Expression profiling of hepatic genes associated with lipid metabolism in nephrotic rats

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Zhou Y, Zhang X, Chen L, Wu J, Dang H, Wei M, Fan Y, Zhang Y, Zhu Y, Wang N, Breyer MD, Guan Y. Expression profiling of hepatic genes associated with lipid metabolism in nephrotic rats. Am J Physiol Renal Physiol 295: F662–F671, 2008. First published July 9, 2008; doi:10.1152/ajprenal.00046.2008.—Hyperlipidemia is one of the major features of nephrotic syndrome (NS). Although many factors have been implicated in the pathogenesis of NS-related dyslipidemia, the underlying mechanisms remain largely uncharacterized. The present study was designed to examine the gene profile associated with lipid metabolism in the livers of nephrotic rats. NS was created in male Sprague-Dawley rats (n = 6) receiving sequential intraperitoneal injections of puromycin aminonucleoside. Analysis by Affymetrix assay, quantitative RT-PCR, and Northern and Western blotting revealed 21 genes associated with cholesterol and fatty acid metabolism. Eight genes involved in cholesterol metabolism, Apo A-I, Acly, Acat, Mpd, Fdps, Ss, Lss, and Nsdhl, were significantly upregulated under NS. Four genes involved in fatty acid biosynthesis, Acc, FAS, ELOVL2, and ELOVL6, and three critical for triglyceride biosynthesis, Gpam, Agpat 3, and Dgat 1, were significantly upregulated, whereas two genes involved in fatty acid oxidation, Dci and MCAD, were downregulated. Expression of several genes in sterol-regulatory element-binding protein (SREBP)-1 activation was also aberrantly altered in nephrotic livers. The expression and transcriptional activity of SREBP-1 but not SREBP-2 were increased in nephrotic rats as assessed by real-time PCR, immunoblotting, and gel shift assays. The upregulation of hepatic genes involved in cholesterol biosynthesis may play an important role in the pathogenesis of hypercholesterolemia, whereas upregulation of genes participating in hepatic fatty acid and triglyceride biosynthesis and downregulation of genes involved in hepatic fatty acid oxidation may contribute to hypertriglyceridemia in nephrotic rats. Activation of SREBP-1 transcription factor may represent an underlying molecular mechanism of hyperlipidemia in NS.

NS is characterized by severe proteinuria, hypoalbuminemia, sodium retention, and hyperlipidemia. Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major abnormality in lipid metabolism (18, 49) and implies a high risk for cardiovascular diseases such as atherosclerosis and coronary heart disease and progressive renal damage in nephrotic patients (7, 32). Increasing evidence suggests that hypercholesterolemia may result from both hepatic overproduction and impaired catabolism, as well as clearance of cholesterol-rich lipoproteins. An early work on hepatic cholesterol synthesis involving 14C-labeled acetate indicated decreased synthesis of cholesterol in nephrotic rats (26). In contrast, Shafrir and Brenner (35) found increased biosynthesis of hepatic cholesterol with increased activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. However, studies by Thabet (43) and Vaziri et al. (24, 47) showed that neither the activity of HMG-CoA reductase nor that of cholesterol 7a-hydroxylase, the rate-limiting enzyme in cholesterol catabolism, was altered in nephrotic rats. Therefore, although many factors appear to be implicated in the pathogenesis of NS-related hypercholesterolemia, the underlying mechanisms remain a matter of debate.

The liver is well known to play a critical role in cholesterol homeostasis. Hepatic cholesterol levels are tightly regulated by multiple regulatory mechanisms involved in de novo synthesis, catabolism, and transport of cholesterol. Endogenous cholesterol synthesis is controlled by a group of microsomal enzymes, including HMG-CoA reductase. Cholesterol levels in the liver are also regulated by a catabolic pathway that converts cholesterol into the primary bile acids. In addition, high-density lipoprotein transports surplus cholesterol from peripheral tissues to the liver via a commonly known reverse cholesterol transport process (9). Recently, a large body of evidence has demonstrated that a family of membrane-bound transcription factors, sterol-regulatory element-binding proteins (SREBPs), play a key role in hepatic cholesterol synthesis (12). However, thus far, whether the SREBP pathway participates in hypercholesterolemia in NS has not been fully characterized.

Hypertriglyceridemia represents another major feature of dyslipidemia in NS. Hypertriglyceridemia has been postulated to result from dysregulation of fatty acid metabolism in the liver. Fatty acid metabolism in hepatocytes is modulated by 1) de novo fatty acid synthesis and uptake of plasma free fatty acids; 2) fatty acid catalysis by oxidation in the mitochondria, peroxisomes, and endoplasmic reticulum; and 3) triglyceride synthesis from fatty acids. Defects in any of these metabolic pathways could lead to hypertriglyceridemia. To date, little is known about the pathogenesis of hypertriglyceridemia in NS. Although enhanced hepatic triglyceride biosynthesis reflected by increased mRNA abundance and activity of hepatic Dgat1 (44), an enzyme catalyzing the final step in the biosynthesis of triglycerides, may play a role, the underlying mechanisms remain largely unknown.
The present study was designed to examine the gene profile associated with lipid metabolism in the livers of nephrotic rats by Affymetrix analysis. We found a set of genes involved in lipid metabolism differentially regulated in nephrotic rats. Dysregulation of genes important for hepatic cholesterol biosynthesis and transport may play an important role in the pathogenesis of hypercholesterolemia in nephrotic rats, whereas aberrant expression of genes involved in hepatic fatty acid and triglyceride biosynthesis and fatty acid oxidation may contribute to hypertriglyceridemia in NS. Activation of SREBP-1 transcription factor, a master regulator of lipid homeostasis, may represent an underlying mechanism.

MATERIALS AND METHODS

Chemical reagents. Puromycin aminonucleoside (PAN) was obtained from Sigma-Aldrich. TRIzol was purchased from Invitrogen. Reverse transcription and probe labeling kits were from Promega. α-32P-labeled dCTP and γ-32P-labeled dATP were from Amersham. Real-time PCR was performed in PTC-200 (MJ Research) with reagents obtained from Tiangen (Beijing, China). Antibodies against SREBP-1 (K-10), MCAD (F-15), Apo A-I (FL-267), and SCAP (C-20) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and tubulin from Sigma (St. Louis, MO); horseradish peroxidase-coupled secondary antibodies were from Zhongshan Golden Bridge (Beijing, China).

Animal treatment. Induction of NS in male Sprague-Dawley rats was as described (47). A total of 200- to 220-g rats were purchased from the Experimental Animal Center at Peking University Health Science Center (Beijing, China). NS rats were given sequential intraperitoneal (ip) injections of PAN (130 mg/kg on day 1 and 60 mg/kg on day 14). Control rats received ip injections of saline. At week 4, the rats were killed, and tissue samples were snap-frozen in liquid nitrogen, then stored at −80°C. Plasma concentrations of total cholesterol, triglycerides, albumin, and creatinine, and blood urea nitrogen were measured by an autoanalyzer (Hitachi 7170A, Tokyo, Japan). Plasma cholesterol and triglyceride profiles were measured by fast-protein liquid chromatography (FPLC). Plasma glucose levels were measured by the glucose oxidase method, and insulin levels were measured by use of an unsensitizing rat insulin ELISA kit (LINCO Research). Twenty-four-hour urinary excretion of protein concentration was determined by a quantitative colorimetric assay by use of a kit from Applygen (Beijing, China). Body and organ weight were also measured. The hepatic lipids were extracted by homogenization of weighed pieces of liver tissue in chloroform:methanol (2:1 vol/vol) solvent (Beijing Chemical Technology). Aliquots from the clear supernatant were used for estimation of cholesterol and triglycerides after the solvent was dried off under a stream of nitrogen. The concentrations of hepatic lipids were expressed as milligrams per gram fresh tissue. All experiments were approved by the Animal Experimentation Committee of Peking University Health Science Center.

RNA extraction and purification. Total RNA from rat livers was isolated by use of TRIzol reagent. A tissue sample (in the amount of 100 mg) was homogenized in 1 ml TRIzol. Phase separation of RNA was performed by adding one-fifth volume of chloroform and then centrifugation at 12,000 g for 10 min. Isopropyl alcohol (0.5 ml/1 ml TRIzol) was added to the aqueous phase to precipitate total RNA, which was followed by two washes with 75% and 100% ethanol. The RNA sample was dried and then dissolved in diethylpyrocarbonate-treated water. RNA quality was determined by optical density (OD) 260/280 ratio. Total RNA extracted was further purified by use of a NucleoSpin RNA Clean-up kit (CapitolBio, Beijing, China).

Affymetrix GeneChip analysis. Total RNA isolated from two control and two nephrotic livers was treated with RNA Clean-up to obtain an OD 260/280 ratio between 1.9 and 2.1 for each sample. The gene expression profile of each sample was examined by use of the Affymetrix chip for rats (AFF-900408). The chip measures the expression of 31,100 probe sets. The Signal Log Ratio (SLR) estimates the magnitude and direction of change in level of a transcript when two arrays are compared (NS vs. control). The gene expression data was analyzed by Affymetrix GeneChip Operating Software Version 1.0. Biological pathway analysis was performed by using GenMAPP Pathway (http://www.genmapp.org/).

Quantitative real-time PCR. Two micrograms of each RNA sample were reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (Promega) and hexadenoxyribose random primers. cDNA samples were then used as templates for quantitative PCR. Real-time PCR analysis involved use of SYBR Green 1 (Invitrogen) according to the manufacturer’s instructions. Primers for the different genes are in Supplemental Table 4 (all supplemental materials are available in the online version of this article on the journal Web site). The PCR reactions were carried out at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 62–64°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 5 min. β-Actin was used as an internal control.

Northern blot analysis. The rat probes for Northern blot analysis were prepared by RT-PCR. The primer sets for Apo A-I were 5'-GGT CCA CTG TTG GTC GCC TAC-3' (sense) and 5'-CTG GCC TTG GTA TGA TAC CCG-3' (antisense) and for Acly 5’-CCT CTT CAG CCG ACA TAC CA-3’ (sense) and 5’-CAC ACT GCC TGG GCG ATA CAG-3’ (antisense). The PCR reactions were for 5-min initial denaturation at 94°C, followed by 35 cycles of 94°C denaturation for 30 s, 60°C (Apo A-I) or 64°C (Acly) annealing for 30 s, and 72°C extension for 30 s with a final extension at 72°C for 5 min in the Geneamp PCR system (Applied Biosystems). PCR products with expected sizes (412 bp for Apo A-I and 499 bp for Acly) were subcloned to the pGEM-T vector (Tiangen) according to the TA cloning method and confirmed by sequencing. The probes were labeled with α-32P-dCTP by use of a DNA labeling kit (Promega) according to the manufacturer’s instructions.

Twenty micrograms of RNA for each sample was diluted in 20 μl of sample buffer (containing 0.8 μl of 1 M triethanolamine, 0.08 μl of 0.5 M EDTA (pH 8.0), 3.33 μl formaldehyde, 10.0 μl deionized formamide, 5.71 μl bromophenol blue, and 0.08 μl diethylpyrocarbonate-treated water), then denatured at 65°C for 10 min and chilled on ice immediately. After 1 μl ethidium bromide (1 mg/ml) was added, samples were electrophoresed on a 1% agarose gel containing 0.04 M triethanolamine, 2 μM EDTA (pH 8.0), 0.05% SDS, and 8% formaldehyde in chilled buffer. After the gel was washed in 10× SSC for 30 min with gentle shaking, RNA was transferred to a Hybond-N+ nylon membrane (Amersham). The membrane was prehybridized for 1 h in ExpressHyb Hybridization Solution (Clontech) at 42°C and then hybridized in the same solution with 100 ng of denatured rat probes for 2 h at 55°C. The membrane was then washed with 2× SSC containing 1% SDS, 1× SSC containing 0.5% SDS, and 1× SSC containing 0.5% SDS for 5 min each at 55°C, then exposed to X-ray film (Kodak) at −70°C for the indicated time.

Western blot analysis. An amount of 100 mg of frozen rat liver tissue was homogenized in 1 ml of 20 mmol/l Tris·Cl (pH 7.4) buffer containing 1 mmol/l EDTA. The crude extract was centrifuged at 12,000 g at 4°C for 2 min. The supernatant was harvested in SDS-PAGE sample buffer (50 mM, pH 6.8, Tris·Cl, 2% β-mercaptoethanol, 5% SDS, 10% glycerol, 0.02% bromophenol blue), followed by repetitive aspiration. One hundred-microgram protein samples were loaded onto 10% SDS-PAGE gels and transferred to nitrocellulose membrane (Applygen, Beijing, China). After being dyed with Ponceau S, the membrane was washed and blocked in PBS (1× PBS with 0.02% Tween 20) supplemented with 5% fat-free powder for 1 h at room temperature with gentle shaking, then incubated with a rabbit anti-human Apo A-I antibody (1:200), a goat anti-human SCAP antibody (1:200), or rabbit anti-human SREBP-1 antibody (1:500) at 4°C. The membrane was washed...
three times for 20 min in PBST and then incubated with horseradish peroxidase-coupled secondary antibodies for 1 h at room temperature. After being washed three times, the membrane was then transferred to the ECL Reagent (Applygen) and exposed to XBT-1 film (Kodak).

**Gel shift assay.** The probes corresponding to nucleotides −79 to −41 of the Acly promoter, which contains a putative SRE site (5′-GTG-AGCTGAT-3′), were chemically synthesized and labeled with γ-[32P]dATP by use of a DNA labeling kit (Promega) (31). The isotope-labeled probe was incubated with liver nuclear extract (6 μg) in a final volume of 20 μl containing 10 mM HEPES (pH 7.9), 75 mM KCl, 1 mM EDTA, 5 mM DDT, 5 mM MgCl2, 0.5% BSA, and 1 μg poly (dl-dC). After a 20-min incubation at room temperature, samples were resolved on 5% polyacrylamide gel in 0.5× TBE at 45 mA for ~2 h at 4°C. For the competition assays, unlabeled oligonucleotides were added to the reactions at an ~250-fold molar excess. The sequences of the oligonucleotides are as follows: Acly −79 to −41, 5′-GCA TGG CCT GTG AGC TGA TGG GGG GCG GGG AGG AGC CCG-3′ (sense); 5′-CGG GCT CCT CCC CGC CCC CCA TCA GCT CAC AGG CCA TGC-3′ (anti-sense).

**Data analysis.** Data are means ± SE. Analysis involved ANOVA and Student’s t-test. P < 0.05 was considered significant.

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**RESULTS**

**Nephrotic and lipid profile of rats.** Compared with controls, nephrotic rats showed predominant proteinuria and hypoalbuminemia and increased plasma cholesterol and triglyceride levels. FPLC analysis showed NS rats with significantly higher levels of VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol, and VLDL-triglycerides than control rats (Fig. 1). Although body weight and the fat-to-body weight ratio were significantly lower in the NS group than in controls, the ratios of body weight to liver weight and kidney weight were significantly greater in the NS group than in controls (Fig. 2), with no significant difference in plasma creatinine level, creatinine clearance rate, and blood urea nitrogen level (data not shown). Plasma glucose and insulin levels were not significantly different between control and NS groups (Supplemental Fig. 1). Although liver cholesterol levels did not differ between control and NS groups, liver triglyceride levels were significantly higher in NS rats than in controls (Supplemental Fig. 2).

**Gene expression profile associated with cholesterol metabolism in the liver.** A total of 389 genes (197 upregulated and 192 downregulated) were identified by Affymetrix assay.
These genes were divided into subgroups according to function as follows: lipid metabolism; transporting; transcription; cell proliferation and apoptosis; receptor and signal transduction; protein modification; and carbohydrate, amino acid, and nucleic acid metabolism (Supplemental Table 1). Twelve hepatic genes associated with cholesterol metabolism were identified by Affymetrix analysis (Supplemental Table 2). Among them, one gene critical to HDL metabolism, Apo A-I, was increased in level in nephrotic rats. Seven upregulated genes involved in cholesterol biosynthesis were ATP-citrate lyase (Acly), acetyl-CoA acetyltransferase (Acat), mevalonate pyrophosphate decarboxylase (Mpd), farnesyl diphosphate synthetase (Fdps), squalene synthetase (Ss), lanosterol synthetase (Lss), and NAD(P)H steroid dehydrogenase-like (Nsdhl). Both quantitative real-time PCR and Northern blot analysis confirmed a marked increase in Apo A-I mRNA level in NS livers (Fig. 3, A and B), and Western blot analysis showed a significant increase in Apo A-I protein expression in NS livers (Fig. 3C). Increased expression of the seven genes involved in cholesterol biosynthesis was also confirmed by real-time PCR analysis (Fig. 4A). Northern blot analysis further demonstrated increased Acly gene expression in nephrotic livers (Fig. 4B).

Gene expression profile associated with fatty acid metabolism in the liver. Affymetrix analysis identified 14 genes associated with fatty acid metabolism (Supplemental Table 3). Quantitative real-time PCR confirmed a significant increase in mRNA levels of four of the genes (Acc, FAS, ELOVL2, and ELOVL6) in nephrotic livers (P < 0.05), with the expression of Scd1, Fads1, and Fads2 not differing between nephrotic rats and controls (Fig. 5A). Three genes critical for triglyceride production (Gpam, Agpat 3, and Dgat 1) identified by Affymetrix analysis were significantly upregulated in NS livers by real-time PCR (P < 0.05) (Fig. 5B). Among four genes involved in fatty acid oxidation identified, decreased expression of Dct and MCAD in NS livers was confirmed by real-time PCR, with no significant difference in Crat and Acox 1 expression between nephrotic rats and controls (Fig. 5C).

Expression of genes involved in SREBP-1 activation. SREBP-1 and three genes essential for its trafficking and cleavage, including SREBP cleavage-activating protein (SCAP), insulin-induced gene 1 (Insig 1), and Insig 2, were differentially regulated; SREBP-1, SCAP and Insig 1 were upregulated and Insig 2 was downregulated in nephrotic livers. Quantitative real-time PCR, Northern blot analysis, and immunoblotting were used to confirm the changes in expression of genes identified by Affymetrix assay. SREBP-1, SCAP, and Insig 1 mRNA level was higher in NS livers than in controls, and Insig 2 mRNA level was markedly reduced (Fig. 6A). Immunoblotting revealed a marked increase in levels of SCAP and the mature form of SREBP-1 (mSREBP-1, 68 kDa) protein in nephrotic livers (Fig. 6, B and C). A gel shift assay further revealed a significantly increased binding of SREBP(s) to the SRE site in the Acly promoter in nephrotic livers (Fig. 6D). Since the levels of SREBP-2 mRNA and protein were not changed in nephrotic livers (Supplemental Fig. 3), these findings further support the activation of SREBP-1 in nephrotic livers.

DISCUSSION

NS is characterized by increased proteinuria, hypoalbuminemia, fluid retention, and hyperlipidemia. As one of the major features of NS, hyperlipidemia is marked by elevated levels of plasma cholesterol, triglycerides, LDL, and VLDL (18, 49), with increased (25) or unchanged level of HDL (18). Maintenance of normal plasma LDL and VLDL levels depends on the rates of their synthesis and catabolism. LDL is cleared primarily via the LDL receptor (LDLR) by the liver (37, 38), and VLDL is removed via the VLDL receptor (VLDLR) by heart, skeletal muscle, and adipose tissue (40, 48). In the present study, nephrotic rats showed elevated levels...
of total cholesterol, LDL-cholesterol, and VLDL-cholesterol but normal hepatic cholesterol content and increased hepatic triglyceride levels. The normality of hepatic tissue cholesterol levels despite severe hypercholesterolemia suggests that LDL clearance in the liver and VLDL clearance in other tissues may be defective in NS rats. In fact, previous studies by Vaziri and Liang (22, 34, 46) have revealed that impaired LDL and VLDL clearance in NS is due in part to downregulation of LDLR expression in the liver and VLDLR expression in skeletal muscle and adipose tissue. In addition, an early study of in vivo hepatic lipogenesis and cholesterol synthesis revealed that the biosynthesis of neutral lipids from both \[^3\]H\(_2\)O and \[^3\]H-palmitate was elevated in nephrotic rats (10). Several other studies further demonstrated that the increased secretion of VLDL in the liver of nephrotic rats appears to be mainly due to posttranscriptional events possibly related to increased lipogenesis (25, 36). Taken together, these findings suggest that NS-associated hypercholesterolemia may result from both hepatic overproduction and impaired catabolism, as well as clearance of cholesterol-rich lipoproteins.

So far, the pathogenesis of hyperlipidemia in NS remains incompletely characterized. Alteration of hepatic cholesterol, fatty acid, and triglyceride metabolism as a result of increased biosynthesis, decreased degradation, or their combination has been considered the major underlying mechanism. Using whole-genome scanning by Affymetrix assay, we revealed 21 genes involved in cholesterol biosynthesis, HDL assembly, fatty acid production and oxidation, and triglyceride biosynthesis showing significant regulation in the livers of nephrotic rats. Increased expression of hepatic enzymes in biosynthetic pathways of cholesterol, fatty acids, and triglycerides was associated with enhanced SREBP-1 rather than SREBP-2 expression at both transcriptional and posttranslational levels.

Affymetrix study of nephrotic rat liver identified 12 genes associated with cholesterol metabolism; seven, i.e., Acly, Acat, Mpd, Fdps, Ss, Lss, and Nsdhl, were upregulated. The cholesterol biosynthetic pathway involves multiple enzymatic steps and is tightly controlled via a negative feedback by some intermediate substrates, as well as the final product, cholesterol. Figure 7 shows the major enzymes and metabolic intermediates in the synthetic pathway for cholesterol. Acly, which was upregulated in nephrotic rat liver, is a homotetrameric cytosolic enzyme that catalyzes the formation of oxaloacetate and acetyl-CoA from citrate in an ATP-dependent manner and was first identified as a phosphoprotein (33). The upregulation of Acly may result in more acetyl-CoA production, leading to enhanced cholesterogenesis and lipogenesis. Acat, also called acetoacetyl-CoA thiolase, is localized in the cytosol and catalyzes the initial step in cholesterol synthesis (3). Acat, together with HMG-CoA synthetase, catalyzes the formation of the substrate HMG-CoA for HMG-CoA reductase to form mevalonate, the rate-limiting enzyme in the pathway. Increased expression of Acat may contribute to more HMG-CoA biosynthesis in nephrotic livers. A series of reactions then converts...
mevalonate to squalene. Mpd catalyzes a bimolecular reaction between mevalonate pyrophosphate and ATP to form isopentenyl pyrophosphate, inorganic phosphate, CO2, and ADP (30). Fdps is a member of the prenyltransferase family and catalyzes the sequential condensation of three 5-carbon isoprene units to form farnesyl pyrophosphate (42), which is further metabolized by Ss to form squalene (29). Upregulation of Mpd, Fdps, and Ss in nephrotic livers may result in more production of squalene, the first specific intermediate in the cholesterol biosynthetic pathway. The nephrotic livers also exhibited significantly increased expression of Lss, an enzyme catalyzing the conversion of oxidosqualene to lanosterol, forming a total of six new carbon-carbon bonds in a single reaction. The regulation of Lss levels in vivo has clinical importance and has been a potential target for the design of hypocholesterolemic drugs (1). Nsdhl encodes a 3β-hydroxysterol dehydrogenase.

Apo A-I, the main structural component of HDL, is synthesized by the liver and intestine and is primarily catabolized by the liver, kidney, skin, and other tissues (11). Our study showed a marked increase in plasma HDL concentration in nephrotic rats, which was accompanied by elevated hepatic Apo A-I mRNA and protein expression, which agrees with results of other studies (23, 27, 41). Increased hepatic Apo A-I production and decreased catabolism of Apo A-I (19, 39) both may contribute to elevated levels of plasma Apo A-I and HDL in nephrotic rats.

The pathogenesis of hypertriglyceridemia in NS is unclear. Multiple mechanisms postulated include 1) de novo fatty acid synthesis and uptake of plasma free fatty acids; 2) fatty acid synthetic pathway. The nephrotic livers also exhibited significantly increased expression of Lss, an enzyme catalyzing the conversion of oxidosqualene to lanosterol, forming a total of six new carbon-carbon bonds in a single reaction. The regulation of Lss levels in vivo has clinical importance and has been a potential target for the design of hypocholesterolemic drugs (1). Nsdhl encodes a 3β-hydroxysterol dehydrogenase. Increased expression of this gene in nephrotic livers may lead to enhanced sterol dehydrogenase activity of Nsdhl enzyme, which is involved in the removal of C-4 methyl groups from the cholesterol precursor lanosterol, finally converting to cholesterol (5). Although it is currently unclear whether Acat, Mpd, Ss, Lss, and Nsdhl are direct target genes of SREBP-1, Acly and Fdps have been previously shown to be under transcriptional control of SREBP-1 (17, 31) (Fig. 7), suggesting SREBP-1 may be involved in overproduction of hepatic cholesterol and hypercholesterolemia. Interestingly, the present study showed that the expression level of neither cholesterol-binding proteins (such as oxysterol-binding protein and caveolin-1) nor cholesterol-catalyzing genes [such as cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in cholesterol catabolism] was significantly altered in nephrotic rat livers.

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catalysis by oxidation in mitochondria, peroxisomes, and endoplasmic reticulum; and 3) triglyceride synthesis from fatty acids (Fig. 7). The present study identified nine genes associated with fatty acid metabolism; four upregulated in nephrotic livers were Acc, FAS, ELOVL2, and ELOVL6. Acc catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, a key molecule in the control of fatty acid metabolism (21). FAS is a multifunctional enzyme that catalyzes de novo biosynthesis of palmitate. The substrates for this anabolic enzyme, such as acetyl CoA and malonyl CoA, are supplied by the serial reactions catalyzed by Acc and Fas. Upregulation of Acc and FAS identified in nephrotic livers may therefore increase long-chain fatty acid biosynthesis. These long-chain fatty acids play an important role in cellular biological functions, including energy metabolism and membrane fluidity. Mammals have long been thought to possess a membrane-bound enzyme that elongates and/or desaturates saturated fatty acyl-CoAs produced by FAS or derived from dietary resources (6). Several enzymes involved in the elongation of long-chain fatty acids include ELOVL2 and ELOVL6. Increased expression of ELOVL2 and ELOVL6 suggests these elongases play an important role in enhanced biosynthesis of long-chain saturated and monosaturated fatty acids in conjunction with FAS and Scds (28). Collectively, the present studies demonstrate that four genes (Acc, FAS, ELOVL2, and ELOVL6) with increased expression levels in nephrotic livers may be involved in overproduction of hepatic fatty acids, thereby contributing to the pathogenesis of hypertriglyceridemia. Since all of these four genes are reported to be regulated by SREBP-1 (15, 16), it is reasonable to speculate that SREBP-1 might represent an underlying mechanism in the pathogenesis of NS-associated hyperlipidemia.

Fatty acids are critical substrates for biosynthesis of triglycerides. Mammals contain two main biochemical pathways for triglyceride biosynthesis: the monoacylglycerol pathway, which plays an important role in nutrient absorption in the small intestine; and the glycerol-3-phosphate pathway, which is responsible for most of de novo triglyceride synthesis. Several genes, especially Gpam, Agpat, and Dgat, are involved in catalyzing the several steps of triglyceride synthesis in turn (Fig. 7). In agreement with a previous report, we found that the mRNA level of hepatic Dgat 1, the enzyme catalyzing the final step in the biosynthesis of triglycerides, was significantly increased in nephrotic rats (44). In the present study, we also identified that two additional genes, Gpam and Agp3, were significantly upregulated in nephrotic livers. Taken together, these findings suggest an important role for Dgat 1, Gpam, and Agp3 in increased hepatic triglyceride production in NS rats. Among the above-mentioned three genes involved in triglyceride biosynthesis, Gpam has been previously proved to be a SREBP-1 target gene (8), suggesting SREBP-1 may play a role in the pathogenesis of hypertriglyceridemia.

Fatty acid degradation in most organisms occurs primarily via β-oxidation. Both mitochondrial and peroxisomal β-oxidation catalyze the chain shortening of acyl-CoA esters, which require the participation of β-oxidation enzymes, including acyl-CoA dehydrogenase, acyl-CoA oxidase, delta 3-, and delta 2-enoyl-CoA isomerase (14). We identified that MCAD and Dei, two genes involved in fatty acid oxidation in mitochondria, were significantly downregulated in the nephrotic liver. Since both are key enzymes in mitochondrial fatty acid β-oxidation (50), their downregulation may therefore result in accumulation of fatty acids in nephrotic livers.
Cholesterol and fatty acid biosynthesis is under tight control by a multivalent feedback mechanism both at transcriptional and posttranscriptional levels (12). The transcriptional regulation is mediated through the action of SREBP5, membrane-bound transcription factors that enhance transcription of genes encoding biosynthetic enzymes of cholesterol, fatty acids, and triglycerides (4). Among three SREBP isoforms, SREBP-1 primarily activates genes involved in lipogenesis. SREBP-1 is synthesized as an inactive precursor protein (preSREBP-1). To be active, the NH2-terminal segment of SREBP-1 (mSREBP) must be released from the membrane so that it can translocate to the nucleus of the cell. The release of active mSREBP requires two sequential cleavages in the Golgi (13). This process begins when SCAP, as a sensor of sterols and an escorter of SREBP-1 in endoplasmic reticulum (ER), binds with the COOH-terminal domain of SREBP-1. When the cellular demand for cholesterol is increased, SCAP escorts SREBP from the ER to the Golgi, where it is cleaved sequentially by two proteases to release mSREBP-1. Our finding of significant elevation in mRNA and protein levels of SCAP in nephrotic rats implies that increased hepatic SCAP levels may facilitate SREBP-1 cleavage and activation. In support of this, the gene expression, maturation, nuclear translocation, and DNA binding activity of SREBP-1 were significantly increased in the livers of NS rats. The increased transcriptional activity of SREBP-1 thereby resulted in marked upregulation of its target.
genes involved in cholesterol and fatty acid biosynthesis in the liver. The importance of SREBP-1 in enhanced lipid synthesis was further supported by our finding of binding of SREBP-1 to the SRE site in the Acly promoter. Interestingly, hepatic expression and maturation of SREBP-2, unlike that of SREBP-1, did not differ between control and NS rats. A similar finding was previously reported by other investigators (20). The underlying mechanism remains unclear. It is possible that, in addition to SCAP, other unidentified factors may be involved in SREBP-1 but not SREBP-2 activation in nephrotic livers.

When the cellular cholesterol level is increased, the SCAP/SREBP complex is retained in the ER by binding to two related ER proteins, Insig 1 and Insig 2, thereby preventing SREBP processing and decreasing lipid synthesis. In our study, Insig 1 expression was increased and Insig 2 level decreased in nephrotic rats. Insig 1, by binding the SCAP/SREBP complex, retains SREBP-1 in the ER, therefore decreasing lipid biosynthesis and lowering the intracellular lipid level. Although it is currently unknown, the underlying mechanism involved in upregulation of the Insig 1 gene in nephrotic livers, increased expression of Insig-1 may be a result of feedback regulation by overproduction of hepatic triglycerides and cholesterol. Although the two isoforms were differentially regulated in nephrotic livers is unclear, the expression of Insig 1 and Insig 2 was not sufficient to override the effect of SCAP, since active SREBP-1 (mSREBP-1) level was significantly increased.

We validated the expression of most of the genes in the present study only at the mRNA level. The change at the transcriptional level does not always predict activity, since it has been repeatedly shown that LDLR and HDLR (SR-B1) protein abundance is significantly reduced in the absence of an mRNA level change in nephrotic livers (23, 45, 46). In addition, although many genes identified are SREBP-regulated genes, several major target genes of SREBP, including HMG-CoA synthase and reductase, were not changed in expression. One possible explanation is that the transcription of HMG-CoA synthase and reductase is mainly regulated by SREBP-2 rather than SREBP-1 (15, 16). The present study clearly demonstrated that the hepatic expression of SREBP-1 rather than SREBP-2 was increased in nephrotic livers (20). Another possible explanation is that the expression of HMG-CoA reductase is transiently increased after the induction of hypercholesterolemia but returns to normal levels during the maintenance phase of hypercholesterolemia in chronic NS. Under a steady-state condition, a sustained increase in cholesterol synthesis is not necessary for hypercholesterolemia, which is maintained by other mechanisms (2, 43, 47). Therefore, in addition to an enhanced SREBP-1 pathway, other mechanisms may be involved in increased hepatic cholesterol synthesis and lipogenesis in NS rats.

In conclusion, our studies have identified a set of hepatic genes involved in the pathogenesis of hypercholesterolemia and hypertriglyceridemia in NS. Marked upregulation of genes involved in hepatic cholesterol biosynthesis and increased expression of the Apo A-I gene in the liver may result in overproduction of cholesterol, leading to hypercholesterolemia. Increased expression of genes involved in hepatic fatty acid and triglyceride biosynthesis and downregulation of genes involved in hepatic fatty acid β-oxidation may account for increased biosynthesis of hepatic fatty acids and triglycerides, thus resulting in hypertri-
glyceridemia. Overactivation of the SREBP-1 pathway represents a possible underlying mechanism for the hyperlipidemia in NS.

REFERENCES


