG conjugated with horseradish peroxidase (Sigma Chemical, Inc.) and developed with 1-step™ Turbo-TMB-ELISA kit (Pierce Inc.) and read at 450 nm after color developed. Purified human plasma LCAT was used as standard for the assay.

### 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 and Figures 1 and 3 to 5. The FGS group exhibited severe proteinuria, hypoalbuminemia, arterial hypertension, elevated serum creatinine concentration, and reduced creatinine clearance. Compared with the control group, the FGS animals showed a fivefold elevation of serum total cholesterol, a 15-fold increased in LDL cholesterol, a threefold rise in VLDL cholesterol, and a fivefold increase in LDL cholesterol/HDL cholesterol ratio. This was coupled with a threefold elevation of serum total triglycerides and a threefold rise in VLDL triglyceride concentrations. Plasma free cholesterol was significantly increased, whereas liver tissue cholesterol concentration was significantly reduced in the Imai rats when compared with the control group.

**Table 1.** Serum concentrations of total triglycerides, very low-density lipoproteins triglycerides (VLDL-TG), albumin, and creatinine, creatinine clearance, urinary protein excretion and systolic blood pressure in normal Sprague-Dawley control rats and 34-week-old Imai rats with focal glomerulosclerosis (FGS)

| | CTL ($N = 6$) | FGS ($N = 6$) | *P* values |
|---|---|---|---|
| Triglyceride *mg/dL* | 73.1 ± 10.2 | 218.1 ± 15.2 | <0.005 |
| VLDL-TG *mg/dL* | 63.0 ± 10.2 | 194.4 ± 16.3 | <0.005 |
| Albumin *g/dL* | 3.70 ± 0.1 | 2.80 ± 0.1 | <0.005 |
| Creatinine *mg/dL* | 0.54 ± 0.29 | 1.05 ± 0.14 | <0.001 |
| Creatinine clearance *mL/min · g kidney weight* | 0.54 ± 0.03 | 0.20 ± 0.04 | <0.001 |
| Urinary protein *mg/24 hours* | 35.2 ± 5.5 | 467.0 ± 27.7 | <0.001 |
| Blood pressure *mm Hg* | 116.7 ± 5.9 | 187.5 ± 12.6 | <0.001 |

Values are mean ± SEM.

### Hepatic HMG-CoA reductase data

Data are depicted in Figure 1. The FGS group showed a twofold elevation of HMG-CoA reductase protein abundance in the liver tissue when compared with the normal control group. A significant direct correlation was found between hepatic HMG-CoA reductase and serum total cholesterol concentration ($r = 0.9$, $P < 0.001$).

### Hepatic cholesterol 7α-hydroxylase data

Results are illustrated in Figure 2. Despite severe hypercholesterolemia, hepatic tissue cholesterol 7α-hydroxylase abundance in the FGS group was virtually identical to that of normocholesterolemic control animals. However, HMG-CoA reductase to cholesterol 7α-hydroxylase ratio (which reflects the balance between hepatic cholesterol biosynthesis and catabolism) was markedly increased in the FGS group as compared to that in the control group.

### Hepatic LDL receptor protein data

Data are given in Figure 3. The FGS group showed an approximately 2.5-fold reduction in hepatic tissue LDL receptor protein abundance as compared to the normal control group. A significant inverse correlation was found between liver tissue LDL receptor protein abundance and serum LDL cholesterol concentration in the study animals ($r = -0.7$, $P < 0.05$).

### Hepatic ACAT-2 results

Data are shown in Figure 4. Liver-specific ACAT-2 protein abundance was nearly 2.5-fold higher in the FGS group as compared with the normal control group. Hepatic ACAT-2 abundance was inversely related to liver free cholesterol ($r = -0.74$, $P < 0.01$) concentration.

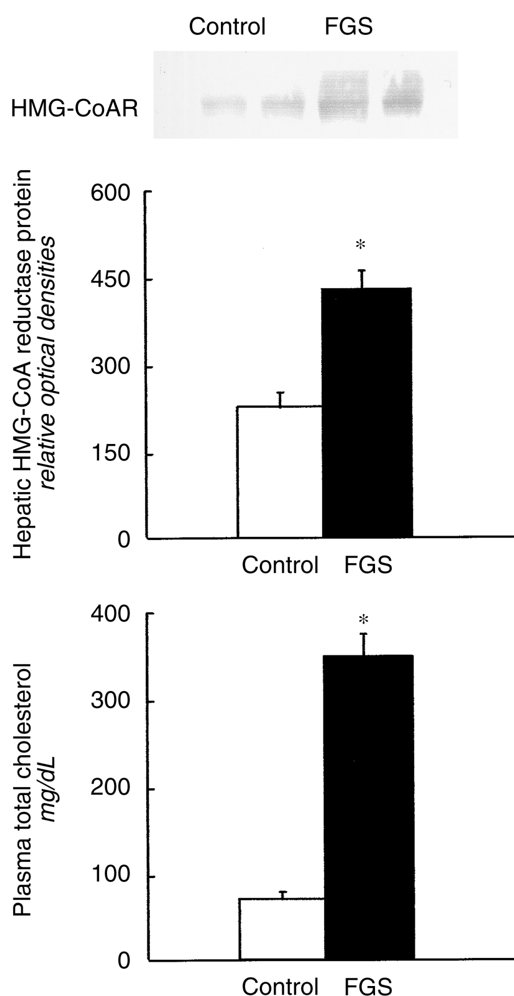


**Fig. 1. Representative Western blot and group data depicting hepatic tissue microsomal 3-hydroxy-3 methyglutaryl coenzyme A (HMG-CoA) reductase protein abundance and plasma cholesterol concentration in the 34-week-old Imai rats with focal glomerulosclerosis (FGS) and the age-matched normal control group.** $N = 6$ in each group. $^*P < 0.01$.

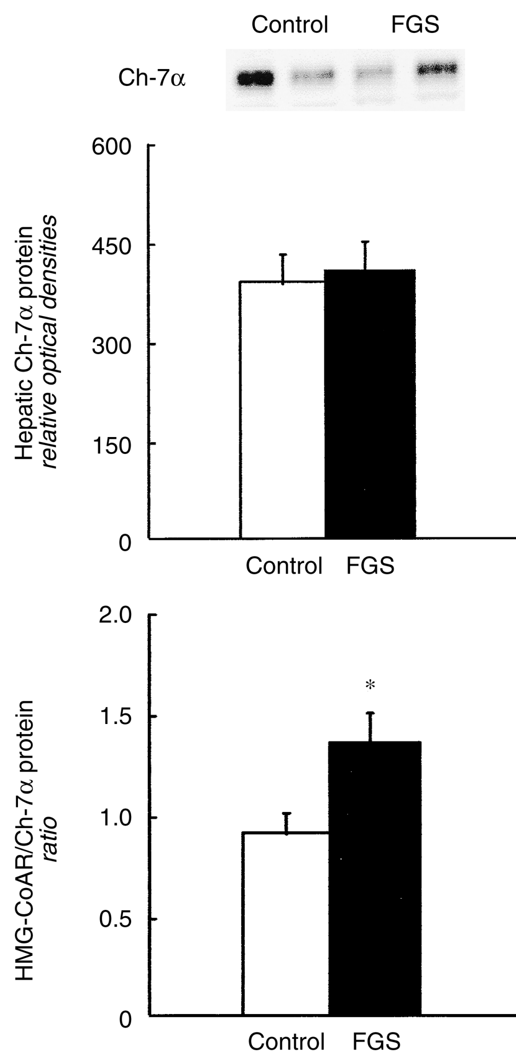


**Fig. 2. Representative Western blot and group data depicting hepatic tissue microsomal cholesterol 7α-hydroxylase (CH-7α) protein abundance and 3-hydroxy-3 methyglutaryl coenzyme A (HMG-CoA) reductase-to-cholesterol 7α-hydroxylase ratio in the 34-week-old Imai rats with focal glomerulosclerosis (FGS) and the age-matched normal control group.** $N = 6$ in each group. No significant difference was found.

## Plasma LCAT data

Results are depicted in Figure 5. Plasma LCAT protein concentration was approximately fourfold lower in the FGS group than that in the normal control group. Plasma LCAT protein concentration was inversely related to serum LDL cholesterol to HDL cholesterol concentration ratio ($r = -0.72$, $P < 0.05$) and serum free cholesterol concentration ($r = -0.84$, $P < 0.01$).

## DISCUSSION

Severe nephrotic proteinuria in our Imai rats with FGS was accompanied by marked elevation of serum total cholesterol and LDL cholesterol concentrations. Earlier studies have pointed to increased LDL synthesis [16] and depressed LDL clearance [17] as the cause of increased plasma LDL cholesterol in nephrotic syndrome. However, the molecular mechanisms responsible for these phenomena were not known.

Hypercholesterolemia in our nephrotic rats with spontaneous FGS was accompanied by an approximately twofold increase in the liver tissue abundance of HMG-CoA reductase. The endoplasmic reticulum-bound enzyme, HMG-CoA reductase is the rate-limiting factor in cholesterol biosynthesis. Although this enzyme is expressed in all tissues, it is most abundantly expressed in the liver, which plays a critical role in regulation of plasma cholesterol level [18]. Thus, marked up-regulation of hepatic HMG-CoA reductase in the FGS group can contribute to hypercholesterolemia in these animals. This supposition is supported by the observed correlation between hepatic HMG-CoA reductase and serum cholesterol in

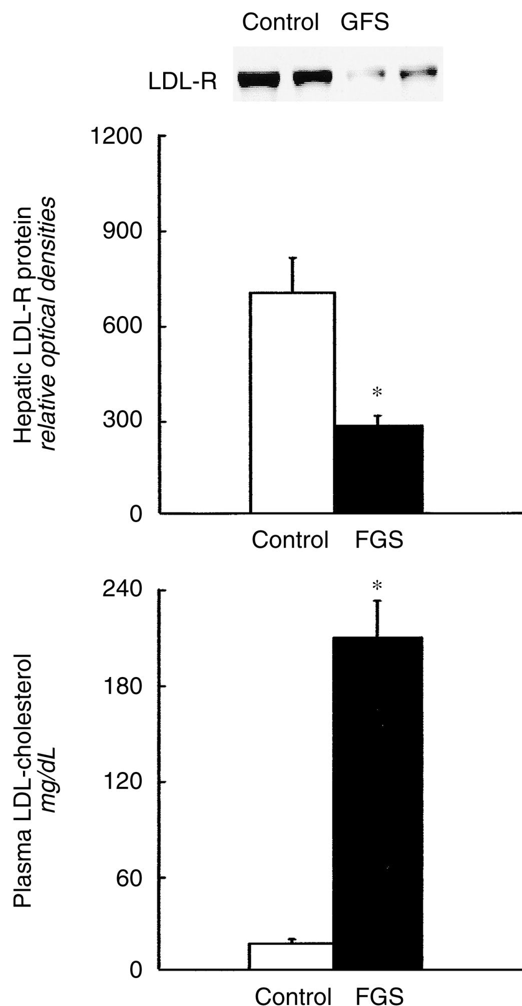


**Fig. 3. Representative Western blot and group data depicting hepatic tissue plasma membrane low-density lipoprotein (LDL) receptor protein abundance and plasma LDL concentration in the 34-week-old Imai rats with focal glomerulosclerosis (FGS) and the age-matched normal control group.** $N = 6$ in each group. *$P < 0.01$.

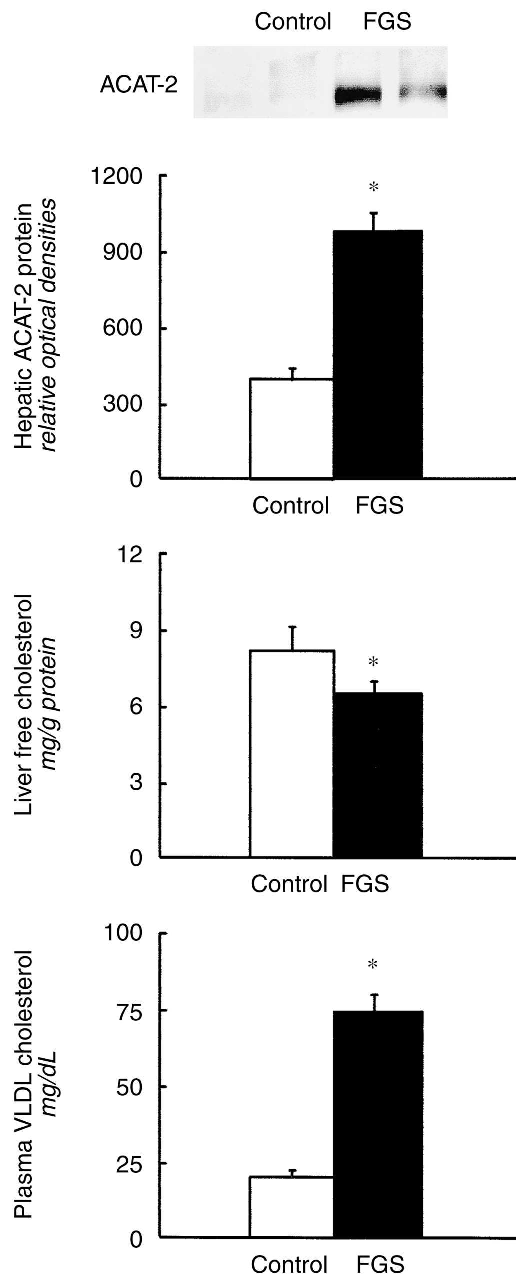


**Fig. 4. Representative Western blot and group data depicting hepatic tissue acyl CoA:cholesterol acyltransferase (ACAT-2) protein abundance, liver-free cholesterol and plasma very low-density lipoprotein (VLDL) concentration in the 34-week-old Imai rats with focal glomerulosclerosis (FGS) and the age-matched normal control group.** $N = 6$ in each group. *$P < 0.01$.

the study animals. In an earlier study, we found up-regulation of hepatic HMG-CoA reductase mRNA abundance and enzymatic activity in rats with puromycin-induced nephrotic syndrome [19]. The present study revealed up-regulation of immunodetectable HMG-CoA reductase protein in spontaneously occurring nephrotic syndrome caused by a glomerulopathy, which closely resembles FGS in humans.

Despite severe hypercholesterolemia, hepatic tissue cholesterol 7α-hydroxylase protein abundance in our FGS animals was virtually identical to that of the normocholesterolemic control rats. Cholesterol 7α-hydroxylase is a unique cytochrome P450 enzyme, which is the first and the rate-controlling step in cholesterol conversion to bile acids. Consequently, this enzyme plays a critical role in cholesterol catabolism. The observed up-regulation of HMG-CoA reductase, which is the determinant of cholesterol biosynthesis [18], coupled with a lack of rise in cholesterol 7α-hydroxylase, which is the determinant of cholesterol catabolism, can support the induction and maintenance of hypercholesterolemia in the FGS animals. The failure of the nephrotic liver to raise cholesterol 7α-hydroxylase expression in rats with spontaneous FGS is consistent with our earlier studies of animals with puromycin-induced proteinuria [12].

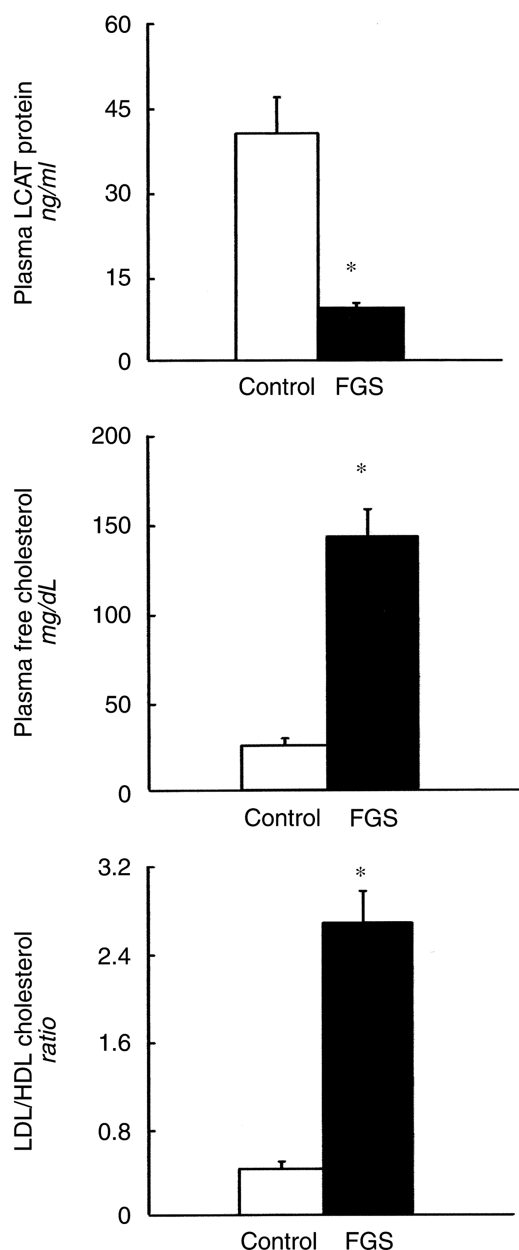


**Fig. 5. Plasma lecithin:cholesterol acyltransferase (LCAT) protein concentration plasma-free cholesterol and high-density lipoprotein (LDL) to high-density lipoprotein (HDL) cholesterol ratio in the 34-week-old Imai rats with focal glomerulosclerosis (FGS) and the age-matched normal control group.** $N = 6$ animals in each group. $^{*}P < 0.01$.

Elevation of serum total cholesterol and LDL cholesterol concentration in our FGS rats was associated with an approximately 2.5-fold reduction in hepatic LDL receptor protein abundance. LDL receptor-mediated hepatic uptake of LDL represents the predominant pathway of clearance of plasma cholesterol. In fact, hereditary LDL receptor deficiency results in severe hypercholesterolemia and premature atherosclerotic cardiovascular disease [20]. It is, therefore, intuitive that acquired LDL receptor deficiency observed in our nephrotic rats with FGS could have contributed to the associated elevation of LDL and total cholesterol concentrations. This viewpoint is supported by the strong inverse correlation between hepatic LDL receptor and plasma total and LDL cholesterol levels founds in the study animals. This phenomenon provides the molecular basis for the previously reported impairment of LDL clearance in nephrotic syndrome [17]. Down-regulation of hepatic LDL receptor protein abundance found in the nephrotic rats with spontaneous FGS is consistent with the results of our earlier study in rats with puromycin-induced nephrotic syndrome [13], thus, pointing to the relevance of the findings to nephrotic syndrome regardless of the underlying cause.

Nephrotic proteinuria in our 34-week-old Imai rats was accompanied by moderate renal insufficiency. This may raise the question as to the possible contribution of renal insufficiency in the pathogenesis of the observed dysregulation of the above cholesterol regulatory proteins in the liver of our FGS rats. In an earlier study of rats with chronic renal insufficiency induced by 5/6 nephrectomy, we found no significant change in hepatic expression of either HMG-CoA reductase, cholesterol 7α-hydroxylase, or LDL receptor [21]. The latter study tends to exclude the possible role of renal insufficiency in the pathogenesis of the above abnormalities.

Liver tissue ACAT-2 protein abundance was nearly 2.5-fold greater in our Imai rats with FGS than that found in the control group. ACAT is a membrane-associated enzyme, which is primarily localized in the endoplasmic reticulum. ACAT catalyzes intracellular esterification of cholesterol and formation of cholesterol ester in nearly all mammalian cells [22]. Two isoforms of ACAT have, thus far, been identified. These include ACAT-1, which is expressed in most tissues, and ACAT-2, which is primarily expressed in the liver and intestine [23–26]. Esterification of free cholesterol by ACAT has been shown to enhance production and secretion of VLDL by the liver [27–29]. Thus, up-regulation of hepatic ACAT-2 in our rats with spontaneous FGS may contribute to elevation of plasma VLDL in these animals. Expression of HGM-CoA reductase is inhibited, while that of cholesterol 7α-hydroxylase is up-regulated by free cholesterol in the hepatocyte [30, 31]. Thus, the observed up-regulation of HGM-CoA reductase and the lack of rise in cholesterol 7α-hydroxylase seen in the FGS rats may be, in part, due to up-regulation of ACAT-2, which helps to lower cellular free cholesterol concentration by catalyzing the formation of cholesterol ester. This is compounded by LDL receptor deficiency, which limits the influx of cholesterol into the hepatocyte.

In a series of recent studies, we have demonstrated marked up-regulation of ACAT-2 in rats with puromycin-induced nephrotic syndrome [14] and rats with chronic renal insufficiency induced by 5/6 nephrectomy

[32]. Accordingly, both severe nephrotic proteinuria and moderate renal insufficiency appear to have contributed to up-regulation of ACAT-2 in our Imai rats with spontaneous FGS.

Plasma LCAT protein concentration in our FGS rats was approximately fourfold lower than that found in the normal control group. LCAT is a 63 kD glycoprotein enzyme that is made and secreted in the plasma by the liver. In the presence of cofactor, ApoA-I, the enzyme catalyses hydrolysis of a Sn2 fatty acid from phospholipid and transesterification of fatty acid to the 3-hydroxyl group of cholesterol to form cholesterol ester [33]. HDL is the main carrier of LCAT in the plasma and the preferred macromolecular platform for the LCAT reactions. LCAT is essential for maturation of cholesterol-poor HDL-3 or nascent HDL to the cardioprotective cholesterol ester-rich HDL-2. The role of LCAT in this process involves generation of a chemical concentration gradient for optimal efflux of free cholesterol from the peripheral cells into the HDL particle through rapid conversion of free cholesterol to esterified cholesterol. Thus, LCAT plays an important role in HDL-mediated retrieval of surplus cholesterol from the peripheral cells for disposal in the liver, a phenomenon commonly referred to as reverse cholesterol transport.

The importance of LCAT in HDL metabolism and reverse cholesterol transport processes is best illustrated by hereditary LCAT deficiency, which is associated with a depressed HDL-2 to HDL-3 ratio, presence of cholesterol-laden foam cells in various tissues, accelerated atherosclerotic cardiovascular disease, progressive renal insufficiency, and corneal opacification [34].

Plasma HDL cholesterol is frequently reduced [35] or unchanged [17], LDL to HDL ratio is markedly elevated and maturation of HDL-3 to HDL-2 is impaired in nephrotic syndrome [36]. Similarly, plasma HDL cholesterol is depressed and HDL maturation is impaired in chronic renal insufficiency [37]. These abnormalities are highly suggestive of LCAT deficiency. In fact, marked elevation of LDL cholesterol to HDL cholesterol ratio in our FGS rats, which had severe nephrotic proteinuria and moderate renal insufficiency, was coupled with severe LCAT deficiency. In a recent study, we found marked down-regulation of hepatic LCAT mRNA abundance and significant reduction of plasma LCAT enzymatic activity in rats with chronic renal insufficiency induced by 5/6 nephrectomy [38]. In a concurrent study, we found severe reduction of plasma LCAT enzymatic activity coupled with heavy urinary losses of LCAT and normal hepatic LCAT mRNA abundance in rats with puromycin-induced nephrotic syndrome [39]. The present study revealed profound reduction of plasma LCAT protein concentration in rats with a spontaneous nephropathy presenting with severe nephrotic proteinuria and moderate renal insufficiency resembling FGS in humans.

Comparative analysis of data obtained in our earlier studies of rats with either nephrotic syndrome or chronic renal insufficiency alone with those of the 34-week old Imai rats exhibiting both conditions revealed predominant effect of nephrotic syndrome on expression of the main cholesterol-regulatory enzymes and receptors. However, our earlier studies of the enzymes and receptors involved in metabolism of triglyceride-rich lipoproteins in this model revealed additive effects of nephrotic syndrome and renal insufficiency on plasma lipids and tissue lipoprotein lipase and VLDL receptor expressions [4].

## CONCLUSION

Imai rats with spontaneous FGS exhibited marked up-regulation of hepatic HMG-CoA reductase and ACAT-2 coupled with severe acquired LDL receptor and LCAT deficiencies. Dysregulation of these key cholesterol regulatory factors can account for the profound alterations of cholesterol metabolism in advanced FGS presenting with a combination of severe nephrotic proteinuria and moderate renal insufficiency.

*Reprint requests to Nosratola D.Vaziri, M.D., M.A.C.P., UCI Medical Center, Division of Nephrology and Hypertension, 101 The City Drive, Bldg 53, Room 125, Rt 81, Orange, CA 92868.*

## REFERENCES

1. Imai Y, Matsumura H, Miyajima H, Oka K: Serum and tissue lipids and glomerulonephritis in the spontaneously hypercholesterolemic rats with a note on the effect of gonadectomy. *Atherosclerosis* 27:165–178, 1977
2. Yoshikawa Y, Yamasaki K: Renal lesions of hyperlipidemic Imai rats: A spontaneous animal model of focal glomerulosclerosis. *Nephron* 59:471–476, 1991
3. Sakemi T, Baba N, Yoshikawa Y: Angiotensin-converting enzyme inhibition attenuates hypercholesterolemia and glomerular injury in hyperlipidemic Imai rats. *Nephron* 62:315–321, 1992
4. Sato T, Liang K, Vaziri ND: Downregulation of lipoprotein lipase and VLDL receptor in rats with focal glomerulosclerosis. *Kidney Int* 61:157–162, 2002
5. Liang K, Vaziri ND: Gene expression of lipoprotein lipase in experimental nephrosis. *J Lab Clin Med* 130:387–394, 1997
6. Liang K, Vaziri ND: Acquired VLDL receptor deficiency in experimental nephrosis. *Kidney Int* 51:1761–1765, 1997
7. Vaziri ND, Liang K: Downregulation of tissue lipoprotein lipase expression in experimental chronic renal failure. *Kidney Int* 50: 1928–1935, 1996
8. Vaziri ND, Wang XQ, Liang K: Secondary hyperparathyroidism downregulates lipoprotein lipase expression in chronic renal failure. *Am J Physiol Renal Physiol* 273:F925–F930, 1997
9. Vaziri ND, Liang K: Downregulation of VLDL receptor expression in chronic experimental renal failure. *Kidney Int* 51:913–919, 1997
10. Lasser NL, Roheim PS, Edelstein D, Eder HA: Serum lipoproteins of normal and cholesterol-fed rats. *J Lipid Res* 14:1–8, 1973
11. Ness GC, Chambers CM, Lopez D: Atorvastatin action involves diminished recovery of hepatic HMG-CoA reductase activity. *J Lipid Res* 39:75–84, 1998
12. Liang KH, Oveisi F, Vaziri ND: Gene expression of hepatic cholesterol 7 alpha-hydroxylase in the course of puromycin-induced nephrosis. *Kidney Int* 49:855–860, 1996
13. Vaziri ND, Liang K: Downregulation of hepatic LDL receptor expression in experimental nephrosis. *Kidney Int* 50:887–893, 1996
14. Vaziri ND, Liang KH: Upregulation of Acyl-coenzyme A: Choles-

terol acyltransferase (ACAT) in nephrotic syndrome. *Kidney Int* 61:1769–1775, 2002
15. Wang J, Gebre AK, Anderson RA, Parks JS: Amino acid residue 149 of lecithin:cholesterol acyltransferase determines phospholipase $A_2$ and transacylase fatty acyl specificity. *J Biol Chem* 272:280–286, 1997
16. de Sain-van der Velden MG, Kaysen GA, Barrett HA, *et al*: Increased VLDL in nephrotic patients results from a decreased catabolism while increased LDL results from increased synthesis. *Kidney Int* 53:994–1001, 1998
17. Joven J, Villabona C, Vilella E, *et al*: Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome. *N Engl J Med* 323:579–584, 1990
18. Dietschy JM, Turley SD, Spady DK: Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J Lipid Res* 34:1637–1659, 1993
19. Vaziri ND, Liang KH: Hepatic HMG-CoA reductase gene expression during the course of puromycin-induced nephrosis. *Kidney Int* 48:1979–1985, 1995
20. Myant NB: Familial hypercholesterolemia: A consequence of LDL-receptor deficiency, in *Cholesterol Metabolism, LDL and the LDL Receptor*, edited by Myant NB, London, Academic Press, Inc., 1990, pp 401–438
21. Liang K, Vaziri ND: Gene expression of LDL receptor, HMG-CoA reductase and cholesterol 7$\alpha$-hydroxylase in chronic renal failure. *Nephrol Dial Transpl* 12:1381–1386, 1997
22. Billheimer JT, Gillies PJ: *Advances in Cholesterol Research*, edited by Esfahani M, Swaney JB, Caldwell, NJ, Telford Press, 1990, pp 7–45
23. Chang CC, Huh HY: Cadigan KM, Chang TY: Molecular cloning and functional expression of human acyl-coenzyme A:cholesterol acyltransferase cDNA in mutant Chinese hamster ovary cells. *J Biol Chem* 268:20747–20755, 1993
24. Anderson RA, Joyce C, Davis M, *et al*: Identification of a form of acyl-CoA:cholesterol acyltransferase specific to liver and intestine in nonhuman primates. *J Biol Chem* 273:26747–26754, 1998
25. Oelkers P, Behari A, Cromley D, *et al*: Characterization of two human genes encoding acyl coenzyme A:cholesterol acyltransferase-related enzymes. *J Biol Chem* 273:26765–26771, 1998
26. Cases S, Novak S, Zheng YW, *et al*: ACAT-2, a second mammalian acyl-CoA:cholesterol acyltransferase. Its cloning, expression, and characterization. *J Biol Chem* 273:26755–26764, 1998
27. Carr TP, Hamilton RL, Rudel LL: ACAT inhibitors decrease secretion of cholesteryl esters and apolipoprotein B by perfused livers of African green monkeys. *J Lipid Res* 36:25–36, 1995
28. Cianflone KM, Yasruel Z, Rodriguez MA, *et al*: Regulation of ApoB secretion from HepG2 cells: Evidence for a critical role for cholesteryl ester synthesis in the response to a fatty acid challenge. *J Lipid Res* 31:2045–2055, 1990
29. Huff MW, Telford DE, Barrett PH, *et al*: Inhibition of hepatic ACAT decreases ApoB secretion in miniature pigs fed a cholesterol-free diet. *Arteriorscler Thromb* 14:1498–1508, 1994
30. Ness GC, Chambers CM: Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: The concept of cholesterol buffering capacity. *Proc Soc Exp Biol Med* 224:8–19, 2000
31. Russell DW, Setchell KD: Bile acid biosynthesis. *Biochemistry* 31:4737–4749, 1992
32. Liang K, Vaziri ND: Upregulation of acyl-CoA:cholesterol acyltransferase in chronic renal failure. *Am J Physiol Endo Metab* 283:E676–E681, 2002
33. Glomset JA: The plasma lecithins:cholesterol acyltransferase reaction. *J Lipid Res* 9:155–167, 1968
34. Kuivenhoven JA, Pritchard H, Hill J, *et al*: The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes. *J Lipid Res* 38:191–205, 1997
35. Gherardi E, Rota E, Calandra S, *et al*: Relationship among the concentrations of serum lipoproteins and changes in their chemical composition in patients with untreated nephrotic syndrome. *Eur J Clin Invest* 7:563–570, 1977
36. Muls E, Rosseneu M, Daneels R, *et al*: Lipoprotein distribution and composition in the human nephrotic syndrome. *Atherosclerosis* 54:225–237, 1985
37. Attman PO, Samuelsson O, Alaupovic P: Lipoprotein metabolism and renal failure. *Am J Kidney Dis* 21:573–592, 1993
38. Vaziri ND, Liang K, Parks JS: Downregulation of hepatic lecithin:cholesterol acyltransferase gene expression in chronic renal failure. *Kidney Int* 59:2192–2196, 2001
39. Vaziri ND, Liang K, Park JS: Acquired lecithin:cholesterol acyltransferase (LCAT) deficiency in nephrotic syndrome. *Am J Physiol Renal Physiol* 49:F823–F829, 2001