Diet-induced Obesity in C57BL/6J Mice Causes Increased Renal Lipid Accumulation and Glomerulosclerosis via a Sterol Regulatory Element-binding Protein-1c-dependent Pathway

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Obesity and metabolic syndrome are associated with glomerulosclerosis and proteinuria, but the mechanisms are not known. The purpose of this study was to determine if there is altered renal lipid metabolism and increased expression of sterol regulatory element-bearing proteins (SREBPs) in a model of diet-induced obesity. C57BL/6J mice that were fed a high fat, 60 kcal % saturated (lard) fat diet (HFD) developed obesity, hyperglycemia, and hyperinsulinemia compared with those that were fed a low fat, 10 kcal % fat diet (LFD). In contrast, A/J mice were resistant when fed the same diet. C57BL/6J mice with HFD exhibited significantly higher levels of renal SREBP-1 and SREBP-2 expression than those mice with LFD, whereas in A/J mice there were no changes with the same treatment. The increases in SREBP-1 and SREBP-2 expression in C57BL/6J mice resulted in renal accumulation of triglyceride and cholesterol. There were also significant increases in the renal expression of plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF), type IV collagen, and fibronecctin, resulting in glomerulosclerosis and proteinuria. To determine a role for SREBPs in modulating renal lipid metabolism and glomerulosclerosis, we performed studies in SREBP-1c−/− mice. In contrast to control mice, in the SREBP-1c−/− mice with HFD the accumulation of triglyceride was prevented, as well as the increases in PAI-1, VEGF, type IV collagen, and fibronecctin expression. Our results therefore suggest that diet-induced obesity causes increased renal lipid accumulation and glomerulosclerosis in C57BL/6J mice via an SREBP-1c-dependent pathway.

Obesity is a known risk factor for cardiovascular disease (1) and type-2 diabetes mellitus (2) and has been proposed to play a role in the pathogenesis of diabetic nephropathy (3). Obesity is one of the defining criteria of the metabolic syndrome as proposed by the National Cholesterol Education Program Adult Treatment Panel III (4) and the World Health Organization (5). The metabolic syndrome, which is characterized by the concurrent existence of obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, and hypertension, has been shown to be a strong and independent risk factor for cardiovascular, and all cause mortality (6, 7) as well as the development of microalbuminuria and chronic kidney disease (8).

Obesity is considered a major generator of metabolic syndrome (9). Early in the course of obesity-initiated metabolic syndrome, structural and functional changes similar to diabetic kidney disease occur (10). These changes include glomerular hyperfiltration, glomerular basement membrane thickening, mesangial cell proliferation, mesangial matrix thickening, and expansion of Bowman’s capsule (10) and are considered precursors to more severe renal injury. Severe obesity has been associated with the eventual development of focal and segmental glomerulosclerosis (11). Although incompletely understood, several hemodynamic, hormonal, and metabolic factors have been proposed to contribute to the pathogenesis of nephropathy associated with metabolic syndrome, including intrarenal hypertension and hyperfiltration, oxidative stress, angiotensin II, inflammatory cytokines, hyperinsulinemia/insulin resistance, hyperglycemia/diabetes, and abnormal lipid metabolism (3, 9, 11–14).

Historically, Virchow (15) first suggested the association between lipids and renal disease in 1856 when he described successive stages of fatty metamorphosis and fatty detritus in the renal epithelium in Bright’s disease. In their classic paper in 1936 describing the pathological sign of nodular sclerosis Kimmelstiel and Wilson (16) also demonstrated the presence of lipid deposits in the kidneys of diabetic patients, and they suggested that these lipids play an important role in the pathogenesis of renal disease. Since then several studies in human subjects and in experimental animals with diabetes have shown a correlation between serum lipids, renal lipids, and proteinuria and progressive decline in renal function (17–20). Our previous work has suggested that renal lipid accumulation associated with increased renal lipid synthesis is involved in the nephropathy seen in the type 1 diabetes animal model (20). Whether a similar alteration in renal lipid metabolism and accumulation of lipids mediates the kidney disease associated with obesity-initiated metabolic syndrome, however, has not been established.

In recent years the sterol regulatory element-binding proteins (SREBPs) (2) have been shown to be master regulators of both fatty acid and cholesterol metabolism. Three SREBP isoforms, SREBP-1a, SREBP-1c, and SREBP-2, have been identified and characterized. SREBPs are...
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synthesized as precursors bound to the endoplasmic reticulum. After a two-step cleavage process, the N-terminal segment of SREBP, also referred to as the mature (active) form, is released from the membrane and translocated into the nucleus, where it binds to enhancer regions of target genes to activate transcription. SREBP-1 preferentially activates genes involved in fatty acid synthesis, whereas SREBP-2 preferentially activates genes involved in cholesterol biosynthesis (21, 22). Although the role of SREBP in lipogenesis has been intensively examined in liver and adipose tissues (23, 24), less is known of its role in renal diseases.

Among the genetic and environmental factors associated with the development and maintenance of obesity, diet composition and caloric intake have been the focus of intense study (25–27). High dietary saturated fat intake has been shown to contribute to the development of obesity, hyperglycemia/diabetes, hyperinsulinemia/insulin resistance, and vascular disease in humans and rodents (28–30), all of which have been implicated in the pathogenesis of renal disease associated with the metabolic syndrome.

The purposes of the present study are to examine in a mouse model of diet-induced obesity (a) whether there is altered renal lipid metabolism and renal accumulation of lipids, (b) whether those changes result in development of renal sclerosis and proteinuria, and (c) whether renal expression of SREBPs per se plays an important role in the pathogenesis of renal disease.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were purchased from Sigma. Antibodies against SREBP-1 and SREBP-2 were obtained from BD PharMingen (San Diego, CA). Antibodies against plasminogen activator inhibitor-1 (PAI-1) and vascular endothelial growth factor (VEGF), and horseradish peroxidase-labeled anti-mouse IgG were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against type IV collagen and fibronectin were obtained from Chemicon (Temecula, CA). All cell culture reagents were purchased from Invitrogen. Chemiluminescence detection reagents were obtained from Pierce.

Animal Models—The first series of studies were performed with 8-week-old male C57BL/6J and A/J mice obtained from the Jackson Laboratory (Bar Harbor, ME), representing differences in susceptibility to the effects of diet on body composition. They were housed in colony cages, maintained on a 12-h light/12-h dark cycle, and fed LFD or HFD for 4 weeks. These mice were also housed in colony cages, maintained on a 12-h light/12-h dark cycle, and fed LFD or HFD for 4 weeks.

Another group of four mice in each experimental and dietary group were anesthetized and perfusion-fixed for 15 min at 4 °C. The nuclei were resuspended in a 0.5-packed nuclear volume of low salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM MgCl₂, 20 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol). A 0.5-packed nuclear volume of high salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM MgCl₂, 0.8 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) was then added. After 30 min of gentle mixing, the nuclei were pelleted by centrifugation (30 min at 25,000 × g). The supernatants were saved, and the protein concentration was determined by the method of Lowry (32).

Kidney homogenates were prepared according to the method of Morooka et al. (31) with modifications. An aliquot of homogenate was centrifuged at 3,300 × g for 15 min at 4 °C. The nuclei were resuspended in a 0.5-packed nuclear volume of low salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM MgCl₂, 20 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol). A 0.5-packed nuclear volume of high salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM MgCl₂, 0.8 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) was then added. After 30 min of continuous gentle mixing, the nuclei were pelleted by centrifugation (30 min at 25,000 × g). The supernatants were saved, and the protein concentration was determined by the method of Lowry (32).

Kidney homogenates were similarly subjected to SDS-PAGE (10% w/v) as described by Laemmli (33) and then transferred to nitrocellulose membranes. Membranes were blocked in 5% dried milk in TBST (0.2% Tween 20 in 1 × Tris-buffered saline), incubated with anti-SREBP-1 (1:1,000 dilution) and anti-SREBP-2 (1:1,000 dilution), followed by horseradish peroxidase-labeled anti-mouse IgG (1:5,000 dilution), washed four times with 1 × Tris-buffered saline, and then developed using a chemiluminescence detection system. The signals were quantified in a Fluor−STM Multimager (Bio−Rad Laboratories).

Blood and Urine Chemistries—Plasma glucose was measured using the glucose CII kit (Wako Chemicals USA, Inc., Richmond, VA). Total plasma cholesterol was measured using the cholesterol CII kit (Wako). Total plasma triglycerides were measured by the L Type TG H kit (Wako). Plasma insulin was determined using a mouse insulin enzyme-linked immunosorbent assay kit (LINCO Research, Inc., St. Charles, MO). Free fatty acids were determined using the NEFA C kit (Wako). Urine albumin concentration was determined by using a competitive enzyme-linked immunosorbent assay via the Albuwell M kit (Exocell, Philadelphia, PA). Urine creatinine concentration was determined by the Creatinine Companion kit (Exocell). Results are expressed as the urine albumin to creatinine ratio (milligrams/mg).

Homogenization and Nuclei Isolation—Kidneys were homogenized at 4 °C in homogenization buffer (20 mM Tris−Cl, pH 7.4, 75 mM NaCl, 2 mM EGTA, 2 mM EDTA, 1 mM Na₃VO₄, 1 mM dithiothreitol), supplemented with protease inhibitor mixture consisting of 104 mM (2-aminoethyl)benzenesulfonylfluoride hydrochloride, 0.08 mM aprotinin, 2 mM leupeptin, 4 mM bestatin, 1.5 mM pepstatin A, and 1.4 mM E-64 (Sigma).

Another group of four mice in each experimental and dietary group were anesthetized and perfusion-fixed for 15 min at 4 °C. The nuclei were resuspended in a 0.5-packed nuclear volume of low salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM MgCl₂, 20 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol). A 0.5-packed nuclear volume of high salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM MgCl₂, 0.8 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) was then added. After 30 min of continuous gentle mixing, the nuclei were pelleted by centrifugation (30 min at 25,000 × g). The supernatants were saved, and the protein concentration was determined by the method of Lowry (32).

Kidney homogenates were similarly subjected to SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were then probed with antibodies for PAI-1 (1:1,000 dilution) and anti-SREBP-1 and SREBP-2 (1:1,000 dilution), followed by horseradish peroxidase-labeled anti-mouse IgG (1:5,000 dilution), washed four times with 1 × Tris-buffered saline, and then developed using a chemiluminescence detection system. The signals were quantified in a Fluor−STM Multimager (Bio−Rad Laboratories).

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**Perfusion Fixation of Kidneys**—Mice were anesthetized and perfused with a fixative buffer that consisted of 3% paraformaldehyde and 0.05% picric acid, in a 6:4 mixture of cacodylate buffer (pH 7.4, adjusted to 300 mosmol with sucrose) and 10% hydroxyethyl starch. After 5 min of fixation, the mice were perfused for an additional 5 min with the cacodylate buffer (20).

**PAS Staining, Oil Red O Staining, and Immunofluorescence Imaging**—Paraffin sections were stained for PAS. Frozen sections were used for Oil Red O staining to determine renal accumulation of neutral fats. The stained kidney sections were imaged with an Olympus microscope and scored semi-quantitatively in a blinded manner by the renal pathologist (M. S. L.).

For immunofluorescence, frozen sections were preincubated for 10 min at room temperature with 3% milk powder in phosphate-buffered saline containing 0.05% Triton X-100. They were then covered overnight at 4 °C with primary anti-mouse type IV collagen or fibronectin antibody diluted 1:200 in the preincubation solution. The sections were rinsed three times with phosphate-buffered saline prior to incubation for 1 h at 4 °C with the secondary antibody, goat anti-rabbit IgG conjugated to Alexa 488 (Molecular Probes, Inc., Eugene, OR). After rinsing with phosphate-buffered saline the sections were mounted using DAKO-Glycergel™ (Dako, Glostrup, Denmark) plus 2.5% 1,4-diazabicyclo[2.2.2]octane (DABCO, Sigma) as a fading retardant. The kidney sections were then imaged with a laser scanning confocal fluorescence microscope (Zeiss LSM 510, Jena, Germany).

**Data Analysis**—Results are presented as the means ± S.E. for at least three independent experiments. Data were analyzed by analysis of variance and Student-Newman-Keuls tests for multiple comparisons or by Student’s t test for unpaired data. Statistical significance was accepted at the p < 0.05 level.

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**TABLE ONE**

| Primers for real-time PCR | Forward | Reverse |
|---------------------------|---------|---------|
| **Cyclophilin**           | TGGAGAGCACCAAGACAGACA | TGCCGGAGTCGACAATGAT |
| **SREBP-1a**              | AGGCGGCTCTGGAAACAGA   | ATGTGCTTCAACGCTGT |
| **SREBP-1c**              | GGCACATATGTTGGAACACTC | GCCACATAGTATCTGGCACAGA |
| **ACC**                   | CCACGAGAATAAGCTACCTTG | TCCCTTTGTGCAACATGAAAGCT |
| **FAS**                   | CTCGCTAGCATTCGGAACCTC | AGCACATCTCGAAGGATACACA |
| **ACO**                   | GCCCAACTATGTTGGAACACATA | ACCAACTCGGCTGACAGAA |
| **SREBP-2**               | CAAATCCTGGCTTGCAGGAAA | ATGTGCTCTGGCCAGCCT |
| **HMGR reductase**        | AGCGAGAAGCAGCACATC    | ATGTGCAATGTTGACGAT |
| **ABCA-1**                | CGTTTCCGGAAATGTCCTCA  | GCAGAGATGCAACAGAGATGGA |
| **ApoE**                  | GGCAGGCTTGTTGACACA    | TCTGTGAGTTCAGCATCAGAT |
| **PAI-1**                 | GGCACCCCTACGATTTCA    | TGAGAGTCGTGTTGCCGAG |
| **VEGF**                  | AACGATGAAGCCCGTGGAGTG | TGAAGAGTCTGGTGCCGAG |
| **Type IV collagen**      | TACCTGCCACTACTGCGTAAC | CGGATGTTGCTGCCTGGAAG |
| **Fibronectin**           | AGACCATACCTGCGGAATGTA | GAGAGCCTTCTGTCCGAGAG |

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**TABLE TWO**

| Comparison of phenotype of A/J and C57BL/6J mice treated with LFD or HFD | Results are expressed as mean ± S.E. Each group includes eight animals. |
|--------------------------------------------------------------------------|-------------------------------------------------|
| LFD                        | HFD                                | LFD                        | HFD                                |
| **Body weight (g)**         | 27.80 ± 0.54                       | 32.32 ± 1.80               | 31.10 ± 0.93                       | 47.89 ± 2.04*                     |
| **Kidney weight (g)**       | 0.30 ± 0.02                        | 0.33 ± 0.01                | 0.32 ± 0.02                        | 0.40 ± 0.02*                      |
| **Plasma glucose (mg/dl)**  | 110.83 ± 10.8                      | 126.67 ± 6.15              | 131.50 ± 14.3                      | 215.67 ± 15.6*                    |
| **Plasma triglycerides (mg/dl)** | 79.15 ± 5.11                   | 82.21 ± 7.66               | 84.76 ± 5.20                       | 105.19 ± 6.52                     |
| **Plasma cholesterol (mg/dl)** | 127.85 ± 15.82                  | 97.47 ± 25.31              | 156.96 ± 6.33                      | 222.78 ± 16.45                    |
| **Plasma insulin (ng/ml)**  | 0.35 ± 0.08                        | 0.47 ± 0.04                | 0.41 ± 0.07                        | 5.48 ± 0.40*                      |
| **Plasma free fatty acid (mM)** | 1.16 ± 0.11                     | 0.86 ± 0.12                | 0.80 ± 0.06                        | 0.88 ± 0.08                       |

* p < 0.01 versus C57BL/6J mice with LFD.
RESULTS

HFD Causes Obesity, Hyperglycemia, and Hyperinsulinemia in C57BL/6J Mice but Not in A/J Mice—To study the effects of HFD on obesity, we fed C57BL/6J and A/J mice with LFD or HFD for 12 weeks and compared the changes in several relevant attributes of mice. As shown in TABLE TWO, the body weight and kidney weight of C57BL/6J mice with HFD were markedly increased as compared with those mice with LFD (p < 0.01). However, this diet-induced obesity was not observed in A/J mice. In addition, the plasma glucose and insulin levels were significantly higher in C57BL/6J mice with HFD, which correlated well with the increased Oil Red O staining (Fig. 1). There were significant increases in renal triglyceride and cholesterol content in C57BL/6J mice with HFD, which correlated well with the increased Oil Red O staining (Fig. 2). However, A/J mice fed a HFD had no increases in renal triglyceride and cholesterol content (Fig. 2).

HFD Causes Increased Renal Lipid Accumulation in C57BL/6J but Not in A/J Mice—To study whether HFD can cause an increase in renal lipid accumulation we performed Oil Red O staining in kidney sections, which revealed the accumulation of neutral lipids in the glomerular and tubulointerstitial cells of HFD-treated C57BL/6J mice (Fig. 1). There were significant increases in renal triglyceride and cholesterol content in C57BL/6J mice with HFD, which correlated well with the increased Oil Red O staining (Fig. 2). However, A/J mice fed a HFD had no increases in renal triglyceride and cholesterol content (Fig. 2).

HFD Causes Increased SREBP-1 and SREBP-2 mRNA and Nuclear Protein Abundance in the Kidneys of C57BL/6J Mice but Not in A/J Mice—Next, we wanted to determine whether the lipid accumulation in the kidneys of HFD-treated C57BL/6J mice was associated with increased expression of SREBPs. SREBPs are members of the basic

FIGURE 2. Triglyceride (A) and cholesterol (B) content in the kidney of LFD or HFD-fed A/J and C57BL/6J mice. Triglyceride and cholesterol content were measured in lipid extracts from kidney homogenate samples. The HFD-fed C57BL/6J mice, not A/J mice, showed statistically significant increases in renal triglyceride and cholesterol content.*, p < 0.05; **, p < 0.01 versus C57BL/6J mice with LFD. Each group includes eight animals.

FIGURE 3. Western blot analysis of the expression of SREBP-1 (A) and SREBP-2 (B) in the kidneys from A/J and C57BL/6J mice treated with LFD or HFD. Nuclear extracts were isolated from kidney homogenates for Western blot analysis (see “Experimental Procedures”). There was a significant increase in renal expression of nuclear SREBP-1 and SREBP-2 proteins in C57BL/6J mice treated with HFD compared with those mice treated with LFD. **, p < 0.01 versus C57BL/6J mice with LFD. Each group includes eight animals.
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FIGURE 4. The expression of SREBP-1a (A), SREBP-1c (B), and SREBP-2 (C) mRNA in the kidneys of C57BL/6J mice with LFD or HFD. Total RNA was isolated from the kidneys using Trizol, and cDNA was synthesized using reverse transcript reagents. The mRNA level was quantified using Bio-Rad iCycler Real-time PCR system. All the data were calculated from triplicate reactions. The C57BL/6J with HFD showed a statistically significant increase in the expression of SREBP-1a and 1c mRNA and showed a trend toward an increase in the expression of SREBP-2 mRNA. *, p < 0.05 versus C57BL/6J mice with LFD. Each group includes eight animals.

| mRNA Level | LFD | HFD |
|------------|-----|-----|
| Fatty acid synthesis | ACC 1.1 ± 0.1 | 4.7 ± 1.2* |
| FAS 1.0 ± 0.1 | 7.2 ± 1.8* |
| Fatty acid oxidation | ACO 7.7 ± 6.7 | 28.6 ± 3.7 |
| Cholesterol synthesis | HMG-CoA Reductase 1.4 ± 0.4 | 7.7 ± 2.5* |
| Cholesterol efflux | ABCA1 1.3 ± 0.4 | 2.8 ± 0.4* |
| ApoE 1.4 ± 0.5 | 4.9 ± 2.5 |

TABLE THREE
mRNA levels of enzymes involved in lipid metabolism in the kidneys of C57BL/6J mice with LFD or HFD

Results are expressed as mean ± S.E. (arbitrary unit). Each group includes eight animals.

The expression of SREBP-1a and SREBP-2 mRNA showed a trend toward an increase, but there was no statistical significance (Fig. 4).

HFD Causes Increased Expression of Lipogenic Enzymes in the Kidneys of C57BL/6J Mice—To determine if the increased abundance of SREBP-1 protein in nuclear extracts induced the transcriptional activation of renal lipogenic enzymes, we measured the mRNA abundance of two key enzymes that mediate fatty acid synthesis, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Compared with C57BL/6J mice with LFD, in those mice with HFD there were significant increases in ACC and FAS mRNA abundance (TABLE THREE), which suggests that fatty acid synthesis may be increased. Because increased renal triglyceride content could also be mediated via decreased fatty acid oxidation, we measured the mRNA level of acetyl-CoA oxidase (ACO), a key enzyme that mediates fatty acid oxidation. We found that ACO mRNA abundance trended to higher level in favor of fatty acid oxidation in C57BL/6J mice with HFD (TABLE THREE), suggesting that increased nuclear SREBP-1 abundance and potential up-regulation of fatty acid synthesis mediate the increased triglyceride accumulation in C57BL/6J mice with HFD (Fig. 2).

To determine if the increased abundance of nuclear SREBP-2 protein induced the transcriptional activation of enzymes that regulate cholesterol metabolism, we measured the mRNA abundance of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, a key enzyme that mediates cholesterol synthesis. In C57BL/6J mice fed an HFD there was a significant increase in HMG-CoA reductase mRNA abundance (TABLE THREE). Because increased renal cholesterol content could also be mediated via decreased cholesterol efflux, we measured the mRNA abundance of ATP-binding cassette A-1 (ABCA1) and ApoE, key mediators of cholesterol efflux. We found that the mRNA abundance of ABCA1 was actually increased and ApoE showed a trend toward increase in C57BL/6J mice fed an HFD (TABLE THREE). Therefore increased nuclear SREBP-2 expression accompanied by increased cholesterol synthesis may mediate the increased cholesterol accumulation in C57BL/6J mice with HFD (Fig. 2).

HFD Causes Glomerulosclerosis and Proteinuria in C57BL/6J but Not in A/J Mice—To evaluate the effects of diet-induced obesity on renal histopathology, we performed the PAS staining on kidney sections. Compared with C57BL/6J mice with LFD, kidney sections of C57BL/6J mice with HFD displayed increased PAS staining, which is a hallmark of mesangial expansion with increased extracellular matrix (ECM) protein content (Fig. 5). Expression of two major ECM proteins, type IV collagen and fibronectin, were indeed markedly increased in HFD-treated C57BL/6J mice as indicated in immunofluorescence microscopic images where the increased expression was most evident in the glomeruli (Fig. 6). Increased mesangial matrix is an indication of glomerulosclerosis. In contrast to C57BL/6J mice, A/J mice, which were resistant to diet-induced obesity, showed no sign of glomerulosclerosis with HFD. The glomerular changes in C57BL/6J mice fed a HFD were also associated with a significant increase of urinary albumin excretion, which is an indicator of glomerular injury with loss of glomerular perm-

helix-loop-helix zipper family of transcription factors that play a critical role in regulating fatty acid and cholesterol synthesis. To become transcriptionally active, SREBPs are cleaved by proteases to release N-terminal segments, their mature and active forms, which can be transported into the nucleus. Immunoblotting of renal nuclei extracts revealed significant increases in SREBP-1 and SREBP-2 protein abundance in C57BL/6J mice with HFD compared with those mice with LFD (Fig. 3). In contrast, there were no significant differences in SREBP-1 or SREBP-2 nuclear protein abundance observed between A/J mice with LFD and HFD (Fig. 3). The increase in the renal expression of nuclear SREBP-1 was regulated at the transcriptional level as HFD caused significantly increased expression of SREBP-1a and SREBP-1c mRNA. SREBP-2 mRNA expression showed a trend toward an increase, but there was no statistical significance (Fig. 4).

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FIGURE 5. Representative photomicrographs of PAS-stained renal sections from LFD or HFD-fed C57BL/6J mice. Kidney slices were embedded in paraffin, stained with PAS reagent, and counterstained with hematoxylin and eosin. Representative glomeruli showed an increase in expansion of mesangium and accumulation of PAS-positive material in HFD-fed C57BL/6J mice compared with LFD-fed mice.

FIGURE 6. Immunofluorescence staining of kidney for fibronectin and type IV collagen of C57BL/6J mice fed a LFD or an HFD. Cryosections were used for immunofluorescence microscopy for fibronectin and type IV collagen. In contrast to C57BL/6J mice fed a LFD, there was increased fibronectin and type IV collagen deposition in those mice fed an HFD.

selectivity (Fig. 7). Again, HFD did not cause proteinuria in A/J mice (Fig. 7).

HFD Causes Increased Renal Expression of PAI-1 and VEGF in C57BL/6J but Not in A/J Mice—Because mesangial expansion, accumulation of ECM proteins, and proteinuria are mediated by increased expression of growth factors such as VEGF (35) and matrix protease inhibitor such as PAI-1 (36), we measured the expression of PAI-1 and VEGF protein in the kidneys. There was a 36% increase in PAI-1 and a 57% increase in VEGF protein expression in the kidneys of HFD-fed C57BL/6J mice compared with those of LFD-fed mice (Fig. 8). In contrast, there were no such changes in the A/J mice (Fig. 8).

SREBP-1c Plays a Major Role in the Regulation of Renal Lipid Accumulation and Expression of PAI-1, VEGF, and ECM Proteins in Response to HFD—To determine a specific causal role for increased renal expression of SREBP-1c in mediating the increased renal lipid accumulation as a result of HFD, we performed experiments in wild-type control and SREBP-1c/H11002 mice with LFD or HFD.

SREBP-1c/H11002 mice are viable, normal in size, and do not display any gross physical or behavioral abnormalities. The targeted deletion of the first exon of SREBP-1c gene results in a total ablation of the SREBP-1c transcript while leaving SREBP-1a transcript intact (37). The basic phenotype of SREBP-1c/H11002 mice is described in TABLE FOUR. Compared with wild-type mice, SREBP-1c/H11002 mice showed lower levels of plasma triglyceride and cholesterol, consistent with the previous report (37). There was no detectable expression of SREBP-1c mRNA in the kidneys of SREBP-1c/H11002 mice, but there were modest compensatory increases in renal mRNA levels of SREBP-1a (normally 21% of SREBP-1c transcript expression in kidney) and SREBP-2 by 29.2% and 39.4% of wild-type, respectively. In wild-type mice where SREBP-1c was present, HFD induced increase in renal SREBP-1 and SREBP-2 nuclear expression (Fig. 9) as well as in renal triglyceride accumulation (TABLE FIVE). In SREBP-1c/H11002 mice with HFD the significant increase in renal triglyceride accumulation seen in HFD-fed wild-type mice was prevented (TABLE FIVE). Interestingly, SREBP-1c deficiency also prevented HFD to increase the renal expression of SREBP-2 nuclear protein that mediates cholesterol synthesis (Fig. 9). To determine the role of SREBP-1c in renal histopathological changes, we measured the renal mRNA levels of PAI-1, VEGF, and two ECM proteins, type IV collagen and fibronectin, all of which have been implicated in glomerulosclerosis and proteinuria. We found that the increased mRNA levels of those molecules induced by HFD in wild-type mice were significantly attenuated in HFD-fed SREBP-1c/H11002 mice (TABLE SIX).

DISCUSSION

The purpose of our current study was to investigate the role of renal lipid metabolism in obesity-induced renal disease. We examined 1) whether there were altered renal lipid metabolism and lipid accumulation associated with renal disease in diet-induced obesity mouse model; 2) whether SREBPs and their regulated lipid synthesis were involved in renal lipid accumulation and how they played roles in the pathogenesis of obesity-induced renal disease by the use of SREBP-1c/H11002 mice.

We observed characteristics of metabolic syndrome such as obesity, hyperglycemia, and hyperinsulinemia in HFD-fed C57BL/6J mice. Our results also showed that in C57BL/6J mice with HFD there were increased renal expression of VEGF, PAI-1, type IV collagen, and fibronectin, and the subsequent development of glomerulosclerosis and proteinuria. These kidney-specific histological changes are in agreement with findings in a hamster model treated with similar dietary interventions (38). However, A/J mice were resistant to the impacts of HFD on C57BL/6J mice. Under HFD condition, they exhibited none of the above characteristics of metabolic syndrome and experienced no altered glomerulosclerosis or proteinuria. The susceptibility of C57BL/6J mice in the setting of HFD highlighted the important role of high caloric intake of dietary fat and genetic background in the development of metabolic syndrome and suggested the potential roles of obesity, hyperglycemia, and hyperinsulinemia in the pathogenesis of renal disease occurred in metabolic syndrome. The variable impacts of diet and genetics seen in A/J and C57BL/6J mice resemble variable
patterns of disease susceptibility seen in human populations such as Native American, African American, and Latin American, which suffer from higher rates of obesity and type 2 diabetes (39–41).

A novel finding of our study was that in HFD-treated C57BL/6J mice, but not in A/J mice, there were increased Oil Red O staining in the kidney sections and significantly higher renal content of triglyceride and cholesterol compared with those mice treated with LFD. Because such increased lipid accumulation might result from renal lipid synthesis, and SREBPs have been shown to be key regulators of the transcriptional activation of lipogenic genes, we determined whether there were diet-induced changes in renal SREBPs expression. We found that the expression of renal SREBP-1 and SREBP-2 nuclear (mature) form was up-regulated, accompanied by the elevated mRNA level of their target enzymes that mediate \textit{de novo} fatty acid and cholesterol synthesis, including ACC, FAS, and HMG-CoA reductase. Although we did not perform cell fractionation studies, we have documented SREBP-1a, SREBP-1c, and SREBP-2 mRNA expression in glomerular mesangial cells, glomerular epithelial cells (podocytes), and renal proximal tubular cells. The regulation of SREBP expression in the kidney has not been well described. Our results show that dietary fatty acids can regulate renal SREBP expression. In our experiments the main source of dietary fatty acid is lard, highly enriched with saturated fatty acid. The data were in parallel with other studies showing high fat feeding stimulated the expression of SREBP in liver (42, 43). Polyunsaturated fatty acids-supplemented fat-free diet has been shown to suppress rat liver expression of SREBPs (44). However, this possibility has not yet been addressed in kidney. In our study HFD induced hyperinsulinemia and hyperglycemia in C57BL/6J mice. Previous studies have suggested that insulin up-regulated SREBP-1c mRNA level in other tissues, such as skeletal muscle (45), adipose tissue (46), and liver (47). It is, therefore, possible that changes in renal SREBPs are secondary to high insulin level in the plasma in HFD-fed C57BL/6J mice. As shown in our previous report hyperglycemia mediated increased renal expression of SREBP-1 in streptozotocin-induced type 1 diabetic rats (20) and may well be a key factor for SREBP regulation in this diet-induced obesity C57BL/6J mice. However, the effect of hyperglycemia is tissue-specific, because in the same type 1 diabetic rats a decline of SREBP-1c expression was observed in liver (47).

HFD-induced increase of SREBPs nuclear protein and their target enzymes mRNA expression in C57BL/6J mice suggested a potential
increase of renal lipid synthesis mediated by SREBPs. The possibility also exists that the increased triglyceride and cholesterol accumulation could also result from decreases in fatty acid oxidation and cholesterol efflux. We found that mRNA levels for ABCA1 and ApoE (proteins involved in cholesterol efflux) and ACO (enzyme that mediates fatty acid re-esterification, may be responsible for increased renal lipid content (48). However, it is noted that HFD did not increase plasma free fatty acid level in our experiments.

The evidence, for a causal role of SREBPs in renal lipid accumulation and in the pathogenesis of glomerulosclerosis and proteinuria associated with diet-induced obesity, reported herein, demonstrates that targeted deletion of SREBP-1c prevented HFD-induced increase in renal triglyceride content and markedly attenuated the mRNA up-regulation of VEGF, PAI-1, type IV collagen, and fibronectin, the factors that have been implicated in glomerulosclerosis and proteinuria. These results are complementary to our previous work to show that in transgenic mice overexpressing SREBP-1a, in the absence of any increases in serum glucose, triglyceride, or cholesterol level, there was a marked up-regulation of ACC and FAS mRNA level, resulting in increased renal triglyceride content, increased expression of transforming growth factor-β, VEGF, type IV collagen, and fibronectin, mesangial expansion, glomerulosclerosis, and proteinuria (20). Therefore, activation of SREBPs resulting in alterations in renal lipid metabolism and renal lipid accumulation may play a critical role in the nephropathy associated with diet-induced obesity.

There is increasing evidence to show the role of lipid metabolism in renal disease. It has been reported that inhibition of cholesterol synthesis by HMG-CoA reductase inhibitors (statins) and of triglyceride synthesis by peroxisome proliferator activated receptor-α agonists (fibrates) protect against diabetic and non-diabetic renal disease (13, 49, 50). A recent meta-analysis of several small scale interventional studies in diabetic and non-diabetic human subjects with glomerulosclerosis and proteinuria indicates that long term treatment with statins and/or fibrates significantly prevent the decline in glomerular filtration rate (29).

The importance and direct effects of lipids per se in mediating kidney cell injury have also been demonstrated in cell culture studies. Studies in renal mesangial and tubular cells grown in culture have shown that incubation, of these cells with low density lipoprotein or very low density lipoprotein, results in increases in cellular triglycerides and cholesterol and causes up-regulation of the growth factors, including transforming growth factor-β and platelet derived growth factor (51); ECM proteins (52); pro-inflammatory cytokines, including interleukins and tumor necrosis factor-α (51); adhesion molecules, including monocoyte

### TABLE FIVE

| Triglyceride and cholesterol content in the kidneys of wild-type and SREBP-1c−/− treated with LFD or HFD |
|-------------------------------------------------|-------------------------------------------------|
| Results are expressed as mean ± S.E. Each group includes four animals. |
| | | | | | | |
| **Wild-type** | **SREBP-1c−/−** | | | | | |
| | | | | | | |
| Triglyceride (µg/mg protein) | | | | | | |
| LFD | 11.0 ± 1.0 | 18.8 ± 1.2<sup>a</sup> | | | | |
| HFD | 10.2 ± 2.3 | 11.0 ± 2.5 | | | | |
| Cholesterol (µg/mg protein) | | | | | | |
| LFD | 9.6 ± 1.6 | 13.0 ± 1.2 | | | | |
| HFD | 11.0 ± 1.5 | 12.5 ± 1.6 | | | | |

<sup>a</sup> *p < 0.05 versus wild-type with LFD.

### TABLE SIX

| mRNA levels of PAI-1, VEGF, type IV collagen, and fibronectin in the kidneys of wild-type and SREBP-1c−/− mice with LFD or HFD |
|-------------------------------------------------|-------------------------------------------------|
| Results are expressed as mean ± S.E. (arbitrary unit). Each group includes four animals. |
| | | | | | | |
| **Wild-type** | **SREBP-1c−/−** | | | | | |
| | | | | | | |
| PAI-1 | | | | | | |
| LFD | 4.3 ± 0.5 | 6.4 ± 0.7<sup>a</sup> | | | | |
| HFD | 3.4 ± 1.2 | 3.9 ± 0.4 | | | | |
| VEGF | | | | | | |
| LFD | 1.3 ± 0.2 | 2.5 ± 0.2<sup>a</sup> | | | | |
| HFD | 1.5 ± 0.1 | 1.8 ± 0.3 | | | | |
| Type IV collagen | | | | | | |
| LFD | 1.4 ± 0.3 | 2.6 ± 0.1<sup>a</sup> | | | | |
| HFD | 2.2 ± 0.5 | 1.8 ± 0.4 | | | | |
| Fibronectin | | | | | | |
| LFD | 3.6 ± 0.4 | 6.0 ± 0.8<sup>a</sup> | | | | |
| HFD | 4.2 ± 1.6 | 3.2 ± 0.4 | | | | |

<sup>a</sup> *p < 0.05 versus wild-type with LFD.
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chymotactant protein-1, intercellular adhesion molecule 1, and vascular cell adhesion molecule-1 (53).

In summary, our results indicate that an HFD administered to C57BL/6J mice, which are susceptible to diet-induced obesity, up-regulates renal expression of the transcriptional factors SREBP-1 and SREBP-2 nuclear forms and mRNA expression of their target enzymes involved in renal fatty acid and cholesterol synthesis. These changes are associated with increased renal accumulation of triglyceride and cholesterol and the development of glomerulosclerosis and proteinuria. In SREBP-1c−/− mice, HFD-induced elevation of renal triglyceride content and increased expression of PAI-1, VEGF, and ECM proteins are attenuated, suggesting that, in this model of diet-induced obesity, SREBP-1c plays an important role in regulation of renal lipid accumulation and the pathogenesis of glomerulosclerosis and proteinuria.

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Diet-induced Obesity in C57BL/6J Mice Causes Increased Renal Lipid Accumulation and Glomerulosclerosis via a Sterol Regulatory Element-binding Protein-1c-dependent Pathway

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