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 follows: 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 hypercholesterolemic 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 (Dewett et al., 2006).

In the present research the potential hypocholesterolemic effect of soybean protein in normolipidemic and high lipemic 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
Table 1: Composition of the semipurified diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Soybean protein diet (%)</th>
<th>Casein diet (%)</th>
<th>Soybean protein/casein diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>0</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Soybean protein</td>
<td>20</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sugar</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Minerals</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

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.](image1)

![Fig. 2: Serum triglyceride concentration (mg dL⁻¹) in control and experimental groups.](image2)
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 hypcholesterolemic effect of soybean protein becomes evident only in moderate (Ciecro 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 triglyceremic effect.

It has been recently reported that many soy products should be beneficial to cardiovascular and overall health because of their high content of polysaturated 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 hypcholesterolemic 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).
REFERENCES


