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## Assessment of Soy Phytoestrogens and Exercise on Lipid Profiles and Menopause Symptoms in Menopausal Women

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**Abstract:** The purpose of this study was to determine the effect of soy phytoestrogens and exercise on lipid profiles and menopausal symptoms in menopausal women. In this study, 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) which were provided daily for 3 months. Subjects were given soymilk (Maxsoy, Co.) which contained 12.5 g of soy protein with Genistein, 13 mg and Daidzein, 4.13 mg day<sup>-1</sup>. Menopausal symptoms was 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 use. Total Antioxidant Status (TAS) of serum was measured using the Ferric Reducing Ability of Plasma (FRAP) assay. 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. Vaginal problem, sexual symptoms and vasomotor significantly reduced (p<0.05) after soymilk consumption. The soymilk treatment and exercise+soymilk decreased total cholesterol and triglyceride levels compared with control group. This study suggests that soymilk isoflavones for 3 months treatment may be a safe and effective alternative therapy for menopausal symptoms and may offer a benefit to enhance antioxidant status to improve women health and quality of life.

**Key words:** Cholesterol, triglyceride, menopausal symptoms, soymilk

### INTRODUCTION

An estimated 40 million women will go through menopause in the next 20 years (Bonnier *et al.*, 1995). The increasing number of middle-aged and older individuals includes a concomitant and continuing rise in the number of women who live most of their lives in a hypoestrogenic state. Today Estrogen Therapy (ERT) and drugs like bisphosphonates, calcitonin and raloxifene is employed to prevent and treat osteoporosis (Scharbo, 1996). However, prolonged exposure to unopposed estrogens stimulates growth of endometrium, thus increasing the risk of endometrial hyperplasia and neoplasia (Boroditsky, 2000). The identification of an alternative agent, which has the beneficial effects of estrogen but has low cancer risk and side effects, would, therefore, be of considerable value. 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. Oxidative stress is caused by a relative overload of oxidants, i.e., reactive oxygen species (Hanachi *et al.*, 2006). Reactive Oxygen

Species (ROS) free radicals production in the body can initiate lipid peroxidation (Hanachi *et al.*, 2004). Antioxidant supplementation can provide extra protection against these ROS. 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 hypothesized that isoflavone supplementation can increase total antioxidant defence systems and reduce the impact of ROS generated due to intensive exercise (Diplock, 1996).

The comparison 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 (Ji, 1993).

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 (Kokkinos *et al.*, 2001), diabetes (Bernsen and Nagelkerke, 2007) other

metabolic disorders, menopausal symptoms and cardiovascular disease (Albright and Thompson, 2006; Larun *et al.*, 2006) joint problems, or mental health. Menopausal women who exercise regularly appear to have a better quality of life than women who do not (Elavsky and McAuley, 2007).

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 (Albertazzi *et al.*, 1998). Soy is a part of Asian traditional diet (Kurzer and Xu, 1997), showing some relationship with the above-mentioned diseases. Women in Asian cultures who regularly consume soy, typically have less severe climacteric symptoms (hot flashes and night sweats) than women in other cultures. It is speculated that soy is the reason for the reduced symptoms (Lock, 1986). There are many varieties of isoflavones extracted from soy protein, especially daidzein and genistein. Daidzein, genistein and their corresponding glycosylated forms account for the major isoflavones in soy.

Many of today's foods for women contain soy, which is being studied for its ability to help prevent cardiovascular disease by lowering the so-called bad cholesterol, or LDLs-low density lipoproteins (Nams, 2000). Epidemiological studies comparing Asian and Western populations suggest that consuming a phytoestrogen-rich diet ameliorates symptoms of estrogen deficiency in postmenopausal women (Adlercreutz, 1990) and may protect against breast cancer, bone loss and cardiovascular disease (Rose *et al.*, 1986; Dalais *et al.*, 1998; Willett, 1998). However, the findings of recent intervention studies support a role for phytoestrogens as an alternative to conventional Hormone Replacement Therapy (HRT) in postmenopausal women (Glazier and Bowman, 2001). The isoflavones may be responsible for the enhancement of triglyceride levels and slight changes in HDL in women who consume soy protein daily (This *et al.*, 2001; Nestel *et al.*, 1999).

Even though there are a lot of publications exist regarding the symptoms of menopause and soy product (i.e., soy milk). As soy products are new in Iranian society, soy does not constitute a part of the general diet in Iran. This study therefore aimed to determine the effect of soy milk consumption and exercise on vasomotor symptom, Total Antioxidant Capacity (TAC), total cholesterol and triglyceride with determined two active compounds (Genistein and Daidzein) in postmenopausal women.

## MATERIALS AND METHODS

**Samples:** To be eligible for this study, Those women years post menopause  $5.47 \pm 3.4$  years 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, soybean-derived products, or herbal supplements. Other inclusion criteria were an intact uterus, Follicle-Stimulating Hormone (FSH) levels in blood serum exceeding  $25 \mu\text{L}$ , estradiol levels less than  $100 \text{ pg m}^{-1}$  and presence of hot flashes. 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 protocol was approved by the Scientific Advisory Committee and Ethical Committee of University. 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.

This study, was carried out on 37 postmenopausal women, age,  $52.2 \pm 4.6$  years were randomly assigned to soymilk consumption ( $n = 15$ ), (exercise+soymilk) one hour walking per day+soymilk ( $n = 12$ ) and control ( $n = 10$ ) which were provided daily for 3 months. Soymilk was provided by Maxsoy, Co, Tehran Iran, which contained, 12.5 g of soy protein with Genistein, 13 mg and Daidzein,  $4.13 \text{ mg day}^{-1}$ . The information on demographic characteristics including age, ethnicity and education level were collected at the start of the study.

**Menopause symptoms:** Women were also queried about menopausal symptoms covered by Kupperman *et al.* (1959). This scale can be used to assess changes in different symptoms, before and after menopause treatment. Height and weight were measured with subjects wearing lightweight clothing and no shoes, body mass index (calculated as  $\text{kg m}^{-2}$ ) was used as an estimate of obesity. 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 flashes was based on number of complaints per day: slight (1-2), moderate (2-3), severe (5-6) and very severe more than 6.

**Total antioxidant assay:** After fasting for 12 h, blood samples were obtained at baseline and 3 months by venipuncture. Total Antioxidant Status (TAS) of serum was measured using the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996). 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 color and can be monitored at 593 nm. products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against  $\mu\text{mol L}^{-1}$  concentration of Fe (II) standard solution. The samples were analyzed for total antioxidant concentrations using an Cecil, CE, 2501 model, 2000, series spectrophotometer.

**Lipid profiles:** Serum total cholesterol and triglyceride levels were measured with enzymatic techniques by using the Hitachi 704 auto-analyzer, assay by using the Randox, Co, kit, UK and Chem-enzyme, Co, Iran.

**Statistical analysis:** Statistical analysis Frequency counts, descriptive statistics, Pearsons correlations, stepwise multiple linear regression and repeated measure were performed using the Statistical Package for the Social Sciences (Windows version 11 SPSS). The result obtained from experiment were analyzed by inferential statistic in terms of t-test and Analysis of Variance (ANOVA) in which post-hoc comparisons were made using the Benferonmis test. In light of multiple comparisons statistical significance will assigned at  $p < 0.05$  for all analysis.

**RESULTS**

Analysis of 37 subjects enrolled, revealed that the mean age of respondents was  $52.2 \pm 4.6$  years, years of post menopause  $5.47 \pm 3.4$  years and the mean height was  $157.4 \pm 7.2$  cm. Comparison of weight, BMI did not reveal any significant changes during different stage of study.

**Menopausal symptoms:** Some of menopausal symptoms such, hot flashes, nervousness, vaginal symptoms and sexual symptoms were significantly changed during different stages of the study (Fig. 1). During the treatment period, the menopausal symptoms of participants using soymilk and exercise were significantly ( $p < 0.05$ ) lower than soymilk treatment group. Soymilk supplementation decreased ( $p < 0.05$ ) of hot flashes by 72%, nervousness by 54%, vaginal symptoms by 70% and sexual symptoms by 62.5% after soymilk consumption. Exercise and soymilk treatment decreased significantly ( $p < 0.05$ ) hot flashes, nervousness, vagina land sexual symptoms by 83, 30, 50 and 45%, respectively.

**Total antioxidant status:** The total antioxidant level in control, soymilk and exercise+soymilk groups increasing of level,  $642.88 \pm 66.9$ ,  $1379.11 \pm 87.4$  and  $1288.75 \pm 98 \mu\text{mol L}^{-1}$ , respectively (Fig. 2).

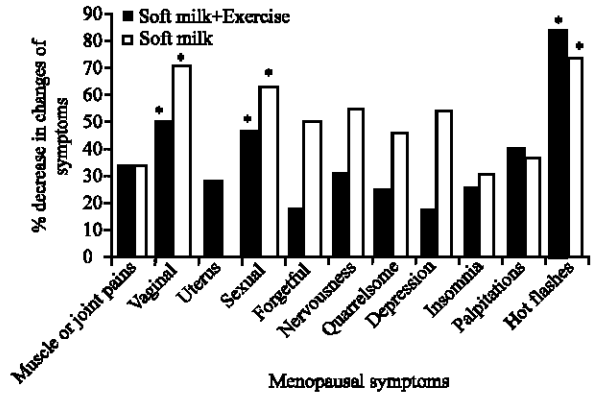


Fig. 1: Percentage changed in menopausal symptoms (Kupperman Index %) in soymilk consumption and soymilk consumption+exercise in menopausal women compared with control group. \*Significant level was set at below 5% ( $p < 0.05$ )

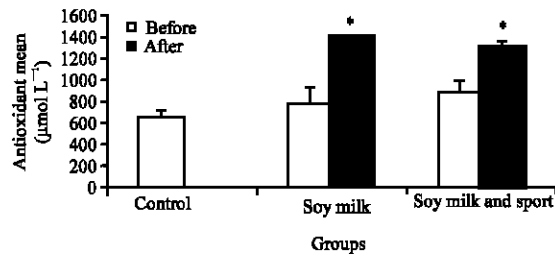


Fig. 2: Effect of soymilk and exercise on FRAP values in menopause women before and after treatment. Data are mean $\pm$ SEM of (n = 10-15) samples obtained from each group. \*Significant level was set at below 5% ( $p < 0.05$ ) compared with control group

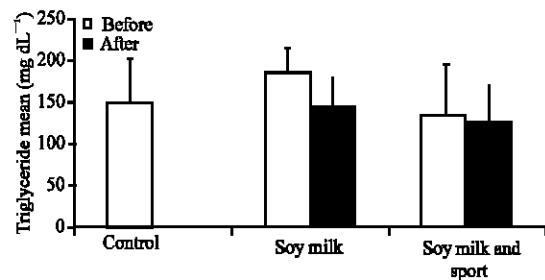


Fig. 3: Effect of soymilk and exercise on total triglycerides values in menopause women before and after treatment. Data are mean $\pm$ SEM of (n = 10-15) samples obtained from each group

**Lipid profiles:** Total cholesterol mean (mg dL<sup>-1</sup>) decreased in soymilk consumption and exercise+soymilk compared with the baseline. However, the mean of total

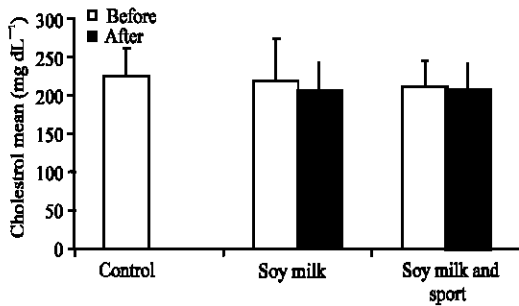


Fig. 4: Effect of soymilk and exercise on total cholesterol values in menopause women before and after treatment. Data are mean±SEM of (n = 10-15) samples obtained from each group

cholesterol and triglycerides levels in two groups decreased compared with the baseline after 3 months but the results were not significant (Fig. 3, 4).

### DISCUSSION

This study demonstrated that 12.5 g of soy protein with Genistein, 13 mg and Daidzein, 4.13 mg day<sup>-1</sup> was effective in alleviating vasomotor symptoms, such as hot flashes, consistent with previous study results (Albertazzi *et al.*, 1998). Additionally, present results showed a decrease of other subjective symptoms, which was not reported by other studies (Upmalis *et al.*, 2000; Glazier and Bowman, 2001). In this study, consumption of Genistein, 13 mg and Daidzein, 4.13 mg day<sup>-1</sup> 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 (Crawford *et al.*, 1996). 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 (Wei *et al.*, 1995). 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 (Ji, 1993). It can be hypothesized that isoflavone supplementation can increase total antioxidant defence systems and reduce the impact of ROS. Soy protein with isoflavones has been reported by Anderson *et al.* (1995) to lower total cholesterol and to inhibit oxidizability of LDL (Tikkanen *et al.*, 1998). Although our data showed a

similar protective effect of isoflavone, such as a decrease in total cholesterol, the mechanisms have not been clearly defined. These effects may be attributed to weak estrogenic effects of isoflavones, which also possess antioxidant properties. Menopausal women who exercise regularly appear to have a better quality of life than women who don't (Scambia *et al.*, 2000). Additionally, our results showed significant (p<0.05) increase of total antioxidant level in control<soymilk consumption<exercise+soymilk groups. These results demonstrate that consumption of soymilk for 3 months was more effective than soymilk+exercise and could reduce some of menopausal symptoms in postmenopausal women. This could be due to the duration of exercise and amount of soymilk consumption.

### CONCLUSION

These results demonstrate that consumption of soymilk with isoflavones for 3 months reduces some of menopausal symptoms and improves quality of life in postmenopausal women. So as to help guide women and clinicians in finding lifestyle changes to prevent symptoms, thus reducing or providing alternatives to use of medications. 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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