

The Effect of Soymilk on Menopausal Symptoms and Total Antioxidant Levels in Menopausal Women

P Hanachi & S Golkho

Women Research Centre, Biomedical Department, Alzahra University, Tehran, Iran

ABSTRACT

Objective: As soy products are new in Iranian society, soya does not constitute a part of the general diet in Iran. This study therefore aimed to determine the effect of soymilk consumption and moderate exercise on vasomotor symptoms and total antioxidant capacity (TAC) in postmenopausal women. **Method:** Thirty-seven postmenopausal women (mean age = 52.2±4.6 years) were randomly assigned to soymilk (n=15), exercise + soymilk (n=12) and control (n= 10) over a 3-month period. Subjects were given soymilk which contained 12.5 gm of soy protein with 13 mg genistein and 4.13 mg daidzein per day. Blood total antioxidant status and menopausal symptoms were measured at zero and 12 weeks. Total antioxidant status (TAS) of serum was measured using the ferric reducing ability of plasma (FRAP) assay. **Results:** Soymilk supplementation, soymilk and moderate exercise significantly ($p<0.05$) improved TAC level. Vasomotor symptoms were improved ($p<0.05$) in soymilk consumption and moderate exercise and soymilk groups compared with control group. However, vaginal, sexual and vasomotor problems significantly changed ($p<0.05$) after soymilk consumption. **Conclusion:** This study suggests that soymilk treatment over a 3-month period may be a safe and effective alternative therapy for menopausal symptoms and may offer enhanced antioxidant status contributing to improved health and quality of life among women.

Keywords: Total antioxidants, menopausal symptoms, isoflavones

INTRODUCTION

Menopause is the time in a woman's life when hormonal changes cause menstruation to stop permanently. The menopause period normally occupies one-third of a woman's life.^[1] Reduced bone density is one of the most prominent symptoms during menopause.^[2] An estimated 40 million women will go through menopause in the next 20 years.^[3,4]

Oxidative stress can be reduced with the provision of additional antioxidants. Antioxidants are closely related with the prevention of degenerative illness such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunctions.^[5,6] Foods of plant origin not only provide us with important antioxidant vitamins (for example, vitamins C and E or provitamin A), but also a complex mixture of other natural substances with antioxidant capacity. Reactive oxygen species (ROS)/free radical production in the body can initiate lipid peroxidation. Current literature suggests that moderate to high intensity exercise can induce oxidative stress in the body because elevated consumption of oxygen can lead to increased generation of ROS. Antioxidant supplementation can provide extra protection against these ROS. It can be hypothesised that isoflavone supplementation can

Corresponding author: hanachi_wrc@yahoo.com

increase total antioxidant defence systems and reduce the impact of ROS generated due to intensive exercise.^[7]

Intensive physical activity has been suggested as one means to induce a surge of ROS in the body, leading to oxidative stress because of the imbalance between antioxidants and oxidants.^[8] Therefore, the combination of dietary isoflavone supplementation and exercise is a way to explore whether isoflavones from soy can counteract the oxidative stress that results from the imbalance between antioxidants and oxidants in the body by their antioxidative capability. Nemoto *et al.*^[9] address the benefits of walking regimens and in so doing reflect a broad movement in the exercise literature and the health intervention community. Menopausal women who exercise regularly appear to have a better quality of life than women who do not.^[10] Although the impact on the number of hot flushes and other symptoms on women could have been due to chance, Elavsky^[10] believes a larger study would have shown a reduction in these symptoms with exercise.

Epidemiologic studies have shown osteoporotic fractures, cardiovascular disease, postmenopausal symptoms and some cancers to be less prevalent in Asians compared to their western counterparts. Hip fracture, for example, is 50–60 % less frequent among Asian compared to western women.^[11] This advantage is gradually annihilated as Asians adapt a western lifestyle.^[12] These observations, prompted researchers to scrutinise Asian dietary habits. Soy is a part of the Asian traditional diet^[13] and shows some relationship with the above-mentioned diseases. Estrogen-like compounds such as isoflavones existing in plant foods especially soy^[14] can inhibit reduced bone density in menopausal women, due to their structural similarity.^[15,16] Isoflavones are phytoestrogens similar to women's estrogens and are bound to cellular estrogen receptors in various organs, thus the affinity of phytoestrogens is weak compared to human estrogens.

An Australian study to determine the effects of soy versus wheat flour supplementation on hot flushes and vaginal cells in postmenopausal women showed benefit with soy after 12 weeks.^[17,18] Another study reported a small reduction in hot flushes in postmenopausal women whose diet was supplemented with soy versus placebo.^[19]

Though there are several publications on the symptoms of menopause and soy products (that is, soy milk), as the product is new in Iranian society, soy is not a part of the general diet of people in Iran. The aim of this study is to determine the effects of soymilk (Maxsoy-Co, Tehran-Iran) consumption and moderate exercise on vasomotor symptoms and total antioxidant capacity (TAC) in post-menopausal women.

MATERIALS AND METHODS

Sample

This study was carried out on 37 post-menopausal women referred to the Women Research Centre of Alzahra University, Tehran aged 5.47 ± 3.4 years, post-menopause years 5.47 ± 3.4 . The subjects were non-smokers and free from diseases, not on any type of hormonal treatment during the previous 12 months, and not currently using lipid-lowering drugs, antidiabetic medications, soybean-derived products, or herbal supplements. Other inclusion criteria were an intact uterus, follicle-stimulating hormone (FSH) levels in blood serum

exceeding 25 U/L, estradiol levels less than 100 pg/m, and presence of hot flushes. Women with a history of uncontrolled hypertension, stroke or transient ischemic attack, cancer diagnosed less than 5 years ago, or previous myocardial infarction were excluded from the study. The study was carried out over a period of three months. The study protocol was approved by the Scientific Advisory Committee and Ethical Committee of the University. All persons gave informed consent for their participation in the study after reading the protocol of this experiment and receiving information about soymilk consumption. The subjects were randomly assigned to soymilk consumption (n=15), (exercise+ soymilk) one hour walking per day + soymilk (n=12) and control (without treatment and exercise) (n= 10). Soymilk which contained 12.5 gm of soy protein with 13 mg genistein and 4.13 mg daidzein per day. The information on demographic characteristics including age, ethnicity, and education level were collected at the start of the study. Gynecologic history, including age at menopause, the use of selected medications, cigarette smoking history, physical activity, and dietary and nutritional habits were also noted down.

Menopause Symptoms

Women were also queried about menopausal symptoms covered by the Kupperman index.^[20] This scale can be used to assess changes in different symptoms before and after menopause treatment. Height and weight were measured with subjects wearing light weight clothing and no shoes; body mass index (calculated as kg/m²) was used as an estimate of obesity. The Kupperman index^[20] is a numerical conversion index and covers 11 menopausal symptoms. Each symptom on the Kupperman index was rated on a scale from 0 to 4 for slight, moderate, severe and very severe complaints. The score of hot flushes was based on number of complaints per day: light (1-2), moderate (2-3), severe (5-6) and very severe (more than 6).

Total Antioxidant Assay

The blood samples of subjects after fasting for 12 hours were obtained at baseline and 3 months by vein puncture to measure total antioxidants using the FRAP assay. This assay was performed using TPTZ reagent as described by Benzie and Strain.^[21] This method measures the ability of the antioxidants contained in the sample to reduce ferric-tripiridyltriazine (Fe³⁺-TPTZ) to a ferrous form (Fe²⁺) which absorbs light at 593 nm. In this reaction, the Fe³⁺ binds to a reagent, that is, TPTZ that is then converted to Fe²⁺-TPTZ complex in the presence of plasma antioxidant factors. The ferro- and ferric-iron formed complex with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against $\mu\text{M/l}$ concentration of Fe (II) standard solution.^[21]

Statistical Analysis

All sample analyses were run in triplicate. Statistical analysis, frequency counts, descriptive statistics, Pearson's correlations, stepwise multiple linear regression and repeated measures were performed using the Statistical Package for the Social Sciences (Windows version 11 SPSS). Statistical significance by treatment group was assessed for percent change and

absolute change by site using analysis for of covariance for repeated measures. Comparison of the changes in parameters between groups as the main outcome was made by ANOVA using the General Linear Model for repeated measures. In light of multiple comparisons, statistical significance will be assigned at $p < 0.05$ for all analysis.

RESULTS

Sample Characteristics

Mean age of respondents was 52.2 ± 4.6 years, years of post menopause 5.47 ± 3.4 and mean height 157.4 ± 7.2 centimeters. Comparison of weight and BMI did not reveal any significant changes during different stages of the study.

Treatment Effects

To evaluate the menopausal symptoms, the menopausal Kupperman index^[20] questionnaire was applied. Some of the menopausal symptoms such as hot flushes, nervousness, vaginal symptoms, and sexual symptoms experienced significant changes during the different stages of the study (Tables 1 and 2).

During the treatment period, the hot flush symptoms of participants in soymilk + exercise group were lower than in soymilk treatment group. Soymilk supplementation substantially decreased significantly ($p < 0.05$) hot flushes by 72.7%, nervousness by 54.6%, vaginal symptoms by 70 % and sexual symptoms by 62.5% compared with control group. In comparison, exercise and soymilk treatment decreased significantly ($p < 0.05$) hot flushes, nervousness, and vaginal, and sexual symptoms by 83.3%, 30%, 50%, and 45.5%, respectively compared with the control group.

Table 1. Percentage change in menopausal symptoms (Kupperman Index %) after exercise and soymilk consumption in menopausal women compared with control group.

Symptoms	% Decrease
Muscle or joint pains	33.3
Vaginal	50*
Uterus	27.3
Sexual	45.5*
Forgetful	16.7
Nervousness	*30
Quarrelsome	25
Depression	16.7
Insomnia	25
Palpitations	40
Hot flushes	83.3*

* significant level was set at below 5 % ($p < 0.05$).

Table 2. Percentage change in menopausal symptoms (Kupperman Index %) after soymilk consumption in menopause women compared with control group.

Symptoms	% Decrease
Muscle or joint pains	33.3
Vaginal	70*
Uterus	-
Sexual	62.5*
Forgetful	50
Nervousness	*54.6
Quarrelsome	45.5
Depression	54.5
Insomnia	30
Palpitations	36.4
Hot flushes	72.7*

* significant level was set at below 5 % ($p < 0.05$).

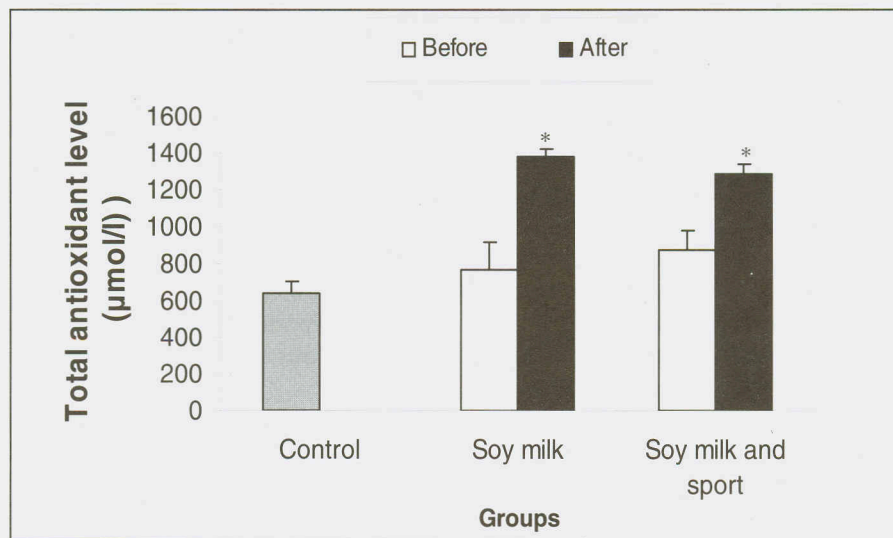


Figure 1: Effect of soymilk and exercise on FRAP values in menopause women before and after treatment. Data are mean \pm S.E.M. of (n=10-15) samples obtained from each group. * Significance level was set at below 5 % ($p < 0.05$) compared with control group.

In the present study, administration of soymilk and exercise plus soymilk was found to increase FRAP level. FRAP was almost within the same range in the three groups before consumption of soymilk. Administration of soymilk with 12.5 gm of soy protein, 13 mg genistein and 4.13 mg daidzein per day caused a differential reduction in FRAP in these groups. The total antioxidant level in control, soymilk consumption and exercise + soymilk groups significantly increased ($p < 0.05$) to 642.88 ± 66.9 , 1379.11 ± 87.4 and 1288.75 ± 98 ($\mu\text{mol/l}$) respectively (Figure 1).

DISCUSSION

Hot flushes are experienced in those periods of female life when estrogen levels are low.^[22] Women who exercised (walking, biking, and swimming) four and a half hours a week and had soy had 49% fewer hot flushes, while those who exercised four or more days a week as well as consumed soy had 46% fewer hot flushes. Those with 30-90 minutes a week of exercise and soy had 26.5% fewer hot flushes. A similar result was obtained if they exercised one to two and half days a week and also consumed soy.^[23]

This study demonstrated that 12.5 gm of soy protein with 13 mg genistein and 4.13 mg daidzein per day was effective in alleviating vasomotor symptoms such as hot flushes, consistent with previous study results.^[24-27] Further, our results showed a decrease in other subjective symptoms which were not reported by other studies.^[26,27] In our study, consumption of 13 mg genistein and 4.13 mg daidzein per day reduced hot flushes, but its real mechanism of action is not known. One possible explanation for isoflavone effect on menopausal symptoms is through its action on the estrogen receptor which is capable of binding several structurally diverse compounds such as natural estrogens and isoflavones.^[28] Another explanation is that isoflavones act through their antioxidant effects. genistein is an inhibitor of tyrosine protein kinases which is seen to play a role in vascular endothelial activity.^[29,30] Finally, the 17β -estradiol enhancement of the isoflavone-group patients suggests that isoflavone supplementation increases estrogen levels. It may have an indirect effect due to isoflavones acting on sex hormone-binding globulin. Isoflavones were observed to have an antioxidant activity *in vitro* and *in vivo*, and augmented the activities of antioxidant enzymes in rats. It can be hypothesised that isoflavone supplementation can increase total antioxidant defence systems and reduce the impact of ROS.^[7]

Additionally, our results showed a significant ($p < 0.05$) increase in total antioxidant level in control < exercise + soymilk < soymilk consumption groups. These results demonstrate that consumption of soymilk for 3 months is more effective than soymilk + exercise and can reduce some of the menopausal symptoms in post-menopausal women. We expected a higher antioxidant activity in the exercise group consuming soy milk. To our surprise, results demonstrated that consumption of soymilk for 3 months was more effective than soymilk + exercise and could reduce some of the menopausal symptoms in post-menopausal women. This could be due to the amount and duration of soymilk consumption. In conclusion, in our future studies we plan to determine the Total Energy Expenditure (TEE) and to include one sport group without soymilk.

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