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# Effects of Dietary Casein, Soy, and Methionine-Supplemented Soy on Serum Lipids Level in Rats

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## Abstract

**The objective of the current study was to determine the influences of dietary proteins and methionine on plasma lipid concentrations. Thirty growing male Sprague-Dawley rats were fed diets similar in all respects except that dietary protein was from either casein, soy protein isolate, or soy protein isolate supplemented with L-methionine (0.24%). The animals were fed experimental diets ad libitum for nine weeks. Plasma total-cholesterol concentrations were unaffected by the protein source or methionine supplementation. Plasma triglyceride concentrations were lower in rats of methionine supplemented soy protein diets (76 mg/dL) than in the rats fed casein or soy diet (120 mg/dL, 109 mg/dL, respectively). These results indicate that soy protein reduces plasma triglycerides relative to casein in rats fed cholesterol free diets, and that methionine-supplemented soy diets decrease plasma triglyceride concentrations more than soy protein alone.**

**Key words:** dietary protein (casein vs soy), methionine, plasma lipids

## INTRODUCTION

The effects of dietary plant and animal proteins on plasma lipid and lipoprotein levels have been examined extensively in recent years. The type of protein in the diet can affect the levels of plasma cholesterol in both humans (1) and experimental animals (2-4). For example, casein has a greater hypercholesterolemic effect in some species than does soy protein (5-7). Soybean protein has been shown to lower blood lipids in humans and some experimental animals (8-12).

Although the mechanism by which dietary proteins influence plasma cholesterol level is not yet fully understood, it is probable that it is mediated by specific amino acids or peptides in the proteins. Several earlier reports have demonstrated a plasma cholesterol-lowering effect of methionine in experimental animals fed low protein or methionine-deficient diets (13,14). When methionine was added to a diet containing adequate protein, the effect of methionine was diminished (15), suggesting that the effect of supplemental methionine is related to protein or methionine status. One study reported that the addition of 0.75% methionine to a 10% casein diet decreased plasma cholesterol, but increased it in those fed 25 and 50% casein diets (16). Thus, the effect of methionine on cholesterol appears to be influenced by the protein content of the animal diet. Addition of sulfur-containing amino acids, and not just methionine, was found to influence serum cholesterol concentrations in another study, but the results of that study were inconclusive (17).

The purpose of this study was to confirm the hypocholesterolemic effects of soy protein and to investigate whether supplementing soy protein with methionine influences plasma lipids.

## MATERIALS AND METHODS

### Animals and analysis

Thirty male Sprague-Dawley rats weighing 141±11 g were obtained from KLEC (Korea Life Engineering Corporation, Seoul, Korea). Animals were housed individually in stainless steel cages (10″×15″×7″) in a temperature-controlled room with an alternating 12 h light and dark cycle. All animals were fed Samyang rat chow and water ad libitum for one week after arrival. Rats were then randomly assigned to one of the three experimental diets (casein protein, soybean protein, or soybean+methionine) described in Table 1. DL-methionine (NRC Teklad diets, Madison, WI) was supplemented in the soy protein+methionine (0.24%) diet to make the methionine concentration equivalent to that in the casein diet. Body weights were recorded weekly. At the end of the 63 days experimental period rats were sacrificed under light anesthesia (ethyl ether). Blood samples were taken from the inferior vena cava, and centrifuged for 30 minutes at 3000 rpm at 4°C to obtain plasma, and frozen at -20°C for later measurements of total cholesterol and triglycerides. Plasma lipids were analyzed by enzymatic methods.

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**Table 1.** Composition of experimental diets

| Ingredient | Dietary groups | | |
|---|---|---|---|
| | Casein | Soybean | Soybean + Methionine |
| Casein[1)] | 20.00 | - | - |
| Soybean[2)] | - | 20.00 | 20.00 |
| Dextrin[3)] | 65.70 | 65.70 | 65.46 |
| Corn-oil[4)] | 5.00 | 5.00 | 5.00 |
| Cellulose[5)] | 3.80 | 3.80 | 3.80 |
| Mineral Mix[6)] | 3.50 | 3.50 | 3.50 |
| Vigamin Mix[7)] | 1.80 | 1.80 | 1.80 |
| Choline[8)] | 0.20 | 0.20 | 0.20 |
| Methionine | - | - | 0.24 |
| Total | 100.00 | 100.00 | 100.00 |

*Gross Energy, kcal/g: 3.878.
[1)]Casein high protein, Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 160030, It contains methionine 0.46%
[2)]Soy Assay Protein, Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 160480, It contains methionine 0.22%
[3)]Dextrin, Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 50740.
[4)]Corn-oil, Dong-Bang Yuo-Ryang Co. Yangpyung-Dong 4-2, Youngdongpo-Gu Seoul: KSH 2102.
[5)]Cellulose, Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 160390.
[6)]Mineral mixture (AIN-76), Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 170915.
[7)]Vitamin mixture(AIN-76), Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 40077.
[8)]Choline Bitartate, Supplied by U.S. Corning Laboratory Services Company, Teklad Test Diets, Madison, Wisconsin. Biological Test Material No. 30190.

### Statistical analysis

SAS (SAS, Cary, NC USA) was used for all statistical analyses. Values are expressed as mean ± SD. Comparison among the three different diet groups was performed using one-way analysis of variance and Duncan's multiple range test.

## RESULTS AND DISCUSSION

This study investigated the effects of the dietary animal protein (casein), plant protein (soybean protein isolate), and soybean protein with supplemental methionine on body weight and plasma lipids in growing male rats. There were no significant differences in initial body weights, but during the experimental period rats fed the casein diet gained significantly more body weight than the other groups and those fed the soy + methionine diet gained significantly more those than fed soy alone (Table 2).

Food intakes and food efficiency ratios (g of body weight gain/g of food ingested) (FER) are shown in Table 3. The casein group had a higher food intake than the groups on soy and soy + methionine diets; food intake was the same for the two soy diet groups. Rats fed the soy + methionine diets had a significantly higher FER than those fed the soy diet but was not significantly different from the casein diet group.

**Table 2.** Initial and final body weights of Sprague-Dawley rats

| Variables | Casein (N=10) | Soy (N=10) | Soy + Methionine (N=10) |
|---|---|---|---|
| Initial weight (g) | 146.9 ± 4.18[1)a2)] | 146.0 ± 4.18[a] | 143.6 ± 4.23[a] |
| Final weight (g) | 368.5 ± 14.2[a] | 307.4 ± 18.1[b] | 339.6 ± 35.5[c] |

[1)]Mean ± SD.
[2)]Means in the same row not sharing a common superscript are significantly different at $p < 0.05$.

**Table 3.** Effect of casein, soy, or soy + methionine on daily food intakes and food efficiency ratios

| Variables | Casein (N=10) | Soy (N=10) | Soy + Methionine (N=10) |
|---|---|---|---|
| Food intake (g/day) | 17.4 ± 0.66[1)a2)] | 15.2 ± 1.05[b] | 15.2 ± 1.26[b] |
| FER | 0.206 ± 0.008[a] | 0.168 ± 0.011[b] | 0.194 ± 0.024[a] |

[1)]Mean ± SD.
[2)]Means in the same column not sharing a common superscript are significantly different at $p < 0.05$.

There were no significant differences among the dietary groups in plasma total cholesterol, LDL-cholesterol, or HDL-cholesterol concentrations (Table 4). The present study does not support a hypocholesterolemic effect of soy protein vs. casein in rats, which is in agreement with other studies that found little or no effect of soy protein in normo-cholesterolemic people. Sugiyama et al. (17) reported that the methyl group of methionine is responsible for the cholesterol-elevating effect of methionine in rats. Furthermore, Morita et al. (18) demonstrated that the addition

**Table 4.** Effect of casein, soy, or soy + methionine on plasma lipids concentrations

| Variables | Casein (N=10) | Soy (N=10) | Soy + Methionine (N=10) |
|---|---|---|---|
| Total-Cholesterol (mg/dL) | 146 ± 29.0[1)a2)] | 131.2 ± 41.2[a] | 125.4 ± 46.2[a] |
| LDL-cholesterol (mg/dL) | 68.5 ± 11.7[a] | 65.7 ± 13.0[a] | 61.2 ± 11.1[a] |
| HDL-chloesterol (mg/dL) | 36.6 ± 14.1[a] | 33.1 ± 15.2[a] | 31.0 ± 11.3[a] |
| Triglyceride (mg/dL) | 120.1 ± 17.8[a] | 109.0 ± 27.7[b] | 76.0 ± 40.4[c] |

[1)]Mean ± SD.
[2)]Means in the same row not sharing a common superscript are significantly different at $p < 0.05$.

of 0.22% methionine to a 25% soybean protein diet, equilibrating the methionine content with a 25% casein diet, increased plasma cholesterol to the levels of rats fed the casein diet. In contrast, Moundras et al. (19) reported that when rats were fed 13% soybean protein, serum cholesterol concentrations were significantly higher than that in rats fed a 13% casein diet, but the addition of 0.4% methionine to the soy protein diet eliminated the effect. The previous study also demonstrated that adding 0.75% methionine to a 10% casein diet decreases plasma cholesterol, but increases plasma cholesterol in rats fed 25% and 50% casein diets (17). Thus the effect of methionine appears to be influenced by the protein status of animals, or perhaps by the amount of total dietary methionine, regardless of source. Serougne and Rukaj (20) reported that serum total and LDL-cholesterol were not altered by methionine supplementation, but Anderson et al. (21) observed that adding methionine to soy protein diets increased HDL-cholesterol in rats that were fed diets high in fat and cholesterol. Studies have fairly and consistently shown that soy protein decreases total and LDL-cholesterol concentrations (22). However, this study did not agree with most earlier studies. Neither LDL-cholesterol nor HDL-cholesterol was altered by methionine supplementation. This discrepancy in the effect of methionine may be attributable to a difference in the kind of methionine used, the amount of methionine in the diets, and / or in the level of supplemental methionine. Also the age and type of rats and lipid contents of diets may be factors that were responsible for the differences.

Plasma triglyceride concentrations were decreased by the addition of methionine to the soy protein diet, which was consistent with the hypolipidemic effect of methionine-supplemented diets previously demonstrated in chemically induced diabetic rats (21). Atherogenic indices are shown in Table 5. It is firmly established that a reduction in LDL cholesterol can prevent both new and recurrent coronary artery disease; increases in HDL cholesterol are also generally considered to be beneficial. Rats fed soy + methionine diets tended to have lower athrogenic indices, HTR (HDL-cholesterol/Total cholesterol) and LHR (LDL-cholesterol/HDL-cholesterol), although the differences were not significant.

**Table 5.** Effect of casein, soy, or soy + methionine on athrogenic indices in rats

| | | | |
|---|---|---|---|
| Athrogenic index[1] | 3.42 ± 1.76[2)a3)] | 3.81 ± 1.93[a] | 3.30 ± 1.09[a] |
| HTR[4] | 49.8 ± 13.5[a] | 57.3 ± 14.4[a] | 47.5 ± 5.8[a] |
| LHR[5] | 2.18 ± 0.93[a] | 2.37 ± 0.94[a] | 2.05 ± 0.93[a] |

[1]Athrogenic index: (Totalcholesterol - HDL-cholesterol) / HDL-cholesterol.
[2]Mean ± SD.
[3]Means in the same row not sharing a common superscript are significantly different at $p < 0.05$.
[4]HTR: (HDL-cholesterol / Total-cholesterol).
[5]LHR: (LDL-cholesterol / HDL-cholesterol).

The most important outcomes of this study are that ad libitum-fed, normo-cholesterolemic, growing rats eating a methionine supplemented soy protein diet had decreased blood triglyceride concentrations and a lower atherogenic index, indicating a reduced risk of heart disease compared with rats fed a casein diet or soy protein diet without supplemental methionine.

## SUMMARY AND CONCLUSION

Effects of dietary proteins and methionine on plasma triglyceride and cholesterol were studied in Sprague-Dawley rats fed diets with proteins from casein, soybean, or soybean + methionine for 9 weeks. The following conclusions can be drawn from the data:

1. Body weight gain was significantly lower for rats fed soy protein compared to those fed either casein or soy + methionine diets.
2. Rats fed soy protein diets supplemented with methionine had significantly higher food efficiency ratios than rats fed soy protein alone.
3. There were no significant differences in mean plasma total cholesterol concentrations among the three dietary groups.
4. Plasma triglyceride concentrations were highest in rats fed casein diets and lowest in those fed soy + methionine diets.

In conclusion, these data demonstrated a hypotriglyceridemic effect of soy protein that is increased with methionine supplementation. These results suggest that soy protein diets, with appropriate amounts of methionine, may be of potential value in treating hypertriglyceridemic individuals. Although the present study demonstrates an effect of methionine on plasma triglyceride levels in growing male rats, the mechanism remains to be elucidated.

## REFERENCES

1. Meinertz H, Nilausen K, Faergeman D. 1989. Soy protein and casein in cholesterol-enriched diet: effects on plasma lipoproteins in normolipidemic subjects. *Am J Clin Nutr* 50: 786-793.
2. Terpstra A, HM, Van Tintelen G, West CE. 1982. The effect of semipurified diets containing different proportions of either casein or soybean protein on the concentration of cholesterol in whole serum, serum lipoproteins and liver in male and female rats. *Atherosclerosis* 42: 85-95.
3. Terpstra, AHM, Laitlnen L, Stucchi AF, Nicolosi RJ. 1994. The effects of semipurified diets containing two levels (20% and 40%) of either casein or soybean protein isolate and concentrate on plasma lipids in hamsters. *Nutr Res* 14: 885-895.

4. Terpstra AHM, Holmee JC, Nicolosi RJ. 1990. The hypocholesteroemic effect of dietary soybean protein vs casein in hamsters fed cholesterol-enriched or cholesterol-free semipurified diets. *J Nutr* 121: 944-947.
5. Forsythe WA. 1986. Comparison of dietary casein or soy protein effects on plasma lipids and hormone concentrations in the gerbil. *J Nutr* 116: 1165-1171.
6. Potter SM, Bakhit R, Essex-Sorlie D, Weingartner K, Chapman K, Winter L, Nelson A, Nelson R, Ham J, Savage W, Prabhudesai M, Erdman JW. 1993. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *Am J Clin Nutr* 58: 501-506.
7. Sirtori CR, Zucchi-Dentone C, Sirtori M, Gatti E, Descovich GC, Gaddi A, Cattin L, DaCol PG, Senin U, Mannarino E, Avellone G, Colombo L, Fragiacomo C, Noseda G, Lenzi S. 1985. Cholesterol-lowering and HDL-raising properties of lecithinated soy proteins in type II hyperlipidemic patients. *Ann Nutr and Metab* 29: 348-357.
8. Carroll KK. 1991. Review of clinical studies on cholesterol-lowering response to soy protein. *J Am Diet Assoc* 91: 820-827.
9. Wong WW, Smith EO, Stuff JE, Hachey DL, Heird WC, Pownell HJ. 1998. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am J Clin Nutr* 68: 1385S-1389S.
10. Tonstad S, Smerud K, Hoie L. 2002. A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins, and plasma total homocysteine in hypercholesterolemic subjects. *Am J Clin Nut* 76: 78-84.
11. Hermansen K, Sondergaard M, Hie L, Carstensen M, Brock B. 2001. Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care* 24: 228-233.
12. Carroll KK, Kurowska EM. 1995. Soy consumption and cholesterol reduction: review of animal and human studies. *J Nutr* 125: 594s-597s.
13. Fillios LC, Mann GV. 1954. Influence of sulfur amino acid deficiency on cholesterol metabolism. *Metabolism* 3: 16-26.
14. Seidel JC, Nath N, Harper AE. 1960. Diet and cholesterolemia; V. Effects of sulfur-containing amino acids and protein. *J Lipids Res* 1: 474-481.
15. Leveille GA, Shockley JW, Sauberlich HE. 1962. Influence of dietary protein level and amino acids on plasma cholesterol of the growing chicks. *J Nutr* 76: 321-324.
16. Sugiyama K, Ohkawa S, Muramatsu K. 1986. Relationship between amino composition of diet and plasma cholesterol level in growing rats fed a high cholesterol diet. *J Nutr Sci Vitaminol* 32: 413-423.
17. Sugiyama K, Kushima Y, Muramatsu K. 1985. Effects of sulfur-containing amino acids and glycine on plasma cholesterol level in rats fed on a high cholesterol diet. *Agri Biol Chem* 49: 3455-3461.
18. Morita T, Oh-hashi A, Takei K, Ikai M, Kasaoka S, Kiriyama S. 1977. Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. *J Nutr* 127: 470-477.
19. Moundras C, Remesy C, Leyrat MA, Cemine C. 1988. Methionine deficiency in rats fed soy protein induces hypercholesterolemia and potentiates lipoprotein susceptibility to peroxidation. *Metabolism* 44: 1146-1152.
20. Serougne C, Rukaj A. 1983. Plasma and lipoprotein cholesterol in rats fed L-amino acid-supplemented diet. *Ann Nutr Metab* 27: 386-395.
21. Anderson JW, Johnstone BM, Cook-Newell ME. 1995. A meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 333: 276-282.
22. Nilausen K, Meinertz H. 1999. Lipoprotein (a) and dietary proteins: Casein lowers lipoprotein (a) concentrations as compared with soy protein. *Am J clin Nutr* 69: 419-425.

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