Original Article

Regulation of Renal Fatty Acid and Cholesterol Metabolism, Inflammation, and Fibrosis in Akita and OVE26 Mice With Type 1 Diabetes

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In Akita and OVE26 mice, two genetic models of type 1 diabetes, diabetic nephropathy is characterized by mesangial expansion and loss of podocytes, resulting in glomerulosclerosis and proteinuria, and is associated with increased expression of profibrotic growth factors, proinflammatory cytokines, and increased oxidative stress. We have also found significant increases in renal triglyceride and cholesterol content. The increase in renal triglyceride content is associated with *1*) increased expression of sterol regulatory element–binding protein (SREBP)-1c and carbohydrate response element–binding protein (ChREBP), which collectively results in increased fatty acid synthesis, *2*) decreased expression of peroxisome proliferator–activated receptor (PPAR)-α and -δ, which results in decreased fatty acid oxidation, and *3*) decreased expression of farnesoid X receptor (FXR) and small heterodimer partner (SHP). The increase in cholesterol content is associated with *1*) increased expression of SREBP-2 and 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, which results in increased cholesterol synthesis, and *2*) decreased expression of liver X receptor (LXR)-α, LXR-β, and ATP-binding cassette transporter-1, which results in decreased cholesterol efflux. Our results indicate that in type 1 diabetes, there is altered renal lipid metabolism favoring net accumulation of triglycerides and cholesterol, which are driven by increases in SREBP-1, ChREBP, and SREBP-2 and decreases in FXR, LXR-α, and LXR-β, which may also play a role in the increased expression of profibrotic growth hormones, proinflammatory cytokines, and oxidative stress. *Diabetes* 55:2502–2509, 2006

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ABCA, ATP-binding cassette transporter; ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; ChREBP, carbohydrate response element–binding protein; FAS, fatty acid synthase; FXR, farnesoid X receptor; HMG, 3-hydroxy-3-methylglutaryl; IL, interleukin; L-PK, liver pyruvate kinase; LXR, liver X receptor; PAI, plasminogen activator inhibitor; PAS, periodic acid Schiff; PPAR, peroxisome proliferator–activated receptor; SCD, stearoyl CoA desaturase; SHP, small heterodimer partner; SREBP, sterol regulatory element–binding protein; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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There is growing evidence that abnormal lipid metabolism and renal accumulation of lipids play a role in the pathogenesis of diabetic nephropathy. Virchow (1) first suggested the association between lipids and renal disease in 1858 when he described successive stages of fatty metamorphosis and fatty detritus in the renal epithelium in Bright's disease. In their classic 1936 article describing the pathological sign of nodular sclerosis, Kimmelstiel and Wilson (2) 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 investigators have shown the accumulation of lipids in the kidneys of diabetic humans and experimental animals (3,4) and have proposed that lipids may play an important role in the pathogenesis of diabetic kidney disease.

In studies in our laboratory in the streptozotocin-induced model of type 1 diabetes in the rat, we have found increased renal expression of the transcriptional factor sterol regulatory element–binding protein (SREBP)-1, which results in increased synthesis and accumulation of triglyceride and correlates with manifestations of obesity and diabetes-related renal sclerosis and proteinuria (5).

Whether the accumulation of lipids per se mediate diabetic renal disease is demonstrated in SREBP-1a transgenic mice that overexpress SREBP-1a in the kidney. In the absence of hyperglycemia or dyslipidemia, increased expression of SREBP-1a in the kidney results in lipid accumulation and increased expression of transforming growth factor (TGF)-β, plasminogen activator inhibitor (PAI)-1, and vascular endothelial growth factor (VEGF), which mediate renal hypertrophy, accumulation of extracellular matrix proteins, and mesangial expansion, resulting in glomerulosclerosis and proteinuria (5). In contrast, in SREBP-1c knockout mice, the effects of a high–saturated fat diet on increased renal expression of TGF-β, PAI-1, and VEGF, as well as the extracellular matrix proteins type IV collagen and fibronectin, are prevented (6). These studies imply that alterations in renal lipid metabolism mediated by SREBPs play an important role in the pathogenesis and progression of renal disease in type 1 diabetes, as well as in obesity and insulin resistance.

Recently, the carbohydrate response element–binding protein (ChREBP) has also been shown to play an important role in regulation of fatty acid synthesis in the liver (7–9). ChREBP may work in concert with SREBP-1c to mediate fatty acid and triglyceride in the liver (7–9).

However, the regulation of ChREBP in the kidney has not been determined.

In addition, another nuclear receptor, the farnesoid X receptor (FXR), has also been shown to have an important role in the regulation of fatty acid metabolism in the liver by inhibiting SREBP-1 and ChREBP, while inducing peroxisome proliferator–activated receptor-α (PPAR-α) (10, 11). However, the regulation of FXR in the kidney has not been determined.

In models of obesity and insulin resistance, as well as type 2 diabetes, we have found that in addition to triglyceride, cholesterol also accumulates in the kidney (6,12). SREBP-2 is the master transcriptional regulator of cholesterol synthesis by way of inducing enzymes that mediate cholesterol synthesis, including cholesterol 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (13,14), whereas the liver X receptor (LXR) modulates cholesterol efflux via inducing ATP-binding cassette transporter (ABCA)-1 (11). The regulation of SREBP-2 and LXR in the kidney of animals with type 1 diabetes, however, has not been determined.

The purpose of our study was to determine the potential role of alterations in renal triglyceride and cholesterol metabolism in two recently described genetic models of type 1 diabetes in mice: the *Ins2*^{Akita} mice on the C57BL/6 background (15,16) and the OVE26 mice on the FVB background (17). Our objective was also to determine the potential roles of the nuclear receptors that regulate fatty acid metabolism (SREBP-1, ChREBP, PPAR-α, and FXR) and cholesterol metabolism (SREBP-2 and LXR) in regulation of renal lipid metabolism and diabetic nephropathy.

Our results indicate that in type 1 diabetes, altered renal lipid metabolism favors net accumulation of triglyceride and cholesterol, which are driven by *1*) increases in the expression and activity of SREBP-1, ChREBP, and SREBP-2 and *2*) decreases in the expression and activity of the nuclear receptors FXR, LXR-α, and LXR-β, which may also play a role in the increased expression of profibrotic growth hormones, proinflammatory cytokines, and oxidative stress.

RESEARCH DESIGN AND METHODS

Male C57BL/6-*Ins2*^{Akita} (Akita) mice in the C57BL/6 background and C57BL/6 control mice were obtained from The Jackson Laboratories (Bar Harbor, ME). *Ins2*^{Akita} is a model of type 1 diabetes. The Akita spontaneous mutation is an autosomal dominant mutation in the insulin II gene (*Ins2*) (15,16). This missense mutation results in an amino acid substitution (cysteine 96 to tyrosine), which corresponds to the seventh amino acid position of the insulin II A chain. The mice were maintained on a 12-h light/dark cycle and fed standard mouse chow (Mouse Chow 5015; Ralston Purina, St. Louis, MO) ad libitum for 3 months until they were killed. The animal studies and the protocols were approved by the institutional review boards at the Denver Veterans Medical Center and the University of Colorado Health Sciences Center.

At the end of the 3 months, spot urine samples were obtained so that albumin and creatinine levels could be measured. In one group of animals (n = 8 in each experimental group), after anesthesia (intraperitoneal injection of pentobarbital; Abbott Laboratories, Chicago, IL), blood was drawn from the inferior vena cava, and then the kidneys were removed and processed for biochemical studies that included *1*) RNA extraction and real-time quantitative PCR, *2*) isolation of nuclei and microsomes and Western blotting, *3*) measurement of stearoyl CoA desaturase-1 (SCD-1) enzyme activity, and *4*) lipid extraction and lipid composition analysis. In another group of animals (n = 4 in each experimental group), after anesthesia, the kidneys were fixed via in vivo perfusion fixation and the kidneys were processed for *1*) periodic acid Schiff (PAS) stain for renal histopathology, *2*) oil red O stain for neutral lipids, and *3*) immunofluorescence microscopy for extracellular matrix proteins (5,6,12,18,19).

Parallel studies were also performed in the OVE26 model of type 1 diabetes. These transgenic mice express a chicken calmodulin minigene controlled by the rat insulin II promoter and develop hyperglycemia within 24 h of age because of decreased pancreatic insulin (17). Male OVE26 mice on the FVB background and control FVB mice were obtained from Dr. Paul Epstein at the University of Louisville, Kentucky, and from The Jackson Laboratories.

Measurement of urine albumin and creatinine. Urine albumin concentration was determined by competitive enzyme-linked immunosorbent assay using an Albuwell M kit (Exocell, Philadelphia, PA). Urine creatinine concentration was determined by Jaffe's reaction of alkaline picrate with creatinine using a Creatinine Companion kit (Exocell). Results were expressed as the urine albumin-to-creatinine ratio (micrograms per milligram) (5,6,12,18,19).

Total RNA extraction and real-time PCR. Total RNA was extracted according to the Trizol protocol (Invitrogen Life Technologies, Carlsbad, CA). Total RNA (2 μg) was subjected to DNase digestion followed by cDNA synthesis using the iScript cDNA Synthesis kit (Bio-Rad, Richmond, CA). We used iQSYBR Green Supermix (Bio-Rad) for real-time PCR according to the manufacturer's instructions. Cyclophilin was used as internal control, and the amount of RNA was calculated by the comparative C_T method. All data were calculated from duplicate reactions, as we have previously described (5,6,12,18,19). Primers used are listed in Table 1.

Nuclei and microsome isolation. Kidneys were homogenized at 4°C in a buffer (20 mmol/l Tris-Cl, pH 7.4, 75 mmol/l NaCl, 2 mmol/l EGTA, 2 mmol/l EDTA, 1 mmol/l Na_3VO_4, and 1 mmol/l dithiothreitol), supplemented with a protease inhibitor cocktail that consisted of 104 mmol/l AEBSF, 0.08 mmol/l aprotinin, 2 mmol/l leupeptin, 4 mmol/l bestatin, 1.5 mmol/l pepstatin A, and 1.4 mmol/l E-64 (Sigma-Aldrich, St. Louis, MO). Nuclear extracts and microsomes were prepared as we have previously described (5,6,12,18,19).

Protein electrophoresis and Western blotting of nuclear extracts and microsomes. Equal amounts of protein samples were subjected to SDS-PAGE (10% wt/vol), and they were then transferred to nitrocellulose membranes. After blockage with 5% fat-free milk powder with 1% Triton X-100 in Tris-buffered saline (20 mmol/l Tris-Cl, 150 mmol/l NaCl, pH 7.4), the blots were incubated with antibodies against SREBP-1 (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1,000), SREBP-2 (Santa Cruz Biotechnology; 1:1,000), and SCD-1 (Santa Cruz Biotechnology; 1:1,000). The SREBP-1 antibody recognizes both SREBP-1a and -1c. Corresponding secondary antibodies were visualized using enhanced chemiluminescence (Pierce, Bradford, IL). The signals were quantified with a Phosphor Imager with chemiluminescence detector and the accompanying densitometry software (Bio-Rad).

Measurement of SCD enzyme activity. SCD enzyme activity was measured in microsomes prepared from individual kidney cortex extracts by determining the conversion of [1-^{14}C] stearoyl-CoA to [1-^{14}C] oleate (20).

Lipid extraction and measurement of lipid composition. Lipids from the renal cortex were extracted by the method of Bligh and Dyer, as we have previously described (5,6,12,18,19). Triglyceride and cholesterol content was measured using kits from Sigma according to instructions of the manufacturer.

Perfusion fixation of mouse kidney. Mice were anesthetized and perfused at a pressure of 180 mmHg through the abdominal aorta, as previously described (5,6,12,18,19).

PAS staining, oil red O staining, and immunofluorescence microscopy. Paraffin sections were stained for PAS. Frozen sections were used for oil red O staining to determine the renal accumulation of neutral fats (5,6,12,18,19). The stained kidney sections were imaged with an Olympus microscope. Immunofluorescence microscopy for type IV collagen and fibronectin were performed as previously described (5,6,12,18,19). The kidney sections were then imaged with a laser scanning confocal microscope (Zeiss LSM 510).

Statistical analysis. SPSS 11.0 for Windows was used for statistical analysis. The results were expressed as the means ± SE. The statistical significance of differences was assessed by unpaired t test.

RESULTS

Ins2^{Akita} mice

Mesangial expansion, podocyte injury, glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. In *Ins2*^{Akita} mice, PAS staining clearly reveals mesangial expansion, increased matrix protein accumulation, and tubulointerstitial fibrosis when compared with age- and sex-matched control mice (Fig. 1*A*). Immunofluorescence microscopy with anti-fibronectin (results not shown) and anti-type IV collagen (Fig. 1*B*) antibodies indicates increased intensity of immunofluorescence in the glomeruli and tubulointerstitial cells in *Ins2*^{Akita} mice, indicating accumulation of extracellular matrix proteins.

TABLE 1
Primers used in quantitative real-time PCR

Gene	Sense	Antisense
Cyclophilin	TGGAGAGCACCAAGACAGACA	TGCCGGAGTCGACAATGAT
ACC	CCCAGCAGAATAAAGCTACTTTGG	TCCTTTTGTGCAACTAGGAACGT
Fatty acid synthase	CCTGGATAGCATTCCGAACCT	AGCACATCTCGAAGGCTACACA
HMG-CoA reductase	AGCCGAAGCAGCACATGAT	CTTGTGGAATGCCTTGTGATTG
SREBP-1c	GGCACTAAGTGCCCTCAACCT	TGCGCAGGAGATGCTATCTCCA
SREBP-2	CAAGTCTGGCGTTCTGAGGAA	ATGTTCTCCTGGCGAAGCT
Acyl-CoA oxidase	GGCCAACTATGGTGGACATCA	ACCAATCTGGCTGCACGAA
ABCA-1	CGTTTCCGGGAAGTGTCCTA	GCTAGAGATGACAAGGAGGATGGA
ChREBP	CGGGACATGTTTGATGACTATGTC	ACCCGCACGCTGCACAACTGG
L-PK	CTTGCTCTACCGTGAGCCTC	ACCACAATCACCAGATCACC
LXR-α	TTGCACCCGACCCTCAGA	AAGCCCGACAACAGCGCTTTGC
LXR-β	CGACTCCAGGACAAGAAGC	TCAAGAAGACACCACCAAGG
Farnesoid X receptor	CCAACCTGGGTTTCTACCC	CACACAGCTCATCCCCTTT
PPAR-α	CTGCAGAGCAACCATCCAGAT	GCCGAAGGTCCACCATTTT
PPAR-δ	TCCAGAAGAAGAACCGCAACA	GGATAGCGTTGTGCGACATG
TGF-β	TAGTAGACGATGGGCAGTGG	TAGTAGACGATGGGCAGTGG
VEGF	AACGATGAAGCCCTGGAGTG	TGAGAGGTCTGGTTCCCGA
PAI-1	GGACACCCTCAGCATGTTCA	TCTGATGAGTTCAGCATCCAAGAT
MCP-1	GTCCCTGTCATGCTTCTGG	CATCTTGCTGGTGAATGAGTAG
TNF-α	AATGGCCTCCCTCTCATCAGT	GCTACAGGCTTGTCACTCGAATT
IL-6	TATGAAGTTCCTCTCTGCAAGAGA	TAGGGAAGGCCGTGGTT
Fibronectin	AGACCATACCTGCCGAATGTAG	GAGAGCTTCCTGTCCTGTAGAG
Nephrin	CGA GGC ACT TCG TGA AAC	GCA CTT GCT CTC CCA GGA CT
Podocin	TTT GCC TTT GCC ATT TGA CA	ATG CTC CCT TGT GCT CTG TTG
Synaptopodin	GTG TTC ATC TGT GCC CAT TCC	AGC TAG CCG AGG CCA TGT TA
Type IV collagen	TACCTGCCACTACTTCGCTAAC	CGGATGGTGTGCTCTGGAAG
Nox4	GTGAAGATTTGCCTGGAAGAAC	GATGATTGATGACTGAGATGATGG
Phoxp22	GCACACCGCCATCCACAC	CCAGCCAACCGAGTCACG
Phoxp47	CCATCATCCTTCAGACCTATCG	CCGCTCTCGCTCTTCTCC
RAGE	CCATCCTACCTTCTCCTG	AGCGACTATTCCACCTTC

RAGE, receptor for advanced glycation end products.

Ins2^{Akita} mice have significant decreases in the mRNA abundance of the podocyte marker proteins synaptopodin and podocin (Table 2). These changes are associated with a significant increase in the urine albumin-to-creatinine ratio in *Ins2*^{Akita} when compared with control mice (Table 2).

Increased renal expression of growth factors and inflammatory cytokines. The glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria in *Ins2*^{Akita} mice are associated with significant increases in renal cortical mRNA abundance of *1*) fibrosis-inducing growth factors TGF-β and PAI-1, *2*) glomerular permeability–inducing growth factor VEGF, and *3*) proinflammatory cytokine tumor necrosis factor (TNF)-α (Table 2).

Increased lipid deposits in kidney. In contrast to minimal or absent oil red O staining in the kidneys of the control mice, there is very strong staining of oil red O in the glomeruli of *Ins2*^{Akita} mice, which indicates accumulation of neutral lipid deposits (Fig. 2*A*). Biochemical analysis of lipid composition of the renal cortex indicates that the accumulation of neutral lipid deposits corresponds to significant increases in triglyceride and cholesterol content (Fig. 2*B*).

Alterations in renal expression of nuclear receptors and genes that regulate triglyceride and cholesterol metabolism. *Ins2*^{Akita} mice have a significant increase in SREBP-1 nuclear protein abundance, a nuclear receptor/transcription factor that is a master regulator of fatty acid and triglyceride synthesis (Table 3). To determine the functional significance of the increase in SREBP-1 protein, we determined the mRNA, protein, and enzyme activity of SCD-1, a key enzyme in regulation of fatty acid and triglyceride synthesis. We found that *Ins2*^{Akita} mice have significant increases in SCD-1 mRNA, SCD-1 protein, and SCD-1 enzyme activity (Table 3).

Interestingly, the expression of FXR, a nuclear receptor that is an important negative regulator of SREBP-1c and TGF-β, and its major target small heterodimer partner (SHP) were significantly decreased in the kidneys of *Ins2*^{Akita} mice (Table 3).

Ins2^{Akita} mice also have a significant increase in SREBP-2 nuclear protein abundance, a nuclear receptor/transcription factor that is a master regulator of cholesterol synthesis (Table 3). In addition, *Ins2*^{Akita} mice have a significant decrease in ABCA-1 mRNA abundance, a key enzyme that mediates cholesterol efflux (Table 3).

Interestingly, the expression of LXR-α and LXR-β, nuclear receptors that are important positive regulators of ABCA-1 and negative regulators of proinflammatory cytokines, were significantly reduced in the kidneys of *Ins2*^{Akita} mice (Table 3).

OVE26 mice

Podocyte injury and increased accumulation of extracellular matrix proteins. Previous studies determined that OVE26 mice on the FVB genetic background develop severe glomerulosclerosis and proteinuria (17). In our study, we confirmed these finding (results not shown), and we have also determined that there is a marked increase in the mRNA abundance for type IV collagen and fibronectin (Table 4), which suggests that the increased expression and accumulation of the extracellular matrix proteins are transcriptionally regulated. In addition, these

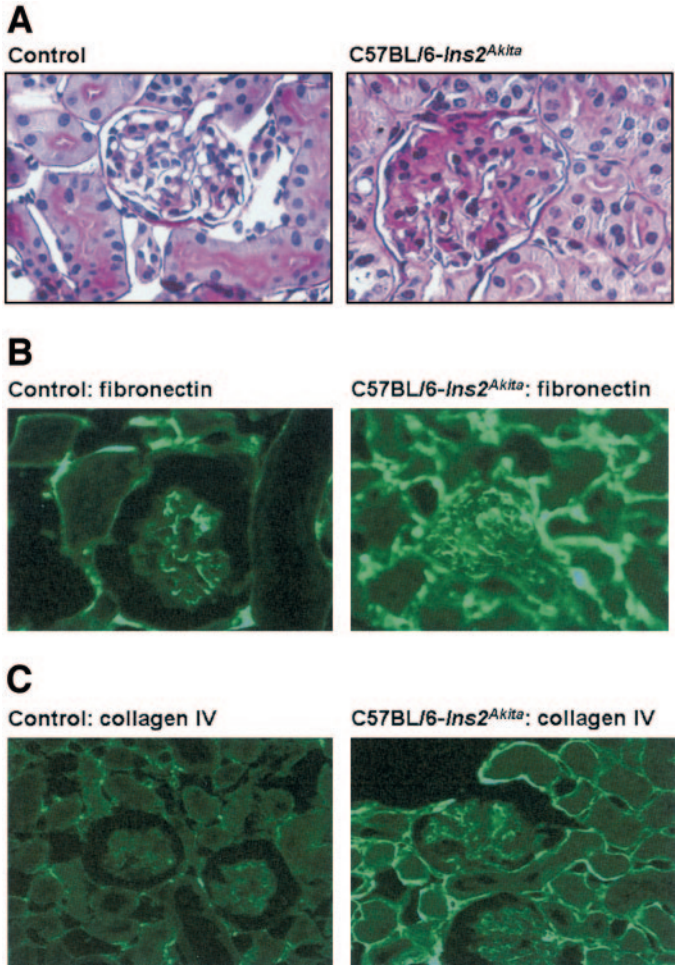

FIG. 1. *A*: **PAS staining of kidney sections indicates increased mesangial expansion and accumulation of matrix proteins in C57BL/6-*Ins2*^{Akita} mice compared with control mice; immunofluorescence microscopy of kidney sections indicates increased glomerular and tubulointerstitial staining for fibronectin (*B*) and type IV collagen (*C*) in Akita mice.**

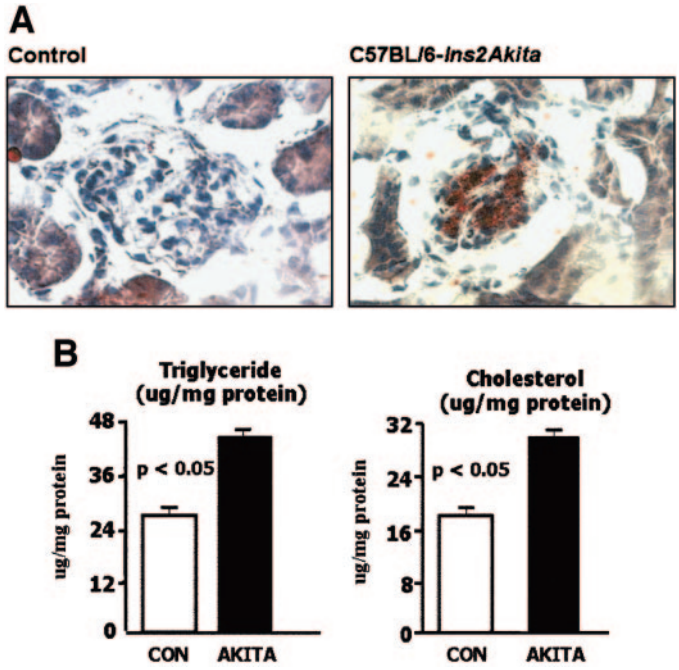

FIG. 2. *A*: **There is a marked increase in glomerular oil red O staining in the kidneys of Akita mice compared with control mice, which indicates an increase in neutral lipid (cholesterol and triglycerides) accumulation.** *B*: **There are significant increases in triglyceride and cholesterol content in the kidneys of Akita mice compared with control (CON) mice.**

mice also have significant decreases in the mRNA abundance for the podocyte marker proteins podocin and synaptopodin (Table 4).

Increased renal expression of inflammatory cytokines and oxidative stress. OVE26 mice have significant increases in renal cortical mRNA abundance of *1*) the proinflammatory cytokines TNF-α and interleukin (IL)-6 (Table 4), *2*) markers of oxidative stress, including components of the NADPH oxidase system (Nox4, p22phox, and p47phox) (Table 4), and *3*) receptor for advanced glycation end products (Table 4).

Alterations in renal expression of nuclear receptors and genes that regulate triglyceride and cholesterol metabolism. OVE26 mice have significant increases in mRNA abundance of *SREBP-1c* target enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Table 5), enzymes that initiate the cascade of fatty acid synthesis. In addition, there is also marked upregulation of ChREBP and its major target enzyme, liver pyruvate kinase (L-PK) (Table 5), which, in concert with the SREBP-1 pathway, mediates fatty acid and triglyceride synthesis.

The increases in SREBP-1– and ChREBP-dependent fatty acid synthesis pathways are paralleled by marked decreases in the expression of PPAR-α, PPAR-δ, and their target enzyme acyl-CoA oxidase (ACO) (Table 5), which

TABLE 2
Urinary albumin excretion, podocyte, profibrotic growth factor, and proinflammatory cytokine mRNA expression in wild-type and Akita mice

	Wild type	Akita	*P* value
Urinary albumin-to-creatinine ratio	0.3 ± 0.1	1.8 ± 0.1	<0.01
Podocyte markers			
Synaptopodin	30.2 ± 1.0	7.1 ± 0.8	<0.01
Podocin	44.9 ± 1.3	11.2 ± 0.7	<0.01
Profibrotic growth factors			
TGF-β	1.9 ± 0.1	4.1 ± 0.2	<0.05
PAI-1	0.9 ± 0.1	6.0 ± 0.2	<0.01
Proinflammatory cytokines			
VEGF	2.1 ± 0.2	3.6 ± 0.2	<0.05
TNF-α	1.7 ± 0.2	4.1 ± 0.3	<0.01

Data are means ± SE (n = 6 mice in each group).

TABLE 3
Nuclear receptor and lipid metabolism enzyme activity, protein, and mRNA expression in wild-type and Akita mice

	Wild type	Akita	*P* value
Fatty acid synthesis			
SREBP-1 protein	3.0 ± 0.2	5.4 ± 0.2	<0.01
SCD-1 mRNA	1.8 ± 0.2	3.6 ± 0.3	<0.05
SCD-1 protein	7.2 ± 0.1	11.6 ± 0.3	<0.05
SCD-1 activity	2.5 ± 0.1	4.2 ± 0.3	<0.05
FXR mRNA	9.1 ± 0.2	2.0 ± 0.4	<0.01
SHP mRNA	3.9 ± 0.2	2.1 ± 0.4	<0.05
Cholesterol synthesis and efflux			
SREBP-2 protein	1.6 ± 0.2	3.4 ± 0.3	<0.01
ABCA1 mRNA	14.8 ± 1.0	5.8 ± 0.9	<0.01
LXR-α	12.3 ± 0.8	3.1 ± 0.7	<0.01
LXR-β	12.9 ± 0.9	2.1 ± 0.8	<0.01

Data are means ± SE (n = 6 mice in each group).

mediate fatty acid oxidation. These marked increases in fatty acid synthesis and decreased fatty acid oxidation are paralleled by a marked decrease in FXR expression (Table 5), a nuclear receptor that has been shown in the liver to be a negative regulator of SREBP-1 and ChREBP and a positive regulator of PPAR-α.

OVE26 mice also have a significant increase in SREBP-2 mRNA abundance, a nuclear receptor and transcription factor that is a master regulator of cholesterol synthesis (Table 5) and its major target enzyme HMG-CoA reductase (Table 5), which is a key enzyme in cholesterol synthesis.

Interestingly, the expression of LXR-α and LXR-β, nuclear receptors that are important positive regulators of ABCA-1 and negative regulators of proinflammatory cytokines, were significantly reduced in the kidneys of OVE26 mice (Table 5).

DISCUSSION

Well-known changes in diabetes-related renal function and structure include mesangial expansion and podocyte injury and loss, which result in glomerulosclerosis, proteinuria, and a decline in glomerular filtration rate (21–29).

TABLE 4
Podocyte, extracellular matrix protein, proinflammatory cytokine, and NADPH oxidase mRNA expression in wild-type and OVE26 mice

	Wild type	OVE26	*P* value
Podocyte markers			
Podocin	86.2 ± 4.3	17.8 ± 6.8	<0.01
Synaptopodin	19.9 ± 3.1	5.6 ± 2.7	<0.01
Extracellular matrix proteins			
Fibronectin	39.2 ± 4.7	204.7 ± 6.1	<0.01
Type IV collagen	2.7 ± 0.3	12.6 ± 0.6	<0.01
Proinflammatory cytokines			
TNF-α	0.2 ± 0.3	1.8 ± 0.1	<0.01
IL-6	0.8 ± 0.4	7.2 ± 1.0	<0.01
Oxidative stress			
Nox4	12.8 ± 1.9	36.7 ± 2.1	<0.05
Phoxp22	2.4 ± 0.4	5.9 ± 0.5	<0.05
Phoxp47	5.8 ± 2.8	44.8 ± 3.9	<0.01
RAGE	16.8 ± 1.8	49.7 ± 3.7	<0.05

Data are means ± SE (n = 5 mice in each group).

TABLE 5
Nuclear receptor and lipid metabolism enzyme mRNA expression in wild-type and OVE26 mice

	Wild type	OVE26	*P* value
Fatty acid synthesis			
SREBP-1c	3.6 ± 0.5	7.1 ± 0.8	<0.05
ACC	3.2 ± 0.8	7.8 ± 0.7	<0.05
FAS	2.1 ± 0.5	4.8 ± 0.4	<0.05
ChREBP	1.4 ± 0.3	4.2 ± 0.4	<0.01
L-PK	7.1 ± 1.0	14.9 ± 1.2	<0.05
Fatty acid oxidation			
PPAR-α	12.1 ± 0.6	3.9 ± 0.9	<0.01
PPAR-δ	14.7 ± 0.5	4.0 ± 0.7	<0.01
ACO	4.6 ± 0.2	1.8 ± 0.3	<0.01
FXR	5.3 ± 0.3	2.3 ± 0.4	<0.05
Cholesterol synthesis			
SREBP-2	3.9 ± 0.6	12.7 ± 0.9	<0.01
HMG-CoA reductase	2.1 ± 0.3	5.1 ± 0.4	<0.05
Cholesterol efflux			
LXR-α	6.3 ± 0.4	3.1 ± 0.5	<0.05
LXR-β	22.1 ± 0.6	7.9 ± 0.9	<0.05
ABCA1	8.9 ± 0.4	3.7 ± 0.4	<0.05

Data are means ± SE (n = 5 mice in each group).

Several hormonal and metabolic factors, including profibrotic growth factors (angiotensin II, TGF-β, and VEGF), proinflammatory cytokines (TNF-α and IL-6), oxidative stress, and advanced glycation end products have been shown to modulate diabetes-related renal disease (30,31). Renal accumulation of lipids has also been thought to play a role in the pathogenesis of diabetic nephropathy (1–6). Our previous study of streptozotocin-induced type 1 diabetes in the rat showed that increased lipid synthesis mediated by increased activity of SREBP-1 plays an important role in the development of diabetic nephropathy (5). Furthermore, we were able to demonstrate that increased renal expression of SREBP-1a per se is the key factor linking increased fatty acid synthesis and accumulation of lipids to development of nephropathy (5).

In this study of two different genetic mouse models of type 1 diabetes, we also demonstrate that the glomerulosclerosis and proteinuria are associated with increased triglyceride and cholesterol accumulation.

A novel finding of our study is that we demonstrate that in addition to increased SREBP-1 activity, increased ChREBP activity most likely plays a role in increased fatty acid synthesis and accumulation of triglycerides. ChREBP has previously been shown to play an important role in triglyceride synthesis in the liver (7–9) and adipose tissue (32). ChREBP mediates insulin-independent, glucose-stimulated gene expression of multiple liver enzymes that are responsible for converting excess carbohydrate to fatty acids for long-term storage (7–9). This is the first demonstration of increased expression of ChREBP and L-PK in diabetic kidneys, which, along with SREBP-1c, may play an important role in mediating increased fatty acid synthesis in the kidney.

In addition to increased fatty acid synthesis induced by coordinate upregulation of SREBP-1 and ChREBP, our study also demonstrates that decreased fatty acid oxida-

tion mediated by decreased expression and activity of PPAR-α, PPAR-δ, and ACO also may play a role in the eventual triglyceride accumulation (33,34). Interestingly, a recent study found accelerated diabetic nephropathy in mice lacking PPAR-α (35).

Another novel finding of our study is that we have found decreased expression of FXR and its immediate target (SHP) in the kidneys of the Akita and OVE26 mice. FXR is highly expressed in liver, kidney, intestine, colon, and adrenal glands. Similar to PPARs, FXR binds DNA as an obligate heterodimer with the retinoid X receptor (RXR) (10,11). The regulation of FXR in the kidney, however, has not been determined.

In the liver, FXR has been shown to play an important role in regulation of triglyceride metabolism by decreasing the expression of SREBP-1 and ChREBP and increasing the expression of PPAR-α (10,11). Indeed, FXR knockout mice have increased liver triglyceride content (36). Our results, therefore, indicate that the decrease in FXR and its major target, SHP, could mediate the increases in SREBP-1 and ChREBP and decreases in PPAR-α and -δ, which result in increased fatty acid and triglyceride synthesis and accumulation of triglyceride in the kidney.

Recent studies indicate that FXR agonists also modulate fibrosis. In rat models of extrahepatic and intrahepatic cholestasis, the FXR agonist GW4064 protects against cholestatic liver damage in part by decreasing the expression of TGF-β (37). In additional models of liver cirrhosis and fibrosis, treatment with the FXR agonist 6-ethyl chenodeoxycholic acid causes decreased expression of *1*) TGF-β1, *2*) tissue metalloproteinase inhibitor-1 and -2, *3*) α1 (I) collagen, *4*) α smooth muscle actin, and *5*) increased expression and activity of matrix metalloproteinase-2. These effects result in resolution of hepatic fibrosis (38). Our results therefore also indicate that the decrease in FXR and its major target (SHP) could also mediate the increased expression of TGF-β in the kidney, resulting in podocyte loss, accumulation of extracellular matrix proteins, renal fibrosis, and proteinuria.

In addition to increased triglyceride content, we have also found a significant increase in renal cholesterol content. The cholesterol accumulation is mediated by SREBP-2– and HMG-CoA reductase–induced increases in cholesterol synthesis. At the same time, we also found evidence for a potential decrease in cholesterol efflux mediated by decreased expression of ABCA-1.

An additional novel finding of this study is that in addition to the regulation of SREBP-1, ChREBP, PPAR-α, PPAR-δ, FXR, and SREBP-2, we have also found significant modulation of LXR-α and LXR-β in the kidneys of the Akita and OVE26 mice.

LXRs are members of the ligand-activated nuclear receptor family of transcription factors, and they function as intracellular sensors of sterols (11,39). LXRs regulate intestinal uptake of dietary cholesterol, cellular efflux of cholesterol in peripheral tissues to HDL, and its eventual excretion from the body via the bile. In response to binding their sterol ligands, LXRs stimulate ABCA-1–mediated transport of cholesterol and phospholipids out of the cell interior to apolipoprotein A1–containing HDL. Hepatic expression of LXR promotes excretion of cholesterol from the body via two processes: *1*) direct transport of cholesterol into biliary canaliculi by way of the ABC transporter proteins ABCG5 and ABCG8 and *2*) upregulation of cytochrome P450 7α-hydroxylase (CYP7A1), which converts cholesterol to bile acids.

In addition to their roles in cholesterol metabolism, LXRs exert important regulatory effects on triglyceride metabolism. These effects are largely caused by their coordinate regulation of SREBP-1c, the master regulator of triglyceride and fatty acid synthesis. LXR transcriptional response elements are present in the promoter of SREBP-1c; however, the net effect on hepatic and serum triglycerides has been variable after treatment with different synthetic LXR agonists (40–42).

The decrease in LXR expression in the diabetic kidney may mediate the decreased expression of ABCA-1 and result in decreased cholesterol efflux. A significant increase in cholesterol accumulation in the kidney is the net result of increased cholesterol synthesis mediated by increased expression of SREBP-2 and HMG-CoA reductase and decreased cholesterol efflux mediated by decreased expression of LXR-α, LXR-β, and ABCA-1.

As modulators of inflammatory cytokines, LXRs may be important in diabetic kidney disease (39). LXRs and their ligands are negative regulators of macrophage inflammatory gene expression. In activated macrophages, LXRs repress expression of the proinflammatory genes IL-6, IL-1β, cyclooxygenase-2, granulocyte colony–stimulating factor, inducible nitric oxide synthase, and matrix metalloproteinase-9, as well as the chemokines monocyte chemoattractant protein-1 and macrophage inflammatory protein-1β. In our studies, we found increased expression of TNF-α and IL-6 in diabetic kidneys of OVE26 and Akita mice, which could be mediated by decreased expression of LXR.

Proinflammatory cytokines in the diabetic kidney can also induce downregulation of LXR and FXR (43,44), which would then modulate renal lipid metabolism and add to the complexity of the interactions between nuclear receptors and proinflammatory cytokines in the pathogenesis of diabetic kidney disease.

We propose that the increases in renal triglyceride and cholesterol accumulation play an important role in the pathogenesis of diabetic nephropathy. Three lines of evidence suggest that altered lipid metabolism is associated with diabetic and nondiabetic renal disease. First, there are a number of genetic abnormalities of lipid metabolism in humans and experimental animals, including Fabry's disease (45); lecithin cholesterol acyltransferase deficiency (46); type IA glycogen storage disease (von Gierke's disease) (47); genetic and acquired lipodystrophy (48); ABCA-1 knockout mice (a murine model of Tangiers disease) and familial HDL deficiency, with defects in ABCA-1– and HDL-mediated reverse cholesterol transport (49); and ApoE knockout mice (50), in which abnormalities in serum and tissue lipids, including renal lipid composition, are associated with glomerular disease and proteinuria. Second, increases in serum lipids have been associated with a faster decline of renal function (51). Third, there is increasing evidence that inhibition of cholesterol synthesis by HMG-CoA reductase inhibitors (statins), inhibition of triglyceride synthesis by PPAR-α agonists (fibrates) (52), or a decrease in LDL achieved by LDL apheresis (29) protect against diabetic and nondiabetic renal disease.

In summary, our findings in Akita and OVE26 mice with type 1 diabetes show renal lipid accumulation, glomerular matrix expansion and sclerosis, podocyte loss, and development of proteinuria (Fig. 3). Our lipid metabolism pathway analysis suggests a global shift in diabetic renal metabolism toward *1*) increased fatty acid synthesis me-

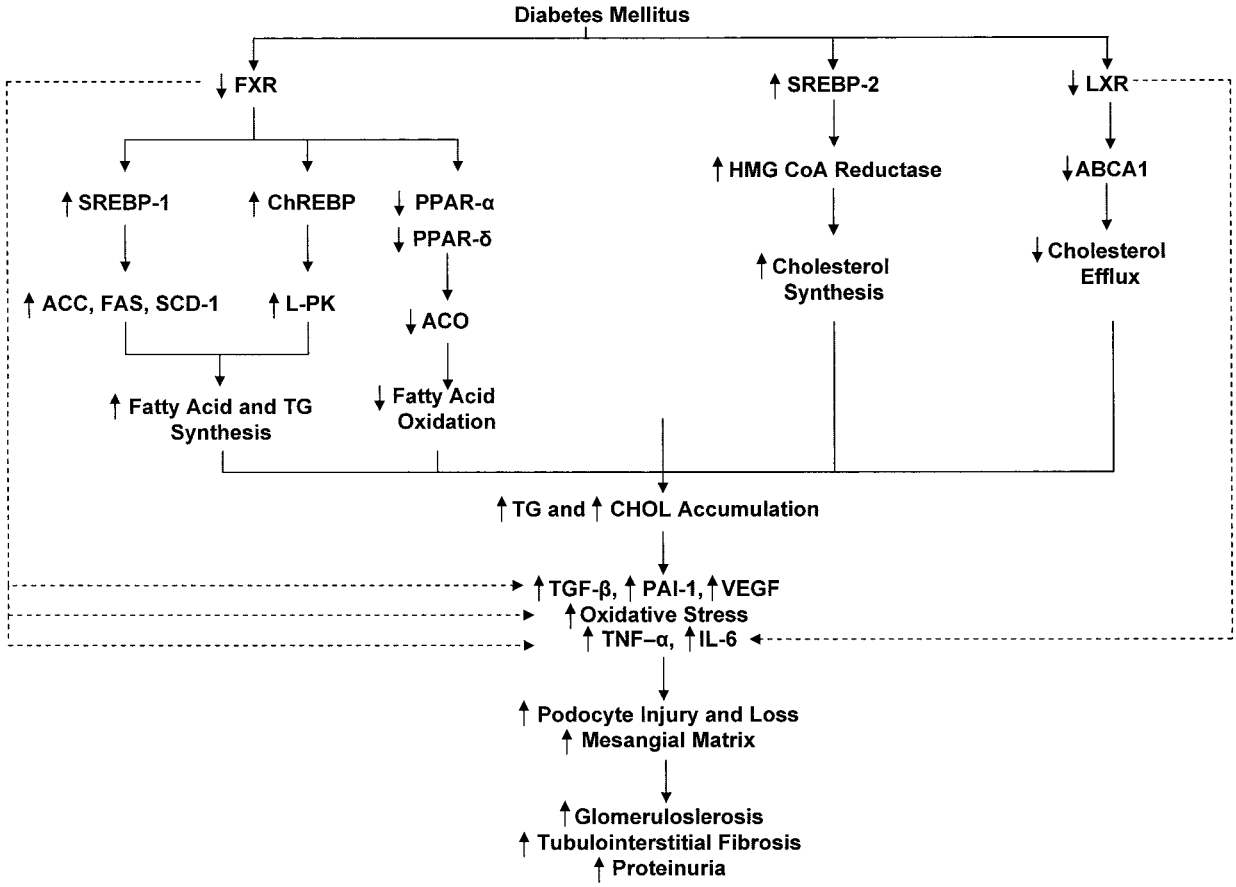

FIG. 3. A summary of our findings in the Akita mice and OVE26 mice indicating significant alterations in nuclear receptor expression and activity resulting in increased lipid synthesis and lipid accumulation, fibrosis, inflammation, and oxidative stress, resulting in glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. CHOL, cholesterol; TG, triglyceride.

diated by increases in SREBP-1, ACC, FAS, SCD-1, ChREBP, and L-PK; *2*) decreased fatty acid oxidation mediated by decreases in PPAR-α, PPAR-δ, and ACO; *3*) increased cholesterol synthesis mediated by increases in SREBP-2 and HMG-CoA reductase; and *4*) decreased cholesterol efflux mediated by decreases in LXR-α, LXR-β, and ABCA-1. We also found increased levels of the profibrotic growth factors TGF-β, PAI-1, and VEGF; the proinflammatory cytokines IL-6 and TNF-α; oxidative stress; and receptor for advanced glycation end products. We have observed a pattern of reciprocal changes in renal lipid biosynthesis and fibrosis/inflammation/oxidative stress in diabetic kidneys of Akita and OVE26 mice, which could be explained mechanistically by the corresponding changes we found in LXR and FXR expression. This parallels similar findings in liver and macrophages by other investigators. Our findings suggest but do not prove a significant role for LXR and FXR in the development of the proinflammatory, profibrotic, and prolipogenic environment characteristic of diabetic kidney disease and warrant future investigations of synthetic agonists of FXR and LXR for their potential roles as therapeutic agents for the treatment of diabetic kidney disease.

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