Beneficial effects of β-conglycinin on renal function and nephrin expression in early streptozotocin-induced diabetic nephropathy rats

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Abstract

The objective of the present study was to investigate the effects of β-conglycinin and soya isoflavones on diabetic nephropathy (DN). DN was induced by an intravenous injection of streptozotocin (25 mg/kg) in spontaneously hypertensive rats. DN rats were divided into a non-diabetic group (C, control group) and three DN groups (D, DN with control diet; B, DN + control diet with one-eighth of casein replaced by β-conglycinin as the protein source; and I, DN + control diet with 0·01 % soya isoflavones). After a 4-week experimental period, we found that fasting blood sugar and plasma and kidney advanced glycation end product levels and 24 h urinary protein excretion of the B group were significantly lower than those of the D group and insulin sensitivity and nephrin expression of the B group were significantly higher than those of the D group. In addition, systolic blood pressure, angiotensin-converting enzyme activity, angiotensin II level and plasma TAG level of the B group were significantly lower than those of the D group, whereas only the levels of plasma TAG and thiobarbituric acid-reactive substances of the I group were lower than those of the D group. In conclusion, β-conglycinin may be beneficial for retarding DN progression and this effect cannot be completely explained by its isoflavone content.

Key words: β-Conglycinin: Soya: Diabetes: Diabetic nephropathy: Hypertension: Nephrin

Diabetic nephropathy (DN) is one of the most serious microvascular complications of diabetes and a major cause of end-stage renal disease[1]. Diabetes mellitus (DM) was the fifth cause among the top ten causes of death in Taiwan in 2010. According to the 2004–8 Nutrition and Health Survey in Taiwan (NHST), the prevalence of DM was 9·2 %, and haemodialysis treatment being required by one-fourth to one-third of the patients is caused by DN.

Systemic hypertension leads to glomerular hypertension, renal microvascular complications and glomerular basement membrane thickening and accelerates renal failure. Renin and angiotensins released from the kidneys during kidney injury lead to elevated blood pressure. These factors may form a vicious cycle and aggravate renal dysfunction. About 50 % of the patients who suffered from type 1 DM for more than 30 years had hypertension[2]. The German Society for Diabetes has also suggested that adjusting the blood sugar levels to a near-normal range and normalising the blood pressure are beneficial for preventing DN.

Hyperglycaemia may also lead to haemodynamic changes[3] and excretion of angiotensin II (Ag II)[4]. Both hyperglycaemia and elevation of Ag II levels induce the formation of reactive oxidative species. Exposure of podocytes to high glucose levels leads to an increase in reactive oxidative species generation and is associated with the up-regulation of cytochrome P450 4A (CYP4A) expression, and CYP4A inhibitors can ameliorate proteinuria in DM mice[5]. In addition, nephrin is a podocyte-specific protein and is a component of the filtration slits in the kidneys. In DN, hyperglycaemia and high Ag II levels are related to the dysregulation of nephrin expression and proteinuria[6,7].

Modification of dietary habits is important in DM treatment. Soyabean is the main source of dietary plant protein source in Oriental diets. Previous studies have shown that soya protein has several health-promoting effects[8], such as improvement of glucose tolerance, insulin sensitivity and hyperlipidaemia[9] and retardation of the progression of hypertension and renal dysfunction[10]. β-Conglycinin, a major

storage protein of soyabeans, has angiotensin-converting enzyme (ACE)-inhibitory activity[11] and can attenuate blood glucose and insulin levels in obese mice[12]. However, the effects of β-conglycinin on DN remain unclear. In addition, soya isoflavones are also considered to be another bioactive component of soya protein. Therefore, the aim of the present study was to investigate the effects of β-conglycinin and soya isoflavones on renal function, blood glucose levels and blood pressure in a rat model of streptozotocin (STZ)-induced DN.

Materials and methods

Animals, diets and experimental design

Male spontaneously hypertensive rats (SHR, 8 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center. The rats were housed in individual cages in a room maintained at 23 ± 1°C with 55 ± 5 % humidity and a 12 h light–12 h dark cycle. Investigators at the Taipei Medical University Laboratory Animal Center followed the protocols described in the 'Guide for the Care and Use of Laboratory Animals'. The rats were fed a standard rat chow diet (Rodent Laboratory Chow 5001; Purina Mills) for 1 week for acclimatisation. β-Conglycinin was purchased from Fuji Oil. Diabetes was induced in the rats by an intravenous injection of 25 mg/kg of STZ (dissolved in pH 4·5 citrate buffer), while the control rats were administered an equal volume of a vehicle. After 48 h, blood samples were collected from the tail vein, and rats with a fasting glucose concentration > 2000 mg/l were divided into three groups: DN rats fed the AIN-93M diet (D group, n 6); DN rats fed the AIN-93M diet containing β-conglycinin (one-eighth of casein was replaced by β-conglycinin as the dietary protein source, B group, n 10); DN rats fed the AIN-93M diet containing soya isoflavones (equivalent to the isoflavone content in the B group, I group, n 9). The control rats were fed the AIN-93M diet (C group, n 8; Table 1). During the 4-week

Table 1. Diet composition

Ingredients (%)*	C	D	B	I
Maize starch	46·6	46·6	46·6	46·6
Dextrin	15·5	15·5	15·5	15·5
Sucrose	10	10	10	10
Casein	14	14	12·25	14
β-Conglycinin			1·75	
L-Cystine	0·2	0·2	0·2	0·2
Soya oil	4	4	4	4
Cellulose	5	5	5	5
Mineral	3·5	3·5	3·5	3·5
Vitamin	1	1	1	1
Choline	0·3	0·3	0·3	0·3
Soya isoflavones				0·01

C, control group; D, diabetic nephropathy (DN) group; B, DN + β-8conglycinin group; I, DN + soya isoflavone group.
* Casein (high N), cellulose (non-nutritive bulk), mineral mixture (AIN-93M) and vitamin mixture (AIN-93M) were obtained from ICN Biochemicals. Dextrin was obtained from Corn Products International, Inc. β-Conglycinin was procured from Fuji Oil Company Limited, and it contained 57 mg/100 g of isoflavones. Soya isoflavones were obtained from Corn Products Korea, Inc. Soya oil and sucrose were obtained from the Taiwan Sugar Corporation.

experimental period, food was provided with free access. The body weight and food intake were recorded daily. At the end of the experimental period, all the rats were killed, and blood and tissue samples were collected for analysis.

Sample collection and analysis

Blood pressure. Systolic blood pressure and diastolic blood pressure were measured by the tail-cuff method (MK-2000ST; Muromachi Kikai). After being starved for 12 h, the rats were put into restrainers, and at least five readings were recorded to calculate the blood pressure.

Blood samples. The rats were killed after being anaesthetised with sodium pentobarbital. Blood samples were collected from the interior vena cava into tubes containing an anticoagulant. Blood samples were immediately centrifuged, and the plasma was stored at −80°C until being analysed. Plasma glucose, insulin, blood urea N, creatinine, uric acid, total cholesterol and TAG concentrations were determined using a Roche Modular P800 Autoanalyzer (Roche, Inc.). Malondialdehyde concentration was measured by the thiobarbituric acid-reactive substances (TBARS) method[13]. ACE activity was analysed as described previously[14]. Advanced glycation end product (AGE) and Ag II concentrations were determined using ELISA kits (STA-317, OxiSelect™; Cell Biolabs, Inc.; EA3501-1; ASSAYPRO). The insulin sensitivity index (ISI) was calculated using the following equation:

$$ISI = \ln 1/(\text{fasting blood sugar} \times \text{fasting insulin}).$$

Urine samples. Using metabolic cages, 24 h urine samples were collected. The 24 h urine volume was recorded, and urinary protein, creatinine and glucose concentrations were determined using a Roche Modular P800 Autoanalyzer (Roche, Inc.). The creatinine clearance rate (Ccr) was calculated as follows:

$$\text{Ccr} = \text{urinary creatinine (mg/dl)}$$
$$\times\, 24\,\text{h urine volume (ml)}/(\text{plasma creatinine (mg/dl)}$$
$$\times\, 1440\,(\text{min})).$$

Kidney samples. Kidney samples were homogenised with a buffer (50 mM-Tris base, 150 mM-NaCl and 1 % Triton-X 100), and suspensions were centrifuged at 1000 *g* at 4°C for 10 min. ACE activity and TBARS and Ag II concentrations in the kidneys were determined using the same method as described previously.

Western blot analysis. Kidney and gastrocnemius muscle samples were homogenised in radioimmunoprecipitation assay (RIPA) buffer (50 mM-Tris–HCl, 150 mM-NaCl, 1 % NP-40 and 0·1 % SDS), and suspensions were centrifuged at 14 000 *g* at 4°C for 10 min. Kidney suspensions were analysed for the expressions of CYP4A (using the polyclonal anti-rat CYP4A antibody, ABR PA3-033), nephrin (using the polyclonal anti-rabbit nephrin antibody, ab58968), tumour growth factor (TGF)-β (using the polyclonal anti-rat TGF-β antibody; Santa Cruz Biotechnology, and using goat anti-rat IgG conjugated with horseradish peroxidase or a goat anti-rabbit

IgG peroxidase-conjugated secondary antibody). Gastrocnemius muscle suspensions were analysed for the expression of PPAR-γ (using the polyclonal anti-rabbit PPAR-γ antibody, D69, and a goat anti-rabbit IgG peroxidase-conjugated secondary antibody). The immune complex was developed using an enhanced chemiluminescence detection system (Western Lighting™; PerkinElmer), and bands were quantified by densitometry using Image-Pro Plus 4.5 (Media Cybernetic). Equal loading of the total protein was verified using a commercially available mAb against glyceraldehyde 3-phosphate dehydrogenase, and the results are expressed as the ratio of protein:glyceraldehyde 3-phosphate dehydrogenase.

Pathological analysis. Dissected kidney samples of the rats were fixed in 10% formaldehyde. The samples were stained with the haematoxylin and eosin stain. Biopsies were examined on a blinded basis by a pathologist, using a 0–3 injury scale, with 0 indicating absent or minimal, 1 indicating mild, 2 indicating moderate and 3 indicating severe injury.

Statistical analysis

Data were analysed using one-way ANOVA and the least significant difference test using the SAS program (version 9.1; SAS, Inc.). Results are expressed as the mean with their standard errors. A *P* value <0·05 was taken as the level of statistical significance.

Results

Body weight, food intake and blood pressure

At the end of the study, body weights of the three diabetic groups (D: 176·1 (SEM 17·6) g; B: 182·6 (SEM 11·2) g; and I: 196·8 (SEM 5·6) g) were lower than that of the C group (350·0 (SEM 6·5) g) (*P* = 0·000), and food intake of the three diabetic groups (D: 32·3 (SEM 1·2) g; B: 30·4 (SEM 1·8) g; and I: 31·1 (SEM 2·0) g) was also higher than that of the C group

(21·3 (SEM 2·5) g) (*P* = 0·003). No difference in body weight and food intake was found among the D, B and I groups. The systolic blood pressure of the B group was significantly lower than that of the D group (*P* = 0·014). There was no difference in diastolic blood pressure among all the groups (*P* = 0·406; Table 2).

Plasma biochemical analysis

At the end of the study, the plasma glucose level of the C group was significantly lower (*P* = 0·000) than those of the DN groups, and the plasma glucose level of the B group was significantly lower (*P* = 0·036) than that of the D group, while no difference was found between the D and I groups (*P* = 0·368) (Table 2). Plasma insulin levels were significantly lower in the three DN groups than in the C group (*P* = 0·004). The ISI of the D group was also lower than that of the C group (*P* = 0·005), while the B group had a significantly higher ISI than the D group (*P* = 0·042) (Table 2). The plasma TAG level of the D group was higher than that of the C group (*P* = 0·025). No difference was found in total cholesterol concentrations among all the groups (Table 2).

Advanced glycation end product levels

Plasma AGE levels of the D and I groups were significantly higher than those of the C and B groups (*P* = 0·005). The kidney AGE level of the D group was significantly higher than that of the C group (*P* = 0·000), and those of both the B and I groups were lower than that of the D group (*P* = 0·003) (Table 2).

Renal function markers in the plasma and urine

Blood urea N levels of the three DN groups were significantly higher than that of the C group (*P* = 0·001). The plasma uric acid concentration of the D group was higher than that of

Table 2. Blood pressure, plasma and kidney biochemical analysis at the end of the study

(Mean values with their standard errors)

	C (*n* 8)		D (*n* 6)		B (*n* 10)		I (*n* 9)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SBP (mmHg)	192·4[c]	3·5	179·0[b]	4·4	165·4[a]	2·9	177·9[b]	3·5
DBP (mmHg)	128·0	7·0	122·4	5·9	114·5	5·5	113·6	8·3
Plasma glucose (mg/l)	1530[a]	81	3816[c]	141	3166[b]	159	4092[c]	296
Plasma insulin (g/l)	0·25[b]	0·04	0·18[a]	0·01	0·17[a]	0·01	0·17[a]	0·01
ISI	0·28[b]	0·01	0·24[a]	0·01	0·26[b]	0·01	0·24[a]	0·01
TAG (mg/l)	315[a]	29	1053[b]	555	450[a]	57	328[a]	49
TC (mg/l)	518	14	680	65	598	30	641	43
Plasma AGE (g/ml)	1·2[a]	0·1	1·6[b]	0·1	1·3[a]	0·0	1·4[b]	0·1
Kidney AGE (mg/g protein)	7·0[a]	0·5	11·1[d]	0·5	8·4[b]	0·5	9·6[b]	0·4
Plasma ACE activity (mU/ml)	103·7	4·9	108·8	6·3	94·4	3·0	98·3	1·6
Kidney ACE activity (U/g protein)	8·1[b]	0·7	7·3[b]	0·3	4·7[a]	0·2	7·7[b]	0·5
Plasma angiotensin II (mg/ml)	140·3[c]	4·4	93·6[b]	2·2	81·7[a]	0·9	86·0[a,b]	0·6
Kidney angiotensin II (mg/g protein)	11·7[a]	0·7	14·6[b]	1·1	11·4[a]	0·3	14·2[b]	0·9
Plasma MDA (mM)	5·5[a]	0·2	6·4[b]	0·6	5·0[a]	0·3	5·1[a]	0·2
Kidney MDA (mmol/g protein)	626·1[a]	20·8	746·7[b]	35·5	629·6[a]	37·6	766·0[b]	45·8

C, control group; D, diabetic nephropathy (DN) group; B, DN + β-conglycinin group; I, DN + soya isoflavone group; SBP, systolic blood pressure; DBP, diastolic blood pressure; ISI, insulin sensitivity index; TC, total cholesterol; AGE, advanced glycation end products; ACE, angiotensin-converting enzyme; MDA, malondialdehyde.
[a,b,c,d] Mean values with unlike superscript letters were significantly different (*P* < 0·05).

Table 3. Renal function markers at the end of the study

(Mean values with their standard errors)

	C (n 8)		D (n 6)		B (n 10)		I (n 9)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma								
BUN (mg/l)	198[a]	5·0	373[b]	65	326[b]	15	336[b]	27
Creatinine (mg/l)	4·0	0·0	4·0	1·0	4·0	0·0	4·0	0·0
Uric acid (mg/l)	4·0[a]	0·0	9·0[b]	2·0	5·0[a]	0·0	3·0[a]	0·0
Urine								
24 h volume (ml/d)	5·3[a]	1·4	18·8[b]	0·5	17·6[b]	1·4	14·5[b]	1·6
Glucose (mg/d)	0·2[a]	0·1	848·2[b]	81·3	858·3[b]	136·8	642·6[b]	117·9
Protein (mg/d)	10·6[a]	0·7	21·0[b]	2·0	10·9[a]	1·4	17·5[b]	2·5
Ccr (ml/min)	1·4[b]	0·2	1·1[a]	0·2	0·9[a]	0·1	1·1[a]	0·1

C, control group; D, diabetic nephropathy (DN) group; B, DN + β-conglycinin group; I, DN + soya isoflavone group; BUN, blood urea N; Ccr, creatinine clearance rate.
[a,b] Mean values with unlike superscript letters were significantly different ($P < 0.05$).

the C group ($P = 0.000$), while no difference was found between the C group and the B and I groups. The 24 h urine volume and urinary glucose excretion of the three DN groups were significantly higher than those of the C group ($P = 0.000$). Urinary protein excretion levels of the D and I groups were significantly higher than that of the C group ($P = 0.002$), while no difference was found between the C and B groups ($P = 0.914$). The Ccr of the DN groups were significantly lower than that of the C group ($P = 0.018$), and there was no difference among the three DN groups (Table 3).

Angiotensin-converting enzyme activity and angiotensin II level

At the end of the study, no differences in plasma ACE activity were found among all the groups ($P = 0.082$). However, the kidney ACE activity of the B group was significantly lower
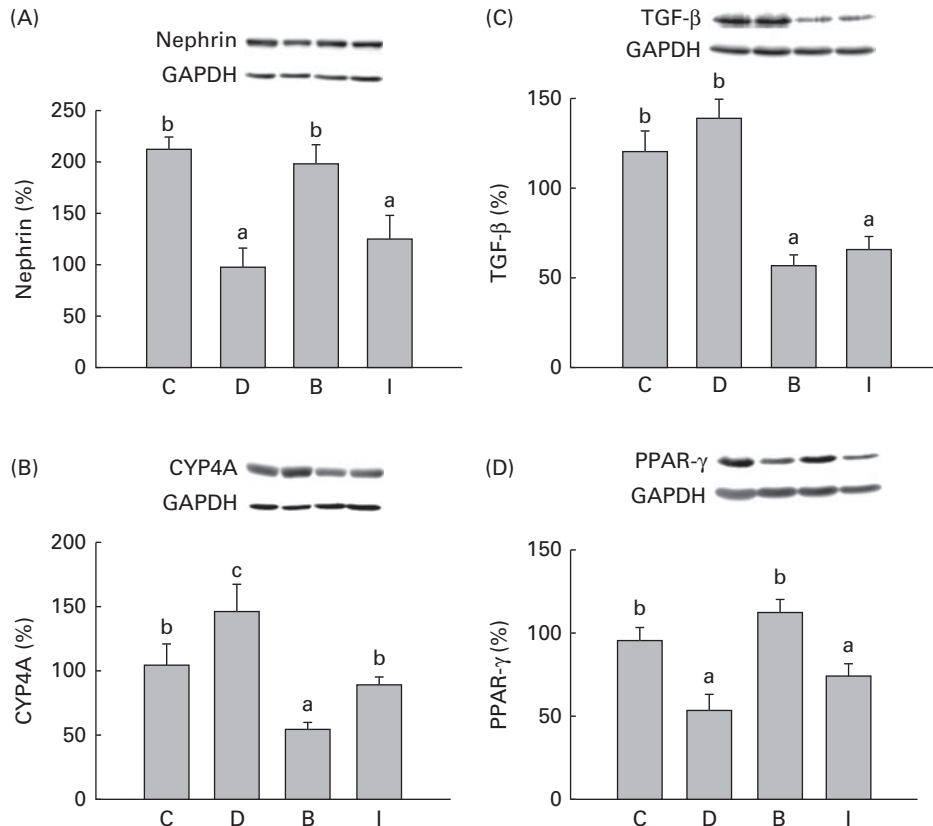

Fig. 1. (A) Nephrin, (B) cytochrome P450 4A (CYP4A) and (C) tumour growth factor (TGF)-β protein expressions in the kidneys and (D) PPAR-γ protein expression in the gastrocnemius muscle at the end of the study. Values are means, with their standard errors represented by vertical bars. [a,b,c] Mean values with unlike letters were significantly different ($P < 0.05$). C, control group (n 8); D, diabetic nephropathy (DN) group (n 6); B, DN + β-conglycinin group (n 10); I, DN + soya isoflavone group (n 9). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

than that of the D group ($P = 0.000$), while no difference was found between the D and I groups ($P = 0.562$). Plasma and kidney Ag II concentrations of the B group were significantly lower than those of the D group ($P = 0.001$), and there was no difference between the I and D groups ($P = 0.738$) (Table 2).

Nephrin, cytochrome P450 4A and PPAR-γ protein expressions

Nephrin protein expression of the D group was significantly lower than those of the C and B groups ($P = 0.004$), and no difference was found between the I and D groups ($P = 0.148$) (Fig. 1(A)). CYP4A protein expression of the D group was significantly higher than that of the C group ($P = 0.024$). CYP4A expressions of the B and I groups were also lower than that of the D group (Fig. 1(B)) ($P = 0.048$). Gastrocnemius muscle PPAR-γ protein expression of the D group was significantly lower than those of the C and B groups ($P = 0.003$), but there was no difference between the I and D groups ($P = 0.119$) (Fig. 1(D)).

### Thiobarbituric acid-reactive substances and tumour growth factor-β protein expressions

Plasma and kidney TBARS levels of the D group were significantly higher than those of the C group ($P = 0.036$). Plasma TBARS levels of both the B and I groups were lower than that of the D group ($P = 0.002$), while only the B group had a lower kidney TBARS concentration than the D group (Table 2). TGF-β protein expressions of the B and I groups were also lower than that of the D group ($P = 0.000$) (Fig. 1(C)).

Pathological analysis

At the end of the study, we found mild renal vessel congestion in the D group. The score of the B group was significantly lower ($P = 0.000$) than that of the D group, but no significant difference was found between the I and D groups ($P = 0.050$) (Fig. 2). No significant glomerulonephritis or interstitial inflammation was observed in all the groups, and the results indicated that our diabetic rats were only undergoing early-stage nephropathy.

Discussion

In the present study, we treated SHR with a single dose of STZ, and the rats showed typical symptoms of diabetes, such as hyperglycaemia, weight loss, polyuria and glycosuria. In addition, the STZ-treated SHR showed higher blood urea N and urinary protein excretion and a lower Ccr, characteristics of renal dysfunction. These results indicate that we have successfully established a rat model of early STZ-induced DN. Although all the diabetic rats exhibited increased food intake, no difference was found among the three groups. The B group orally ingested the diet containing 1·75 % β-conglycinin with 0·01 % soya isoflavones and the D group consumed the same amount of soya isoflavones since both the groups exhibited the same degree of food intake.
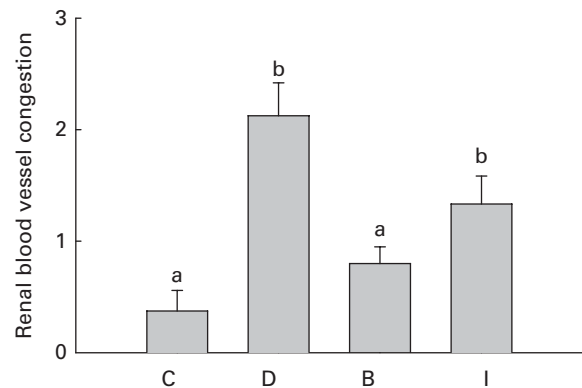
(A)
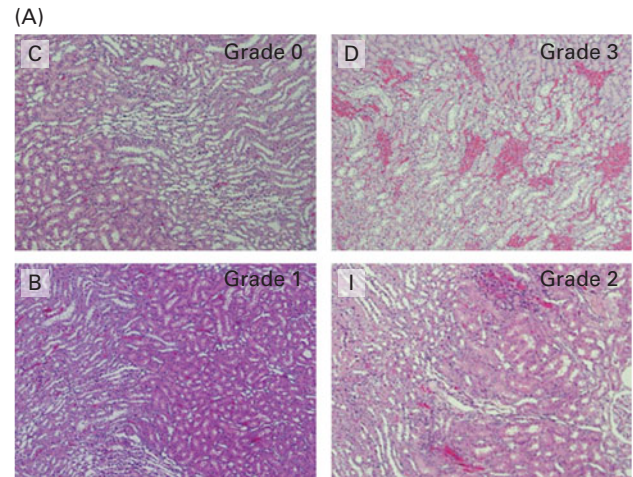
(B)

Fig. 2. (A) Histopathology of renal blood vessel congestion (haematoxylin and eosin stain, 40 ×) and (B) score at the end of the study. Values are means, with their standard errors represented by vertical bars. [a,b] Mean values with unlike letters were significantly different ($P < 0.05$). C, control group (*n* 8); D, diabetic nephropathy (DN) group (*n* 6); B, DN + β-conglycinin group (*n* 10); I, DN + soya isoflavone group (*n* 9). Grade 0, absent or minimal; grade 1, mild; grade 2, moderate; grade 3, severe. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)

Therefore, we can confirm that the effects β-conglycinin on diabetic rats are due to its protein composition or soya isoflavones through a comparison of the results of the two groups.

Many studies have reported that soya protein can retard the progression of renal failure[15]. β-Conglycinin is the major storage protein of soyabeans and is considered to be one of the bioactive components in soya protein. In the present study, β-conglycinin was used to replace one-eighth of casein as the dietary protein, and we found that β-conglycinin decreased urinary protein excretion. Nephrin is a protein that helps maintain the filtration barrier integrity in the kidneys[16]. A human study has indicated that diabetic patients, before the onset of albuminuria, exhibited increased urinary nephrin excretion[17]. Thus, nephrin may be an early indicator of renal injury[18]. In animal experiments, renal nephrin expression was also lower in diabetic SHR[19]. We found that β-conglycinin intervention significantly increased nephrin expression and decreased urinary protein excretion in the DN

rats. However, we did not find changes in nephrin expression or urinary protein excretion in the I group, which consumed the same amount of isoflavones as the B group. These results suggest that β-conglycinin supplementation retards renal injury in diabetic rats, and this effect cannot be completely explained by its isoflavone content.

A previous study has reported that β-conglycinin improves glucose tolerance by increasing insulin sensitivity[20]. In the present study, β-conglycinin decreased the fasting blood glucose levels in the DN rats, but it did not affect plasma insulin concentrations. In addition, we also found lower plasma and kidney AGE concentrations in rats consuming the diets containing β-conglycinin. AGE can also interrupt renal nephrin expression[6]. Therefore, β-conglycinin might ameliorate hyperglycaemia by increasing insulin sensitivity and thus decrease plasma and kidney AGE levels, which may help to preserve nephrin expression in DN rats. Thiazolidinedione, a PPAR-γ ligand, is a common diabetic medication that can increase insulin sensitivity and ameliorate hyperglycaemia[21]. However, thiazolidinedione is still restricted in clinical use because of its side effects, such as liver toxicity[22]. PPAR-γ is found in many tissues, and PPAR-γ expression in skeletal muscle is important for regulating insulin sensitivity[23] and carbohydrate metabolism[24]. In the present study, gastrocnemius muscle PPAR-γ expression was significantly lower in the DN rats than in the control rats, and the ISI was also lower in the DN rats. A previous study has found that soya protein may stimulate PPAR-γ expression and improve insulin sensitivity[25]. In the present study, gastrocnemius muscle PPAR-γ expression in the β-conglycinin diet-fed rats was significantly higher than that in the DN group and the ISI was also higher than that of the DN group. The present results suggest that β-conglycinin supplementation may increase insulin sensitivity via the stimulation of gastrocnemius muscle PPAR-γ expression. Although some studies have also reported that soya isoflavones stimulate insulin secretion *in vivo* and *in vitro*[26], we did not find significant effects of soya isoflavones on insulin or blood sugar levels.

In the present study, rats with STZ-induced DN exhibited lower blood pressure than the control rats, which was consistent with the finding in a previous study that a STZ injection may interfere with the development of hypertension in SHR[27]. Studies have suggested that the renin–angiotensin system (RAS) in local tissues is more important for long-term blood pressure maintenance[28] and cardiorenal remodelling[29]. Glomerular hypertension leads to increased permeability in Bowman's capsule and urinary albumin excretion, leading to the progression of DN[30]. In clinical studies, ACE inhibitors can reduce blood pressure and urinary protein excretion and retard the progression of DN[31,32]. In addition, high concentrations of Ag II *in vivo* may also inhibit renal nephrin expression[33]. We found that the β-conglycinin diet-fed rats exhibited lower systolic blood pressure, kidney ACE activity and Ag II concentrations. These results suggest that the ACE-inhibitory effect of β-conglycinin in the kidneys leads to a decrease in the production of Ag II and consequently plays a role in decreasing blood pressure and increasing nephrin expression. CYP4A expression in the rat kidney converts arachidonic acid to hydroxyeicosatetraenoic acid. Hydroxyeicosatetraenoic acid is a vasoactive substance that stimulates Ag II release from renal vessels and leads to an elevation in blood pressure[34]. In the present study, both β-conglycinin and soya isoflavones reduced kidney CYP4A expression, and thus, the effect of β-conglycinin on blood pressure may partially have been caused by the inhibition of CYP4A expression and the inhibitory effect of β-conglycinin on CYP4A may partially have been caused by its isoflavone content.

Hyperglycaemia increases oxidative stress and reactive oxygen species generation, inflammatory factor release[35] and RAS activation[36] and promotes AGE generation[37]. High blood glucose, Ag II and AGE concentrations also stimulate TGF-β expression[3]. TGF-β overexpression may lead to inflammation, basement membrane extracellular matrix accumulation and renal injury[4]. In the present study, β-conglycinin intervention decreased TBARS concentration and TGF-β expression and soya isoflavones also reduced oxidative and inflammatory indicators in the DN rats. Previous studies have indicated that soya protein exhibits renal protective effects in animal models subjected to various renal diseases, which may be related to the effects of its isoflavone content, such as anti-inflammatory and antioxidative effects[15]. For example, genistein down-regulated *TGF-β* gene expression at the transcriptional level[38]. Therefore, in addition to improving hyperglycaemia and inactivating the RAS, the soya isoflavones of β-conglycinin may also normalise oxidative stress and decrease TGF-β expression in DN rats.

Hyperlipidaemia also plays an important role in the progression of renal diseases[39]. Hyperlipidaemia may increase glomerular vascular permeability and lead to renal dysfunction. Improvement of plasma lipid profiles can retard renal failure[40]. In the present study, the DN rats exhibited hypertriacylglycerolaemia. β-Conglycinin was reported to reduce TAG levels by decreasing sterol regulatory element-binding protein 1 (*SREBP1*) mRNA expression[12] or regulating adiponectin levels[20], and these two mechanisms are affected by insulin. Therefore, β-conglycinin can reduce plasma TAG concentrations in DN rats and may improve insulin sensitivity. Soya isoflavones may act as ligands of oestrogen receptors[41] or regulate lipid metabolism via protein signalling[25]. In the present study, we also found that soya isoflavones lowered plasma TAG concentrations in the DN rats. The results suggest that β-conglycinin may also reduce plasma levels because of its isoflavone content.

In summary, β-conglycinin increased PPAR-γ expression and improved insulin sensitivity and thus ameliorated hyperglycaemia and AGE generation. In addition, β-conglycinin inhibited the activity of ACE, decreased the generation of Ag II and retarded the elevation of blood pressure and oxidative stress. It also improved renal nephrin expression and may be beneficial for maintaining the renal filtration barrier. Although soya isoflavones can protect against oxidative stress and hyperlipidaemia in DN, they alone were unable to retard the progression of renal dysfunction in the present study. Therefore, the beneficial effects of β-conglycinin cannot be completely explained by its isoflavone content.

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