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Hypocholesterolemic Effects of Dietary Soybean vs. Casein Proteins in a Crossed Over Diets in Rat

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Abstract: To investigate the effects of dietary proteins on the level of serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL), 32 male Wistar rats were randomly divided in control and 3 experimental groups (E1, E2 and E3). The feeding regimes of rats were as follow: control, standard diet; E1, a cholesterol free diet containing 20% soybean protein; E2, a cholesterol free diet containing 20% casein and E3, a cholesterol free diet containing 10% soybean protein and 10% casein. The experimental period was 11 weeks but at the end of 7th week the diets of E1 and E2 groups were crossed over for the next 4 weeks. Blood samples were collected weekly, via the ophthalmic sinus and the serum levels TC, TG and HDL were measured. In comparison with control group, the results show that at the end of 7th week TC levels in E1 and E2 groups were significantly ($p<0.05$) increased while HDL level unchanged and the TC value of E2 was bigger (not significant) than E1. However by crossing over the diets, the TC level was significantly ($p<0.05$) diminished in E2 while TG value remarkably ($p<0.05$) increased. These results indicate that soybean protein may insert its hypocholesterolemic effect in hypercholestrolemic condition than in normolipidemic condition.

Key words: Soybean, casein, cholesterol, diet, rat

INTRODUCTION

This is about one century that the cholesterol-lowering effect of soybean protein has been recognized (Ignatowsky, 1908). The dietary intake of soybean protein is probably associated with reduced plasma cholesterol level and lower risk of coronary heart disease (Matthan *et al*., 2007; Adams *et al*., 2005).

Epidemiological, clinical and animal studies have been shown that dietary consumption of soybean protein significantly decrease the TC, low density lipoprotein cholesterol (LDL) and triglyceride levels and with a non significant increase in serum HDL concentration (Frank *et al*., 2006; Zhuo *et al*., 2004; Greaves *et al*., 2000; Crouse *et al*., 1999; Anderson *et al*., 1995). Since phytoestrogens, particularly the isoflavones have structural similarities to estrogen, it has been thought that isoflavones are probably the active components of soy and responsible for its beneficial effects (Sacks *et al*., 2006; Song *et al*., 2003; Demonty *et al*., 2002). However, the results of controlled clinical trials in human populations have been inconsistent and raise serious questions regarding the hypothesis that soybean and/or isoflavones lower serum cholesterol in a clinically relevant way (Dewell *et al*., 2006).

In the present research the potential hypocholesterolemic effect of soybean protein in normolipidemic and high lipidemic conditions was investigated in rat.

MATERIALS AND METHODS

Animal and diets: Male Wistar rats (~6 months old) were obtained from Razi Institute (Mashhad, Iran). The animals were fed a commercial nonpurified diet (Javaneh Khorasan, Mashhad, Iran) on arrival. After an adaptation period of 1 week, they were divided in control (n = 7) and 3 experimental groups (E1, E2 and E3; n = 8). The feeding of control group was continued by commercial diet while the experimental groups were transferred to semipurified diet added soybean and/or casein as follows; E1 group purified diet + 20% soybean protein, E2 group semipurified diet + 20% casein, E3 group purified diet + 10% soybean protein and 10% casein (Table 1). The feeding period was 11 weeks but in E1 and E2 groups, at the beginning of 8th week, the diets of both groups were crossed over for the last 4 weeks.

Animals were housed in plastic cages, with a bedding of wood chips, in a temperature controlled room (20°C) and a 12 h light:dark cycle. They were allowed access to

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Table 1: Composition of the semipurified diets

Ingredients	Amount: Soybean protein diet (%)	Casein diet (%)	Soybean protein/casein diet (%)
Casein	0	20	10
Soybean protein	20	0	10
DL-methionine	0	0.3	0.3
Corn starch	15	15	15
Sugar	50	50	50
Cellulose	5	5	5
Corn oil	5	5	5
Minerals	3.5	3.5	3.5
Vitamins	1	1	1
Choline chloride	0.2	0.2	0.2

food and water *ad libitum*. Animals were cared for and handled in accordance with the Iranian Society of Animal Care (member of International Animal Care Society) and also local Ferdowsi University of Mashhad animal ethics directions.

Analytical methods: Blood samples were collected weekly via the retroorbital sinus and the serum concentrations of TC and TG were measured enzymatically (Reactive Co., Iran). Serum very low density lipoprotein cholesterol (VLDL) + LDL were precipitated by a HDL Cholesterol Precipitating Reagent (Analyze Co., Iran) and the supernatant (the HDL fraction) was assayed for HDL. The concentration of cholesterol in the VLDL + LDL fraction was calculated as the difference between TC and HDL cholesterol.

Statistical analyses: One-way single factor ANOVA was used to compare more than two groups followed by student test (Microsoft Office Excel software) to detect differences between groups. For all test, $p<0.05$ was considered statistically significant. All results are expressed as the mean±SEM.

RESULTS

Intra group statistical analyses of data belong to control group show no significant differences in the TC, TG and HDL during the experimental period.

As shown in Fig. 1, the serum level of TC in E1 (from 69.4 at week 0 to 107 mg dL^{-1} at 7th week) and E2 (from 67.4 at week 0 to 117.6 mg dL^{-1} at 7th week) were significantly increased ($p<0.05$). After crossing over the E1 and E2 diets, although the serum level of TC still increased in E1 (up to 122 mg dL^{-1} at 11th week) but the level of TC in E2 significantly decreased (106 mg dL^{-1}) at 11th week when compared by 7th week.

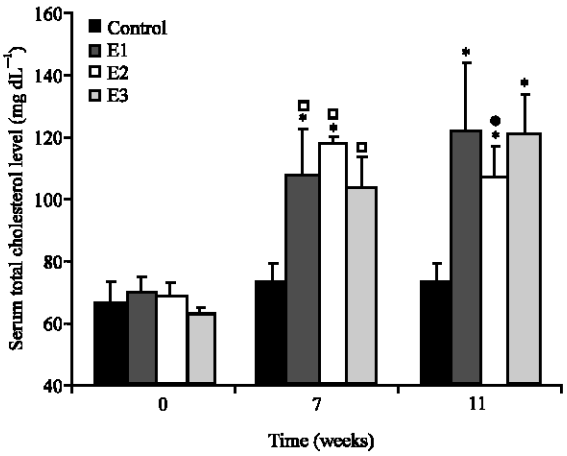

Fig. 1: Serum total cholesterol level (mg dL^{-1}) in control and experimental groups. Inter and intra groups statistical analysis comparisons (ANOVA and t-test) have been applied. Data are presented as mean±SEM. *$p<0.05$ compare experimental groups with control, ●$p<0.05$ compare week 0 with week 7, □$p<0.05$ compare week 7 with week 11. E1, E2, E3 are experimental groups treated by 20% soybean protein, 20% casein and 10% soybean protein+10% casein diets, respectively

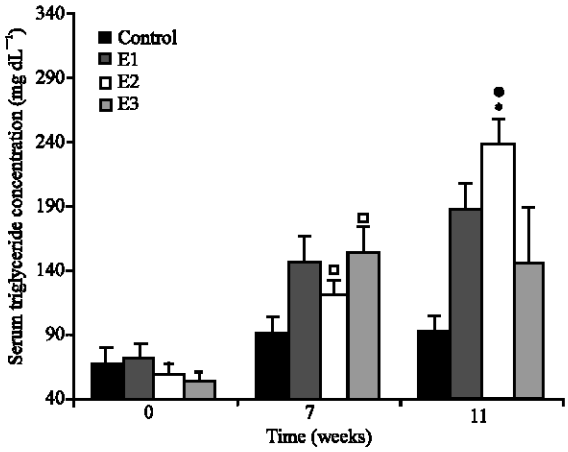

Fig. 2: Serum triglyceride concentration (mg dL^{-}) in control and experimental groups. Inter and intra groups statistical analysis comparisons (ANOVA and t-test) have been applied. Data are presented as mean±SEM. *$p<0.05$ compare experimental groups with control, □$p<0.05$ compare week 0 with week 7, ● $p<0.05$ compare week 7 with week 11. E1, E2, E3 are experimental groups treated by 20% soybean protein, 20% casein and 10% soybean protein+10% casein diets, respectively. with weeks 7 and 11

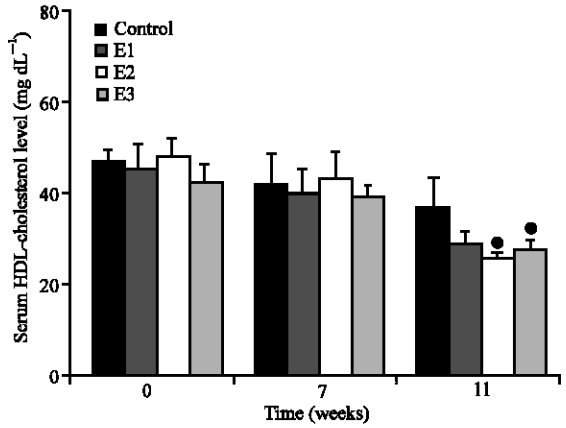

Fig. 3: Serum HDL-cholesterol level (mg dL^{-1}) in control and experimental groups. Inter and intra groups statistical analysis comparisons (ANOVA and t-test) have been applied. Data are presented as mean±SEM. ●p<0.05 compare week 0 with week 7 and 11. E1, E2, E3 are experimental groups treated by 20% soybean protein, 20% casein and 10% soybean protein+10% casein diets, respectively

Table 2: Mean body weights (g) of rats in control and experimental groups at weeks 0, 7 and 11

	Groups			
Weeks	Control	E1	E2	E3
0	350±16 n = 7	365±16 n = 8	321±21 n = 8	312±16 n = 8
7	365±13 n = 6	377±15 n = 7	369±19 n = 7	378±19 n = 7○
11	367±13 n = 6	381±15 n = 7	371±19 n = 7	382±19 n = 6○

Inter and intra groups statistical analysis comparisons (ANOVA and t-test) have been applied. Data are presented as mean±SEM. ○P<0.05 compare week 0 with week 7 and 11. E1, E2, E3 are experimental groups treated by 20% soybean protein, 20% casein and 10% soybean protein+10% casein diets, respectively

Intra group comparison (week 0 vs. week 7) of TG show that this parameter increased significantly ($p<0.05$) in E2 and E3 groups (Fig. 2). Moreover, after E1 diet crossed over by E2 diet, the serum level of TG increased significantly in E2 group (from 123 mg dL^{-1} at 7th week to 241 mg dL^{-1} at 11th week; $p<0.05$).

The data of HDL measurement (Fig. 3) show that the fluctuation of HDL level in control and experimental groups are almost similar. Intra group comparisons of HDL levels at week 7 by week 11 in E2 group show that after crossing over the E1 and E2 diets, the reduction of HDL level in E2 group is significant ($p<0.05$).

The body weight gain was similar for the animals of control and experimental groups which mean that the diets were well accepted by rats throughout the experiment (Table 2).

DISCUSSION

It has been well known that the type of protein in the diet can affect the levels of plasma cholesterol in both humans and animal models. High blood cholesterol concentration is a major risk factor for vascular disease and coronary heart disease (Grundy, 1995). There are numerous reports on beneficial effects of soybean protein which suggest that consumption of soy protein decreases serum total cholesterol and LDL concentrations in humans (Frank *et al*., 2006; Greaves *et al*., 2000; Teixeira *et al*., 2000; Crouse *et al*., 1999; Wong *et al*., 1998; Meinertz *et al*., 1989), rat (Fukui *et al*., 2002), hamsters (Potter *et al*., 1996) and monkey (Adams *et al*., 2005; Wagner *et al*., 2000; Greaves *et al*., 2000). However other studies have shown that different hypocholesterolemic effect of soybean protein becomes evident only in moderate (Cicero *et al*., 2002) or high hypercholesterolemic conditions (Meinertz *et al*., 1989).

Present results showed that dietary soy protein in hypercholesterolemic condition and not in normolipidic condition, lessened serum total cholesterol levels (Fig. 1; group E2, compared week 7 with week 11). Furthermore, the type of protein had little effect when low cholesterol diets were used and fed to normolipidemic animal (Demonty *et al*., 2003; Meinertz *et al*., 1989). These findings indicate that in normolipidemic rats soybean protein did not decrease the level of cholesterol but in hyperlipidemic condition it may lower the level of cholesterol. In E2 group, fed by cholesterol-free casein diet, the level of TG significantly increased at the end of week 11, therefore replacement of E1 and E2 diets with each other not only ceased TG elevation but also attenuated it, which means that soybean protein probably has hyper triglycedemic effect.

It has been recently reported that many soy products should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins and minerals and low content of saturated fat (Frank *et al*., 2006). However, in an extensive review (Dewell *et al*., 2006) it has been mentioned that, in regard to the hypocholesterolemic benefits of soybean protein and soy isoflavone, conclusions made by researchers and health agencies are perhaps too premature to make any recommendation for their use as an alternative to established therapies in the management of hypercholesterolemia in populations at risk for coronary artery disease (Cho *et al*., 2007; Clerici *et al*., 2007; Vega-López and Lichtenstein, 2005; Dewell *et al*., 2002).

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