



ORIGINAL ARTICLE

The Effect of Soya on some Hormone levels in Women with Polycystic Ovary Syndrome (balance diet): a cross over Randomized clinical trial

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ABSTRACT

Hormonal effects of soya and isoflavones have been investigated in various studies revealing inconsistent results. The present study aimed to assess the effects of soya on sexual hormones includes FSH, Estradiol, and Testosterone in the women with polycystic ovary syndrome (PCOS). A total of 42 women with PCOS participated in this study. The patients received 70 g/d soy flour (as a loaf of soy bread) for 2 months. At four stages (before the study, after the first two months, after the wash out period, and after the second two months), blood sample were taken from the participants on days 3-5 of the menstrual cycle. Soy bread consumption caused no statistically significant changes in the levels of FSH, Estradiol, and Testosterone. The possible advantages derived from the therapeutic use of soy bread in PCOS may limit to long-term consumption.

Keywords: Soy bread, FSH, Estradiol, Testosterone

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INTRODUCTION

Poly Cystic Ovarian Syndrome (PCOS) is the most common endocrine disorder of the reproductive tract. The prevalence of PCOS has been reported to be about 5-10% among the women in the reproductive age [1-3]. Besides, the prevalence of PCOS among the reproductive age Iranian women was 14.6% using the Rotterdam criteria definition [4].

Biochemical abnormalities in the patients with PCOS can lead to an increase in testosterone and androgen levels, ratio of Luteinizing Hormone (LH) to Follicle Stimulating Hormone (FSH), insulin level, and insulin resistance and a decrease in *Sex Hormone-Binding Globulin (SHBG)* level [5]. The therapeutic approaches for PCOS consist of two types of interventions: drug treatment and change in lifestyle with exercise and weight loss. The available common drugs are effective in prevention of the prevalence of PCOS, but they have side effects namely amenorrhea, decreased bone mineral density, depression, and overweight [6].

Many women with this syndrome require long-term treatment. Nevertheless, most medical treatments for infertility seem to be less effective in obese women [7-9]. Therefore, the use of non-pharmacological treatment strategies is considered in most studies [10]. Soy is a plant of the Leguminosae family which contains isoflavones, such as genistein and daidzein [11]. The effects of some isoflavones, in particular soy isoflavones, have been studied by several researchers and their biological activity related to estrogen receptor-mediated mechanisms have been observed [12-14]. Numerous other biological effects, including antioxidant capacity and antiproliferative as well as anti-inflammatory effects independent of estrogen receptors, have been determined, as well [13-16].

Up to now, studies have shown inconsistent hormonal effects of isoflavone consumption in premenopausal women [17-19]. The majority of these studies have been conducted in normal women, while metabolic syndrome and hormonal disturbances are predominant findings in the women with PCOS [20]. Furthermore, the use of cheap and accessible forms of soy foods have been less investigated in the previous studies. Thus, the current Crossover, Randomized, Controlled Trial (RCT) aims to clarify the hormonal effects of soya in the premenopausal women suffering from PCOS.

MATERIALS AND METHODS

Subjects

The present study was conducted on 42 women whose age ranged from 18 to 35 years old. They suffered from the PCOS and had referred to Motahari clinic affiliated to Shiraz University of Medical Sciences since August 2012 to December 2012. The participants were selected through convenience sampling and were randomly divided into two groups each including 28 subjects using block randomization. The inclusion criteria of the patients were having two of the three clinical-biochemical Rotterdam criteria; i.e., hyperandrogenism symptoms, oligomenorrhea, and anovulation, and sonographic evidence of PCOS.

These women should not have used steroids or other drugs that affected the metabolic system three months before the study. The women suffering from liver disease, hyperthyroidism, hypothyroidism, hyperprolactinemia, hyperplasia of adrenal glands, kidney disease, cardiovascular disease, endocrinoneoplasia, nervous system disorders, and mental disorders were excluded from the study.

Study design

This crossover RCT aimed to investigate the effectiveness of balanced bread of soybean flour in some sexual hormones in premenopausal women with PCOS.

The research project protocol and the advantages and limitations of the diet were described for each volunteer. Then, the individuals signed written informed consents to participate in the study and completed the demographic questionnaires. All the participants started their diets 3 days before the study. In each step, the experimental and control groups respectively consumed soy bread and wheat bread with their common diets for 2 months. Each loaf of soy bread was made of 75 grams of soy flour and 40 grams of wheat flour (HadisBread Company, Shiraz, Iran). On the other hand, the control group was asked not to consume the intended list of foods that contained high amounts of soya. It should be mentioned that the study participants were aware of their diets.

All the participants were contacted once a week and were asked about the amount of calories and types of consumed foods. In the first step of the study, group A was considered as the experimental group and group B as the control group. The two groups followed their diets for two months. Afterwards, both groups were advised to use their usual diets for a month (wash out period). In the second step of the study, the diets were exchanged between the two groups. During the study, the participants were asked not to alter their common diets and not to use hormonal drugs, such as LD, HD, and metformin. However, they could use low amounts of acetaminophen or aspirin. None of the participants had any important medical problems and they did not use the medications that might affect the hormonal profiles.

Biochemical measurements

At four stages (before the study, after the first two months, after the wash out period, and after the second two months), 10 ml blood samples were taken from the participants on days 3-5 of the menstrual cycle (some subjects had taken oral *medroxy progesterone* acetate tablets for *inducing menstrual periods*). The blood samples were investigated for FSH, estradiol, and testosterone levels. Serum and plasma were separated by centrifugation at $1,465 \times g$ (3,200 rpm) at 4° C for 15 minutes. Then, they were stored at -80°C until further analysis. Serum levels of testosterone and estradiol were determined by radioimmunoassay (RIA) using commercial reagents (Immunotech Company, France). In addition, FSH was measured by *electroimmuno assay* (EIA) using commercial reagents (Cambridge Isotope Laboratories, Inc., USA). Besides, analytical sensitivity for the assayed factors was as follows: testosterone 2.5 ng/dl, estradiol 0.6 ng/dl, and FSH 0.02 IU/l.

Statistical analysis

The analyses were performed using the SPSS statistical software (version 16). Descriptive variables, such as mean and standard deviation, were used. The data were compared using paired and independent t-test, Wilcoxon, and Mann Whitney u test. Besides, $P < 0.05$ was considered as statistically significant.

RESULTS

The study results revealed no significant difference between the two groups in the terms of age, BMI, testosterone, FSH, and estradiol before the intervention ($P > 0.05$). The basic characteristics of the subjects are presented in Table 1. The results of the study also indicated that the transitional period was not significantly effective in the results ($P > 0.05$). The group effect had no impact on the results, as well.

Moreover, a slight decrease was found in circulating total testosterone, FSH, and estradiol concentrations following soy bread consumption; however, these were not statistically significant ($P > 0.05$) (Table 2).

	Group A	Group B	P value
Age (mean \pm S.D)	24.33 \pm 4.78	23.29 \pm 4.16	0.454
Weight (mean \pm S.D)	63.67 \pm 24.60	64.90 \pm 23.38	0.868
Number of pregnancies med(IQR*)	1(1-1)	1(1-1)	0.638
Number of abortions med (IQR)	1(1-1)	1(1-1)	0.973
Number of children med (IQR)	1(1-1)	1(1-1)	1.000
Prevention of pregnancy n (%)	19(90.50)	17(81.00)	0.519
Use of hormones n (%)	20(95.20)	19(90.50)	0.549
FSH (mIU/ml)	14.4 \pm 23.85	7.93 \pm 10.83	0.598
Estradiol (pg/ml)	23.97 \pm 16.05	22.64 \pm 11.43	0.759
Testosterone (ng/ml)	0.74 \pm 0.33	0.67 \pm 0.34	0.529

* Interquartile range

		Before the first intervention	After the first intervention	After the second intervention	Effect of soy bread diet after two phases (totally)	Effect of soy bread diet (P*value)
FSH(mIU/ml)	Group A	14.4 \pm 23.8	13.07 \pm 19.45	14.04 \pm 27.7	- 0.29 \pm 6.27	0.827
	Group B	7.93 \pm 10.93	6.9 \pm 8.97	7.3 \pm 8.75		
Estradiol (pg/ml)	Group A	23.97 \pm 16.05	22.42 \pm 13.07	26.31 \pm 18.34	-0.519 \pm 11.24	0.648
	Group B	22.64 \pm 11.43	19.7 \pm 14.03	22.55 \pm 13.51		
Testosterone (ng/ml)	Group A	0.74 \pm 0.33	0.69 \pm 0.22	0.74 \pm 0.32	0.006 \pm 0.21	0.807
	Group B	0.67 \pm 0.34	0.61 \pm 0.32	0.68 \pm 0.34		

P value was yielded from paired t test or Wilcoxon test

DISCUSSION

In the last recent decade, many international studies have estimated the effects of phytoestrogens on different aspects of health topics. However, the effects of phytoestrogens on health have not been clearly established. Nevertheless, the possible estrogenic and antiestrogenic effects of isoflavones, phytoestrogens contained in soy foods, have been identified [12-16]. Studies have shown positive epidemiologic evidence linking soy food consumption to reduced risk of coronary heart disease and cancers, such as breast cancer [21-25]. Nevertheless, some other studies have presented conflicting results, including increased risk of developing estrogen-sensitive cancers, impaired cognitive function, and reproductive problems [26]. These inconsistencies in the results of soy studies have been justified using a number of possible explanations, such as using various forms of products including usual soy foods, Isolated Soy Protein (ISP), and isolated isoflavones, investigation of pre- and or post-menopausal women, and dose response relationships. Furthermore, most of these studies have been conducted among the normal women, while metabolic syndrome and hormonal disturbances are predominant findings in the women with PCOS [20]. The gonadotropins pulse in the women with PCOS may also show resistance to the effects of genistein [27]. The confusion regarding the researches on soy and health often starts when researchers do not provide sufficient details about the products and compounds that they use or do not relate the used amounts or concentrations to real-life consumption patterns [26]. Considering these controversies, we decided to evaluate the effect of soy, as soy bread, in the premenopausal women suffering from PCOS.

Maskarinec *et al.* conducted a randomized 2-year dietary intervention on 220 healthy premenopausal women and the intervention group consumed two daily servings of soy foods. The results of that study indicated that the intervention had no significant effects on E1, E2, SHBG, and rostenedione, and progesterone [28]. Although some studies have shown a decrease in estradiol levels after use of soy foods [29-32], other studies have revealed amino increase [17;19;33] or no changes in the levels of this hormone [34-36]. Likewise the studies have shown a significant decrease [20], increase [37] or no

changes (17-19) in the levels of testosterone after use of soy foods. We found no significant effects of soy consumption on serum levels of estradiol and testosterone.

The systematic review by Hooper *et al.*, [38] investigated 47 studies that assessed the effects of soy isoflavones on hormone concentrations in pre- and post-menopausal women. The results of this review showed that consumption of soy isoflavones had no effects on circulating total estradiol, estrone, and SHBG in premenopausal women. This is in agreement with the findings of the current study regarding circulating total estradiol. Of course, the premenopausal women with PCOS were investigated in the present study.

The effect of soy consumption on FSH serum levels in the current study is in agreement with the result of a similar study by Khani *et al.*, [20] on 146 women with PCOS. That study showed the non-significant effect of genistein after 3 months. Nevertheless, the systematic review by Hooper *et al.* [38] indicated that consumption of soy isoflavones led to a significant reduction in FSH (by ~22%, $P=0.01$) in pre- and post-menopausal women with normal hormonal profiles.

The previous studies often used ISP (17;19;33-36) or one of the soy metabolic products (20;29;31) to investigate the effects of soya on the study subjects. These approaches were employed to evaluate the effects of each component of soya with more accuracy. Nevertheless, there are several reasons why the food-based approach is a more realistic test for investigation of this intervention such as whether soy foods can be introduced into the usual diet of women and or the effects of soy foods with different macronutrients and micronutrients examined, not just of the isoflavonoid-related effect [28].

In conclusion, according to the results of the present study and those of the recent studies conducted on the issue, the effect of soya (as soy bread) on the sexual hormones in the PCOS women who are usually involved with hormonal disturbances cannot be confirmed. Further robust studies are required to pay attention to other dimensions, such as different ages, different types of soy foods or metabolites, and other sexual hormones, in the women with PCOS in order to come to more promising results.

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