Original Article

Effects of Probiotic Supplementation on Metabolic Status in Pregnant Women: a Randomized, Double-blind, Placebo-Controlled Trial

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Abstract

Background: Limited data is available on the effects of multispecies probiotic supplementation on metabolic status in pregnant women in the first half of pregnancy. The current study was carried out to determine the effects of multispecies probiotic capsule supplementation on metabolic status among pregnant women in the first half of pregnancy.

Methods: A randomized clinical trial was conducted among 60 pregnant women aged 18–37 years. The participants were randomly divided into two groups: group A (n = 30) received multispecies probiotic supplements containing three probiotic bacteria spices *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* (2 × 10^9 CFU/g each) and group B (n = 30) received placebo from 9 weeks of gestation for a duration of 12 weeks. Fasting blood samples were taken at the beginning of the study and after 12 weeks of intervention to determine metabolic profiles, inflammatory cytokines and biomarkers of oxidative stress.

Results: After 12 weeks of intervention, compared to the placebo group, the pregnant women who consumed probiotic capsule had significantly decreased serum insulin concentrations (-1.5 ± 4.8 vs. +1.3 ± 5.2 μIU/mL, *P* = 0.03), the homeostasis model of assessment-estimated insulin resistance (HOMA-IR) (-0.3 ± 0.9 vs. +0.3 ± 1.1, *P* = 0.04), the homeostasis model of assessment-estimated b cell function (HOMA-B) (-7.2 ± 23.1 vs. +5.3 ± 22.6, *P* = 0.03) and increased quantitative insulin sensitivity check index (QUICKI) (+0.01 ± 0.05 vs. -0.01 ± 0.02, *P* = 0.03). In addition, changes in serum triglycerides levels (-14.7 ± 46.5 vs. +37.3 ± 74.2 mg/dL, *P* = 0.002), high-sensitivity C-reactive protein (hs-CRP) (-1.0 ± 2.6 vs. +1.7 ± 4.3 mg/L, *P* = 0.004), plasma nitric oxide (NO) (+6.8 ± 9.3 vs. -4.7 ± 7.4 μmol/L, *P* < 0.001), total antioxidant capacity (TAC) (+171.9 ± 187.6 vs. -51.9 ± 208.8 mmol/L, *P* < 0.001) and glutathione (GSH) concentrations (+34.3 ± 71.6 vs. -36.9 ± 108.3 μmol/L, *P* = 0.004) in supplemented women were significantly different from those of the placebo group. However, after controlling for baseline levels, age and BMI at the study baseline, the changes in plasma GSH were not significantly different between the groups.

Conclusion: Overall, probiotic supplementation for 12 weeks among pregnant women in the first half of pregnancy had beneficial effects on markers of insulin metabolism, triglycerides, biomarkers of inflammation and oxidative stress.

Keywords: Metabolic status, pregnant, probiotic, supplementation

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Introduction

Pregnant women are susceptible to insulin resistance, dyslipidemia, inflammation and oxidative stress due to micronutrient deficiency,[1] increased maternal adipose tissue and production of hormones by placenta.[2–3] Elevated circulating levels of markers of insulin resistance and lipid profiles in pregnant women are implicated in the pathogenesis of ischemic heart disease, type 2 diabetes mellitus (T2DM), and essential hypertension.[4] In addition, increased inflammatory cytokines and biomarkers of oxidative stress during pregnancy might predict future development of both metabolic and cardiovascular diseases.[5]

Previous studies have shown that gut microbiota play an important role in energy homeostasis, inflammation and glucose metabolism.[6–7] In addition, recent evidence suggests that manipulation of the maternal gut microbiota during pregnancy may have important benefits in terms of improving metabolic profiles[8] and pregnancy outcomes.[9] Our previous studies among pregnant women have demonstrated that administration of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* with a total of min 1 × 10^7 CFU/g for 9 weeks decreased inflammation and oxidative stress.[10] Furthermore, consumption of *Lactobacillus sporogenes* (1 × 10^8 CFU/g) and inulin resulted in a significant decrease of serum triglycerides and VLDL-cholesterol levels in patients with type-2 diabetes after 8 weeks.[11] However, consumption of probiotic Lactobacillus did not affect pregnancy outcomes, including the number of spontaneous abortions, pre-

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term births and low birth weight.[12]

Improvement of metabolic profiles, biomarkers of inflammation and oxidative stress by probiotics might be due to their effects on increasing concentrations of GSH,[13] scavenging superoxide and hydroxyl radicals,[14] reduced inflammatory signaling[15] and decreased adiposity.[16] We are aware of no study indicating the effects of probiotic supplementation on metabolic profiles, inflammation and oxidative stress among pregnant women in the first half of pregnancy. The aim of the current study was, therefore, to investigate the effects of a probiotic supplementation on metabolic profiles, inflammatory factors and biomarkers of oxidative stress among pregnant women in the first half of pregnancy.

Materials and Methods

Participants

The participants of this randomized double-blind placebo-controlled clinical trial study consisted of 60 pregnant women in the first half of pregnancy at the age range of 18–37 years who agreed to participate in the current study between March 2015 and July 2015. The exclusion criteria were as follows: pregnant women with a recognized cause of recurrent miscarriages or a structural uterine abnormality distorting the cavity as well as those with a history of rheumatoid arthritis, thyroid, parathyroid or adrenal diseases and hepatic or renal failure. In the sample size formula suggested for randomized clinical trials, considering the type I error of 5% ($\alpha = 0.05$) and type II error of 20% ($\beta = 0.20$; Power = 80%) and serum insulin levels as key variable,[17] we used 8.72 as SD and 7.00 μIU/mL as the change in mean (d) of serum insulin levels as the main variable. Based on this, we needed 25 participants in each group. However, we recruited 30 pregnant women in each group (totally, 60 subjects) to compensate for the probable loss to follow-up.

Ethics statements

The present study protocol was confirmed in accordance with the principals of the Declaration of Helsinki and approved by the Research Council and the ethics committee of AUMS (reference number: 93167). Informed consent was taken from all participants. The current trial was registered at the Iranian registry of clinical trials (http://www.irct.ir: IRCT201503035623N38), which is a primary registry in the World Health Organization (WHO) Registry Network.

Study design

At the beginning of the study, the pregnant women were stratified one-by-one according to BMI (<25 and ≥25 kg/m^2) and age (<25 and ≥25 years). The participants of the present study were randomly allocated into two groups (probiotic and placebo). The probiotic group (n = 30) received three probiotic bacteria species, including *Lactobacillus acidophilus* 2×10^9, *Lactobacillus casei* 2×10^9 and *Bifidobacterium bifidum* 2×10^9 CFU/g prepared by Tak Gen Zist Pharmaceutical Company (Tehran, Iran). It is well known that it would be more appropriate if the strains used in probiotic supplements for human consumption are derived from the human intestinal tract, well characterized, able to outlive the rigors of the digestive tract and possibly colonisz, biologically active against the target as well as to be stable and amenable to commercial production and distribution.[18] Due to lack of evidence about the appropriate dosage of probiotics for pregnant women, we used the above-mentioned doses based on few previous studies in healthy subjects.[19–20] The placebo (starch) group (n = 30) received one placebo capsule per day for 12 weeks which was identical in color, shape, size and package to the probiotic capsules and also produced by the same pharmaceutical company. Subjects were advised to keep their life style habits such as usual diet and levels of physical activity during the study period. Compliance to the consumption of probiotic or placebo capsules was assessed by unused containers of the probiotic and placebo capsules which were returned to the researchers. Furthermore, we sent a reminder message on the participants' cell phones regarding consumption of supplements. Three dietary records (two week days and one weekend) at weeks 3, 6, and 9 of the intervention were obtained from each participant. To determine average daily macro- and micro-nutrient intakes, we used modified Nutritionist IV software (First Databank, San Bruno, CA). Physical activity was defined as metabolic equivalents (METs) in hours per day in this study. To quantify the METs for each participant, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables.[21] A questionnaire was used to measure physical activity.

Randomization

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

Assessment of anthropometric measures

Weight and height (Seca, Hamburg, Germany) were quantified without shoes in light clothing in the gynecology clinic by a trained midwife, at the beginning of the study and after 3 months. BMI was calculated as weight (kg) divided by height squared (m^2).

Biochemical measurements

A 10-mL fresh blood sample was taken from each participant after 10–12 h overnight fast, pre- and post-intervention at Arak reference laboratory. Then, the samples were centrifuged and stored at -80°C until analyzed further. Fasting plasma glucose (FPG) concentrations were measured by the glucoseoxidase method (Pars Azmoon Co, Tehran, Iran). To determine serum triglycerides, total-, LDL-, HDL- and VLDL-cholesterol concentrations, we used enzymatic kits (Pars Azmoon Co, Tehran, Iran). In the current study, all inter- and intra-assay coefficient variances (CVs) for FPG and lipid concentrations were less than 5%. Fasting insulin levels were quantified by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Monobind, California, USA). HOMA-IR, β-cell function (HOMA-B) and the quantitative insulin sensitivity check index (QUICKI) were calculated based on the suggested formulas.[22] Serum hs-CRP concentrations were measured by a commercial ELISA kit (LDN, Nordhorn, Germany). The plasma NO concentrations were assessed using Griess method.[23] Plasma TAC concentrations were measured by the method of ferric reducing antioxidant power (FRAP) developed by Benzie and Strain,[24] total glutathione (GSH) using the method of Beutler et al.[25] and malondialdehyde (MDA) concentrations by the thiobarbituric acid reactive substances (TBARs) spectrophotometric test.[26]

Statistical methods

To evaluate the normality of variables, we used Kolmogrov-Smirnov test. To detect differences in the general characteristics of participants and dietary intakes between the two groups, independent samples student's *t*-test was applied. One-way repeated measures ANOVA was used to determine the effects of probiotic consumption on glycemic status, lipid concentrations, biomarkers of inflammation and oxidative stress. To adjust results for confounders, ANCOVA test was used to compare the mean changes of the outcome variables between the groups while adjusting for baseline values, age and baseline BMI. In all analyses, *P*-value <0.05 was considered as statistically significant. Statistical analyses were performed using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

At the baseline, we recruited 70 participants; however, 10 subjects were excluded from the study because of not fulfilling the inclusion criteria. In the current study, 60 pregnant women [probiotic (n = 30) and placebo (n = 30)] completed the trial. On average, the rate of compliance in the present study was high, such that 100% of capsules were taken throughout the study in both groups. No side effects were reported following the consumption of probiotic supplements in pregnant women throughout the study.

No significant difference existed in the anthropometric measurements before and after the intervention or the abortion rate after probiotic supplementation (Table 1).

Comparison of total calorie intake, macro- and micro-nutrients between the two groups based on the three-day dietary records throughout the study showed no statistically significant difference (Data not shown).

After 12 weeks of intervention, compared to the placebo group, pregnant women who consumed probiotic capsules had significantly decreased serum insulin concentrations (-1.5 ± 4.8 vs. +1.3 ± 5.2 μIU/mL, $P = 0.03$), HOMA-IR (-0.3 ± 0.9 vs. +0.3 ± 1.1, $P = 0.04$), HOMA-B (-7.2 ± 23.1 vs. +5.3 ± 22.6, $P = 0.03$) and increased QUICKI (+0.01 ± 0.05 vs. -0.01 ± 0.02, $P = 0.03$) (Table 2). In addition, changes in serum triglycerides levels (-14.7 ± 46.5 vs. +37.3 ± 74.2 mg/dL, $P = 0.002$), VLDL-cholesterol (-2.9 ± 9.3 vs. +7.4 ± 14.8 mg/dL, P=0.002), hs-CRP (-1.0 ± 2.6 vs. +1.7 ± 4.3 mg/L, $P = 0.004$), plasma NO (+6.8 ± 9.3 vs. -4.7 ± 7.4 μmol/L, $P < 0.001$), TAC (+171.9 ± 187.6 vs. -51.9 ± 208.8 mmol/L, $P < 0.001$) and GSH concentrations (+34.3 ± 71.6 vs. -36.9 ± 108.3 μmol/L, $P = 0.004$) in supplemented women were significantly different from those in the placebo group. We did not observe any significant change in other lipid concentrations or MDA levels.

The baseline concentrations of hs-CRP ($P = 0.01$) and GSH ($P < 0.001$) differed significantly between the two groups. Therefore, baseline concentrations, age and baseline BMI were controlled for in the analyses. After adjustment for baseline levels, age and baseline BMI, no significant changes in our findings occurred, except for plasma GSH concentrations ($P = 0.40$) (Table 3).

Discussion

This randomized clinical trial demonstrated that probiotic supplementation for 12 weeks among pregnant women in the first half of pregnancy had beneficial effects on markers of insulin metabolism, triglycerides, biomarkers of inflammation and oxidative stress; however, it did not have any effect on other lipid profiles. To the best of our knowledge, the current study is the first evaluating the effects of multispecies probiotic supplementation on metabolic profiles in pregnant women in the first half of pregnancy.

Pregnant women are susceptible to some metabolic disorders.[27–28] Findings from the present study exhibited that consumption of probiotic supplements by pregnant women for 12 weeks led to a significant reduction in fasting serum insulin levels, HOMA-IR, HOMA-B, triglycerides, and VLDL-cholesterol and increased QUICKI score compared with the placebo, while FPG and other lipid concentrations remained unchanged. Supporting our study, in a meta-analysis by Ruan *et al*.[29] probiotic consumption was shown to reduce fasting plasma insulin and HOMA-IR significantly. A 6-week supplementation with probiotic VSL#3 among overweight adults also improved insulin sensitivity and decreased triglycerides, total-, LDL- and VLDL-cholesterol levels.[30] In addition, consumption of the synbiotic bread containing heat-resistant probiotic *Lactobacillus sporogenes* (1×10^8 CFU/g) and inulin decreased serum triglycerides and VLDL-cholesterol levels in patients with type-2 diabetes after 8 weeks.[11] However, the administration of *Lactobacillus rhamnosus* among mildly or moderately hypercholesterolemic men for 4 weeks[31] and supplementation with *Lactobacillus fermentum* among individuals with elevated serum cholesterol for a period of 10 weeks[32] did not affect serum lipid levels. Probiotics intake may improve markers of insulin metabolism, triglycerides and VLDL-cholesterol levels by reducing cytokines and suppressing the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway,[33] the impact on gene expression [16] and gut microbiota-short chain fatty acids (SCFA)-hormone axis.[34]

The current study revealed that probiotic capsule intake by pregnant women for 12 weeks could significantly decrease serum hs-CRP and significantly increase plasma NO, TAC and GSH levels compared with placebo, although it did not influence plasma MDA levels. However, after controlling for baseline age and BMI, the

Table 1. General characteristics of study participants.[1]

	Placebo group ($n = 30$)	Probiotic group ($n = 30$)
Age (y)	28.4 ± 5.3	27.1 ± 5.1
Height (cm)	163.3 ± 5.1	163.3 ± 5.9
Weight at study baseline (kg)	68.1 ± 12.4	68.4 ± 12.2
Weight at end-of-trial (kg)	72.7 ± 11.7	73.9 ± 11.6
Weight change (kg)	4.6 ± 1.6	5.5 ± 2.9
BMI at study baseline (kg/m^2)	25.5 ± 4.1	25.6 ± 4.2
BMI at end-of-trial (kg/m^2)	27.2 ± 3.8	27.7 ± 4.1
BMI change (kg/m^2)	1.7 ± 0.7	2.1 ± 1.1

[1] Data are means ± SDs.

Table 2. Metabolic profiles, biomarkers of inflammation and oxidative stress at study baseline and after 12-week intervention in pregnant women that received either probiotic supplements or placebo [1]

	Placebo group (n = 30)			Probiotic group (n = 30)			P[2]
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
FPG (mg/dL)	83.0 ± 6.7	82.8 ± 6.9	-0.2 ± 4.9	81.6 ± 7.9	80.3 ± 8.7	-1.2 ± 8.3	0.57
Insulin (µIU/mL)	12.8 ± 9.5	14.1 ± 9.3	1.3 ± 5.2	11.1 ± 5.3	9.6 ± 4.7	-1.5 ± 4.8	0.03
HOMA-IR	2.6 ± 2.0	2.9 ± 1.9	0.3 ± 1.1	2.3 ± 1.0	2.0 ± 1.0	-0.3 ± 0.9	0.04
HOMA-B	52.8 ± 41.6	58.1 ± 40.9	5.3 ± 22.6	46.1 ± 25.2	38.9 ± 21.0	-7.2 ± 23.1	0.03
QUICKI	0.35 ± 0.04	0.34 ± 0.03	-0.01 ± 0.02	0.34 ± 0.02	0.35 ± 0.05	0.01 ± 0.05	0.03
Triglycerides (mg/dL)	141.5 ± 84.6	178.8 ± 101.7	37.3 ± 74.2	156.1 ± 78.8	141.4 ± 64.3	-14.7 ± 46.5	0.002
VLDL-cholesterol (mg/dL)	28.3 ± 16.9	35.7 ± 20.3	7.4 ± 14.8	31.2 ± 15.7	28.3 ± 12.9	-2.9 ± 9.3	0.002
Total cholesterol (mg/dL)	182.0 ± 54.6	189.6 ± 44.6	7.6 ± 43.4	182.5 ± 32.3	174.4 ± 35.9	-8.1 ± 25.4	0.09
LDL-cholesterol (mg/dL)	95.5 ± 37.9	95.8 ± 29.6	0.3 ± 32.9	91.8 ± 26.1	87.9 ± 29.5	-3.9 ± 17.7	0.53
HDL-cholesterol (mg/dL)	58.2 ± 10.1	58.0 ± 9.4	-0.2 ± 10.7	59.4 ± 9.4	58.2 ± 8.7	-1.2 ± 6.7	0.64
hs-CRP (mg/L)	4.8 ± 5.1	6.5 ± 5.0	1.7 ± 4.3	7.9 ± 4.5	6.9 ± 4.3	-1.0 ± 2.6	0.004
NO (µmol/L)	50.0 ± 7.3	45.3 ± 4.9	-4.7 ± 7.4	46.0 ± 8.7	52.8 ± 10.8	6.8 ± 9.3	<0.001
TAC (mmol/L)	893.0 ± 210.8	841.1 ± 223.7	-51.9 ± 208.8	859.3 ± 114.2	1031.2 ± 150.2	171.9 ± 187.6	<0.001
GSH (µmol/L)	604.3 ± 155.6	567.4 ± 150.9	-36.9 ± 108.3	446.4 ± 44.4	480.7 ± 57.5	34.3 ± 71.6	0.004
MDA (µmol/L)	2.8 ± 1.1	2.7 ± 1.2	-0.1 ± 1.3	3.2 ± 0.8	2.6 ± 0.8	-0.6 ± 1.2	0.17

[1]All values are means± SDs. [2]Obtained from repeated measures ANOVA test. FPG = fasting plasma glucose; GSH = total glutathione; HOMA-IR = homeostasis model of assessment-estimated insulin resistance; HOMA-B = homeostasis model of assessment-estimated b cell function; hs-CRP = high-sensitivity C-reactive protein; MDA = malondialdehyde; NO = nitric oxide; QUICKI = quantitative insulin sensitivity check index; TAC = total antioxidant capacity.

Table 3. Adjusted changes in metabolic variables in pregnant women that received either probiotic or placebo.[1]

	Placebo group (n = 30)	Probiotic group (n = 30)	95% CI	Effect size	P[2]
FPG (mg/dL)	-0.03 ± 6.0	-1.4 ± 6.0	-1.74, 4.53	0.01	0.37
Insulin (µIU/mL)	1.5 ± 4.9	-1.8 ± 4.9	0.89, 5.83	0.12	0.008
HOMA-IR	0.3 ± 1.1	-0.3 ± 1.1	0.16, 1.16	0.11	0.01
HOMA-B	6.6 ± 20.8	-8.5 ± 20.8	4.21, 26.16	0.12	0.008
QUICKI	-0.008 ± 0.04	0.01 ± 0.04	-0.04, 0.000	0.06	0.04
Triglycerides (mg/dL)	36.8 ± 59.7	-14.2 ± 59.7	19.90, 82.06	0.16	0.002
VLDL-cholesterol (mg/dL)	7.4 ± 12.1	-2.8 ± 12.1	3.98, 16.41	0.16	0.002
Total cholesterol (mg/dL)	7.4 ± 30.8	-7.8 ± 30.8	-1.18, 31.48	0.05	0.06
LDL-cholesterol (mg/dL)	0.8 ± 23.0	-4.4 ± 23.0	-6.96, 17.26	0.01	0.39
HDL-cholesterol (mg/dL)	-0.5 ± 7.7	-0.9 ± 7.7	-3.68, 4.41	.001	0.85
hs-CRP (mg/L)	1.3 ± 3.3	-0.6 ± 3.3	0.11, 3.64	0.07	0.03
NO (µmol/L)	-3.6 ± 7.7	5.8 ± 7.7	-13.42, -5.34	0.28	<0.001
TAC (mmol/L)	-47.1 ± 176.5	167.1 ± 176.5	-306.09, -122.23	0.28	<0.001
GSH (µmol/L)	-12.0 ± 89.9	9.4 ± 89.9	-72.31, 29.53	0.01	0.40
MDA (µmol/L)	-0.3 ± 1.1	-0.4 ± 1.1	-0.40, 0.66	0.004	0.63

[1] All values are means± SDs. [2] Obtained from analysis of covariance adjusted for baseline values+ age and baseline BMI. FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated b cell function; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity.

changes in plasma GSH were not significantly different between the groups. Consistent with our study, Zarrati *et al*.[35] demonstrated that consumption of 200 g/day yogurt, enriched by *Lactobacillus acidophilus*, *Bifidobacterium langum*, and *Lactobacillus casei* 10^8 CFU/g by overweight and obese individuals for 8 weeks decreased inflammatory cytokines. The administration of soy milk fermented with *Lactobacillus plantarum* or *Streptococcus thermophilus* for 48 hours in human umbilical vein endothelial cells also resulted in increased production of NO.[36] In contrast, such beneficial effects of probiotic supplementation on biomarkers of inflammation and oxidative stress were not reported by others. For instance, a 8-week multispecies probiotics supplementation did not influence CRP in PCOS patients.[37] Furthermore, supplementation with *Lactobacillus GG* among children with active inflammatory bowel disease for 4 weeks did not increase intestinal NO concentrations.[38] In addition, our previous study among pregnant women indicated that synbiotic food consumption for 9 weeks led to a significant rise in plasma GSH levels.[39] Also, no significant intra- and inter-group differences were seen for MDA and TAC levels following the consumption of capsules containing 10^8 CFU/g of *Lactobacillus casei* in rheumatoid arthritis (RA) for 8 weeks.[40] Improved inflammatory factors by probiotics may be due to SCFA produced in the colon,[41] increased generation of NO [42] and decreased production of hydrogen peroxide radicals.[43] In addition, the beneficial effects of probiotics on plasma TAC levels might result from the production of butyrate in the gut,[44] and its impact on decreased lipid peroxidation such as oxidized LDL, 8-isoprostanes and glutathione redox ratio.[45] The difference in our findings compared to others might be explained by different study designs, different dosages of probiotics used, as well as different participants of the study.

This study had some limitations. In the current study, we did not measure fecal bacteria loads before and after probiotic supplementation. Another limitation was that we could not assess other inflammatory cytokines and biomarkers of oxidative stress.

Overall, probiotic supplementation for 12 weeks in pregnant women in the first half of pregnancy had beneficial effects on markers of insulin metabolism, triglycerides, biomarkers of inflammation and oxidative stress; however, it did not have any effect on other lipid profiles. This suggests probiotic supplementation may confer advantageous therapeutic potential for pregnant women. Further research is needed in other participants and for longer periods to determine the safety of probiotic supplementation. Moreover, further studies should measure the expressed levels of related variables with insulin, inflammation and oxidative stress to explore the plausible mechanism and confirm our findings. It must be kept in mind that we evaluated the effects of probiotic supplementation on metabolic status among healthy pregnant in the current study. Since the evaluation of probiotic supplementation in women with gestational diabetes mellitus (GDM) women is interesting, we recommend its performance in future studies.

Conflicts of interest

None declared.

Author contributions

ZA contributed to conception, design, statistical analysis and drafting of the manuscript. MJ, FB, ZV, AS, MT-E and PJ contributed in conception, data collection and manuscript drafting. All authors read and approved the final version of the paper.

Clinical registration

www.irct.ir as IRCT201503035623N38.

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