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

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Abstract

Studies have demonstrated that isolated soy protein (ISP) can slow progression of renal injury, reduce blood pressure, and improve serum lipid profile in experimental animals and human subjects. The mechanism and components of soy responsible have not been fully established. The present study was designed to evaluate the effects of the low-molecular-weight fraction (LMF) and the high-molecular-weight fraction (HMF) isolated from soy protein hydrolysate on renal function, lipid metabolism, and blood pressure in five-sixths nephrectomized rats. Experimental animals were subjected to a nephrectomy and allocated into four groups (180 g/kg casein, 180 g/kg ISP, 100 g/kg casein with 80 g/kg LMF, and 100 g/kg casein with 80 g/kg HMF). The LMF group had the most-significant decreases in blood pressure, and total cholesterol (TC), as well as significantly retarded progression of the experimentally induced renal disease than did the other groups. The HMF group exhibited significantly increased fecal excretion of total steroids. The serum creatinine, proteinuria, TC, and low-density-lipoprotein cholesterol concentrations, and blood pressure were significantly reduced and high-density-lipoprotein cholesterol was significantly increased in the ISP and HMF groups compared with the casein group, but no significant differences were observed between the ISP and HMF groups. These results suggest that both soy protein hydrolysate fractions favorably affected chronic renal failure induced by a five-sixths nephrectomy, and the low-molecular-weight fraction of soy protein hydrolysate had most-pronounced effect on attenuating hypertension and slowing the progression of renal disease.

Key words: soy protein hydrolysate: renal failure: cholesterol: blood pressure

Introduction

Chronic kidney disease (CKD) is a public health problem and affects a substantial portion of the world's population. Several therapeutic strategies to slow CKD progression have been reported including dietary protein restriction, control of systemic hypertension, angiotensin-converting enzyme (ACE) therapy, reduction of proteinuria, and treatment of hyperlipidemia (Taal & Brenner 2001). Soy protein has been investigated for its potential health benefits in preventing and treating hypercholesterolemia (Anderson *et al.* 1995) and hypertension (He *et al.* 2005; Yang *et al.* 2005). Studies have also shown that soy 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 soy protein can reduce proteinuria, hypercholesterolemia, and systolic blood pressure, and retard the progression of CKD in five-sixths nephrectomized rats (Chen *et al.* 2003). The constituents of soy protein which possess renal protective effects remain to be identified. Research has shown that the undigested high-molecular-weight fraction (HMF) of soy protein hydrolysate has a hypocholesterolemic effect (Sugano *et al.* 1990; Gatchalian-Yee *et al.* 1997), and the soluble low-molecular-weight fraction (LMF) 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 soy 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 soy protein hydrolysate on ameliorating disease progression.

Materials and Methods

Preparation of soy protein hydrolysate

Isolated soy protein (ISP, Fujipro WR, Fujioil Co., Tokyo, Japan) was exhaustively hydrolysated with 3% pepsin (Sigma Chemical, St. Louis, MO) 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 supernatant and precipitate were respectively collected and lyophilized, ground to a powder, and stored at 4°C. The supernatant was identified as the low-molecular-weight fraction (LMF) of soy protein hydrolysate, and the precipitate was identified as the high-molecular-weight fraction (HMF).

Animals and diets

Fifty male Wistar rats (250~280 g) were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Animals were housed in individual cages which were in a room under controlled lighting 08:00~20:00 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 wk. After 1 wk adaptation, forty rats underwent a five-sixths nephrectomy (experimental animals), and ten rats underwent a sham operation (control animals) as described previously (Chen *et al.* 2003). After the operation, the experimental animals were randomly assigned to 1 of 4 groups and received a different diet for 14 wk: group A (Casein) was fed a standard diet containing 180 g/kg casein as the protein source; group B (ISP) was fed a diet containing 180 g/kg isolated soy protein; group C (LMF) was fed a diet containing 100 g/kg casein and 80 g/kg of the low-molecular-weight fraction of soy protein hydrolysate; and group D (HMF) was fed a diet containing 100 g/kg casein and 80 g/100g of the high-molecular-weight fraction of soy protein hydrolysate. Control animals were assigned to 2 groups which were fed either the 180 g/kg casein or 180 g/kg isolated soy protein diet. The diets were isoenergetic and contained equal amounts of fat, mineral, and vitamin supplements (AIN-93; ICN Biochemicals, Aurora, OH). The compositions of the diets are shown in Table 1. During the experimental period, food intake was recorded daily. 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 and urine sampling After overnight fasting, tail venous blood and 24-h urine were collected at the beginning of the study and at 0, 6, and 12 wk after the operation. Plasma samples were analyzed for albumin, total cholesterol (TC), triacylglycerol (TG), creatinine, and blood urea nitrogen (BUN); urine was analyzed for creatinine, urea nitrogen, and protein. All analyses were carried out on a Hitachi 7170 Autoanalyser (Tokyo, Japan). The creatinine clearance rate was calculated by the following equation: (urine creatinine

concentration(mg/dL) × urine output (mL))/(plasma creatinine concentration (mg/dL) × 1440 (min)).

Blood pressure The systolic blood pressure (SBP) and mean blood pressure (MBP) were measured at the beginning and at 7 and 14 wk after the operation by the tail-cuff method using an electro-sphygmomanometer (Blood pressure Analyzer, 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 (DBP) was calculated by the following equation:(3 × MBP - SBP)/2.

Liver lipids and fecal steroids At the end of the feeding period, rats were sacrificed by exsanguination from the abdominal aorta under light diethyl ether anesthesia. The liver was excised and weighed. Liver lipids were extracted by the method of Folch *et al.* (1957). Cholesterol and TG concentration in the liver were determinded with diagnostic kits (Randox, Antrim, UK). Feces were collected at 0 and 12 wk after the operation and lyophilized until analyzed. Bile acids and steroids were separated from the feces according to the method of Folch *et al.* (1957) and were measured with commercial kits (Randox, Antrim, UK).

Statistical analysis

Statistical analyses were performed using the SAS software (version 8.2; SAS Institute, Cary, NC). Data were analyzed by one-way ANOVA and Fisher's least significant different test. Results are expressed as mean values with the standard deviation. Significance for all analyses was set at $p < 0.05$. Any animal which needed to be killed prematurely was excluded from those comparisons.

Results

Body weight and feeding efficiency

Daily food intake of the experimental group and control groups did not differ (Table 2). At the end of study, the weight gain, feeding efficiency, and serum albumin of the control groups were significantly higher than those of the experimental groups (Tables 2 and 3). In experimental animals, the casein group gained significantly less weight and had a lower feeding efficiency compared with those of the other groups (Table 2). There were no differences in weight gain and food efficiency among the ISP, LMF and HMF groups (Table 2). No significant difference was found in serum albumin among the experimental groups (Table 3).

Plasma lipid and lipoprotein

Plasma total cholesterol (TC) and lipoproteins were significantly increased in the experimental groups after the nephrectomy (Fig. 1), and no significant difference was found in plasma triacylglycerol (TG) among all groups (Table 3). In the experimental animals, TC of the casein group was significantly increased compared with those of the other groups (Fig. 1a). The LMF group had lower TC levels than did the ISP and HMF groups (Fig. 1a). The low-density-lipoprotein cholesterol (LDL-C) concentration also significantly increased in the casein group of experimental animals (Fig. 1b). There was no difference in LDL-C among the ISP, LMF and HMF groups (Fig. 1b). The high-density-lipoprotein cholesterol (HDL-C) of the ISP and HMF groups were significantly higher than the casein group, and there was no difference between the LMF and casein groups (Fig. 1c). No differences were found in HDL-C among the ISP, LMF and HMF groups (Fig. 1c). The ratio of HDL-C to TC of the ISP, LMF and HMF groups were significantly higher than the casein group(0.83 ± 0.10, 0.84 ± 0.10, 0.79 ± 0.12, and 0.50 ± 0.08, respectively), and there were no differences among the ISP, LMF and HMF groups.

Renal function

Serum creatinine, BUN, and urine 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 (Table 3). There were no differences in serum creatinine, BUN, or urine protein excretion among the ISP, LMF and HMF groups. The value of urine urea nitrogen excretion of the ISP group was significantly higher than t the other groups, whereas the LMF and the HMF groups were significantly lower than the casein group in the experimental animals. No difference was found in urine urea excretion between the LMF and HMF groups. There was a significantly decreased creatinine clearance rate in the casein group of experimental animals compared to the other groups. The creatinine clearance rate of the LMF group was significantly higher than those in the other experimental groups, and there were no differences between the ISP and HMF groups.

Liver lipid and fecal total steroids

Results for liver weight, the liver TC concentration, and fecal total steroids excretion are shown in Table 4. There was no difference in liver weight among the experimental groups. The liver TC concentrations of the LMF and HMF groups were significantly lower than the casein group, and no differences were found among the ISP, LMF and HMF groups or between the ISP and casein groups. The fecal cholesterol excretion of the HMF group was the highest among the four groups. The casein group had lower fecal cholesterol excretion, but there was no significant difference compared with the ISP and LMF groups. The ISP group exhibited significantly increased fecal bile acid excretion compared with the other groups. The fecal bile acid excretion of the HMF group was significantly higher than those of the LMF and casein groups. There was no difference in fecal bile acid excretion between the LMF and casein groups.

Blood pressure

The blood pressures at the end of study are shown in Figure 2. Blood pressures significantly increased in the experimental groups after the nephrectomy. The LMF group had the lowest mean blood pressure (MBP) and diastolic blood pressure (DBP) among the four experimental groups. The MBP and DBP of the ISP and HMF groups were significantly lower than the casein groups in experimental animals. The systolic blood pressure (SBP) of the LMF group was lower than those of the ISP and casein groups, whereas those of the ISP and the HMF group were lower than the casein group. There was no difference in SBP between the LMF and HMF groups. No difference was found in blood pressure between the ISP and HMF groups.

Discussion

Results from this study showed that both LMF and HMF of soy protein hydrolysates had beneficial effects on slowing the disease progression, reducing blood pressure, and improving serum lipid profile, and LMF had the most-pronounced renal protective effects in five-sixths nephrectomized rats. Replacing 80 g/kg LMF with casein in a standard diet of nephrectomized rats for 14 wk, produced significantly lower blood pressures (MBP and DBP) and serum TC concentration compared with those of rats fed the HMF substitution. Furthermore, the creatinine clearance rate was significantly higher in the LMF than in the HMF group.

When rats are subjected to surgical ablation of five-sixths of their renal mass, they develop hypertension, proteinuria, and a progressive loss of the glomerular filtration rate, features similar to those of human CKD. The LMF of soy protein hydrolysate has been shown to prevent the development of hypertension in spontaneously hypertensive rats and to have angiotensin-converting enzyme (ACE) inhibitory activity (Chen JR 2002;Yang *et al.* 2004). Results of the present study showed that the LMF group had the lowest MBP and DBP among the four experimental groups. Studies have found that hypertension is important in the pathogenesis of chronic renal disease progression (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 the creatinine clearance rate in the LMF group may indicate substantial protection from progressive renal injury.

A number of studies have demonstrated that the HMF of soy protein hydrolysate is more hypocholesterolemic than soy protein itself (Sugano *et al.* 1990; Gatchalian-Yee *et al.* 1997), and LMF of soy protein hydrolysate had no hypocholesterolemic effect in rats fed a cholesterol-enriched diet(Sugano *et al.* 1988). In the present study, the LMF had a greater cholesterol-lowing effect than the HMF, which may have been due to the different animal model we used. Mechanisms responsible for the hypocholesterolemic effects of soy protein include increasing bile acid excretion, decreasing steroid absorption, and changing hepatic lipid metabolism (Potter 1995). Enhanced fecal steroid excretion with the HMF was shown by several studies, and this may be the major mechanism for the hypocholesterolemic effect of the HMF (Sugano *et al.* 1988;Gatchalian-Yee *et al.* 1997;Chen *et al.* 2003). The results of the present study showed that the fecal cholesterol and bile acid excretion significantly increased in the HMF group compared with the LMF group; thus another mechanism must be responsible for the cholesterol-lowering effect of the LMF. Chronic renal disease is associated with abnormal lipid metabolism (Appel 1991). The mechanism of hypercholesterolemia in nephrectomized rats may contribute to renal injury. Serum TC and lipoprotein concentrations were significantly elevated in the experimental animals after the nephrectomy, and were positively associated with serum creatinine in this study. These data suggest that attenuation of renal injury may have resulted from the reduction in

hyperlipidemia. Therefore, the hypocholesterolemic effect in LMF-fed rats being greater than in HMF-fed rats can possibly be attributed to the antihypertensive effect and slowing of the disease progression.

Urine protein excretion was reduced with soy protein consumption in several models of chronic kidney disease (Aukema *et al.* 1999; Tovar *et al.* 2002; Chen *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 soy protein hydrolysate fractions significantly reduced proteinuria. There were no significant differences in serum albumin among the groups at the end of the study, and there were significantly decreased blood urea nitrogen levels in all soy protein and soy protein hydrolysate groups compared with the casein group, indicating that both the LMF and HMF of soy protein hydrolysates may be quite effective in ameliorating ureamic symptoms, and while maintaining an adequate nutritional status.

Few data are available for defining the mechanism and constituents of soy protein responsible for its renal protective effects. This study showed that serum creatinine, blood pressure, plasma lipids, and proteinuria of nephrectomized rats significantly decreased, and there was a significantly increased creatinine clearance rate with administration of both soy protein hydrolysates. The renal protective effects of the LMF and HMF appear to be due to different mechanisms. Current therapeutic strategies for achieving maximal renal protection include control of hypertension, ACE therapy, reduction of proteinuria, treatment of hyperlipidemia, and dietary protein restriction (Taal & Brenner 2001). The HMF group exhibited significantly increased fecal steroids excretion, and liver cholesterol tended to lower than in the other groups. Clinical studies have shown that renal function declines more rapidly among patients with renal disease who have hyperlipidemia (Maschio *et al*. 1991). These data suggest that the hypocholesterolemic effect of HMF may be one of possible effects of slowing the disease progression. However, the creatinine clearance rate was significantly greater in rats fed the LMF compared with those fed the other diets. Furthermore, blood pressure was significantly lower in the LMF group than in the other groups. These results indicate that the antihypertensive effect of LMF may play an important role in the renal protective effects of soy protein.

In conclusion, by comparing the effects of the two fractions isolated from soy protein hydrolysate prepared by peptic hydrolysis, our findings demonstrate that the low-molecular-weight fraction 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 low-molecular-weight soy protein hydrolysate affects blood pressure, and the results of those studies may possibly be used in dietary management in the future to prevent the progression of renal disease.

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Table1. Composition of the experimental diets (g/kg)

Ingredient	Diet group				
	Standard	Casein	ISP	LMF	HMF
Maize starch	550	550	550	550	550
Casein	180	180	0	100	100
Isolated soy protein	0	0	180	0	0
SSP	0	0	0	80	0
PSP	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; LMF, low-molecular-weight fraction of soy protein hydrolysate; HMF, high-molecular-weight 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)

Table 2. Food intake, body weight and feeding efficiency of five-sixths nephrectomized rats and sham-operated rats fed different protein diets. (Mean values and standard deviation)

	Five-sixths nephrectomy								Sham operation			
	Casein (*n* 6)		ISP (*n* 5)		LMF (*n* 6)		HMF (*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/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

[a,b] Mean values within a row without a common superscript letter significantly differ at $p < 0.05$.

§ Feeding efficiency (%)= (daily weight gain/daily food intake)×100%.

Casein group (180 g casein/kg); ISP group (180 g isolated soy protein /kg); LMF group (100 g casein and 80 g low-molecular-weight fraction of soy protein hydrolysate/kg); HMF group (100 g casein and 80 g high-molecular-weight fraction of soy protein hydrolysate/kg).

Table 3. Biochemical results of five-sixths nephrectomized rats and sham-operated rats fed differing protein diets. (Mean values and standard deviation)

	Five-sixths nephrectomy								Sham operation			
	Casein (*n* 6)		ISP (*n* 5)		LMF (*n* 6)		HMF (*n* 6)		Casein (*n* 5)		ISP (*n* 4)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Serum Albumin (g/L)												
Baseline	44.0	1.2	43.7	1.0	43.9	0.8	43.8	1.1	44.3	1.2	43. 7	1.9
Initial	36.1[a]	0.7	36.2[a]	2.7	36.2[a]	2.1	36.0[a]	2.7	39.1[b]	1.8	39.4[b]	2.0
Final	39.5[a]	4.6	41.0[a]	2.4	42.8[a]	3.6	43.1[a]	2.5	45.0[b]	3.4	45.3[b]	3.3
Serum Triacylglycerol (mmol/L)												
Baseline	0.26	0.06	0.27	0.03	0.26	0.07	0.27	0.07	0.27	0.01	0.27	0.05
Initial	0.25	0.09	0.25	0.10	0.28	0.15	0.26	0.08	0.27	0.09	0.28	0.10
Final	0.36	0.28	0.25	0.06	0.26	0.12	0.28	0.07	0.25	0.14	0.21	0.07
Serum Creatinine (μ mol/L)												
Baseline	30.9	4.6	29.5	4.8	28.7	4.8	30.9	4.7	30.9	4.8	30.9	5.1
Initial	84.0[a]	4.6	84.6[a]	6.3	85.1[a]	6.7	84.0[a]	8.8	44.2[b]	6.3	40.7[b]	8.8
Final	125.5[a]	15.2	74.3[b]	10.8	73.4[b]	16.8	75.1[b]	4.7	53.0[c]	8.8	53.0[c]	7.2
Serum Urea nitrogen (mmol/L)												
Baseline	3.7	0.1	3.6	0.2	3.6	0.1	3.6	0.3	3.8	0.5	3.9	0.4
Initial	10.5[a]	1.5	10.7[a]	3.9	10.7[a]	2.9	11.3[a]	3.9	3.8[b]	0.2	3.9[b]	0.2
Final	15.7[a]	4.4	8.2[b]	1.4	8.4[b]	0.9	8.2[b]	1.9	5.4[c]	2.9	4.9[c]	1.3
24-h urine protein (mg/d)												
Baseline	6.4	0.6	6.5	0.6	6.3	0.7	6.2	0.8	6.0	0.05	6.8	0.3
Initial	13.1[a]	2.5	12.8[a]	3.6	12.0[a]	5.5	12.2[a]	3.0	7.8[b]	3.3	7.0[b]	0.3
Final	27.9[a]	12.8	14.9[b]	1.8	14.2[b]	5.4	12.0[b]	1.7	8.1[c]	1.5	6.1[c]	0.1
Urine Urea nitrogen excretion (mmol/d)												
Baseline	65.3	2.7	65.4	2.5	64.3	3.6	65.2	3.6	64.7	3.8	65.1	2.1
Initial	75.9	18.0	76.7	10.6	75.5	18.9	75.9	4.8	69.2	01.7	69.4	2.7
Final	52.3[a]	8.5	88.5[b]	13.2	22.3[c]	7.8	26.3[c]	6.9	58.6[a]	13.5	53.3[a]	1.4
Creatinine clearance rate (mL/mim)												
Baseline	3.28	0.37	3.38	0.15	3.34	0.12	3.11	0.10	3.18	0.10	3.37	0.27
Initial	0.80[a]	0.18	0.88[a]	0.03	0.83[a]	0.01	0.80[a]	0.03	2.30[b]	0.03	2.57[b]	0.04
Final	0.54[a]	0.06	1.28[b]	0.16	1.42[c]	0.28	1.21[b]	0.13	2.08[d]	0.13	2.16[d]	0.13

[a,b] Mean values within a row without a common superscript letter significantly differ at $p < 0.05$. Casein group (180 g casein/kg); ISP group (180 g isolated soy protein /kg); LMF group (100 g casein and 80 g low-molecular-weight fraction of soy protein hydrolysate/kg); HMF group (100 g casein and 80 g high-molecular-weight fraction of soy protein hydrolysate/kg).

Table 4. Liver weight and liver lipids of five-sixths nephrectomized rats and sham-operated rats fed different protein diets. (Mean values and standard deviation)

	Five-sixths nephrectomy							
	Casein (*n* 6)		ISP (*n* 5)		LMF (*n* 6)		HMF (*n* 6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver								
Liver weight (g/rat)	9.5	42.2	9.3	0.8	9.3	0.5	8.8	0.6
TC (μ mol/g liver)	26.4 [a]	0.7	23.9 [ab]	4.1	20.6 [b]	1.7	19.0 [b]	7.6
Feces								
TC (μ mol/g feces)	1.95 [a]	0.36	2.27 [a]	1.45	2.13 [a]	1.23	9.76 [b]	2.20
Total bile acid (μ mol/g feces)	804 [a]	247	3663 [b]	689	1009 [a]	635	2360 [c]	1210

[a,b] Mean values within a row without a common superscript letter significantly differ at $p < 0.05$.

Casein group (180 g casein/kg); ISP group (180 g isolated soy protein/kg); LMF group (100 g casein and 80 g low-molecular-weight fraction of soy protein hydrolysate/kg); HMF group (100 g casein and 80 g high-molecular-weight fraction of soy protein hydrolysate/kg).

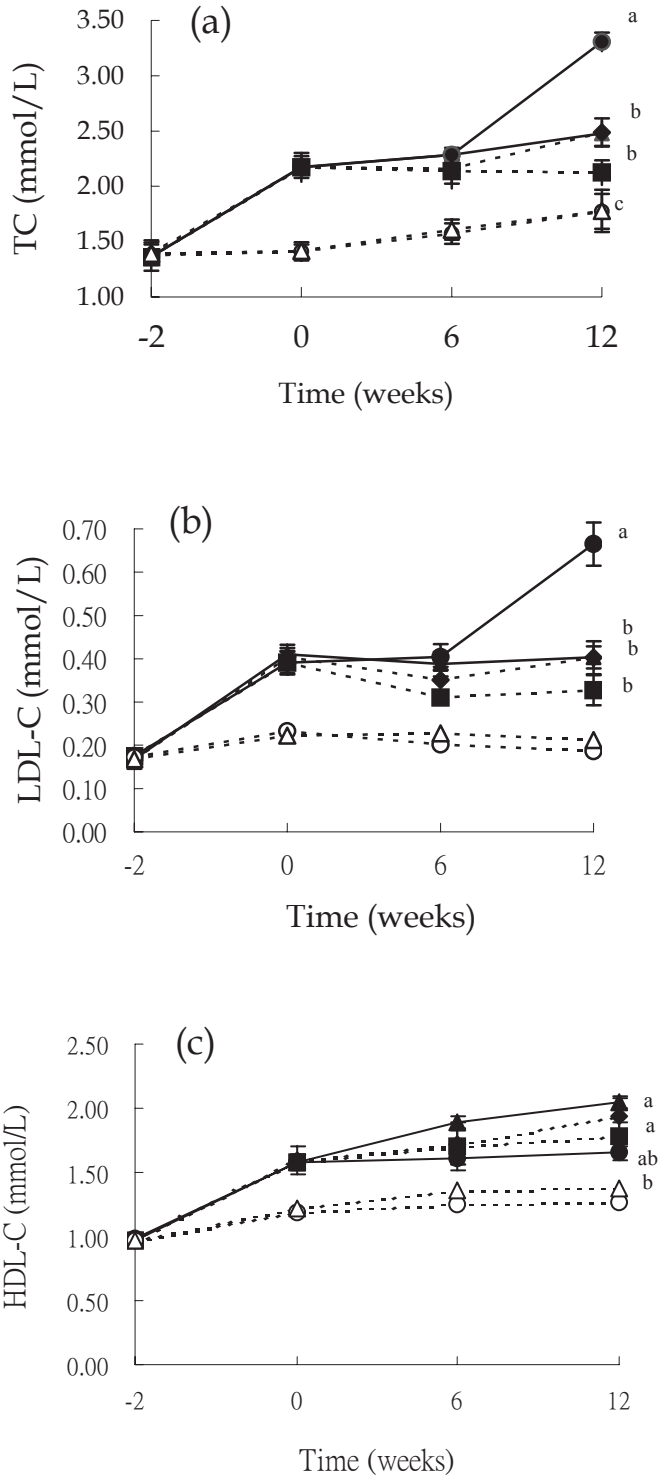

Figure 1. Plasma lipid and lipoprotein of five-sixths nephrectomized rats and sham-operated rats fed different protein diets.

-●- Casein group (nephrectomized rats with 180 g casein/kg); -▲- ISP group(nephrectomized rats with 180 g isolated soy protein/kg); -■- LMF group(nephrectomized rats with 100 g casein and 80 g low-molecular-weight fraction of soyabean protein hydrolysate/kg); - ◆ - HMF group (nephrectomized rats with 100 g casein and 80 g high-molecular-weight fraction of soy protein hydrolysate/kg)

Mean values within a row without a common superscript letter are significantly different at $p < 0.05$.

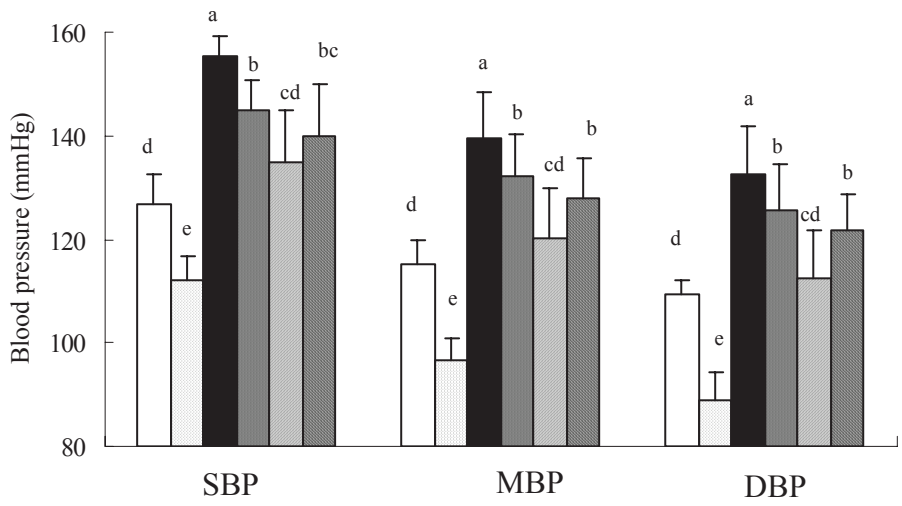

Figure 2. **S**ystolic blood pressure (a), mean blood pressure (b), and diastolic blood pressure (c) of five-sixths nephrectomized rats and sham-operated rats fed different protein diets at week 14.

Casein group (nephrectomized rats with 180 g casein/kg); ISP group(nephrectomized rats with 180 g isolated soy protein /kg); LMF group(nephrectomized rats with 100 g casein and 80 g low-molecular-weight fraction of soy protein hydrolysate/kg); HMF group (nephrectomized rats with100 g casein and 80 g high-molecular-weight fraction of soy protein hydrolysate/kg); S Casein group (sham-operated rats with 180 g casein/kg); S ISP group (sham-operated rats with 180 g isolated soy protein /kg). SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure. Mean values within a row without a common superscript letter significantly differ at $p < 0.05$.