Effect of synbiotic pomegranate juice on glycemic, sex hormone profile and anthropometric indices in PCOS: A randomized, triple blind, controlled trial

Z. Esmaeilinezhad [a], S. Babajafari [a,*], Z. Sohrabi [a], M.-H. Eskandari [c], S. Amooee [b], R. Barati-Boldaji [a]

[a] Nutrition Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Iran

Abstract *Background and Aims:* Polycystic Ovarian Syndrome is a common reproductive, endocrine, and metabolic disease in women. Pomegranate juice, known as a rich source of phytochemicals with high antioxidant activity, enriched with probiotic may improve PCOS.
Methods and Results: A randomized, controlled, triple-blinded, parallel trial study was performed in PCOS patients (n = 92). Three treatment groups (23 patients each) received 2 L of synbiotic pomegranate juice (SPJ), pomegranate juice (PJ), and synbiotic beverage (SB) weekly. The control group (23 patients) received 2 L of placebo beverage weekly. Primary outcome was any change in insulin resistance and secondary outcomes were fasting blood sugar (FBS), insulin sensitivity, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), body mass index (BMI), waist and hip circumference, from baseline to the end of the trial.
    At the end of the study, 86 patients were analyzed. There was significant change in insulin resistance in the SPJ and SB groups. Insulin sensitivity increased significantly in the SPJ and SB groups. Insulin also changed significantly in the SPJ and SB groups. BMI, weight and waist circumference decreased significantly in the SPJ and SB groups. Testosterone level also decreased significantly in the SPJ and SB groups. There was no significant change in FPG, LH and FSH in any of the groups.
Conclusion: SPJ in the form of a new beverage can improve insulin resistance, insulin, testosterone level, BMI, weight and waist circumference in PCOS. This trial was registered in *Iranian Registry of Clinical Trials*, with number: 25272.
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Introduction

Polycystic ovarian syndrome (PCOS), one of the most common endocrine diseases [1], affects up to 18% of women in reproductive age [2]. The hyperandrogenic condition of these people indicates that gonadotropin is not properly secreted from the pituitary gland and hence leads to an increase in LH and the ratio of LH to FSH,

* Corresponding author. Nutrition Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Razi Blvd, Shiraz, Iran. Fax: +98 71 37257288.
    E-mail address: jafaris@sums.ac.ir (S. Babajafari).

anovulation, incomplete follicles maturation, multiple cysts, and increased ovarian volume [1,3]. PCOS is also associated with a wide range of other disorders including obesity, insulin resistance, dyslipidemia, metabolic changes, and ultimately diabetes and cardiovascular disease. Furthermore, the risk of endometrial cancer are higher in these individuals [1,2]. Therefore, PCOS is not only a problem of beauty, fertility, and ovarian disease, but is also a metabolic disease which affects life expectancy [4], and has economic burden [2].

Unfortunately, the exact cause of this syndrome has not been completely determined [1]. Although genetic, metabolic, and neuroendocrine interactions are as important as environmental factors [5]. Insulin resistance is one of the important factors in PCOS etiology [1], and hyperinsulinemia leads to reproductive dysfunction, hyperandrogenemia, and increasing LH [1,6]. Obesity, especially abdominal obesity, is a factor which affects insulin resistance, insulin secretion, androgen level, and ovarian volume in PCOS patients [1]. Recently, data showed that, dysbiosis of gut microbiota due to a high fat-sugar diet in PCOS patients caused intestinal permeability. With increase in permeability, lipopolysaccharides produced by bacteria could move into the circulatory system and cause mild chronic inflammation. This inflammation activates the immune system against the action of insulin receptors and increases serum insulin, which in turn increases the production of ovarian androgens. Moreover, bidirectional interaction between sex hormones and intestinal microflora has been shown; therefore, as a result, dysbiosis can affect all three components of PCOS (anovulation, increase of androgens, and ovarian cysts) [4,7,8]. So, the regulation of intestinal microflora may be beneficial in the treatment of PCOS.

Today, functional foods like prebiotics and probiotics are used widely because of their health related benefits such as reduction of insulin resistance, inflammations, and oxidative stress (which exist in PCOS) [9]. They can help in weight loss, improvement in lipid profiles, reduction in the risk of cardiovascular disease, and reduction in the risk of cancer as well [10].

Nowadays, due to increase in vegetarianism, allergies to dairy proteins, lactose intolerance, and dairy cholesterol effects, demand for probiotics in non-dairy products has increased. Fruit juices have fresh taste and high nutritional value, including vitamins and antioxidants [11,12], which made them desirable for most people. However, they consider energy dense items with high sugar content which made them a risk factor for increasing BMI [13,14], beside they are low in fiber [15].

Pomegranate juice, known as a rich source of phytochemicals with high antioxidant activity, its main antioxidative compounds are anthocyanins and ellagic acid derivatives, which are the main constituents of the juice, giving the fruit its color [16], can affect the growth of probiotic bacteria and produce short-chain fatty acids that exert anti-inflammatory effects, improve glycemic index, lipid profiles, and obesity by influencing peroxisome proliferator-activated receptors (PPARs) [16]. Other beneficial effects of pomegranate include the improvement of cardiovascular complications [16].

Therefore, due to the new demands for functional foods, and especially those made from fruit, this study aimed to assess the effect of synbiotic pomegranate juice on glycemic indices, sex hormone profile and anthropometric measurements in PCOS patients for the first time.

Methods

Study design and patients' characteristics

This is a randomized controlled parallel, triple-blind trial that conformed to the declaration of Helsinki and Good Clinical Practice Guidelines. The ethics committee of Shiraz University of Medical Sciences in Iran reviewed and approved the study protocol.

Eligible patients were those with polycystic ovarian syndrome, aged 15−48 years who referred to Motahari Clinic which is a clinic affiliated to Shiraz University of Medical Sciences, with health care providers including nurses, nutritionists, gynecologists, and physicians. Patients were selected according to the Rotterdam criteria, which include two of the following features:

1. Oligomenorrhea (interval between two menstrual periods more than 35 days) or amenorrhea (no vaginal bleeding for at least 6 months).
2. Clinical findings of increased blood androgen levels (hirsutism scores greater than 7 or obvious acne), or increased blood testosterone levels (testosterone levels above 2 nmol).
3. Polycystic ovaries in ultrasound scan (≥ 12 follicles measuring 2−9 mm in diameter, or ovarian volume >10 ml in at least one ovary).

All the aforementioned criteria are considered acceptable in the absence of other medical conditions related to excess androgens such as increased blood prolactin, nonclassical congenital adrenal hyperplasia, Cushing's syndrome, androgen-producing tumors and acromegaly [17]. In the current study, patients treated with corticosteroids, antibiotics, vitamin, minerals, antioxidants supplements, and hormonal pills as well as patients with severe diseases of the stomach, intestine, heart, kidney, liver, lung, autoimmune diseases, and AIDS, were excluded. Patients undergoing chemotherapy, smokers, lactating women, and people with special diet and physical activity, were also excluded.

Sample size

The sample size was determined according to insulin resistance with homeostatic model assessment of insulin resistance (as our primary outcome) in a previous study of PCOS women that received probiotic supplement [18]. Sample size was determined to be 21 patients in each group with S1 = 0.6, S2 = 0.05, d = 0.049 for insulin resistance and test strength of 80% and $\alpha = 0.05$. To allow

almost 10% drop out in each group, 23 patients per group were determined as the final sample size. 135 PCOS women were screened and 92 patients who signed the informed consent participated in this trial.

Randomization

Participants were randomly assigned in a 1:1:1:1 ratio, to 4 groups.

1. Synbiotic pomegranate juice (SPJ): Patients in this group received 2 L (2 × 1-L) of pomegranate juice containing inulin and lactobacillus per week, for 8 weeks in disposable bottles. (each liter of pomegranate juice contains 20 g of inulin $+2 \times 10^8$ CFU/g lactobacillus)
2. Pomegranate juice (PJ): Patients in this group received 2 L (2 × 1-L) of pomegranate juice per week, for 8 weeks in disposable bottles.
3. Synbiotic beverage (SB): Patients in this group received 2 L (2 × 1-L) of synbiotic beverage per week, for 8 weeks in disposable bottles. (each liter of beverage contains 1 L of water + 20 g of inulin $+2 \times 10^8$ CFU/g lactobacillus + pomegranate flavoring)
4. Control group: Patients in this group received 2 L (2 × 1-L) of placebo beverage per week, for 8 weeks in disposable bottles. (each liter of beverage contains 1 L of water + pomegranate flavoring).

Randomization was done by random allocation software to assign patients using blocked randomization with a fixed block size of 4 [19]. Randomization was conducted by an investigator who had no clinical involvement in the trial. In addition, other procedures including enrollment, sequence generation, allocation concealment, and randomization process were all done by the principal investigators.

Blinding

In order to blind patients to the samples, the drinks in all the groups were identical in appearance, color, and taste. The drinks were coded differently in each group to blind the investigator. Finally, the statistician was also blinded to the data by special codes for each group.

Intervention

Choice of pomegranate juice
Accordingly, pomegranate juice was obtained from specific industry which also was extracted from one kind of pomegranate fruit.

The viability of five kinds of bacteria in pomegranate juice were measured over two weeks. Three species of bacteria (*Lactobacillus rhamnosus* GG, *bacillus koagolans and indicous*) with the highest survival rate in pomegranate juice were selected (Table 1), and 10^8 CFU/ml of each selected bacteria plus 20 gr of inulin were added to each liter of pomegranate and flavored beverage to

produce SPJ and SB. These bottles of beverage were prepared freshly every week and were delivered to patients immediately after the preparation.

Outcome
Primary outcome of this study was insulin resistance and secondary outcome included blood glucose, insulin, total testosterone, LH and FSH.

Before the study, the demographic questionnaires were filled through face-to-face interview by the main investigator. To assess dietary intake and monitor dietary compliance, 3-day dietary recalls (including two-week days and one weekend day) were collected from subjects at baseline and at the end of the study phase. Blood samples in the fasting 12-h period at the beginning and end of the intervention at Motahari Clinic were collected by a laboratory expert. Blood samples were centrifuged and serum were kept at $-70\,^{\circ}$C in the freezer until final measurement for blood glucose, insulin, testosterone, LH and FSH.

Measurement
Plasma glucose levels were assessed using an enzymatic colorimetric (GOD-PAP) methodology (Pars Azmoon Inc, Iran). Serum insulin levels were measured by Enzyme-linked Immunosorbent Assay (ELISA) (monobind, US). Total testosterone, LH, and FSH were measured by Enzyme-linked Immunosorbent Assay (ELISA) (Pishtazteb, Iran). Insulin resistance was calculated with the Homeostatic model assessment of insulin resistance (HOMA-IR index) and insulin sensitivity was calculated with quantitative insulin sensitivity check index (QUICKI) as follows:

HOMA-IR: Fasting plasma insulin (μu/ml) × fasting plasma glucose (mmol/L)/22.5.

QUICKI: 1/(log (fasting insulin μU/mL) + log (fasting glucose mg/dL)).

Body weight was measured with minimal clothing and without shoes by standard scale (Seca, Germany) to the nearest 100 g. Height was measured with the individuals standing without shoes to the nearest 0.5 cm. BMI was computed as the weight in kilogram divided by the height squared in meters [20]. Waist circumference (WC) was measured with non-stretchable midway measuring tape in the iliac crest [21]. Dietary intakes were analyzed by Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods.

Statistical methods

Data were analyzed using SPSS software (Version 22; IBM, Armonk, USA)). Normality of data was assessed by Kolmogorov−Smirnov test. To compare changes between the start and end of the intervention in each group, paired t-test was used for the data with normal distribution and Wilcoxon signed-rank test was used for skewed data. One-way ANCOVA test was used for variations among groups and Bonferroni post-hoc test was used to compare the four groups with normal distribution. Kruskal−Wallis test was used in the case of abnormal distribution. $P < 0.05$ was considered significant.

Table 1 The viability of bacteria in pomegranate juice for two weeks.

Lactobacillus in pomegranate juice	First day	Seventh day	Fourteenth day
Lactobacillus casei	28×10^8	174×10^6	15×10^5
Lactobacillus ramnosous[a]	42×10^8	57×10^7	37×10^7
Lactobacillus plantroum	35×10^8	48×10^7	25×10^5
bacillus koagolans and indicous[a]	29×10^9	18×10^9	94×10^8

[a] Selected samples.

Results

Eligible patients were recruited from January to July, 2017. During the treatment phase of the study, 6 patients left the study due to different reasons (Fig. 1). Baseline demographic and laboratory-measured parameters of patients are shown in Table 2. There was no statistically significant difference between the baseline measured parameters among groups.

Changes in laboratory parameters are shown in Table 3. The primary outcome "HOMA-IR" decreased significantly in SPJ and SB groups as compared to the baseline ($P < 0.05$). Changes in SPJ and SB groups when compared with the control group were significant ($P > 0.05$) (Table 3).

In comparison with the baseline BMI, weight and waist circumferences decreased in SPJ and SB groups ($P < 0.05$) and waist to hip ratio decreased in the SPJ group and
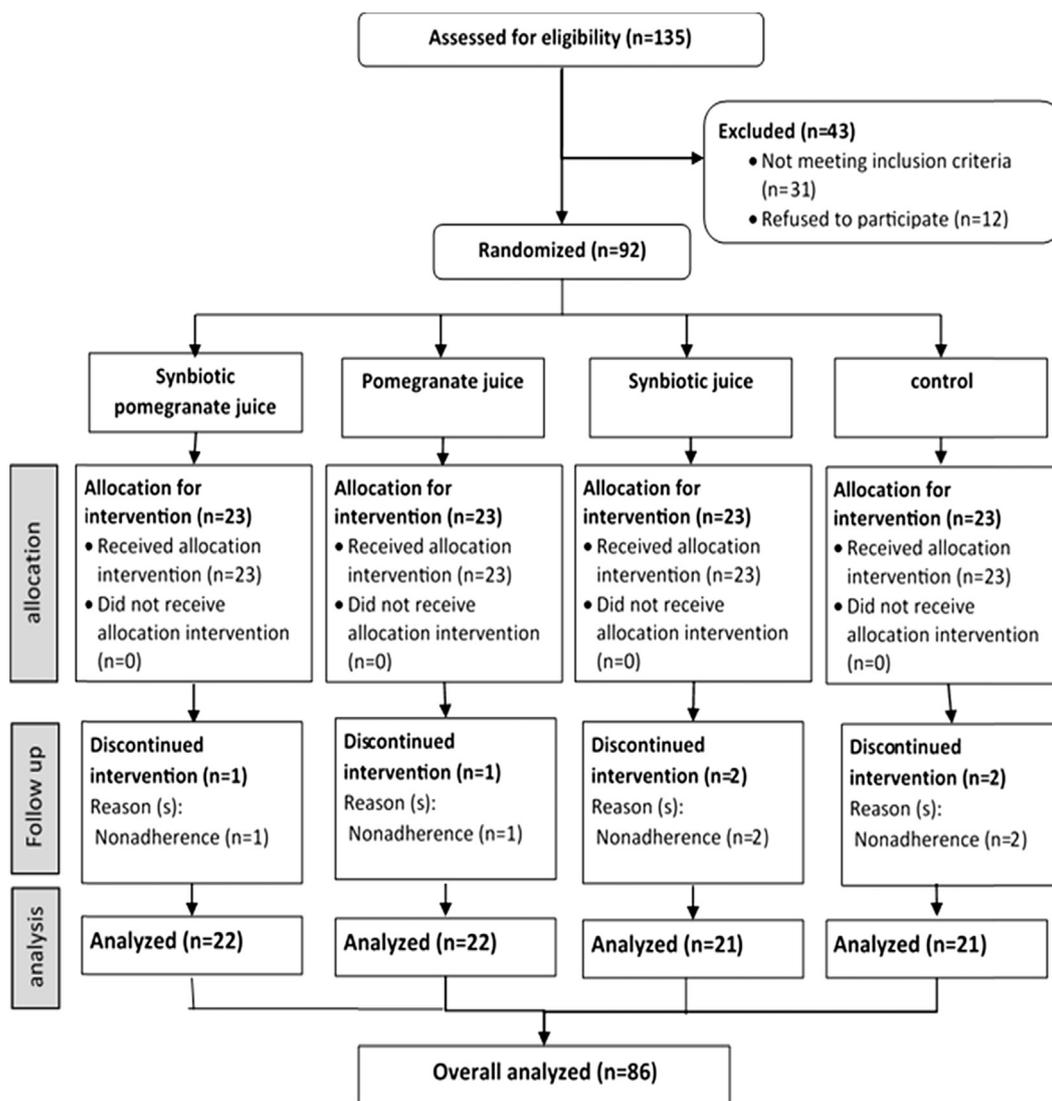

Figure 1 Flow diagram of the trial.

Table 2 Baseline demographic characteristics and measured parameters in patients.

	Group 1: Synbiotic pomegranate juice (n = 23)	Group 2: Pomegranate juice (n = 23)	Group 3: Synbiotic beverage (n = 23)	Group 4: Control (n = 23)	P-value
Age (years)	30.04 ± 6.39	29.30 ± 7.46	29.52 ± 5.82	30.60 ± 7.43	0.91
Height (cm)	162.82 ± 4.64	163.21 ± 4.35	162.82 ± 4.72	162.21 ± 4.81	0.92
Weight (kg)	68.26 ± 6.77	69.89 ± 7.90	69.65 ± 4.52	70.47 ± 5.62	0.67
BMI (kg/m^2)	25.75 ± 2.45	26.25 ± 2.93	26.29 ± 1.70	26.77 ± 1.70	0.50
Waist (cm)	94.21 ± 7.60	95.30 ± 7.30	93.65 ± 5.59	96.13 ± 5.40	0.58
Hip (cm)	98.21 ± 7.09	101.00 ± 8.08	101.30 ± 5.78	102.30 ± 6.75	0.06
Waist/hip	0.95 ± 0.05	0.94 ± 0.03	0.92 ± 0.04	0.94 ± 0.03	0.06
FBS (mg.dl)	112.04 ± 9.41	112.82 ± 12.61	112.65 ± 8.46	114.56 ± 8.16	0.84
Insulin (μiu.ml)	22.80 ± 3.97	22.15 ± 3.48	22.02 ± 4.32	24.66 ± 3.33	0.07
HOMA.IR	6.32 ± 1.32	6.16 ± 1.17	6.11 ± 1.22	6.95 ± 0.91	0.06
QUICKI	0.294 ± 0.008	0.295 ± 0.007	0.295 ± 0.008	0.290 ± 0.004	0.05
Testosterone (ng.ml)	0.69 ± 0.17	0.66 ± 0.12	0.63 ± 0.10	0.61 ± 0.12	0.17
LH (iu.L)	11.02 ± 5.82	11.84 ± 4.17	13.62 ± 4.87	10.26 ± 4.80	0.09
FSH (iu.L)	6.71 ± 3.41	6.71 ± 2.56	8.08 ± 2.94	6.13 ± 2.87	0.13
LH/FSH	1.62 ± 0.10	2.00 ± 1.62	1.68 ± 0.07	1.67 ± 0.04	0.10

Note: values for continuous variables, as mean $\pm$ standard deviation with normal distribution.
Baseline parameters are compared using one-way ANOVA.
$P < 0.05$ is considered significant.
Abbreviations: BMI: Body Mass Index, FBS: Fasting Blood Sugar, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, QUICKI: Quantitative insulin sensitivity check index, LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone.

Table 3 Effect of interventions on levels of measured parameters in patients after 8 weeks.

Measured parameter	Group 1: Synbiotic pomegranate juice (n = 23)	Group 2: Pomegranate juice (n = 23)	Group 3: Synbiotic beverage (n = 23)	Group 4: Control (n = 23)	P-value[a]
Primary outcome					
HOMA-IR	5.75 ± 1.22[a]	6.20 ± 1.23	5.61 ± 0.99[a]	7.33 ± 0.92	>**0.001**
Secondary outcome					
Weight (kg)	67.34 ± 6.25[a]	69.97 ± 7.69	68.71 ± 4.91[a]	71.57 ± 5.60	**0.002**
BMI (kg/m^2)	25.38 ± 2.21[a]	26.30 ± 2.90	25.92 ± 1.82[a]	27.04 ± 1.77	**0.002**
Waist (cm)	91.59 ± 5.15[a]	95.04 ± 5.73	91.47 ± 5.69[a]	96.85 ± 5.03	>**0.001**
Hip (cm)	97.81 ± 6.09	100.68 ± 7.74	100.61 ± 5.24	101.61 ± 6.49	**0.88**
Waist/hip	0.93 ± 0.04[a]	0.94 ± 0.04	0.90 ± 0.04[a]	0.95 ± 0.03	**0.002**
FBS (mg.dl)	110.36 ± 6.57[a]	113.68 ± 10.63	111.47 ± 6.58[a]	114.85 ± 7.51	>**0.001**
Insulin (μiu.ml)	21.03 ± 3.94	22.07 ± 3.74	20.36 ± 3.35[a]	25.89 ± 3.11	>**0.001**
QUICKI	0.29 ± 0.008[a]	0.29 ± 0.007	0.29 ± 0.007[a]	0.28 ± 0.004	>**0.001**
Testosterone (ng.ml)	0.56 ± 0.23[a]	0.63 ± 0.08	0.54 ± 0.12[a]	0.67 ± 0.13	**0.002**
LH (iu.L)	10.77 ± 5.25	11.28 ± 4.24	13.28 ± 4.58	11.03 ± 4	**0.27**
FSH (iu.L)	6.67 ± 3.26	6.68 ± 2.52	7.91 ± 2.27	6.62 ± 2.40	**0.88**
LH/FSH	1.65 ± 0.33	1.68 ± 0.07	1.67 ± 0.04	1.66 ± 0.02	**0.91**

NOTE: data are expressed as mean $\pm$ standard deviation.
[a]$P < 0.05$: significantly different to control beverage.
Abbreviations: BMI: Body Mass Index, FBS: Fasting Blood Sugar, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, QUICKI: Quantitative insulin sensitivity check index, LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone.
[a] ANCOVA test adjusted for baseline.

increased in the control group $(P < 0.05)$ .The changes in BMI, weight, waist, and waist to hip ratio among the groups were statistically different in the SPJ and SB groups as compared to the control group $(P < 0.05)$.

Hip circumference did not change significantly within and between any group (Table 3).

Regarding the FBS levels the changes in SPJ and SB groups compared with control group were significant $(P < 0.05)$. Insulin decreased in SPJ and SB groups when compared to the baseline $(P < 0.05)$, but in comparison between groups, only the change between SB and the control groups was significant $(P < 0.05)$. Insulin sensitivity increased in the SPJ and SB groups $(P < 0.05)$ and among the groups; a statistically significant difference was observed between SPJ and SB groups versus control group $(P < 0.05)$ (Table 3).

Testosterone reduced in SPJ and SB groups significantly in comparison with the baseline $(P < 0.05)$ and increased significantly in the control group $(P < 0.05)$. Comparing the changes in testosterone among the groups, statistically significant changes in SPJ and SB groups when compared with the control group, was observed. There was no significant change in LH and FSH in any of the groups (Table 3).

Discussion

To the best of the authors' knowledge, this is the first study that examined the effect of synbiotic pomegranate juice on PCOS treatment. In this randomized, triple-blind, placebo-controlled trial, oral consumption of SPJ and SB improved insulin resistance, anthropometric indices, and testosterone levels in patients with PCOS.

Insulin resistance is an important feature in the pathology and pathogenesis of PCOS, with a 65–70% prevalence, and should be noticed in PCOS therapy [22]. In the present study, insulin resistance reduced in the SPJ and SB groups as reflected by a significant decrease in the HOMA-IR and increase in QUICKI. Consistent with the results obtained in the present study, Eslamparast et al. [23] reported significant improvements in QUICKI and FBS after synbiotic supplementation in subjects with metabolic syndrome. Shoaei et al. [18] reported similar findings in patients with PCOS after probiotic supplementation. Few studies have examined the effects of synbiotic on insulin resistance [23,24], and no available study examined such an effect on patients with PCOS, and exact mechanisms are yet to be known. These findings can be possibly explained via the effect of synbiotic on production of SCFA which binds to G protein-coupled receptors and cause various biological effects like, increasing glucagon-like peptide 1 secretion from enteroendocrine L-cells which may improve carbohydrate metabolism, reduce glucotoxicity, and improve insulin sensitivity of target cells and β-cell function following probiotic consumption. Furthermore, SCFAs prevent low grade chronic inflammation and improve gut permeability, which diminishes the Toll-like receptor pathway, and in turn reduces entrance of proinflammatory cytokines and lipopolysaccharide into bloodstream [25]. Moreover, since inflammation is considered as one of the causes of insulin resistance, it is stated that probiotic can improve insulin resistance by increasing liver natural killer T (NKT) cells which can reduce inflammatory signaling and regulate pro-inflammatory and anti-inflammatory imbalance of cytokines [26].

Pomegranate components, specially punicic (an isomer of conjugated α-linolenic acid that can upregulating PPAR γ and decrease TNF-α [29]) and catalpic acids, can improve insulin resistance, insulin levels and fasting glucose concentrations. They also normalize glucose levels through expression of PPAR-related genes in abdominal adipose tissue and suppressing expression of the inflammatory cytokine [16]. In this study, the improvement of glycemic profile is approximately equal in magnitude, in SPJ and SB and there is no superiority. This indicated that this finding might be due to the synbiotic formulation and there were not any synergic effects between pomegranate juice and synbiotic. We think that might due to the short duration of intervention.

Similar to the current findings, some prior studies did not show significant decrease in FBS [24,27]. In the present study, lack of significant change in FBS could be partially explained by the fact that the aforementioned studies used different strains of probiotics, hence contributing to the different results in FBS as compared to the present study [18,23].

The results of the present study indicated significant reductions in BMI, weight and WC in SPJ and SB groups. In agreement with our findings, study by Ahmadi et al. [28] showed that probiotics supplementation caused significant reduction in weight and BMI of women with PCOS after 12 weeks. Different mechanisms are suggested in relation to these anthropometric changes after synbiotics consumption. It is stated that some strains of probiotics (such as Lactobacillus) can alter sugar and lipid metabolism, and produce conjugated linoleic acid, which leads to reduction in size and number of small adipocytes in adipose tissue [29]. Furthermore, it is suggested that the increased production of short-chain fatty acids from probiotic consumption, might lead to decreased food intake and weight through alterations in intestinal hormones secretion, Glucagon-Like Peptide 1, and Peptide YY [30]. It also increased the oxidation of fatty acids and energy consumption by increasing brown fat, expression of UCP1, and 5′-AMP-activated protein kinase (AMPK), thereby, inhibiting acetyl-CoA carboxylase (ACC) [30]. In a holistic look, it is mentioned that probiotics consumption decreased metabolic effects of dysbiosis (microbial imbalance) present in gastrointestinal system of patients with metabolic disorders such as obesity, metabolic syndrome and diabetes [31]. Since PCOS has a common feature of etiology and pathogenesis with these diseases, synbiotics can be beneficial in improving microflora changes in women with PCOS, leading to improvement in metabolic state. In the present study, a significant change in the ratio of waist/hip was observed only in the SPJ group which can be attributed to the synergistic effects of pomegranate juice and synbiotic compounds. Data showed that pomegranate extract (PE) can reduce ATP citrate lyase (ACLY) expression (key enzymes in Lipogenesis), and so inhibit lipogenesis and Acetyl-CoA production. Furthermore, PE can stimulate the expression of PPARα, PGC-1α,[1] and beta-oxidation-related genes. Beside this action, data showed that in the mitochondria, PE can increase complex II and IV activities in the electron transport chain. As a result, all these mechanisms might lead to increase in fatty acid oxidation, energy production and weight reduction [32].

The obtained results showed that there was significant decrease in testosterone level in SPJ and SB groups when compared to the control group. In agreement with the current findings, Guo et al. [4] believed that interventions that alter the intestinal flora and can modify dysbiosis, could lead to changes in sex hormones in women with PCOS. In this study, treatment of PCOS rats with Lactobacillus reduced androgen biosynthesis, and their ovarian morphologies were normalized. As mentioned earlier, synbiotics consumption as nutritional substances can be a promising intervention to normalize sexual hormones level in women with PCOS. Furthermore, improvement of endocrine disorders in patients with PCOS can be due to

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[1] Peroxisomal proliferator-activated receptor–mma coactivator-1α.

significant weight loss and changes in levels of insulin secretion in these groups. This insulin sensitivity improvement reduces the production of testosterone by decreasing cytochrome P450C17 [33]. Previous studies have shown a positive effect of weight loss on metabolic and hormonal parameters in women with PCOS [34,35]. All of them are consistent with the present findings with regards to sex hormones.

Although, the results of our study showed statistically significant differences with respect to some factors such as HOMA-IR, weight, BMI, waist, waist/hip, FBS, insulin, QUICKI, and testosterone after treatment with different products, however, these differences were not clinically significant. We think that the longer treatment with the products as well as increasing the probiotic count maybe change these non-significant clinically results to clinically significant.

The current study has some limitations. First, the authors were not aware of bioavailability of probiotics and polyphenolic compound of pomegranate juice during digestion and absorption process in gut. Second, inability to measure fasting plasma glucose level repeatedly and finally, fecal bacteria loads could not be measured before and after probiotic supplementation.

Conclusion

This study is the first to assess the effect of synbiotic and pomegranate juice consumption alone and together, on insulin sensitivity, sexual hormonal changes and anthropometric measures among PCOS patients. In summary, this randomized, triple-blind, controlled trial showed that SPJ and SB consumption improved some metabolic syndrome features including dysglycemia and anthropometric indices, and also reduced testosterone level in women with PCOS. Since there were no adverse effects during this study, added synbiotic to fruit juice might be a good option to promote health and reduce the risk of metabolic diseases in these women. But we should aware that, most of fruit juices are rich in sugar, calories and low in fiber. So moderate consumption is recommended.

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Conflict of interest

None.

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Each author accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. SB takes responsibilities that this study has been reported honestly, accurately, and transparently; that no important aspect of study has been omitted, and that any discrepancies from the study as planned (and, if relevant registered) has been explained.

Contributions: Research idea and study design: ZE, SB, ZS, RBB; Data acquisition: ZE; Data analysis/interpretation: ZE, RBB, ZS; Statistical analysis: ZE, RB; Supervision or mentorship: SB, MHE, ZS.

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