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ORIGINAL ARTICLE

Effects of Soy Protein Isoflavones on Serum Lipids, Lipoprotein Profile and Serum Glucose of Hypercholesterolemic Rabbits

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The main objective of this research was to determine the effects of soy protein isoflavones on serum lipids, lipoproteins and fasting blood sugar levels in hypercholesterolemic rabbits.

Materials and Method: Twenty-four male New Zealand rabbits received the basic pellet diet for two weeks and were then placed on a hypercholesterolemic diet (pellets plus 1% cholesterol) for three weeks. After elevation of total cholesterol, the rabbits were randomly allocated into four experimental groups. Groups 1 to 3 received 100g soy protein containing 200mg (SPI+), 100mg (SPI50%) soy protein diet and without isoflavones (SPI-), respectively, for six weeks. The fourth group was kept on the hypercholesterolemic diet (HC).

Results: Findings showed that cholesterol rich diet produced significant increase in total, LDL- and HDL-cholesterol concentrations. In SPI+ group these parameters remained unchanged, compared with SPI- and SPI50% groups (P<0.0001). HDL-cholesterol was significantly elevated after administration of HC diet and remained high (almost three fold) in all soy diets relative to baseline. However, its level was significantly lower in SPI50% compared with SPI-group (p<0.01). Triglycerides and VLDL concentrations were significantly increased in SPI50% compared with baseline and HC groups (P<0.03). Fasting blood sugar levels were not changed in all soy treatment groups.

Conclusions: These results suggest that intact soy protein isoflavones ameliorate the lipid profile in spite of high-cholesterol intake, but has no obvious effect on blood sugar levels and can therefore be useful in hyperlipidemias especially when cholesterol intake is simultaneously decreased. Moreover, there is no direct dose-response relationship between soy isoflavone content and its lipid-lowering effect.

Key Words: Soy protein, Isoflavones, Lipid, Glucose

Introduction

Prospective, epidemiological studies have established that lipid and lipoproteins play a role as risk factors for atherosclerotic cardiovascular diseases (CVD). It is generally recognized that the higher the total cholesterol, the higher the mortalities of CVD.[1-4]

In recent years, a great deal of interest has emerged in the role of soy-bean isoflavones in reducing heart diseases, and isoflavones might be responsible, in part, for the ability of soybean to lower the risk of CVD and

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atherosclerosis.[4-5] Anderson in his review of 38 trials suggested that about 60-70% of the cholesterol lowering effect of soy protein may be due to its isoflavone content.[5] Isoflavones are a group of phytoestrogens which occur mainly in soy and its products receiving great scrutiny as food supplements for the purpose of both enhancing health and preventing several chronic diseases, including coronary heart disease, cancers of reproductive organs and osteoporosis.[6-9] Aglycone forms of soy isoflavones especially genistein and daidzein have been greatly studied because of their greater estrogenic and antioxidant activities.[10]

Soy isoflavones have been shown to decrease total, VLDL and LDL cholesterol levels while increasing HDL cholesterol levels in peripubertal rhesus monkeys fed soy protein-based diets.[11]

Anthony et al. in their study on young cynomolgus monkeys, fed the animals with diets containing either casein and lactalbumin, alcohol extracted soybean-protein isolate (S-), or unextracted soybean protein isolate (S+) as the source of protein.[12] The extent of coronary artery atherosclerosis was quantified on a subset of monkeys from each treatment group (S-, n18, S+, n15). At the end of study, the authors concluded that the average lesion size in the S+ group was approximately 70% smaller than in the S- group.[12]

In a randomized clinical trial, mildly hypercholesterolemic men and women were treated with protein supplements (25 g protein in each) which contained casein, alcohol-extracted soybean protein isolate (3mg isoflavones), or soybean protein isolate that contained 27, 37, or 62mg isoflavones per day. The treatment phase lasted 9 weeks and at the end, the 62 mg isoflavone group had significantly lower LDL- cholesterol concentrations than in the casein group while the alcohol- extracted soybean protein had no effect. The authors also reported a dose-response relationship between lowering of total and LDL-cholesterol concentrations with increasing isoflavone dose.[13] An additional support for the lipid lowering effect of soy isoflavones came from the Baum et al research in which two doses of isoflavones (56 and 90 mg/d) were given to postmenopausal women and it was seen that LDL+VLDL cholesterol was lower and HDL cholesterol was significantly higher in both groups than the control group.[14]

On the other hand, it is claimed that purified isoflavones have no effect on plasma lipid and lipoprotein concentrations in normolipidemic subjects.[15,16] At present, there is no general agreement about the effect of soy protein isoflavones (SPI) on lipid profiles and moreover, it is not clear that which part of the soy protein has lipid-lowering effects. In this study, an animal model was designed to assess the effect of SPI on serum lipid, lipoprotein profile, and blood sugar of experimentally-induced hypercholesterolemic rabbits, and to detect any dose-response effect of SPI on the above mentioned variables.

Materials and Methods

Animals: 24 male New Zealand rabbits, three months of age and mean body weight of 1650 +/- 100 g were purchased from the Razi Institute, Tehran, Iran. After two weeks of an adaptation period and feeding with standard pellets they were placed on the hypercholesterolemic diet (HC: 1% purified cholesterol, Merck, Germany) for the next three weeks. After HC diet, rabbits were then divided randomly into four groups as follows (six rabbits in each group):

SPI+ group: which received 100 g soy protein with 200 mg isoflavones (intact soy protein) and 1% cholesterol.

SPI- group: which received 100 g alcohol-extracted soy protein with trace amounts(< 5 mg) of isoflavones and 1% cholesterol.

SPI 50% group: which received 100 g soy protein with 100 mg isoflavones (half-dose isoflavone) and 1% cholesterol.

HC group: which continued the same HC diet. The soy treatment period lasted for 45 days.

Soy isoflavones: Textured soy protein concentrate was purchased from Karoon Soya factory, Ahvaz, its isoflavone content was determined by a modified HPLC method[17] and alcohol extracted SPI was prepared from it. The isoflavones contents of soy were: Genistin 51.2, malonyl genistin 42.4, genistein 2.4, daidzin 54.5, malonyl deidzin 34.4 and daidzein 13.5 mg in each 100 g textured soy protein concentrate. The rest of soy isoflavones were acetyl derivatives.

Analytical procedure for the determination of isoflavones: Textured soy protein concentrate was extracted with 80% methanol, and 20µl of filtered extracts were injected to HPLC (Cecil, CE 1000, UK). A reverse phase 125×4mm Eurospher-100 C18-5 column with a gradient system of elution was employed.

Mobile phase consisted of solvent A (5% acetic acid in water) and solvent B (methanol: acetonitrile+ dichloromethane, 100:50:10). Gradient system started with 90% solvent A, and reduced to 25% in 25 minutes and then increased to 90% in the next 5 minutes. Desmethylangolensin was used as an internal standard because it has some advantages over the flavone and flavanone which have been used previously.[17] Detection was carried out at 260nm. All 12 members of soy isoflavones were isolated successfully within 20 minutes.

Characteristics of assays: Extraction efficiencies and HPLC conditions were evaluated and optimized, leading to precision and spiking recovery of 3-7 % and 94-104 %, respectively.

Laboratory tests: Serum triglycerides, total and HDL – cholesterol concentrations of each rabbit were measured by enzymatic methods (Mann Kit, Iran) at base line, after HC period and after 45 days of soy treatments. VLDL and LDL-cholesterol levels were calculated. Fasting blood sugar (FBS) concentrations were measured after six weeks of triple soy treatments only. Body weights of rabbits were recorded in three phases of study for comparison of their actual intakes. Temperature was set at 24 degree C and water consumption was unrestricted.

Statistics: SPSS software version #10 was used for statistical analysis and ANOVA followed by Tukey's post hoc tests for comparison between and within groups and a 0.05 cut-off point was considered as the significant level.

Results

Table 1 presents the serum lipids and lipoprotein profile at three stages of the study. After the three week HC period, serum total cholesterol concentrations increased significantly. LDL- and HDL-cholesterol levels were also elevated ($P<0.0001$). In the SPI+ group, after six weeks treatment, serum total and LDL-cholesterol concentrations remained unchanged but in both SPI- and SPI50% these variables ($P<0.0001$) and also triglycerides levels ($P<0.03$) significantly increased. Blood sugar concentrations showed no changes in any of the three SPI treatment groups (Fig.1). Weight changes during triple soy diets treatments were similar and showed no significant differences.

Discussion

Isoflavones are a group of phytoestrogens which are mainly found in soy and soybean-based foods in the human diet. The major dietary isoflavones are the glycosides of genistein and daidzein which have been shown to have estrogenic or anti-estrogenic activity in vitro[18] and in vivo.[19,20] Hypocholesterolemic effects of soy protein have been demonstrated in experimental animals and human subjects,[21,22] and it has been suggested that isoflavones could mainly be responsible.[5] Extensive evidence has been derived from researches which applied isoflavones in conjunction with soy protein but the effect of soy protein supplementation after removal of isoflavones remains unclear.

In this research, the effect of soy protein containing 200 mg, 100 mg and a trace amount of both glycoside and aglycone forms

Table 1. Comparison of lipid profile of rabbits at the end of each stage (mg/dl)*

		D	I	E	T	S
Variables	**Baseline**	**HC**	**SPI +**	**SPI -**		**SPI 50%**
TC	43.1(12.6)	415.3(122.6)†	517.2(162.1)	1050.4(202.8)‡		1243.5(122.4)
TG	42.2(17.4)	39.3(21.1)‖	51.6(23.3)	84(39.2)		113.5(27.5)§
LDL-C	15.1(8.9)	367.4(125.9)†	450.2(168.4)	963.8(196.9)‡		1187.8(121.9)
HDL-C	18.2(4.3)	40.1(9.9)†	56.6(12.6)	58.8(16.1)		33(5.9)‖
VLDL-C	8(2.7)	7.8(4.2)	10.4(4.7)	17(7.6)		22.6(5.3)§

* Values are means (SD); †- P<0.0001 Compared with baseline; ‡- P<0.0001 Compared with SPI+; §- P<0.03 Compared with baseline HC and SPI+; ‖- P<0.01 Compared with SPI-
TC: Total Cholesterol; TG: Triglycerides; HC: Hypercholesterolemic diet
SPI + : Soy protein with intact isoflavones (200mg)
SPI - : soy protein without isoflavones (<5 mg)
SPI 50%: Soy protein with half- dose isoflavones (100mg)

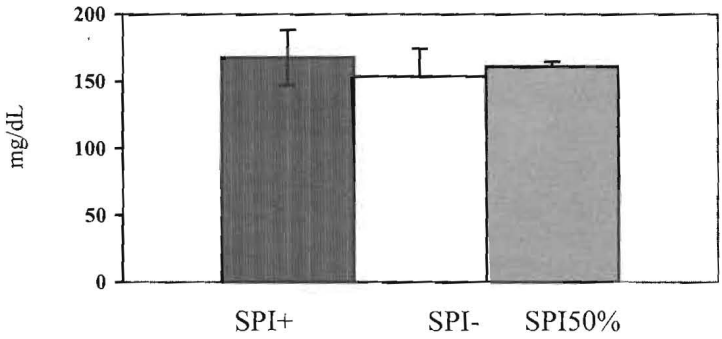

Fig.1. Serum glucose levels in the three soy treatments at the end of study

of soy isoflavones were assessed in hyper cholesterolemic male rabbits. Although the rabbits had a cholesterol-rich diet, the serum total and LDL-cholesterol remained unchanged in the SPI+ group (i.e. with intact soy protein diet). The lipid profile showed a deterioration in both SPI- and SPI50% groups and unanticipated findings were seen in the latter group. Some researchers have suggested that 30-50mg isoflavones per day is necessary for achieving a biological effect in humans.[23] Hence, the effective dose in experimental animals, due to their faster blood circulation, should be higher.

Wangen et al also have reported a small (6.5%) but significant reduction in LDL-cholesterol concentration in the high-isoflavone diet (132 mg/d) and an almost significant decrease in the low isoflavone diet (65 mg/d) compared with the control group in normo- and mildly hypercholesterolemic postmenopausal women.[24] Nestel[15] and Hodgson[16] however have shown that 80 and 55 mg purified isoflavones have no significant cholesterol-lowering effect on normolipidemic subjects. It is important to remember aglycon forms of soy isoflavones i.e. genistein and daidzein have more estrogenic activity,[25] but in the present research the amounts of free forms were much lower than the contents of glycosidic forms. The final effect therefore may be different when the isoflavones content varies.

Kirk et al. evaluated the effects of diets containing either intact or phytoestrogen-extracted soybean protein; on atherosclerosis, in two strains of mice, LDL receptor-deficient and wild-type (C57BL/6) mice and observed that the lesion area in wild-type mice was significantly smaller in those fed on intact or unextracted soybean protein as compared with phytoestrogen-extracted soybean protein, no differences however in the extent of atherosclerosis was seen in the LDL receptor-deficient mice.[26]

Anthony also reported that soybean isoflavones inhibit atherosclerotic plaque progression in surgically postmenopausal cynomolgus monkeys, which is comparable with those, treated with conjugated equine estrogens.[27] Both these researches have indicated that isoflavones modulate plasma cholesterol concentrations by increasing LDL receptor activity and thereby inhibiting atherosclerosis.

In our study, in spite of the Crouse et al. findings,[13] total, LDL- and VLDL-cholesterol levels were higher in SPI50% than that of both the SPI+ and SPI- groups, suggesting that there was no dose-response relationship between isoflavones intake and serum lipids variations. Therefore, the effect of isoflavones may be in association with soy protein or its other components. Weggemans and Trautwein in their meta-analysis article of ten clinical trials also reported that the changes in LDL- and HDL-cholesterol levels were not correlated to soy-associated isoflavones.[28] HDL-cholesterol elevation after the HC period may be due to high cholesterol intake and it remained elevated in both the SPI+(intact) and SPI-(alcohol extracted) groups but it was attenuated in the SPI50% group. The triglycerides changes in the latter group were reciprocal to HDL-cholesterol variations. Anthony et al. have shown that HDL-cholesterol increased 50% after +soy isoflavones treatment in monkeys but in the – soy isoflavones group the increment was 20%.[2] Furthermore, in gerbils, apoprotein A-1 levels significantly increased after one month soy protein isoflavones treatment.[29]

At present, the findings about HDL-cholesterol and triglycerides concentrations are inconclusive.

In this study, it is shown that soy isoflavones have no significant effect on serum sugar concentrations. Vedavanam et al. showed that soybean phytochemical extract (SPE) may act as an inhibitor of glucose uptake in the rabbit intestinal brush border membrane vesicles in vitro.[30] However, the effect of soy isoflavones on blood sugar levels has not been clearly mentioned in literature and needs further research.

The results of this research have indicated that soy protein isoflavones maintained the serum lipid and lipoprotein levels in hypercholesterolemic rabbits kept on a high-cholesterol diet, but alcohol-extracted (even half-dose isoflavones) soy protein diets do not have positive effect. Moreover, the hypocholesterolemic effect of isoflavones is not in a dose-response manner and it is suggested that isoflavones activity is closely related to soy protein.

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