Effects of Soy Flour Fortified Bread Consumption on Cardiovascular Risk Factors According to APOE Genotypes in Overweight and Obese Adult Women: A Cross-over Randomized Controlled Clinical Trial

Elham Sharifi-Zahabi¹, Mohammad H Entezari¹,*, Mohammad R Maracy²

¹Food Security Research Center and Department of Clinical Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran  
²Department of Epidemiology and Biostatistics, School of Public Health, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran

Recent studies suggest that inclusion of soy product in the diet may have favorable effects on relief of cardiovascular diseases (CVDs) and risk factors. These effects might be associated with the presence of specific polymorphism in gene. The aim of this study was to examine the effects of consumption of soy flour fortified bread on cardiovascular risk factors in overweight and obese women according to APOE genotype. In a randomized cross-over clinical trial 30 overweight and obese women received a mild weight loss diet and assigned to a regular diet and a soy bread diet, each for 6 weeks and a washout period for 20 days. Subjects in the soy bread diet were asked to replace 120 grams of their daily usual bread intake with equal amount of soy bread. No significant effects of soy bread on serum lipid, systolic blood pressure and anthropometric indices were observed compared to the regular diet (p > 0.05). For diastolic blood pressure (DBP), comparison of mean differences between two groups showed a marginally significant effect of soy bread (p = 0.06). Compared to regular diet, soy bread had a significant effect on DBP in E2 genotype group (ɛ2/ɛ2) (p = 0.03). Having ɛ2 allele may influences responses of CVD risk factor to soy bread consumption. However more nutrigenetic studies are required.

**Key Words:** Soy, Obesity, Cardiovascular diseases, Apolipoprotein E

*Corresponding author* Mohammad Hasan Entezari  
**Address** Food Security Research Center and Department of Clinical Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran  
Tel +98-311-7922775  Fax +98-311-6682509  
E-mail entezari@hlth.mui.ac.ir

Received July 8, 2015  
Revised September 29, 2015  
Accepted October 3, 2015

© 2015 The Korean Society of Clinical Nutrition

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Persistence obesity can lead to metabolic abnormality including dyslipidemia, dysglycemia, hypertension and procoagulant state. The cluster of these conditions is immediate initiator of CVD and type 2 diabetes [1,3,4]. Increasing rate of CVD in developing world has created an urgent need for effective strategy to reduce the risk of obesity and CVD. Dietary interventions are thought to be successful strategies to improve serum lipid profile and reduce the risk of CVD [5]. Consumption of a diet high in phytoestersols, antioxidants and isoflavones has been shown to affect plasma lipid and risk of CVD [6]. In this regard consumption of soy as a rich source of phytoestrogens and antioxidants has attracted more attentions. Epidemiological studies demonstrated that soy intake is associated with reduced risk of CVD [7-9]. Clinical trials have also shown the improvement effects of soy on body weight and serum lipid profile [10-14]. The replacement of soy nut in the diet has been shown to reduce the serum lipid [15]. Soy bean is a unique source of phytochemicals, specific amino acids, saponins, lecithins, phytoestrogens, dietary fiber and high quality protein. These chemical properties of whole soy make it more effective even than other soy components such as isolated soy protein or isoflavone [16].

However evidences on serum lipid responses to dietary interventions vary dramatically according to individual’s genotype [17]. Apolipoprotein (Apo) E has an important role in the normal metabolism of lipid [18]. The apolipoprotein E gene is a highly polymorphic gene, but three common APOE alleles (ε2, ε3, and ε4) produce three major APOE isoforms. Previous studies have shown that subjects with various APOE alleles have different serum lipid responses to therapeutic diets [19]. Therefore it is valuable to consider subject genetic variation when assessing the impact of a dietary intervention.

Since regular consumption of soy especially in the form of whole soy is thought to have cardioprotective effects, incorporation of soy in cereal product (i.e., bread) is a viable way to increase the daily consumption of soy by individuals at high risk for CVD. It is also a cost-effective alternative to improve the protein quality of these products (cereal grains are deficient in lysine) [20]. Therefore the aim of this study was to examine the effects of bread fortified with soy flour on serum lipid profile, blood pressure and anthropometric indices according to APOE genotype in overweight and obese women.

Introduction

The trend of noncommunicable diseases (NCDs) such as diabetes and cardiovascular disease (CVD) is increasing mainly due to nutritional and epidemiological transition. NCDs remain the major cause of death and disability in both developed and developing countries. They are responsible for approximately 80% of death in low and middle income countries [1]. According to WHO report, NCDs will account for nearly three-quarter of all deaths in developing countries by 2020 [2]. Obesity and overweight are common preventable risk factors of NCDs.

Materials and Methods

Participants

Overweight and obese women in Isfahan University of Medical Sciences, were recruited in the study via an advertisement. Women were eligible if they have age range of 19-35 years old, body mass index (BMI) between 25 and 35, no history of any serious medical conditions including; diabetes, CVD, hepatic diseases, renal diseases and cancer, no history of food allergies, not adherence to a specific diet or other medically prescribed diet, no lactation or pregnancy. The exclusion criteria were; use of drugs known to interfere with the study protocol, incidence of any chronic or acute disease after starting the project, smoking and adherence to a specific regimen. The formula for cross-over trials [21], n = [(Z_{1-α/2} + Z_{1-β})^2 / 2(Δ)^2], and LDL cholesterol (LDL-C) as the principle variable [22], were used for calculating the study sample size; where S was the variance of LDL-C and Δ was the differences in mean of LDL-C. Given the Z_{1-α/2} = 1.96, Z_{1-β} = 0.85, S = 1 and Δ = 0.4, the sample size needed for the study was 24 subjects.

A total of thirty five women volunteered to participate in the study, after screening for eligibility 5 subjects were excluded because of having allergy to soy (n = 2), use of medication (n = 1) and personal reasons (n = 2). Thirty women met the inclusion criteria and enrolled in the study. All subjects signed written informed consent before their participation.

Study procedures

This study was a cross-over randomized clinical trial conducted among overweight and obese women in Isfahan University of Medical Sciences, Isfahan, Iran. After two weeks of run-in, using randomized block design method, participants were randomized into a treatment diet (a diet containing soy bread) and a control diet (regular diet); duration of each diet
Soy Bread and Cardiovascular Diseases

was six weeks. Each patient who received two diets and a 20
day washout period was set between the two periods of the
study. Therefore all measurements were determined at base-
line, after 6, 9, and 15 weeks (Figure 2). Due to differences in
color and texture of the bread, we were unable to blind the
participants. All participants received a weight loss diet and
they were recommended not to change their usual physi-
cal activity level during the study. Every 2 weeks a three day
physical activity record was taken from subjects to ensure
subjects adherence to recommendation. The soy bread was
formulated using substituting 30% of the wheat flour in white
bread with roasted soy flour. Every week enough packages of
fresh bread were given to participants and they were instruct-
ed to replace 120 g of soy bread with identical amount of their
daily bread and if necessary other carbohydrate-rich foods
such as pasta, rice and breakfast cereals. Participants were
also asked to use fresh or to freeze the bread and defreeze it
before consumption. Women in the control diet phase were
on their regular diet and asked not to consume soy products.
To ensure consumption of soy bread and maintenance of usual
lifestyle, subjects were visited by the researcher every two
weeks.

Diets

Two diets were prescribed for each participant: 1) control
diet: a regular diet with 300-500 kcal/day deficit, subjects in
this diet were instructed to continue their usual bread intake
and other cereal products and not to consume soy product, 2)
treatment diet: this regimen was identical to the control diet
except that 120 grams of the usual bread intake was replaced
with the equal amount of soy bread and where necessary
other carbohydrate rich products (i.e., pasta, rice). The regi-
mens providing acceptable levels of macronutrient as follows:
50-60% carbohydrate, 15-20% protein and < 30% fat were

Figure 1. The enrollment of study participants.
prescribed for two groups. Prescribed regimens were adjusted for total fat, protein and carbohydrate, according to macro nutrient composition of breads. Daily caloric requirement were calculated based on formula suggested by the Institute of Medicine, Food and Nutrition Board [23].

Each participant received food menus, exchange list and written instruction and benefits of each diet were described for all of them. Every two week a three-day food record (two working and one off days) was taken from participants to determine their dietary intake. Adherence to the study protocol was determined based on participant’s attendance at the periodic visits and also by analyzing the three-day food record. Table 1 shows the nutrient content of soy bread.

Variables assessment

After an overnight fasting for 10–12 h, a venous blood sample was obtained from each subject. Serum was separated after centrifuging at 2,500 × g for 10 min. Serum total cholesterol (TC) and triglyceride (TG) concentrations were measured enzymatically using BioSystems kit (BioSystem S.A. Costa Brava30, Barcelona, Spain). HDL cholesterol (HDL-C) was measured by direct enzymatic method using BioSystems kit. Since none of participants had serum TG higher than 400 mg/dL the Friedewald equation [24], LDL-C = TC – (HDL-C + (TG/5)), was used to calculate the serum levels of LDL-C.

While subjects were barefoot and minimally clothed, body weight was measured using a calibrated scale and recorded to the nearest 0.1 kg. Height was measured by using a tape measure at the standing position while the subjects were not wearing shoes and had the shoulders in a normal position. BMI was calculated as weight (in kg) divided by height (in m²).

DNA extraction and genotyping

Genomic DNA was extracted from 200 μl of whole blood by High Pure PCR Template preparation kit (Roche, Mannheim, Germany).

Oligonucleotide primers design

Human APOE sequence (NG_007084.2, NCBI reference assembly sequence) was used to design the primers according to the method described by Calero et al. [25].
Soy Bread and Cardiovascular Diseases

DNA amplification

DNA was amplified using the method described by Calero et al. [25], with the use of a real-time PCR (Corbett Research 6000 system, Qiagen, Hilden, Germany). Three reaction mixtures of combined primers were designed to obtain a predicted amplification product of 173 bp: 1) Reaction APOE2 (APOE_112C and APOE_158C as primers), 2) Reaction APOE3 (APOE_112C and APOE_158R as primers), 3) Reaction APOE4 (APOE_112R and APOE_158R as primers). Same reaction mixtures without DNA were used to perform negative controls.

Statistical analysis

Dietary records were analyzed by using the Nutritionist four (Nut4) software (for windows, 1994, First Databank, San Bruno, CA). SPSS software version 18 was used to statistical analyses of all data. Comparison of the mean differences of variables in two groups (soy diet and regular diet) and examination of the main effects according to APOE genotype were completed using paired t-test. Period effect and carry over effect were checked by t-test. Results are described as mean ± standard deviation (SD). A p-value < 0.05 was considered as significant for all the analyses.

Results

The study had a good compliance, all subjects completed both periods of the study. Identification of APOE genotype in 29 subjects was performed. The mean (± SD) age and BMI of the subjects at baseline were 22.1 ± 3.1 years and 28.8 ± 2.6 kg/m² respectively. Ten percent of participants were married and 90% of them were single. No serious complaints were reported after consumption of soy bread. Nutrient intakes of participants according to the analysis of three day food records are shown in Table 2. No significant differences regarding energy, macronutrient and fiber intake were observed between two groups during the study. The activity level of the subjects was similar during the two periods of the study (33.2 ± 2.9 Metabolic Equivalent of Task (MET)-h/d and 33.5 ± 3.0 MET-h/d in control diet and soy bread diet respectively, p = 0.39).

For the APOE genotype, the identified distribution were 17.2% for ε3/ε3 genotype (n = 5); 52% for the ε2/ε2 genotype (n = 15); 24% for the ε2/ε3 genotype (n = 7); 3.4% for ε2/ε4 genotype (n = 1) and 3.4% for ε4/ε4 genotype (n = 1). Subjects were classified into following groups: 1) E2 genotype group; participants with genotype ε2/ε2 assigned in this group; 2) E3 genotype group; participants with at least one ε3 allele (ε3/ε3 or ε2/ε3) were allocated in this group. Two Individuals with ε4/ε4 and ε2/ε4 genotype were excluded from the analysis. Period effect and carry over effect for majority of variables showed no significant results (p > 0.05). For weight, BMI, and systolic blood pressure (SBP) period effect was significant (p < 0.05), therefore we adjusted these variables for period effect. The effects of two diets on anthropometric measurements, blood pressure, and serum lipids are shown in Table 3 and 4. Results for weight, BMI and SBP showed significant treatment effects (p < 0.05) but not when analysis were adjusted for period effect (p > 0.05). A marginally significant difference for diastolic blood pressure (DBP) was observed (p = 0.06). Comparison of the mean change of WC, HC, waist to

Table 2. Energy and nutrient intake of subjects during the study*

| Nutrient          | Soy bread diet † (n = 30) | Regular diet † (n = 30) | Differences ‡ | p value ‡ |
|-------------------|--------------------------|--------------------------|----------------|-----------|
| Energy, kcal      | 2,063.2 ± 342.2          | 2,050.3 ± 329.0          | 16.2 ± 75.0    | 0.26      |
| Carbohydrate, g   | 290.8 ± 46.4             | 287.4 ± 45.5             | 4.4 ± 13.6     | 0.12      |
| Protein, g        | 91.7 ± 15.0              | 89.8 ± 15.2              | 2.1 ± 6.4      | 0.11      |
| Total fat, g      | 64.2 ± 12.6              | 64.5 ± 11.5              | -0.2 ± 4.8     | 0.83      |
| Saturated fat, g  | 15.1 ± 3.5               | 18.4 ± 4.1               | -3.3 ± 3.1     | 0.08      |
| Monounsaturated fat, g | 16.9 ± 4.6             | 18.5 ± 4.7               | -1.6 ± 2.8     | 0.36      |
| Polyunsaturated fat, g | 25.1 ± 5.8            | 21.1 ± 4.6               | 3.8 ± 4.8      | 0.47      |
| Fiber, g          | 24.1 ± 6.0               | 20.1 ± 3.2               | 4.3 ± 3.1      | 0.56      |

*All values are shown in mean ± SD; †Soy bread diet: all subjects in this group received a weight loss diet and were also asked to replace 120 g of their usual bread intake with identical amount of soy flour fortified bread; ‡Regular diet: all participant were on their regular diet; subjects were asked not to consume soy products; ‡Differences of variable between two groups (soy bread – regular diet); ‡p values for differences between two groups (paired t-test).
hip ratio (WHR) and body fat percent showed no significant differences between two groups. As shown in Table 4 serum lipids including TG, TC, LDL-C and HDL-C did not significantly differ between two groups indicating that although soy bread could reduce the serum levels of TG, TC, and LDL-C more than the regular diet, the reductions were not significant. In case of HDL-C, a slight decrease was observed after consumption of soy bread in comparison to the control diet, but this change was not significant.

The effect of soy bread on DBP was significantly influenced by presence of ε2/ε2 genotype (Table 5). DBP was significantly decreased following consumption of soy bread compared to DBP after soy bread consumption in E2 genotype group but not in E3 genotype group (p=0.03).

### Discussion

The current study evaluated the effects of soy fortified bread consumption on CVDs risk factors among obese and overweight women. In this work we also concurrently considered the influence of genetic variation on the responses to soy bread through identification of APOE polymorphism. The result of the study indicated significant reduction in regard to weight, BMI and SBP after consumption of soy bread, but adjusting for period effect covered these significant changes and the effect of soy bread diet in compared to regular diet was not significant. Also a mild and favorable but insignificant effect on WC, HC and body fat was observed. Data regarding the effect of soy on anthropometric values are conflicting [26-28]. Some experimental studies have reported beneficial effects of soy on weight and fat mass reduction [27-30]. Clinical trials on the effects of different soy product on body composition and weight reduction in obese adults are limited [26].

### Table 3. Effect of soy bread and regular diet on anthropometric values

| Variables | First phase (n = 30) | Second phase (n = 30) | Mean change difference\(^a\) | p value\(^b\) |
|-----------|---------------------|-----------------------|-----------------------------|--------------|
|           | Week 0 | Week 6 | Week 9 | Week 15 |                                    |                |
| Weight, kg |         |         |         |         |                                    |                |
| SBD\(^c\) | 75.9 ± 8.9 | 73.1 ± 9.3 | 73.5 ± 10.2 | 71.1 ± 10.5 | 0.6 ± 1.0 | 0.12 |
| RD\(^c\) | 73.2 ± 10.4 | 71.0 ± 10.5 | 75.3 ± 8.9 | 73.4 ± 8.9 |                      |                |
| BMI, kg/m\(^2\) |         |         |         |         |                                    |                |
| SBD | 28.5 ± 2.4 | 27.4 ± 2.7 | 29.1 ± 2.8 | 27.9 ± 3.0 | 0.2 ± 0.4 | 0.13 |
| RD | 29.2 ± 2.8 | 28.1 ± 3.0 | 28.2 ± 2.5 | 27.5 ± 2.5 |                      |                |
| Waist, cm |         |         |         |         |                                    |                |
| SBD | 93.3 ± 8.2 | 91.4 ± 8.1 | 91.1 ± 10.0 | 89.0 ± 10.0 | 0.7 ± 1.2 | 0.60 |
| RD | 90.3 ± 10.2 | 89.0 ± 10.5 | 93.0 ± 8.6 | 91.9 ± 8.4 |                      |                |
| HC, cm |         |         |         |         |                                    |                |
| SBD | 101.4 ± 4.8 | 100.2 ± 4.6 | 100.3 ± 4.9 | 99.1 ± 5.0 | -0.2 ± 0.9 | 0.19 |
| RD | 99.8 ± 5.1 | 98.6 ± 5.1 | 101.3 ± 4.7 | 99.7 ± 4.7 |                      |                |
| WHR |         |         |         |         |                                    |                |
| SBD | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.0 ± 0.0 | 0.32 |
| RD | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 |                      |                |
| Body fat, % |         |         |         |         |                                    |                |
| SBD | 35.9 ± 2.6 | 34.6 ± 3.2 | 38.4 ± 2.6 | 36.8 ± 3.3 | 0.1 ± 1.6 | 0.72 |
| RD | 38.3 ± 2.5 | 36.8 ± 2.7 | 36.0 ± 2.4 | 34.7 ± 2.9 |                      |                |

HC: hip circumference, WHR: waist to hip ratio, SBD: soy bread diet, RD: regular diet.

\(^a\)All values are shown in mean ± SD; \(^b\)Mean change differences of variable = mean differences in soy bread diet–mean differences in regular diet; \(^c\)p values for mean change differences between two groups (Paired t-test); \(^d\)All subjects in this group received a weight loss diet and were also asked to replace 120 g of their usual bread intake with identical amount of soy bread; \(^e\)All participant were on their regular diet; subjects were asked not to consume soy products.
Table 4. Effect of soy bread and regular diet on serum lipids and blood pressure*

| Variables | First phase (n = 30) | Second phase (n = 30) | Mean change difference | p value
|-----------|----------------------|----------------------|------------------------|-------|
|           | Week 0   | Week 6  | Week 9 | Week 15 | Week 0   | Week 6  | Week 9 | Week 15 |
| TC, mg/dL |          |         |        |         |          |         |        |         |
| SBD       | 170.3 ± 28.3 | 162.1 ± 35.3 | 179.4 ± 27.1 | 171.7 ± 22.7 | 3.1 ± 13.8 | 0.23    |
| RD        | 183.3 ± 24.8 | 177.0 ± 28.5 | 159.3 ± 21.8 | 155.8 ± 22.7 |        |        |
| TG, mg/dL |          |         |        |         |          |         |        |         |
| SBD       | 111.9 ± 38.9 | 101.0 ± 42.4 | 112.9 ± 54.3 | 106.7 ± 50.4 | 5.3 ± 16.8 | 0.01    |
| RD        | 108.7 ± 45.5 | 105.3 ± 46.8 | 104.6 ± 33.4 | 101.6 ± 39.1 |        |        |
| LDL-C, mg/dL |        |         |        |         |          |         |        |         |
| SBD       | 106.4 ± 23.8 | 99.2 ± 28.7 | 115.3 ± 25.5 | 108.4 ± 22.5 | 2.2 ± 10.8 | 0.27    |
| RD        | 119.4 ± 21.9 | 113.2 ± 24.2 | 98.0 ± 18.7 | 94.6 ± 19.6 |        |        |
| HDL-C, mg/dL |        |         |        |         |          |         |        |         |
| SBD       | 41.5 ± 7.4  | 42.7 ± 7.2  | 41.5 ± 9.2  | 42.1 ± 9.3  | 0.0 ± 2.5  | 0.90    |
| RD        | 42.1 ± 8.6  | 42.8 ± 8.4  | 40.3 ± 8.0  | 41.2 ± 7.5  |        |        |
| SBP, mmHg |          |         |        |         |          |         |        |         |
| SBD       | 113.6 ± 8.3 | 107.9 ± 7.6 | 114.5 ± 7.3 | 108.1 ± 6.5 | 3.6 ± 5.9  | 0.90    |
| RD        | 113.0 ± 5.9 | 110.1 ± 7.4 | 113.3 ± 5.7 | 113.6 ± 6.3 |        |        |
| DBP, mmHg |          |         |        |         |          |         |        |         |
| SBD       | 75.2 ± 5.8  | 70.8 ± 6.4  | 77.6 ± 4.8  | 74.6 ± 6.6  | 2.0 ± 5.7  | 0.06    |
| RD        | 78.3 ± 4.9  | 75.5 ± 7.6  | 73.9 ± 6.7  | 73.4 ± 6.04 |        |        |

TC: total cholesterol, TG: triglycerides, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure, SBD: soy bread diet, RD: regular diet.

*All values are showed in mean ± SD; †Mean change differences of variable = mean differences in soy bread diet – mean differences in regular diet; ‡p values for mean change differences between two groups (Paired t-test); §All subjects in this group received a weight loss diet and were also asked to replace 120 g of their usual bread intake with identical amount of soy bread; ††All participant were on their regular diet; subjects were asked not to consume soy products. ¶Marginally significant difference for DBP between SBD and RD (p = 0.06).

Liao et al. [31] and Deibert et al. [32] in their study on obese adults found favorable effects of a soy based low calorie diet on body composition and fat mass during 2 and 6 months intervention. The capacity of soy bean to regulate body weight and fat mass reduction might be related to its isoflavone content and other soy ingredients such as specific peptides and amino acids [31,33]. Certain peptides in soy protein like β-conglycinin have also effects on weight reduction. They can suppress food intake by increasing circulating levels of cholecystokinin [11]. However others found no significant effects of soy protein on body composition and fat mass reduction [34]. In this study soy bread diet had a marginally significant effect on DBP. Beneficial effects of soy product on blood pressure have been shown on some clinical trials [35,36]. Azadbakht and Nurbakhsh [35] in their study on obese and overweight women showed a significant effect of 6 weeks soy milk consumption on both diastolic and systolic blood pressure. Bioactive components such as isoflavones, soy protein or unique amino acid profile (higher arginine to lysine and methionine ratio) of soy bean might be responsible for these favorable effects [37]. Enhancement of serum nitric oxide level after consumption of soy product is associated with blood pressure reduction [38]. Angiotensin converting enzyme (ACE) inhibitory peptides derived from soy bean also have a role in blood pressure reduction through their ability to limit the vasoconstrictory effects of angiotensin II and potentiate the vasodilatory effects of Bradikinin [39]. Most studies in field of soy and CVDs have focused mainly on soy protein or soy isoflavone [30,32,33]. Recent papers indicated that a complete form of soy such as soy bean may have more favorable effects than other single soy components [16]. However there are also some clinical trials indicated no significant effects of either...
Table 5. Differences of variables between soy bread and regular diet in E2 and E3 genotype groups* †

| Variables | E2 (ɛ2/ɛ2) (n = 15) | p value | E3 (ɛ3/ɛ3, ɛ2/ɛ3) (n = 12) | p value |
|-----------|---------------------|---------|---------------------------|---------|
| Weight    | 0.3 ± 0.7           | 0.01    | 0.5 ± 0.8                 | 0.28    |
| WC        | 0.7 ± 0.9           | 0.40    | 0.5 ± 1.2                 | 0.09    |
| HC        | -0.3 ± 0.6          | 0.10    | -0.1 ± 1.0                | 0.54    |
| WHR       | 0.0 ± 0.0           | 0.12    | 0.0 ± 0.0                 | 0.09    |
| Body fat  | 0.2 ± 1.5           | 0.50    | -0.0 ± 1.7                | 0.91    |
| DBP       | 2.2 ± 3.5           | 0.03    | 1.1 ± 5.5                 | 0.32    |
| SBP       | 3.3 ± 7.2           | 0.01    | 3.9 ± 6.5                 | 0.82    |
| TC        | 3.0 ± 9.6           | 0.25    | 3.5 ± 12.0                | 0.21    |
| TG        | 5.7 ± 17.7          | 0.23    | 5.9 ± 17.3                | 0.12    |
| LDL-C     | 2.1 ± 7.3           | 0.30    | 2.2 ± 10.0                | 0.33    |
| HDL-C     | -0.3 ± 2.1          | 0.50    | 2.3 ± 0.5                 | 0.82    |

WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, DBP: diastolic blood pressure, SBP: systolic blood pressure, TC: total cholesterol, TG: triglycerides, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol.

*Values are showed in mean ± SD; †Mean change differences of variable = mean differences in soy bread diet – mean differences in regular diet.

soy protein or soy bean on SBP and DBP [37].

Polymorphisms in APOE gene may affect the responses to soy bread diet [19,40]. When the effect of having the e2/e2 genotype was evaluated, a significant reduction in DBP was observed after consumption of soy bread compared to regular diet in E2 genotype group. Although to our knowledge no other soy intervention study has examined the influence of genetic variation on the blood pressure and body composition responses, some studies have considered APOE genotypes. In a study by Egert et al. [40], quercetin supplementation showed blood pressure lowering effect in overweight and obese subjects with e3/e3 genotype. Moreover most studies in field of APOE and body composition are cross sectional studies, in which the relationship between various APOE isoforms, anthropometric values, and CVDs was examined [41]. Experimental nutrigenetic studies are also limited. In a study of Kuhel et al. [42] a high fat and high cholesterol diet resulted in increased adiposity in APOE2 (ɛ2/ɛ2) mice compared to APOE3 (ɛ3/ɛ3) mice. The limited number of soy-related intervention studies considering the genetic variation, support the need of more nutrigenetic studies in future. The present study also measured fasting serum lipids in response to consumption of soy bread. Our results showed that serum lipids were not significantly affected by soy bread consumption compared with the regular diet. These findings are consistent with the Liu et al.'s [43] study in which TC and LDL-C were not significantly changed by moderate intake of soy protein with isoflavone during 3 and 6 months. Campbell et al. [44] also showed that one year consumption of soy protein, did not improve the TC and TG in overweight post menopausal women with moderate hypercholesterolemia. In contrast, some clinical trials suggest beneficial effects of soy products including soy milk [45], soy protein or soy nut [37] on serum lipid. Several mechanisms are suggested for lipid lowering effects of soy including soy isoflavones, soy protein, dietary fiber, 7s globulin protein of soy that up-regulate LDL receptors and thereby can reduce the serum LDL-C level, polyphenols, plant sterols and specific amino acid profile of soy [37]. Overall we did not see significant effects of soy bread diet on serum lipid profile. This may be related to the characteristic of our study population. The subjects in present study were young and healthy women without any chronic disease. Most study in field of soy and CVDs are conducted mainly in postmenopausal women or hypercholesterolemic or diabetic subjects [7,12,22]. Previous studies revealed that favorable effects of soy product on lipid profile are more noticeable in individuals with hypercholesterolemia [13]. The amount of soy is also an important factor. Although higher amounts of soy flour may cause better effects on mentioned variables, we had limited ability to increase the soy flour in bread over 30% mainly due to unfavorable changes occurred in texture and taste of the bread. Genotype variability is also a factor that may affect the lipid and lipoprotein responses to dietary interventions. In this study comparison of the effects of soy bread and regular diet within APOE isoforms showed no
significant effect of various APOE isoforms. Studies regarding the influence of APOE on the responses of serum lipid to soy are limited. Our result is inconsistent with those of Gaddi et al. [46], who reported greater reducing effect of soy protein on TC in subject with ε3/ε3 or ε3/ε4 genotype. Sanchez-Muniz et al. [17] in their study showed that consumption of plant sterol result in more reduction in TC and LDL-C concentrations in ε2 and ε3 carriers (ε2/ε3 or ε2/ε2) and (ε3/ε3), and TG in only ε2 carriers compared to control. Others indicated no association between APOE and serum lipid response to soy and soluble fiber [47].

The present work has both limitation and strength. Consideration of the effect of APOE genotype on fasting serum lipid, anthropometric values, and blood pressure responses to dietary intervention was strength of this study while, the retrospective genotyping of study participants lead to small and unbalanced number of subjects in various APOE subgroups, which brings further reason to interpret the findings with caution. However our ability to acquire the significant differences based on APOE genotype showed that this study was not underpowered. Another limitation of the study was its open labeled characteristic. Although this characteristic might cause some bias in the results, laboratory and statistical personnel of the research were blinded to reduce the bias. The strengths of current study include a randomized cross over design, inclusion of genetic variability, excellent compliance of the individuals, and the selection soy-based bread as a functional food.

Conclusion

The findings of present study indicate that having ε2 allele may affect the responses of CVDs risk factors to soy bread. However more studies are needed to assess the effect of soy on cardiovascular risk factors while considering the influence of genotype.

Acknowledgment

This study was extracted from Msc dissertation which was approved by School of Nutrition and Food Sciences, Isfahan University of Medical Sciences (code: 391432). The authors are grateful to all the women who participated in the study. We also appreciate the Mohammad Reza Khajeh for preparing the soy bread.

Conflict of Interest

The authors have declared no conflict of interest.

ORCID

Mohammad Hasan Entezari http://orcid.org/0000-0003-4321-4124

References

1. Kankeu HT, Saksema P, Xu K, Evans DB. The financial burden from non-communicable diseases in low- and middle-income countries: a literature review. Health Res Policy Syst 2013;11:31.
2. Sadeghi-Bazargani H, Jafarzadeh H, Failah M, Hekmat S, Bashiri J, Hosseini-Gilizadeh GH, Solimanamohamadzadeh MS, Mortezaazadeh A, Shaker A, Danehzan M, Zohouri A, Khorasani H, Ghasemnejad B, Malekpour N, Kharazmi E, Babaie M, Madinatmohammadi M, Mashadi-Abdollahi H. Risk factor investigation for cardiovascular health through WHO STEPS approach in Ardabil, Iran. Vasc Health Risk Manag 2011;7:417-24.
3. Mirza A, Khurana L. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 2008;93:59-30.
4. Wang H, Peng DQ. New insights into the mechanism of low high-density lipoprotein cholesterol in obesity. Lipids Health Dis 2011;10:176-86.
5. Pipe EA, Gobert CP, Capes SE, Darlington GA, Lampe JW, Duncan AM. Soy protein reduces serum LDL cholesterol and the LDL cholesterol:HDL cholesterol and apolipoprotein B:apolipoprotein A-I ratios in adults with type 2 diabetes. J Nutr 2009;139:1700-6.
6. Carmignani LO, Pedro AO, da Costa-Paiva LH, Pinto-Neto AM. The effect of soy dietary supplement and low dose of hormone therapy on main cardiovascular health biomarkers: a randomized controlled trial. Rev Bras Ginecol Obstet 2014;36:251-8.
7. Goodman-Gruen D, Kritz-Silverstein D. Usual dietary isoflavone intake is associated with cardiovascular disease risk factors in postmenopausal women. J Nutr 2001;131:1202-6.
8. Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 1998;128:209-13.
9. Zhang X, Shu XO, Gao YT, Yang G, Li Q, Li H, Jin F, Zheng W. Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. J Nutr 2003;133:2874-8.
10. Guo Y, Wu G, Su X, Yang H, Zhang J. Antioxidant activity of a daidzein derivative on male obese mice induced by a high-fat diet. Nutr Res 2009;29:656-63.
11. Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. Int J Med Sci 2007;4:72-82.
12. Oldewage-Theron W, Egal A. The effect of consumption of soy foods on the blood lipid profile of women: a pilot study from Qwa-Qwa. J Nutr Sci Vitaminol (Tokyo) 2013;59:431-6.
13. Taku K, Umemaki K, Sato Y, Taki Y, Endoh K, Watanabe S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. Am J Clin Nutr 2007;85:1148-56.
14. Reynolds K, Chin A, Lees KA, Nguyen A, Bujnowski D, He J. A meta-analysis of the effect of soy protein supplementation on serum lipids. Am J Cardiol 2006;98:633-40.
15. Yazdekhisti N, Zaitoun Y, Norhaizan ME, Najafpour Boushehri S. Effects of roasted soy-nut supplementation on lipid profile of Iranian post-menopausal women. Middle East J Sci Res 2011;8:186-22.
16. Reinwald S, Akabas SR, Weaver CM. Whole versus the piecemeal approach to evaluating soy. J Nutr 2010;140:23355-2343S.
17. Sanchez-Muniz FJ, Maki KC, Schaefrer EJ, Orodivas JM. Serum lipid and antioxidant responses in hypercholesterolemic men and women.
receiving plant sterol esters vary by apolipoprotein E genotype. J Nutr 2009;139:13-9.

18. Mendivil CO, Rimm EB, Furtado J, Sacks FM. Apolipoprotein E in VLDL and LDL with apolipoprotein C-III is associated with a lower risk of coronary heart disease. J Am Heart Assoc 2013;2:e001350.

19. Morbois-Trabut L, Chabrolle C, Garrigue MA, LasFargues G, Lecomte P. Apolipoprotein E genotype and plasma lipid levels in Caucasian diabetic patients. Diabetes Metab 2006;32:270-5.

20. Ivanovski B, Seetharaman K, Duizer LM. Development of soy-based bread with acceptable sensory properties. J Food Sci 2012;77:S71-6.

21. Fleiss JL. The design and analysis of clinical experiments. New York (NY): Wiley; 1986.

22. Onuegbu AJ, Olisekodiaka JM, Onibon MO, Adesiyan AA, Igbeneghu CA. Consumption of soymilk lowers atherogenic lipid fraction in healthy individuals. J Med Food 2011;14:257-60.

23. Mahan LK, Escott-Stump S, Raymond JL. Krause's food and the nutrition care process. 13th ed. St. Louis (Mo): Elsevier/Saunders; 2012.

24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

25. Calero O, Hortiguela R, Bullido MJ, Calero M. Apolipoprotein E genotyping method by real time PCR, a fast and cost-effective alternative to the TaqMan and FRET assays. J Neurosci Methods 2009;183:238-40.

26. Bakhtiari A, Yassin Z, Hanachi P, Rahmat A, Ahmad Z, Jalali F. Effects of soy on metabolic biomarkers of cardiovascular disease in elderly women with metabolic syndrome. Arch Iran Med 2012;15:462-8.

27. Davis J, Higginbotham A, O’Connor T, Mountsdait-Moussa N, Tebbé A, Kim YC, Chou WK, Shay N, Adler S, Peterson R, Banz W. Soy protein and isoflavones influence adiposity and development of metabolic syndrome in the obese male ZDF rat. Ann Nutr Metab 2007;51:42-52.

28. Cheik NC, Rossi EA, Guerra RL, Tenório NM, Oller do Nascimento CM, Viana FP, Manzoni MS, Carlos IZ, Leão da Silva P, Vendramini RC, Dâmaso AR. Effects of a fermented soy product on the adipocyte area reduction and dyslipidemia control in hypercholesterolemic adult male rats. Lipids Health Dis 2008;7:50.

29. Frigolet ME, Torres N, Uribe-Figueroa L, Rangel C, Jimenez-Sanchez G, Tovar AR. White adipose tissue genome wide-expression profiling and adipocyte metabolic functions after soy protein consumption in rats. J Nutr Biochem 2011;22:118-29.

30. Torre-Villalvazo I, Gonzalez F, Aguilar-Salinas CA, Tovar AR, Torres N. Dietary soy protein reduces cardiac lipid accumulation and the ceramide concentration in high-fat diet-fed rats and ob/ob mice. J Nutr 2009;139:2237-43.

31. Liao FH, Shihe MJ, Yang SC, Lin SH, Chien YW. Effectiveness of a soy-based compared with a traditional low-calorie diet on weight loss and lipid levels in overweight adults. Nutrition 2007;23:551-6.

32. Debet P, König D, Schmidt-Trucksaesa A, Zaneker KS, Frey I, Landmann U, Berg A. Weight loss without losing muscle mass in pre-obese and obese subjects induced by a high-soy-protein diet. Int J Obes Relat Metab Disord 2004;28:1349-52.

33. Crespio A, Alonso M, Vida M, Pavón FJ, Serrano A, Rivera P, Romero-Zerbo Y, Fernández-Lluberes P, Martínez A, Pérez-Valero V, Bermúdez-Silva FJ, Suárez J, de Fonseca FR. Reduction of body weight, liver steatosis and expression of stearyol-CoA desaturase 1 by the isoflavone daidzein in diet-induced obesity. Br J Pharmacol 2011;164:1899-915.

34. St-Onge MP, Claps N, Wolper C, Heymsfield SB. Supplementation with soy-protein-rich foods does not enhance weight loss. J Am Diet Assoc 2007;107:500-5.

35. Azadbakht L, Nurbakhsh S. Effect of soy drink replacement in a weight reducing diet on anthropometric values and blood pressure among overweight and obese female youths. Asia Pac J Clin Nutri 2011;20:383-9.

36. Simão AN, Azadbakht L, Bakhtiary A, Yassin Z, Hanachi P, Rahmat A, Ahmad Z, Jalali F. Effects of soy on metabolic biomarkers of cardiovascular disease in elderly women with metabolic syndrome. Arch Iran Med 2012;15:462-8.

37. Simão AN, Azadbakht L, Bakhtiary AN, Dichi JB, Matsuo T, Dichi I. Nitric oxide enhancement and blood pressure decrease in patients with metabolic syndrome using soy protein or fish oil. Arq Bras Endocrinol Metabol 2010;54:404-5.

38. Le De F, Panarese S, Gallerani R, Cerci LR. Angiotensin converting enzyme (ACE) inhibitory peptides: production and implementation of functional food. Curr Pharm Des 2009;15:3622-43.

39. Egert S, Boetsch-Saadatmandi C, Wolftram S, Rimbach G, Müller MJ. Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. J Nutr 2010;140:278-84.

40. Petkeviciene J, Smalinkiene A, Luksiene DI, Jureniene K, Ramazauskien E, V, Klimbiene J, Lesauskaite V. Associations between apolipoprotein E genotype, diet, body mass index, and serum lipids in Lithuanian adult population. PLoS One 2012;7:e141525.

41. Kuhel DG, Konaniah ES, Basford JE, MeVey C, Goodin CT, Chatterjee TK, Weintraub NL, Hui DY. Apolipoprotein E2 accentuates postprandial inflammation and diet-induced obesity to promote hyperinsulinemia in mice. Diabetes 2013;62:382-91.

42. Liu ZM, Ho SC, Chen YM, Ho YP. The effects of isoflavones combined with soy protein on lipid profiles, C-reactive protein and cardiovascular risk among postmenopausal Chinese women. Nutr Metab Cardiovasc Dis 2012;22:712-9.

43. Campbell SC, Khalil DA, Payton ME, Arjmandi BH. One-year soy protein supplementation does not improve lipid profile in postmenopausal women. Menopause 2010;17:587-93.

44. Takatsuka N, Nagata C, Kurisu Y, Inaba S, Kawakami N, Shimizu H. Hypcholesterolemic effect of soymilk supplementation with usual diet in premenopausal normolipidemic Japanese women. Prev Med 2000;31:308-14.

45. Gaddi A, Carroccchi A, Matteucci A, Rimondi S, Ravaglia G, Desovich GC, Sirtori CR. Dietary treatment for familial hypercholesterolemia- differential effects of dietary soy protein according to the apolipoprotein E phenotypes. Am J Clin Nutri 1991;53:1911-6.

46. Torres N, Guevara-Cruz M, Granados J, Vargas-Alarcón G, González-Palacios B, Ramos-Barragan VE, Quiróz-Olíguin G, Flores-Islas HM, Tovar AR. Reduction of serum lipids by soy protein and soluble fiber is not associated with the ABCGS/G8, apolipoprotein E, and apolipoprotein AT polymorphisms in a group of hyperlipidemic Mexican subjects. Nutr Res 2009;29:728-35.