Soymilk Intake Is Associated With Plasma and Liver Lipid Profiles in Rats Fed a High-Cholesterol Diet

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OBJECTIVE: This study investigated the effects of soymilk on lipid metabolism in Sprague-Dawley rats fed a cholesterol-enriched (0.3%) diet.

METHODS: Thirty male Sprague-Dawley rats weighing 230.0 ± 9.8 g were randomly assigned to one of three groups: control, S1 (containing 15% soymilk powder in the diet), and S2 (22.5%). After 8 wk, lipid profiles of the plasma, liver, and feces were determined.

RESULTS: Body weight gain, daily food intake, and feeding efficiency showed no differences across groups ($P > 0.05$). The experimental groups had significantly lower plasma levels of cholesterol, triacylglycerol, and low-density lipoprotein cholesterol than the control group ($P < 0.05$) at weeks 4 and 8. However, total fecal excretion of neutral steroid did not significantly differ across groups ($P > 0.05$).

CONCLUSION: Soymilk affects the metabolism of plasma cholesterol in Sprague-Dawley rats. *Nutrition* 2004;20:929–933. ©Elsevier Inc. 2004

KEY WORDS: soybean, soymilk, cholesterol, rat, lipid metabolism

INTRODUCTION

Recently, evidence from animal and clinical studies has suggested the beneficial health effect of soybeans,[1–5] including improvement of plasma lipid profiles[6] and decreases in hormone-dependent conditions or diseases such as postmenopausal disorder,[7] breast cancer[8,9], and osteoporosis.[10]

Western diets have resulted in an increased consumption of animal lipids. Cardiovascular diseases are the leading cause of death in the West.[8,9,11] Many factors have been related to the incidence of cardiovascular diseases. Previous studies have shown that atherosclerosis is the major cause of those diseases. Dietary lipids and cholesterol are the major factors that affect lipid metabolism. However, the effects of other components of foods, in addition to lipids, on plasma lipids should not be ignored.[12–14] In general, instead of using hypolipidemic medicine to treat abnormal lipidemia in patients who are initially diagnosed with hyperlipidemia, dietary therapy, especially long-term modification of dietary behavior, is often suggested to decrease and control blood lipids. Therefore, to emphasize that patients with hyperlipidemia should carefully consider their dietary intake and health, many foods with the property of mediating hyperlipidemia are noted.

Soybeans have high nutritional value and certain special physical properties. Soy foods, such as tofu and soymilk, are important food sources for Asian populations.[15] Epidemiologic studies have shown that Asian populations have a lower incidence of coronary heart disease.[6,16] In addition, soymilk is the best food to replace dairy products for those who have lactose intolerance. This study investigated the effects of different doses of soymilk on lipid metabolism in rats that consumed a cholesterol-enriched diet.

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MATERIALS AND METHODS

Materials

The soymilk used in this study was purchased from Uni-President Enterprises Corp. (Tainan, Taiwan). The soymilk was freeze-dried to prepare a soymilk powder to add to the experimental diet. The composition of the soymilk powder consisted of 46.5% carbohydrate, 27.9% protein, 12.8% fat, and 2.8% ash.

Animals and Diets

Thirty 4-wk-old male Sprague-Dawley rats were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The initial average body weight was 175.7 ± 11.8 g. During the second week, animals were fed Rodent Laboratory Chow 5001 (LabDiet®, Brentwood, MO) and then their average body weight increased to 230.0 ± 9.8 g. Animals were randomly assigned to one of three groups ($n = 10$/group): control; S1, which also consumed 15% soymilk powder; and S2, which also consumed 22.5% soymilk powder. Animals were individually housed in stainless steel cages, and food and water were given ad libitum. The room temperature was maintained at 23 ± 2°C, and the relative humidity was 55 ± 5%. We used a 12-h light, 12-h dark cycle, and 6:00 AM to 6:00 PM was the light period. During the experimental period, food intake was recorded daily, and body weights were recorded weekly. The composition of the experimental diets of each group is presented in Table I, and AIN-93 M was used as a reference.[17] The control and experimental diets were isocaloric, and carbohydrate, protein, and fat were proportionally deducted from the experimental diet to compensate for the added soymilk powder. All components of the diets were mixed in the proper proportions every week and stored at 4°C.

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TABLE I.

COMPOSITION OF THE EXPERIMENTAL DIETS (%)

Group/Ingredient*	Control	S1	S2
Casein	19.7	15.9	14.0
Cornstarch	51	46.1	43.6
Sucrose	10.9	8.8	7.7
Soybean oil	10.8	8.7	7.6
Mineral mixture*	4	4	4
Cellulose	2	2	2
Vitamin mixture	1	1	1
Cholesterol	0.3	0.3	0.3
Methionine	0.3	0.3	0.3
Choline bitartrate	0.1	0.1	0.1
Cholic acid	0.1	0.1	0.1
Soymilk powder†	0	15	22.5

* Casein (high nitrogen), sucrose (food grade), soybean oil, mineral mixture (AIN-93M mineral mixture), cellulose (non-nutritive bulk), and vitamin mixture (AIN-93M vitamin mixture) were obtained from ICN Biochemicals (Aurora, OH). Cornstarch was purchased from Samyang Genex (Seoul, Korea). Cholesterol, choline bitartrate, and cholic acid were obtained from Sigma (St. Louis, MO).
† Purchased from Uni-President Enterprises (Tainan, Taiwan).

Data Collection

BLOOD. After the preliminary feeding, the animals' blood was collected from the tail vein as a baseline. Then animals were anesthetized after a 12-h fast, and their blood was drawn at weeks 2, 4, and 8. Blood was collected in heparinized tubes. After centrifugation (1500*g*, 4°C, 15 min), plasma was separated out and stored at −70°C until analysis. Plasma concentrations of total cholesterol and triacylglycerol were assayed enzymatically by using the methods described by Allain et al.[18] and Mcgowan et al.,[19] respectively. Plasma from animals was collected in tubes coated with ethylene-diaminetetra-acetic acid for lipoprotein separation by the method described by Lindgren et al.[20] Ultracentrifugation was performed at 4°C. Samples were added with different densities of sodium bromide (d = 1.006, 1.063, and 1.210). Centrifugation was performed at 120 000*g* for 6 h. The fractions were then stored at −20°C until analysis.

LIVER. At the end of the experiment, animals were injected with 6% sodium pentobarbital (100 mg/kg body weight, intraperitoneal). After perfusion, livers were collected and stored at −70°C. Liver lipids were extracted according to the method of Folch et al.[21] The liver triacylglycerol concentration was determined by the method described by Soloni.[22] The total cholesterol concentration was based on the method of Carlson and Goldfarb.[23]

FECES. A 2-d fecal collection was made in the initial and final weeks. After recording the weight, the feces were dried at 60°C for 12 h. The feces were ground and screened with a nylon mesh (no. 40). Samples were collected and stored at −20°C until analysis. The total concentration of neutral steroids was determined by the method of Carlson and Goldfard.[23]

Statistical Analysis

Results were analyzed with SAS statistical software (SAS Institute, Cary, NC, USA). All values are presented as mean ± standard deviation. Data were analyzed by one-way analysis of variance and Duncan's multiple range tests. $P < 0.05$ was considered statistically significant.

TABLE II.

CHANGES IN BODY WEIGHT, DAILY FOOD INTAKE, FEEDING EFFICIENCY, AND LIVER WEIGHT OF RATS FED DIFFERENT DIETS*

	Diet group		
	Control	S1	S2
Initial body weight (g)	230.8 ± 13.6	226.5 ± 8.0	226.3 ± 7.9
Final body weight (g)	512.6 ± 37.4	523.9 ± 36.2	504.6 ± 33.6
Daily weight gain (g)	5.0 ± 0.7	5.3 ± 0.6	5.0 ± 0.7
Daily food intake (g)	24.0 ± 1.3	23.4 ± 1.2	24.2 ± 1.5
Feeding efficiency† (%)	21.0 ± 2.7	22.8 ± 2.8	20.5 ± 3.3
Liver weight (g)	25.4 ± 3.2^{a}	22.7 ± 2.5^{ab}	22.2 ± 2.5^{b}
Hepatosomatic index‡ (%)	4.9 ± 0.5^{a}	4.4 ± 0.7^{ab}	4.3 ± 0.4^{b}

* Data are expressed as the mean ± SD (n = 10); and values in a row with different superscript letters significantly differ ($P < 0.05$) as analyzed by one-way analysis of variance test.
† Feeding efficiency: (daily weight gain/daily foot intake) × 100%.
‡ Relative liver weight: (liver weight/body weight) × 100%.

RESULTS

Body Weight, Food Intake, and Feeding Efficiency

The initial average body weight of animals was 227.9 ± 10.1 g. After the 8-wk experimental period, the average body weight of each group had increased to 514.0 ± 35.5 g. The initial and final body weights showed no significant differences across groups ($P > 0.05$). Different amounts of soymilk powder in each experimental diet did not significantly affect food intake or body weight gain (Table II). Further, the S2 group showed a lower liver weight and hepatosomatic index than did the control group ($P < 0.05$).

Plasma Lipid Profiles

Plasma concentrations of total cholesterol increased significantly ($P < 0.05$) after feeding a cholesterol-rich diet. However, the addition of different amounts of soymilk inhibited the increase of plasma cholesterol (Figure 1a). Soymilk had no significant effects on plasma total cholesterol at week 2. However, the plasma levels of total cholesterol in groups S1 and S2 were lower than those in the control group at weeks 4 and 8. Plasma triacylglycerol concentrations showed significant differences after consumption of the experimental diets. However, the addition of different amounts of soymilk inhibited the increase of plasma triacylglycerols. Soymilk had no significant effects on plasma triacylglycerols at week 2. Nevertheless, a significant difference was observed at weeks 4 and 8 (Figure 1b). During the experimental period, plasma levels of high-density lipoprotein cholesterol did not change significantly. In contrast, levels of high-density lipoprotein cholesterol in the S2 group were significantly lower than those in the control group at week 8 ($P < 0.05$; Figure 1c). In addition, plasma levels of low-density lipoprotein in all groups slowly increased during the experimental period, and levels in the S1 and S2 groups were significantly lower than those in the control group at weeks 4 and 8 ($P < 0.05$; Figure 1d).

Liver Lipid Profiles

Data are presented in Table III. Liver cholesterol levels (μM/g liver) were not affect by the diet ($P > 0.05$). Triacylglycerol levels

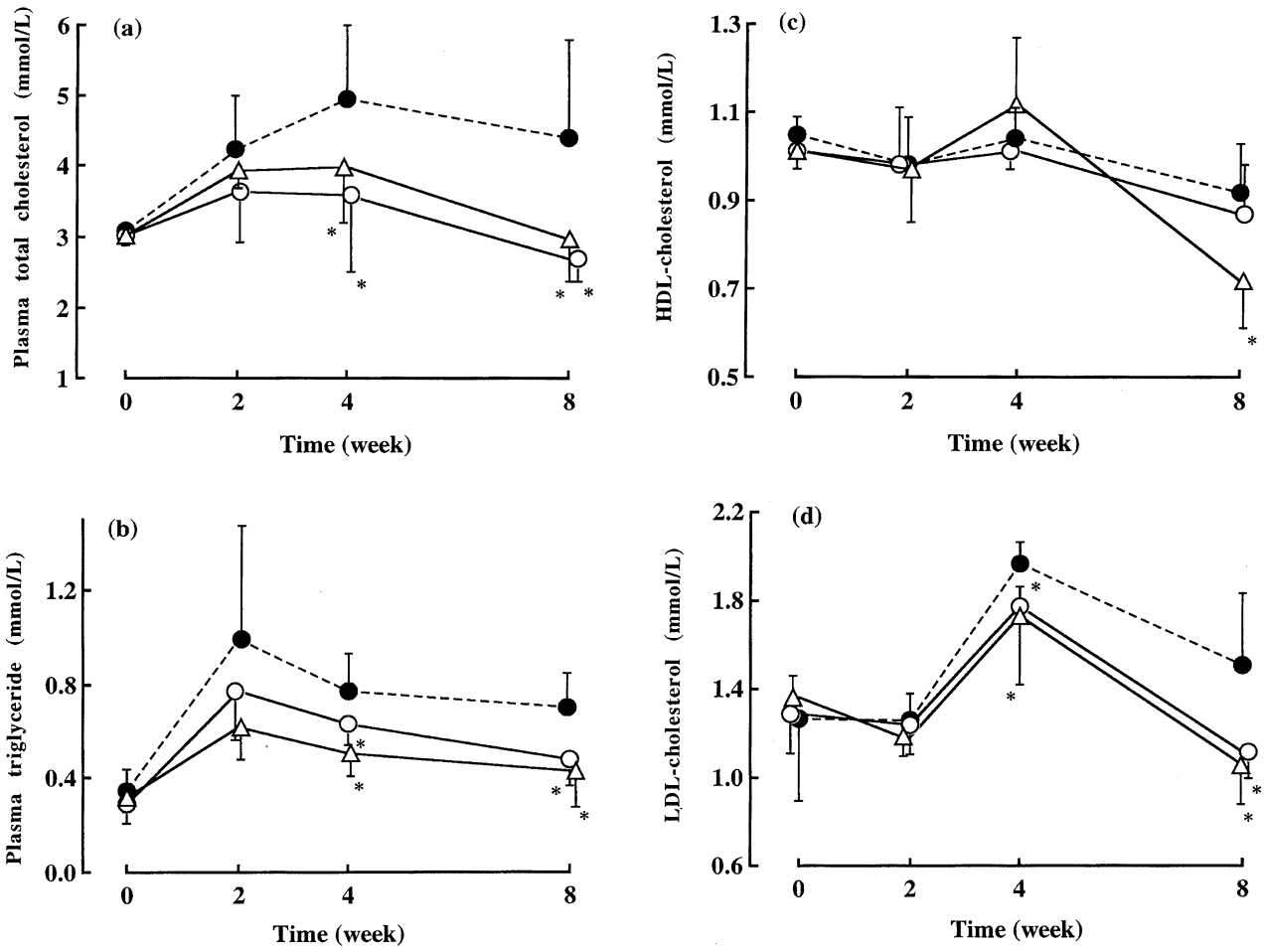

FIG. 1. Effects of different amounts of soymilk on plasma concentrations (mM/L) of total cholesterol (a), triacylglycerol (b), HDL cholesterol (c), and LDL cholesterol (d) in Sprague-Dawley rats. Vertical bars represent the mean ± standard deviation (n = 10/group). *Significantly different from the control group at the same time ($P < 0.05$). HDL, high-density lipoprotein; LDL, low-density lipoprotein; open circles, S1 group; solid circles, control group; triangles, S2 group.

(μM/g liver) did not differ significantly across groups ($P > 0.05$). However, the concentration of total cholesterol in the whole liver decreased significantly in the S1 and S2 groups compared with the control group ($P < 0.05$). The S1 and S2 groups had similar levels of total cholesterol in the whole liver.

TABLE III.

EFFECTS OF DIFFERENT AMOUNTS OF SOYMILK POWDER ON LIVER TOTAL CHOLESTEROL AND TRIGLYCERIDE CONCENTRATIONS IN SD RATS*

	Diet group		
	Control	S1	S2
Total cholesterol			
μmol/g liver	23.9 ± 5.1	18.8 ± 6.0	21.7 ± 4.3
μmol/liver	601.0 ± 123.1[a]	423.3 ± 121.5[b]	480.7 ± 67.7[b]
Triglyceride			
μmol/g liver	20.3 ± 2.9	19.7 ± 2.3	21.7 ± 3.0
μmol/liver	509.3 ± 113.1	452.7 ± 80.7	475.8 ± 57.7

* Data are expressed as the mean ± SD (n = 10); and values in a row with different superscript letters significantly differ ($P < 0.05$) as analyzed by one-way analysis of variance test.

Total Neutral Steroids in Feces

At the beginning of the experiment, there were no obvious differences across groups. However, fecal excretion of neutral steroids increased after animals had ingested the cholesterol-rich diet. The excretion of neutral steroids in the S1 and S2 groups tended to be higher than in the control group, although it did not differ significantly across groups at the end of the experiment (Table IV).

DISCUSSION

In this study, soymilk powder was added to the experimental diet, and lipid metabolism was studied in rats fed a cholesterol-rich diet. According to the results of this study, soymilk inhibited the increase of plasma total cholesterol and triacylglycerols, even with a high-cholesterol diet. The data of the control group were similar to those of previous studies.[1] Plasma concentrations of total cholesterol and low-density lipoprotein cholesterol in the control group increased significantly, whereas those in the S1 and S2 groups decreased. Clinical and animal experiments have shown that soybean protein has a cholesterol-lowering effect.[24,25] In 1999, the

TABLE IV.

LEVELS OF TOTAL NEUTRAL STEROIDS IN FECES OF RATS FED DIFFERENT DIETS*

	Diet group		
	Control	S1	S2
Total neutral steroids (μmol/day)			
Initial	14.3 ± 3.3	12.7 ± 2.5	13.5 ± 2.8
Final	43.6 ± 19.9	42.8 ± 11.6	54.3 ± 9.9

* Data are expressed as the mean ± SD (n = 10).

U.S. Food and Drug Administration approved a claim that soy products decrease the risk of coronary heart disease;[26] the health claim does not specify a requirement for the presence or quantity of the non-protein constituents such as saponins, phytate, trypsin inhibitors, and isoflavones that influence plasma cholesterol.

The present data confirmed the cholesterol- and triacylglycerol-lowering effects of soymilk in rats. The results showed that addition of soymilk significantly decreased blood lipids and inhibited hepatic fat accumulation; however, there were no significant differences between the S1 and S2 groups, in which 20% and 30% casein, respectively, were replaced. The data suggested that increasing soybean protein in feed does not have an additional lipid-lowering effect. Recently, many scientists have become interested in the clinical effects of soybean on lipid metabolism. The recommended intake of soybean protein for humans is 25 g,[26] which is equivalent to 5% of the daily total food intake of 500 g of dry weight. The converted daily soybean protein intake for rats is 1 g based on 5% of daily feed intake (mean, 20 g). Therefore, we used the ratio of 15% soymilk powder in this study. In addition, to consider the application to daily life, we replaced casein with 20% to 30% soybean protein to observe its hypolipidemic effect. It may be difficult to include such a tremendous soybean protein intake in daily life, but a suitable soybean protein intake may prevent cardiovascular diseases. This study investigated whether lipid metabolism would be affected when animal protein in the diet was replaced with plant protein. We also considered its application to daily life.

Studies citing the protective effects of soybeans for cardiovascular diseases have been in the literature since the 1940s. It is still unclear what component of soybean has a beneficial effect on plasma lipids, but studies have suggested that the lipid-lowering effect is related to soy isoflavone.[25,27] However, Nestel et al.[28] reported the administration of genistein (45 mg/d) had no significant effect on blood lipid concentrations over a 5- to 10-wk period. Further, Sirtori et al.[29] found a marked decrease in plasma cholesterol in hypercholesterolemic patients who consumed isoflavone-poor soybean protein. Based on extrapolation of the data in the U.S. Department of Agriculture Iowa State University Isoflavone Database, the isoflavone concentrations in the experimental diets in this study were rather low (84 μg/g diet for the S1 group and 126 μg/g diet for the S2 group). Therefore, the effect of isoflavones in our study was ignored.

The minor components of soybean, such as oligosaccharides (stachyose and raffinose), phytosterols, saponin, and phytate, may also affect lipid metabolism. Dietary fibers have been reported to decrease plasma cholesterol by binding bile acids and decreasing their recycling through the enterohepatic circulation. In addition, certain fibers may delay the digestion and absorption of fat. To observe the changes in blood lipids and fecal excretion of steroids, Kikuchi-Hayakawa et al.[2] found that adding 30% soymilk powder in the feed has hypolipidemic effects in hamsters. Further, there was an inverse relation between plasma levels of very low-density lipoprotein plus low-density lipoprotein cholesterol and bile acid excretion in the feces. In general, fermentation may increase the release of aglycons from isoflavonoid glucosides, but Kikuchi-Hayakawa et al.[2] showed that the antiatherogenic effect of soymilk is not diminished by prefermentation.

Soymilk may enhance the excretion of neutral steroids, resulting in a long-term effect of decreasing blood lipids from fecal neutral steroids. We speculate that the major mechanisms by which soymilk decreases plasma cholesterol are to inhibit cholesterol absorption and to stimulate cholesterol excretion. The data suggested that the hypolipidemic effect of soymilk occurs through the inhibition of cholesterol absorption. However, we found no increase in neutral steroids in the feces. The excretion of neutral steroids in the feces collected for 2 d did not significantly differ, suggesting that the effect of dietary factors on the excretion of neutral steroids was long term instead of short term.

The triacylglycerol-lowering effects of soybean protein or its hydrolysate on the liver are ubiquitous not only in animal but also in human studies. Iritani et al.[30] indicated that plasma triacylglycerols differed as a consequence of dietary patterns. They also pointed out that a deficiency or imbalance of specific amino acids in the diet could decrease plasma levels of triacylglycerol due to the decrease of very low-density lipoprotein synthesis in the liver. Several studies have indicated that the amino acid composition of proteins is involved in their effects on plasma cholesterol. However, the relation between specific amino acids and plasma lipid profile has not been conclusive.[8,31,32] A previous study reported that intact plant proteins are more hypolipidemic than their corresponding amino acid mixtures,[33] a report that has been confirmed.[34]

In this study, plasma concentrations of total cholesterol and triacylglycerol were markedly lower in the S1 and S2 groups. We suspect that the hypolipidemic effect was due to high concentrations of soymilk powder in the experimental diet. Chen et al.[1] suggested that the pepsin-undigested fraction of soybean protein has an effect on fecal excretion of steroids or bile acids, which, as a consequence, may influence cholesterol metabolism. The undigested fragments in soybean protein can inhibit cholesterol diffusion in the gastrointestinal tract. Huff et al.[35] indicated that hypercholesterolemia could be decreased by replacing 25% casein of total protein with soybean protein. The hypolipidemic components of soybean protein have not been identified. The hydrophobic domain of the high-molecular-weight protein particle could combine with cholesterol and bile acids to further decrease the absorption of cholesterol and bile acids. Sugano et al.[36] reported that only the soybean protein hydrolysate, which has a molecular weight greater than 5000 Da, has a hypolipidemic effect. In addition, Iwami et al.[37] indicated that the hydrophobic character of the protein was related to its hypocholesterolemic property. The results showed that approximately 15% to 22.5% soymilk decreased plasma concentrations of total cholesterol and triacylglycerol compared with the control group. It may be that soymilk disturbs the absorption of lipids, resulting in the decreased synthesis of liver lipoproteins such as very low-density lipoprotein. The metabolism of very low-density lipoprotein was also decreased; hence, plasma triacylglycerol concentration also decreased[38]. In the present study, we believe that there was more lipid accumulation in the liver of the control group, and the addition of soymilk powder might have inhibited fat deposits, thus decreasing the formation of fatty liver. Moreover, the extra lipids can be used to form lipoproteins or stored in the liver. Therefore, the liver plays a crucial role in the mediation of plasma lipid profiles.

The detailed mechanisms for the hypocholesterolemic effect of soymilk require further study. It is hypothesized that non-absorbed nitrogen-containing substances are correlated with the lipid-lowering effect. Short-term intake of soymilk might not significantly affect plasma lipid profiles, but the hypolipidemic effect was observed after a 4-wk intake of soymilk.

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