ORIGINAL ARTICLE

Effects of Soy Protein Isoflavones on Lipid Profile and Serum Hormones in Hypercholesterolemic Men

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Isoflavones are a group of phytoestrogens found mainly in soy bean and its products. Their actions on various tissues have motivated researchers to assess the possible related mechanisms and functions. The main objective of this study is to determine the metabolic effects of purified alcohol-extracted soy protein isoflavones (SPI) on serum lipoproteins and hormones of mild to moderate hypercholesterolemic male subjects. The effects of SPI on this type of patients and on the serum hormone levels have not been evaluated previously.

Materials and methods: 30 male volunteers were randomly divided into two groups in a double-blind parallel randomized placebo-controlled trial (n=15). Group 1 received 50mg purified alcohol-extracted soy isoflavones solution and group 2 received placebo in a similarly colored solution for an 8-week period. Both groups were matched for age, duration of illness, medications, and diet. Data on other variables of each subject i.e. body mass index, smoking habits, blood pressure, and past medical history were also collected.

Results: LDL-, VLDL-, and HDL-cholesterol, Tg and thyroxine, triiodothyronine, FSH, testosterone and fasting blood sugar levels did not change significantly compared to their baseline levels. Total cholesterol decreased by 10 percent, ($p=0.055$). TSH levels in SPI group showed a significant rise after intervention ($p<0.05$), remaining, however, within normal range.

Conclusion: These results indicate that alcohol-extracted SPI without soy protein does not affect serum lipid profiles and hormones in hypercholesterolemic men. More trials with larger number of subjects conducted over a longer period of study are needed to confirm these findings.

Key words: Soy protein isoflavones, Lipid, Hormones, Hypercholesterolemic men

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Introduction

In recent years, there has been a great interest in the role of soy bean isoflavones in reducing cardiovascular diseases (CVD), and isoflavones might be responsible in part for this ability of soy bean to lower the risk of CVD and atherosclerosis.[1-4] Anderson (1995) in his review of 38 trials has suggested that about 60-70% of cholesterol lowering effect of soy protein may be due to its isoflavone content.[5] Isoflavones as a group of phytoestrogens which occur mainly in soy and its products are being carefully scrutinized 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 intensively studied because of their remarkable 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]

In another study of young Cynomolgus monkeys, the animals were fed on diets containing either casein and lactalbumin, alcohol extracted soy bean protein isolate (S-), or unextracted soy bean protein isolate (S+) as the source of protein.[12] Coronary artery atherosclerosis extent was quantified on a subset of monkeys from each treatment group. At the end, 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 (n=94) and women (n=62) were treated daily with protein supplements (25 g protein in each) which contained casein, alcohol-extracted soy bean protein isolate (3mg isoflavones), or isolated soy bean protein that contained 27, 37, or 62mg isoflavones. The treatment phase lasted 9 weeks and at the end, the 62 mg isoflavone group had significantly lower LDL-cholesterol concentrations than the casein group while the alcohol- extracted soy bean protein had no effect. The authors also reported a dose-response relationship between progressively lower total and LDL-cholesterol concentrations and increases in isoflavone doses.[13]

Additional support for the lipid lowering effect of soy isoflavones comes from the Baum et al research in which two doses of isoflavones (56 and 90 mg/d) were given to postmenopausal women and the results of the study showed that LDL+VLDL cholesterol was lower and HDL cholesterol increased significantly in both groups as compared to 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] Because of lack of data on the effect of purified soy protein isoflavone (SPI) in hypercholesterolemic men, the present research aims at determining the effect of SPI on serum lipid profiles and certain hormones in this type of patients.

Materials and Methods

Subjects

30 mild to moderate hypercholesterolemic males (serum total cholesterol level between 200 to 300 mg/dL and serum triglyceride levels below 400 mg/dL) men, referring to a private cardiologist's clinic, were recruited for the study and randomly divided into two groups (n1, n2=15) in a double-blind randomized placebo-controlled trial (RCT) design. Group 1 received 50 mg purified alcohol-extracted soy protein isoflavone (SPI) solution and group 2 took placebos in a similarly colored solution for 8 weeks. Subjects did not consume any other soy products during the study, the amounts of isoflavone intake from foods being controlled. All cases were also matched for alcohol consumption, blood lipid- and sugar-lowering drugs, duration of cigarette smoking and body mass index (BMI). Food frequency and 24-hour recall questionnaires were also completed. All subjects were requested to follow their usual dietary and activity habits. There was no history of hepatic and renal disease in any of subjects. One subject in the SPI group had mild and controlled diabetes mellitus. No significant weight changes were seen in either group during the study. The protocol of study was officially approved by the Medical Ethics Committee of Ahwaz University of Medical Sciences (project No.173).

Soy isoflavones

Textured soy protein concentrate was purchased from the Karoon Soya Factory, Ahwaz and then alcohol extracted SPI was prepared using ethanol 70%. Cherry essence was added to both SPI and placebo solutions. Soy isoflavone contents were detected by HPLC and they included: 18 mg daidzin, 3 mg gly-

citin and 25 mg genistin, 1 mg genistein, and 3 mg daidzein.

Analytical procedure for measurement of isoflavones

Samples were extracted with 80% methanol, and 20µl of filtered extracts were injected to HPLC (Cecil, CE 1000, UK). A reverse phase 125×4 mm 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 increased to 90% in 5 minutes. Desmethylangolensin was used as internal standard. This agent has some advantages over the flavone and flavanone, which have been used as internal standards previously.[17] Detection was carried out at 260 nm. All 12 members of soy isoflavones were isolated successfully within 20 minutes.

Variables

For all subjects, weight, height, body mass index, systolic and diastolic blood pressure, smoking habits, disease and drug histories were obtained. Food frequency and 24-hour food recall questionnaires were completed for all subjects at baseline and at the end of the 8-week intervention period.

Laboratory tests

Serum triglycerides, fasting blood sugar (FBS), total and HDL-cholesterol concentrations were measured by enzymatic methods (Mann Kit, Iran) at base-line and after 8 weeks. VLDL and LDL-cholesterol levels were then calculated and serum thyroxine (T_4) and triiodothyronine (T_3), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), and testosterone levels were measured by the enzyme immunoassay (EIA) method using Opticoat kit (Biotecx Lab. Inc, USA).

Statistics

SPSS software version 10 was used for statistical analysis and independent and paired t-tests were performed for comparisons between and within groups, respectively. 0.05 cut-off point was considered significant.

Results

Table 1 represents the baseline characteristics which indicate no differences between the two groups. Table 2 shows the lipids and lipoprotein profiles in the SPI and placebo groups before and after 8 weeks treatment. There were no statistically significant changes in any lipid profiles. However, the reduction in total cholesterol concentrations in the SPI group as compared to baseline levels reached borderline significance (p=0.055).

Table 1. Baseline characteristics

	SPI (n=15)	Placebo (n=15)
Age (y)	50.3±7.5	42.8±10.3
BMI (Kg/m^2)	26.4±3.2	25.9±2.3
SBP (mm Hg)	130±26.8	124.3±8.7
DBP (mm Hg)	80.8±17.3	82.9±7.5

Values are mean±SD

SPI: Soyprotein isoflavones; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure

Independent t-test showed no significant difference in baseline values between SPI and placebo groups

Table 3 shows serum hormones values. There is a significant increase in serum TSH concentrations after 8 weeks of soy isoflavone treatment (p<0.05). Serum thyroxine, triiodothyronine, FSH and testosterone levels did not change significantly after SPI intervention. There were no statistically significant mean weight changes in either group. Dietary habits of all subjects remained unchanged during the eight weeks of the study (data not shown here).

Discussion

Isoflavones belong to a class of phytoestrogens that are constituents of soy and soy bean-based foods in the human diet. The major dietary isoflavones are the glycosides of

Table 2. Comparison of lipid profiles and FBS levels at baseline and 8 weeks post intervention

	SPI (n=15)		Placebo (n=15)	
	Baseline	Post	Baseline	Post
Total cholesterol (mg/dL)	241±41	229±29	234±36	234±39
LDL-Cholesterol (mg/dL)	134±44.6	113.5±30.8	152.2±34	137.8±48.2
HDL-Cholesterol (mg/dL)	46.7±8.9	48.2±10	42.3±8	44.2±4.9
VLDL-Cholesterol (mg/dL)	56.5±26.6	54.5±26.4	44.5±15.7	51.8±23.8
Triglycerides (mg/dL)	301±164	234±176	222.6±78.6	261±119.3
Risk factor	2.9±1	2.4±0.8	3.7±1	3.1±1.2
FBS (mg/dL)	99±44	106.3±47	101.6±53	116.9±66.4

Values are means±SD

SPI: Soyprotein isoflavones; FBS: Fasting blood sugar; Risk factor :LDL-C/HDL-C

Independent t-test showed no significant difference in baseline values between SPI and placebo groups.

Table 3. Comparison of serum hormones levels at baseline and 8 weeks post intervention

	SPI (n=15)		Placebo (n=15)		
	Baseline	Post	Baseline	Post	P value*
T_4 (μg/dL)	7.7±3.2	6.9±2.7	8.4±1.3	7.9±1.5	0.1
T_3 (ng/dL)	1.5±0.6	1.3±0.4	1.2±0.4	1.2±0.3	0.1
TSH (μIU/mL)	0.9±0.3	1.4±0.3	1.1±0.7	0.7±0.4	0.03†
FSH (IU/L)	4.8±4	4.6±3.3	4.8±2.6	8±6.9	0.02
Testosterone (ng/mL)	4.8±1.7	4.6±1.4	4.6±2.4	5.1±1.8	0.2

Values are means±SD

SPI: Soyprotein isoflavones; T_4: Thyroxine; T_3: Triiodothyronine; TSH: Thyroid stimulating hormone; FSH: Follicle stimulating hormone

Independent t-test showed no significant difference in baseline values between SPI and placebo groups. * P values are for paired t-test

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] A large body of evidence is available regarding application of isoflavones in soy protein; however the effect of purified isoflavonoid supplementation on serum lipids and hormones in human (especially hypercholesterolemic subjects) remains unclear.

In this research, 50 mg of both glycoside and aglycone forms of soy isoflavones were used for about 8 weeks in mild to moderately hypercholesterolemic male subjects but there was no significant difference between the SPI and control groups after intervention. Although the subjects were free-living, the diets did not differ significantly. Setchel has suggested that 30-50 mg isoflavones per day is necessary for achieving a biological effect.[23] It is possible that these results may be due to the number of subjects in each group. However, it is also possible that isoflavones have a cholesterol-lowering effect when they are accompanied with other components in soy, especially soy protein, as some researchers have indicated.[6] Wangen et al also have reported a small (6.5%) but significant reduction in LDL-cholesterol concentration in high-isoflavone diets (132 mg/d) and near significant decrease in low isoflavone diets (65 mg/d) compared with the control group in

normo- and mildly hypercholesterolemic postmenopausal women.[24]

Nestel et al in a randomized, placebo - controlled cross-over trial gave 80 mg purified isoflavones (45 mg genistein, 34 mg daidzein, 3 mg glycitin) or placebos to normolipidemic women for a 5-week period and found improved systemic arterial compliance with isoflavone pill treatment, but no change in plasma lipids.[15] In another randomized, placebo-controlled, double blind trial, forty-six men and thirteen postmenopausal women with normal lipid patterns were treated for 8 weeks with 55 mg purified isoflavones (16 mg biochanin A, 30mg genistein, 8 mg formononetin, 1 mg daidzein) isolated from Subterranean clover, and the researcher found no significant improvement in lipid and lipoprotein profiles in the isoflavone group.[16]

Aglycon forms of soy isoflavones i.e. genistein and daidzein have greater estrogenic activity,[25] but in the present study the amounts used of both were less than those of the contents of glycoside forms.

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

Anthony also reported that soy bean isoflavones inhibit atherosclerotic plaque progression in surgically postmenopausal Cynomolgus monkeys, an occurence which is comparable with those treated with conjugated equine estrogens.[27] Both studies mentioned above indicate that isoflavones modulate plasma cholesterol concentrations by increasing LDL receptor activity, thereby inhibiting atherosclerosis.

The results of the present research also show that soy isoflavones have no effect on serum thyroxine, triiodothyronine, FSH and testosterone concentrations but increase the serum TSH levels (Table3). Anthony et al have reported that intact soy protein has no effect on free thyroxine, sex hormone-binding globulin, dehydroepiandrosterone sulfate, estradiol and testosterone levels in peripubertal rhesus monkeys after 6 months.[11] Forsythe et al hypothesized that the differences in plasma aminoacid composition due to consumption of soy or animal proteins could affect cholesterol metabolism by altering hormone concentrations, especially insulin, glucagon and thyroxine that are involved in the metabolism of cholesterol.[28,29] Moreover, gerbils fed casein had significantly lower plasma thyroxine and TSH concentrations than did the gerbils fed soy protein and there was no difference in triiodothyronine between the two groups.[28]

At present, there is little data of the effect of soy isoflavones on serum hormones, particularly in humans and it remains to be clarified whether soy protein can affect the thyroid function per se or acts in association with isoflavones. However, the results obtained here do not support the hypothesis that 50 mg soy protein isoflavones as a purified solution can ameliorate lipid and lipoprotein profiles in mild to moderately hypercholesterolemic male subjects. It is recommended that serum hormone levels, especially thyroid hormones, be evaluated over a longer period of time in a larger population.

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