Curcumin decreases renal triglyceride accumulation through AMPK–SREBP signaling pathway in streptozotocin-induced type 1 diabetic rats[☆]

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Abstract

Diabetic kidney disease has been associated with the presence of lipid deposits. We assumed that curcumin, a polyphenol, would attenuate the tissue dyslipidemic condition through activation of 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK) phosphorylation and suppression of sterol regulatory element-binding protein (SREBP)-1c in the kidney and would prevent renal progression in experimental type 1 diabetic rats. Diabetes was induced with streptozotocin (STZ) (55 mg/kg) by intraperitoneal injection in male Sprague–Dawley rats. Three weeks after STZ injection, rats were divided into three groups, namely, control, diabetic and diabetic treated with curcumin (100 mg/kg/day) by gavage for 8 weeks. We found that curcumin decreased plasma triglyceride and the amount of renal triglyceride significantly. Furthermore, treatment of diabetic rats with curcumin increased the phosphorylation of AMPK and prevented the increased renal expression of SREBP-1c and, as a result, decreased the expression of acetyl CoA carboxylase and fatty acid synthase as well as adipose differentiation-related protein, a marker of cytoplasmic droplets. We also demonstrate that curcumin significantly suppressed the increased expression of transforming growth factor β , vascular endothelial growth factor and extracellular matrix proteins such as type IV collagen and fibronectin. In addition, curcumin treatment increased nephrin expression to near-normal levels in diabetic rats. These results demonstrated that curcumin protects against the development of diabetic nephropathy through the AMPK–SREBP pathway and the reduction of renal triglyceride accumulation which could be a possible mechanism by which curcumin preserves renal function in diabetes.

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1. Introduction

Diabetic nephropathy (DN) is characterized by early vascular dysfunction and increasing matrix accumulation in the kidney, eventually leading to proteinuria, glomerulosclerosis and interstitial fibrosis [1]. Since the description by Kimmelstiel and Wilson [2] of classical nodular glomerulosclerosis and the presence of lipid deposits in diabetic kidney, several investigators have shown the presence of lipid deposits in the kidney 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 in addition to the notorious role played by hyperglycemia, hypertension, growth factors, inflammatory cytokines, oxidative stress and advanced glycation end products [3,4]. That the deposition of lipids per

se may mediate diabetic renal disease is supported by up-regulated renal expression of the transcriptional factor sterol regulatory element-binding protein (SREBP)-1c, which causes increased fatty acid synthesis and accumulation of triglycerides, lipid bodies and cholesterol in the kidneys [5,6].

The SREBPs have been described as master regulators of both fatty acid and cholesterol metabolism [7–9]. Studies have shown that increased renal expression of a transcriptional factor, SREBP-1, resulted in increased synthesis and accumulation of triglyceride and was correlated with renal sclerosis and proteinuria [6,10]. The role of SREBP-1 in DN was demonstrated in a study using SREBP-1c knockout mice. The absence of SREBP-1c completely abolished the increase in urinary albumin excretion induced by diabetes in the streptozotocin (STZ)-treated mice, revealing the crucial role of SREBP-1c in DN [11].

Another important modulator of lipid metabolism is 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK). AMPK is a heterotrimeric enzyme complex consisting of a catalytic subunit (α) and two regulatory subunits (β and γ) [12]. Previous studies demonstrated that increased phosphorylation and activation of AMPK lead to suppression of SREBP-1c protein expression and, as a

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result, down-regulation of target genes of SREBP-1c which preferentially controls the expression of genes involved in triglyceride synthesis and accumulation, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [13,14]. It has been reported that AMPK is regulated by cytokines in the control of whole-body energy balance, by antidiabetic drugs and by natural plant products such as berberine, resveratrol and green tea catechin epigallocatechin-3-gallate [12].

Previous investigations have shown that curcumin, a yellow coloring component of the rhizome of *Curcuma longa* L, has complex pharmacological effects, such as antiinflammatory, antioxidant, anticarcinogenic and hypolipidemic activities [15,16]. Study showed that some genes, such as low-density-lipoprotein receptor, SREBP and CD36, involved in cholesterol homeostasis were influenced by curcumin [17–20]. In an *in vitro* study, curcumin activated AMPK and suppressed hepatic gluconeogenic gene expression [21]. Furthermore, curcumin has been shown to protect against nephropathy, retinopathy and islet damage in STZ-induced diabetic rats [22,23]. However, the molecular mechanisms that mediate the triglyceride-lowering activity of curcumin are poorly understood. In this study, we investigated the effect of curcumin on activating AMPK, decreasing tissue triglyceride as well as SREBP-1c expression in the kidney and on the attenuation of renal dysfunction in STZ-induced type 1 diabetes.

2. Methods and materials

2.1. Animal model

Male Sprague–Dawley rats (250–280 g; obtained from Charles River Japan Inc.) were housed in colony cages, maintained on a 12-h light/12-h dark cycle. Rats in the STZ group were injected intraperitoneally with 55 mg/kg body weight of 20 mM sodium citrate solution (pH 4.5), and rats in the control group were injected with 20 mM sodium citrate solution. Tail vein blood glucose levels were measured every week, and rats with blood glucose of greater than 300 mg/dl were considered diabetic. After 3 weeks of induction of diabetes, rats were randomized to receive either vehicle or curcumin (100 mg/kg body weight; Sigma-Aldrich, Tokyo, Japan) by oral gavage and followed for 8 weeks ($n=5$ per group). Curcumin was suspended in 0.5% carboxymethyl cellulose. Before sacrifice, individual rats were placed in metabolic cages to collect 24-h urine samples for the measurement of urine protein and creatinine concentrations, which were determined by the Bradford and Jaffe methods, respectively [24,25]. All animal studies were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Niigata University.

2.2. Estimation of biochemical parameters

Blood samples were drawn via heart puncture in each rat at the time of death into EDTA Vacutainer tubes. EDTA blood was centrifuged at 3000g, 4°C, for 15 min for separation of plasma. The plasma was used for the estimation of plasma lipids and creatinine. Creatinine clearance was calculated in individual rats as follows: creatinine clearance = urine creatinine $\times$ urine volume / plasma creatinine $\times$ time [26].

2.3. Lipid extraction and triglyceride analysis

Briefly, total lipids were extracted from rat kidney by the method of Bligh and Dyer [27]. Triglyceride in the kidneys was analyzed using a triglyceride determination kit.

2.4. Preparation of kidney homogenates, membrane fraction and nuclear extracts

Kidney cortex was homogenized in ice-cold buffer A (20 mmol/L Tris-HCl, pH 7.5; 2 mmol/L EDTA; 10 mmol/L EGTA; and 0.25 mol/L sucrose containing complete protease and phosphatase inhibitors). To prepare membrane fractions, tissue homogenates were centrifuged at 1000g for 10 min at 4°C, and the supernatant was then ultracentrifuged at 100,000g for 1 h at 4°C. This supernatant was retained as the cytosolic fraction, and the pellet was resuspended in buffer B (buffer A with 1% Triton X-100) and ultracentrifuged at 100,000g for 1 h at 4°C. This supernatant was retained as the membranous fraction [28]. To prepare nuclear fraction, kidney cortex was homogenized in ice-cold HEPES buffer (10 mM HEPES, 0.2% Triton X-100, 50 mM NaCl, 0.5 mM sucrose, 0.1 mM EDTA, and protease and phosphatase inhibitors) and homogenized with an ice-chilled Dounce homogenizer at 4°C. This was spun at 10,000 rpm for 10 min. The pellet was suspended in ice-cold buffer (10 mM HEPES, 500 mM NaCl, 10% glycerol, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% IGEPAL, and protease and phosphatase inhibitors), vortexed at 4°C for 15 min and centrifuged for 10 min at 14,000 rpm. The resulting supernatant was aliquoted and stored as the nuclear extract at -80°C . The absence of cross-reactivity with β -actin in Western blots confirmed the

purity of nuclear extracts. A small aliquot of kidney homogenate, membrane fraction and nuclear extract was kept at 4°C for protein estimation [29].

2.5. Western blot analyses

Protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. Membranes were blocked in 5% powdered milk in Tris-buffered saline with Tween (0.2% Tween 20 in 1 $\times$ Tris-buffered saline) (TBS-T) and incubated using the following antibodies: Antibodies against SREBP-1c, AMPK α , p-AMPK α (Thr 172), adipose differentiation-related protein (ADRP) and vascular endothelial growth factor (VEGF) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibody against transforming growth factor (TGF)- β was obtained from Promega. Antibody against nephrin was obtained from ProSci. All antibodies were used at a dilution of 1:1000. After washing for three times with TBS-T, incubation with appropriate horseradish-peroxidase-conjugated secondary antibodies was performed for 1 h at room temperature. Next, samples were washed three times with 1 $\times$ Tris-buffered saline and then developed using a chemiluminescence detection system (Amersham Biosciences, Buckinghamshire, UK). The signals were quantified with densitometric analysis using Scion Image program (Epson GT-X700, Tokyo, Japan). Antibodies to Lamin A (Santa Cruz) and β -actin (Cell Signaling) served as housekeeping proteins for nuclear and cytosolic target proteins, respectively.

2.6. Quantitative real-time polymerase chain reaction (PCR)

Kidney tissues were preserved by immersion in RNAlater (Ambion Inc., Austin, TX, USA) immediately after sampling. Total RNA was extracted after homogenization using Ultra TurraxT8 (IKA Labortechnik, Staufen, Germany) in TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the standard protocol. Then, cDNA was synthesized with Invitrogen SuperScript preamplification system. To investigate the expression of ACC and FAS mRNA, real-time PCR (QIACube real-time analyzer, Qiagen, Germantown, MD, USA) was performed with TaqMan probe (TaqMan Gene expression assays; Applied Biosystems, Foster City, CA, USA) according to the following protocol: 95°C for 3 min, 95°C for 15 s, 60°C for 30 s, 72°C for 30 s, 40 cycles. Primer sequences were as follows: ACC (forward), CCCAGCAGAATAAGCTACTTTGG, (reverse), TCCTTTTGTGCACTAGGAACGT; FAS, (forward), CCTGGATAGCATTCCGAACCT, (reverse), AGCACATCTCGAAGGCTACACA; GAPDH, (forward), GCTCATTTCTGGTATGACACG, (reverse), AGGGGTCTACATGGCAACTG. Relative standard curves representing several 10-fold dilutions (1:10:100:1000:10,000:100,000) of cDNA from kidney tissue samples were used for linear regression analysis of other samples. Results were normalized to GAPDH mRNA as an internal control and are thus shown as relative mRNA levels.

2.7. Histology and oil red O staining

Paraffin sections were stained with periodic acid–Schiff, Azan–Mallory, and hematoxylin and eosin (H&E). The stained kidney sections were imaged with an Olympus microscope, and 100 glomeruli showing global and/or segmental sclerosis and tubules showing dilatation and epithelial hyperplasia were scored semiquantitatively in a blinded manner. Frozen sections were used for oil red O staining to determine renal accumulation of neutral fats [30]. Briefly, kidney transverse frozen sections were cut at 8 μm and placed on glass slides. To determine lipid deposition, sections were fixed in 10% formalin for 10 min, rinsed in distilled water and stained with oil red O solution (Muto Pure Chemicals, Tokyo, Japan) which was preheated to 60°C, for 5 min. Sections were rinsed with 60% isopropanol for 2 min and distilled water and counterstained with Mayer's hematoxylin for 1 min. These were subsequently used to assess the area of lipid deposition.

2.8. Immunofluorescence staining for fibronectin, type IV collagen and ADRP

Immunofluorescence for type IV collagen and fibronectin was done on frozen sections. Briefly, frozen sections were preincubated for 10 min at room temperature with 3% milk powder in phosphate-buffered saline containing 0.05% Triton X-100. Nonspecific binding sites were blocked with 2% goat serum for 60 min and washed for three times for 5 min each. They were then incubated with rabbit polyclonal serum against type IV collagen (1:50; Abcam, Cambridge, England) and mouse monoclonal serum against fibronectin (1:50; Santa Cruz, CA, USA) at 1:500 overnight at 4°C. The sections were rinsed three times with phosphate-buffered saline before incubation for 1 h with the secondary antibody, fluorescein isothiocyanate-conjugated (FITC) goat anti-rabbit IgG, at 1:100 in the dark at room temperature. After rinsing with phosphate-buffered saline, the sections were mounted using 3:1 Vectashield:DAPI. The kidney sections were then imaged with a fluorescence microscope at 400-fold magnification (CIA-102; Olympus) [5]. For ADRP imaging, paraffin-embedded sections were used. The sections were deparaffinized in xylene and rehydrated in graded ethanol and distilled water, and the antigen was unmasked with sodium citrate (10 mM, pH 6.0), followed by microwave heat source on high power for 3 min and then with low power for 10 min. After phosphate-buffered saline wash, the nonspecific background was blocked with 3% normal rabbit serum at room temperature for 1 h. Incubation with anti-ADRP (1:50; Santa Cruz, CA, USA) was performed at room

temperature for 60 min. Sections were then washed in phosphate-buffered saline and incubated with FITC-conjugated rabbit anti-goat secondary antibody (1:50; Sigma-Aldrich, Tokyo, Japan). After extensive washing with phosphate-buffered saline, slides were mounted in 3:1 Vectashield:DAPI and examined with an Olympus CIA-102 fluorescence microscope equipped with an Olympus camera. The integrated fluorescence intensity was quantified and measured with the HPIAS-1000 Image-Analysis System. The relative fluorescence intensity was calculated by dividing the total integrated optical density by the total number of cells in each field. Mean fluorescence intensity measurements were obtained from six separate experiments in each group [31–33].

2.9. Statistical analysis

Data are shown as mean±S.E.M. and were analyzed using one-way analysis of variance followed by Tukey's methods for post hoc analysis and two-tailed *t* test when appropriate. A value of *P*<0.05 was considered statistically significant. For statistical analysis, GraphPad Prism 5 software (San Diego, CA, USA) was used.

3. Results

3.1. Biochemical parameters in experimental animals

As shown in Table 1, the mean plasma glucose levels were significantly higher in vehicle-treated diabetic rats than in control nondiabetic rats (*P*<0.01). Chronic treatment with curcumin in diabetic rats altered plasma glucose levels significantly compared with those in vehicle-treated diabetic rats. To evaluate the effect of curcumin on preventing hyperfiltration induced by STZ, we measured 24-h urinary protein excretion. Although vehicle-treated diabetic rats showed a marked elevation of 24-h urinary protein excretion, curcumin treatment significantly improved this alteration. Moreover, plasma triglyceride was significantly increased in the vehicle-treated diabetic rats compared with that in the control nondiabetic rats. The administration of curcumin markedly decreased plasma triglyceride levels. In addition, vehicle-treated diabetic rats also exhibited increased plasma creatinine and plasma urea, and decreased creatinine clearance, which were all ameliorated by curcumin treatment. At 11 weeks of STZ-induced type 1 diabetes, kidney weight corrected for body weight was significantly increased by 45% in vehicle-treated diabetic rats (Fig. 1A; *P*<0.01), which was prevented by curcumin treatment.

3.2. Curcumin inhibits lipid accumulation in kidney

There were significant increases in renal triglyceride content in vehicle-treated diabetic rats, which were reversed by curcumin treatment (Fig. 1B). ADRP is a marker of lipid droplets. Western blot analysis clearly showed a 2.2-fold increase of ADRP protein expression in vehicle-treated diabetic rats (Fig. 2A), which was attenuated by curcumin treatment. In agreement with Western blot analysis, immunofluorescence staining of ADRP revealed typical lipid droplets as ring-shaped in the tubules of vehicle-treated diabetic rats (Fig. 2C). In contrast, there were almost no lipid droplets in the

Fig. 1. (A) Effect of curcumin treatment on the kidney weight to body weight ratio. (B) The vehicle-treated diabetic rats showed statistically significant increases in renal triglyceride content, which were reduced by curcumin treatment. Results are expressed as mean±S.E.M. *n*=5 per group. C, age-matched normal rats; D, diabetic-treated rats administered with vehicle; Cur, diabetic rats treated with curcumin 100 mg/kg/day. ***P*<0.01 vs. C; ##*P*<0.01 vs. D.

curcumin-treated diabetic rats (Fig. 2D). Likewise, there is very strong staining of oil red O in the glomerular and tubulointerstitial cells of vehicle-treated diabetic rats, which indicates accumulation of neutral lipids deposits (Fig. 2F) and almost no staining in the curcumin-treated diabetic rats (Fig. 2G). Lipid accumulation was also shown by the unstained area in renal tissue under H&E staining in the vehicle-treated diabetic rats (Fig. 2I) which was reduced by curcumin treatment (Fig. 2J). These data strongly indicate that there were excessive amounts of lipid deposits in the kidneys of vehicle-treated diabetic rats. Curcumin treatments improved this lipid accumulation in the kidneys of diabetic rats.

3.3. Renal histologic change in experimental animals

Periodic acid–Schiff staining reveals ~56% sclerosis of glomeruli in vehicle-treated diabetic rats (Fig. 2L), whereas the curcumin-treated diabetic rats developed only 28.3% (*P*<0.05) (Fig. 2M) when compared with control nondiabetic rats (Fig. 2K). In addition to pronounced glomerular sclerosis, severe tubulointerstitial changes were observed in the vehicle-treated diabetic rats. Histological evaluations revealed that the vehicle-treated diabetic rats had 58.3% dilated tubules (Fig. 2L). Curcumin treatment significantly reduced this to 34% (*P*<0.05) compared with that in vehicle-treated diabetic rats (Fig. 2M). Interstitial collagen deposition was studied in Azan–Mallory-stained renal sections as an index of interstitial fibrosis. Vehicle-treated diabetic rats showed higher interstitial fibrosis (Fig. 2O) than control nondiabetic rats (Fig. 2N), which was improved in the curcumin-treated diabetic rats (Fig. 2P). Immunofluorescence microscopy with anti-fibronectin and anti-type IV collagen antibodies revealed increased intensity of immunofluorescence in the glomeruli and tubulointerstitial cells in vehicle-treated diabetic rats (Fig. 2R, 2U), indicating accumulation of extracellular matrix (ECM) proteins and glomerulosclerosis and tubulointerstitial fibrosis, whereas curcumin

Table 1
Biochemical parameters of the experimental animals at the end of intervention study (*n*=5)

	C	D	Cur
24-h urine protein (g/day)	12.5±0.7	42±5.2*	15.2±3.7***
Plasma glucose (mg/dl)	118.6±6	744.4±9.3*	597.1±9.9***
Plasma urea (mg/dl)	21.9±0.6	45±1.9*	33.2±2.5***
Plasma creatinine (mg/dl)	0.3±0.01	2.1±0.1*	0.8±0.2***
Creatinine clearance (ml/min)	3.9±0.5	0.8±0.1*	2.7±0.5***
Plasma triglyceride (mg/dl)	104.8±2.1	320.3±2.1*	114±3.7***

Data are means±S.E.M.

* *P*<0.01.

** *P*<0.05 vs. C.

*** *P*<0.01 vs. D.

Fig. 2. (A) Western blot and densitometric quantification of ADRP. (B–D) Immunofluorescence staining of kidney for ADRP (glomeruli and tubules) ($\times 400$). (E–G) Representative photomicrograph of the oil-red-O-stained renal tissues. Lipid droplets appear as red spots. There is increased oil red O staining in the glomerular and tubular cells of diabetic rats, and treatment with curcumin 100 mg/kg/day reverses these changes ($\times 400$). (H–J) Hematoxylin and eosin staining of the cross-sectional tissue slices of kidney depicted lipid accumulation, which was indicated by the unstained area in renal tissues ($\times 200$). (K–M) Periodic acid–Schiff staining of kidney depicted glomerular sclerosis and tubular dilatation ($\times 200$). (N–P) Azan–Mallory staining for fibrosis of the cross-sectional tissue slices of kidney. Fibrosis is indicated by the blue area ($\times 200$). (Q–S) Immunofluorescence microscopy of frozen sections stained with fibronectin antibody ($\times 400$). (T–V) Immunofluorescence microscopy of frozen sections stained with type IV collagen antibody ($\times 400$). Kidney tissues were harvested from age-matched normal rats as a control (B, E, H, K, N, Q, T), diabetic-treated rats administered with vehicle (C, F, I, L, O, R, U) and diabetic rats treated with curcumin 100 mg/kg/day (D, G, J, M, P, S, V). $n=5$ rats in each experimental group. ** $P<.01$ vs. C; ## $P<.05$ vs. D.

suppressed the increased intensity of fibronectin and type IV collagen expression in the diabetic rats (Fig. 2S, V).

3.4. Effect of curcumin on renal expression of AMPK

To understand the mechanism by which curcumin attenuated lipid accumulation in the kidneys of diabetic rats, we measured the effect of curcumin on AMPK activation on renal tissues. Since phosphorylation of the catalytic subunit AMPK- α at Thr172 position is essential for AMPK activation, AMPK activation was monitored by using a specific antibody that recognizes the phosphorylated AMPK- α at Thr172. As shown in Fig. 3A, AMPK phosphorylation was reduced in the vehicle-treated diabetic rats by nearly 60% ($P<.01$). Curcumin prevented the reduction in AMPK phosphorylation in diabetic rats, suggesting that it may protect the AMPK activity.

3.5. Effect of curcumin on renal expression of nuclear receptors and genes that regulate triglyceride and cholesterol metabolism

SREBP-1c is a transcription factor that activates the transcription of genes involved in fatty acid and triglyceride synthesis, such as ACC and FAS [34]. To assess the SREBP-1c precursor and the nuclear form, cell lysates were fractionated into a separate membrane fraction containing SREBP precursors and a nuclear fraction containing active nSREBP [35]. Fig. 3 (B and C) shows that both precursor (2.6-fold) and mature forms (2.7-fold) of SREBP-1c were increased in the vehicle-

treated diabetic rats. It is noteworthy that curcumin administration significantly reduced both precursor and mature forms of SREBP-1c and was well correlated with mRNA encoding of the SREBP-1c target genes, ACC and FAS (Fig. 4A and B).

3.6. Curcumin decreases proteinuria and renal expression of profibrotic growth factors

Because mesangial expansion and accumulation of ECM proteins are mediated by increased expression of growth factors such as TGF- β [36] and VEGF [37], we measured the expression of TGF- β 1 and VEGF in the kidney cortex of experimental animals. There were a 1.8-fold increase in TGF- β 1 (Fig. 5A) and a 2.4-fold increase in VEGF (Fig. 5B) expression, and curcumin treatment ameliorated these increases by 1.2- and 1.4-fold, respectively. Next, we analyzed the expression pattern of the slit diaphragm protein nephrin. Fig. 5C shows that the down-regulation of nephrin occurred in the vehicle-treated diabetic rats compared with that in the control nondiabetic rats. This decrease in renal nephrin protein expression was markedly increased by curcumin treatment ($P<.05$).

4. Discussion

The most important findings of this study were as follows: (1) Curcumin can activate AMPK by increasing AMPK phosphorylation in renal tissue in STZ-induced type 1 diabetes. (2) This increase of AMPK

Fig. 3. (A) Representative Western blots and group data depicting protein abundance of the phosphorylated AMPK in the renal tissues. (B) Western blots analysis of the expression of SREBP-1c nuclear extracts and membrane (precursor) (C) isolated from kidney tissues. STZ-induced type 1 diabetes causes increased SREBP-1c expression in both nuclear and membrane extracts, and these changes were reversed upon treatment with curcumin. Data are means±S.E.M. $n=5$ per group. C, age-matched normal rats; D, diabetic-treated rats administered with vehicle; Cur, diabetic rats treated with curcumin 100 mg/kg/day. ** $P<.01$ vs. C; ## $P<.01$ vs. D.

activation, consecutively, sharply suppresses renal SREBP-1c and the target genes such as ACC and FAS. (3) As a result, curcumin attenuates proteinuria, glomerulosclerosis, and tubulointerstitial dilatation and fibrosis. In the present study, we also found that, in STZ-induced type 1 DN rats, there were excessive amounts of lipid deposits in the kidney sections, as shown by oil red O and H&E staining, the presence

of lipid bodies, as shown by ADRP immunofluorescence microscopy and ADRP protein levels, using Western blot analysis; and significantly higher renal content of triglyceride compared with those in control nondiabetic rats; all of these abnormalities were ameliorated by curcumin treatment.

Accumulating evidence indicates that curcumin (diferuloylmethane), a polyphenol natural product of the plant *C. longa*, exerts effects on a wide range of molecular targets controlling lipid accumulation and has antidiabetic and anticancer activities [15,16]. Our previous study in STZ-induced type 1 DN showed that curcumin exhibited renoprotective effects to lessen proteinuria and diabetic pathologic changes through inhibition of macrophage infiltration, which suggests that curcumin has an antifibrotic and anti-inflammatory effect [38]. Diabetic rats with curcumin diet showed that curcumin has a hypocholesterolemic action via stimulation of hepatic cholesterol-7 α -hydroxylase and HMG-CoA reductase [16]. Curcumin also inhibits ox-LDL-induced cholesterol accumulation in cultured vascular smooth muscle cells through inhibition of nuclear translocation of SREBP-1 [18]. It has also been shown that curcumin improved muscular insulin resistance through the up-regulation of phosphorylated AMPK [39]. Furthermore, curcumin increases the phosphorylation of AMPK and its downstream target as well as suppresses gluconeogenic gene expression in hepatoma cells [21,40]. Although several lines of evidences strongly suggest that curcumin could regulate glucose and lipid homeostasis, the potential mechanism of curcumin in regulating triglyceride accumulation in type 1 DN has not been studied. In the present study, we show for the first time that curcumin protects renal triglyceride accumulation in STZ-induced type 1 diabetes through activation of AMPK and suppresses lipogenic gene expression.

AMPK is a heterotrimeric protein consisting of a combination of $\alpha_{1/2}$ -, $\beta_{1/2}$ - and $\gamma_{1/2/3}$ -subunits [41], with the α_1 - rather than α_2 -subunit expressed in the kidney [42]. The enzymatic activity of AMPK is dependent on phosphorylation of Thr172 of the α -subunit [43]. On

Fig. 4. (A and B) Effects of curcumin on renal mRNA levels of ACC and FAS in rats with DN were determined by quantitative reverse transcriptase PCR. The expression level of each sample is expressed relative to the expression level of GAPDH gene. Data are means±S.E.M. $n=5$ per group. C, age-matched normal rats; D, diabetic-treated rats administered with vehicle; Cur, diabetic rats treated with curcumin 100 mg/kg/day. ** $P<.01$ vs. C; ## $P<.05$ vs. D.

Fig. 5. Representative Western blots and group data depicted protein abundance of TGF- β (A), VEGF (B) and nephrin (C) in the renal tissues of diabetic rats, curcumin-treated diabetic rats and control nondiabetic rats. $n=5$ in each group. Data are means $\pm$ S.E.M. ** $P<.01$ vs. C; ## $P<.05$ vs. D.

activation, AMPK inhibits energy-consuming reactions, such as synthesis of fatty acids and sterols, and activates adenosine-triphosphate-generating processes, such as fatty acid oxidation [44]. A very recent study also demonstrated that activation of AMPK inhibits hepatic fatty acid synthesis by suppressing SREBP-1c, a master regulator of lipogenic gene expression, in rat hepatoma cells [45]. Lee et al. have shown that, in STZ-induced insulin-deficient diabetes, a model of type 1 diabetes, AMPK phosphorylation was reduced at the time when the kidneys were hypertrophic [46]. In line with previous reports, we have also observed that both total and phosphorylated AMPK expressions were reduced in vehicle-treated

diabetic rats (Fig. 3A) and that these decreases under diabetic conditions were ameliorated by curcumin treatment, which in turn attenuated the expression of SREBP-1c in the renal tissue of type 1 diabetic rats and, as a consequence, caused decreases in the mRNA abundance of two SREBP-1-activated enzymes that mediate fatty acid and triglyceride synthesis, ACC and FAS.

Abnormal lipid deposits have been observed in tubules of human diabetic kidney [47] and have been proposed to participate in the pathogenesis of DN and other proteinuric renal diseases [3]. Lipid droplets were also detected in the cortical tubules of the STZ-induced diabetic rats [5]. Increased deposition of lipid in the kidney contributes to cellular damage and to the progression of diabetic kidney disease. ADRP, also called adipophilin in humans, is a ~50-kDa fatty-acid-binding protein that is transcriptionally activated when preadipocytes differentiate into mature adipocytes [48]. ADRP belongs to the perilipin family of lipid storage proteins [49] and, similar to other perilipin members, localizes to the cytoplasmic surface of lipid storage droplets in distinct cell types. Induction of the ADRP gene is apparently an important aspect of the response to diabetes and formation of lipid storage droplets in the diabetic kidney. We found that the ADRP protein (Fig. 2A) and ADRP immunofluorescence (Fig. 2C) were significantly induced in vehicle-treated diabetic rat kidneys and corresponded with the presence of lipid droplets in oil red O (Fig. 2F) and H&E staining (Fig. 2I) and increase in renal triglyceride content (Fig. 1B), and that these increases under diabetic conditions were ameliorated by curcumin treatment.

In vivo and *in vitro* studies have shown that low-density lipoprotein or very low density lipoprotein induces up-regulation of growth factors, including TGF- β and platelet-derived growth factor, ECM [50], lipid peroxidation and glycoxidation [51], and change in expression of podocyte-specific proteins such as nephrin [52], indicating a direct role for lipids in activating the mediators of glomerulosclerosis and proteinuria. Ishigaki et al. indicated that activation of renal SREBP-1c was consistently associated with pathohistological fibrosis and TGF- β activation both *in vivo* and *in vitro*, accompanying induction of fibrosis markers such as type IV collagen and fibronectin [11]. It has also been reported that renal hypertrophy in STZ-induced type 1 diabetic rats was associated with reduction in AMPK phosphorylation [53]. Our study indicates that accumulation of renal triglyceride in STZ-treated diabetic rats caused increased expression of TGF- β and VEGF as well as ECM proteins such as type IV collagen and fibronectin and decreased expression of nephrin, resulting in glomerulosclerosis and proteinuria, respectively. Curcumin treatment markedly decreased TGF- β and VEGF and increased nephrin expression in renal tissue of diabetic rats. Curcumin also reduced the augmented expressions of type IV collagen and fibronectin. This effect might be mediated by curcumin's function as an AMPK activator, which further down-regulated the SREBP-1c expression.

We have shown that curcumin inhibits renal triglyceride accumulation in STZ-induced type 1 DN by activation of AMPK and suppresses SREBP-1c expression, as well as inhibits the expression of lipogenic genes, which may explain some of the lipid-lowering effects of curcumin. Since AMPK is considered one of the preventive or therapeutic targets for metabolic diseases such as diabetes, obesity and cancer, the effective modulation of AMPK by curcumin could be a promising effective approach for treating these diseases.

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