The Effects of Soy Isoflavones on Metabolic Status of Patients With Polycystic Ovary Syndrome

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Context: Limited data are available evaluating the effects of soy isoflavones on metabolic status of patients with polycystic ovary syndrome (PCOS).

Objective: The current study was performed to determine the effects of soy isoflavones on metabolic status of patients with PCOS.

Methods: This randomized, double-blind, placebo-controlled trial was performed on 70 women diagnosed with PCOS according to the Rotterdam criteria who were 18–40 years old. Participants were randomly allocated into two groups to take either 50 mg/d soy isoflavones (n = 35) or placebo (n = 35) for 12 weeks. Metabolic, endocrine, inflammation, and oxidative stress biomarkers were quantified at the beginning of the study and after the 12-week intervention.

Results: After 12 weeks of intervention, compared to the placebo group, soy isoflavone administration significantly decreased circulating serum levels of insulin (-1.2 ± 4.0 vs $+2.8 \pm 4.7$ μIU/mL; $P < .001$) and homeostasis model of assessment-estimated insulin resistance (-0.3 ± 1.0 vs $+0.6 \pm 1.1$; $P < .001$) and increased the quantitative insulin sensitivity check index ($+0.0009 \pm 0.01$ vs -0.01 ± 0.03; $P = .01$). Supplementation with soy isoflavones resulted in significant reductions in free androgen index (-0.03 ± 0.04 vs $+0.02 \pm 0.03$; $P < .001$) and serum triglycerides (-13.3 ± 62.2 vs $+10.3 \pm 24.5$ mg/dL; $P = .04$) compared to the placebo group. There was a significant increase in plasma total glutathione ($+96.0 \pm 102.2$ vs $+22.7 \pm 157.8$ μmol/L; $P = .04$) and a significant decrease in malondialdehyde levels (-0.7 ± 0.8 vs $+0.8 \pm 2.3$ μmol/L; $P = .001$) by soy isoflavone intake compared with the placebo group. We did not observe any significant effect of soy isoflavone intake on other lipid profiles and inflammatory and oxidative stress markers.

Conclusion: Soy isoflavone administration for 12 weeks in women with PCOS significantly improved markers of insulin resistance, hormonal status, triglycerides, and biomarkers of oxidative stress. **(*J Clin Endocrinol Metab* 101: 3386–3394, 2016)**

Polycystic ovary syndrome (PCOS), a common endocrine disorder among women of reproductive age, is mainly associated with hyperinsulinemia, impaired glucose metabolism, hyperandrogenism, and dyslipidemia (1). Moreover, emerging evidence has shown that elevated levels of inflammatory factors and biomarkers of oxidative stress in the blood and histological samples of women with PCOS might play important roles in the pathogenesis of PCOS (2, 3). These disorders can increase the risk of type 2 diabetes mellitus (T2DM), coronary heart disease, endometrial cancer (4), and ovulatory dysfunction (5, 6).

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Abbreviations: BMI, body mass index; CV, coefficient variance; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; FPG, fasting plasma glucose; GSH, glutathione; HC, hip circumference; HDL, high-density lipoprotein; HOMA-B, homeostasis model of assessment-B cell function; HOMA-IR, homeostasis model of assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; HSD, hydroxysteroid dehydrogenase; LDL, low-density lipoprotein; MDA, malondialdehyde; MET, metabolic equivalent; mFG, modified Ferriman-Gallwey score; NO, nitric oxide; PCOS, polycystic ovary syndrome; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity; T2DM, type 2 diabetes mellitus; VLDL, very LDL; WC, waist circumference.

Recently, some human studies have shown the relation between dietary pattern and metabolic profiles, inflammatory factors, and biomarkers of oxidative stress among women with PCOS (7–9). There is growing interest in using the soy isoflavones in diseases related to metabolic syndrome (10). The basis of this interest derives mostly from the results of epidemiological surveys, and nutritional intervention studies in human and animal models suggest that dietary isoflavones have protective effects against menopausal symptoms, coronary heart disease, cancer, hyperlipidemia, osteoporosis, and various forms of chronic renal disease (11–13). In a pilot study without the placebo group by Romualdi et al (14), it was shown that taking 36 mg/d of genistein for 6 months among patients with PCOS decreased low-density lipoprotein (LDL)-cholesterol levels but did not affect anthropometric features, other lipid profiles, the hormonal milieu, menstrual cyclicity, and glycoinsulinemic metabolism. In addition, consumption of genistein (an isoflavone in soybean products) with a dose of 18 mg twice a day orally for 3 months among women with PCOS decreased LH, triglycerides, LDL-cholesterol, dehydroepiandrosterone sulfate (DHEAS), and T but did not change other lipid profiles and FSH (8). Furthermore, further studies have reported a positive impact of phytoestrogens on the glycoinsulinemic assessment of postmenopausal women, obese subjects, and patients with T2DM (15, 16).

Favorable effects of soy isoflavones on metabolic profiles may result from a direct inhibitory activity on several enzymes, which accounts for the positive metabolic effects associated with their dietary intake (14), glucose metabolism (17), and through the inhibition of insulin discharge by the pancreatic islets (18). Limited data are available assessing the effects of soy isoflavones on glucose homeostasis parameters, hormonal status, lipid concentrations, markers of inflammation and oxidative stress of patients with PCOS. The aim of the present study was to evaluate the effects of soy isoflavone supplementation on metabolic status in women with PCOS.

Subjects and Methods

This study was a prospective randomized, double-blind, placebo-controlled clinical trial.

Participants

This trial was conducted among 70 participants with PCOS aged 18–40 years who were referred to the Kosar Clinic in Arak, Iran, from December 2015 to February 2016. Diagnosis of PCOS was performed according to the Rotterdam criteria (19). Those with two of the following criteria were considered as having PCOS: 1) oligo- and/or anovulation (defined as menses delayed $>$ 35 days or $<$ eight spontaneous hemorrhagic episodes per year); 2) clinical (hirsutism using modified Ferriman-Gallwey score [mFG] of $\geq$ 8) (19) and/or biochemical signs of hyperandrogenism (20); and 3) polycystic ovaries (12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume $>$ 10 mL^3) (19). We excluded pregnant women and subjects with elevated levels of prolactin, thyroid disorder, endocrine diseases including diabetes or impaired glucose tolerance, and gastrointestinal problems from the study.

Ethics statements

This study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all participants. The research was approved by the ethics committee of Arak University of Medical Sciences and was recorded in the Iranian web site for registration of clinical trials (http://www.irct.ir: IRCT201601025623N62).

Study design

At the beginning of the study, all participants were matched for age, phenotypes of PCOS, and body mass index (BMI). Participants were then randomly divided into two groups to receive either soy isoflavone supplements (n = 35) or placebo (n = 35) for 12 weeks. Participants were requested not to change their ordinary physical activity and not to take any nutritional supplements during the 12-week trial. All patients completed 3-day food records and three physical activity records at the study baseline; at weeks 3, 6, and 9 of the intervention; and at the end of the trial. Daily macro- and micronutrient intakes were analyzed by nutritionist IV software (First Databank). In the current study, physical activity was described as metabolic equivalents (METs) in hours per day. To determine the MET for each patient, we multiplied the time reported for each physical activity (in hours per day) by its related MET coefficient by standard tables (21).

Intervention

In the intervention group, participants received 50 mg/d soy isoflavones containing 37.5 mg genistein, 10 mg daidzein, and 2.5 mg glycitein for 12 weeks. Due to the existence of limited data about the appropriate dosage of soy isoflavones for PCOS, we used the above-mentioned dosage of soy isoflavones based on a previous study in PCOS patients (14). It must be considered that this level (50 mg soy isoflavones) is achievable by consuming 500 mL soy milk daily in a real dietary intake. Soy isoflavone supplement and placebo capsules were similar in shape and size and were manufactured by Soygol.

Treatment adherence

Every 4 weeks, participants were given enough supplements to last until 3 days after their next scheduled visit, and they were instructed to return all the unused supplements at each visit. Patients were scheduled for follow-up visits every 2 weeks for an intermediate evaluation. To evaluate compliance, the remaining supplements were counted and subtracted from the amount of supplements provided to the participants. To increase compliance, all participants received short messages on their cell phones every day to remind them about taking the capsules.

Assessment of anthropometric measures

Weight and height of participants were determined in an overnight fasting status using a standard scale (Seca) at the beginning

of the study and after the 12-week intervention. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was assessed at the minimum circumference between the iliac crest and the last rib. Hip circumference (HC) was quantified at the maximum protuberance of the buttocks. All anthropometric measures were done by a trained midwife.

Assessment of outcomes

In our study, markers of insulin resistance and androgens were considered as the primary outcome, and lipid profiles and biomarkers of inflammation and oxidative stress were considered as the secondary outcomes.

Clinical assessments

In the current study, clinical assessments included determinations of hirsutism using a mFG scoring system (22), of acne score (23), and of alopecia based on assessment guidelines collated by Olsen et al (24). Acne was marked by a four-point scale: 0, no acne; 1, minor acne on face; 2, moderate acne on face only; and 3, severe acne on face and back or chest (23).

Biochemical assessment

Ten-milliliter fasting blood samples were collected at the beginning and end of the study at the Arak reference laboratory in a fasting status and centrifuged to separate serum. Then, the samples were stored at −80°C before analysis. Serum insulin concentrations were assessed using available ELISA kit (Dia Metra) with inter- and intra-assay coefficient variances (CVs) of 3.3 to 4.9%, respectively. The homeostasis model of assessment-insulin resistance (HOMA-IR), homeostasis model of assessment β-cell function (HOMA-B), and quantitative insulin sensitivity check index (QUICKI) were determined according to the suggested formulas (25). Serum total T with inter- and intra-assay CVs of 4.0 to 5.9%, SHBG with inter- and intra-assay CVs of 3.5 to 5.1%, free T with inter- and intra-assay CVs of 3.9 to 5.8%, and DHEAS concentrations with inter- and intra-assay CVs of 4.1 to 6.2% were determined using commercial kits (DiaMetra). Free androgen index (FAI) was calculated as the ratio of total T to SHBG. Enzymatic kits (Pars Azmun) were used to quantify fasting plasma glucose (FPG), serum triglycerides, and very LDL (VLDL)-, total-, LDL-, and high-density lipoprotein (HDL)-cholesterol concentrations. All inter- and intra-assay CVs for FPG and lipid concentrations were < 5%. Serum high-sensitivity C-reactive protein (hs-CRP) concentrations were evaluated by commercial ELISA kit (LDN) with inter- and intra-assay CVs of 4.5 to 6.4%, respectively. The plasma nitric oxide (NO) concentrations were assessed using Griess method (26). Plasma total antioxidant capacity (TAC) concentrations were determined by the method of ferric-reducing antioxidant power developed by Benzie and Strain (27), total glutathione (GSH) concentrations were determined using the method of Beutler and Gelbart (28), and malondialdehyde (MDA) concentrations were determined by the thiobarbituric acid reactive substances spectrophotometric test (29). All inter- and intra-assay CVs for NO, TAC, GSH, and MDA concentrations were < 5%.

Sample size

Using a formula suggested for clinical trials, having 30 participants in each group was adequate while considering a type 1 error (α) of 0.05 and type 2 error (β) of 0.20 (power = 80%), 4.3 pg/mL as SD, and 3.0 pg/mL as the mean distinction (d) of total T as the key variable (30). Assuming five dropouts in each group, the final sample size was determined to be 35 participants in each group.

Randomization

Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation were concealed from the researchers and participants until the final analyses were completed. The randomized allocation sequence, enrolling participants, and allocating them to interventions were conducted by a trained midwife at the gynecology clinic.

Statistical methods

To evaluate whether the study variables were normally distributed or not, we used the Kolmogorov-Smirnov test. For non-normally distributed variables (total T, DHEAS, and GSH), we applied log transformation. To detect differences in anthropometric measures as well as in macro- and micronutrient intakes between the two groups, we applied Student's *t* test to independent samples. The Pearson χ^2 test was used to compare categorical variables. To determine the effects of soy isoflavone administration on markers of insulin resistance, hormonal status, lipid profiles, biomarkers of inflammation and oxidative stress, we used one-way repeated measures ANOVA. In this analysis, the treatment (soy isoflavones vs placebo group) was regarded as a between-subject factor, and time with two time-points (the onset of the study and after 12-week intervention) was considered as a within-subject factor. To identify within-group differences (baseline and end of trial), we used paired-samples *t* tests. Adjustment for changes in baseline values of biochemical parameters, age, and BMI at the baseline was performed by analysis of covariance using general linear models. *P* value < .05 was considered statistically significant. All statistical analyses used the Statistical Package for Social Science version 18 (SPSS Inc).

Results

In the current study, all 70 participants (soy isoflavones [n = 35] and placebo [n = 35]) completed the trial (Figure 1). On average, the rate of compliance in the present study was high, such that > 90% of capsules were taken throughout the study in both groups. No side effects were reported after the consumption of soy isoflavone supplements in women with PCOS throughout the study.

Mean age, height, weight, BMI, WC, HC, and METs at the baseline and the end of the trial were not statistically different between the two groups (Table 1). After 12 weeks of intervention, alopecia (31.6 vs 4.3%; *P* = .01) decreased following the consumption of soy isoflavone supplements compared with the placebo, but acne was unchanged (31.8 vs 13.0%; *P* = .13).

Based on the 3-day dietary records obtained at the baseline, at the end of the trial, and throughout the trial, we found no significant difference in mean dietary macro- and micronutrient intakes between the two groups (Table 2).

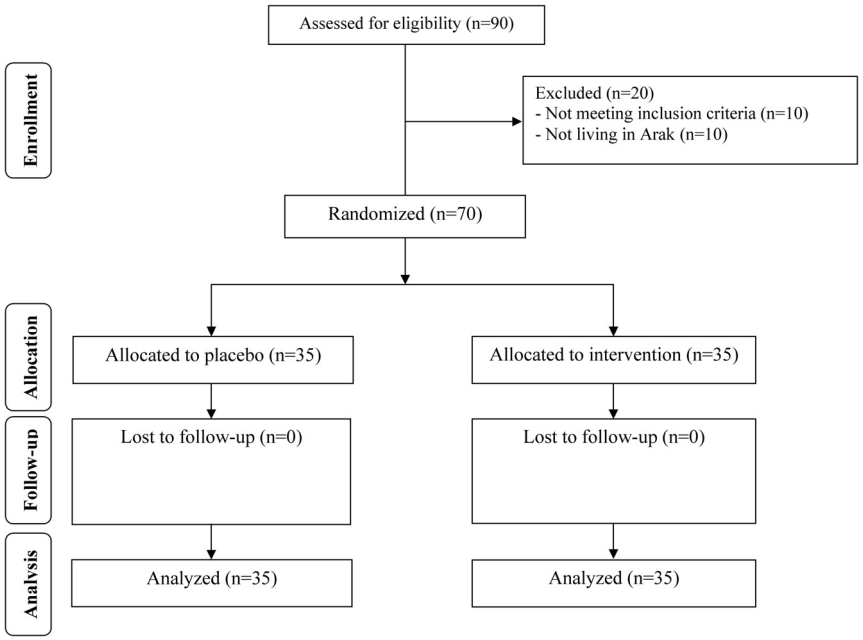

Figure 1. Summary of patient flow diagram.

After 12 weeks of intervention, compared to the placebo group, soy isoflavone administration significantly decreased circulating serum levels of insulin (−1.2 ± 4.0 vs +2.8 ± 4.7 μIU/mL; $P < .001$), HOMA-IR (−0.3 ± 1.0 vs +0.6 ± 1.1; $P < .001$), and HOMA-B (−4.4 ± 15.0 vs +10.7 ± 18.2; $P < .001$) and increased QUICKI (+0.0009 ± 0.01 vs −0.01 ± 0.03; $P = .01$) (Table 3). Supplementation with soy isoflavones resulted in significant reductions in serum total T (−0.2 ± 0.4 vs +0.1 ± 0.3 ng/mL; $P = .01$), FAI (−0.03 ± 0.04 vs +0.02 ± 0.03; $P < .001$), mFG scores (−1.1 ± 0.9 vs −0.2 ± 0.8; $P < .001$), triglycerides (−13.3 ± 62.2 vs +10.3 ± 24.5 mg/dL; $P = .04$), and VLDL-cholesterol (−2.7 ± 12.4 vs +2.0 ± 4.9 mg/dL; $P = .04$) compared to the placebo group. There was a significant increase in SHBG (+3.9 ± 6.2 vs −1.3 ± 3.5 nmol/L; $P < .001$) and plasma GSH (+96.0 ± 102.2 vs +22.7 ± 157.8 μmol/L; $P = .04$) and a significant decrease in MDA levels (−0.7 ± 0.8 vs +0.8 ± 2.3 μmol/L; $P = .001$) by soy isoflavones intake compared with the placebo group. We did not observe any significant effect of soy isoflavone intake on other lipid profiles and inflammatory and oxidative stress markers.

After adjustment for baseline values of biochemical markers, age, and baseline BMI, no significant changes in our findings occurred, except for plasma NO levels ($P = .02$) (Table 4).

Discussion

In the current trial, which to the best of our knowledge is the first of its kind, we evaluated the effects of soy isoflavone supplementation on glycemia status, hormonal status, lipid concentrations, and biomarkers of inflammation and oxidative stress among patients with PCOS. We demonstrated that soy isoflavone administration for 12 weeks among women with PCOS had beneficial effects on markers of insulin resistance, serum total T, SHBG, FAI, trig-

Table 1. General Characteristics of Study Participants

	Placebo Group	Soy Isoflavones Group	*P* Value[a]
n	35	35	
Age, y	25.9 ± 4.8	27.5 ± 6.4	.25
Height, cm	162.5 ± 6.9	163.8 ± 4.9	.36
Weight at study baseline, kg	70.4 ± 13.4	66.9 ± 12.7	.26
Weight at end of trial, kg	69.6 ± 13.3	66.2 ± 12.5	.27
Weight change, kg	−0.8 ± 1.3	−0.7 ± 0.7	.77
BMI at study baseline, kg/m^2	26.7 ± 4.7	24.9 ± 5.6	.12
BMI at end of trial, kg/m^2	26.4 ± 4.6	24.7 ± 4.5	.12
BMI change, kg/m^2	−0.3 ± 0.5	−0.3 ± 0.3	.71
WC at study baseline, cm	83.7 ± 11.8	80.5 ± 9.7	.21
WC at end of trial, cm	83.0 ± 12.0	80.0 ± 9.4	.24
WC change, cm	−0.7 ± 1.4	−0.5 ± 1.7	.52
HC at study baseline, cm	98.9 ± 10.6	100.4 ± 9.7	.52
HC at end of trial, cm	98.1 ± 10.5	99.8 ± 9.6	.48
HC change, cm	−0.7 ± 1.7	−0.6 ± 1.1	.71
MET-h/day at study baseline	29.7 ± 2.1	30.1 ± 2.1	.36
MET-h/day at end of trial	29.6 ± 2.2	30.1 ± 2.2	.24
MET-h/day change	−0.1 ± 0.7	0.02 ± 0.7	.38

Data are expressed as mean ± SD.

[a] Obtained from independent *t* test.

Table 2. Dietary Intakes of Participants Throughout the Study

	Placebo Group	Soy Isoflavones Group	*P* Value[a]
n	35	35	
Energy, kcal/d	2396 ± 182	2371 ± 295	.67
Carbohydrates, g/d	333.2 ± 35.4	327.4 ± 58.8	.61
Protein, g/d	84.5 ± 10.1	87.9 ± 16.6	.30
Fat, g/d	84.1 ± 11.4	82.7 ± 14.6	.64
SFAs, g/d	24.8 ± 4.8	25.4 ± 4.5	.64
PUFAs, g/d	25.9 ± 6.2	26.4 ± 7.1	.76
MUFAs, g/d	22.4 ± 5.8	23.1 ± 5.9	.66
Cholesterol, mg/d	215.3 ± 109.9	197.0 ± 94.9	.45
TDF, g/d	18.4 ± 5.0	19.3 ± 5.0	.48
Magnesium, mg/d	271.6 ± 65.1	273.7 ± 55.4	.88
Zinc, mg/d	10.1 ± 2.2	10.6 ± 2.7	.46
Manganese, mg/d	2.1 ± 0.7	2.1 ± 0.9	.92
Selenium, μg/d	54.2 ± 10.8	53.0 ± 10.6	.62
Calcium, mg/d	1130.7 ± 215.7	1134.2 ± 161.2	.94

Abbreviations: MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; TDF, total dietary fiber. Data are expressed as mean ± SD.

[a] Obtained from independent *t* test.

lycerides, VLDL-cholesterol, GSH, and MDA levels, but it did not have any effect on FPG, other lipid profiles, and biomarkers of inflammation and oxidative stress.

Patients with PCOS are susceptible to several aberrations, including metabolic disorders, inflammation, and oxidative stress (2, 5, 6, 31). Our study indicated that soy isoflavone intake for 12 weeks in women with PCOS resulted in a significant decrease in serum insulin levels, HOMA-IR, and HOMA-B and a significant increase in QUICKI compared with the placebo. Supporting our findings, a 2-month supplementation with a soy food containing 50 mg isoflavones/d led to a significant decrease of insulin levels among postmenopausal women (30). Furthermore, preliminary data from epidemiological surveys and nutritional intervention studies in animals and humans have reported that phytoestrogens may improve glycemic and insulinemic concentrations (15, 32). Few studies have shown an amelioration of insulin sensitivity in animal and human models after soy isoflavone administration (17, 33). However, a 6-month administration of soy protein with or without isoflavone supplementation did not influence glycemic control and insulin sensitivity among Chinese postmenopausal women (34). The results of the current study may support a direct pharmacological effect of soy isoflavones on the markers of insulin metabolism. Furthermore, soy isoflavones may improve markers of insulin resistance through the inhibition of protein tyrosine kinases, which play an important role in the regulation of insulin secretion by pancreatic β-cells (15).

The current study demonstrated that soy isoflavone intake for 12 weeks in women with PCOS led to a significant decrease in FAI and mFG scores compared with the placebo, but there was no change in other hormonal profiles.

Table 3. Metabolic Profiles and Biomarkers of Inflammation and Oxidative Stress at Baseline and After 12 Weeks of Intervention in Patients With PCOS

	Placebo Group (n = 35)				Soy Isoflavones Group (n = 35)				
	Baseline	End of Trial	Change	*P* Value[a]	Baseline	End of Trial	Change	*P* Value[a]	*P* Value[b]
FPG, mg/dL	93.9 ± 6.1	93.7 ± 7.0	−0.2 ± 4.5	.73	91.3 ± 8.8	92.8 ± 5.1	1.5 ± 6.7	.18	.19
Insulin, μIU/mL	10.1 ± 6.0	12.9 ± 7.2	2.8 ± 4.7	.002	11.6 ± 5.6	10.4 ± 2.9	−1.2 ± 4.0	.08	<.001
HOMA-IR	2.4 ± 1.5	3.0 ± 1.8	0.6 ± 1.1	.002	2.7 ± 1.4	2.4 ± 0.8	−0.3 ± 1.0	.08	<.001
HOMA-B	35.1 ± 22.5	45.8 ± 26.9	10.7 ± 18.2	.001	42.1 ± 20.3	37.7 ± 10.8	−4.4 ± 15.0	.09	<.001
QUICKI	0.34 ± 0.03	0.33 ± 0.03	−0.01 ± 0.03	.01	0.33 ± 0.02	0.33 ± 0.01	0.0009 ± 0.01	.83	.01
Total T, ng/mL	1.2 ± 0.7	1.3 ± 0.6	0.1 ± 0.3	.03	1.4 ± 1.0	1.2 ± 0.9	−0.2 ± 0.4	.19	.01
SHBG, nmol/L	38.9 ± 9.9	37.6 ± 9.1	−1.3 ± 3.5	.03	38.0 ± 8.7	41.9 ± 7.9	3.9 ± 6.2	.001	<.001
FAI	0.10 ± 0.06	0.12 ± 0.05	0.02 ± 0.03	.004	0.12 ± 0.08	0.09 ± 0.06	−0.03 ± 0.04	.001	<.001
mFG scores	14.1 ± 5.3	13.9 ± 5.2	−0.2 ± 0.8	.08	14.7 ± 4.6	13.5 ± 4.1	−1.1 ± 0.9	<.001	<.001
Free T, pg/mL	5.6 ± 3.3	5.2 ± 3.4	−0.4 ± 1.9	.19	4.7 ± 3.8	4.0 ± 2.7	−0.7 ± 2.3	.06	.54
DHEAS, μg/mL	4.3 ± 2.1	4.2 ± 2.1	−0.1 ± 1.4	.49	4.7 ± 3.8	4.0 ± 2.7	−0.7 ± 2.3	<.001	.96
Triglycerides, mg/dL	106.3 ± 41.0	116.6 ± 5.1	10.3 ± 24.5	.01	97.0 ± 76.3	83.7 ± 32.0	−13.3 ± 62.2	.21	.04
VLDL-cholesterol, mg/dL	21.3 ± 8.2	23.3 ± 10.0	2.0 ± 4.9	.01	19.4 ± 15.3	16.7 ± 6.4	−2.7 ± 12.4	.21	.04
Total cholesterol, mg/dL	170.8 ± 32.3	170.6 ± 34.0	−0.2 ± 14.8	.93	149.1 ± 34.0	152.5 ± 29.4	3.4 ± 19.4	.30	.38
LDL-cholesterol, mg/dL	96.3 ± 29.6	93.9 ± 31.5	−2.4 ± 12.8	.27	85.0 ± 32.6	89.9 ± 27.6	4.9 ± 24.5	.24	.12
HDL-cholesterol, mg/dL	53.1 ± 9.3	53.3 ± 10.4	0.2 ± 4.0	.81	44.7 ± 8.0	45.9 ± 7.8	1.2 ± 5.3	.21	.38
Total-/HDL-cholesterol ratio	3.3 ± 0.8	3.3 ± 0.8	−0.01 ± 0.3	.80	3.4 ± 0.8	3.4 ± 0.8	0.005 ± 0.5	.94	.86
hs-CRP, mg/mL	5.3 ± 4.8	5.5 ± 4.3	0.2 ± 2.9	.82	4.8 ± 4.0	4.6 ± 3.6	−0.2 ± 3.6	.82	.75
NO, μmol/L	54.2 ± 16.1	56.9 ± 16.8	2.7 ± 20.2	.44	42.5 ± 3.2	45.5 ± 4.3	3.0 ± 5.1	.001	.93
TAC, mmol/L	977.9 ± 167.1	1011.3 ± 200.1	33.4 ± 251.6	.43	1081.6 ± 124.7	1111.7 ± 130.9	30.0 ± 68.0	.01	.93
GSH, μmol/L	440.0 ± 103.1	462.7 ± 135.4	22.7 ± 157.8	.75	493.2 ± 90.6	589.2 ± 63.6	96.0 ± 102.2	<.001	.04
MDA, μmol/L	2.5 ± 1.0	3.3 ± 2.1	0.8 ± 2.3	.05	2.9 ± 0.6	2.2 ± 0.5	−0.7 ± 0.8	<.001	.001

All values are expressed as mean ± SD.

[a] *P* values represent paired-samples *t* test.

[b] *P* values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA).

Table 4. Changes (Means ± Standard Errors) in Metabolic Profiles of Patients With PCOS Adjusted for Baseline Values of Biochemical Parameters, Age, and Baseline BMI

	Placebo Group	Soy Isoflavones Group	*P* Value[a]
n	35	35	
FPG, mg/dL	0.5 ± 0.8	0.8 ± 0.8	.75
Insulin, μIU/mL	2.5 ± 0.7	−1.0 ± 0.7	.001
HOMA-IR	0.6 ± 0.2	−0.2 ± 0.2	.001
HOMA-B	9.5 ± 2.6	−3.3 ± 2.6	.001
QUICKI	−0.01 ± 0.003	−0.001 ± 0.003	.02
Total T, ng/mL	0.1 ± 0.05	−0.1 ± 0.05	.008
SHBG, nmol/L	−1.4 ± 0.8	4.0 ± 0.8	<.001
FAI	0.01 ± 0.005	−0.02 ± 0.005	<.001
mFG scores	−0.3 ± 0.1	−1.1 ± 0.1	<.001
Free T, pg/mL	−0.3 ± 0.3	−0.8 ± 0.3	.28
DHEAS, μg/mL	−0.2 ± 0.3	−0.6 ± 0.3	.21
Triglycerides, mg/dL	12.2 ± 5.5	−15.2 ± 5.5	.001
VLDL-cholesterol, mg/dL	2.4 ± 1.1	−3.0 ± 1.1	.001
Total cholesterol, mg/dL	1.9 ± 2.9	1.3 ± 2.9	.89
LDL-cholesterol, mg/dL	−1.0 ± 3.2	3.6 ± 3.2	.31
HDL-cholesterol, mg/dL	0.7 ± 0.9	0.6 ± 0.9	.95
Total-/HDL-cholesterol ratio	−0.01 ± 0.07	0.01 ± 0.07	.80
hs-CRP, mg/mL	0.001 ± 0.5	−0.02 ± 0.5	.97
NO, μmol/L	6.8 ± 2.3	−1.2 ± 2.3	.02
TAC, mmol/L	−5.2 ± 28.2	68.7 ± 28.2	.08
GSH, μmol/L	3.9 ± 18.4	114.8 ± 18.4	<.001
MDA, μmol/L	0.6 ± 0.3	−0.5 ± 0.3	.004

The values of changes refer to the difference between pre- and post-treatment.

[a] Obtained from analysis of covariance.

In vitro studies have indicated that phytoestrogens are able to increase SHBG mRNA levels (35). In addition, two in vivo studies confirmed that dietary intake of soy isoflavones in postmenopausal women resulted in a significant increase of circulating levels of SHBG (36, 37). However, few studies were able to observe any change in androgen and SHBG levels in subjects with PCOS during the isoflavone treatment (14, 38). Soy isoflavones, mainly by binding to the estrogen receptor, may modulate the activity of steroidogenic enzymes such as P450 aromatase, 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β- HSD (39, 40). Inhibiting expression and activity of 3β-HSD by phytoestrogens, which catalyzes the conversion of androstenediol to T, may result in decreased levels of T (41). In addition, phytoestrogens may increase SHBG levels through increased SHBG mRNA levels (35) and stimulating SHBG production (37).

Findings of the present study indicated that supplementation with soy isoflavones in PCOS patients significantly decreased serum triglycerides and VLDL-cholesterol levels compared with the placebo but did not influence other serum lipid profiles. Beneficial effects of soy isoflavones on blood lipids were the most consistently reported findings. Two recent meta-analyses have demonstrated that the isoflavone content of soy may be responsible for its lipid-lowering effects (42, 43). In addition, triglyceride levels were significantly lower after 6 months of high-dose isoflavone treatment (300 mg/d) in healthy postmenopausal women, but the difference did not persist after 1 year (44). However, a daily supplement of 84 or 126 mg soy germ isoflavones did not affect lipid profiles in early postmenopausal Chinese women after 24 weeks of treatment (45). Soy isoflavones may reduce triglycerides and VLDL-cholesterol levels by the reduction of glucose incorporation into lipids (46), dose-dependent inhibition of glucose conversion into lipids, and increased lipolysis and decreased lipid synthesis (47).

We found that, compared with the placebo, soy isoflavone intake in women with PCOS did not affect inflammatory markers. In agreement with our study, soy milk consumption for 4 weeks had no significant effect on inflammatory markers among T2DM patients with nephropathy (48). Similar findings were seen among postmenopausal Chinese women with early hyperglycemia for 6 months (49) and among postmenopausal Caucasian and African American women for 3 months (50). In addition, a high soy diet for 4 weeks had no effect on vascular NO production in animal models (51). However, C-reactive protein concentrations were decreased after the intake of 0.5 cup of soy nuts (25 g of soy protein and 101 mg of aglycone isoflavones) was replaced with daily intake of 25 g of non-soy protein for 8 weeks among postmenopausal women (52). Increased inflammatory cytokines in women with PCOS render them at a potential increased

risk for the development of atherosclerosis, T2DM, infertility, and other comorbidities (53). The discrepancies regarding this issue between our study and other studies might be explained by different study designs, different types and durations of phytoestrogen supplementation, and general characteristics of the study participants.

The current study demonstrated that taking soy isoflavones among women with PCOS was associated with a significant rise in plasma GSH and a significant reduction in plasma MDA levels compared with the placebo, but it did not influence plasma TAC concentrations. In line with our results, the consumption of isoflavone-enriched pasta by patients with T2DM for 8 weeks resulted in a significant rise in GSH levels (54). Moreover, Azadbakht et al (55) observed that soya consumption for 8 weeks reduced plasma MDA concentrations in postmenopausal women with the metabolic syndrome (55). In another study, soy protein with or without isoflavones showed no significant effect on the antioxidant capacity concentrations (56). However, few researchers observed any beneficial effects after the intake of soy isoflavones on GSH and MDA levels. For instance, soy milk supplementation for 4 weeks did not alter plasma biomarkers of oxidative stress in postmenopausal women (57). In a study by Yang et al (58), it was also observed that in rats fed peptic-digested soy protein, no changes in circulating MDA were found. Oxidative stress levels are correlated with obesity, insulin resistance, hyperandrogenemia, and chronic inflammation (59). In addition, increased oxidative stress is considered a potential inducement of PCOS pathogenesis (60). Oxidative stress could also directly induce genetic variation by DNA damage, such as DNA chain rupture and base modification, and epigenetic change including elevated DNA methylation levels, which both play important roles in the pathogenesis of cancer (61, 62). Phytoestrogen intake may result in decreased oxidative stress through inhibiting oxidative modification of LDL-cholesterol (63). In addition, isoflavones may decrease oxidative stress through their ability to modulate gene expression and the activity levels of enzymes involved in antioxidant defense and the metabolism of xenobiotics including nicotinamide adenine dinucleotide phosphate hydrogen quinone oxidoreductase 1 and glutathione S-transferase (64).

Limitations of the current study include the lack of testing for a dose-response relationship between soy isoflavone intake and occurred changes in the metabolic profiles and biomarkers of oxidative stress. Furthermore, we did not evaluate soy isoflavone administration on estrogen levels and other biomarkers of inflammation and oxidative stress. It must be noted that we quantified total T and other steroids using the method of the ELISA. Due to limited funding for research projects in developing countries, we were unable to make more accurate determination of total T and other steroid levels with liquid chromatography-tandem mass spectrometry methods. Furthermore, we did not validate ELISA kits against the "gold standard" liquid chromatography-tandem mass spectrometry. Therefore, our findings should be interpreted with caution.

In conclusion, soy isoflavone administration for 12 weeks in women with PCOS significantly improved markers of insulin resistance, hormonal status, triglycerides, and biomarkers of oxidative stress.

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