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Original Article

Synbiotic-Containing *Bacillus coagulans* (GBI-30) and the Effects on Glycemic Control, Androgen Hormones and Anthropometric Indices in Women with Polycystic Ovarian Syndrome

Zahra Hariri [1], ***Zahra Yari*** [2], ***Maryam Nilghaz*** [1], ***Monirsadat Mirhoseini*** [3], ***Sedighe Hosseini*** [4], ***Khadijeh Abhari*** [5], ****Golbon Sohrab*** [1]

1. *Department of Clinical Nutrition and Dietetics, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Department of Nutrition Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Department of Cellular and Molecular Nutrition, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Department of Obstetrics and Gynecology, Preventative Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
5. *Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: golbonsohrab@yahoo.com

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Abstract

Background: We aimed to investigate the effect of synbiotic supplement-containing spore-forming Bacillus coagulans (GBI-30) on the glycemic, hormonal and anthropometric parameters in patients with PCOS.

Methods: In this randomized, triple-blind, placebo-controlled trial, 72 patients with PCOS were randomly and equally assigned to receive a daily sachet of synbiotic (containing Bacillus coagulans (GBI-30), Lactobacillus rhamnosus, Lactobacillus helveticus, and fructooligosaccharide (FOS)) or placebo for 12 wk. Glycemic, hormonal and anthropometric parameters were measured at baseline and after the 12-week of intervention.

Results: At the end, 60 patients completed the study. After adjustment for potential confounders, significant decrease in insulin (−3.38±20.20 vs. +2.33, *P*=0.042), homeostatic model assessment for insulin resistance (HOMA-IR) (−0.43±3.61 vs. +0.73 ± 2.91, *P*=0.044) and dehydroepiandrosterone sulfate (DHEAS) (−1.47±6.62 vs. + 0.03 ± 2.06, *P*=0.047) was observed in the intervention group compared to the control group. Supplementation with synbiotic failed to show a significant effect on anthropometric parameters and fasting blood sugar.

Conclusion: Overall, 12 wk supplementation with synbiotic-containing Bacillus coagulans (GBI-30) can offer additional benefit to lifestyle intervention on metabolic status of women with polycystic ovarian syndrome.

Keywords: Polycystic ovary syndrome; Bacillus coagulans; Synbiotics; Insulin resistance; Hyperandrogenism

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Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of infertility in women from the endocrine aspect, associated with an increase in androgens biosynthesis and subsequently, lack of ovulation (1, 2). PCOS affects about 6- 20% of women of reproductive age, depending on the diagnostic criteria (3, 4). Hyperandrogenism (HA) is one of the major endocrinopathies that appears to be associated with PCOS complications including acne, androgenic hair loss (androgenic alopecia), hirsutism, changes in the hypothalamus-pituitary-ovary axis, defects in the production of follicles by the ovary, and finally, it leads to menstrual disorders and infertility (5-7). Although the etiology of this syndrome is still not fully understood, insulin resistance (IR) has been introduced as one of the possible mechanisms, therefore at least part of the treatments of the disease have been proposed based on the reduction of insulin resistance (8). Recently, gut microbiome dysbiosis have been shown to cause various endocrine disorders attributed to insulin resistance (9). Moreover, the bidirectional interaction between sex hormones and intestinal microflora has been reported in previous studies (10). Accordingly, and based on a recent meta-analysis, regulation of gut microflora through synbiotic supplementation may be beneficial in the treatment of PCOS, especially improving glycemic indices (11).

Common probiotics include various species of Bifidobacterium and Lactobacillus (12). However, the high sensitivity of these bacteria to the acidic pH of the stomach and bile salts of the intestine has made their supplemental use problematic. On the other hand, Bacillus coagulans species has recently been introduced as a valuable probiotic due to its spore production and consequent resistance to heat, stomach acid and bile salts and having a greater chance of crossing barriers (13).

Considering the health beneficial effects of synbiotics, and since the data investigating the effect of synbiotics on hormonal status, anthropometric measurements and glycemic indicators of patients with PCOS are few and contradictory, therefore, this study was conducted with the aim of determining the effect of synbiotic supplementation on the metabolic factors of women with polycystic ovaries.

Materials and Methods

Participants

This study was a parallel-arm randomized triple-blind, placebo-controlled trial, registered in the Iranian website for registration of clinical trials (http://www.irct.ir: IRCT20150815023617N6) and was carried out according to the Declaration of Helsinki and Good Clinical Practice guidelines. The research was approved by the ethics committee of Nutrition and Food Technology Research Institute (NNFTRI) of Shahid Beheshti University of Medical Sciences (ethics ID: IR.SBMU.nnftri.Rec.1401.017).

Participants were recruited from the obstetrics & gynecology clinic of Ayatollah Taleqani Hospital, between Jun and Aug 2022. To be included in the study, participants were required to be newly diagnosed with PCOS according to the Rotterdam criteria (14), aged 18 to 45 years old, and had a BMI range of 18.5-35 kg/m^2. The diagnosis of PCOS was done by an experienced gynecologist (SH). Exclusion criteria were: Pregnancy, Smoking, having history of liver diseases, kidney or heart failure, infectious or inflammatory diseases, thyroid gland disorders, diabetes, types of cancer and hyperprolactinemia, taking supplements or products containing synbiotics or probiotics in the last month, taking antibiotic in the last three months, adherence to weight loss or any special diets during the last three months and taking corticosteroids or omega-3.

Study design

Prior to the initiation of the trial, all participants completed a written informed consent form. Then the main investigator (ZH) randomly assigned the participants to either take synbiotic (n = 36) or placebo (n = 36) groups for 12 wk based on block randomization stratified by BMI (<25 kg/m^2 or ≥ 25 kg/m^2). The two grams sachets of synbiotic contained 10^{11} spores of Bacillus coagulans (GBI-30), 10^{10} colony-forming units (CFU) of Lactobacillus Rhamnus, 10^{10} CFU of Lactobacillus helveticus, 500 mg of fructooligosaccharides (FOS) and 0.7% Natural orange flavoring. The placebo sachets also contained 2 g of starch and 0.7% Natural orange flavoring. The sachets were coded as A or B by the manufacturing company. All researchers, patients and statistician were blinded until the end of the intervention. Both types of sachets were provided by the same company and had the same shape, color, packaging and taste.

Dietary assessment

At the beginning and end of the intervention, the patients undertook 3-day 24-h recall (two workdays and one weekend) in order to investigate the dietary confounding factors. These values were converted into grams and then entered the Nutritionist IV (N4) software to assess the macronutrients and micronutrients.

Anthropometric Measurement

All anthropometric measurements were performed at the beginning and end of the study according to National Health and Nutrition Examination Survey (NHANES): Anthropometry Procedures Manual (15). Both body weight and height were determined by Seca 70 Mechanical Column Scale with light clothing and no shoes, to the nearest 0.1 kg and 0.1 cm, respectively. Waist and hip circumference were measured with a flexible measuring tape to the nearest 0.1 cm. BMI was calculated as weight in kg divided by height in meters squared (16).

Biochemical assessments

At the first and the end of the intervention, after 10 to 12 h of fasting, 5 cc of blood was taken from each patient. Blood samples were centrifuged for 10 min at a speed of 2000 revolutions per minute to separate the serum stored in a freezer at -80 degrees Celsius until the biochemical tests were performed.

Glucose measurement was done using Parsazmoon kit (Tehran, Iran) and through enzyme colorimetric method in order to quantify serum insulin, Monobind kit (CA, USA) and ELISA method with a sensitivity of 0.182 μIU/ml were applied. Accordingly, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Homeostasis Model Assessment of β-cell function (HOMA-β) and Quantitative Insulin Sensitivity Check Index (QUICKI) were calculated using the following formulas:

$$\text{HOMA-IR} = \frac{\text{Fasting Blood Glucose } (\frac{\text{mg}}{\text{dL}}) \times \text{Fasting Insulin } (\frac{\text{lU}}{\text{ml}})}{405} \quad (17)$$

$$\text{HOMA- ß} = \frac{360 \times \text{Fasting Insulin } (\frac{\text{lU}}{\text{ml}})}{\text{glucose} - 63} \% \quad (18)$$

$$\text{QUICKI} = \frac{1}{\log \text{fasting insulin } (\frac{\text{lU}}{\text{ml}}) + \log \text{fasting glucose } (\frac{\text{mg}}{\text{dl}})} \quad (19)$$

Both serum total testosterone and dehydroepiandrosterone sulfate (DHEAS) values with inter- and intra-assay CVs of below 7% were determined using ELISA kits (DiaMetra, Milano, Italy).

Sample size

The sample size calculation was based on our previous publication (20), considering the key factor C-reactive protein (CRP) in PCOS patients supplemented with synbiotics. In each group the sample size was set at 36 participants, having regard to type 1 error (α) of 0.05, type 2 error (β) of 0.20 (power=80%), and 20% dropout.

Statistical analysis

All statistical analyzes were done using Statistical Package for Social Science software ver. 26 (IBM Corp., Armonk, NY, USA). First of all, the normal distribution of variables was evaluated by the

Kolmogorov–Simonov test. Paired t-test and independent t-test were performed to compare variables inter and intra groups, respectively. Analysis of covariance (ANCOVA) test was applied to compare the effect of supplementation with considering the confounders. All quantitative variables were reported as mean and standard deviation (SD) and qualitative variables were reported as frequency (percentage). $P<0.05$ was considered statistically significant.

Results

Overall, 80 patients were screened for eligibility, 72 of them entered the study, and statistical analysis was performed on 60 patients who completed the study (Fig. 1).

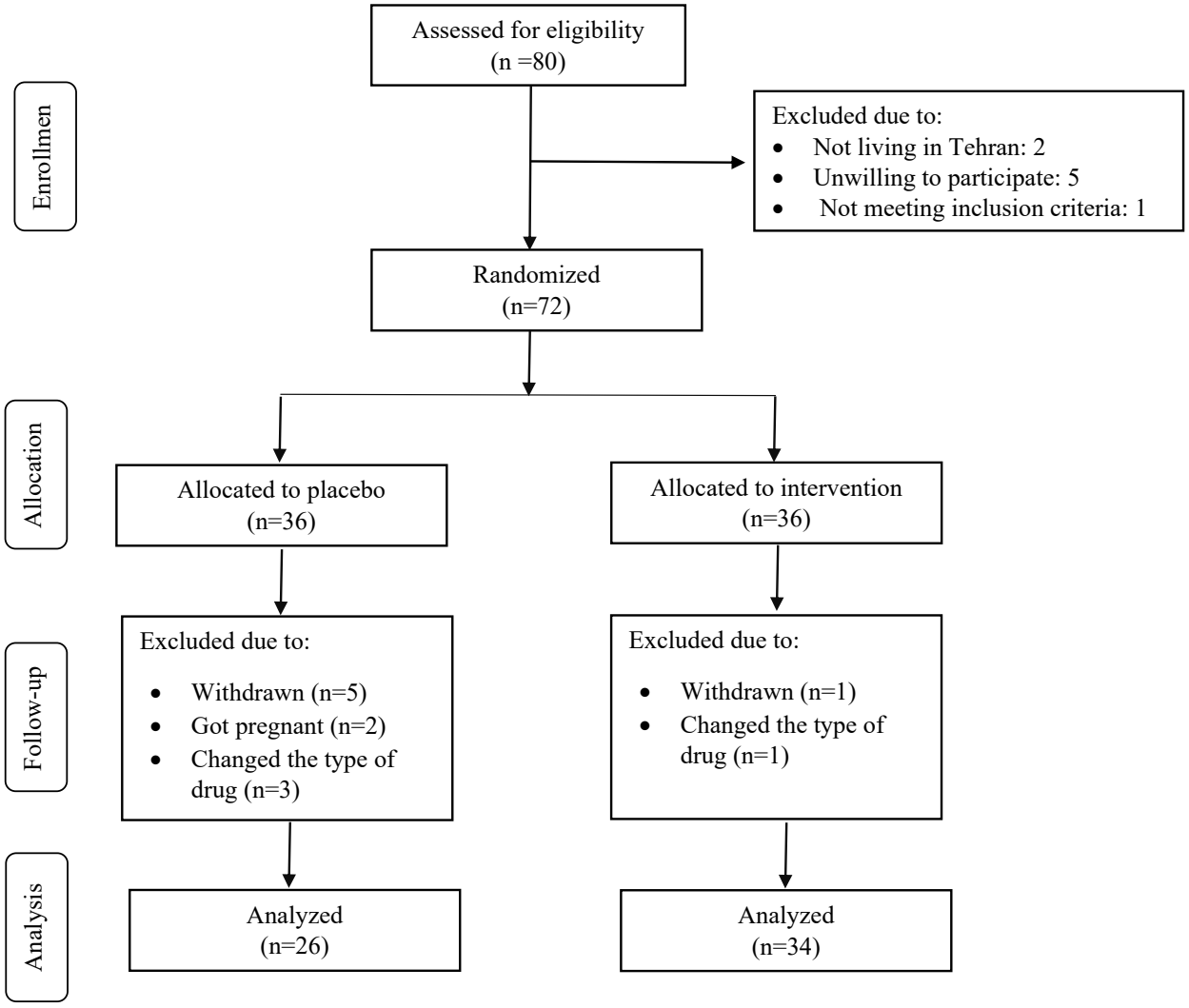

Fig. 1: Summary of patient flow diagram

Baseline demographic and clinical features are shown in Table 1. There was no significant difference between the two groups at the beginning according to age, BMI, marriage, history of previous pregnancy, duration of PCOS and receiving hypoglycemic and antiandrogen agents.

Table 1: Baseline characteristics of participants in both groups

Characteristics	synbiotic group N=34	placebo group N=26	*P*-value
Age (years)	28.14 ± 5.74	28.38 ± 6.39	0.880*
Basic BMI (kg/m²)	24.74 ± 4.14	25.02 ± 4.23	0.796*
Marriage (%) Single Married Widow Divorced	19 (55.9%) 14 (41.2%) 0 (0%) 1 (2.9%)	13 (50.0%) 10 (38.5%) 0 (0%) 3 (11.5)	0.416†
History of previous pregnancy (%) Yes No	3 (8.8%) 31 (91.2%)	3 (11.5%) 23 (88.5%)	0.728†
Duration of PCOS (year)	9.28 ± 6.15	7.49 ± 4.72	0.228*
Hypoglycemic agents (%) Yes No	5 (14.7%) 29 (85.3%)	5 (19.2%) 21 (80.8%)	0.641†
Antiandrogen agents (%) Yes No	6 (17.6%) 28 (82.4%)	5 (19.2%) 21 (80.8%)	0.875†

*Obtained from independent sample t-test (mean ± SD). † Obtained from Chi-Square test (percentage).
P< 0.05 is considered statistically significant.
Abbreviations: BMI: Body mass index; PCOS: polycystic ovarian syndrome.

As presented in Table 2, there was no significant differences in terms of dietary intakes (average energy, macronutrients, vitamin D, vitamin C, vitamin E and fiber intake) during the intervention between the two groups and among each group.

Table 3 summarizes the anthropometric indices at baseline and after the 12-week intervention in participants with polycystic ovary syndrome. Based on within-group analysis, there was no statistically significant difference in the initial and final values of each group. In addition, no statistically significant difference in terms of anthropometric indices between the two groups at the beginning and end of the study was observed (*P*>0.05). Analysis of covariance did not change the results.

Mean of glycemic indices including FBG, fasting insulin, HOMA-IR, HOMA-β and QUICKI and androgens (e.g., Total testosterone and DHEA-S) at baseline and after the 12-week intervention are displayed in Table 4. Statistical analysis showed that changes in insulin (−3.38±20.20 vs. +2.33 ±12.79, *P*=0.042), HOMA-IR (− 0.43±3.61 vs. + 0.73±2.91, *P*=0.044) and DHEA-S (−1.47±6.62 vs. +0.03 ±2.06, *P*=0.047) turned out to be significant after confounders adjustment. Moreover, the decrease of total testosterone in the synbiotic group was marginally significant compared to the control group (−3.38±20.20 vs. +2.33±12.79, *P*=0.056). Moreover, within-group analysis in the synbiotic group showed that total testosterone decreased meaningfully compared to its baseline value (0.28 ± 0.16 vs. 0.63 ± 0.96, *P*=0.034).

Table 2: Dietary intakes in the Intervention and control group

Variable	synbiotic group N=34	placebo group N=26	95% CI		*P***
			Lower	Upper	
Energy (kcal/d)	1727.19±429.69	1622.83±396.05	-113.70	322.42	0.342
Baseline	1613.87±488.47	1510.74±359.31	-130.55	336.80	0.380
Post	-127.55±376.45	-107.67±275.72	-199.73	159.98	0.825
Change	0.065	0.063			
P*					
Carbohydrates (g/d)	213.92±85.60	194.13±64.85	-20.74	60.32	0.332
Baseline	194.93±78.31	179.68±68.14	-24.35	54.86	0.444
End of trial	-21.67±84.30	-13.87±75.98	-51.00	35.41	0.719
Change	0.156	0.370			
P*					
Protein (g/d)	68.05±29.38	62.34±23.72	-8.57	19.83	0.431
Baseline	62.49±27.68	62.02±32.36	-15.47	16.42	0.953
End of trial	-6.07±27.50	-0.25±28.71	-20.81	9.18	0.440
Change	0.221	0.965			
P*					
Fat (g/d)	77.25±17.20	76.19±17.74	-8.09	10.22	0.817
Baseline	72.76±19.44	70.47±15.74	-7.29	11.87	0.634
End of trial	-4.94±17.57	-5.66±12.80	-7.66	9.10	0.864
Change	0.122	0.037			
P*					
Fiber (g/d)	19.65±6.97	19.82±7.15	-3.87	3.53	0.927
Baseline	18.03±7.85	17.42±7.58	-3.52	4.75	0.767
End of trial	-1.71±6.70	-1.99±7.97	-3.62	4.17	0.888
Change	0.158	0.224			
P*					
Vitamin D (mcg/d)	11.81±18.73	22.92±44.67	-28.29	6.09	0.641
Baseline	17.00±18.63	19.34±26.21	-14.24	9.57	0.696
End of trial	4.81±16.34	-3.06±40.2	-7.70	23.48	0.315
Change	0.105	0.705			
P*					
Vitamin C (mg/d)	80.47±80.43	166.06±215.45	-166.91	-4.24	0.040
Baseline	149.38±199.05	98.23±139.48	-38.98	141.29	0.281
End of trial	67.78±205.65	-52.97±193.22	13.60	227.92	0.028
Change	0.072	0.183			
P*					
Vitamin E (mg/d)	38.22±56.17	55.26±90.56	-55.51	21.43	0.379
Baseline	22.52±6.65	35.20±54.71	-35.36	10.02	0.260
End of trial	-16.17±55.86	-10.22±55.86	-35.83	23.93	0.691
Change	0.112	0.369			
P*					

Variables are mean ± SD;
*P**: *P*-values for comparison within groups via paired t-test.
*P*** : *P*-values for comparison between groups via independent t-test.
P< 0.05 is considered statistically significant.
Abbreviation: CI: confidence interval.

Table 3: Anthropometric indices at baseline and after the 12-week intervention in subjects with polycystic ovary syndrome

Variables	synbiotic group N=34	placebo group N=26	95% CI		*P***	*P****
			Lower	Upper		
Weight (mg/dl)	65.51±11.74	66.46±11.06	-6.92	5.02	0.752	
Baseline	65.30±12.35	65.60±10.08	-6.43	5.82	0.922	
End of trial	-0.20±3.05	-0.10±2.34	-1.59	1.37	0.884	0.387
Change	0.693	0.836				
P*						
BMI (kg/m²)	24.74±4.14	25.02±4.23	-2.46	1.89	0.796	
Baseline	24.68±4.29	24.66±3.65	-2.08	2.14	0.985	
End of trial	-0.05±1.20	-0.06±0.87	-0.560	0.580	0.973	0.463
Change	0.781	0.704				
P*						
WC (cm)	81.68±11.23	82.76±9.83	-6.70	4.54	0.702	
Baseline	80.33±11.24	82.28±10.18	-7.68	3.79	0.500	
End of trial	-0.70±5.29	-0.04±5.66	-3.61	2.27	0.650	0.181
Change	0.462	0.972				
P*						
HC (cm)	102.57±8.97	102.92±8.35	-4.94	4.25	0.881	
Baseline	101.60±10.56	101.56±7.87	-5.03	5.09	0.985	
End of trial	-0.75±5.77	-1.00±3.34	-2.36	2.85	0.853	0.843
Change	0.470	0.148				
P*						
WHR	0.79±0.06	0.80±0.05	-0.04	0.02	0.606	
Baseline	0.79±0.06	0.80±0.07	-0.05	0.01	0.307	
End of trial	0.00±0.05	0.00±0.05	-0.03	0.02	0.587	0.235
Change	0.959	0.470				
P*						
WHtR	0.50±0.06	0.50±0.06	-0.03	0.02	0.733	
Baseline	0.49±0.06	0.50±0.06	-0.04	0.02	0.539	
End of trial	-0.004±0.03	0.00±0.03	-0.02	0.01	0.620	0.210
Change	0.445	0.997				
P*						

*P**: *P*-values for comparison within groups via paired t-test.
*P*** : *P*-values for comparison between groups via independent t-test.
*P**** : *P*-values based on an ANCOVA model adjusted with baseline value of the outcome, age, marital status, PCOS duration and mean changes in BMI, dietary energy, carbohydrate protein, fat, total fiber, vitamin D, vitamin C and vitamin E.
P< 0.05 is considered statistically significant.
Abbreviations: CI: confidence interval; BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist to hip ratio; WHtR: waist to height ratio

Table 4: Glycemic indices and Hormonal status at baseline and after the 12-week intervention in subjects with polycystic ovary syndrome

Variables	synbiotic group N=34	placebo group N=26	95% CI		*P***	*P****
			Lower	Upper		
FBG (mg/dl)	91.08±12.45	92.73±6.93	-6.71	3.06	0.519	0.187
Baseline	94.00±6.87	98.16±22.56	-12.36	4.04	0.380	
End of trial	2.91±11.98	5.04±22.25	-11.13	6.88	0.638	
Change	0.166	0.269				
P*						
Fasting insulin (Mu/ml)	13.86±24.13	9.14±11.49				
Baseline	10.48±9.10	11.54±12.32	-5.54	15.00	0.361	
End of trial	-3.38±20.20	2.33±12.79	-6.63	4.52	0.741	0.042
Change	0.336	0.370	-14.93	3.49	0.219	
P*						
HOMA-IR	2.87±4.41	2.10±2.72	-1.20	2.74	0.438	
Baseline	2.43±2.15	2.86±3.26	-1.84	0.98	0.547	
End of trial	-0.43±3.61	0.73±2.91	-2.93	0.58	0.187	0.044
Change	0.485	0.485				
P*						
HOMA-β	330.79±811.72	121.35±176.41	-115.52	534.41	0.202	
Baseline	127.07±107.55	137.17±130.61	-72.25	52.05	0.746	
End of trial	-203.72±761.59	16.44±211.35	-534.32	94.00	0.166	0.332
Change	0.128	0.701				
P*						
QUICKI	0.49±0.01	0.49±0.01	-0.01	0.006	0.672	
Baseline	0.49±0.01	0.49±0.02	-0.005	0.01	0.525	
End of trial	-0.001	-0.005	-0.006	0.01	0.410	0.328
Change	0.641	0.223				
P*						
Total testosterone (ng/ml)	0.63±0.96	0.57±1.20	-0.50	0.62	0.832	
Baseline	0.28±0.16	0.30±0.31	-0.14	0.11	0.802	
End of trial	-0.34±0.97	-0.26±1.24	-0.67	0.49	0.766	0.056
Change	0.034	0.290				
P*						
DHEA-S (µg/dl)	4.36±7.62	2.15±2.29	-0.89	5.31	0.159	
Baseline	2.88±2.13	2.23±1.29	-0.31	1.62	0.216	
End of trial	-1.47±6.62	0.03±2.06	-4.26	1.24	0.277	0.047
Change	0.205	0.729				
P*						

P*: p-values for comparison within groups via paired t-test.
P**: p-values for comparison between groups via independent t-test.
P***: P***: p-values based on an ANCOVA model adjusted with baseline value of the outcome, age, marital status, PCOS duration and mean changes in BMI, dietary energy, carbohydrate protein, fat, total fiber, vitamin D, vitamin C and vitamin E.
P< 0.05 is considered statistically significant.
Abbreviations: CI: confidence interval; FPG: fasting plasma glucose; HOMA-IR: homeostasis model of assessment of insulin resistance; HOMA- β: homeostasis model of assessment of β-cell function; DHEA: dehydroepiandrosterone-sulfate

Discussion

To our knowledge, this study was the first randomized, triple-blind, placebo-controlled trial to investigate the effects of synbiotic-containing Bacillus coagulans (GBI-30), Lactobacillus rhamnosus, Lactobacillus helveticus, and fructooligosaccharide (FOS) on glycemic control, androgen hormones and anthropometric measurements of women with polycystic ovary syndrome. Our findings demonstrated that this supplementation has advantageous effects on insulin, insulin resistance, and male hormones testosterone and

DHEA, but it did not significantly affect anthropometric or other glycemic indices.

The enhancing effect of Bacillus coagulans (GBI-30) on glycemic indices was seen similarly in our previous study, conducted on patients with type 2 diabetes (21). In this study, a 12-week supplementation with a synbiotic containing 10^{11} spores of Bacillus coagulans (GBI-30), 10^{10} CFU Lactobacillus rhamnosus, 10^{9} CFU Lactobacillus acidophilus and 500 mg FOS was able to reduce FBG, insulin, HOMA-IR and HOMA- β significantly in the treatment group compared with the control group. The ineffectiveness of Bacillus coagulans on anthropometric factors, which was evident in our findings, also happened in this study. Moreover, in Abhari et al.'s study, supplementing with 10^{9} spores of Bacillus coagulans plus 400 mg inulin in people with non-alcoholic fatty liver was able to reduce insulin, body weight, BMI, WC and HC only in the synbiotic group compared to baseline, but did not cause a significant difference against control group (22).

Our results and the two mentioned studies, suggest B. coagulans may reduce insulin resistance in metabolic diseases, a relevant outcome for PCOS where pathophysiology is linked to insulin resistance. Excess insulin stimulates LH secretion from the pituitary, triggering adrenal and theca cells to produce androgens like testosterone, androstenedione, DHEA, and DHEAS (23).

The gut microbiome improves mitochondrial activity, energy metabolism, fatty acid oxidation, and intestinal barrier function via short-chain fatty acids (SCFAs), especially butyrate (24-26). Disrupted gut permeability increases endotoxins like LPS, promoting insulin resistance. Synbiotics may mitigate this by restoring microbiota balance (27). In Samimi et al.'s trial, synbiotic capsules (2×10^{9} CFU of L. acidophilus, L. casei, and B. bifidum + 800 mg inulin) decreased insulin and HOMA-IR and increased QUICKI, while FBG, weight, and BMI remained unchanged (28). Similarly, a meta-analysis of 7 PCOS trials found probiotics/synbiotics lowered insulin and HOMA-IR but not FBG or anthropometric indices (29). However, another 12-week trial found no effect on glycemic indices (30), supporting the idea that synbiotics may not consistently affect FBG or anthropometric measures in PCOS.

DHEAS and total testosterone—hallmarks of PCOS—were the androgens assessed in our study (31). The 12-week intervention of Karamali et al. in PCOS women with probiotic had a significant effect on reducing total testosterone and increasing sex hormone-binding globulin (SHBG), but did not change the DHEA-S (32) . This is while Nasri et al. conducted a similar study with the same bacterial composition, time period and sample size as the study of Karamali et. al, but their results did not reveal any significant decrease in testosterone or DHEA-S (33). Moreover, a meta-analysis which investigated the effects of probiotic/synbiotic on total testosterone and DHEA-S in people with PCOS didn't demonstrate any significant change. This inconsistency between this study and previous studies can be attributed to the difference in bacterial strain (34).

The current study has few limitations as follows: First, ovarian cyst status was not compared in within-group and between-group analysis. Second, there were heterogeneities among participants in terms of the severity of the disease. Third the exclusion of 16.66% of the participants at the end of the trial which is a relatively significant number. Fourth, physical activity was not assessed as a potential confounder. Fifth, the running period was not carried out in this study.

Conclusion

The present randomized, triple-blind, placebo-controlled trial demonstrated that taking synbiotic containing Bacillus coagulans (GBI-30) can have remarkably ameliorating effects for the glycemic status and androgen levels of women with polycystic ovarian syndrome. However, this supplement did not have a significant effect on anthropometric or other glycemic indicators.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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