

The Effect of Soymilk on Alkaline Phosphatase, Total Antioxidant Levels, and Vasomotor Symptoms in Menopause Women

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Abstract

Objective

The aim of this study was to determine the effect of revival soymilk consumption and moderate exercise on vasomotor symptoms, total antioxidant capacity (TAC) and alkaline phosphates activity in postmenopausal women.

Materials and Methods

In this study, daily for 3 months, 37 postmenopausal women (mean age = 52.2± 4.6 years) were randomly assigned to soymilk (n=15), exercise +soymilk (n=12) and control (n= 10). The daily soymilk consisted of Revival brand Maxsoy, which contained 12.5 g of soy protein with Genistein (13 mg) and Daidzein (4.13 mg). Menopausal symptoms were measured using the Kupperman index, a standard measure of climacteric symptoms that has been validated in menopausal women at baseline and 3 months of daily consumption of soymilk. Total antioxidant status (TAS) of serum was measured using the ferric reducing ability of plasma (FRAP) assay. Total alkaline phosphatase (TALP) was assayed calorimetrically with Hitachi 902 autoanalyzer assay.

Results

Soymilk supplementation, soymilk and moderate exercise significantly ($p < 0.05$) improved TAC level and TALP activity. Vasomotor symptoms were improved ($p < 0.05$) in the soymilk consumption plus moderate exercise and soymilk groups as compared with the control group. The data showed a decrease in menopausal symptoms $p < 0.05$, paired t test, two-tailed, between the baseline and soymilk groups, and $p < 0.05$, ANOVA test, between the control and two groups. Vaginal problem, sexual symptoms and vasomotor significantly changed ($p < 0.05$) after soymilk consumption.

Conclusion

These data may suggest that consumption of soymilk for 3 months enhances antioxidant and TALP status and reduces menopausal symptoms in postmenopausal women.

Keywords: Alkaline phosphatase, Isoflavones, Menopausal symptoms, Soymilk, Total antioxidant

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Introduction

Menopause is a period normally occupying one-third of women's life (1). Menopause is the time in a woman's life when hormonal changes cause menstruation to stop permanently. Reduced bone density is one of the most prominent symptoms during menopause (2). This usually occurs between 45 and 55. An estimated 40 million women will go through menopause in the next 20 years (3, 4).

Antioxidants are closely related with the prevention of degenerative illnesses, such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunctions (5, 6). Foods of plant origin not only provide us with important antioxidant vitamins (e.g. vitamin C, vitamin E or provitamin A), but also a complex mixture of other natural substances with antioxidant capacity. It is possible to measure all of the antioxidant components in a sample individually, but this is expensive and time-consuming. Reactive oxygen species (ROS)/ free radicals production in the body can initiate lipid peroxidation. Antioxidant provides protection against these ROS. *in vitro* and *in vivo* studies suggest that isoflavones have antioxidant activity and augment the activities of antioxidant enzymes in rats. The isoflavone supplementation can increase the total antioxidant defence systems and reduce the impact of ROS, generated due to intensive exercise (7).

The comparison of dietary isoflavone supplementation and exercise is a way to explore whether isoflavones from soya can counteract the oxidative stress that results from the imbalance between antioxidants and oxidants in the body by their antioxidative capability (8).

Nemoto *et al.* (2007) address the benefits of walking regimens and in so doing reflect a broad movement in the exercise literature and the health intervention community. The health benefits associated with walking,

whether the outcome measurement is blood pressure (9), diabetes (10), other metabolic disorders, menopausal symptoms and cardiovascular disease (11, 12), joint problems, or mental health (13). Menopausal women who exercise regularly appear to have a better quality of life than women who don't (14).

Hip fracture, is 50–60% less frequent among Asian compared to western women (15). This advantage is gradually annihilated as Asian adapt western lifestyle (16). These observations, prompted researchers to scrutinize Asian dietary habits. Soya is a part of Asian traditional diet (17), showing some relationship with the osteoporotic fractures, postmenopausal symptoms and cardiovascular diseases. Estrogen-like compounds such as isoflavones existing in plant foods especially soy (18) can curb reduced bone density in menopausal women, due to their structural similarity (19, 20).

Isoflavones are phyto-estrogens similar to women's estrogens and are bound to cellular estrogen receptors in various organs, thus phytoestrogens affinity is weak compared to human's estrogens. Recent studies have shown that cells have two types estrogen receptors α and β - Human estrogens have more affinity to α -receptors, whereas, isoflavones have high affinity to β -receptors. Receptors exist in brain, bone, bladder and vascular epithelium, being important in the function of non-steroid estrogens (21).

The results demonstrated soy protein consumption to have caused increase in total alkaline phosphatase (TALP) in menopausal women with osteopenia (22). Subsequently, reports of effects of phytoestrogen supplementation on postmenopausal vasomotor symptoms have been consistent. An Australian study of the effects of soy versus wheat flour supplementation on hot flushes and vaginal cells in postmenopausal women showed benefit with soy after 12 weeks

(23). A study reported a small reduction in hot flushes in postmenopausal women whose diet was supplemented with soy versus placebo (24).

Even though there are a lot of publications exist regarding the symptoms of menopause and soy product (i.e. soy milk), since the product is new in Iranian society and soya, it is not general diet in Iran. The aim of this study was to determine the effect of revival soymilk Maxsoy- Co (Tehran-Iran) consumption and moderate exercise on vasomotor symptom with determined two active compounds (Genistein and Daidzein)s and total antioxidant capacity (TAC) and alkaline phosphates activity in postmenopausal women.

Materials and Methods

This study, was carried out daily for 3 months, on 37 postmenopausal women, aged 52.2 ± 4.6 , years of post menopause 5.47 ± 3.4 and randomly assigned to soymilk consumption (n=15), one hour walking per day plus soymilk (n=12) and control (n= 10). Soymilk which contained 12.5 gr of soy protein with Genistein (13 mg) and Daidzein (4.13 mg) per day.

The Information on demographic characteristics such as age, ethnicity, and education level were collected at the start of the study. Gynecologic record, including age at menopause, the use of selected medications, cigarette smoking history, physical activity, dietary and nutritional habits were collected. Height and weight were measured with subjects wearing lightweight clothing and no shoes; body mass index (calculated as kg/m^2) was used as an estimate of obesity.

To be eligible for this study, those women recruited, who 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, and 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 the 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 length of the study was three months. All persons gave informed consent for their participation in the study, after reading the protocol of this experiment and receiving information about soymilk consumption.

Women were also, queried regarding menopausal symptoms covered by Kupperman index, 1959 (25). This scale can be used to assess changes in different symptoms, before and after menopause treatment. The Kupperman index 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 no, slight, moderate, severe and very severe complaints. The score of hot flushes was based on the number of complaints per day: slight (1-2), moderate (2-3), severe (5-6) and very severe (more than 6).

After fasting for 12 hours, blood samples were obtained at baseline and 3 months by venipuncture to measure the total antioxidant, using the FRAP assay, which depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. (Fe (II)-TPTZ has an intensive blue colour and can be monitored at 593 nm (26). Total alkaline phosphatase (TALP) was assayed calorimetrically using the Hitachi 902 autoanalyzer assay kit.

Statistical Analysis

All sample analyses were run in triplicate. Statistical analysis Frequency counts, descriptive statistics, pearson's correlations,

stepwise multiple linear regression and repeated measurements were performed using the Statistical Package for the Social Sciences (Windows version 11 SPSS). Statistical significance for the percentage change was assessed by the treatment group and the absolute change by site using the covariance analysis for repeated measurements. Comparison of the changes in parameters between groups, as the main outcome, was made by ANOVA, using the General Linear Model for repeated measurements. In the light of multiple comparisons, statistical significance was assigned at a $p < 0.05$ for all analysis.

Results

Mean age was 52.2 ± 4.6 years, post menopause 5.47 ± 3.4 years and mean height 157.4 ± 7.2 centimeters. Comparison of weight, BMI did not reveal any significant changes during different stages of the study. The total alkaline phosphatase activity in soymilk consumption and exercise plus soymilk was significantly ($p < 0.05$) increased, compared with the baseline time, 190.82 ± 42.3 , 211.91 ± 43.1 , 170.58 ± 39.6 , 183.67 ± 58.9 (IU/L) respectively (Figure 1).

To evaluate the menopausal symptoms, the menopausal Kupperman index questionnaire was applied during different stages of the study. The total antioxidant level in control, soymilk consumption and exercise plus soymilk groups was significantly ($p < 0.05$) increased, to 642.88 ± 66.9 , 1379.11 ± 87.4 and 1288.75 ± 98 ($\mu\text{mol/l}$) respectively (Figure 2).

Some of the menopausal symptoms such as hot flushes, nervousness, vaginal symptoms, and sexual symptoms were significantly changed (Table 1 and 2).

Soy milk supplementation or consumption, significantly ($p < 0.05$) decreased the hot

flushes by 72%, nervousness by 54%, vaginal symptoms by 70%, and sexual symptoms by 62.5%. In comparison exercise plus soymilk treatment, significantly ($p < 0.05$) decreased the hot flushes, nervousness, vaginal, and sexual symptoms by 83%, 30%, 50% and 45% respectively.

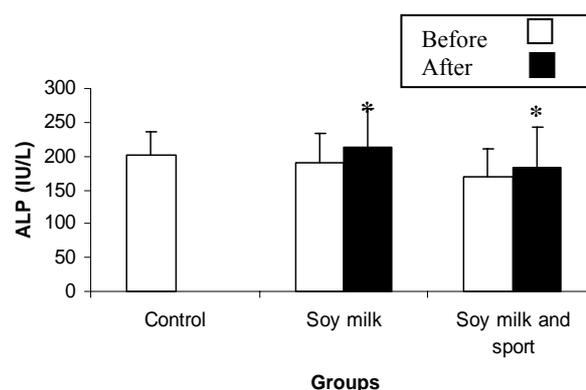


Figure 1. Effect of soymilk and sport on ALP activity in menopause women after and before treatment. * Significant level was set at below 5% ($p < 0.05$).

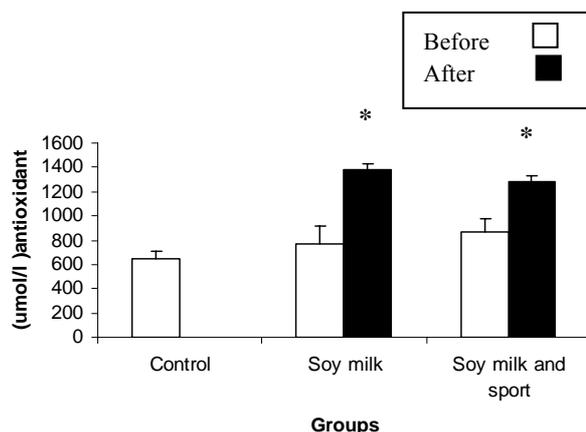


Figure 2. Effect of soymilk and sport on FRAP values in menopause women after and before consumption of soymilk. * significant level was set at below 5% ($p < 0.05$).

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Table 1. Comparison of changes in percentage for menopausal symptoms (Kupperman Index %) after exercise and soymilk consumption in menopause women.

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 flashes	83.3*

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

Table 2. Comparison of changes in percentage for menopausal symptoms (Kupperman Index %) after soymilk consumption in menopause women.

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 flashes	72.7*

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

Discussion

Hot flashes are experienced in those periods of the female life, when estrogen levels are low. The women who exercised (walking, biking, and swimming) four and a half hours a week and consumed soy, had 49% fewer hot flashes, and those who exercised four or more days a week plus soy, had 46% fewer hot flashes (27,28). This study demonstrated that 12.5 g of soy protein with Genistein=13 mg and Daidzein= 4.13 mg per day was effective in alleviating vasomotor symptoms, such as hot flashes, consistent with previous studies results (29-32). Additionally, the

present results showed a decrease of other subjective symptoms, which was not reported by previous studies (31, 32). In this study, consumption of soymilk (Genistein 13 mg Daidzein 4.13 mg per day) reduces hot flashes, 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 (33). 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 (34, 35). The isoflavone supplementation may increase estrogen levels and may have an indirect effect due to isoflavones acting on sex hormone-binding globulin. The results of this study show that isoflavone supplementation can increase total antioxidant defence systems (7). It seems isoflavones of soymilk can be effective on ALP activity through curbing bone resorption especially in menopause women groups as was demonstrated in the subjects of present study. To be effective different studies have reported the intake of 20–90 milligrams of isoflavones per day. The soymilk in this study provided, 13 mg of Genistein and 4.13 mg of Daidzein per day, which is in accordance with other studies (36, 37). Some of studies have reported the lesser or more amounts can be effective in different periods of time (38). The Soya protein consumption, thus, is a valuable plant estrogen, which can be recommended to reduce the vasomotor symptoms in menopause women. Additionally, the present results showed significant ($p < 0.05$) increase of total antioxidant level in the control < exercise + soymilk < soymilk consumption groups. These results demonstrate that consumption of soymilk for 3 months is more effective than soymilk plus exercise and can reduce some of

menopausal symptoms in postmenopausal women. A higher antioxidant activity was expected in the exercise group consuming soy milk.

However, surprisingly, the results demonstrated that consumption of soymilk for 3 months was more effective than soymilk plus exercise and could reduce some of menopausal symptoms in postmenopausal women. This could be due to the amount and duration of soymilk consumption. In conclusion, the future studies, should

determine the total energy expenditure (TEE), include one sport group without soymilk and more antioxidant assay methods for blood samples are required.

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