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Effects of Probiotic Supplementation on Hormonal Profiles, Biomarkers of Inflammation and Oxidative Stress in Women With Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: To the best of our knowledge, data on effects of probiotic administration on hormonal profiles, biomarkers of inflammation and oxidative stress in women with polycystic ovary syndrome (PCOS) are scarce. This investigation was conducted to assess the effects of probiotic supplementation on hormonal profiles, biomarkers of inflammation and oxidative stress in women with PCOS.

Methods: This randomized, double-blind, placebo-controlled trial was conducted on 60 women with PCOS, aged 18-40 years old. Subjects were randomly assigned into 2 groups to receive either probiotics or placebo (n=30 each group) for 12 weeks. Metabolic profiles were quantified at baseline and after a 12-week intervention.

Results: After the 12-week intervention, compared with placebo, probiotic supplementation significantly increased serum sex hormone-binding globulin (SHBG) (+25.9 ± 32.5 vs. +0.5 ± 15.6 nmol/L, $P < 0.001$) and plasma total antioxidant capacity (TAC) (+8.8 ± 120.5 vs. -98.3 ± 246.4 mmol/L, $P = 0.04$), and significantly decreased serum total testosterone (-0.2 ± 0.7 vs. +0.2 ± 0.6 ng/mL, $P = 0.03$), modified Ferriman-Gallwey (mF-G) scores (-1.7 ± 1.5 vs. -0.2 ± 1.0, $P < 0.001$), serum high-sensitivity C-reactive protein (hs-CRP) (-1150.0 ± 1295.2 vs. +202.5 ± 1426.3 ng/mL, $P < 0.001$) and plasma malondialdehyde (MDA) concentrations (-0.2 ± 0.6 vs. +0.9 ± 1.3 μmol/L, $P < 0.001$). We did not observe any detrimental effect of probiotic supplementation on other metabolic profiles.

Conclusion: Overall, probiotic supplementation of PCOS women for 12 weeks had beneficial effects on total testosterone, SHBG, mFG scores, hs-CRP, TAC and MDA levels but did not affect other metabolic profiles.

Keywords: Hormonal profiles, Inflammation, Oxidative stress, Polycystic ovary syndrome, Probiotic

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Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder in reproductive years affecting 5%–10% of premenopausal women.[1] Subjects with PCOS frequently have metabolic disorders including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), dyslipidemia, insulin resistance, hyperandrogenemia, visceral obesity and endothelial dysfunction.[2,3] Low-grade inflammation can induce production of inflammatory factors in PCOS as well as directly stimulate excess ovarian androgen production, which in turn would result in increased oxidative stress.[4]

Nowadays, there is a growing interest to use probiotics in patients with metabolic diseases such as T2DM.[5] The basis of this interest derives mainly from the results of studies suggesting that dysbiosis of gut microbiota has been implicated in multiple disease states such as diabetes, obesity and CVD.[6,7] Few studies have evaluated the effects of probiotic supplementation on metabolic profiles in PCOS women. In a study by Guo et al,[8] it was

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observed that dysbiosis of gut microbiota correlated with pathogenesis of PCOS and microbiota interventions through microbiota transplantation and *Lactobacillus* transplantation were effective for treatment of PCOS rats. We have previously shown that taking probiotic supplements for 12 weeks by PCOS women had favorable effects on markers of insulin metabolism, triglycerides and VLDL-cholesterol values, but did not affect other lipid profiles.[9] In addition, probiotic supplementation for 6 months could serve as a novel therapeutic option in the clinical management of knee osteoarthritis likely through reducing high sensitivity C-reactive protein (hs-CRP) concentrations.[10] Probiotic soy milk administration for 8 weeks to diabetic kidney disease patients could improve few biomarkers of oxidative stress.[11]

This evidence suggests the importance of probiotic supplementation for patients with PCOS. Probiotic intake may affect metabolic profiles, biomarkers of inflammation and oxidative stress through production of short chain fatty acid (SCFA), decreased expression of inflammation-relevant genes,[12] and up-regulation of oxidative pentose pathway activity.[13] To the best of our knowledge, data on the effects of probiotic supplementation on hormonal profiles, biomarkers of inflammation and oxidative stress in women with PCOS are limited. The objective of this study was to evaluate the effects of probiotic supplementation on hormonal profiles, biomarkers of inflammation and oxidative stress in these patients.

Subjects and Methods

Trial Design and Participants

This randomized, double-blind, placebo-controlled clinical trial, registered in the Iranian registry of clinical trials (identifier: IRCT201704235623N112, http://www.irct.ir), conducted at a Akbarabadi clinic affiliated to Iran University of Medical Sciences (IUMS), Tehran, Iran, among 60 women with PCOS, aged 18–40 years old between January 2017 and August 2017. This investigation was conducted in accordance with the Declaration of Helsinki and informed consent form was taken from all individuals. The research was approved by the ethics committee of IUMS. Diagnosis of PCOS was carried out according to the Rotterdam criteria.[14] Biochemical hyperandrogenism defined based on total testosterone levels >2 nmol/L. Exclusion criteria were as follows: smokers, taking probiotic supplements, pregnant women, endocrine diseases including thyroid, diabetes and/or impaired glucose tolerance.

Study Design

At first, all subjects were randomly divided into 2 groups to receive either probiotic supplements containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum* (2×10^9 CFU/g each) or placebo (starch) (n = 30 each group) for 12 weeks. The appearance of the placebo was similar in color, shape, size, and packaging, smell and taste from the probiotic capsule. All capsules were produced by Tak Gen Zist Pharmaceutical Company (Tehran, Iran). All patients completed 3-day food records and three physical activity records as metabolic equivalents (METs) at weeks 0, 3, 6, 9, and 12 of the intervention. Daily macro- and micro-nutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA) adapted for the Iranian food pattern.[15]

Treatment Adherence

To evaluate the compliance the remaining supplements were counted and subtracted from the amount of supplements provided to the individuals. To increase compliance, all individuals received short messages on their cell phones every day to remind them about taking the capsules.

Clinical Assessments

Clinical assessments included determinations of hirsutism using a mFG scoring system,[16] of acne score[17] and of alopecia based on assessment guidelines collated by Olsen et al.[18]

Assessment of Outcomes

Hs-CRP was considered as the primary outcomes, and nitric oxide (NO), biomarkers of oxidative stress and hormonal profiles were defined as the secondary outcomes. Ten milliliters fasting blood samples were collected at baseline and after the 12-week treatment at Akbarabadi reference laboratory, Tehran, Iran. Serum total testosterone with inter- and intra-assay coefficient variances (CVs) of 4.1 to 6.0%, sex hormone-binding globulin (SHBG) with inter- and intra-assay CVs of 3.5 to 5.5% and dehydroepiandrosterone sulfate (DHEAS) values with inter- and intra-assay CVs of 4.4 to 6.4% were determined using Elisa kits (DiaMetra, Milano, Italy). Free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG. Serum hs-CRP levels were assessed by an ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs of 4.9 to 6.8%, respectively. The plasma NO using Griess method,[19] total antioxidant capacity (TAC) by the use of ferric reducing antioxidant power developed by Benzie and Strain,[20] total glutathione (GSH) using the method of Beutler et al.[21] and malondialdehyde (MDA) values by the thiobarbituric acid reactive substances spectrophotometric test[22] were determined with inter- and intra-assay CVs less than 5%.

Sample Zize

Using a formula suggested for clinical trials, having 25 subjects in each group were adequate while considering

a type one error (α) of 0.05 and type 2 error (β) of 0.20 (power = 80%), 441.7 ng/mL as SD and 335.0 ng/mL as the mean distinction (d) of hs-CRP as the main outcome.[23] The correlation of hs-CRP between subsequent 8-week periods was $r = 0.29$. Assuming 5 dropouts in each group, the final sample size was determined to be 30 subjects in each group.

Randomization

Randomization assignment was performed using computer-generated random numbers. Randomization and allocation were concealed from the investigators and participants until the final analyses were completed. The randomized allocation sequence, enrolling participants and allocating them to interventions were conducted by a trained staff at the clinic. Another person, who was not involved in the trial and not aware of random sequences, assigned the subjects to the numbered bottles of capsules.

Statistical Methods

To evaluate whether the study variables were normally distributed or not, we used the Kolmogrov-Smirnov test. The Pearson chi-square test was used to compare categorical variables. To detect differences in anthropometric measures and in macro- and micro-nutrient intakes between the 2 groups, we applied independent *t* test. To assess the effects of probiotic supplementation on hormonal profiles, biomarkers of inflammation and oxidative stress, we used one-way repeated measures analysis of variance. Adjustment for changes in baseline values of biochemical parameters was performed by analysis of covariance (ANCOVA) using general linear models. The *P* value of <0.05 were considered statistically significant. All statistical analyses used the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

Firstly, we invited 71 participants with PCOS; however, 11 subjects were excluded in the eligibility phase because of not meeting inclusion criteria. All 60 subjects (probiotic [n = 30] and placebo [n = 30]) completed the trial (Figure 1). On average, the rate of compliance ranged between 90% to 100% both groups. No side effects were reported following the consumption of probiotic supplements in subjects with PCOS throughout the study.

Mean age, height, and weight, body mass index (BMI) and METs at baseline and end-of-trial were not significant between the 2 groups (Table 1). After 12 weeks of intervention, the frequency of decreased alopecia (15.8 vs. 5.6%, $P = 0.31$) and acne (26.3 vs. 9.5 %, $P = 0.16$) did not change following the consumption of probiotic supplements compared with the placebo.

No significant difference in mean dietary macro- and micro-nutrient intakes between the 2 groups was observed (Data not shown).

After a 12-week intervention, compared with the placebo, probiotic supplementation significantly increased serum SHBG (+25.9 ± 32.5 vs. +0.5 ± 15.6 nmol/L, $P < 0.001$) and plasma TAC (+8.8 ± 120.5

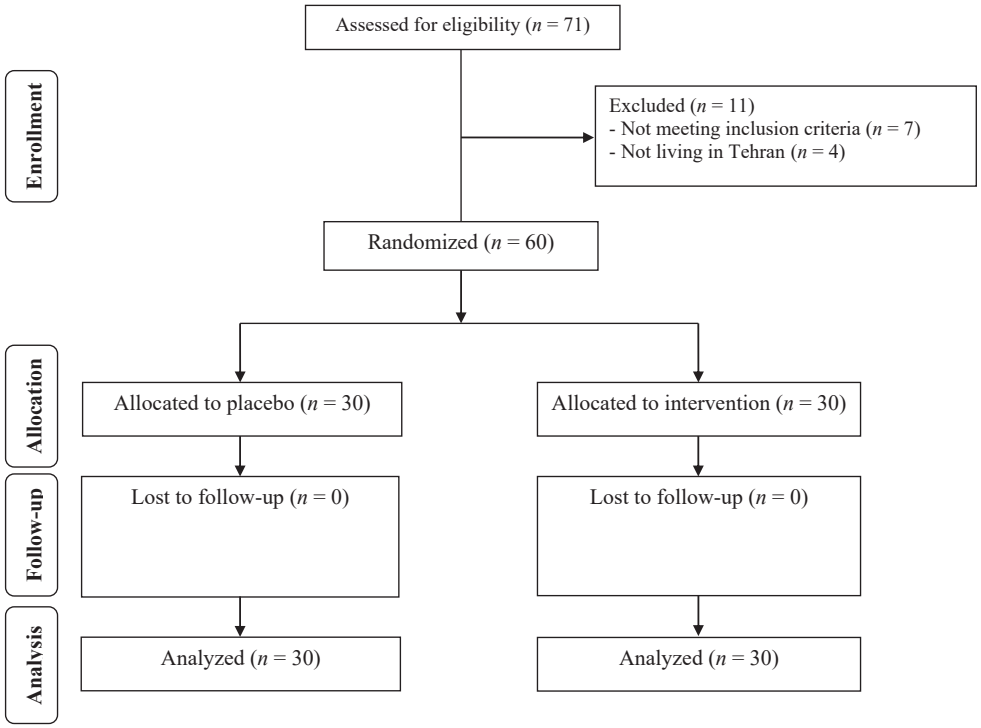

Figure 1. Summary of Patient Flow Diagram.

Table 1. General Characteristics of Study Participants

	Placebo group (*n* = 30)	Probiotic Group (*n* = 30)	*P*[a]
Age (y)	27.7 ± 4.7	27.2 ± 4.6	0.70
Height (cm)	164.5 ± 5.5	163.2 ± 6.0	0.36
Weight at study baseline (kg)	63.7 ± 8.0	62.9 ± 7.8	0.68
Weight at end-of-trial (kg)	63.8 ± 8.0	62.7 ± 7.8	0.57
BMI at study baseline (kg/m^2)	23.6 ± 3.5	23.7 ± 3.6	0.91
BMI at end-of-trial (kg/m^2)	23.6 ± 3.5	23.6 ± 3.6	0.98

Data are shown as mean ± SD.
[a] Obtained from independent *t* test.

vs. -98.3 ± 246.4 mmol/L, $P = 0.04$) and significantly decreased serum total testosterone (-0.2 ± 0.7 vs. +0.2 ± 0.6 ng/mL, $P = 0.03$), mF-G scores (-1.7 ± 1.5 vs. -0.2 ± 1.0, $P < 0.001$), serum hs-CRP (-1150.0 ± 1295.2 vs. +202.5 ± 1426.3 ng/mL, $P < 0.001$) and plasma MDA concentrations (-0.2 ± 0.6 vs. +0.9 ± 1.3 µmol/L, $P < 0.001$) (Table 2). We did observe no detrimental effect of probiotic supplementation on other metabolic profiles.

Baseline levels of serum testosterone levels ($P < 0.001$) were significantly different between the 2 groups. Therefore, we controlled the analyses for the baseline levels. When we adjusted the analyses for baseline values of biochemical variables, significant changes in plasma GSH levels ($P = 0.001$) were observed, but other findings did not alter (Table 3).

Discussion

To the best of our knowledge, this investigation is the first report of probiotic supplementation on hormonal profiles, biomarkers of inflammation and oxidative stress in women with PCOS. We found that taking probiotic supplements for 12 weeks by PCOS women had beneficial effects on total testosterone, SHBG, mFG scores, hs-CRP, TAC and MDA levels, but did not affect other metabolic profiles.

PCOS women are susceptible to multiple metabolic disturbances such as hyperandrogenemia, increased inflammation and oxidative stress.[24,25] In addition, micronutrients deficieny is common in women childbearing age,[26] which in turn may increase metabolic complications in women with PCOS. We found that probiotic administration for 12 weeks to PCOS women led to a significant increase in serum SHBG and a significant decrease in serum total testosterone and mFG scores but did not affect serum DHEAS levels compared with placebo. However, to the best of our knowledge, data on the effects of probiotic supplementation on hormonal profiles in women with PCOS are limited. Multiple studies have evaluated the effects of probiotic supplementation on markers of insulin resistance among subjects without PCOS. In a meta-analysis study, we have previously shown that synbiotic supplementation in patients with metabolic diseases to improve glucose homeostasis parameters was useful. In addition, some investigators indicated that taking probiotic supplements for 8 weeks by people with T2DM[23] and patients with rheumatoid arthritis was beneficial in improving markers of insulin metabolism, whereas other studies concluded that this approach had no detrimental effects among overweight men and women[27] and healthy volunteers.[28] Probiotic intake might improve total testosterone, SHBG and mFG scores through improved insulin sensitivity, elevation of fecal pH[29] and reduction of pro-inflammatory cytokine production.[30]

Our study demonstrated that, compared with the placebo, taking probiotic supplements for 12 weeks by subjects with PCOS decreased serum hs-CRP and plasma MDA and increased plasma TAC but did not change plasma NO and GSH concentrations. In line with our study, in people with major depressive disorder,

Table 2. Hormonal Profiles, Biomarkers of Inflammation and Oxidative Stress at Baseline and After the 12-Week Intervention in Subjects With Polycystic Ovary Syndrome

	Placebo Group (n = 30)			Probiotic Group (n = 30)				*P* [a]
	Baseline	End-of-Trial	Change	Baseline	End-of-Trial	Change	95% CI	
Total testosterone (ng/mL)	1.9 ± 0.7	2.1 ± 0.8	0.2 ± 0.6	1.3 ± 0.7	1.1 ± 0.8	-0.2 ± 0.7	0.02, 0.7	0.03
SHBG (nmol/L)	52.5 ± 18.4	52.9 ± 16.2	0.5 ± 15.6	46.3 ± 10.3	72.2 ± 31.9	25.9 ± 32.5	-38.6, -12.2	<0.001
FAI	0.14 ± 0.07	0.15 ± 0.08	0.005 ± 0.06	0.09 ± 0.05	0.07 ± 0.09	-0.02 ± 0.09	-0.01, 0.07	0.16
mF-G scores	12.6 ± 4.5	12.4 ± 4.5	-0.2 ± 1.0	14.1 ± 4.9	12.4 ± 3.8	-1.7 ± 1.5	1.1, 2.3	<0.001
DHEAS (µg/mL)	1.3 ± 0.4	1.2 ± 0.5	-0.1 ± 0.3	1.1 ± 0.6	1.2 ± 0.7	0.07 ± 0.4	-0.3, 0.1	0.19
hs-CRP (ng/mL)	3281.9 ± 1983.5	3384.4 ± 2404.9	202.5 ± 1426.3	3546.7 ± 1003.1	2396.7 ± 1588.6	-1150.0 ± 1295.2	648.4, 2056.5	<0.001
NO (µmol/L)	41.0 ± 8.8	39.3 ± 13.2	-1.6 ± 8.8	42.9 ±2.2	43.1 ± 1.8	0.2 ± 2.7	-5.2, 1.5	0.28
TAC (mmol/L)	904.6 ± 308.9	806.2 ± 339.3	-98.3 ± 246.4	935.5 ± 344.8	948.3 ± 380.2	8.8 ± 120.5	-207.4, -6.9	0.04
GSH (µmol/L)	456.7 ± 115.7	418.6 ± 129.1	-38.0 ± 154.9	494.6 ± 121.2	527.4 ± 98.1	32.8 ± 146.1	-148.7, 7.0	0.07
MDA (µmol/L)	2.3 ± 0.9	3.2 ± 1.1	0.9 ± 1.3	2.1 ± 0.4	1.9 ± 0.6	-0.2 ± 0.6	0.6, 1.7	<0.001

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; mF-G, modified Ferriman Gallwey; MDA, malondialdehyde; NO, nitric oxide; SHBG, sex hormone-binding globulin; TAC, total antioxidant.
All values are shown as mean ± SD.
[a] *P* values represent independent *t* test.

Table 3. Adjusted Changes in Metabolic Profile of Women With Polycystic Ovary Syndrome

	Placebo Group (n = 30)	Probiotic Group (n = 30)	95% CI	*P* [a]
Total testosterone (ng/mL)	0.2 ± 0.1	-0.3 ± 0.1	0.2, 0.9	0.008
SHBG (nmol/L)	2.1 ± 4.5	24.3 ± 4.5	-34.9, -8.8	0.001
FAI	0.01 ± 0.01	-0.03 ± 0.01	-0.002, 0.09	0.06
mF-G scores	-0.3 ± 0.2	-1.7 ± 0.2	0.4, 1.8	<0.001
DHEAS (µg/mL)	-0.05 ± 0.06	0.07 ± 0.06	-0.3, 0.1	0.19
hs-CRP (ng/mL)	197.2 ± 251.1	-1143.2 ± 251.1	660.6, 2066.1	<0.001
NO (µmol/L)	-1.5 ± 1.2	0.2 ± 1.2	-5.1, 1.7	0.32
TAC (mmol/L)	-99.5 ± 35.5	10.0 ± 35.5	-208.3, -7.2	0.03
GSH (µmol/L)	-53.9 ± 20.9	48.7 ± 20.9	-164.5, -43.1	0.001
MDA (µmol/L)	1.0 ± 0.2	-0.3 ± 0.2	0.9, 1.8	<0.001

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; mF-G, modified Ferriman Gallwey; MDA, malondialdehyde; NO, nitric oxide; SHBG, sex hormone-binding globulin; TAC, total antioxidant.
All values are shown as mean ± SD. Values are adjusted for baseline values.
[a] Obtained from ANCOVA.

probiotic supplementation for 8 weeks was effective in reducing serum hs-CRP concentrations.[31] In addition, in critically-ill patients, administration of probiotics was demonstrated to reduce cardiovascular risk factors such as hs-CRP.[32] However, in another study, an 8-week probiotic supplementation had no beneficial effect on CRP levels in PCOS patients.[33] Kullisaar et al[34] observed that 2 *Lactobacillus fermentum* strains, E-3 and E-18, have antioxidative properties such as increased levels of GSH. Moreover, Songisepp et al[35] found significant improvement in total antioxidant status after probiotics supplementation for 3 weeks in healthy people. However, synbiotic supplementation for 6 weeks in subjects with a low serum enterolactone concentration did not influence serum CRP values.[36] A previous investigation has reported that probiotic yogurt intake for 6 weeks could improve antioxidant status in people with T2DM.[37]

Increased CRP concentrations are positively correlated with insulin resistance and the incidence of T2DM.[38] Therefore, high CRP concentrations are considered a potential cause of long-term consequences of PCOS.[39] Toulis et al[40] found that CRP levels were significantly increased in subjects with PCOS compared with controls. In addition, accumulating evidence has reported that increased oxidative stress induced by oxygen species (ROS) may contribute to the progress of insulin resistance and hyperandrogenism which are main features of PCOS.[41] It was also reported by multiple previous investigations, subjects with PCOS indicate reduction in antioxidant status and increased oxidative stress status,[42] physiological concentrations of ROS have an important function in female reproductive system during folliculogenesis and oocyte maturation.[43] Therefore, to manage and control parameters of inflammation and oxidative stress in PCOS women, these are important factors in decreasing events related to metabolic disorders. Anti-inflammatory and antioxidant effects of probiotics may be explained by their production of saturated fatty acids in the gut[44] and its impact on decreasing lipid peroxidation such as oxidized LDL and 8-isoprostanes.[45]

Strengths of the present study include its double-blinded design and consideration of the confounding parameters including dietary nutrient intake and physical activity. Its limitations include absence of fecal sample data to demonstrate transit of the specific probiotic through the gastrointestinal tracts of study subjects in the intervention group.

Overall, probiotic supplementation for 12 weeks in PCOS women had beneficial effects on total testosterone, SHBG, mFG scores, hs-CRP, TAC and MDA levels, but did not affect other metabolic profiles.

Authors' Contribution

ZA contributed to the conception, design, statistical analysis and drafting of the manuscript. MK, SE, SR, MJ, FB, MT-E, FK, S-MM, MCh and SH-G contributed to data collection and manuscript drafting. ZA supervised the study.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Clinical Trial Registration

Identifier: IRCT201704235623N112, http://www.irct.ir.

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References

1. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet. 2007;370(9588):685-97. doi: 10.1016/S0140-6736(07)61345-2.
2. Dokras A. Cardiovascular disease risk in women with PCOS. Steroids. 2013;78(8):773-6. doi: 10.1016/j.steroids.2013.04.009.
3. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev. 2013;14(2):95-109. doi: 10.1111/j.1467-789X.2012.01053.x.
4. Artimani T, Karimi J, Mehdizadeh M, Yavangi M, Khanlarzadeh E, Ghorbani M, et al. Evaluation of pro-oxidant-antioxidant balance (PAB) and its association with inflammatory cytokines in polycystic ovary syndrome (PCOS). Gynecol Endocrinol. 2018;34(2):148-52. doi: 10.1080/09513590.2017.1371691.

5. Akram Kooshki A, Tofighiyan T, Rakhshani MH. Effects of Synbiotics on Inflammatory Markers in Patients With Type 2 Diabetes Mellitus. Glob J Health Sci. 2015;7(7 Spec No):1-5. doi: 10.5539/gjhs.v7n7p1.
6. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472(7341):57-63. doi: 10.1038/nature09922.
7. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013;339(6123):1084-8. doi: 10.1126/science.1233521.
8. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, et al. Association between Polycystic Ovary Syndrome and Gut Microbiota. PLoS One. 2016;11(4):e0153196. doi: 10.1371/journal.pone.0153196.
9. Ahmadi S, Jamilian M, Karamali M, Tajabadi-Ebrahimi M, Jafari P, Taghizadeh M, et al. Probiotic supplementation and the effects on weight loss, glycaemia and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Hum Fertil (Camb). 2017;20(4):254-61. doi: 10.1080/14647273.2017.1283446.
10. Lei M, Guo C, Wang D, Zhang C, Hua L. The effect of probiotic Lactobacillus casei Shirota on knee osteoarthritis: a randomised double-blind, placebo-controlled clinical trial. Benef Microbes. 2017;8(5):697-703. doi: 10.3920/bm2016.0207.
11. Miraghajani M, Zaghian N, Mirlohi M, Feizi A, Ghiasvand R. The Impact of Probiotic Soy Milk Consumption on Oxidative Stress Among Type 2 Diabetic Kidney Disease Patients: A Randomized Controlled Clinical Trial. J Ren Nutr. 2017;27(5):317-24. doi: 10.1053/j.jrn.2017.04.004.
12. Voltolini C, Battersby S, Etherington SL, Petraglia F, Norman JE, Jabbour HN. A novel antiinflammatory role for the short-chain fatty acids in human labor. Endocrinology. 2012;153(1):395-403. doi: 10.1210/en.2011-1457.
13. Matthews GM, Howarth GS, Butler RN. Short-chain fatty acid modulation of apoptosis in the Kato III human gastric carcinoma cell line. Cancer Biol Ther. 2007;6(7):1051-7.
14. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25.
15. Asemi Z, Jamilian M, Mesdaghinia E, Esmaillzadeh A. Effects of selenium supplementation on glucose homeostasis, inflammation, and oxidative stress in gestational diabetes: Randomized, double-blind, placebo-controlled trial. Nutrition. 2015;31(10):1235-42. doi: 10.1016/j.nut.2015.04.014.
16. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 1981;140(7):815-30.
17. Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. Fertil Steril. 2000;73(6):1149-54.
18. Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines--Part II. National Alopecia Areata Foundation. J Am Acad Dermatol. 2004;51(3):440-7. doi: 10.1016/j.jaad.2003.09.032.
19. Tatsch E, Bochi GV, Pereira Rda S, Kober H, Agertt VA, de Campos MM, et al. A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. Clin Biochem. 2011;44(4):348-50. doi: 10.1016/j.clinbiochem.2010.12.011.
20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6. doi: 10.1006/abio.1996.0292.
21. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med. 1985;105(5):581-4.
22. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990;9(6):515-40.
23. Asemi Z, Zare Z, Shakeri H, Sabihi SS, Esmaillzadeh A. Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. Ann Nutr Metab. 2013;63(1-2):1-9. doi: 10.1159/000349922.
24. Asemi Z, Foroozanfard F, Hashemi T, Bahmani F, Jamilian M, Esmaillzadeh A. Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. Clin Nutr. 2015;34(4):586-92. doi: 10.1016/j.clnu.2014.09.015.
25. Foroozanfard F, Jamilian M, Bahmani F, Talaee R, Talaee N, Hashemi T, et al. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D-deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Clin Endocrinol (Oxf). 2015;83(6):888-94. doi: 10.1111/cen.12840.
26. Asemi Z, Taghizadeh M, Sarahroodi S, Jazayeri S, Tabasi Z, Seyyedi F. Assessment of the relationship of vitamin D with serum antioxidant vitamins E and A and their deficiencies in Iranian pregnant women. Saudi Med J. 2010;31(10):1119-23.
27. Ivey KL, Hodgson JM, Kerr DA, Lewis JR, Thompson PL, Prince RL. The effects of probiotic bacteria on glycaemic control in overweight men and women: a randomised controlled trial. Eur J Clin Nutr. 2014;68(4):447-52. doi: 10.1038/ejcn.2013.294.
28. Naruszewicz M, Johansson ML, Zapolska-Downar D, Bukowska H. Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers. Am J Clin Nutr. 2002;76(6):1249-55.
29. Compare D, Coccoli P, Rocco A, Nardone OM, De Maria S, Carteni M, et al. Gut--liver axis: the impact of gut microbiota on non alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis. 2012;22(6):471-6. doi: 10.1016/j.numecd.2012.02.007.
30. Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology. 2003;37(2):343-50. doi: 10.1053/jhep.2003.50048.
31. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, Jafari P, Akbari H, Taghizadeh M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition. 2016;32(3):315-20. doi: 10.1016/j.nut.2015.09.003.
32. Sanaie S, Ebrahimi-Mameghani M, Mahmoodpoor A, Shadvar K, Golzari SE. Effect of a Probiotic Preparation (VSL#3) on CardiovascularRisk Parameters in Critically-Ill Patients. J Cardiovasc Thorac Res. 2013;5(2):67-70. doi: 10.5681/jcvtr.2013.014.
33. Shoaei T, Heidari-Beni M, Tehrani HG, Feizi A, Esmaillzadeh A, Askari G. Effects of Probiotic Supplementation on Pancreatic beta-cell Function and C-reactive Protein in Women with Polycystic Ovary Syndrome: A Randomized Double-blind Placebo-controlled Clinical Trial. Int J Prev Med. 2015;6:27. doi: 10.4103/2008-7802.153866.
34. Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, Kairane C, et al. Two antioxidative lactobacilli strains as promising probiotics. Int J Food Microbiol. 2002;72(3):215-24.
35. Songisepp E, Kals J, Kullisaar T, Mandar R, Hutt P, Zilmer M, et al. Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. Nutr J. 2005;4:22. doi: 10.1186/1475-2891-4-22.
36. Holma R, Kekkonen RA, Hatakka K, Poussa T, Vapaatalo H, Adlercreutz H, et al. Low serum enterolactone concentration is associated with low colonic Lactobacillus-Enterococcus counts in men but is not affected by a synbiotic mixture in a randomised, placebo-controlled, double-blind, cross-over intervention study. Br J Nutr. 2014;111(2):301-9. doi: 10.1017/s0007114513002420.
37. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition. 2012;28(5):539-43. doi: 10.1016/j.nut.2011.08.013.
38. Odegaard AO, Jacobs DR, Jr., Sanchez OA, Goff DC, Jr., Reiner AP, Gross MD. Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. Cardiovasc Diabetol. 2016;15:51. doi: 10.1186/s12933-016-0369-6.
39. Repaci A, Gambineri A, Pasquali R. The role of low-grade inflammation in the polycystic ovary syndrome. Mol Cell

Endocrinol. 2011;335(1):30-41. doi: 10.1016/j.mce.2010.08.002.
40. Toulis KA, Goulis DG, Mintziori G, Kintiraki E, Eukarpidis E, Mouratoglou SA, et al. Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. Hum Reprod Update. 2011;17(6):741-60. doi: 10.1093/humupd/dmr025.
41. Deepika ML, Nalini S, Maruthi G, Ramchander V, Ranjith K, Latha KP, et al. Analysis of oxidative stress status through MN test and serum MDA levels in PCOS women. Pak J Biol Sci. 2014;17(4):574-7.
42. Moti M, Amini L, Mirhoseini Ardakani SS, Kamalzadeh S, Masoomikarimi M, Jafarisani M. Oxidative stress and anti-oxidant defense system in Iranian women with polycystic ovary syndrome. Iran J Reprod Med. 2015;13(6):373-8.
43. Nunez-Calonge R, Cortes S, Gutierrez Gonzalez LM, Kireev R, Vara E, Ortega L, et al. Oxidative stress in follicular fluid of young women with low response compared with fertile oocyte donors. Reprod Biomed Online. 2016;32(4):446-56. doi: 10.1016/j.rbmo.2015.12.010.
44. Sadrzadeh-Yeganeh H, Elmadfa I, Djazayery A, Jalali M, Heshmat R, Chamary M. The effects of probiotic and conventional yoghurt on lipid profile in women. Br J Nutr. 2010;103(12):1778-83. doi: 10.1017/s0007114509993801.
45. Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M. Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr. 2003;90(2):449-56.