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Dietary Soy Protein and Calcium Reduce Serum Lipid and Cholesterol in Rats Fed Fat-Enriched Diets

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Abstract

This study investigated the effects of dietary soy protein and Ca on the lipid profiles of rats fed fat-enriched diets. Rats were divided into two groups and fed either a casein-low Ca (Exp I) or ISP-high Ca diet (Exp II) for a control period of 4 weeks. The two groups of rats were again subdivided into 4 groups and fed one of 4 experimental diets for another 4 weeks. The experimental diets consisted of 18% beef tallow and 1% cholesterol, in which either 20% casein or ISP with one of two levels of Ca, high (1%) or low (0.1%). The concentrations of total lipid, cholesterol and triglyceride in serum, liver and feces were determined. At the end of the control period, the serum total lipid and cholesterol concentrations were low in the rats fed ISP-high Ca diet (67~76% and 83~86%). During the next 4 week period, these concentrations remained significantly lower in rats fed the diets containing ISP and high Ca compared with those on casein and low Ca diets ($p<0.05$). Total lipid and cholesterol concentrations in feces were significantly higher in the ISP-high Ca dietary group at 4 weeks, and high in both high Ca groups at 8 weeks. This study demonstrates that both soy protein and Ca reduce serum and liver cholesterol, triglyceride, and total lipid in rats fed fat-enriched diet, and that they have an additive effect when combined.

Key words: soy protein, calcium, hypocholesterolemic effect, additive effect

INTRODUCTION

Coronary vascular disease (CVD) is the leading cause of death in many developed countries, and is the subject of ongoing intensive research (1). Several clinical trials have demonstrated that the incidence of atherosclerosis can be modified by diet, and of the dietary proteins, plant proteins, particularly soy protein, can lower the concentrations of atherogenic lipoproteins and sometimes to increase anti-atherogenic HDL (2). Numerous studies have shown that soy consumption improves plasma lipid profiles in animals and humans (1,3-7). A meta-analysis of human studies showed that soy protein intake is inversely associated with plasma concentrations of total cholesterol, LDL cholesterol and triacylglycerol (7). Supplimentation of diets with soy or substitution of soy protein for animal protein improved the lipid profiles in a variety of animal species (3,4), particularly in those with elevated total cholesterol concentrations (5,6).

Ca is also known to be a cholesterol-lowering agent. We previously demonstrated that increased dietary Ca reduced hyperlipidemia induced by a high cholesterol diet in mature rats (8). High dietary Ca intake also lowered the cholesterol and lipid in serum and tissues of several experimental animals. But the mechanism has not been clarified (9). An inverse association between stroke mortality and water hardness first led to the suggestion of an antihypertensive effect of calcium carbonate (10). Subsequent epidemiological studies across several countries and age groups presented a fairly consistent pattern of inverse associations between seated blood pressure and calcium intake from food (11-13).

The purpose of this study was to examine the effect of consumption of soy protein and calcium on the improvement of lipid profiles in serum and liver of animals with cholesterol-induced hyperlipidemia.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats, weighing about 200 g were housed in a controlled environment at 23±3°C, with equal 12h periods of light and dark, and had free access to deionized water and diet. Four kinds of experimental diet consisted of 18% beef tallow and 1% cholesterol with 20% protein from either casein (Maeil Dairy Co., Korea) or

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Weeks	1	2	3	4	5	6	7	8
Exp. I	Control period with casein-low Ca diet				Test period with four kinds of experimental diets			
Exp. II	Control period with ISP-high Ca diet				Test period with four kinds of experimental diets			

Fig. 1. Scheme of experimental design.

isolated soy protein (ISP : PP 500E, Ralston Purina International Co.) and one of two levels of Ca: high (1%) or low (0.1%). For the first control period of 4 weeks, rats were divided into two groups and fed the casein-low Ca diet to induce hyperlipidemic-like state (Exp. I) or ISP-high Ca diet to induce hypolipidemic-like state (Exp. II) (Fig. 1). After the control period, the two groups of rats were again subdivided into four groups (6~8 rats each) and fed one of the four kinds of experimental diets (casein-low Ca, casein-high Ca, ISP-low Ca, ISP-high Ca) for another period of 4 weeks *ad libitum*. Weight gain and diet intake were determined every other day.

The compositions of the diets are shown in Table 1. The mineral mixture was prepared according to AIN-93M diet, except for calcium. Calcium content was adjusted in accordance with the experimental protocol by the addition of calcium carbonate. Vitamin and cellulose were purchased from ICN (USA).

Sample preparation

Before sacrificing, all animals were fasted overnight and then fed for 1.5 hr to normalize for a post-absorptive state. One hour after feeding, blood was collected from aorta and portal vein, kept at 4°C for overnight. Serum was separated by centrifugation at 3,000 rpm for 20 min and stored at -20°C until analysis. After collecting the blood, the liver was immediately removed, rinsed with cold isotonic saline (NaCl 9 g/L), blotted, and freeze dried (Freeze-dryer 18, Labcon Co.). Feces were collected for 4 days before sacrifice and freeze dried. Total lipids in serum was determined as described by Fringe and Dunn (14). Serum cholesterol level was determined by the method of Zlatkis and Zak (15), and triglyceride in serum by the method of Biggs et al. (16). Total cholesterol and triglyceride in tissue and feces were determined as the same methods after extracting the total lipid as by Folch et al. (17).

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

Ingredients	Casein		ISP	
	low Ca	high Ca	low Ca	high Ca
Starch	510.0	487.5	510.0	487.5
Casein	200.0	200.0	-	-
ISP	-	-	200.0	200.0
Beef tallow	180.0	180.0	180.0	180.0
Cholesterol	10.0	10.0	10.0	10.0
Choline bitartrate	2.5	2.5	2.5	2.5
Cellulose	50.0	50.0	50.0	50.0
Vitamin mixture	10.0	10.0	10.0	10.0
Mineral mixture (Ca-free)[1)]	35.0	35.0	35.0	35.0
Ca carbonate	2.5	25.0	2.5	25.0

[1)]Mineral mixture (Ca-free) was prepared according to AIN-93M composition.

Statistical analysis

Data are expressed as the mean ± standard error, and analyzed for two kinds of protein and the two levels of calcium using Duncan's multiple range test and Student's t-test.

RESULTS AND DISCUSSION

Body weight and food intake

There were no significant differences in final body weight, weight gain or food intake among the groups (Table 2).

Lipid profile in aortic serum

The aotic serum lipid concentrations of the rats fed experimental diets are shown in Table 3. The concentrations of total lipid, cholesterol, and triglyceride in aortic serum were significantly lower in the ISP-high Ca group than in the other groups. Feeding the casein-low Ca diet for 8 weeks increased the concentrations of cholesterol, total lipid and triglyceride by 218%, 147% and 123%, respectively; while the substitution of ISP-high Ca diet for casein-low Ca diet after 4 weeks, decreased concentrations of total lipid and triglyceride, and only slightly increased cholesterol, suggesting a lipid-lowering effect of ISP-high Ca diet.

In Exp II, the rats were fed the ISP-high Ca diet for the 4 week control period, instead of the casein-low Ca diet, to examine the effect of ISP-high Ca diet more clearly. Total lipid, cholesterol and triglyceride concentrations in aortic serum were lower in ISP-high Ca group than in the other groups. Feeding ISP-high Ca diet for 8 weeks resulted in a lower total lipid concentration, however, substitution of casein-low Ca diet or ISP-low Ca diet after 4 weeks reversed the trend and caused a significant increase (65,59%) in total lipid concentration. The lipid-lowering effect was more pronounced in the high Ca diet than in the ISP diet ($p < 0.05$). There have been several reports suggesting a cholesterol-lowering effect of Ca (9,18), and we also found dietary Ca intake was effective to moderate

Table 2. Body weight and food intake of the rats fed experimental diets

	Initial body weight (g)	Final body weight (g)	Food intake (g/d)	Weight gain (g/d)
Exp I				
Control period				
Casein-low Ca	198.6±7.2[1)]	357.2±17.0	18.6±0.7	5.7±0.7
Test period				
Casein-low Ca	198.5±7.0	424.0±13.5	18.3±0.7[a2)]	4.1±0.4
Casein-high Ca	199.0±4.8	454.0±24.2	21.2±0.7[b]	4.6±0.4
ISP-low Ca	197.0±7.6	412.7±20.9	18.8±0.1[ab]	3.9±0.4
ISP-high Ca	196.4±1.4	435.0±14.3	18.9±0.7[ab]	4.3±0.3
Exp II				
Control period				
ISP-high Ca	196.3±5.3	339.3±12.4	21.2±0.9	5.1±0.3
Test period				
Casein-low Ca	196.3±6.5	441.7±10.1	18.5±0.5[a]	3.9±0.6
Casein-high Ca	197.8±2.8	445.4±24.3	20.2±0.7[ab]	3.5±1.0
ISP-low Ca	197.6±7.6	421.6±23.3	19.8±1.0[ab]	4.3±0.4
ISP-high Ca	195.2±3.5	440.4±15.3	20.8±0.2[b]	4.4±0.3

[1)]Mean ± SE.
[2)]Values with different superscript within the column are significantly different at $p<0.05$.

Table 3. The concentration of lipids in aortic serum of the rats fed experimental diets

	Total lipid (mg/100 mL)	Cholesterol (mg/100 mL)	Triglyceride (mg/100 mL)
Exp I			
Control period			
Casein-low Ca	483.3±10.4[1)]	137.2± 9.9	57.8±10.9
Test period			
Casein-low Ca	709.7±12.9[a2)]	298.9±35.0[a]	71.2± 7.8[a]
Casein-high Ca	671.0±15.9[a]	221.2±25.6[ab]	44.1± 4.5[b]
ISP-low Ca	632.3±34.1[a]	203.9±27.1[b]	42.7± 5.0[b]
ISP-high Ca	438.7±34.2[b]	168.6±19.3[b]	33.9± 1.7[b]
Protein sources	p<0.05	p<0.05	p<0.05
Ca levels	p<0.05	p<0.05	p<0.05
Protein * Ca	p<0.05	p<0.05	p<0.05
Exp II			
Control period			
ISP-high Ca	367.5±19.2	118.5±11.2	37.5±2.7
Test period			
Casein-low Ca	605.5±46.5[a]	275.4±29.3[a]	59.8±8.7[a]
Casein-high Ca	516.1±12.9[ab]	183.8±10.6[b]	47.0±3.2[ab]
ISP-low Ca	586.0±23.0[a]	178.4± 8.3[b]	44.5±2.4[ab]
ISP-high Ca	400.0±38.2[b]	140.0±14.3[b]	32.2±1.7[b]
Protein sources	NS[3)]	p<0.05	p<0.05
Ca levels	p<0.05	p<0.05	NS
Protein * Ca	p<0.05	p<0.05	p<0.05

[1)]Mean ± SE.
[2)]Values with different superscript within the column are significantly different at $p<0.05$.
[3)]Not significantly different at $p<0.05$.

the hyperlipidemia induced by a high cholesterol diet in mature rats (8). Cholesterol and triglyceride concentrations also were lowest in ISP-high Ca group and were highest in casein-low Ca group. These results demonstrate that ISP and high Ca can improve the lipid profiles in aortic serum of the rat fed fat-enriched diet, and that the effects are additive.

Lipid profile in portal serum

The concentrations of lipids in portal serum of the rats are shown in Table 4. In Exp I, total lipid and cholesterol concentration in portal serum were significantly lower in the ISP-high Ca group than in the other groups. ISP was shown to be more effective at lowering total lipid and cholesterol than casein, but dietary Ca did not significantly af-

Table 4. The concentration of lipids in portal serum of the rats fed experimental diets

	Total lipid (mg/100 mL)	Cholesterol (mg/100 mL)	Triglyceride (mg/100 mL)
Exp I			
Control period			
Casein-low Ca	548.6±43.2[1)]	146.7± 3.8	51.6±2.7
Test period			
Casein-low Ca	856.6±49.3[a2)]	293.5±11.1[a]	84.9±7.9[a]
Casein-high Ca	824.6±35.5[a]	277.4±25.7[a]	41.3±4.8[b]
ISP-low Ca	759.7±59.6[a]	212.6±31.0[b]	64.6±1.8[a]
ISP-high Ca	586.9±59.5[b]	173.4±12.4[b]	66.4±7.8[a]
Protein sources	NS[3)]	p<0.05	NS
Ca levels	NS	NS	p<0.05
Protein * Ca	p<0.05	p<0.05	p<0.05
Exp II			
Control period			
ISP-high Ca	369.1±23.8	122.0± 0.6	32.8±2.7
Test period			
Casein-low Ca	905.5±42.8[a]	268.4±19.4[a]	57.4±3.0[a]
Casein-high Ca	775.8±12.9[a]	224.5±19.5[ab]	37.7±6.2[b]
ISP-low Ca	774.8±48.0[a]	188.5± 8.7[b]	37.7±3.1[b]
ISP-high Ca	517.2±58.3[b]	171.8± 4.7[b]	33.3±4.0[b]
Protein sources	p<0.05	p<0.05	p<0.05
Ca levels	p<0.05	p<0.05	p<0.05
Protein * Ca	p<0.05	p<0.05	p<0.05

[1)]Mean ± SE.
[2)]Values with different superscript within the column are significantly different at $p<0.05$.
[3)]Not significantly different at $p<0.05$.

fect cholesterol in portal serum. Triglyceride concentration in portal serum was significantly lower in the casein-high Ca group than in the other groups. Further study is needed to explain why triglyceride was lower in portal serum of rats fed the casein-high Ca diet.

In Exp II, total lipid concentration in portal serum was significantly higher in the casein-low Ca group, casein-high Ca group and ISP-low Ca group than in the ISP-high Ca group, suggesting casein and/or low Ca diet increased total lipid concentration significantly. Cholesterol and triglyceride concentrations in portal serum was significantly increased up to 56% and 72% in the casein-low Ca group than in the ISP-high Ca group.

While total lipid, cholesterol and triglyceride concentrations were increased by continuous feeding of a casein-low Ca diet by 56%, 100% and 65%, respectively, as compared with those that continued on that diet for 8 weeks; rats that were switched to the ISP-high Ca diet after 4 weeks showed only a small increase (7%, 29% and 18%, Exp I). However, while total lipid and cholesterol concentration in rats continuously fed the ISP-high Ca diet increased only 40% and 41% respectively, the substitution of casein-low Ca diet for ISP-high Ca diet after 4 weeks resulted in increase of 145% and 120%, respectively (Exp II). Portal blood has been known to be the main excretion pathway of cholesterol synthesized endogenously, but in this study, the lipid profile of portal serum was similar to that of aortic serum.

Lipid profile in liver

Total lipid, cholesterol and triglyceride increased by 30%, 7% and 23% respectively, in rats continuously fed the casein-low Ca diet for 8 weeks compared with the levels after 4 weeks. However, the substitution of ISP-high Ca diet for casein-low Ca diet after 4 weeks resulted in a decrease of 13%, 9% and 24%, respectively (Table 5, Exp I).

Total lipid, cholesterol and triglyceride concentration in liver were significantly lower in the ISP-high Ca groups after switching from the casein-low Ca diet. Ca alone did not ignificantly affect liver lipids, even though there were trends showing decreased lipid concentrations with increased dietary Ca, suggesting ISP was more effective than Ca at lowering liver lipid concentrations. This result is not in accord with the results of Dougherty and Iacono (19), which demonstrated that cholesterol concentration in liver was significantly decreased by increased dietary Ca in a long term study. The difference between their study and ours may be explained by different time periods of the experiments. We also found that feeding high Ca diet for 8 weeks in Exp II was more effective for preventing the development of hyperlipidemia. In Exp II, total lipid and cholesterol concentrations were significantly low in the ISP-high Ca group than in casein-low Ca, casein-high Ca and ISP-low Ca groups, suggesting ISP and high Ca had an additive effect in the moderation the lipid profiles in liver of rats fed fat-enriched diet.

Table 5. The concentration of lipids in liver of the rats fed experimental diets

	Dry weight (g)	Total lipid (mg/g)	Cholesterol (mg/g)	Triglyceride (mg/g)
Exp I				
Control period				
Casein-low Ca	4.88±0.46	397.7±39.4	23.5±0.5	288.4±48.3
Test period				
Casein-low Ca	6.52±0.30[a]	519.0±22.6[a]	25.2±0.5[a]	353.5±17.9[a]
Casein-high Ca	6.63±0.17[a]	476.8±21.4[a]	25.5±1.2[a]	330.0±16.0[a]
ISP-low Ca	5.89±0.41[ab]	369.3±34.7[b]	23.7±0.8[ab]	312.8±30.1[a]
ISP-high Ca	5.09±0.44[a]	350.0±36.1[b]	21.0±1.3[b]	220.4±30.6[b]
Protein sources	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
Ca levels	NS[3)]	NS	NS	NS
Protein * Ca	NS	NS	NS	NS
Exp II				
Control period				
ISP-high Ca	3.91±0.18	313.5±20.9	20.6±0.8	174.6±22.5
Test period				
Casein-low Ca	6.35±0.26[a]	477.8±22.8[a]	27.2±1.2[a]	369.3±41.1[a]
Casein-high Ca	5.59±0.37[ab]	438.8±15.4[a]	25.5±0.9[a]	236.3±40.7[bc]
ISP-low Ca	4.91±0.27[b]	379.3±11.4[b]	25.3±0.9[a]	290.3±9.5[ab]
ISP-high Ca	5.06±0.21[b]	280.2±21.3[c]	20.1±1.4[b]	173.5±7.6[c]
Protein sources	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
Ca levels	NS	$p<0.05$	$p<0.05$	$p<0.05$
Protein * Ca	NS	$p<0.05$	$p<0.05$	$p<0.05$

[1)]Mean±SE.
[2)]Values with different superscript within the column are significantly different at $p<0.05$.
[3)]Not significantly different at $p<0.05$.

Lipid profile in feces

Table 6 shows fecal lipid concentrations from rats fed experimental diets. The difference between the protein sources was not significant, but high dietary Ca intake increased fecal total lipid and cholesterol concentrations significantly. That is, dry weight of the feces, fecal total lipid and cholesterol concentrations were significantly higher in the high Ca dietary groups than those on a low Ca diet. Therefore, the reduction in serum cholesterol by high dietary Ca might be explained by increased fecal excretion of lipids, which would be consistent with other studies (18,20). Drenick (20) reported that dietary Ca increased fecal total lipid; and Fleischman et al. (18) found that increased dietary Ca reduced serum cholesterol, phospholipid and triglyceride concentrations, and postulated that the effect was due to the observed increase in the excretion of lipids and bile acid in feces.

Numerous studies have shown that the incidence of atherosclerosis can be modified by diet, and that plant-derived proteins, especially soy, have a beneficial effect (21-23); however, the components of soy responsible for those beneficial effects and their mechanisms of action remain unclear. Terpstra et al. (24) suggested that the hypocholesterolemic action of soy protein might be due to increased fecal steroids. Also, recently, it was observed that the absorption of cholesterol was significantly lower from diets containing soy protein, but bile acid excretion was not significantly affected, supporting the hypothesis that the hypocholesterolemic effect of soy protein is mediated in part by decreased cholesterol absorption (25). However, in this study, there was no effect of soy protein on fecal cholesterol. Therefore, our results suggest that the cholesterol-lowering effect of ISP in serum and liver is not due to decreased cholesterol absorption, but appears to be related with the metabolism in the liver.

Table 6. Fecal lipid contents of the rats fed experimental diets

	Dry feces (g/d)	Total lipid (mg/d)	Cholesterol (mg/d)
Exp I			
Control period			
Casein-low Ca	1.09±0.07[1)]	111.2±15.7	23.6±0.5
Test period			
Casein-low Ca	0.90±0.07[a]	327.4±35.5[a]	26.1±2.2[a]
Casein-high Ca	3.30±0.19[b]	863.7±39.4[b]	52.9±3.0[b]
ISP-low Ca	1.18±0.13[a]	426.2±60.1[a]	29.8±3.4[a]
ISP-high Ca	3.60±0.19[b]	864.3±49.5[b]	58.4±8.2[b]
Protein sources	NS[3)]	NS	NS
Ca levels	$p<0.05$	$p<0.05$	$p<0.05$
Protein * Ca	NS	NS	NS
Exp II			
Control period			
ISP-high Ca	3.43±0.25	298.7±24.8	56.9±2.7
Test period			
Casein-low Ca	1.12±0.15[a]	254.0±49.2[a]	24.6±2.2[a]
Casein-high Ca	3.44±0.29[b]	950.9±49.3[b]	46.7±7.9[b]
ISP-low Ca	1.46±0.13[a]	375.9±30.4[a]	29.0±1.1[a]
ISP-high Ca	3.52±0.24[b]	856.1±44.8[b]	55.1±3.3[b]
Protein sources	NS	NS	NS
Ca levels	$p<0.05$	$p<0.05$	$p<0.05$
Protein * Ca	NS	$p<0.05$	NS

[1)] Mean ± SE.
[2)] Values with different superscript within the column are significantly different at $p<0.05$.
[3)] Not significantly different at $p<0.05$.

It has also been suggested that the decreased cholesterol concentration, relative to those observed with a casein diet, may be due to soy polypeptides or to non-protein impurities such as soy isoflavones or saponin (26). Phytoestrogenic isoflavones have been the subject of particular interest (1). Isoflavone from soybean are believed to be a most plausible factor in cholesterol-lowering, and several studies have reported a cholesterol-lowering effect of isoflavones (21,27). However, the positive effects have not been consistent in all studies. For example, a semipurified soy extract, rich in isoflavones, added to casein protein did not have lipid-lowering effects, and suggested that other components of soy such as saponins, phytic acid or the amino acid composition itself may be involved in the hypocholesterolemic effects (25,28). Another study showed that the isoflavone aglycon and saponin-rich extracts did not affect the serum lipid profile of cholesterol-fed rabbits even though the atherosclerotic lesion area of the aortic arch was significantly reduced in the isoflavone groups (22). Recently, Lovati et al. (29) suggested that if one or more soy peptides could reach the liver after avoiding intestinal digestion, they may elicit a cholesterol-lowering effect, and moreover, the protein moiety, devoid of the isoflavone components, would likely be responsible for major biochemical effect of soy protein. The soy protein isolates we used in this study were alcohol extracted and expected to contain minimal amounts of isoflavones and other alcohol extractable constituents. Therefore, the difference in lipid profiles in this study, between the ISP diet and casein diet, may have been due to the protein moieties and not due to the nonprotein impurities, suggesting that isoflavones can be ruled out as the active agents.

This study demonstrates that both soy protein and Ca reduce serum and liver cholesterol, triglyceride, and total lipid in rats fed a fat-enriched diet, and that they have an additive effect when combined. Although the mechanisms for the effects of Ca and soy protein on serum lipids remain elusive, the increased fecal fat excretion in the rats fed high Ca diet lends support for decreased lipid absorption as the mechanism for hypolipidemic effect of Ca.

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