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Effects of soy components on blood and liver lipids in rats fed high-cholesterol diets

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Abstract

AIM: To assess the effects of soy protein, isoflavone, and saponin on liver and blood lipid in rats that consumed high-cholesterol diets.

METHODS: High-cholesterol diets (1%) with or without soy material were fed to 6-wk-old male Sprague-Dawley rats for 8 wk. Blood lipids, liver lipids, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) levels were measured. The *in vitro* bile acid-binding ability of soy materials was analyzed.

RESULTS: The results of *in vitro* studies showed that soy protein isolate had a significantly higher bile acid-binding ability ($8.4 \pm 0.8\%$) than soy saponin ($3.1 \pm 0.7\%$) and isoflavone ($1.3 \pm 0.4\%$, $P < 0.05$). On the other hand, at the end of the experimental period, rats that consumed soy protein diets had lower GOT and GPT levels than rats that consumed casein under high-cholesterol diets. Rats that consumed soy protein also had lower total cholesterol (TC) levels in the liver than those that consumed casein under high-cholesterol diets. Rats that consumed the soy protein diet containing both saponin and isoflavone had lower hepatic TC level than those that consumed the soy protein diet without isoflavone alone. The effect of different types of proteins on triglyceride was not significant.

CONCLUSION: Consumption of soy provided benefits to control lipid levels under high-cholesterol dieting conditions in this rat model of hypercholesterolemia. The major component that reduced hepatic TC was not saponin, but possibly isoflavone.

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Key words: Soy; Isoflavone; Saponin; Triglyceride; Cholesterol

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INTRODUCTION

Soybeans contain many ingredients that are considered to have health benefits. Among them, soy protein, soy saponin, and isoflavone are most commonly noted. All of them consist of aglycones and glycosides. Soy protein has been shown to have a cholesterol-lowering ability because some ingredients in soy protein bind with cholesterol so that the complex is absorbed^[1]. Wang and Ng^[2] also demonstrated that the alcohol-extractable materials in soy protein were effective in lowering cholesterol. Previous studies showed that soy protein loses its cholesterol-lowering effect if it is extracted with alcohol^[3-5]. Saponin and isoflavone are alcohol-soluble components in soy beans. Unlike isoflavone, soy saponin is amphiphilic. Recent studies suggest that soy saponin is effective in lowering cholesterol^[6] and inhibiting tumor because it binds with cholesterol^[1,7]. Venter^[8] indicated that soy protein decreases LDL cholesterol in people with elevated serum cholesterol^[9]. The objective of this study was to identify the effect of individual components of the alcohol-soluble portion of soy protein on bile acid binding and lipid oxidation in order to delineate their potential role in the health benefits from soy consumption.

MATERIALS AND METHODS

Extraction of saponin

Soybeans were ground up in a blender for 2 min. The isoflavone/saponin mixture was then extracted from soy powder (1:20, soy powder:methanol) with 80% methanol at 40 °C for 2 h. After the methanol solution had been removed in a rotary evaporator, a certain amount of distilled water (e.g., 5 g of soy powder as starting material would need 10 mL of distilled water) was added to separate saponin from isoflavone. A quick test for saponin was performed by shaking the liquid sample for 1 min. Foaming for 10 min indicated a positive result of saponin. Saponin samples were analyzed for isoflavone residues with HPLC before further application. Commercial saponin was also purchased from Wako (Japan) for use as the HPLC standard.

In vitro bile acid binding of saponin

Ten grams of soy saponin was mixed with 5 mL of bile salt (10^{-4} mol)-containing buffer solution (0.1 mol/L of Tris-HCl and 0.1 mol/L Na₂N₃) at pH 4.0 and 37 °C for 2 h, followed

by dialysis (Spectra/POR, MW cut-off 6 000-8 000, Spectrum Medical, Los Angeles, CA, USA) for 4 d. The content of the bile salt was determined by enzymatic analysis as previously described^[10].

Animals

Thirty-two 6-wk-old male Sprague-Dawley rats (Animal Center, National Scientific Council, Taipei, Taiwan) were randomly assigned to five groups according to the isoflavone and saponin contents in their diets: (1) soy protein (with isoflavone and saponin)+1% cholesterol (SP); (2) alcohol-washed soy protein (no isoflavone or saponin)+1% cholesterol (ASP); (3) alcohol-washed soy protein+soy saponin+1% cholesterol (SS); (4) casein+1% cholesterol (PC); and (5) casein (negative control, NC). The composition of each diet is shown in Table 1. Guidelines for the ethical care and treatment of animals from the Animal Care Committee at Taipei Medical University were strictly followed. Rats were individually housed and maintained in a temperature-controlled (23 ± 2 °C) room with a 12-h light/dark cycle. They were fed a chow diet for 1 wk before switching to the experimental diets. Five rats were randomly chosen and killed after completing the 1 wk of chow diet to acquire the baseline total cholesterol (TC) and triglyceride (TG) values of the liver. Water and food were available *ad libitum*. Two milliliters of blood samples were collected from the tail vein during wk 2, 4, and 6 after the start of the experimental diets. After wk 8, all rats were killed, and liver and blood samples were collected.

Table 1 Composition of the experimental diets

	NC ³	CC	SP	ASP	SS
Corn starch	588	588	588	588	588
Dextrin	191.1	191.1	191.1	191.1	191.1
Casein	294	294	0	0	0
Soy protein ¹	0	0	294	0	0
Soy protein ²	0	0	0	294	294
Sucrose	147	73.5	73.5	73.5	66.15
Saponin	0	0	0	0	7.35
Soybean oil	88.2	147	147	147	147
Mineral mix	58.8	58.8	58.8	58.8	58.8
Cellulose	73.5	73.5	73.5	73.5	73.5
Vitamins	14.7	14.7	14.7	14.7	14.7
Cholesterol	0	14.7	14.7	14.7	14.7
Methionine	14.7	14.7	14.7	14.7	14.7
Choline	0	0.735	0.735	0.735	0.735
Cholic acid	0	0.735	0.735	0.735	0.735

¹Soy protein with isoflavone and saponin; ²Alcohol-extracted soy protein; ³NC, negative control (casein); PC, positive control (casein+1% cholesterol); SP, soy protein diet; ASP, alcohol-extracted soy protein diet; SS, soy saponin diet.

Blood lipid and liver function analyses

TC, TG, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were measured with commercial kits (Randox).

Statistical analysis

Data are presented as the mean \pm SD. One- and two-way ANOVA were performed using SAS[®] 8.1 software. Least

significant difference test was performed to determine within-group differences.

RESULTS

Bile acid binding

The percentages of bile acid binding were as follows: soy saponin, $3.1\pm 0.7\%$; soy isoflavone aglycones, $1.3\pm 0.4\%$; and soy protein isolate, $8.4\pm 0.8\%$. The differences in bile acid-binding percentages between each group were all significant ($P<0.05$).

In vivo studies

Table 2 shows the body weight changes of rats in each group during the feeding period. None of the mean body weights differed significantly among the groups. The effects of different diets on serum GOT, GPT, TG, and TC are shown in Table 3. In wk 0, the GOT and TC in five groups did not differ from each other. The GPT in the NC group was a bit, but significantly higher than that in the ASP group ($P<0.05$). The TG in the PC group was significantly higher (though the differences were not very big) than those in the ASP and SS groups ($P<0.05$). In wk 2, the GPT and TG levels did not differ among all groups, while the TC level in the SS group and the GOT level in the PC group were the highest ($P<0.05$). In wk 4, the PC group had higher GOT and GPT levels than the other groups ($P<0.05$), but the TC and TG levels still did not significantly differ among all groups. In wk 6, the PC group had higher GOT, GPT, and TG levels than the ASP, AS, and NC groups ($P<0.05$). The SS and NC groups also had higher TG levels than the SP and ASP groups ($P<0.05$). In wk 8, the PC group still had higher GPT levels than the other groups ($P<0.05$). The SS group had a lower TG level than the SP, NC, PC ($P<0.05$), and ASP ($P>0.05$) groups. The groups fed 1%-cholesterol diets had higher TC values than the NC group ($P<0.05$). Generally speaking, dietary consumption of soy protein either with or without alcohol extraction resulted in lower GOT and GPT levels than casein and showed a tendency to lower serum TG levels.

Table 2 Body weight (g) changes of rats during the experiment (all $n = 9$)

	Wk 1	Wk 2	Wk 4	Wk 6	Wk 8
NC ¹	349 \pm 23	376 \pm 37	421 \pm 51	455 \pm 59	482 \pm 59
PC	357 \pm 18	392 \pm 19	444 \pm 32	488 \pm 33	521 \pm 33
SP	352 \pm 26	376 \pm 36	426 \pm 35	471 \pm 39	494 \pm 41
ASP	358 \pm 23	396 \pm 27	449 \pm 40	491 \pm 44	522 \pm 49
SS	364 \pm 12	399 \pm 20	448 \pm 29	489 \pm 24	518 \pm 22

¹NC, negative control (casein); PC, positive control (casein+1% cholesterol); SP, soy protein; ASP, alcohol-extracted soy protein; SS, alcohol-extracted soy protein plus saponin.

The changes in GOT, GPT, TG, and TC during the experiment are also presented in Table 3. In the NC group (regular AIN76), GOT, GPT, and TC levels did not significantly change throughout the experiment. By contrast, the TG level gradually increased from wk 2 to 6

Table 3 Effects of experimental diets on serum GOT, GPT, TG, and TC through the experiment (all $n=9$)

		Wk 0	Wk 2	Wk 4	Wk 6	Wk 8
ASP ¹	GOT ²	67.8±20.8 ^a	49.9±11.2 ^a	73.8±13.2 ^a	65.1±22.0 ^a	70.7±22.8 ^a
	GPT	13.8±5.5 ^a	13.8±7.4	21.0±9.8 ^a	21.1±9.8 ^a	20.5±6.2 ^a
	TC	57.9±15.1 ^a	39.1±10.4 ^a	54.2±9.3 ^a	62.3±12.0 ^a	64.0±7.8 ^a
	TG	32.0±14.2 ^a	52.1±14.7 ^a	34.4±12.4 ^a	30.4±6.6 ^a	35.2±23.4 ^a
SS	GOT	61.0±16.7	52.3±12.1 ^a	61.6±27.7 ^a	88.8±77.3 ^a	103.7±90.8 ^a
	GPT	16.9±3.7 ^a	15.7±12.8 ^a	18.2±7.6 ^a	18.2±7.6 ^a	26.8±11.6 ^a
	TC	62.1±31.6 ^a	53.2±11.4 ^a	52.3±9.6 ^a	58.6±14.7 ^a	74.7±5.7 ^a
	TG	33.5±14.1 ^a	42.3±15.5	42.1±17.3	34.3±26.3 ^a	22.3±14.5 ^a
NC	GOT	68.7±13.5 ^a	49.9±12.7 ^a	60.4±15.1 ^a	54.4±10.1 ^a	55.0±20.8 ^a
	GPT	19.8±4.2 ^a	25.8±13.6	17.2±7.0 ^a	17.2±7.0 ^a	16.8±8.2 ^a
	TC	46.5±12.9	46.4±12.4 ^a	49.4±16.2	53.6±15.6	50.6±13.3 ^a
	TG	43.2±12.5 ^a	68.5±19.6 ^a	40.7±21.2 ^a	76.6±29.0 ^a	52.2±25.1 ^a
PC	GOT	71.0±11.9 ^a	59.8±11.6 ^a	109.5±41.8 ^a	125.2±48.0 ^a	114.6±38.4 ^a
	GPT	16.3±5.3 ^a	21.5±12.5 ^a	65.1±37.9 ^a	65.1±37.9 ^a	49.5±29.0 ^a
	TC	43.2±19.0 ^a	43.7±10.6 ^a	55.8±16.0 ^a	68.7±18.6 ^a	69.4±11.2 ^a
	TG	53.2±12.1 ^a	60.3±34.5	56.2±20.4	63.1±35.3 ^a	42.5±14.0 ^a
SP	GOT	79.7±10.4 ^a	44.6±14.9 ^a	64.5±10.5 ^a	61.5±15.8 ^a	93.3±35.1 ^a
	GPT	17.5±5.4 ^a	19.5±6.5 ^a	25.8±11.3 ^a	25.8±11.3 ^a	35.6±15.7 ^a
	TC	47.5±12.8 ^a	46.9±11.5 ^a	50.2±8.0 ^a	64.0±9.1 ^a	71.9±10.5 ^a
	TG	43.4±10.2 ^a	43.8±22.6 ^a	45.0±12.2 ^a	34.7±12.2 ^a	52.3±8.9 ^a

¹ASP, alcohol-extracted soy protein; SS, alcohol-extracted soy protein plus saponin; NC, negative control (casein); PC, positive control (casein+1% cholesterol); SP, soy protein. ²GOT and GPT, IU/dL; TG and TC, mg/dL. ^a $P<0.05$ within.

($P<0.05$). In the SP (intact soy protein) group, GOT, GPT, and TC levels had significantly increased ($P<0.05$) by the end of the experiment. Changes in the TG level did not show a clear trend. In the ASP (alcohol-washed soy protein) group, GOT and GPT levels did not change significantly during the experiment. The TC level significantly increased from wk 2 to 8 ($P<0.05$), while the TG level significantly decreased ($P<0.05$) during the experiment. In the SS group (ASP+saponin), the GOT level did not change throughout the experiment and the GPT level gradually increased toward the end of the experiment ($P<0.05$). By contrast, the TG level reached a maximum in wk 6 ($P<0.05$). In the PC group (high-cholesterol casein diet), the GOT, GPT, and the TC levels were elevated from wk 4 until the end of the feeding period ($P<0.05$).

Table 4 shows the hepatic TC and TG levels at baseline and at the end of the feeding period. The hepatic TG level in the SS group was the highest among the five groups in wk 8 ($P<0.05$), and was significantly higher than at baseline ($P<0.05$). TG levels in the other groups did not change

significantly from baseline. Hepatic TC levels in all groups increased ($P<0.05$) compared to baseline. Rats that consumed soy protein (SP, SS, and ASP groups) had lower TC levels in the liver than those consumed casein (PC group) under high-cholesterol diets ($P\leq 0.05$). The SS and PC groups had higher hepatic TC levels than the other groups ($P<0.05$). Hepatic TC level in the SP and NC groups were significantly lower than those of the other groups ($P<0.05$). No significant differences were observed between the effects of casein and soy protein on hepatic TC and TG levels.

DISCUSSION

In this study, different types of dietary protein and the addition of saponin did not affect the growth of rats. We found that alcohol-extracted soy proteins (without isoflavone and saponin) with or without the addition of saponin had a protective effect on maintaining GOT and GPT under elevated cholesterol conditions. A diet high in cholesterol causes an increase in oxidative stress in the liver^[11] and results in increases in GOT and GPT levels. In this study, the increasing levels of GOT and GPT in the rats fed casein with 1% cholesterol also produced elevated TC levels at the end of the feeding period compared to baseline.

The serum cholesterol content in the body is balanced by dietary cholesterol absorption, cholesterol synthesis in the body, and biliary cholesterol excretion. The cholesterol-lowering effect of saponin is due to the increase in bile acid excretion^[12], which occurs through interference with the absorption of cholesterol^[13]. Potter^[14] also indicated that saponin might alter the absorption of cholesterol and bile acid. One of the possible mechanisms for this effect is that saponin forms micelles with bile acid^[4]. Our *in vitro* data also suggested that soy protein and saponin bind a certain amount of bile acid, while the TC levels did not differ among

Table 4 TC and TG levels in the liver at the baseline and at the end of the feeding period

	TC (mg/g liver)	TG (mg/g liver)
Baseline	1.4±1.2	18.1±5.3
NC ¹	2.5±1.7 ^a	15.8±5.9 ^a
PC	5.3±1.1 ^a	14.8±5.1 ^a
SP	3.3±0.6 ^a	17.1±3.5 ^a
SS	4.8±1.1 ^a	25.8±3.7 ^a
ASP	3.6±0.9 ^a	18.9±2.4 ^a

¹NC, negative control (casein); PC, positive control (casein+1% cholesterol); SP, soy protein; SS, alcohol-extracted soy protein plus saponin; ASP, alcohol-extracted soy protein. ^a $P<0.05$ vs others.

groups. Jimenez *et al.*^[15], reported that soy saponin in the diet of rats did not have the same blood cholesterol-lowering effect of another amphiphilic compound, lecithin. This may have been due to soy saponin not being absorbed well in the small intestine through oral intake and reaching the colon directly^[16]. Thus, the cholesterol-lowering effect of saponin appears to occur mainly through its bile acid- or cholesterol-binding ability in the digestive tract. In addition, the cholesterol-lowering effect may be changed if amino acids appear together^[8]. Sautier *et al.*^[17], also showed that the effect of soy saponin on lowering serum cholesterol might be greatly depressed if soy protein is added. In our experiments, soy saponin was added with soy protein and, therefore, no significant effect on TC was observed. Yamakoshi *et al.*^[18], found that soy isoflavone had more significant cholesterol-lowering effects in plasma compared to saponin because saponin is not absorbed in the small intestine.

Soy isoflavone has been shown to have a cholesterol-lowering effect in the liver^[19,20]. Soy protein also increases the activity of cholesterol receptors in the liver^[21]. In our study, only the soy protein diet containing both isoflavone and saponin (SP group) lowered TC levels. Since we used healthy rats as the animal model, the liver cholesterol receptors should have been normal. This implies that the cholesterol-lowering effect in the liver in our study was from the effect of isoflavone rather than from saponin or soy protein. Sugano *et al.*^[10], also suggested that the cholesterol-lowering effect of saponin might be reduced if it was ingested with soy protein.

In this study, we used SD rats as the animal model. It has been suggested that hypercholesterolemia is more easily induced in hamsters than in SD rats^[22]. However, high cholesterol levels were observed in our SD rats fed high-cholesterol diets, which suggests that this animal model is also suitable for use in research. Since the hypercholesterolemic effect was not as apparent as that of hamsters; however, the results might have been affected. On the other hand, Gao *et al.*^[22], suggested that SD rats might be a better animal model for investigating TG levels. In this study, the effect of different diets on blood TG levels of SD rats was highly significant.

In conclusion, this study found that soy protein was better than casein for liver protection in terms of GOT and GTP under high-cholesterol diet conditions. Soy protein and saponin showed some bile acid-binding abilities *in vitro*. However, the major component that reduced liver TC was isoflavone. The effects of various protein types on lowering TG still need further investigation. In addition, the SD rat is a useful animal model for studying hypercholesterolemia.

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