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Effects of various soya protein hydrolysates on lipid profile, blood pressure and renal function in five-sixths nephrectomized rats

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Studies have demonstrated that isolated soya protein (ISP) can slow the progression of renal injury, reduce blood pressure and improve the serum lipid profile in experimental animals and human subjects. The mechanisms and components of soya responsible have not been fully established. The present study was designed to evaluate the effects of the hydrophilic supernatant fraction (SF) and the hydrophobic precipitate fraction (PF) isolated from soya protein hydrolysate on renal function, lipid metabolism and blood pressure in five-sixths nephrectomized rats. Experimental animals were subjected to a nephrectomy and allocated to four groups (180 g casein/kg, 180 g ISP/kg, 100 g casein/kg with 80 g SF/kg, and 100 g casein/kg with 80 g PF/kg). The SF group had the most significant decreases in blood pressure and total cholesterol, as well as a significantly retarded progression of the experimentally induced renal disease, compared with the other groups. The PF group exhibited a significantly increased faecal excretion of total steroids. The serum creatinine, level of proteinuria, total cholesterol and LDL-cholesterol concentrations, and blood pressure were significantly reduced, and HDL-cholesterol was significantly increased, in the ISP and PF groups compared with the casein group, but no significant differences were observed between the ISP and PF groups. These results suggest that both soya protein hydrolysate fractions favourably affected chronic renal failure induced by a five-sixths nephrectomy, and the hydrophilic fraction of soya protein hydrolysate had the most pronounced effect on attenuating hypertension and slowing the progression of renal disease.

Soya protein hydrolysate: Renal failure: Cholesterol: Blood pressure

Chronic kidney disease is a public health problem and affects a substantial portion of the world's population. Several therapeutic strategies to slow the progression of chronic kidney disease have been reported, including dietary protein restriction, the control of systemic hypertension, angiotensin-converting enzyme therapy, the reduction of proteinuria and the treatment of hyperlipidaemia (Taal & Brenner, 2001). Soya protein has been investigated for its potential health benefits in preventing and treating hypercholesterolaemia (Anderson *et al.* 1995) and hypertension (He *et al.* 2005; Yang *et al.* 2005). Studies have also shown that soya protein substitution is effective in reducing proteinuria in nephrotic syndrome (D'Amico *et al.* 1992) and in ameliorating the progression of diabetic nephropathy (Azadbakht *et al.* 2003; Teixeira *et al.* 2004) and polycystic kidney disease (Tomobe *et al.* 1998; Aukema *et al.* 1999). Our previous study demonstrated that soya protein can reduce proteinuria, hypercholesterolaemia and systolic blood pressure, and retard the progression of chronic kidney disease in five-sixths nephrectomized rats (Chen ST *et al.* 2003).

The constituents of soya protein that possess renal protective effects remain to be identified. Research has shown that the hydrophobic precipitate fraction (PF) of soya protein hydrolysate has a hypocholesterolaemic effect (Sugano *et al.* 1990; Gatchalian-Yee *et al.* 1997), and that the hydrophilic supernatant fraction (SF) can attenuate the development of hypertension in spontaneously hypertensive rats (Yang *et al.* 2004). The objectives of this study were to investigate the effect of the two fractions of soya protein hydrolysate on renal function, blood pressure and lipid metabolism in rats with chronic renal failure induced by a five-sixths nephrectomy, and to examine the active components of soya protein hydrolysate on ameliorating disease progression.

Materials and methods

Preparation of soya protein hydrolysate

Isolated soya protein (ISP; Fujipro WR, Fujioil Co., Tokyo, Japan) was exhaustively hydrolysated with 3 % pepsin (w/w) (Sigma Chemical, St Louis, MO, USA) at pH 2·0 and 37°C for 24 h. The hydrolysate solution from pepsin digestion was heated to 100°C for 10 min and centrifuged at 7500 ***g*** for 20 min after being neutralized. The SF and PF were respectively collected and lyophilized, ground to a powder and stored at 4°C.

Abbreviations: ISP, isolated soya protein; PF, precipitate fraction; SF, supernatant fraction.

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Animals and diets

Fifty male Wistar rats (weight 250–280 g) were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The animals were housed in individual cages that were kept in a room under controlled lighting from 08.00 to 20.00 hours at 24 ± 1°C and a relative humidity of 55 % ± 5 %. All rats were fed a standard diet and had free access to tap water for 1 week. After 1 week's adaptation, forty rats underwent a five-sixths nephrectomy (experimental animals), and ten rats underwent a sham operation (control animals), as previously described (Chen ST *et al.* 2003).

After the operation, the experimental animals were randomly assigned to one of four groups and received a different diet for 14 weeks: group A (casein) was fed a standard diet containing 180 g casein/kg as the protein source; group B (ISP) was fed a diet containing 180 g ISP/kg; group C (SF) was fed a diet containing 100 g casein/kg and 80 g SF of soya protein hydrolysate/kg; group D (PF) was fed a diet containing 100 g casein/kg and 80 g PF of soya protein hydrolysate/100 g. Control animals were assigned to two groups that were fed either the 180 g casein/kg or the 180 g ISP/kg diet. The diets were isoenergetic and contained equal amounts of fat, minerals and vitamin supplements (AIN-93; ICN Biochemicals, Aurora, OH, USA). The compositions of the diets are shown in Table 1.

During the experimental period, food intake was recorded daily. The animals were weighed each week. All animals were treated in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985).

Data collection

Blood, urine and faecal sampling. The animals were placed in metabolic cages for 3 d for 24 h urine and faecal collections. After overnight fasting, tail venous blood was collected at the beginning of the study and at 0, 6 and 12 weeks after the operation. Plasma samples were analysed for albumin, total cholesterol, triacylglycerol, creatinine and blood urea N; urine was analysed for creatinine, urea N and protein. All analyses were carried out on a Hitachi 7170 Autoanalyser (Tokyo, Japan). The creatinine clearance rate was calculated by the following equation:

$$(\text{urine creatinine concentration}_{(\text{mg/dl})} \times \text{urine output}_{(\text{ml})})/(\text{plasma creatinine concentration}_{(\text{mg/dl})} \times 1440_{(\text{min})}).$$

Liver lipids and faecal steroids. At the end of the feeding period, the rats were killed by exsanguination from the abdominal aorta under diethyl ether anaesthesia. The liver was excised and weighed. Liver lipids were extracted by the method of Folch *et al.* (1957). Cholesterol and triacylglycerol concentration in the liver were determined with diagnostic kits (Randox, Antrim, UK). Faeces were collected at 0 and 12 weeks after the operation and lyophilized until analysed. Bile acids and steroids were separated from the faeces according to the method of Folch *et al.* (1957) and were measured with commercial kits (Randox).

Blood pressure. The systolic blood pressure and mean blood pressure were measured at the beginning of the experiment and at 7 and 14 weeks after the operation by the tail-cuff method using an electro-sphygmomanometer (blood pressure analyser, model 179; IITC Life Science, Woodland Hill, CA, USA). Rats were kept in a dark, warm and quiet environment during the measurements. At least five readings were recorded. The maximum and minimum values were discarded, and the average blood pressure values were calculated from the remaining three values. The diastolic blood pressure was calculated by the following equation: $(3 \times \text{mean blood pressure} - \text{systolic blood pressure})/2$.

Table 1. Composition of the experimental diets (g/kg)

	Diet group				
Ingredient	Standard	Casein	ISP	SF	PF
Maize starch	550	550	550	550	550
Casein	180	180	0	100	100
ISP	0	0	180	0	0
SF	0	0	0	80	0
PF	0	0	0	0	80
Sucrose	60	60	60	60	60
Soyabean oil	60	60	60	60	60
Cellulose	70	70	70	70	70
Mineral mixture	60	60	60	60	60
Vitamin mixture	20	20	20	20	20
L-Methionine	3	3	3	3	3

ISP, isolated soy protein; SF, supernatant fraction of soy protein hydrolysate; PF, precipitate fraction of soy protein hydrolysate.

Casein (high-N), sucrose (food-grade), soyabean oil, cellulose (non-nutritive bulk), mineral mixture (AIN-93M mineral mixture) and vitamin mixture (AIN-93M vitamin mixture) were obtained from ICN Biochemicals (Aurora, OH, USA). Maize starch was purchased from Samyang Genex (Seoul, Korea). Methionine was obtained from Wako Pure Chemical (Osaka, Japan). ISP was obtained from Fujipro WR, Fujioil Co. (Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using SAS software (version 8.2; SAS Institute, Cary, NC, USA). Data were analysed by one-way ANOVA and Fisher's least significant difference test. Results are expressed as means and standard deviations. Significance for all analyses was set at $P<0{\cdot}05$. Any animal that needed to be killed prematurely was excluded from these comparisons.

Results

Body weight and feeding efficiency

The daily food intake of the experimental and control groups did not differ (Table 2). At the end of study, the weight gain, feeding efficiency and serum albumin level of the control groups were significantly higher than those of the experimental groups (Table 2, Fig. 1(A)). Of the experimental animals, the casein group gained significantly less weight and had a lower feeding efficiency than other groups (Table 2). There were no differences in weight gain and food efficiency among the ISP, SF and PF groups (Table 2). No significant difference was found in serum albumin level between the experimental groups (Fig. 1(A)).

Table 2. Food intake, body weight, liver lipids, faecal steroids and blood pressure of five-sixths nephrectomized rats and sham-operated rats fed different protein diets
(Mean values and standard deviations)

	Five-sixths nephrectomy								Sham operation			
	Casein (*n* 6)		ISP (*n* 5)		SF (*n* 6)		PF (*n* 6)		Casein (*n* 5)		ISP (*n* 4)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight gain (g/rat)	138·3[a]	14·8	148·4[b]	21·9	145·6[b]	17·1	145·2[b]	16·7	188·3[c]	19·4	184·6[c]	7·5
Food intake (g/rat per d)	24·7	1·0	24·5	0·9	24·9	1·7	24·1	2·4	24·8	0·5	24·5	0·0
Feeding efficiency (%)*	4·67[a]	1·2	5·1[b]	0·6	5·0[b]	0·9	5·3[b]	1·4	6·3[c]	0·5	6·2[c]	0·6
Liver												
Liver weight (g/rat)	9·5	0·2	9·3	0·8	9·3	0·5	8·8	0·6	9·8	1·6	9·1	0·8
Total cholesterol (μmol/g liver)	26·4[a]	0·7	23·9[ab]	4·1	20·6[b]	1·7	19·0[b]	7·6	21·7[b]	5·5	13·1[c]	0·5
Faeces (μmol/g faeces)												
Total cholesterol	1·95[a]	0·36	2·27[a]	1·45	2·13[a]	1·23	9·76[b]	2·20	11·72[c]	0·50	16·33[d]	0·51
Total bile acid	804[a]	247	3663[b]	689	1009[a]	635	2360[c]	1210	1041[a]	212	3220[b]	623
Systolic blood pressure (mmHg)	155·2[a]	8·9	144·8[b]	5·9	135·0[cd]	9·8	140·0[bc]	9·9	126·9[d]	5·6	112·2[e]	4·5

ISP, isolated soya protein; SF, supernatant fraction of soya protein hydrolysate; PF, precipitate fraction of soya protein hydrolysate.
Casein group (180 g casein/kg); ISP group (180 g isolated soya protein/kg); SF group (100 g casein and 80 g supernatant fraction of soya protein hydrolysate/kg); PF group (100 g casein and 80 g precipitate fraction of soya protein hydrolysate/kg).
[a,b,c,d,e] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
*Feeding efficiency (%) = (daily weight gain/daily food intake) × 100 %.

Plasma lipids and lipoproteins

Plasma total cholesterol and lipoproteins were significantly increased in the experimental groups after nephrectomy, and no significant difference was found in plasma triacylglycerol concentration between all the groups (Fig. 2).

In the experimental animals, the concentration of total cholesterol in the casein group was significantly increased compared with that of the other groups. The SF group had lower total cholesterol levels than the ISP and PF groups (Fig. 2(A)). The LDL-cholesterol concentration also significantly increased in the casein group of experimental animals (0·66 (SD 0·07) mmol/l). There was no difference in LDL-cholesterol between the ISP, SF and PF groups (0·40 (SD 0·06), 0·33 (SD 0·08) and 0·40 (SD 0·07) mmol/l, respectively; Fig. 2(B)). The HDL-cholesterol levels in the ISP and PF groups were significantly higher than those in the casein group, and there was no difference between the SF and casein groups. No differences were found in HDL-cholesterol between the ISP, SF and PF groups (Fig. 2(C)). The ratios of HDL-cholesterol to total cholesterol in the ISP, SF and PF groups were significantly higher than those in the casein group (0·83 (SD 0·10), 0·84 (SD 0·10), 0·79 (SD 0·12) and 0·50 (SD 0·08), respectively), and there were no differences between the ISP, SF and PF groups.

Liver lipid and faecal total steroids

Results for liver weight, liver total cholesterol concentration and faecal total steroid excretion are shown in Table 2. There was no difference in liver weight between the experimental groups. The liver total cholesterol concentrations of the SF and PF groups were significantly lower than those of the casein group, and no differences were found between the ISP, SF and PF groups or between the ISP and casein groups.

The faecal cholesterol excretion of the PF group was the highest among the four groups. The casein group had lower faecal cholesterol excretion, but there was no significant difference compared with the ISP and SF groups. The ISP group exhibited significantly increased faecal bile acid excretion compared with the other groups. The faecal bile acid excretion of the PF group was significantly higher than that of the SF and casein groups. There was no difference in faecal bile acid excretion between the SF and casein groups.

Blood pressure

The blood pressures at the end of study are shown in Table 2. Blood pressures significantly increased in the experimental groups after nephrectomy. Of the four experimental groups, the SF group had the lowest mean blood pressure and diastolic blood pressure. The systolic blood pressure of the SF group was lower than that of the ISP and casein groups, whereas systolic blood pressure was lower in the ISP and the PF groups than the casein group. There was no difference in systolic blood pressure between the SF and PF groups. No difference in blood pressure was found between the ISP and PF groups.

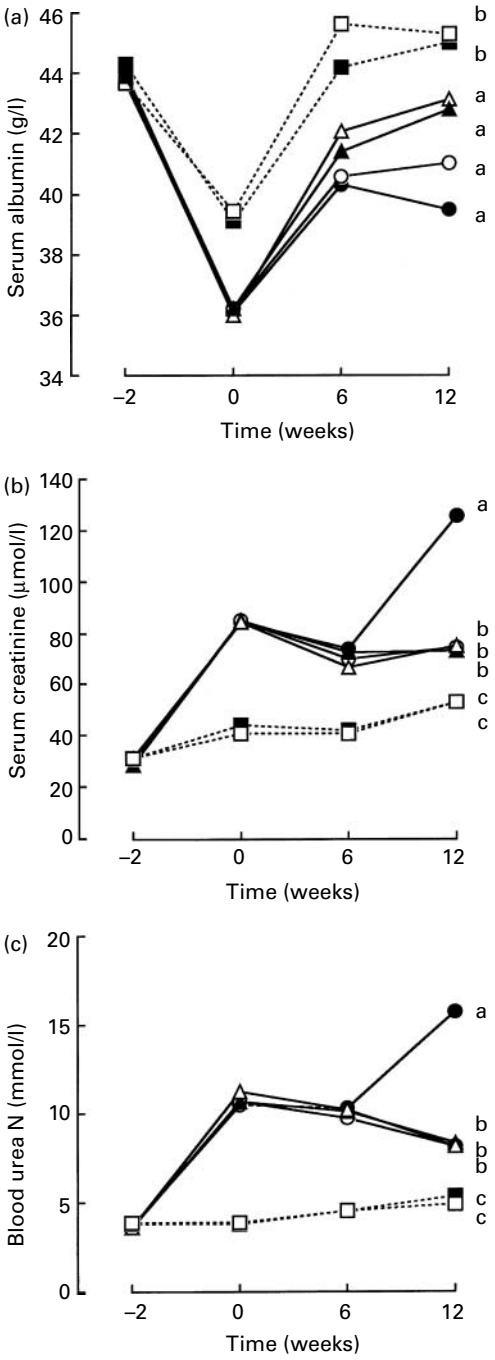

Fig. 1. Serum biochemical results of five-sixths nephrectomized rats and sham-operated rats fed different protein diets. Mean values with unlike superscript letters were significantly different ($P<0{\cdot}05$). ●, Casein group (nephrectomized rats with 180 g casein/kg); ○, ISP group (nephrectomized rats with 180 g isolated soya protein/kg); ▲, SF group (nephrectomized rats with 100 g casein and 80 g supernatant fraction of soya protein hydrolysate/kg); △, PF group (nephrectomized rats with 100 g casein and 80 g precipitate fraction of soya protein hydrolysate/kg); ■, sCasein group (sham-operated rats with 180 g casein/kg); □, sISP group (sham-operated rats with 180 g isolated soya protein/kg).

Renal function

Serum creatinine, blood urea N and urinary protein excretion were significantly increased in the experimental animals after the nephrectomy, and were significantly higher in the casein group than in the other experimental groups. There were no differences in serum creatinine (Fig. 1(B)), blood urea N (Fig. 1(C)) or urinary protein excretion (Fig. 3(A)) between the ISP, SF and PF groups.

The value of urinary urea N excretion for the ISP group was significantly higher than for the other groups, whereas values for the SF and PF groups were significantly lower than for the casein group in the experimental animals. No difference was found in urinary urea excretion between the SF and PF groups (Fig. 3(B)).

There was a significantly decreased creatinine clearance rate in the casein group of experimental animals compared with the other groups. The creatinine clearance rate of the SF group was significantly higher than that of the other experimental groups, and there were no differences between the ISP and PF groups (Fig. 3(C)).

Discussion

The results from this study showed that both the hydrophilic and the hydrophobic fraction of soya protein hydrolysate had a beneficial effect on slowing disease progression, reducing blood pressure and improving serum lipid profile, the hydrophilic fraction having the most pronounced renoprotective effects in five-sixths nephrectomized rats. Replacing 80 g SF/kg with casein in the standard diet of nephrectomized rats for 14 weeks produced significantly lower blood pressures and serum total cholesterol concentrations compared with those of rats fed PF. Furthermore, the creatinine clearance rate was significantly higher in the SF than in the PF group.

When rats are subjected to surgical ablation of five-sixths of their renal mass, they develop hypertension, proteinuria and a progressive fall in glomerular filtration rate, features similar to those of human chronic kidney disease. The SF of soya protein hydrolysate has been shown to prevent the development of hypertension in spontaneously hypertensive rats and to have angiotensin-converting enzyme inhibitory activity (Chen *et al.* 2002; Yang *et al.* 2004). The results of the present study showed that the SF group had the lowest blood pressure among the four experimental groups. Studies have found that hypertension is important in the pathogenesis of the progression of chronic renal disease (Peterson *et al.* 1995; Klag *et al.* 1996), and antihypertensive therapy has a major role in slowing the progression of renal disease (Wright *et al.* 2002). Significant decreases in blood pressure and increases in creatinine clearance rate in the SF group may indicate substantial protection from progressive renal injury.

A number of studies have demonstrated that the PF of soya protein hydrolysate is more hypocholesterolaemic than soya protein itself (Sugano *et al.* 1990; Gatchalian-Yee *et al.* 1997), and SF from soya protein hydrolysate had no hypocholesterolaemic effect in rats fed a cholesterol-enriched diet (Sugano *et al.* 1988). In the present study, SF had a greater cholesterol-lowing effect than PF, which may have been due to the different animal model we used.

Mechanisms responsible for the hypocholesterolaemic effects of soya protein include increasing bile acid excretion, decreasing steroid absorption and changing hepatic lipid metabolism (Potter, 1995). Enhanced faecal steroid excretion with PF has been shown by several studies, and this may be

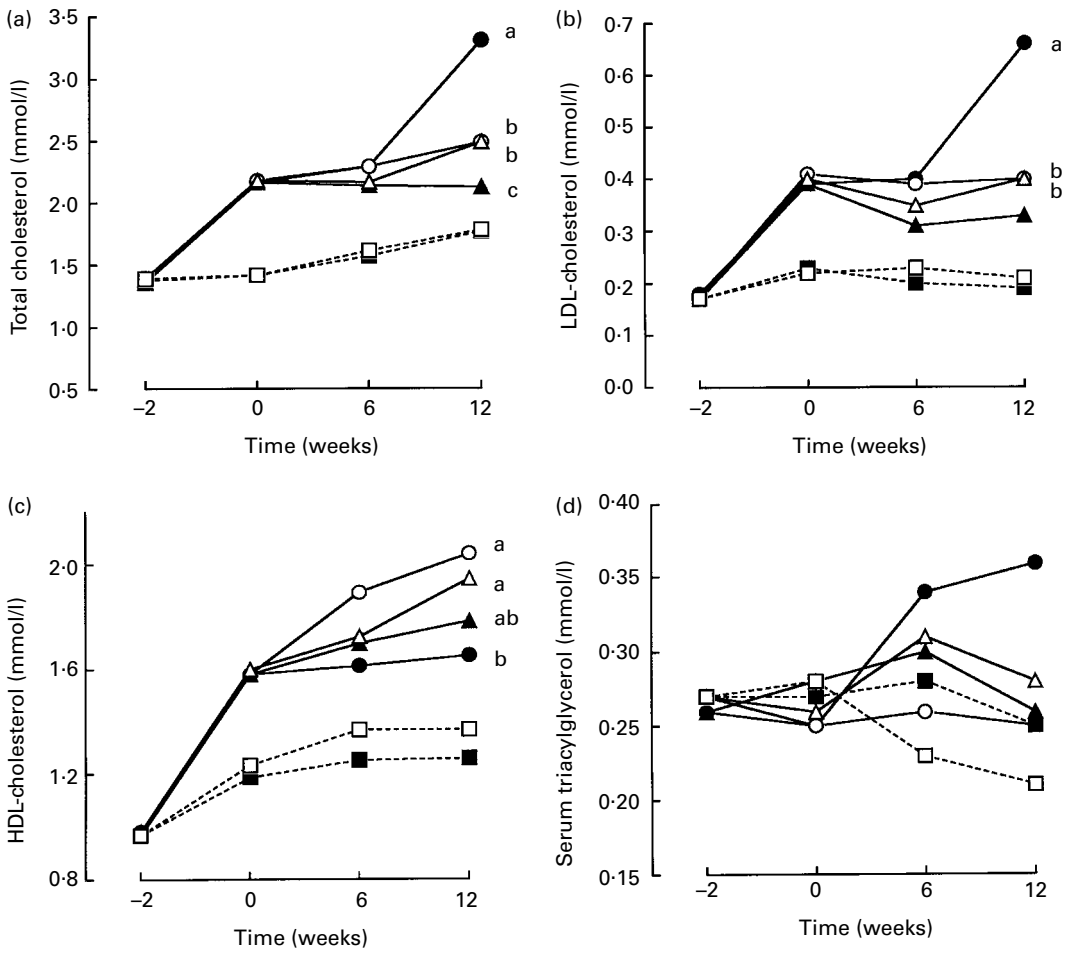

Fig. 2. Plasma lipid and lipoprotein levels of five-sixths nephrectomized rats and sham-operated rats fed different protein diets. Mean values with unlike superscript letters were significantly different ($P<0·05$). ●, Casein group (nephrectomized rats with 180 g casein/kg); ○, ISP group (nephrectomized rats with 180 g isolated soya protein/kg); ▲, SF group (nephrectomized rats with 100 g casein and 80 g supernatant fraction of soya protein hydrolysate/kg); △, PF group (nephrectomized rats with 100 g casein and 80 g precipitate fraction of soya protein hydrolysate/kg); ■, sCasein group (sham-operated rats with 180 g casein/kg); □, sISP group (sham-operated rats with 180 g isolated soya protein/kg).

the major mechanism for the hypocholesterolaemic effect of PF (Sugano *et al.* 1988; Gatchalian-Yee *et al.* 1997; Chen JR *et al.* 2003). The results of the present study showed that the faecal cholesterol and bile acid excretion increased significantly in the PF group compared with the SF group; thus, another mechanism must be responsible for the cholesterol-lowering effect of SF. Chronic renal disease is associated with abnormal lipid metabolism (Appel, 1991). The mechanism of hypercholesterolaemia in nephrectomized rats may contribute to renal injury. Serum total cholesterol and lipoprotein concentrations were significantly elevated in the experimental animals after nephrectomy, and were positively associated with serum creatinine level in the present study. These data suggest that the attenuation of renal injury may have resulted from the reduction in hyperlipidaemia. Therefore, the greater hypocholesterolaemic effect in SF-fed rats than PF-fed rats can possibly be attributed to the antihypertensive effect and slowing of disease progression.

Urinary protein excretion is reduced with soya protein consumption in several models of chronic kidney disease (Aukema *et al.* 1999; Tovar *et al.* 2002; Chen ST *et al.* 2003) and in human studies (D'Amico *et al.* 1992; Azadbakht *et al.* 2003; Teixeira *et al.* 2004). The results of the present study showed that both soya protein hydrolysate fractions significantly reduced proteinuria. There were no significant differences in serum albumin level between the groups at the end of the study, and there were significantly decreased blood urea N levels in all soya protein and soya protein hydrolysate groups compared with the casein group, indicating that both the SF and the PF of soya protein hydrolysate may be quite effective in ameliorating uraemic symptoms while maintaining an adequate nutritional status.

Despite these observations, our findings have the following limitations. First, the glomerular filtration rate was estimated from the creatinine clearance rate, and there is day-to-day variability in 24 h creatinine clearance (Walser, 1990). Second, mean blood pressure was measured by the tail-cuff method, but it is controversial whether the technique is reliable for mean blood pressure measurements (Ikeda *et al.* 1991; Kramer & Remie, 2004).

Few data are, however, available for defining the mechanism and constituents of soya protein responsible for its renoprotective effects. This study showed that serum creatinine, blood pressure, plasma lipids and proteinuria in nephrectomized rats

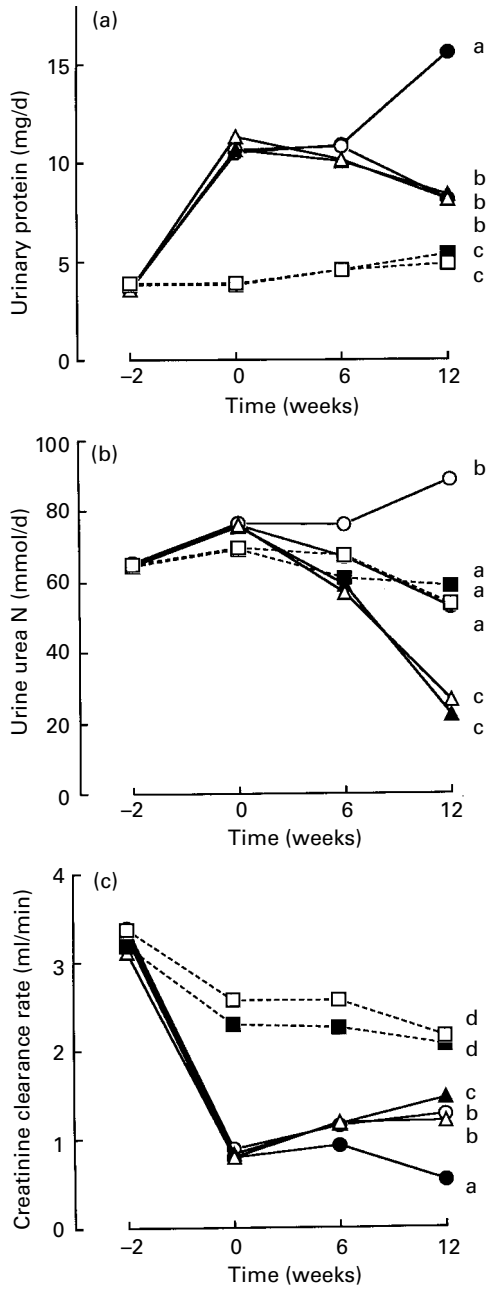

Fig. 3. Urine biochemical results and creatinine clearance rate of five-sixths nephrectomized rats and sham-operated rats fed different protein diets. Mean values with unlike superscript letters were significantly different ($P<0{\cdot}05$). ●, Casein group (nephrectomized rats with 180 g casein/kg); ○, ISP group (nephrectomized rats with 180 g isolated soya protein/kg); ▲, SF group (nephrectomized rats with 100 g casein and 80 g supernatant fraction of soya protein hydrolysate/kg); △, PF group (nephrectomized rats with 100 g casein and 80 g precipitate fraction of soya protein hydrolysate/kg); ■, sCasein group (sham-operated rats with 180 g casein/kg); □, sISP group (sham-operated rats with 180 g isolated soya protein/kg).

decreased significantly, and there was a significantly increased creatinine clearance rate, with the administration of both soya protein hydrolysates. The renoprotective effects of SF and PF appear to be due to different mechanisms. Current therapeutic strategies for achieving maximal renal protection include control of hypertension, angiotensin-converting enzyme therapy, reduction of proteinuria, treatment of hyperlipidaemia and dietary protein restriction (Taal & Brenner, 2001). The PF group exhibited significantly increased faecal steroid excretion, and liver cholesterol level tended to be lower than in the other groups. Clinical studies have shown that renal function declines more rapidly among patients with renal disease who have hyperlipidaemia (Maschio *et al.* 1991). These data suggest that the hypocholesterolaemic effect of PF may be one of possible effects underlying the slowing of disease progression. The creatinine clearance rate was, however, significantly greater in rats fed SF compared with those fed the other diets. Furthermore, blood pressure was significantly lower in the SF group than in the other groups. These results indicate that the antihypertensive effect of SF may play an important role in the renoprotective effects of soya protein.

In conclusion, by comparing the effects of the two fractions isolated from soya protein hydrolysate prepared by peptic hydrolysis, our findings demonstrate that the hydrophilic SF was more effective in modulating blood pressure and had the most pronounced effect on slowing the progression of renal disease. Further studies are needed to clarify how the hydrophilic fraction of soya protein hydrolysate affects blood pressure, and the results of those studies may possibly then be used in dietary management to prevent the progression of renal disease.

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