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Original Article

Lycopene ameliorates renal function in rats with streptozotocin-induced diabetes

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Abstract: Aim: To study the effect of lycopene on ameliorating renal function of diabetic nephropathy. Methods: Sixty male SD rats were divided into four groups: normal untreated (NC-U), normal treatment (NC-L), diabetes untreated (DM-U) and diabetes treatment (DM-L). DM was prepared by a single injection of STZ (70 mg/kg, intraperitoneally) dissolved in 0.1 M citrate buffer (pH 4.5). DM-U and NC-U rats received control diet; DM-L and NC-L rats received lycopene. After treated with lycopene for 8 weeks, blood was obtained for analyzing plasma lipid profiles, glucose and renal function. The kidneys were used to determine SOD activity, malondialdehyde (MDA) level, processed for histological examination and western blot. Results: Treatment of diabetic rats with lycopene decreased the values of blood urea nitrogen, 24 h urea protein and creatinine. The serum lipids like TC, TG, and LDL were decreased and HDL was increased in DM-L rats when compared with those of diabetic rats. Administration of lycopene decreased the levels of MDA content and expression of CTGF, increased Akt/PKB phosphorylation and SOD activity in diabetic renal tissues. Conclusions: Lycopene protects against development of diabetic nephropathy and ameliorates renal function via improving oxidative status and regulating p-Akt and CTGF.

Keywords: Diabetic nephropathy, lycopene, oxidative stress

Introduction

Diabetic nephropathy, one of the major complications in patient with type 1 and 2 diabetes, is characterized by specific renal morphological and functional alterations, such as the increased basement membrane thickeness, mesangial expansion, tubulointerstitial fibrosis and manifested microalbuminuria [1, 2]. Although the pathogenesis of diabetic cardiomyopathy is still far from being fully elucidated, hyperglycemia is the major factor precipitating renal injury in this setting [3-6]. Hyperglycemia can lead to oxidative stress via glucose autooxidation, increased formation of advanced glycation end products [7, 8]. High glucose facilitating the glycolysis and adenosine triphosphate generation causes significant reactive oxygen species (ROS) production [9]. Experi mentally, hyperlipidemia contributes to the progression of diabetic renal disease. LDL modification, such as oxidation, glycation, and formation of advanced glycated end products, induces glomerulosclerosis include the glomerular infiltration of LDL, and hypercholesterolemia triggers proinflammatory events [10-12]. It is well known that transforming growth factor-β (TGF-β) is the central cytokine for the development of diabetic nephropathy mediating glomerular hypertrophy, matrix expansion and glomerulosclerosis [13]. Connective tissue growth factor (CTGF), a cytokine discovered recently, has been demonstrated to play an important role in fibrotic response through a TGF-β1-dependent or independent pathway [14].

Lycopene, a kind of carotenoid found abundantly in tomatoes and tomato products, has a high antioxidant capacity and anti free-radical action. Previous studies have shown that intake of tomatoes and tomato products strengthens the antioxidant system, and inhibits lipid peroxidation in humans [15]. It has been postulated that many chronic diseases, such as cardiovascular disease, cancer, diabetes and eye diseases etc, are the result of long-term oxidative stress. Antioxidants (for example, vitamin E, lycopene and tocopherols) have an Important

role to play in protection against oxidative damage [16]. Several studies have demonstrated that lycopene which possesses many conjugated double bonds is an effective antioxidant and a free radical scavenger [16]. Recent data suggest that lycopene reduced lipid peroxidation and atherogenesis in hemodialysis patients with chronic renal failure [17]. However, there is no data demonstrating the effect of lycopene on renal function in rats with diabetes.

Therefore, the aim of this study was to evaluate the role of lycopene in the progression of diabetic nephropathy and the relevant mechanisms involving endogenous antioxidants, the serum lipid profile, CTGF in a type 1 diabetic rat model.

Materials and methods

Experimental animals and treatments

Sixty male Sprague-Dawley rats weighing 220-260 g were obtained from Experimental Center of Wannan Medical College, were raised in standard laboratory with 12-hour light-dark cycle at 22±2°C. Animals received a standard pellet diet and water ad libitum. After 2 weeks of acclimatization, thirty rats were injected intraperitoneally streptozotocin (STZ, 70 mg/kg) dissolved in 0.1 M citric acid-citrate sodium buffer (pH 4.5) for diabetes. Blood glucose concentrations of peripheral blood from the tail vein were measured (One Touch SureStep Meter, LifeScan, Calif, USA) after 48 h of STZ injection. Animals with blood glucose level > 15 mmol/L were considered as diabetic. The diabetic rats were randomly divided into diabetes untreated group (DM-U) and diabetes treatment with lycopene group (DM-L). The other were injected the same volume of buffer, and were randomly divided into normal untreated group (NC-U) and normal treatment group (NC-L) after measure of glucose. Rats from normal treatment group and diabetes treatment group received lycopene (20 mg/kg per day) by oral gavage tube.

After eight weeks of drug dosing, all rats were anaesthetized with pentobarbital sodium (65 mg/kg i.p.). Blood was obtained for analyzing plasma lipid profiles, glucose and renal function. The kidneys were placed on the ice quickly and homogenized with lysis buffer. Aliquots were stored in a -70°C refrigerator. A 24 h urine sample was collected on the day before the blood sample and aliquots were taken.

Plasma lipid profile and kidney function analyses of blood samples

At the end of experiment, blood samples were collected from the arteria carotis for biochemical analyses. Blood samples were centrifuged at 1300 × g for separation of plasma. Kidney function were analysed by measure of creatinine, urea protein and blood urea nitrogen (BUN) by enzymatic colorimetric methods using automatic analyzer. Plasma lipid profiles were determined by the enzymatic colorimetric methods (commercial kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Antioxidant measurement

For analyses of antioxidant, kidney tissue was lysed and homogenized with 0.1 M PBS and centrifuged at 12,000 g for 10 min at 4°C. Oxidative stress biomarkers measured were superoxide dismutase activity (SOD) and malonaldehyde (MDA) as lipid peroxidation index with commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Pathological histology of kidney

At the end of laboratory operations, all rats were killed. The kidneys were instantly removed and put into narrow-mouth bottle with 10% buffered formaldehyde and proceeded for morphological analysis by conventional methods with H&E stain under a light microscope at magnifications of 400 ×.

Western blot

For analysis of CTGF, phosphorylated AKT protein expression, kidneys were dissected out, and homogenized in lysis buffer with sodium orthovanadate (2 mM), phenylmethylsulfonyl fluoride (0.2 mM), leupeptin (2 μg/mL), and aprotinin (2 μg/mL) on ice for 30 min, then were centrifugated at 13,000 g for 15 min at 4°C. Proteins from homogenization (50 μg protein) were electrophoretically separated by 8% or 12% SDS-PAGE and then transferred onto nitrocellulose membrane. After blockade of nonspecific sites with 5% nonfat milk for 1 h at room temperature, membranes were incubated with a rabbit polyclonal anti-CTGF, AKT/PKB,

Table 1. Change in Body Weight (BW), Heart Weight (HW) and Heart Weight/Body Weight (HW/BW) ratio at eight weeks after streptozotocin or vehicle injection in rats (mean±SD)

	Body weight (g)	Kidney weight (g)	KW/BW*1000	Blood glucose (mmol/L)
NC-U	457±21	1.72±0.22	3.76±0.41	6.01±0.66
NC-L	439±19	1.67±0.21	3.83±0.49	5.89±0.72
DM-U	191±14*	2.04±0.24*	10.56±1.17*	28.21±3.60*
DM-L	242±33#	1.79±0.19#	7.55±1.54#	24.86±3.39#

*Significantly different: $P < 0.02$ vs. NC-U; #Significantly different: $P < 0.04$ vs. DM-U; n=10 per group.

Table 2. Physiological and biochemical parameters of rats in various groups (mean±SD)

	BUN (mmol/L)	Creatinine (μmol/L)	Urea protein (mg/L)
NC-U	6.15±0.77	65.91±6.77	6.84±0.55
NC-L	6.34±0.90	67.22±7.50	6.53±0.61
DM-U	19.42±2.42*	83.28±9.18*	26.44±3.98*
DM-L	11.40±2.09#	72.39±7.49#	14.79±2.71#

*Significantly different: $P < 0.001$ vs. NC-U; #Significantly different: $P < 0.02$ vs. DM-U; n=10 per group.

phospho-AKT/PKB, β-actin antibody (1:500 dilution) overnight at 4°C. Membranes were incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody after 3 washing steps (20 mM Tris, 500 mM NaCl, 0.1% Tween-20) for 1 hour. After rinsing with wash buffer for three times, the reaction was visualized by DAB.

Statistical analysis

The paper presents values as means±STD and statistical significances were determined by Duncan's multiple range tests using SPSS 16.0. The differences among the means were examined by post hoc tests for ONE-WAY ANOVA. Differences were treated as significance if $P < 0.05$.

Results

Effects of lycopene on blood glucose, body weight (BW) and kidney weight (KW)

After STZ administration, animals presented polyuria, increased water consumption. Rats showed a significant increase in level of blood glucose in diabetes untreated group and diabetes treatment group when compared with normal untreated group ($P < 0.01$), and developed uncontrolled type 1 diabetes mellitus (**Table 1**). Diabetic rats presented marked BW loss ($P < 0.01$) and KW gain ($P < 0.05$). In addition, diabetic rats had an increased KW/BW ratio, a marker for the development of diabetic nephropathy. Treatment with lycopene did not significantly effect on the elevated blood glucose value, but prevented BW loss and increase in BW/KW ratio.

Change of biochemical parameters profiling in various rats

At the end of experiment, we measured BUN, 24 h urea protein, and creatinine for evaluating kidney function. The results showed that diabetic rats treated with or without lycopene presented increased BUN, 24 h urea protein and creatinine levels compared with normal untreated group ($P < 0.01$) (**Table 2**), but treatment with lycopene to diabetic rats corrected the elevations in BUN, 24 h urea protein, and creatinine levels ($P < 0.01$).

Improvements in hyperlipidemia by lycopene

As shown in **Table 3**, plasma lipid profile was evaluated in all experimental groups. The results indicated that diabetic state increased the levels of plasma TC, TG, and LDL, but the level of HDL was significantly decreased in diabetic rats when compared with normal untreated group ($P < 0.01$). The diabetic rats treated with lycopene reduced TC, TG, and LDL concentrations, and enhanced HDL level ($P < 0.01$).

Antioxidant effects of lycopene

MDA in renal tissues of STZ diabetic rats was significantly increased compared with that of control rats. Treatment of diabetic rats with lycopene reduced MDA formation. Renal SOD activity was significantly decreased in diabetic rats ($P < 0.01$), and increased prominently in treatment group rats ($P < 0.01$) (**Figure 1**).

Attenuation on kidney lesions in STZ-induced diabetic rats by lycopene

On histological examination, we observed the morphological changes of renal tissue under

Table 3. Effects of lycopene on serum lipid profile in control and diabetic groups of rats (mean±SD)

	Cholesterol (mmol/L)	Triglyceride (mmol/L)	LDL (mmol/L)	HDL-c (mmol/L)
NC-U	2.51±0.45	1.64±0.35	1.48±0.31	0.97±0.22
NC-L	2.29±0.35	1.56±0.20	1.43±0.27	0.94±0.18
DM-U	3.73±0.61*	4.29±0.49*	4.76±0.68*	0.64±0.16*
DM-L	2.72±0.46#	2.84±0.37#	2.97±0.66#	1.09±0.23#

*Significantly different: $P < 0.001$ vs. NC-U; #Significantly different: $P < 0.001$ vs. DM-U; n=10 per group.

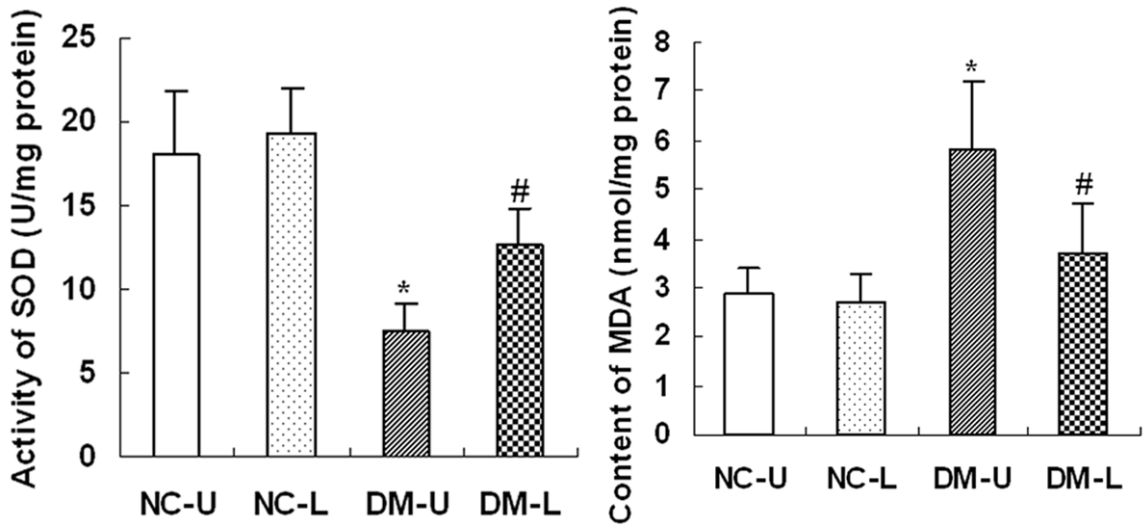

Figure 1. Lycopene-induced changes in SOD activity and MDA level in kidney homogenates of rats. SOD activity was increased and MDA level was decreased by lycopene. Data are means±SD from 10 rats. *Significantly different: $P < 0.001$ vs NC-U; #Significantly different: $P < 0.01$ vs. DM-U.

microscope. As shown in **Figure 2**, characteristic changes in glomerular from diabetes untreated group included hypertrophy, excessive accumulation of ECM and mesangial matrix expansion. Furthermore, lycopene ameliorated these changes in diabetes treatment group.

Effects of lycopene on p-Akt and CTGF

We measured the expression of Akt signaling cascade. Rats in diabetes untreated group presented a remarkable decrease in level of phosphorylated Akt, and increased CTGF protein expression compared with normal untreated group. Lycopene treatment elevated the level of phosphorylated Akt and reduced expression of CTGF in diabetic rat kidneys compared with diabetes untreated group (**Figure 3**).

Discussion

Diabetic nephropathy is a chronic complication of diabetes and one cause of death patients with diabetes mellitus, and thus, preventing or delaying it, has been a major goal in biomedical research. The development of promising therapy seems more likely to be beneficial from reducing oxidative stress induced by hyperglycaemia [18]. Our present study reports the progression of renal disease in STZ diabetic rats and demonstrates that a daily chronic administration of lycopene markedly reduces renal injury in this model.

Clinical observations and experimental animal studies have suggested that hyperlipidemia aggravates the progression of diabetic nephropathy [11, 12, 19]. Increase in renal deposition of LDL causes severe glomerulosclerosis in a variety of glomerular diseases [10]. Hypercholesterolemia itself triggers proinflammatory events through the activation of pathways. Therefore, the high level of serum lipids in DM increases the risk of diabetic nephropathy [20]. In our study, plasma cholesterol, TG and LDL levels are increased significantly in STZ diabetic rats, but plasma HDL-C value is reduced

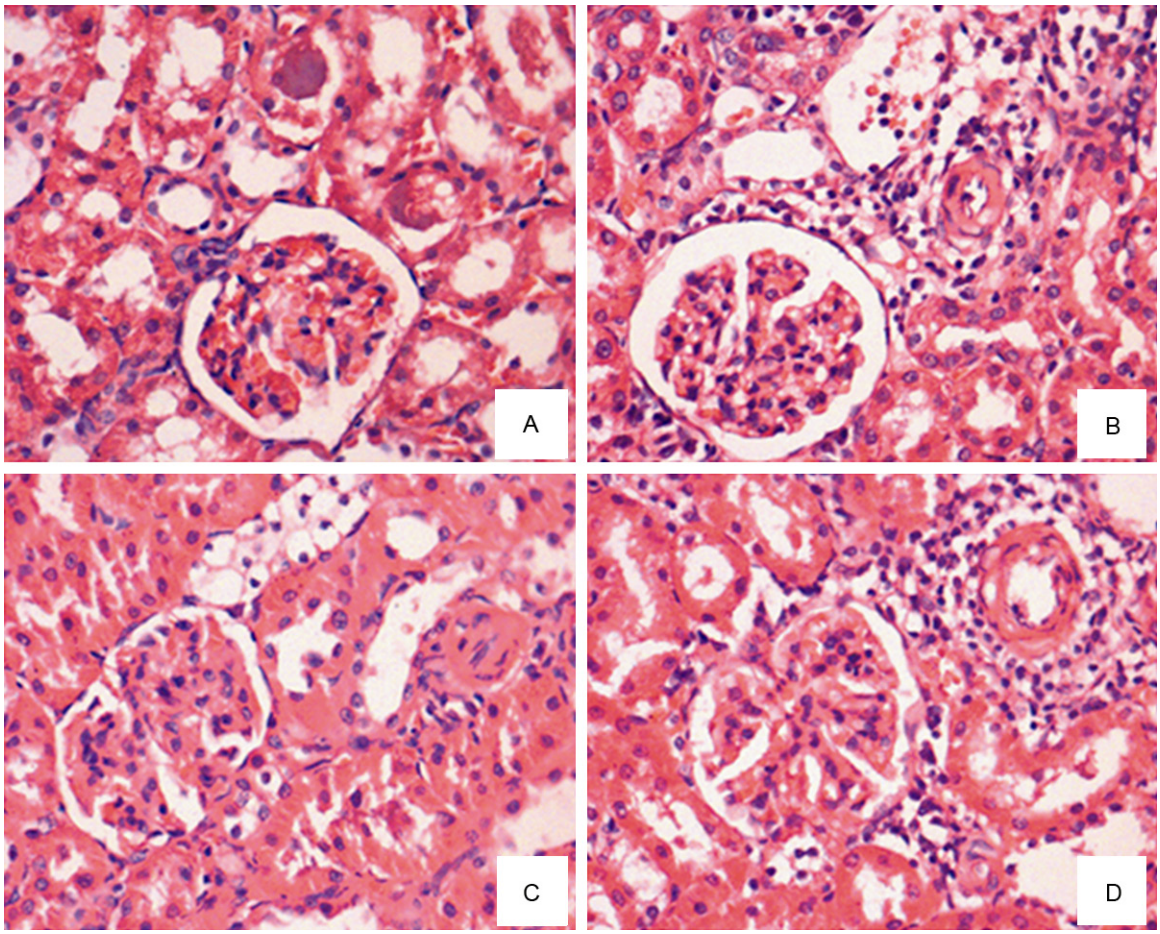

Figure 2. Photomicrographs of H-E staining in the kidney of each group. (A) Normal untreated rat; (B) Normal treatment rat with lycopene; (C) Diabetic untreated rat; (D) Diabetes treatment rats with lycopene. The kidney specimen of the diabetic group showed markedly severe destruction in glomerular and tubulointerstitial lesions such as glomerular sclerosis atrophy, interstitial expansion, and interstitial cellular infiltration (C). General morphology of glomerulus and tubulointerstitial lesions of diabetic rat with lycopene was much improved and showed quite normal appearance (D).

prominently in diabetic rats. This result is consonant with previous studies of diabetic rats [21]. Our data show that administering lycopene decreases TC, TG and LDL significantly and increases HDL in STZ diabetic rats. The previous studies suggest that lycopene has the capacity of improving serum lipids [22]. It is implied that lycopene might possess a promising effect on deceleration of metabolic syndrome in diabetes.

Diabetic nephropathy is a serious and important microvascular complication that occurs frequently in patients with diabetes. The pathogenetic mechanisms for the micro-vascular complications may be associated with oxidative stress which is regarded as the major factor that couples hyperglycemia with vascular complications. Oxidative stress causes an increase of reactive oxygen species (ROS) which can assault at various target organ systems [23-25]. Increased level of plasma MDA and low SOD activity are found in diabetes [26]. Lycopene, a polyunsaturated hydrocarbon containing lots of conjugated double bonds, is one of the most efficient antioxidants among the natural carotenoids [27]. Clinical study has demonstrated that consumption of tomato products which contain lycopene may decrease biomarkers level of oxidative stress in healthy subjects, smokers, and type 2 diabetics [28]. In present study, our data show that lycopene could reduce MDA level and increase SOD activity significantly in kidney homogenate. The results show that lycopene possesses potent antioxidant effects on diabetes.

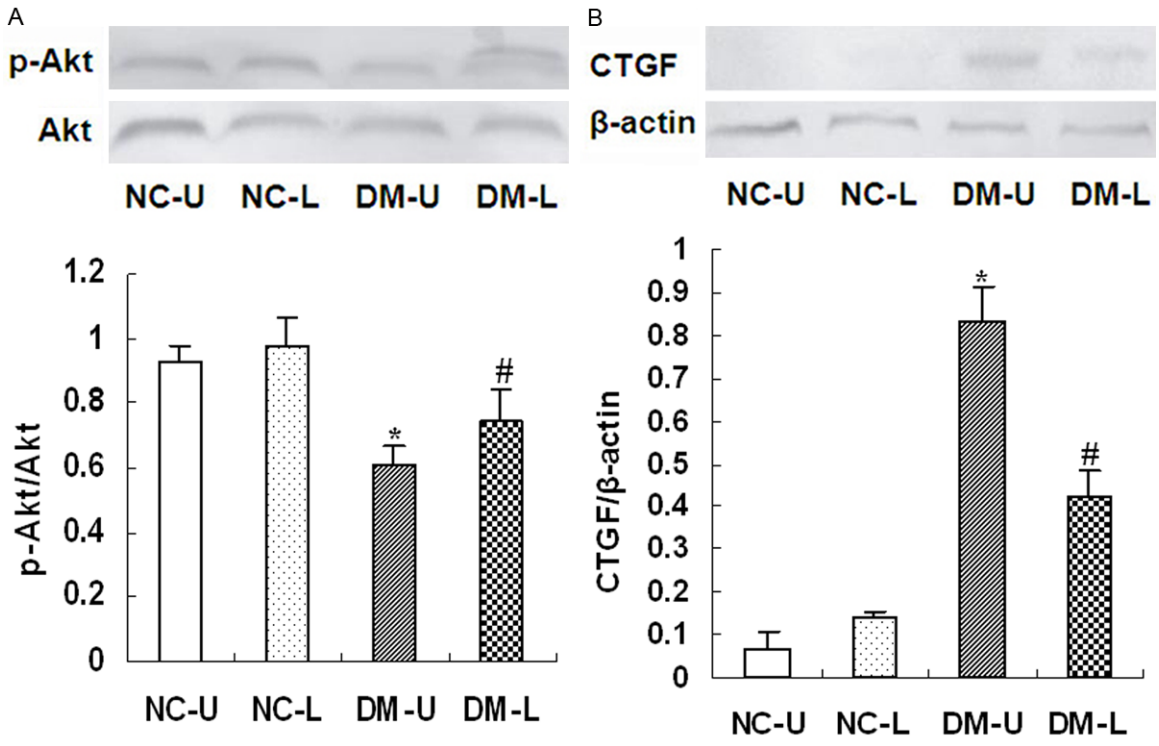

Figure 3. Effects of lycopene on p-Akt and CTGF expression. Immunoblot showing levels of p-Akt (A) and CTGF (B) in the kidney of normal untreated, normal treatment rat with lycopene, diabetic untreated and diabetes treatment rats with lycopene rats. *Significantly different: $P < 0.001$ vs. NC-U; #Significantly different: $P < 0.01$ vs. DM-U; n=5 per group.

Akt is an important downstream effector of insulin signaling, and regulates a variety of cellular functions, including glucose metabolism, cell proliferation, cell hypertrophy, and cell apoptosis [29, 30]. Many studies show a decrease in Akt phosphorylation and activity in myocardiocytes and renal tissues in STZ-diabetic rats, and administration of insulin tends to bring Akt activity and phosphorylation of Akt to near normal [31, 32]. As a matter of fact, oxidative stress is a distinctly important factor involved in inhibition of Akt activation in hyper-glycemia status. Previous study shows that excess production of H_2O_2 in adipocytes would result in significant decrease of Akt phosphorylation [33]. It has been indicated that high glucose-induced oxidative stress by peroxynitrite could increase the activity of p38MAP kinase and inhibit activity of Akt-1 kinase [34]. Our result indicated that expression of p-Akt protein is decreased in diabetic rats, and it is found to recover after lycopene treatment. It is reasonably to suppose that lycopene could restore p-Akt level and subsequent signaling cascade through ameliorating oxidative stress in diabetic rats.

Hyperglycemia can change growth factors by means of metabolic and hemodynamic pathways. In response to hyperglycaemia, the generation of the powerful profibrotic factor, TGF-β1 increases significantly, which leads to fibrotic consequences [35]. With the accumulation of ECM, CTGF may play the part of a downstream mediator of TGF-β1 [36]. CTGF has been confirmed to play an important role in fibration through transforming growth factor-β1-dependent or -independent pathway [14]. Indeed, CTGF levels in tissue or blood have been demonstrated to be significant correlation with the degree of fibrosis in many diseases [37]. Previous study showed that PKC upregulated CTGF expression by inhibiting the PI3K/Akt pathway [38]. Our study showed that lycopene could increase p-Akt expression and reduce CTGF expression. These suggest that lycopene's protective effect against diabetic nephropathy is at least in part via increasing

antioxidant and reducing fibrosis. In addition, our results show that lycopene treatment prevented the development of diabetic nephropathy by remarkably decreasing creatinine and BUN in diabetic rats. Lycopene-fed rats had less renal injury. This may be explained that there was an increased elimination of blood creatinine and urea by kidney or reduced protein degradation. Lycopene also prevented the increase in 24 h urea protein in diabetic rats.

In summary, the results from this study show that lycopene possesses strong antioxidant properties as well as lipid-lowering effect. Lycopene not only can significantly decrease TC, TG and LDL-C levels but also increase HDL-C value. Moreover, lycopene may prevent morphological destruction of kidney due to diabetes mellitus through its anti-oxidative and reducing fibrosis mechanisms. Limited by the inability to use relatively large sample of animals, we need further studies to expound the relationship between lycopene and Akt pathway in diabetes and to clarify the circumstantial mechanisms of lycopene on improvement in diabetic nephropathy. From our results, lycopene shows therapeutic promise in amelioration of hyperlipidemia and prevention of metabolic syndrome in diabetic patients.

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Disclosure of conflict of interest

None.

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