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Effects of Probiotic Supplementation on Pancreatic β-cell Function and C-reactive Protein in Women with Polycystic Ovary Syndrome: A Randomized Double-blind Placebo-controlled Clinical Trial

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a polygenic endocrine disorder in women of reproductive age that lead to infertility. The aim of this study was to investigate the effects of probiotic on pancreatic β-cell function and C-reactive protein (CRP) in PCOS patients.

Methods: This randomized double-blind placebo-controlled clinical trial was conducted among 72 women aged 15–40 years old diagnosed with PCOS. Participants were randomly assigned to two groups receiving: (1) Probiotic supplements ($n = 36$), (2) placebo ($n = 36$) for 8-week. Fasting blood samples were taken at baseline and after 8-week of intervention.

Results: Probiotic supplementation, compare with placebo, reduced fasting blood sugar (−4.15 ± 2.87 vs. 2.57 ± 5.66 mg/dL, respectively $P = 0.7$), serum insulin levels in crude model (−0.49 ± 0.67 vs. 0.34 ± 0.82 μIU/mL, respectively, $P = 0.09$), homeostasis model of assessment-insulin resistance score (−0.25 ± 0.18 vs. −0.05 ± 0.18, respectively, $P = 0.14$) nonsignificantly. Serum insulin levels after adjustment with covariates reduced significantly in probiotic group ($P = 0.02$). We did not found any significant differences in mean changes of CRP between groups (−0.25 ± 0.18 vs. −0.05 ± 0.18, respectively, $P = 0.14$).

Conclusions: A 8-week multispecies probiotics supplementation had nonsignificantly beneficial effect on pancreatic β-cell function and CRP in PCOS patients. After adjustment for some covariates, serum insulin changes were significantly different between groups.

Keywords: C-reactive protein, pancreatic β-cell function, polycystic ovary syndrome, probiotic supplementation

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a polygenic endocrine disorder in women of reproductive age that lead to infertility.[1,2] Complications of PCOS are ovarian enlargement, hyperandrogenism and hirsutism, acne, alopecia, menstrual irregularity,[3] endometrial cancer,[4]

and some metabolic complications including glucose metabolism disturbance,[5] β-cell dysfunction[6] and elevated C-reactive protein (CRP) as an inflammation marker.[7] The prevalence of PCOS was estimated 12–21% in Western countries[8,9] and 15.2% in Iranian women.[10] Although the pathogenesis of PCOS is not completely known, the potential factors involve in gonadotropin releasing hormone secretion abnormalities and insulin resistance.[11]

Lifestyle modification, weight loss, and exercise would be beneficial in the management of PCOS.[12] According to previous findings, it seems that lifestyle change and diet manipulation improve most chronic diseases.[13,14] Probiotics as living microorganisms[15] synergism with gut microbiota and probably influence on metabolic and inflammatory conditions.[16] Dairy products including yoghurts, fermented foods and some cheeses are known as a valuable resources of probiotic cultures in diet. However, the gut microbiome alteration and biological effects of each source are ambiguous. Available probiotic supplements are influenced by initial dose strain, quality, temperature and anaerobic storage conditions.[17] Previous investigations have indicated the beneficial role of probiotics in various diseases as gastrointestinal diseases, infections, diabetes and atopic diseases.[14] Several animal studies reported that probiotics improved blood sugar and insulin resistance in diabetic rats.[18,19] A meta-analysis study showed that probiotic ameliorated insulin resistance in nonalcoholic fatty liver disease patients.[20] Despite many evidences about the association between insulin resistance and probiotics in various diseases, a little information is available about probiotic and CRP.

Considering the effects of probiotic supplementation on some metabolic disorders including insulin resistance and inflammation and these disorders are associated with PCOS, it seems that probiotic supplementation has favorable effects on PCOS. To the best of our knowledge and belief, this study is the first that investigated the effect of probiotic supplementation on pancreatic β-cell function and CRP in women with PCOS.

METHODS

Study design

Seventy-two PCOS patients aged 15–40 years old were assigned to this randomized double-blind placebo-controlled clinical trial that was performed in Isfahan, Iran, during May 2013 to December 2013.

Exclusion criteria were age below 15 and more than 40 years, those with history of chronic heart, kidney, liver, lung or pancreatic disease specially cardiovascular diseases, thyroid disorder, small bowel syndrome, autoimmune disease, allergy to probiotic capsules or placebo, current or previous (within the last 6 months) use of chemotherapy, corticosteroid (insulin injection, statins, diuretics), antibiotic, multivitamin mineral supplements and omega-3 medications and women with specific diet or physical activity programs.

Participants were stratified according to body mass index (BMI) (<18.5, 18.5–24.9, 25–29.9, >30 kg/m^2) and age (15–20, 21–25, 26–30, 31–35, 36–40 years) in order to matching participants and after getting informed consent were randomly allocated to one of the two groups: (1) Probiotic supplement (n = 32), (2) placebo (n = 33) for 8-week. Diagnosis of PCOS was done according to the 2003 Rotterdam criteria:[10] those with two of the following features were considered as PCOS: Oligo-ovulation and/or anovulation, clinical and biochemical hyper- and rogenism, and polycystic ovaries in ultrasonography. Subjects that admitted to the infertility centers of two hospitals affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, as Beheshti and the Alzahra Hospital were screened for PCOS. Those who have inclusion criteria were entered to the study. Their blood samples were transferred on dry ice to laboratory of Sedigheh Tahereh Metabolism and Endocrinology Research Center, Isfahan, Iran for analysis.

Patients in probiotic group received one Familact probiotic capsule (500 mg) daily that each capsule contained the following bacterial strains: *Lactobacillus casei* 7×10^9 CFU/g, *Lactobacillus acidophilus* 2×10^9 CFU/g, *Lactobacillus rhamnosus* 1.5×10^9 CFU/g, *Lactobacillus bulgaricus* 2×10^8 CFU/g, *Bifidobacterium breve* 2×10^{10} CFU/g, *Bifidobacterium longum* 7×10^9 CFU/g, *Streptococcus thermophiles* 1.5×10^9 CFU/g. Participants in the placebo group received the placebo that contained starch and maltodextrins but no bacteria. The placebo was indistinguishable in color, shape, size, and packaging, smell and taste from the probiotic supplement. All capsules were provided by fermented biological company of Tehran University of Medical Sciences that was recorded and approved by Food and Drug Administration. The study was approved by the Ethical Committee of Isfahan University of Medical Sciences, Isfahan, Iran. Patients take capsules with water after lunch.

Demographic characteristics of participants were collected by questionnaire. Three days food records were taken (2-week days and 1-week end) and Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods was used to obtain nutrient intake. Three physical activity records were taken at 2, 4, and 6 weeks. Compliance to supplements was monitored by: (1) Participant interview (2) follow-up by frequent

short message service and phone call (3) bring the medication containers. The registration ID in the Iranian website (www.irct.ir) was: IRCT2013081111763N11.

Anthropometric assessment

Body weight was assessed with minimal clothing and without shoes by standard scale (Seca, Germany) to the nearest 0.1 kg. Height was measured by a wall mounted stadiometer to the nearest 0.5 cm. BMI was computed as the weight in kilogram divided by the height in meters squared. Waist circumference (WC) was measured in the middle of the lowest gear and the top of the iliac crest with a nonstretched tape.

Biochemical assessment

The blood samples (10 cc) were taken at the baseline and after 8-week intervention after 12 h of fasting. Blood samples were analyzed at Laboratory of Metabolism and Endocrinology Research Center, Isfahan, Iran. Blood was immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate serum and stored at −70°C before analysis. Commercial kits were used to determine fasting plasma glucose (FPG) and CRP, (Pars Azmun, Tehran, Iran). The inter-assay coefficients of variations (CVs) for serum CRP were 4.00%. The intra- and inter-assay CVs for FPG were 1.74 and 1.19%, respectively. Serum insulin was assayed by immunoassay system (Advia Centaur Up, USA). The homeostatic model of assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) was calculated with formulas: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL)/405, QUICKI = 1/(log (fasting insulin μU/mL) + log (fasting glucose mg/dL).[21] Systematic error and inter-assay variability decreased by blinded fashion, duplicate, pairs (during intervention) at the same time, the same analytical run, and random order for glucose, insulin and CRP measurements.

Statistical analysis

T-test was used to detect differences in general characteristics and dietary intakes between the two groups. The effects of probiotic supplementation on fetal bovine serum (FBS), Insulin, HOMA, QUICKI, CRP, and variations were compared by paired *t*-test. The percentage of changes in variables after intervention determined by of the following formula: ([after values – before values]/before values) ×100. MANCOVA was applied to identify any differences between the two groups at the end of the study that analysis was adjusted with baseline values and some covariates including age, BMI, waist, marriage status, education and physical activity to omit the probably potential bias. All statistical analyses were done by the Statistical Package for Social Science software (SPSS Inc., Chicago, IL, USA version 16.0). All results were expressed as means ± standard error. $P < 0.05$ was considered as statistically significant. Smirnov–Kolmogorov test was used to check the normality of our data.

RESULTS

A total of 85 women were screened for PCOS in the infertility center of Beheshti and Alzahra Hospital Affiliated to Isfahan University of Medical Sciences, Isfahan, Iran. Totally, 72 patients had all the inclusion criteria and were ultimately included in the trial. Finally, 65 subjects (probiotic [$n = 32$], placebo [$n = 33$]) completed our study. Among individuals in the placebo group, three women (unwilling to continue [$n = 2$], become pregnant [$n = 1$]) and in the probiotic group, four women (unwilling to continue [$n = 2$], become pregnant [$n = 1$], health problems [$n = 1$]) did not complete the study [Figure 1]. There were no significant differences in terms of mean age, weight, BMI, WC, marriage status and physical activity between probiotic and placebo groups at the baseline of the study [Table 1].

No statistically significant differences were seen between the two groups in terms of dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids, polyunsaturated fatty acid, monounsaturated fatty acid, cholesterol, dietary fiber, Vitamin E, Vitamin C, calcium and magnesium based on dietary records that obtained throughout the intervention [Table 2].

We found within probiotic group a nonsignificant reduction in FBS (85.7 ± 2.6 vs. 81.5 ± 2.1 mg/dL, respectively, $P = 0.2$), serum insulin levels (9.8 ± 0.9 vs. 9.3 ± 0.71 μIU/mL, $P = 0.5$) and HOMA-IR score (2.11 ± 0.21 vs. 1.9 ± 0.2, respectively, $P = 0.2$) in before intervention compared with after intervention. Fasting blood sugar (−4.15 ± 2.87 vs. 2.57 ± 5.66 mg/dL, respectively $P = 0.7$), serum insulin levels (−0.49 ± 0.67 vs. 0.34 ± 0.82 μIU/mL, respectively, $P = 0.09$) and HOMA-IR score (−0.25 ± 0.18 vs. −0.05 ± 0.18, respectively, $P = 0.14$) reduced

Table 1: General characteristics of study participants in two groups at baseline

	Placebo group ($n=33$)	Probiotic group ($n=32$)	P^b
Age (year)	25.72±0.1[a]	26.5±0.1	0.6
Physical activity (METh/day)	32.5±0.2	34.9±0.2	0.06
Weight (kg)	67.22±2.5	67.5±2.5	0.94
BMI[c] (kg/m^2)	25.8±0.1	26.06±0.1	0.83
WC[d] (cm)	86.31±2.09	88.81±2.6	0.5
Married (%)	26 (78.8)	19 (59.4)	0.4
Education (%)			
Under academic	8 (24.2)	3 (9.4)	0.2
Academic	25 (90.6)	29 (75.8)	

[a]Data are means±SE, [b]Resulted from independent *t*-test, [c]BMI=Body mass index, [d]WC=Waist circumference, SE=Standard error

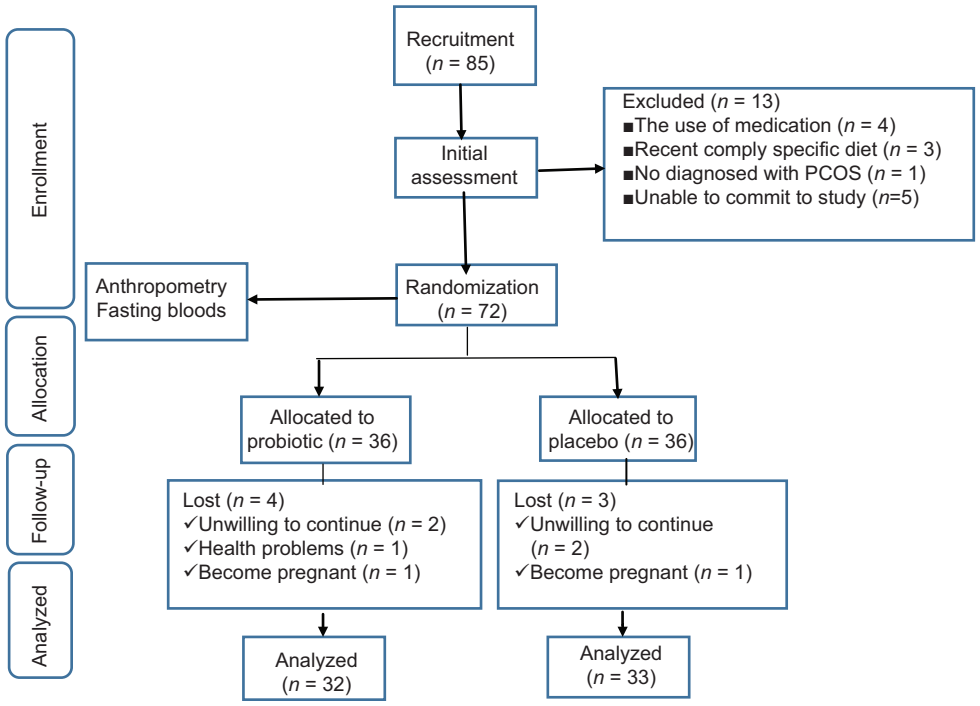

Figure 1: Summary of patient flow diagram, individuals received 500 mg probiotic supplements daily, polycystic ovary syndrome

Table 2: Dietary intakes of study participants throughout the study[a]

	Placebo group (*n*=33)	Probiotic group (*n*=32)	*P*[b]
Energy (kcal/day)	993.1±44.1	1010.6±47.34	0.8
Carbohydrates (g/day)	159.64±6.13	168.81±8.4	0.4
Protein (g/day)	35.8±1.3	37.9±1.9	0.4
Fat (g/day)	25.4±1.8	27.08±1.9	0.51
SFA[c] (g/day)	6.9±0.54	7.3±0.52	0.6
PUFA[d] (g/day)	4.1±0.5	5.2±0.5	0.7
MUFA[e] (g/day)	6.12±0.5	6.5±0.5	0.6
Cholesterol (mg/day)	161.6±6.7	170.7±10.11	0.5
Dietary fiber (g/day)	9.4±0.33	9.9±0.5	0.4
Vitamin C (mg/day)	63.51±2.14	69.6±3.4	0.13
Vitamin E (mg/day)	4.1±0.62	5.2±0.54	0.8
Vitamin B9 (μg/day)	96.13±3.4	101.2±4.1	0.4
Calcium (mg/day)	336.5±15	359.13±18.12	0.33
Magnesium (mg/day)	103.04±3.1	110.43±5.2	0.3

[a]Data are means±SE, [b]Resulted from independent *t*-test, [c]SFA=Saturated fatty acid, [d]PUFA=Polyunsaturated fatty acid, [e]MUFA=Monounsaturated fatty acid, SE=Standard error

nonsignificantly in probiotic group compared with placebo. Supplementation with probiotic or placebo had no significant effects on pancreatic β-cell function and CRP (0.29 ± 0.12 vs. −0.05 ± 0.13 mg/dL, respectively $P = 0.08$). We found a significant difference in mean change of serum insulin ($P = 0.02$) after adjustment with some covariate including age, BMI, waist, education, marriage status, physical activity. Adjustment with the above-mentioned covariates did not affect our findings about other variables. When we adjusted the analysis for baseline values findings remained nonsignificant [Table 3].

DISCUSSION

The current study demonstrated that taking probiotic supplements for 8-week had no significant effect on CRP and pancreatic β-cell function among PCOS women. However, after adjustment with some covariates, only serum insulin level decreased significantly in the probiotic group compare with placebo group. Previous studies have assessed the effect of probiotic supplementation on insulin resistance and inflammation marker. To the best of our knowledge, the present study is the first study that investigated the effect of probiotic supplementation on pancreatic β-cell function and CRP in PCOS women.

Patients with PCOS are predisposed to insulin resistance and metabolic dysfunctions.[22] In agreement with our findings, previous studies showed supplementation with probiotic reduced insulin resistant in type II diabetes patients.[23-27] Several animal studies showed probiotics improved gut permeability, plasma endotoxin levels, inflammation, and insulin resistance.[28] According to Alokail *et al*., study, supplementation with probiotics verified circulating endotoxin and inflammation markers during 26 weeks in type II diabetes patients.[15] Nitert *et al*., showed probiotic supplementation with >1 × 109 CFU each of *L. rhamnosus* GG and *Bifidobacterium lactis* BB-12 per capsule from 16 weeks of gestation until delivery prevented gestational diabetes in high-risk group

Table 3: The effect of probiotic supplementations on glucose metabolism and CRP

	Placebo group ($n=33$)	P*	Probiotic group ($n=32$)	P*	$P^{¥}$	$P^{\#}$	$P^{§}$
FBS (mg/dL)							
Week 0	89.6±2.08	0.7	85.7±2.6	0.2	0.7	0.2	0.33
Week 8	92.2±5.7		81.5±2.1				
Change	2.57±5.66		−4.15±2.87				
Insulin (μIU/mL)							
Week 0	10.03±0.9	0.7	9.8±0.9	0.5	0.09	0.2	0.02
Week 8	10.4±0.9		9.3±0.71				
Change	0.34±0.82		−0.49±0.67				
HOMA-IR							
Week 0	2.3±0.21	0.8	2.11±0.21	0.2	0.14	0.12	0.06
Week 8	2.2±0.2		1.9±0.2				
Change	−0.05±0.18		−0.25±0.18				
QUICKI							
Week 0	0.34±0.006	0.9	0.4±0.007	0.7	0.11	0.4	0.2
Week 8	0.34±0.006		0.4±0.005				
Change	0.0009±0.0068		0.002±0.005				
CRP (mg/dL)							
Week 0	1.3±0.1	0.71	1.08±0.08	0.02	0.08	0.2	0.2
Week 8	1.21±0.11		1.4±0.1				
Change	−0.05±0.13		0.29±0.12				

All values are means±SE. *Significantly different with respect to values at the beginning of the study; P value resulted from paired t-test, ¥Significantly different with respect to placebo group, #Significantly different with respect to placebo group, adjusted with the baseline values, §Significantly different with respect to placebo group, adjusted with age, BMI, waist, education, marriage status and physical activity. HOMA-IR=Homeostasis model of assessment for insulin resistance, QUICKI=Quantitative insulin sensitivity check index, SE=Standard error, CRP=C-reactive protein, FBS=Fasting blood sugar, BMI=Body mass index

of pregnant women.[29] Laitinen *et al*., reported a risk reduction of elevated glucose concentration, (odds ratio: 0.31, 95% confidence interval 0.12–0.78, P = 0.013), Insulin concentration (P = 0.032), HOMA-IR and (P = 0.028) and QUICKI (P = 0.028) during probiotics supplementation compared with the placebo group in normo-glycemic pregnant women.[30] Study on 54 diabetic patients aged 35–70 years showed, multispecies probiotic administration for 8-week increased fasting blood sugar decreased serum high-sensitivity CRP (hs-CRP) and increased plasma total GSH, compared with placebo on.[31] Consumption of probiotic yoghurt containing *L. acidophilus* and *Bifidobacterium animalis* among pregnant women after 9 weeks led to decrease serum hs-CRP[32] as the same probiotic supplementation in colorectal cancer,[33] autoimmune[34,35] and chronic kidney disease.[36] However, different findings can be explained by the probiotics dosage and strains used in different studies. Combination of gut microbiota and probiotics can influence glucose metabolism via immune system modulation and prevent pancreatic β-cell dysfunction via bacteria endotoxins inhibition resulted from lipopolysaccharide and inflammation reduction[37,38] and produced short chain fatty acids explained enzymatic synthesis of hepatic CRP.[38]

This study was the first study that assessed the effect of probiotic supplementation on CRP and pancreatic β-cell function among PCOS women. Some limitations of our study included: We were not able to assay the effect of probiotic supplementation on oral glucose tolerance tests and hormonal tests in subjects. In addition, long-term intervention might verify the results in terms of probiotic beneficial effects on inflammatory or insulin resistance markers. Probiotic dosage that was used in the present study was not assured by any guidelines.[39] Treatment noncompliance is the risk of all randomized controlled trials.

CONCLUSIONS

Probiotic supplementation for 8-week had no beneficial effects on FBS, HOMA-IR, QUICKI and CRP significantly. After adjustment with some covariates, serum insulin changes were significantly different between groups.

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