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Hepatic tissue sterol regulatory element binding protein 2 and low-density lipoprotein receptor in nephrotic syndrome

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Abstract

Hypercholesterolemia is a main feature of nephrotic syndrome (NS) and is, in part, caused by acquired low-density lipoprotein (LDL) receptor deficiency. The LDL receptor deficiency in NS is accompanied by normal hepatic LDL receptor messenger RNA (mRNA) abundance. Expression of LDL receptor, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and several other cholesterol-regulatory factors is regulated by sterol regulatory element binding protein 2 (SREBP-2). This study tested the hypothesis that nephrotic hypercholesterolemia may be associated with dysregulation of hepatic tissue SREBP-2 abundance or activity. Protein and mRNA abundance of SREBP-2, LDL receptor, and HMG-CoA reductase was determined in the livers of rats with chronic puromycin-induced NS and of control rats. The nephrotic group showed heavy proteinuria, hypoalbuminemia, severe hypercholesterolemia, and normal liver tissue total and free cholesterol concentrations. Despite severe hypercholesterolemia, the inactive microsomal and the active nuclear SREBP-2 levels were unchanged in the liver of the nephrotic animals. This was associated with a marked reduction in LDL receptor protein abundance. In confirmation of our earlier studies, LDL receptor and HMG-CoA reductase mRNA levels were unchanged in nephrotic animals. Hepatic SREBP-2 abundance and activity in hypercholesterolemic nephrotic rats were similar to those found in the normocholesterolemic control animals, representing a maladaptive response. This paradox may be, in part, due to acquired LDL receptor deficiency that helps sustain SREBP-2 expression/activity and maintain hypercholesterolemia by limiting hepatic cholesterol uptake. This is because SREBP-2 expression and activity are, in part, regulated by intracellular as opposed to plasma cholesterol.
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1. Introduction

Hypercholesterolemia is one of the major features of nephrotic syndrome (NS) [1-4]. Hypercholesterolemia in NS is associated with marked reductions of hepatic low-density lipoprotein (LDL) receptor [5,6] and high-density lipoprotein (HDL) receptor (SRB-1) [7], significant upregulation of hepatic acyl coenzyme A (CoA): cholesterol acyltransferase [8,9], and dysregulation of 3-hydroxy-3-methylglutaryl (HMG)–CoA reductase and cholesterol 7-α hydroxylase [10,11]. Together, these abnormalities contribute to the genesis and maintenance of hypercholesterolemia by (*a*) limiting the hepatic uptake of plasma cholesterol (LDL receptor and SRB-1 deficiencies); (*b*) raising esterification and packaging of cholesterol in apolipoprotein B–containing lipoproteins for secretion in the circulation (upregulation of acyl CoA:cholesterol acyltransferase); and finally, (*c*) impeding the liver's ability to lower cholesterol biosynthesis (HMG-CoA reductase) and raise cholesterol catabolism to bile acids (cholesterol 7-α hydroxylase) for disposal in the gut.

Expression of several key proteins involved in cholesterol and fatty acid metabolism is regulated by the sterol regulatory element binding proteins (SREBPs) [12-14]. The SREBPs are members of the basic helix-loop-helix leucine zipper family of transcription factors that regulate expression of the target genes by binding to the sterol regulatory elements (SREs) in their promoter regions. The SREs have been identified in the promoter regions of HMG-CoA reductase [15], LDL receptor [16], fatty acid synthase [17], and SREBP-2 [18] genes. The SREBPs are synthesized as 125-kd precursor proteins that are stored in the endoplasmic reticulum as inactive complexes bound to SREBP cleavage activating protein (SCAP) [19]. The SCAP molecule that contains a sterol sensor domain regulates the activation of SREBPs according to the cellular sterol concentration. The precursor SREBPs undergo a unique

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Table 1
Primer sequences used in the study

Target mRNA	Forward	Reverse
RNA34	AGCCCCCTCACTCCCAAA	GGGTGCTGTGCTTCTGTGAAC
β-Actin	TCTGTGTGGATTGGTGGCTCTA	CTGCTTGCTGATCCACATCTG
HPRT	GCGAAAGTGGAAAAGCCAAGT	CCACATCAACAGGACTCTTGTAGATT
HMG-CoA reductase	ACCACCATATCAGTCAATGTGGAT	ACCACCTTGGCTGGAATGAC
SREBP-2	GGTACGCTGGTTACTCAAAAAGGT	CCCTCGCACTGCTCTTAGCT
LDL receptor	GCCCACTCACCCAAGTTCAC	GGTCGGTACAGTGTCGACTTCTG

posttranslational modification process to become active transcriptional factors [13,14]. In the presence of high sterol concentration, SREBP/SCAP complexes remain in the endoplasmic reticulum [20]. However, in the presence of low sterol concentrations, the complexes translocate to the Golgi apparatus. In the Golgi apparatus, the SREBPs undergo a 2-step sequential proteolytic cleavage that leads to the release of the biologically active 68-kd transcription factors [21]. The active/mature SREBPs, in turn, translocate to the nucleus and bind to SRE in the promoter region of target genes. Three different SREBP isoforms have been thus far identified: SREBP-1a, SREBP-1c, and SREBP-2 [12]. The SREBP-1a and SREBP-1c are synthesized by alternative splicing of messenger RNA (mRNA) transcript derived from a single gene, whereas SREBP-2 is encoded by a separate gene. Previous reports have suggested that SREBP-2 is predominantly involved in the regulation of cholesterol metabolism [22,23], SREBP-1c is preferentially involved in lipogenesis, and SREBP-1a is involved in the activation of SREBP-responsive genes related to lipid and glucose metabolism [24-26].

At high concentrations, intracellular sterols inhibit SREBP-2 gene expression [18] and limit maturation of its inactive precursor molecule [27]. These conclusions are based on the observation that SREBP-2 gene expression is down-regulated by cholesterol-loading and up-regulated by statin treatment in cultured cells and in experimental animals [18,23]. The proteolytic activation of SREBP-2 is enhanced, whereas that of SREBP-1 is reduced by cholesterol-lowering therapies (inhibition of cholesterol synthesis by statins or use of bile acid–sequestering agents) [23,28]. In contrast, polyunsaturated fatty acids inhibit SREBP-1 expression and maturation but have no effect on SREBP-2 activation [29,30]. Given the critical role of SREBP-2 in the regulation of cholesterol metabolism, we tested the hypothesis that hypercholesterolemia in NS may be associated with altered hepatic expression and/or activation of this transcription factor.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats were rendered nephrotic by sequential intraperitoneal injections of puromycin aminonucleoside (130 mg/kg on day 1 and 60 mg/kg on day 14). Placebo-injected rats served as controls. The rats were observed for 2 weeks after the second puromycin/placebo injection. The rats were housed in a temperature-controlled facility with 12-hour light/dark cycles and were provided free access to regular rat chow and water. At the end of the 2-week observation period, animals were placed in metabolic cages for a 24-hour urine collection. They were then anesthetized (pentobarbital 50 mg/kg IP) and euthanized by exsanguinations using cardiac puncture. The liver was immediately removed, frozen in liquid nitrogen, and stored at −70°C until processed. All experiments were approved by the University of California, Irvine Institutional Committee for the Use and Care of Experimental Animals. Serum creatinine, albumin, total cholesterol, triglyceride, HDL and LDL cholesterol concentrations, urinary protein excretion, and liver tissue total and free cholesterol were measured as described in our previous studies [31].

2.2. mRNA determinations

Frozen liver tissues were suspended in the lysis buffer containing a cocktail of antisense primers for the target mRNA and extracted with Polytron (Kinematica AG, Littau, Switzerland). Fifty microliters of each extract was transferred to 4 wells of the oligodeoxythymidine-immobilized microplate (quadruplicate) (GenePlate; RNAture, Irvine, CA) for mRNA purification [32,33] followed by complementary DNA synthesis [34]. Four microliters of

Table 2
Plasma concentrations of total cholesterol, LDL cholesterol, VLDL cholesterol, albumin, creatinine, and triglyceride, and total cholesterol–HDL cholesterol ratio, creatinine clearance, urinary protein excretion, and body weight in the NS and control groups

Groups	CTL	NS	*P*
Total cholesterol (mg/dL)	59 ± 2.2	264 ± 17	<.05
Free cholesterol (mg/dL)	17 ± 2.0	71 ± 8.1	<.05
LDL cholesterol (mg/dL)	27 ± 2.7	173 ± 16	<.05
VLDL cholesterol (mg/dL)	9.3 ± 0.9	33 ± 2.9	<.05
Total cholesterol–HDL cholesterol ratio	2.7 ± 0.3	4.8 ± 0.5	<.05
Triglycerides (mg/dL)	47 ± 4.5	167 ± 15	<.05
Albumin (g/dL)	3.8 ± 0.1	2.3 ± 0.2	<.05
Creatinine (mg/dL)	0.38 ± 0.02	0.43 ± 0.03	NS
Ccr (mL/min)	2.7 ± 0.2	2.4 ± 0.1	NS
Urine protein (mg/24 h)	11 ± 1.3	217 ± 25	<.05
Body weight (g)	346 ± 8.3	329 ± 7.0	<.05

CTL indicates control groups; Ccr; creatinine clearance; NS, not significant.

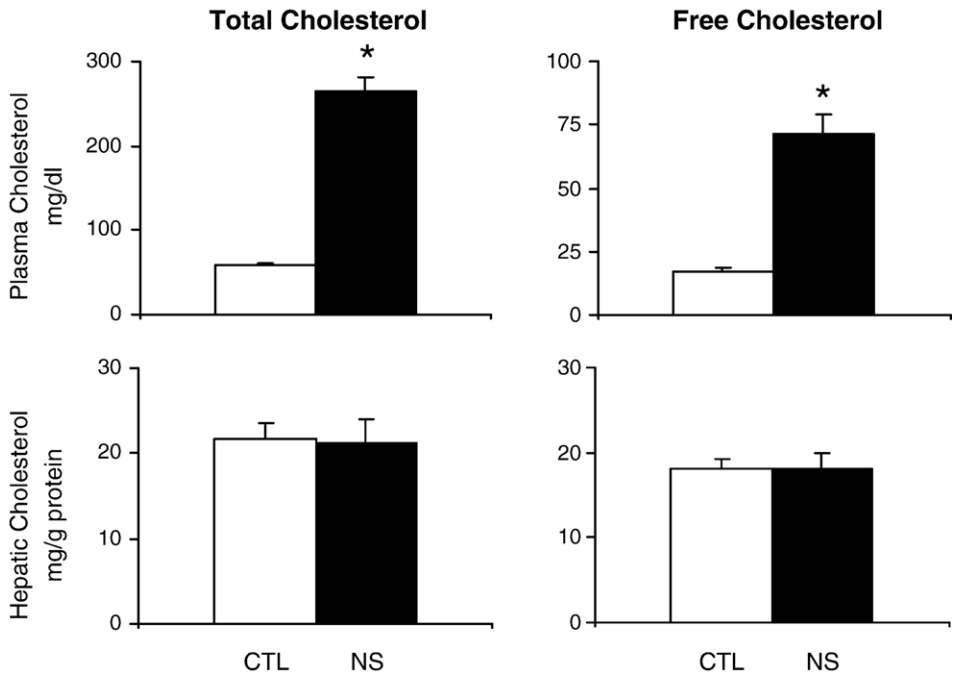

Fig. 1. Bar graphs depicting total and free cholesterol concentrations in the plasma and liver tissues of nephrotic and control rats. n = 6 animals in each group; *$P < .01$.

complementary DNA was then directly transferred to the 384-well polymerase chain reaction (PCR) plates, to which 5 μL iTaq SYBR master mix (BioRad, Hercules, CA) and 1 μL oligonucleotide cocktail (15 μmol/L each of forward and reverse primer) were applied; and PCR was conducted in PRISM 7900HT (Applied Biosystems, Foster City, CA), with 1 cycle of 95°C for 10 minutes followed by 45 cycles of 95°C for 30 seconds and 60°C for 1 minute each. The 1× reverse transcriptase buffer was used as negative control to confirm the absence of primer dimerization. Moreover, the melting curve was analyzed in each case to confirm that the PCR signals were derived from the single PCR product. The cycle threshold was determined by the SDS ABI analytical software. Hypoxanthine guanine phosphoribosyl transferase (HPRT) and β-actin were used as internal controls, and RNA34 was used as external control. Because the values of the 3 controls were very similar between the control and nephrotic rats, the SREBP-2, LDL receptor, and HMG-CoA reductase mRNA values were normalized against HPRT. Nucleotide sequences for various target genes were retrieved from the UniGene database in the GenBank. The PCR primers for each gene were designed by Primer Express (Applied Biosystem, Foster City, CA) and HYB simulator (RNAture) and synthesized by IDT (Coralville, IA). The primer sequences are depicted in Table 1.

2.3. Microsomal and nuclear protein extractions

Liver tissue was homogenized in solution A containing 0.25 mol/L sucrose, 50 mmol/L Tris-HCl (pH 7.5), 3 mmol/L $MgCl_2$, and protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 3 μg/mL pepstatin, 5 μg/mL leupeptin, and 10 μg/mL aprotinin). The homogenate was centrifuged at 800*g* for 5 minutes at 4°C. A portion of the supernatant was stored at −70°C and used for measurement of total tissue protein. The remaining supernatant was centrifuged at 100 000*g* for 45 minutes at 4°C to precipitate microsomes. Microsomal pellets were resuspended in solution A, and aliquots were stored at −70°C until processed. The pellet was stored at −70°C and subsequently used for preparation of the nuclear extract.

Nuclear extracts were prepared by a minor modification of the method described by Haddad et al [35]. Briefly, the pellet obtained from the first centrifugation was resuspended in solution B (0.25 mol/L sucrose, 50 mmol/L Tris-HCl [pH 7.5], 1 mmol/L EDTA, and protease inhibitors) using a glass pestle homogenizer. The homogenate was filtered through a 53-μm nylon mesh (Spectrum Laboratories, Rancho Dominguez, CA), and filtrate was then centrifuged at 800*g* for 5 minutes. The pellet was collected, resuspended in 0.5% Triton X–supplemented solution B, and centrifuged at 800*g* for 10 minutes. The resulting pellet was resuspended in nuclear storage buffer (20 mmol/L Tris-HCl [pH 7.5], 25% glycerol, 1 mmol/L $MgCl_2$, 1 mmol/L EDTA, 1 mmol/L dithiothreitol, and protease inhibitors) supplemented with 0.4 mol/L KCl and extracted for 45 minutes on ice with sporadic vortex. The mixture was then centrifuged at 5000*g* for 30 minutes, and the supernatant was collected and diluted with 3 vol of the nuclear storage buffer. The nuclear extract was then loaded onto the Centrion-10 column (Millipore, Bedford, MA) and centrifuged at 5000*g*, and the resulting concentrate was stored at −70°C in aliquots until used.

2.4. Western blot analyses

The SREBP-2 protein abundance in the microsomal and nuclear fractions of the liver tissue was measured by Western blot analysis using a rabbit anti–rat SREBP-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Briefly, aliquots containing 50 μg proteins were fractionated on 4% to 20% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 2 hours and transferred to Hybond-ECL membrane (Amersham Life Science, Arlington Heights, IL).

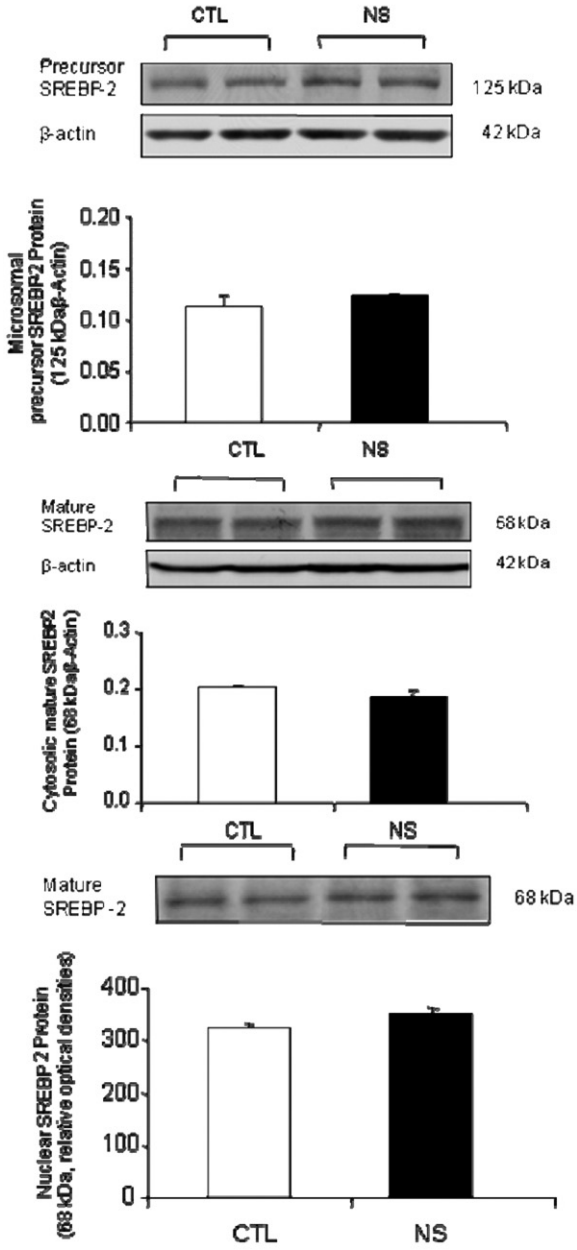

Fig. 2. Representative Western blots and group data depicting protein abundance of the microsomal SREBP-2 precursor molecule and active/mature cytosolic and nuclear SREBP-2 fractions in the livers of the NS and control rats. n = 6 in each group. CTL indicates control rats.

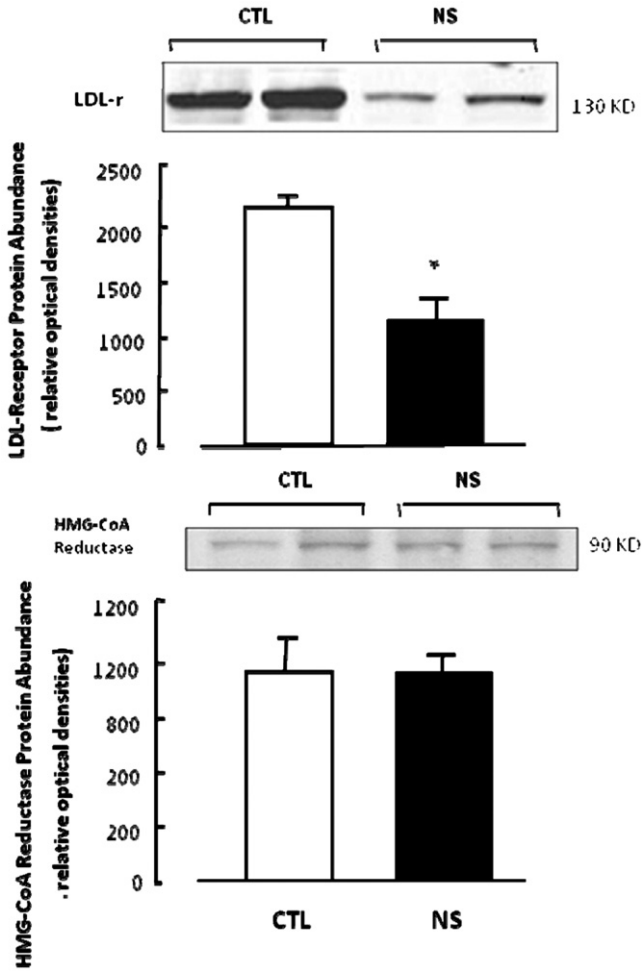

Fig. 3. Representative Western blots and group data depicting protein abundance of hepatic HMG-CoA reductase and LDL receptor in the NS and control groups. n = 6 in each group; *$P < .01$.

The membrane was incubated for 1 hour in blocking buffer (1× Tris-buffered saline [TBS], 0.05% Tween-20, and 5% nonfat milk) and then overnight in the same buffer containing 1:1000 polyclonal anti–SREBP-2 antibody (Santa Cruz Biotechnology). Membrane was washed 3 times for 5 minutes in 1× TBS and 0.05% Tween-20 before 2 hours of incubation in a buffer (1× TBS, 0.05% Tween-20, and 3% nonfat milk) containing horseradish peroxidase–linked anti–rabbit immunoglobulin G (Amersham Life Science) at 1:3000 dilution. The membrane was washed 4 times and developed by autoluminography using the ECL chemiluminescent agents (Amersham Life Science).

Hepatic tissue HMG-CoA reductase and LDL receptor protein abundance was measured by Western blot as described in our earlier studies [31] using a polyclonal anti–HMG-CoA reductase antibody (generously supplied by Prof GC Ness, Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, FL) and an anti–bovine LDL receptor antibody purchased from Cortex Biochem (Davis, CA).

Table 3
Hepatic HMG-CoA reductase, LDL receptor, SREBP-1, and SREBP-2/HPRT mRNA ratios in the NS and normal control rats

	Control	Nephrotic	*P*
HMG-CoA reductase	2.01 ± 0.76	1.47 ± 1.07	NS
SREBP-2	0.51 ± 0.21	0.58 ± 0.23	NS
LDL receptor	1.12 ± 0.30	0.96 ± 0.41	NS

Data are presented as mean ± SD of results obtained from 5 rats.

2.5. Data analysis

Student *t* test was used in statistical evaluation of the data, which are shown as mean ± SEM. *P* values less than .05 were considered significant.

3. Results

3.1. General data

Data are shown in Table 2 and Fig. 1. The nephrotic group exhibited severe proteinuria, hypoalbuminemia, and elevation of plasma free and total cholesterol, LDL, very low-density lipoprotein (VLDL), triglyceride, free fatty acid, and total cholesterol–HDL ratio.

In contrast to plasma cholesterol level, liver tissue free and total cholesterol concentrations were not significantly different between nephrotic and control groups.

3.2. SREBP-2, LDL receptor, and HMG-CoA reductase data

Data are illustrated in Figs. 2 and 3 and Table 3. No significant difference was found in hepatic tissue SREBP-2 mRNA abundance between the nephrotic and control rats. Protein abundance of the 125-kd SREBP-2 precursor molecule in the microsomal fraction of the liver was similar among the nephrotic and control groups. Likewise, abundance of the active/mature (68 kd) SREBP-2 molecule in the cytosolic and nuclear fractions was comparable among the nephrotic and control rats.

The LDL receptor protein abundance was markedly reduced, but LDL receptor mRNA abundance was unchanged in the nephrotic animals. The HMG-CoA reductase protein and mRNA abundance was similar among the nephrotic and control groups.

4. Discussion

As noted earlier, SREBPs regulate expression of a number of genes involved in lipid metabolism by binding to SRE in their promoter regions [28]. The SREBP was initially identified as a transcriptional factor binding to the SRE in the promoter region of the LDL receptor gene [16]. Subsequently, SRE was found in the promoter regions of genes encoding several other lipid-regulatory factors, such as HMG-CoA reductase [15], fatty acid synthase [17], ATP-binding cassette A-1 (ABCA-1) [31], and SREBP-2 itself [18]. Among the identified SREBP isoforms, SREBP-2 plays a key role in the regulation of cholesterol homeostasis [22,23] Expression of SREBP-2 seems to be regulated by intracellular cholesterol both at the transcriptional and posttranslational levels [18,28,36].

Despite severe hypercholesterolemia, liver tissue SREBP-2 mRNA abundance, microsomal SREBP-2 precursor protein abundance, and active nuclear SREBP-2 fraction were unchanged in animals with chronic NS. Given the existence of severe hypercholesterolemia, the absence of change in hepatic SREBP-2 seems to represent a maladaptive response in the nephrotic animals. It should be noted however that SREBP-2 expression and activation are regulated by intracellular as opposed to plasma cholesterol concentration. Unlike plasma cholesterol, the intracellular cholesterol concentration was unchanged in the liver of nephrotic animals. This phenomenon can, in part, contribute to the lack of change in the abundance of both precursor and active forms of SREBP-2 in the study animals.

The lack of rise in hepatic tissue cholesterol concentration despite severe hypercholesterolemia in the NS is due to the diminished uptake of cholesterol-rich lipoproteins by the liver. The latter abnormality is primarily caused by acquired LDL receptor and SRB-1 deficiencies [5,8]. It is of note that the reductions of hepatic LDL receptor and SRB-1 protein abundance in NS are accompanied by a normal mRNA abundance, transcription rate, and hence gene expression of these proteins [5,7]. In contrast to animals with NS, animals with hereditary analbuminemia show normal hepatic LDL receptor and SRB-1 protein abundance [31]. These observations suggest that heavy proteinuria (but not hypoalbuminemia) is responsible for the reduction in hepatic LDL receptor and SRB-1 by either limiting synthesis or accelerating degeneration of these proteins.

The HMG-CoA reductase gene expression is regulated by SREBP-2. Hepatic HMG-CoA reductase gene expression and activity rise during the induction of hypercholesterolemia but return to normal during the maintenance phase of hypercholesterolemia in chronic NS [11]. Because SREBP-2 regulates gene expression of LDL receptor and HMG-CoA reductase, the lack of change in hepatic LDL receptor and HMG-CoA reductase mRNA in nephrotic animals shown here and in our earlier studies [5,11] is consistent with the findings of the present study that showed no significant change in hepatic SREBP-2 abundance or activity in this model.

In conclusion, despite severe hypercholesterolemia, animals with puromycin-induced NS exhibit normal SREBP-2 expression and activation, representing a maladaptive response. This was associated with and, perhaps in part, due to the lack of rise in hepatocellular cholesterol concentration occasioned by diminished uptake of cholesterol-rich lipoproteins by the nephrotic liver.

References

[1] Joven J, Villabona C, Vilella E, Masana L, Alberti R, Valles M. Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome. N Engl J Med 1990;323:579-84.

[2] Kaysen GA. Hyperlipidemia of the nephrotic syndrome. Kidney Int Suppl 1991;31:S8-S15.
[3] Vaziri ND. Molecular mechanisms of lipid disorders in nephrotic syndrome. Kidney Int 2003;63:1964-76.
[4] Wheeler DC, Bernard DB. Lipid abnormalities in the nephrotic syndrome: causes, consequences, and treatment. Am J Kidney Dis 1994;23:331-46.
[5] Vaziri ND, Liang KH. Downregulation of hepatic LDL receptor expression in experimental nephrosis. Kidney Int 1996;50:887-93.
[6] Vaziri ND, Sato T, Liang K. Molecular mechanism of altered cholesterol metabolism in focal glomerulosclerosis. Kidney Int 2003;63:1756-63.
[7] Liang K, Vaziri ND. Downregulation of hepatic high-density lipoprotein receptor, SR-B1, in nephrotic syndrome. Kidney Int 1999;56:621-6.
[8] Vaziri ND, Liang K. Acyl–coenzyme A:cholesterol acyltransferase inhibition ameliorates proteinuria hyperlipidemia, LCAT, SRB-1 and LDL receptor deficiencies in nephrotic syndrome. Circulation 2004; 110:419-25.
[9] Vaziri ND, Liang KH. Upregulation of acyl–coenzyme A: cholesterol acyltransferase (ACAT) in nephrotic syndrome. Kidney Int 2002;61: 1769-75.
[10] Liang KH, Oveisi F, Vaziri ND. Gene expression of hepatic cholesterol 7 alpha-hydroxylase in the course of puromycin-induced nephrosis. Kidney Int 1996;49:855-60.
[11] Vaziri ND, Liang KH. Hepatic HMG-CoA reductase gene expression during the course of puromycin-induced nephrosis. Kidney Int 1995;48:1979-85.
[12] Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331-40.
[13] Edwards PA, Tabor D, Kast HR, Venkateswaran A. Regulation of gene expression by SREBP and SCAP. Biochim Biophys Acta 2000;1529: 103-13.
[14] Weber LW, Boll M, Stampfl A. Maintaining cholesterol homeostasis: sterol regulatory element-binding proteins. World J Gastroenterol 2004;10:3081-7.
[15] Vallett SM, Sanchez HB, Rosenfeld JM, Osborne TF. A direct role for sterol regulatory element binding protein in activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene. J Biol Chem 1996;271: 12247-53.
[16] Smith JR, Osborne TF, Goldstein JL, Brown MS. Identification of nucleotides responsible for enhancer activity of sterol regulatory element in low density lipoprotein receptor gene. J Biol Chem 1990;265:2306-10.
[17] Bennett MK, Lopez JM, Sanchez HB, Osborne TF. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. J Biol Chem 1995;270: 25578-83.
[18] Sato R, Inoue J, Kawabe Y, Kodama T, Takano T, Maeda M. Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein–2. J Biol Chem 1996;271:26461-4.
[19] Hua X, Nohturfft A, Goldstein JL, Brown MS. Sterol resistance in CHO cells traced to point mutations in SREBP cleavage activating protein (SCAP). Cell 1996;87:415-26.
[20] Sakai J, Nohturfft A, Cheng D, Ho YK, Brown MS, Goldstein JL. Identification of complexes between the COOH-terminal domains of sterol regulatory element-binding proteins (SREBPs) and SREBP cleavage-activating protein. J Biol Chem 1977;272: 20213-21.
[21] Sakai J, Duncan EA, Rawson RB, Hua X, Brown MS, Goldstein JL. Sterol regulated release of SREBP-2 from cell membranes require two sequential cleavages, one within a transmembrane segment. Cell 1996;85:1037-46.
[22] Horton JD, Shimomura I, Brown MS, Hammer RE, Goldstein JL, Shimano H. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element binding protein–2. J Clin Invest 1998;101:2331-9.
[23] Sheng Z, Otani H, Brown MS, Goldstein JL. Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. Proc Natl Acad Sci U S A 1995;92:935-8.
[24] Bécard D, Hainault I, Azzout-Marniche D, Bertry-Coussot L, Ferré P, Foufelle F. Adenovirus-mediated overexpression of sterol regulatory element binding protein–1c mimics insulin effects on hepatic gene expression and glucose homeostasis in diabetic mice. Diabetes 2001;50:2425-30.
[25] Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, et al. Sterol regulatory element-binding protein–1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999;274:35832-9.
[26] Amemiya-Kudo M, Shimano H, Hasty AH, Yahagi N, Yoshikawa T, Matsuzaka T, et al. Transcriptional activities of nuclear SREBP-1a, -1c, and -2 to different target promoters of lipogenic and cholesterogenic genes. J Lipid Res 2002;43:1220-35.
[27] Brown AJ, Sun L, Feramisco JD, Brown MS, Goldstein JL. Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. Mol Cell 2002;10:237-45.
[28] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109:1125-31.
[29] Ou JF, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, et al. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein–1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci U S A 2001;98: 6027-32.
[30] Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, et al. A crucial role of sterol regulatory element-binding protein–1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 1999;274:35840-4.
[31] Liang K, Vaziri ND. HMG-CoA reductase, cholesterol 7α-hydroxylase, LCAT, ACAT, LDL receptor and SRB-1 in hereditary analbuminemia. Kidney Int 2003;64:192-8.
[32] Mitsuhashi M, Tomozawa S, Endo K, Shinagawa A. Quantification of mRNA in whole blood by assessing recovery of RNA and efficiency of cDNA synthesis. Clin Chem 2006;52:634-42.
[33] Hamaguchi Y, Aso Y, Shimada H, Mitsuhashi M. Direct reverse transcription-PCR on oligo(dT)-immobilized polypropylene microplates after capturing total mRNA from crude cell lysates. Clin Chem 1998;44:2256-63.
[34] Mitsuhashi M, Keller C, Akitaya T. Gene manipulation on plastic plates. Nature 1992;357:519-20.
[35] Haddad F, Bodell PW, McCue SA, Baldwin KM. Effects of diabetes on rodent cardiac thyroid hormone receptor and isomyosin expression. Am J Physiol Endocrinol Metab 1997;272:E856-63.
[36] Zeng L, Liao H, Liu Y, Lee T-S, Zhu M, Wang X, et al. Sterol-responsive element-binding protein (SREBP) 2 down-regulates ATP-binding cassette transporter A1 in vascular endothelial cells. J Biol Chem 2004;279:48801-7.