Role of Sterol Regulatory Element-binding Protein 1 in Regulation of Renal Lipid Metabolism and Glomerulosclerosis in Diabetes Mellitus*

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Diabetic renal disease is associated with lipid deposits in the kidney. The purpose of our study was to determine whether there is altered regulation of the sterol regulatory element-binding proteins (SREBPs) in the diabetic kidney and whether SREBPs mediate the abnormal renal lipid metabolism and diabetic renal disease. In streptozotocin-induced diabetes in the rat, there were marked increases in SREBP-1 and fatty acid synthase (FAS) expression, resulting in increased triglyceride (TG) accumulation. Treatment of diabetic rats with insulin prevented the increased renal expression of SREBP-1 and the accumulation of TG. The role of hyperglycemia in the up-regulation of SREBP-1 was confirmed in renal cells cultured in a high glucose media. High glucose induced increased expression of SREBP-1a and -1c mRNA, SREBP-1 protein, and FAS, resulting in increased TG content. To determine a direct role for SREBP in mediating the increase in renal lipids and glomerulosclerosis, we studied SREBP-1a transgenic mice with increased renal expression of SREBP-1. The increase in SREBP-1 was associated with increased expression of FAS and acetyl CoA carboxylase, resulting in increased TG content, increased expression of transforming growth factor β1 and vascular endothelial growth factor, mesangial expansion, glomerulosclerosis, and proteinuria. Our study therefore indicates that renal SREBP-1 expression is increased in diabetes and that SREBP-1 plays an important role in the increased lipid synthesis, TG accumulation, mesangial expansion, glomerulosclerosis, and proteinuria by increasing the expression of transforming growth factor β and vascular endothelial growth factor.

Diabetes mellitus is the leading cause of cardiovascular and renal disease in the United States and most of the world. In the United States, diabetes mellitus now accounts for more than 50% of the patients who develop end-stage renal disease, necessitating dialysis or transplantation for the maintenance of life (1). In addition to the prominent role played by hypertension, hyperglycemia, growth factors (including angiotensin II, transforming growth factor (TGF)-β, and vascular endothelial growth factor (VEGF)), inflammatory cytokines, oxidative stress and advanced glycation end products, abnormal lipid metabolism, and renal accumulation of lipids have also been proposed to play a role in the pathogenesis of diabetic nephropathy (2, 3). Since the description by Kimmelstiel and Wilson (4) of the classical nodular glomerulosclerosis and presence of lipid deposits in the diabetic kidney, several investigators have shown the presence of lipid deposits in the kidneys of diabetic humans and experimental animals, and they have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease (5, 6). The major assumption has been that these lipid deposits originate solely from increased levels of serum lipids. However, the possibility of an increase in renal lipid synthesis also resulting in renal lipid accumulation has not been entertained. Furthermore, whether the accumulation of lipids per se may mediate diabetic renal disease also has not been directly determined.

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate fatty acid and cholesterol synthesis. SREBP's belong to basic helix-loop-helix-leucine zipper family and activate the entire program of fatty acid and cholesterol synthesis in liver (7, 8). SREBPs are synthesized as ~1150-amino acid precursors bound to the endoplasmic reticulum and nuclear envelope (7). To be activated, the NH2-terminal segment must be released from the membrane by a sequential two-step cleavage process (9, 10). After the second cleavage, the ~500-amino acid NH2-terminal segment of SREBP is released from the membrane and translocated to the nucleus, where it binds to enhancer regions of target genes to activate transcription.

Currently, there are three SREBP isoforms that have been identified and characterized (7, 11). SREBP-1a and -1c are derived from a single gene through the use of alternative transcription start sites that produce alternate forms of exon 1 (12). The third SREBP isoform, SREBP-2, is derived from a separate gene and is ~45% identical to SREBP-1a (13). In most cultured cell lines, the predominant SREBP-1 isoform is SREBP-1a (14). In contrast, in most animal tissues, SREBP-1c is the predominant SREBP-1 isoform (14). Multiple lines of evidence suggest that SREBP-1 and SREBP-2 have different relative effects on target genes. SREBP-1 preferentially activates genes involved in fatty acid synthesis, including acetyl CoA carboxylase and

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¶ The abbreviations used are: TGF, transforming growth factor; SREBP, sterol regulatory element-binding protein; VEGF, vascular endothelial growth factor; FAS, fatty acid synthase; TG, triglyceride; STZ, streptozotocin; MCT, murine cortical tubule; TBS, Tris-buffered saline; PAS, periodic acid Schiff; ECM, extracellular matrix; HG, high glucose; LG, low (normal) glucose; TgSREBP-1a, SREBP-1a transgenic; WT, wild-type; MOPS, 4-morpholinepropanesulfonic acid.

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fatty acid synthase (FAS), whereas SREBP-2 preferentially activates genes involved in cholesterol biosynthesis such as hydroxymethylglutaryl CoA synthase, hydroxymethylglutaryl CoA reductase, farnesyl diphosphate synthase, and squalene synthase (15–18).

SREBP activity can be regulated at both the transcriptional and posttranslational level. The proteolytic process can be blocked by sterols and activated by sterol depletion (9, 10). Transcriptional activation of SREBP-1 can be up-regulated by insulin (19), glucose (20), and liver X receptor (21) and suppressed by fatty acids (22, 23).

Because SREBP-1 plays important roles in the regulation of lipid homeostasis, altered regulation of this transcription factor may be involved in diabetic nephropathy by modulating renal lipid metabolism. In the present study, we found that in streptozotocin-induced diabetes, a type I diabetes model characterized by hyperglycemia and no changes in serum lipids, SREBP-1 expression was increased in the kidney cortex, resulting in up-regulation of enzymes responsible for fatty acid synthesis and, as a consequence, high renal triglyceride content, and was associated with mesangial expansion and glomerulosclerosis. Treatment of diabetic rats with insulin to correct the hyperglycemia prevented the increase in renal expression of SREBP-1 and the renal accumulation of triglyceride. The effect of hyperglycemia per se on up-regulation of SREBP-1 was confirmed in a cell culture model, in which we demonstrated that high glucose levels stimulated the expression of both SREBP-1α and -1c, which in turn augmented expression of FAS and acetyl CoA carboxylase, resulting in a higher triglyceride content. To directly prove a role of SREBP-1 in renal disease, we studied the SREBP-1α transgenic mice that overexpress the transcriptionally active form of SREBP-1α in kidney, adipose tissue, and liver (24). Although they have decreased serum glucose and triglyceride levels, these mice have an elevated renal content of triglycerides, increased expression of TGF-β1 and VEGF, mesangial expansion, accumulation of the extracellular matrix proteins type IV collagen and fibronectin, glomerulosclerosis, and proteinuria. We therefore propose that activation of renal SREBP-1 resulting in triglyceride accumulation plays a direct and an important role in the pathogenesis of diabetic nephropathy.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were purchased from Sigma. Antibodies against SREBP-1 and -2 were obtained from Santa Cruz Biotechnology; anti-collagen type IV and anti-fibronectin monoclonal antibodies were obtained from Sigma. All cell culture reagents were purchased from Invitrogen. Chemiluminescence detection reagents were obtained from Pierce.

**Streptozotocin-induced Diabetes**—Male Harlan Sprague-Dawley rats (200–250 g; obtained from Harlan Sprague-Dawley) were housed in...
colonies, maintained on a 12-h light/12-h dark cycle, and fed Teklad 4% Mouse/Rat Diet 7001 from Harlan Teklad Premier Laboratory Diets (Madison, WI). Rats in the streptozotocin (STZ) group were injected intravenously with 60 mg/kg body weight STZ in 50 mM sodium citrate solution (pH 4.5), and rats in the control group were injected with 50 mM sodium citrate. Tail vein blood glucose levels were measured every day, and animals were sacrificed 2 weeks after the induction of diabetes. One group of STZ rats was treated with regular and neutral protamine Hagedorn (NPH) insulin to achieve normal glucose levels. Of this purpose was homogenized in the same buffer. Nuclei were harvested once with a 21-gauge needle and incubated on ice for 15 min. Kidney cortex for this purpose was homogenized in the same buffer. Nuclei were harvested once with a 21-gauge needle and incubated on ice for 15 min. Kidney cortex and livers of rats were homogenized, and membrane and nuclear extract were isolated (see “Experimental Procedures”). 10 μg of protein was subjected to SDS-PAGE (10% w/v) and transferred to nitrocellulose membrane. Blots were blocked in 5% dried milk in TBST, washed four times with 1× TBS, and incubated with mouse anti-SREBP-1 (1:1,000) or goat anti-SREBP-2 (1:500) followed by horseradish peroxidase-labeled anti-mouse or anti-goat IgG (1:10,000 dilution), washed four times with 1× TBS, and developed using the chemiluminescence detection system. Equal loading and transfer were confirmed by reprobing membranes with anti-β-actin. The signals were quantified by densitometry. Whereas there was no change in renal expression of SREBP-1 in diabetic rats (A), SREBP-1 expression in the liver was decreased (B), indicating a tissue-specific effect of diabetes on SREBP-1 regulation. C, renal SREBP-2 expression was not altered in diabetic rats.

SREBP-1a Transgenic Mice—Transgenic mice overexpressing amino acids 1–460 of human SREBP-1a under the control of the phosphoenolpyruvate carboxyl kinase promoter have been described previously (24). All mice were housed in colony cages with a 12-h light/12-h dark cycle and maintained on Teklad 6% (w/w) Mouse/Rat Diet 7002 from Harlan Teklad Premier Laboratory Diets. For these experiments, we studied eight male SREBP-1a transgenic mice and eight littermate male wild-type mice.

Cell Culture—Murine cortical tubule (MCT) cells were a kind gift from Fuad Ziyadeh (University of Pennsylvania). The cells were maintained in Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum, 100 μg/ml streptomycin, 100 units/ml penicillin, and 4 mM glutamine, with a normal D-glucose concentration of 100 mg/dl. The cells were incubated in a humidified atmosphere of 5% CO₂ at 37 °C and passed every 3 days by trypsinization. For experiments, cells at 80% confluence were made quiescent by incubation in serum-free Dulbecco’s modified Eagle’s medium for 24 h and then cultured in Dulbecco’s modified Eagle’s medium containing 100 mg/dl glucose (normal glucose) or 450 mg/dl glucose (high glucose) for 24–48 h.

Preparation of Membranes and Nuclei—Cells were washed with ice-cold phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 1.7 mM KH₂PO₄, pH 7.4) and scraped with lysis buffer (50 mM Tris-Cl, pH 7.5, 1 mM pepstatin A, 1 μM leupeptin, 0.1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 mM EGTA, and 2 mM EDTA, pH 8.6). Kidney cortex was homogenized in the same buffer. To prepare membrane fractions, cell lysates or tissue homogenates were passed through a 25-gauge needle 10 times and centrifuged at 50,000 g at 4 °C in lysis buffer containing 1% Triton X-100 and re-centrifuged at 100,000 g for 60 min. Pellets were incubated under constant shaking for 30 min at 4 °C in lysis buffer containing 1% Triton X-100 and re-centrifuged at 100,000 × g for 60 min at 4 °C, and supernatants were collected as membrane fraction. To prepare nuclear extracts, cells were scraped into ice-cold 10 mM Tris-Cl, pH 7.4, 1.5 mM MgCl₂, 0.25% (v/v) Nonidet P-40, 1 mM pepstatin A, 1 μM leupeptin, 0.1 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, and 0.5 μg/ml leupeptin, passed through a 21-gauge needle, and incubated on ice for 15 min. Kidney cortex for this purpose was homogenized in the same buffer. Nuclei were harvested by centrifugation (1,000 × g, 10 min), the pellet was washed twice with the above-mentioned buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM MgCl₂, 0.25% (v/v) Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, 0.5 μg/ml leupeptin), and nuclear protein was extracted with 0.3 M NaCl, 10 mM Tris-Cl, pH 7.4, 1 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, 0.1% (v/v) SDS, and 0.5 μg/ml leupeptin at 4 °C.
for 1 h. The chromatin material was pelleted by centrifugation at 10,000 \( \times g \) for 30 min at 4 °C. The supernatant (nuclear extract) was collected and stored at −70 °C. All protein concentrations were determined by the method of Lowry (25).

**Immunoblotting—**Protein samples were subjected to SDS-PAGE (10% w/v), as described by Laemmli (26) and then transferred to nitrocellulose membranes. Membranes were blocked in 5% dried milk in TBST (0.2% Tween 20 in 1× TBS), incubated in the respective primary antibodies (the antibody titer was optimized for each antibody) followed by horseradish peroxidase-labeled anti-rabbit IgG (1:10,000 dilution), washed four times with 1× TBS, and then developed using the chemiluminescence detection system (Pierce).

**Blot Hybridization of RNA—**Total RNA was isolated as described previously (27). Absorbance at 260 and 280 nm was obtained to quantify the purity of the RNA fraction. cDNA probes were made using a digoxigenin labeling kit (Roche Molecular Biochemicals), and hybridizations were performed according to the manufacturer’s protocol.

**RNase Protection Assay—**A plasmid containing fragments of SREBP-1a and -1c was linearized with HindIII, and antisense RNA probe was synthesized using a digoxigenin RNA labeling kit (Roche Molecular Biochemicals). RNase protection assays were performed with a kit from Ambion according to the manufacturer’s protocol.

**Measurement of Lipid Composition—**Totals lipids were extracted from 100 mg of tissue by the method of Folch et al. (28). The contents of triglyceride and cholesterol were measured with respective kits from Sigma.

**Perfusion Fixation of Rat and Mouse Kidneys—**Rats and mice were anesthetized and perfused at a pressure of 180 mm Hg through the abdominal aorta 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 rats were perfused for an additional 5 min with the cacodylate buffer.

**PAS Staining, Oil-Red O Staining, and Immunofluorescence Microscopy—**Paraffin sections were stained for (a) hematoxylin and eosin and (b) PAS. Frozen sections were used for Oil-Red O staining to determine the renal accumulation of neutral fats (29). The stained kidney sections were imaged with an Olympus microscope and scored semiquantitatively in a blinded manner by the renal pathologist.

**Results**

Streptozotocin-induced Diabetes Modulates Renal SREBP-1 Expression and Lipid Composition—To study the potential association between hyperglycemia and altered renal SREBP-1 expression and lipid metabolism in diabetes, we treated Harlan Sprague-Dawley rats with a single dose of streptozotocin (60 mg/kg body weight). This dose resulted in an elevation of blood glucose levels the second day after injection (data not shown) that persisted for at least 2 weeks, at which point the animals were sacrificed. As shown in Table I, the glucose levels of streptozotocin-treated rats (469 ± 108 mg/dl) were markedly higher than those of the control animals (109 ± 11 mg/dl; \( p < 0.01 \)). However, there were no significant changes in the serum lipid levels, including triglyceride, cholesterol, low density lipoprotein, and very low density lipoprotein.

Compared those of with control animals, glomeruli of STZ-treated rats displayed increased PAS staining, which is a hallmark of mesangial expansion with higher extracellular matrix
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Glucose induces up-regulation of SREBP-1 protein expression. Cultured under LG (100 mg/dl) and HG (450 mg/dl) conditions. High glucose induces up-regulation of both SREBP-1α and SREBP-1c.

The representative results are from three independent experiments. Western blot and quantification were performed as described in the Fig. 3 legend. High glucose induces up-regulation of SREBP-1 protein expression.

To study whether short-term (2 weeks) hyperglycemia can cause alterations in lipid metabolism, we performed Oil-Red O staining, which revealed the accumulation of neutral lipids in the tubular and tubulointerstitial cells of STZ-treated rats (Fig. 1, left middle panel).

SREBP-1 expression, accumulation of neutral fat, and sclerosis of the kidney with increased matrix protein accumulation, were indeed mediated by insulin deficiency and the resulting hyperglycemia.
glycemia, we treated the STZ diabetic rats with insulin to achieve normal blood glucose levels. Insulin treatment resulted in prevention of increased renal SREBP-1 expression (Fig. 5 A), neutral fat accumulation (Fig. 1, bottom left panel), increased PAS staining (Fig. 1, bottom right panel), and matrix protein accumulation (Fig. 2, bottom panels). Insulin treatment also corrected the decreased SREBP-1 expression in the liver (Fig. 5 B).

**Table II**

| Parameter                  | WT (n = 8) | TgSBP-1a (n = 8) | p     |
|----------------------------|------------|-----------------|-------|
| Glucose (mg/dl)            | 121 ± 12   | 98 ± 13         | <0.05 |
| Serum TG (mg/dl)           | 172 ± 78   | 138 ± 37        | NS±   |
| Serum cholesterol (mg/dl)  | 58 ± 14    | 51 ± 18         | NS±   |
| Liver TG                   | 18 ± 5     | 362 ± 68        | <0.005|
| Liver cholesterol          | 45 ± 2     | 174 ± 31        | <0.005|

± NS, nonsignificant

**Fig. 8.** A, abundance of SREBP-1 protein in the kidney cortex of WT and TgSBP-1a mice. 20 μg of kidney cortex protein was loaded in each lane for immunoblot as described under “Experimental Procedures.” B, mRNA abundance of FAS, acetyl CoA carboxylase (ACC), and acyl CoA oxidase (ACO) in the kidney cortex of WT and TgSBP-1a mice. FAS and acetyl CoA carboxylase mRNA abundance are increased in TgSBP-1a. Total triglyceride (C) and total cholesterol (D) contents in the kidney cortex of WT and TgSBP-1a mice. There is a significant increase in triglyceride content in TgSBP-1a mice.

Increased Renal SREBP-1a Expression Modulates Renal Lipid Composition, Expression of Growth Factors, and Glomerulosclerosis—The results above strongly suggested that increased expression of SREBP-1 and stimulated fatty acid synthesis resulted in lipid accumulation and glomerulosclerosis in STZ diabetic rats. To directly address this possibility, we studied the kidneys of SREBP-1a transgenic (TgSBP-1a) mice. Due to the ectopic expression of SREBP-1a mature forms, which can bypass the proteolytic processing step and translocate directly to nuclei to activate target genes, lipid synthesis in the liver of TgSBP-1a mice was dramatically augmented, as was reported previously (24) and confirmed in this study by the 20-fold increase of triglyceride content and the 5-fold increase of cholesterol content in the liver of transgenic animals compared with those of wild-type controls (Table II). However,
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DISCUSSION

Since the description by Kimmelstiel and Wilson (4) of the classical nodular glomerulosclerosis and presence of lipid deposits in the diabetic kidney, several investigators have shown the presence of lipid deposits in the kidneys of diabetic humans and experimental animals, and they have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease (5, 6). The major assumption has been that these lipid deposits originate from increased levels of serum lipids. However, the possibility of an increase in renal lipid synthesis also resulting in renal lipid accumulation has not been entertained. Furthermore, whether the accumulation of lipids per se may mediate diabetic renal disease has also not been directly determined.

Our study demonstrates that in the 2-week model of streptozotocin-induced type I diabetes in the rat, lipid deposits occur in the absence of any alterations in serum cholesterol or triglyceride concentration. Furthermore, the increase in renal lipid accumulation is associated with a tissue-specific increase in renal SREBP-1 expression, which results in activation of enzymes of fatty acid synthesis and an increase in triglyceride accumulation. The effect of streptozotocin-induced diabetes on SREBP-1 expression in the kidney is in contrast to that observed in the liver, where there is decreased expression of SREBP-1 (Ref. 31; Figs. 3B and 5B). This clearly indicates that there is tissue-specific regulation of SREBP-1 by hyperglycemia and/or insulin deficiency.

When streptozotocin-treated diabetic rats were treated with insulin to achieve normoglycemia, there was almost complete...
normalization of kidney and liver SREBP-1 expression, increased renal neutral fat (triglyceride) content, increased PAS staining, and accumulation of extracellular matrix proteins. These results indicate that the changes in renal expression of SREBP-1 in STZ rats are mediated by hyperglycemia and/or insulin deficiency and are not due to nonspecific toxic effects of streptozotocin per se.

To determine whether hyperglycemia per se induced the increase in SREBP-1 expression in the kidney, we incubated human mesangial cells or mouse proximal tubular cells in the presence of a high glucose medium (450 mg/dl), a glucose level almost identical to that measured in the serum of streptozotocin-treated diabetic rats (Table I). We found that after 24 and 48 h of incubation of MCT cells with a high glucose medium, there was a marked up-regulation of the SREBP-1a and SREBP-1c mRNA level. The increased expression of SREBP-1a and SREBP-1c further resulted in stimulation of FAS mRNA and accumulation of triglycerides. These results therefore indicate that in the kidney, a high glucose concentration per se can directly up-regulate the transcriptional factor SREBP-1 and result in increased accumulation of triglycerides. Because the same experimental conditions also result in cell hypertrophy, increased expression of TGF-β, and extracellular matrix proteins (34, 35), our results suggest that accumulation of triglycerides can mediate the cellular changes characteristic of diabetic nephropathy.

We next performed studies in SREBP-1a transgenic mice to determine whether increased renal expression of SREBP-1a per se results in increased renal lipid accumulation and mediates glomerulosclerosis. We found that in transgenic mice over-expressing SREBP-1a in the kidney, despite decreased serum glucose and triglyceride concentration, there is a marked up-regulation of the mRNA of enzymes that mediate fatty acid synthesis, resulting in the accumulation of mainly triglycerides. Furthermore, the increase in SREBP-1a and triglycerides is associated with increased expression of TGF-β and VEGF, growth factors that have been implicated to play an important role in the pathogenesis of diabetic nephropathy (32, 33). The SREBP-1a transgenic mice also have increased expression of the extracellular matrix proteins type IV collagen and fibronectin.

Previous 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 causes up-regulation of growth factors, including TGF-β and platelet-derived growth factor (36), plasminogen activator inhibitor 1 (37, 38), extracellular matrix proteins (36, 39), pro-inflammatory cytokines including interleukins and tumor necrosis factor α (36), adhesion molecules including monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (40, 41), and lipid peroxidation and glycoxidation (42). Our studies in vivo indicate that accumulation of renal triglycerides in streptozotocin-treated diabetic rats and SREBP-1a transgenic mice causes increased expression of TGF-β and VEGF as well as collagen and fibronectin, resulting in glomerulosclerosis and proteinuria.

The clinical significance of increased renal triglyceride content in renal disease is illustrated in patients with type IA glycogen storage disease (von Gierke’s disease), who develop...
focal glomerular sclerosis with prominent lipid deposits (mainly triglycerides) progressing to advanced renal insufficiency (43). In addition, a potential role for triglycerides in mediating human diabetic renal disease is illustrated by studies in diabetic patients in which treatment with an oral fibrate, a peroxisome proliferator-activated receptor α agonist that lowers serum triglyceride concentration, also results in a significant decrease in proteinuria, a clinical marker of diabetic renal injury (44, 45).

In summary, our results indicate that in diabetes mellitus, hyperglycemia per se directly up-regulates renal expression of the transcriptional factor SREBP-1, which causes increased fatty acid synthesis and accumulation of triglycerides. This is associated with up-regulation of TGF-β and VEGF expression and accumulation of collagen and fibronectin, which result in mesangial expansion and accumulation of extracellular matrix proteins, resulting in glomerulosclerosis and proteinuria (Fig. 13). We therefore propose that activation of renal SREBP-1 resulting in alterations in renal lipid metabolism and renal lipid accumulation plays an important role in the pathogenesis of diabetic nephropathy.

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