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±0.8%) than soy saponin (3.1±0.7%) and isoflavone (1.3±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 soy protein 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.

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

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 [9]. Wang and Ng [10] 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 amphphilic. Recent studies suggest that soy saponin is effective in lowering cholesterol [6] and inhibiting tumor because it binds with cholesterol [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 (10-3 mol)-containing buffer solution (0.1 mol/L of Tris-HCl and 0.1 mol/L NaN3) 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 ℃) 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.

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±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±0.7%; soy isoflavone aglycones, 1.3±0.4%; and soy protein isolate, 8.4±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 1 Composition of the experimental diets |
|---------------------------------------------|
| NC | CC | SP | ASP | SS |
|---|---|---|---|---|
| Corn-starch | 588 | 588 | 588 | 588 |
| Dextrin | 191.1 | 191.1 | 191.1 | 191.1 |
| Casein | 294 | 294 | 0 | 0 |
| Soy protein | 0 | 0 | 294 | 0 |
| Soy protein | 0 | 0 | 0 | 294 |
| Sucrose | 147 | 73.5 | 73.5 | 73.5 |
| Saponin | 0 | 0 | 0 | 7.35 |
| Soybean oil | 88.2 | 147 | 147 | 147 |
| Mineral mix | 58.8 | 58.8 | 58.8 | 58.8 |
| Cellulose | 73.5 | 73.5 | 73.5 | 73.5 |
| Vitamins | 14.7 | 14.7 | 14.7 | 14.7 |
| Cholesterol | 0 | 14.7 | 14.7 | 14.7 |
| Methionine | 14.7 | 14.7 | 14.7 | 14.7 |
| Choline | 0 | 0.735 | 0.735 | 0.735 |
| Cholic acid | 0 | 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.

| 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 | 349±23 | 376±37 | 421±51 | 455±59 | 482±59 |
| PC | 357±18 | 392±19 | 446±32 | 488±33 | 521±33 |
| SP | 352±26 | 376±36 | 426±35 | 471±39 | 494±41 |
| ASP | 358±23 | 396±27 | 449±40 | 491±44 | 522±49 |
| SS | 364±12 | 399±20 | 448±29 | 489±24 | 518±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
from wk 2 to 8 (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 throughout the experiment and the GPT level gradually increased (ASP+saponin), the GOT level did not change. In the SS group was the highest among the five groups in the end of the experiment. Changes in the TG level 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<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 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, 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. These micelles may have a higher affinity for bile acid in the intestinal tract, leading to increased bile acid excretion and decreased cholesterol absorption. This mechanism may explain the cholesterol-lowering effect of saponin.

### 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    | 67.8±20.8* | 49.9±11.2* | 73.8±13.2* | 65.1±22.0* | 70.7±22.8* |
| GPT    | 13.8±5.5  | 13.8±4.5  | 21.0±9.8  | 21.1±9.8  | 20.5±6.2  |
| TC     | 57.9±15.1* | 39.1±10.4* | 54.2±9.3*  | 62.3±12.0* | 64.0±7.8*  |
| TG     | 32.0±14.2 | 52.1±14.7 | 34.4±12.4 | 30.4±6.6  | 35.2±6.3  |
| SS     | 61.0±16.7 | 52.3±12.1 | 61.6±27.7 | 88.8±77.3* | 103.7±89.0* |
| GOT    | 16.9±3.7  | 15.7±12.8 | 18.2±7.6  | 18.2±7.6  | 26.8±11.6  |
| GPT    | 62.1±31.6 | 53.2±11.4 | 52.3±6.9  | 58.6±14.7* | 74.7±5.7*  |
| TC     | 33.5±14.1 | 42.3±15.5 | 42.1±17.3 | 34.3±26.3 | 22.3±14.5* |
| TG     | 68.7±15.5 | 49.9±12.7 | 60.4±15.1 | 54.4±10.1* | 55.0±20.8* |
| NC     | 19.8±4.2  | 25.8±13.6 | 17.2±7.0  | 17.2±7.0  | 16.8±2.8   |
| PC     | 46.5±12.9 | 46.4±12.4 | 49.4±16.2 | 53.6±15.6 | 50.6±13.3  |
| GOT    | 43.2±12.5 | 68.5±19.6 | 40.7±21.2 | 76.6±29.0 | 52.2±25.1* |
| GPT    | 71.0±11.9 | 59.8±11.6 | 109.5±41.8 | 125.2±48.0 | 114.6±38.4 |
| TC     | 16.3±5.3  | 21.5±12.5 | 65.1±37.9 | 65.1±37.9 | 49.5±29.0  |
| TG     | 43.2±10.0 | 43.7±10.6 | 55.8±16.0 | 68.7±18.6 | 69.4±11.2  |
| SP     | 53.2±12.1 | 60.3±34.5 | 56.2±20.4 | 61.3±35.3 | 42.5±14.0  |
| GOT    | 79.7±10.4 | 44.6±14.9 | 64.5±10.5 | 61.5±15.8 | 93.3±35.1  |
| GPT    | 17.5±5.4  | 19.5±6.5  | 25.8±11.3 | 25.8±11.3 | 35.6±15.7  |
| TC     | 47.5±12.8 | 46.9±11.5 | 50.2±8.0  | 64.0±9.1  | 71.9±10.5  |
| TG     | 43.4±10.2 | 43.8±22.6 | 45.0±12.2 | 34.7±12.2 | 52.3±8.9   |

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. 1GOT and GPT, IU/dL; TC and TG, mg/dL. *P<0.05 within.

### 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      | 2.5±1.7  | 15.8±5.9  |
| PC      | 5.3±1.1  | 14.8±5.1  |
| SP      | 3.3±0.6  | 17.1±3.5  |
| SS      | 4.8±1.1  | 25.8±3.7  |
| ASP     | 3.6±0.9  | 18.9±2.4  |

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. *P<0.05 vs others.
groups. Jimenez et al. 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. 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 in the digestive tract. In addition, the soy isolavone 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., found that soy isolavone 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. Soy protein also increases the activity of cholesterol receptors in the liver. 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., 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. 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., 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.

REFERENCES

1. Potter JD, Topping DL, Oakenfull D. Soya, saponins and plasma cholesterol. Lancet 1979; 1: 223
2. Wang HX, Ng TB. Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antiinflammatory activities. Life Sci 1995; 65: 2663-2677
3. Lucas EA, Khalil DA, Daggy BP, Arjmandi BH. Ethanol-extracted soy protein isolate does not modulate serum cholesterol in golden Syrian hamsters: a model of postmenopausal hypercholesterolemia. J Nutr 2001; 131: 211-214
4. Oak enfull D. Soy protein, saponins and plasma cholesterol. J Nutr 2001; 131: 2971-2972
5. Arjmandi BH. Reply to Dr. David Oakenfull. J Nutr 2001; 131: 2972
6. Anderson RL, Wolf WJ. Compositional changes in tryptophan inhibitors, phytic acid, saponins and isoflavones related to soybeen processing. J Nutr 1995; 125: 5815-5885
7. Messina M, Barnes S. The role of soy products in reducing risk of cancer. J Nutr Cancer Inst 1991; 83: 541-546
8. Venter CS. Health benefit of soybeans and soy products: a review. J Family Ecol Consumer Sci 1999; 127: 24-33
9. Carroll KK. Review of clinical studies on cholesterol-lowering response to soy protein. J Am Diet Assoc 1991; 91: 820-827
10. Sugano M, Goto S, Yamada Y, Yoshida K, Hashimoto Y, Matsuo T, Kimoto M. Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. J Nutr 1990; 120: 977-985
11. Al Kanhal MA, Ahmad F, Al Othman AA, Arif Z, Al Orf S, Al Mushed KS. Effect of pure and oxidized cholesterol-rich diets on some biochemical parameters in rats. Int J Food Sci Nutr 2002; 53: 381-388
12. Erdman JW. AHA Science Advisory: Soy protein and cardiovascular disease: A statement for healthcare professionals from the Nutrition Committee of the AHA. Circulation 2000; 102: 2555-2559
13. Shin DH, Heo HJ, Lee YJ, Kim HK. Amaranth squalene reduces serum and liver lipid levels in rats fed a cholesterol diet. Br J Biomed Sci 2004; 61: 11-14
14. Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr 1995; 125: 6065-6115
15. Jimenez MA, Scarino ML, Vignolini F, Mengheri E. Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats. J Nutr 1990; 120: 659-667
16. Oh YJ, Sung MK. Soybean saponins inhibit cell proliferation by suppressing PKC activation and induce differentiation of HT-29 human colon adenocarcinoma cells. Nutcr Cancer 2001; 50: 132-138
17. Sautier C, Doucet C, Flamant C, Lemonnier D. Effects of soy protein and saponins on serum, tissue and feces steroids in rat. Atherosclerosis 1979; 34: 233-241
18. Yamakoshi J, Piskula MK, Izumi T, Tobe K, Saito M, Kataoka S, Obata A, Kikuchi M. Isolavone aglycone-rich extract without soy protein attenuates atherosclerosis development in cholesterol-fed rabbits. J Nutr 2000; 130: 1887-1893
19. Iqbal MJ, Yaegashi S, Ahsan R, Lightfoot DA, Banz WJ. Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. Physiol Genomics 2002; 11: 219-226
20. Xiao CW, L’Abbe MR, Gilani GS, Cooke GM, Curran IH, Papademetriou SA. Dietary soy protein isolate and isoflavones modulate hepatic thyroid hormone receptors in rats. J Nutr 2004; 134: 743-749
21. Sirri CR, Bosisio R, Pazzucconi F, Bondioli A, Gatti E, Lovati MR, Murphy P. Soy milk with a high glycitein content does not reduce low-density lipoprotein cholesterol in type II hypercholesterolemic patients. Ann Nutr Metab 2002; 46: 88-92
22. Gao Y, Li K, Tang S, Xiao Y. Study on animal models for hyperlipidemia. Weisheng Yanjiu 2002; 31: 97-99