Original Article

Effects of Soy Protein Hydrolysate on Blood Pressure and Angiotensin-Converting Enzyme Activity in Rats with Chronic Renal Failure

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We investigated the effects of soy protein and soy protein hydrolysate on blood pressure control, angiotensin-converting enzyme (ACE) activity, and renal function in a rat chronic renal failure model. Rats that had undergone a 5/6 nephrectomy were separated into three groups and fed different experimental diets for 14 weeks. At the end of the study, rats that fed a diet containing soy protein as the protein source had better blood pressure control and renal function, as well as lower circulating ACE activity and renal tumor-necrosis factor-α (TNF-α) concentration than rats fed a casein protein diet. Soy protein hydrolysate was shown to be as effective as soy protein in preventing the elevation of blood pressure, the progression of renal failure, and decreases in kidney TNF-α level, plasma ACE activity, and insulin concentration. In conclusion, the beneficial effects of consuming soy protein on blood pressure and renal function may be mediated mostly by its pepsin-digested hydrolysate through its ACE inhibitory activity. (*Hypertens Res* 2008; 31: 957–963)

***Key Words*: soy protein, renal failure, angiotensin-converting enzyme, blood pressure**

Introduction

Chronic renal failure is a major problem worldwide. The loss of renal function is related to a vicious cycle of hypertension. Many studies have emphasized the importance of managing high blood pressure in renal disease (*1*). The renin-angiotensin system (RAS) helps regulate blood pressure *in vivo*. Studies have also demonstrated that the local RAS might be effective independently of the systemic RAS (*2*). Angiotensin-converting enzyme (ACE) can catalyze angiotensin I (Ag I) into bioactive angiotensin II (Ag II) in the circulatory system and in tissues, and can lead to an elevation of blood pressure and to tissue damage by increased oxidative stress; its proinflammatory properties and inflammation are associated with related complications in renal failure patients (*3*). Investigators have also found that intrarenal ACE plays an important role in the progression of nephropathy (*4*).

Many clinical trials have shown that lowering blood pressure may reduce the rate of loss of renal function and that RAS blockade has additional renoprotective effects over the control of blood pressure alone (*5*). In rats with nephritic syndrome, intrarenal RAS blockade may ameliorate renal function impairment (*6*). In a diabetic nephropathy mouse model, treatment with captopril and imidapril led to decreases in blood pressure, renal ACE activity, and urine protein (*7*).

Our previous studies found that soy protein may lower blood pressure and retard the loss of renal function in rats with chronic renal failure (*8*). In a spontaneously hypertensive rat model, we found that the ACE inhibitory activity of the hydrophilic portion of soy protein hydrolysate (SPH) retarded the development of hypertension (*9*). Soy protein was also reported to have beneficial effects on inflammation and on the nutritional status of patients with end-stage renal failure (*3*). Thus, we prepared SPH by pepsin digestion to investigate whether or not this SPH can preserve renal func-

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tion and lower blood pressure in a chronic renal failure model by inhibiting circulating and renal ACE activities.

Methods

Preparation of SPH

Soy protein isolate (SPI; Fuji Oil, Osaka, Japan) was dissolved in 0.05 mol/L phosphate buffer (pH 7.6) and adjusted to pH 2.0 with 1 Eq/L HCl. The protein solution was pepsin-hydrolyzed for 24 h at 37°C. The solution was then adjusted to pH 7.0 and centrifuged. The hydrophobic precipitate was discarded, and the hydrophilic supernatant was lyophilized and ground into powder as our SPH sample.

Animals and Diets

Thirty-four male Wistar rats (350–400 g, 16 week old) were purchased from the Laboratory Animal Center of the College of Medicine, National Taiwan University. The animal experiment was approved by the University Committee for Animal Care and Use, and followed the guidelines of Taiwan's National Animal Research Center. Rats were housed in individual cages that were kept in a room under a 12 h light-dark cycle at 22±1°C and a relative humidity of 55±5%. Twenty-four rats underwent a 5/6 nephrectomy (*8*), including the surgical excision of two-thirds of the left kidney and the entire right kidney. The rats were then randomly divided into three experimental groups (8 rats per group), each of which was fed a different experimental diet for 14 weeks: a casein (C) group, a soy protein (S) group, and an SPH (H) group. The other 10 rats underwent a sham operation, consisting of a laparotomy and manipulation of kidneys without destruction of renal tissue, and were then divided into two control groups (5 rats per group): a casein (SC) group and a soy protein (SS) group. The diets for all these groups are shown in Table 1. Food and water were provided with free access. Body weight and food intake were recorded every week.

Table 1. Diet Composition (g/kg)

Ingredient*	Group				
	C	S	H	SC	SS
Cornstarch	550	550	550	550	550
Casein	180	0	100	180	0
SPI	0	180	0	0	180
SPH	0	0	80	0	0
Cellulose	70	70	70	70	70
Soybean oil	60	60	60	60	60
Mineral mix	60	60	60	60	60
Sucrose	60	60	60	60	60
Vitamin mix	20	20	20	20	20
L-Methionine	3	3	3	3	3

C, casein group; S, soy protein group; H, soy protein hydrolysate (SPH) group; SC, rats underwent sham operation and fed with casein diet; SS, rats underwent sham operation and fed with soy protein diet. *Casein (high-N), cellulose (non-nutritive bulk), mineral mixture (AIN-93M) and vitamin mixture (AIN-93M) were obtained from the ICN Biochemicals (Aurora, USA). Cornstarch was from the Samyang Genex (Seoul, Korea). Soybean oil and sucrose were from the Taiwan Sugar Corporation (Tainan, Taiwan). Soy protein isolate (SPI) were obtained from the Fuji Oil Co. Ltd. (Osaka, Japan).

Measurement of Blood Pressure

Blood pressure was measured at the beginning (0 week) and end (14 weeks) of the experiment during 1,800–2,100 h by the tail-cuff method with an electrosphygmomanometer (Model 179, Blood Pressure Analyzer IITC; Life Science Instruments, Woodland Hills, USA) in awake rats. After being starved for 12 h, the rats were put into restrainers, and at least five readings were recorded. The maximum and minimum values were discarded, and the blood pressure was calculated as the average of the remaining three values.

Blood, Urine, and Tissue Sampling

In the 14th week after surgery, rats were placed in a metabolic cage for 24 h urine collection. They were then anesthetized with sodium pentobarbital and sacrificed. Blood samples were collected from the inferior vena cava into tubes containing anticoagulant. The samples were immediately centrifuged, and the plasma was stored at −80°C until analysis. Plasma albumin, creatinine, urea nitrogen, glucose, urine total protein, and urea nitrogen were determined using an autoanalyzer (model 7170; Hitachi, Tokyo, Japan). The creatinine clearance rate was calculated as follows:

$$\text{Creatinine clearance rate} = [\text{urine creatinine (mg/dL)} \times 24\text{ h (1,440 min) urine volume (mL)}]/[\text{plasma creatinine (mg/dL)} \times 1{,}440\text{ (min)}]$$

Plasma insulin was measured with a commercial kit (10-1124-01; Mercodia, Uppsala, Sweden). The remaining portion of the kidney of each rat was collected, weighed, and stored at −80°C for various kinds of analysis.

ACE Activity

The kidney was homogenized in phosphate buffer (pH 7.2), and the supernatant was used for analyzing ACE activity. ACE activities in plasma and kidney homogenates were measured by a spectrophotometric method (*10*). Briefly, we used Hip-His-Leu as the substrate and incubated our samples in it at 37°C for 80 min. The reaction was stopped with 1 Eq/L HCl. The hippuric acid obtained was extracted with ethyl acetate, and its concentration was determined at 228 nm. ACE activity was expressed as IU/mg protein, and the protein contained was quantified by the Lowry method.

Table 2. Body Weight, Food Intake, and Plasma, Urine and Kidney Analysis of Rats with or without 5/6 Nephrectomy

	C	S	H	SC	SS
Body weight (g)	424.5±18.7	443.4±14.2	449.8±10.5	471.1±72.1	460.2±69.5
Food intake (g)	25.0±0.1	24.8±0.2	25.1±0.1	24.7±3.9	25.3±3.6
Blood					
Alb (g/dL)	3.74±0.22	3.94±0.11	4.08±0.10	4.10±0.63	4.28±0.62
UN (mg/dL)	45.50±23.09	23.04±1.43	24.98±1.22	13.84±3.73	15.22±2.06
Cr (mg/dL)	1.90±0.71	0.94±0.04	1.10±0.07	0.60±0.16*	0.60±0.07*
Glucose (mg/dL)	160.25±11.06	171.20±7.49	146.50±7.64	143.0±22.72	150.75±21.94
Insulin (μg/L)	2.93±0.25	1.88±0.23*	1.45±0.25*	1.51±0.38*	1.17±0.56*
MDA (μmol/L)	60.81±9.25	74.47±4.88	54.01±8.58	48.27±6.89	40.94±9.09
Urine					
UN (mg/d)	146.54±11.86	196.41±35.71	62.35±10.94*	164.18±24.67	149.29±2.84
Protein (mg/d)	117.22±25.76	19.92±2.87*	14.23±2.73*	8.11±0.77*	6.05±0.03*
CCr (mL/min)	0.83±0.19	1.48±0.13*	1.44±0.12*	1.83±0.44*	1.83±0.44*
Kidney					
MDA (μmmol/L)	90.47±5.07	99.11±5.53	80.24±2.84	71.12±12.71	63.83±10.21*
SOD (U/mg protein)	61.79±8.64	56.67±5.84	55.20±5.77	88.81±15.66	89.43±13.87*
CAT (U/mg protein)	5.05±1.23	3.33±0.59	3.79±0.46	6.74±1.46	6.68±1.37*

C, casein group; S, soy protein group; H, soy protein hydrolysate (SPH) group; SC, rats underwent sham operation and fed with casein diet; SS, rats underwent sham operation and fed with soy protein diet; Alb, albumin; UN, urea nitrogen; Cr, creatinine; MDA, malonaldehyde; CCr, creatinine clearance; SOD, superoxide dismutase; CAT, catalase. Data were expressed as mean±SEM. *Mean values were significantly different from those of group C ($p<0.05$).

Antioxidative Enzyme Activities and Malonaldehyde Levels

Malonaldehyde (MDA) in plasma and in the kidney homogenate was measured as a thiobarbituric acid reactive substance (TBARS) (*11*). Superoxide dismutase (SOD) activity in the kidney homogenates was analyzed with a commercial kit (Randox Laboratories, Crumlin, UK). Catalase (CAT) activity was analyzed by the method of Aebi (*12*).

TNF-α Levels of the Kidney

Kidneys were homogenized with buffer containing 50 mmol/L Tris-base, 150 mmol/L NaCl, 1% Triton-X (pH 7.2), and protease inhibitors. The tumor necrosis factor-α (TNF-α) level was measured with an enzyme-linked immunosorbent assay (ELISA) kit (rat TNF-α/TNFSF1A; R&D Systems, Minneapolis, USA) and the protein contained was quantified by the Lowry method.

Statistical Analysis

Data were analyzed by one-way ANOVA and Fisher's least-significant difference test using Statistical Analysis System software (SAS Institute, Cary, USA). Results are expressed as the mean±SEM. A value of $p<0.05$ was taken as the level of statistical significance.

Results

Body Weight Change and Food Intake

The average body weights of the rats were 339.7±8.6 g (with 5/6 nephrectomy) and 374.9±11.4 g (with sham operation) at 0 week, and no significant difference was found among the groups. At the end of the study, no differences were found in body weight or food intake among all groups (Table 2). This result indicated that the consumption of soy protein and SPH did not affect normal growth and appetite compared with the control casein group.

Blood Pressure

Figure 1 shows the systolic and diastolic blood pressures (SBP and DBP) of all rats at 0 week and of each group at the end of the study. In the 14th week, SBP and DBP of the SC group were higher than the baseline and SS group values. The 5/6 nephrectomy caused a significant increase in blood pressure, which was significantly higher in the control group than in the S and H groups.

Renal Function

Plasma creatinine was higher in the C group than in both sham-operated groups, and no significant difference was found in the S or H group compared with the sham-operated

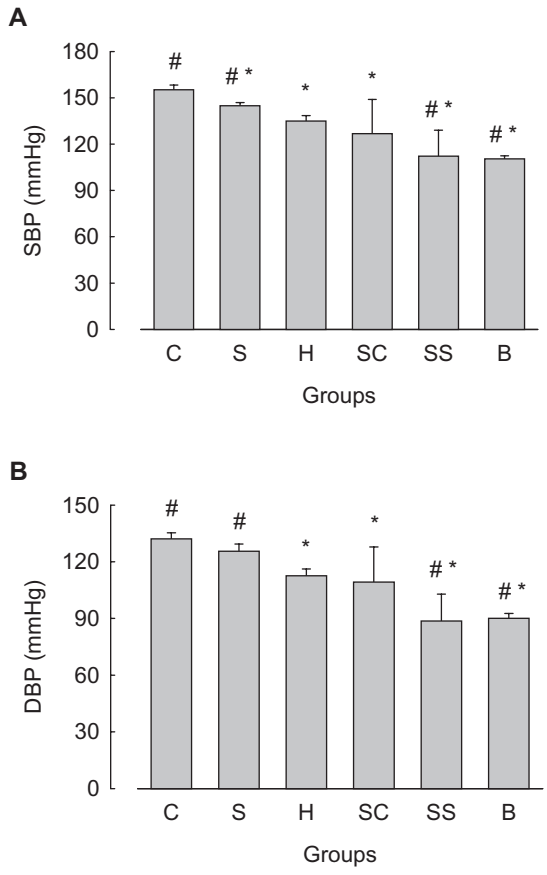

Fig. 1. *A: Systolic blood pressure (SBP) and B: diastolic blood pressure (DBP) in rats with or without 5/6 nephrectomy. C, casein group; S, soy protein group; H, soy protein hydrolysate group; SC, rats underwent sham operation and fed with casein diet; SS, rats underwent sham operation and fed with soy protein diet; B, blood pressure of all rats at the baseline before any operation. Data were presented as mean±SEM. *Mean values were significantly different from those of group C ($p < 0.05$). #Mean values were significantly different from those of group SC ($p < 0.05$).*

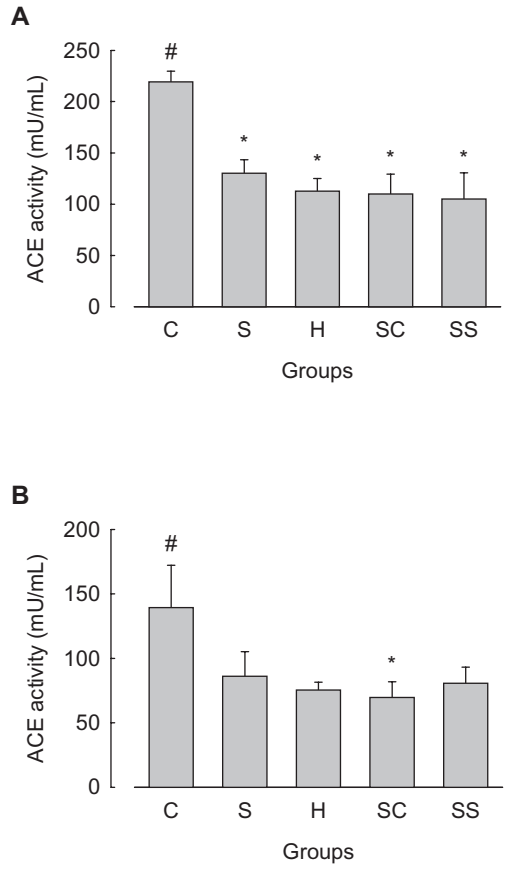

Fig. 2. *Plasma (A) and renal (B) ACE activity in rats with or without 5/6 nephrectomy. C, casein group; S, soy protein group; H, soy protein hydrolysate group; SC, rats underwent sham operation and fed with casein diet; SS, rats underwent sham operation and fed with soy protein diet. Data were presented as mean±SEM. *Mean values were significantly different from those of group C ($p < 0.05$). #Mean values were significantly different from those of group SC ($p < 0.05$).*

groups. Urine protein of the C group was significantly higher and the creatinine clearance rate was lower than those of the other groups. Plasma glucose did not differ among the groups. The plasma insulin concentration was significantly higher in the C group than in the sham-operated groups. In the 5/6 nephrectomy groups, rats fed the soy protein and SPH diets had lower plasma insulin concentrations than rats fed the control diet.

SOD, CAT, and MDA Levels

Plasma MDA concentration did not differ among the groups. Rats subjected to the 5/6 nephrectomy had higher MDA levels and lower levels of SOD and CAT activity in the kidney compared with the sham-operated groups. The different diets had no significant effects on MDA, SOD, or CAT activity levels in rats with either 5/6 nephrectomy or the sham operation.

ACE Activity and TNF-α Level

Plasma and renal ACE activity levels (Fig. 2) were significantly higher in the C group than in the SC group, and this showed that the 5/6 nephrectomy significantly elevated both circulating and tissue ACE activities. No difference between the SC and SS groups was found. In addition, neither plasma nor renal ACE activity in rats fed the soy protein or SPH diet differed from those of the sham-operated groups. Rats with the 5/6 nephrectomy in the C group also had higher kidney TNF-α levels, and there were no differences between the S and H groups and the sham-operated groups (Fig. 3).

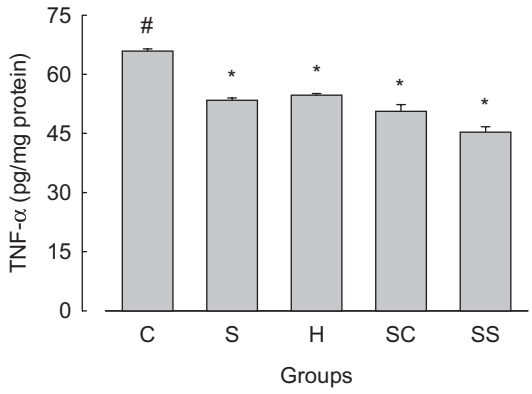

Fig. 3. *Kidney TNF-α level in rats with or without 5/6 nephrectomy. C, casein group; S, soy protein group; H, soy protein hydrolysate group; SC, rats underwent sham operation and fed with casein diet; SS, rats underwent sham operation and fed with soy protein diet. Data were presented as mean±SEM. *Mean values were significantly different from those of group C ($p < 0.05$). #Mean values were significantly different from those of group SC ($p < 0.05$).*

Discussion

In the present study, we found that consumption of soy protein or its hydrophilic hydrolysate retarded the elevation in blood pressure and progression of renal failure in a 5/6 nephrectomized animal model. We also found that the hydrophilic SPH was as effective as soy protein in decreasing blood pressure, preserving renal function, and inhibiting ACE activity. Our results demonstrate that the beneficial effects of soy protein toward renal failure may originate, at least in part, from mediation by its pepsin-digested hydrophilic hydrolysate with ACE inhibitory activity.

Lowering blood pressure and proteinuria by ACE inhibitors (ACEIs) may diminish the risk of disease progression in patients with nondiabetic renal diseases (*13*). Many animal and human studies have demonstrated that the RAS plays an important role in blood pressure control and renal function with renal failure (*14*, *15*). The present study showed that 5/6 nephrectomized rats consuming diets with soy protein or its hydrophilic hydrolysate, SPH, showed reductions in SBP and DBP, and consistently exhibited significant reductions in plasma ACE activity as well as a trend toward decreasing renal ACE activity. Although DBP did not differ between the S and C groups ($p = 0.2389$), the average DBP in the S group was lower than that in the C group. Previous studies also demonstrated that SBP might be a better predictor of related cardiovascular events and mortality than DBP (*16*). The SPI and SPH groups also showed reduced urinary protein excretion and elevated creatinine clearance rates. Recent studies have shown that treatment with ACEIs delays the progression of proteinuric nephropathy (*17*). In progressive chronic nephropathy, the ACEI enalapril exhibited beneficial effects on blood pressure control, renoprotection, and minimization of proteinuria (*18*). An *in vitro* study showed that many different peptides with ACE inhibitory activities were produced after pepsin-pancreatin digestion (*19*). Our previous studies also showed that SPH inhibited ACE activity in spontaneously hypertensive rats (*9*). Separation of the hydrophilic hydrolysate from peptic-digested soy protein afforded some ACE inhibitory peptides, including Ile-Ala, Tyr-Leu-Ala-Gly-Asn-Gln, Phe-Phe-Leu, Ile-Tyr-Leu-Leu, and Val-Met-Asp-Lys-Pro-Gln-Gly (*20*). Although plasma creatinine in the C group did not differ from that in the S and H groups, both S and H had lower average values than C. One reason for the absence of significant differences may be the severe injury caused by the 5/6 nephrectomy. Many clinical trials also revealed that in patients with renal insufficiency (high serum creatinine level at baseline) treated with ACEIs, there is a strong association between an early or moderate rise in serum creatinine and a slowing of renal disease progression (*21*). In addition, there was also no significant difference among the S, H, and sham-operated groups, and we found beneficial effects of soy protein and SPH on blood pressure, urinary protein excretion, and the creatinine clearance rate. These results suggested that, in chronic renal failure, the hydrophilic protein hydrolysate of soy protein might retard the progression of renal damage and the elevation of SBP by inhibiting ACE activity *in vivo*.

In addition, soy isoflavones included in soy protein products may have a role in renal disease protection (*22*). In spontaneously hypertensive rats, soy isoflavone (78 mg/d) had an antihypertensive effect, possibly through the reduction of oxidative stress (*23*). Soy isoflavone intake (10 mg/d) also led to reductions in serum and aorta ACE activities and downregulated aortic ACE mRNA expression (*24*) in normotensive Sprague-Dawley rats. Isoflavone-containing soy supplements were also shown to have beneficial effects in patients with end-stage renal disease (*3*). In our H group, isoflavone consumption (0.71 mg/d) was lower than soy protein consumption (4.69 mg/d). However, no difference was found in the hypotensive and renoprotective effects between soy protein and SPH. In addition, isoflavone consumption in our H group was much lower than that in another study (*23*). Thus, the physiological functions might not have been evident. These results suggested that the beneficial effects of soy protein in a chronic renal failure animal model might not result from the isoflavones but may instead be related to the enzyme-digested hydrophilic components.

Ag II, which causes inflammation and produces reactive oxygen species, might not only cause an elevation in blood pressure but also be involved in hypertension-induced tissue damage. In an animal experiment, Ag II infusion led to elevation of TNF-α synthesis and concentration, and TNF-α was found mainly in the renal tissues (*25*). The effects of Ag II on renal disease may be mediated by TNF-α, and the use of

ACEIs in experimental animals thereby may interfere with its action (*26*). One study demonstrated that blocking the formation of Ag II by ACEIs may result in beneficial organ protective effects in addition to the actions of ACEIs in controlling blood pressure (*27*). In the present study, we determined the renal TNF-α concentration and found that consumption of soy protein or SPH significantly lowered the renal TNF-α level in rats with chronic renal failure. These results are consistent with those of a previous study, which showed that treating chronic renal failure subjects with an ACEI decreased the plasma TNF-α level, whereas treatment with a β-blocker or calcium antagonist did not (*6*). Increases in Ag II and TNF-α levels in specific tissues may also cause injury by enhancing the local generation of free radicals (*28*). This suggests that ACE inhibition might have beneficial effects on reducing tissue injury from inflammation and oxidative stress during renal failure. Gallardo *et al*. found that the inhibition of TNF-α decreased oxidative products in rats with chronic renal failure (*29*). Although we found that consumption of either soy protein or SPH lowered renal TNF-α, there were no differences in plasma or renal MDA levels at the end of the study. The absence of effects of soy protein or SPH on the circulating and renal antioxidative statuses may be attributable to the severe injury caused by the 5/6 nephrectomy and the long duration of the experimental period. Further study may be required to investigate the protective effects of soy protein against inflammation and oxidative damage in the early stage of renal failure.

Hyperglycemia and hyperinsulinemia in patients with chronic renal failure may increase risk factors related to cardiovascular disease and have other adverse effects, which may be caused by insulin resistance or an impaired metabolic clearance rate of insulin and glucose uptake (*30*). Our study showed no differences in plasma glucose concentrations among the groups. However, plasma insulin concentrations were lower in the S and H groups than in the control group and did not statistically differ from those of the sham-operated groups. Although patients with chronic renal insufficiency showed abnormalities in glucose metabolism, some patients with chronic renal failure maintained normoglycemia in the presence of hyperinsulinemia (*30*). In hemodialysis patients, substituting soy protein for 30 g of dietary protein was also found to significantly lower the serum insulin concentration when compared with milk protein (*31*). A recent study also demonstrated that consumption of soy protein might ameliorate hyperinsulinemia with its amino acid patterns as well as its isoflavones (*32*). Further investigations may be required to clarify the effects and mechanisms of soy protein consumption on insulin metabolism and its advanced effects on nephropathy.

Conclusions

In conclusion, SPH had beneficial effects on preventing both the progression of renal failure and the elevation of blood pressure, and those effects may be mediated by its angiotensin-converting enzyme inhibitory activity to reduce Ag II–induced inflammation. Further investigation is needed to clarify other possible mechanisms underlying the beneficial effects of soy consumption. Such information may be helpful in modifying dietary compositions to retard the progression of renal failure.

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