Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-018-9490-z

The Effects of Probiotic Supplementation on Genetic and Metabolic Profiles in Patients with Gestational Diabetes Mellitus: a Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

This study was carried out to evaluate the effects of probiotic supplementation on genetic and metabolic profiles in patients with gestational diabetes mellitus (GDM) who were not on oral hypoglycemic agents. This randomized, double-blind, placebo-controlled clinical trial was conducted in 48 patients with GDM. Participants were randomly divided into two groups to intake either probiotic capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Lactobacillus fermentum* (2×10^9 CFU/g each) ($n = 24$) or placebo ($n = 24$) for 6 weeks. Probiotic intake upregulated peroxisome proliferator-activated receptor gamma ($P = 0.01$), transforming growth factor beta ($P = 0.002$) and vascular endothelial growth factor ($P = 0.006$), and downregulated gene expression of tumor necrosis factor alpha ($P = 0.03$) in peripheral blood mononuclear cells of subjects with GDM. In addition, probiotic supplementation significantly decreased fasting plasma glucose (β, − 3.43 mg/dL; 95% CI, − 6.48, − 0.38; $P = 0.02$), serum insulin levels (β, − 2.29 μIU/mL; 95% CI, − 3.60, − 0.99; $P = 0.001$), and insulin resistance (β, − 0.67; 95% CI, − 1.05, − 0.29; $P = 0.001$) and significantly increased insulin sensitivity (β, 0.009; 95% CI, 0.004, 0.01; $P = 0.001$) compared with the placebo. Additionally, consuming probiotic significantly decreased triglycerides ($P = 0.02$), VLDL-cholesterol ($P = 0.02$), and total-/HDL-cholesterol ratio ($P = 0.006$) and significantly increased HDL-cholesterol levels ($P = 0.03$) compared with the placebo. Finally, probiotic administration led to a significant reduction in plasma malondialdehyde ($P < 0.001$), and a significant elevation in plasma nitric oxide ($P = 0.01$) and total antioxidant capacity ($P = 0.01$) was observed compared with the placebo. Overall, probiotic supplementation for 6 weeks to patients with GDM had beneficial effects on gene expression related to insulin and inflammation, glycemic control, few lipid profiles, inflammatory markers, and oxidative stress.

Keywords Probiotic supplementation · Gestational diabetes mellitus · Metabolic status · Insulin resistance · Inflammation

Introduction

Gestational diabetes mellitus is defined as impaired carbohydrate and lipid metabolism during pregnancy and is characterized by progressive hyperglycemia and insulin resistance and compensatory hyperinsulinemia [1]. In the USA, the prevalence of gestational diabetes mellitus (GDM) was reported up to 14% of pregnancies, accounting for 200,000 cases annually [2]. The prevalence of this condition in Iran was reported 4.7% [3]. Several studies have reported metabolic and genetic disorders in women with GDM [4]. In a study by Zhao et al. [5], it was seen the downregulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) in placenta that may predict hyperglycemia in offspring at young adulthood. In addition, GDM is correlated with inflammatory process and increased oxidative damage compared with normal pregnancy, which in turn are predictive of future type 2 diabetes mellitus [6] and cardiovascular disease (CVD) and other atherosclerotic events [7].

It was documented that probiotic administration to pregnant women had favorable effects on metabolic profiles and biomarkers of inflammation and oxidative stress. Few studies

This study was registered in the Iranian website (www.irct.ir) for registration of clinical trials (http://www.irct.ir: IRCT20171010036697N1).

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Published online: 08 December 2018

[8, 9] aimed to evaluate the impact of probiotic supplementation on metabolic profiles in patients with GDM and have demonstrated that probiotic administration had beneficial effects on glycemic status, rather than lipid metabolism. In addition, Taylor et al. [10] reviewed the effects of probiotics on metabolic outcomes in women with GDM. Consistently, it was seen that improved glucose metabolism with a considerable decrease in homeostasis model of assessment-insulin resistance (HOMA-IR) was observed following the supplementation of probiotic [10]. However, in a study conducted by Lindsay et al. [11], there were no differences in metabolic profiles or pregnancy outcomes in obese pregnant women that may be due to the limited number of relevant studies. Taken all together, the abovementioned studies have indicated that probiotic administration during pregnancy has beneficial effects on metabolic outcomes in pregnant women such as patients with GDM and even healthy pregnant women.

However, data from studies investigating the effect of probiotics on glycemic control, lipid profiles, biomarkers of inflammation, and oxidative stress in patients with GDM are inconclusive. The dose or CFU of a probiotic is an important factor for the efficacy of probiotic supplementation on metabolic profiles in pregnant women. It was documented that the dose of more than 10^7 CFU probiotic counts could show beneficial effects of probiotic supplementation on metabolic profiles in pregnant women [12]. However, studies about specific doses of probiotics are limited and further studies about optimal dose of a probiotic supplementation in women with GDM are required. In addition, probiotic strains are also variable among the studies, and it is difficult to evaluate the effects of a specific probiotic species on metabolic profiles. Most studies have widely used *Lactobacillus* and *Bifidobacterium*. Currently, studies about the effects of specific probiotic strains are also limited. There is no consensus on the specific dose of probiotics and the ideal probiotic strains for the clinical intervention. Therefore, further randomized controlled trial studies that fully evaluate and compare the efficacy among variable CFU doses and different probiotic strains are warranted, which are critically important to determine the optimal dose and ideal probiotic strain supplementation during pregnancy. In the current study, we hypothesized that probiotic supplementation might affect metabolic and genetic status of pregnant women with GDM. This study was aimed to evaluate the effects of probiotic supplementation on metabolic and genetic profiles in patients with GDM who were not on oral hypoglycemic agents.

Methods

Trial Design and Participants

The current study, registered in the Iranian website for clinical trials (http://www.irct.ir: IRCT20171010036697N1), was a randomized, double-blind, placebo-controlled clinical trial. This study was conducted in 48 patients with GDM at 24–28 weeks of gestation referred to the Naghavi Clinic in Kashan, Iran, between December 2017 and June 2018. The investigation was performed in accordance with the Declaration of Helsinki and informed consent was taken from all participants. This study was approved by the ethics committee of Kashan University of Medical Sciences (KAUMS). Eligible subjects were primigravida and aged 18–40 years (at weeks 24–28 of gestation) who were diagnosed with GDM by a "one-step" diagnosed based on the American Diabetes Association guidelines [13]. Subjects with clinical characteristics at enrollment including pre-eclampsia, eclampsia, thyroid disorders, smokers, those with kidney or liver diseases and required commencing insulin therapy during intervention, and taking probiotic products, including probiotic yogurt and kefir during the intervention, were our exclusion criteria.

Study Design

To decrease potential confounding effects, random stratification was used to assign participants to two test groups with matching for BMI and age**.** Each test group was randomly assigned to test the probiotic or placebo ($n = 24$ each group). Patients were requested not to change their routine physical activity or usual diets throughout the study and not to take any anti-inflammatory and antioxidant medications or supplements that might affect their nutritional status during the 6-week intervention. Consumption of probiotic supplements and placebos throughout the study was checked through asking subjects to return the medication containers. Furthermore, a short message was sent to the cell phones of all patients every day to remind participants to use the supplements. A 3-day food record (one weekend day and two weekdays) and physical activity records were completed by all participants. The individual's nutrient intake was then calculated and averaged at weeks 0, 3, and 6 of the intervention using Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods. Physical activity was described as metabolic equivalents (METs) in hours per day. To determine the METs for each participant, we multiplied the times (in hour per day) reported for each physical activity by its related MET coefficient by standard tables [14]. After diagnosis of GDM in patients attending the center, they were first instructed about the healthy diet; however, they were not given a specific menu and they were just participating in a nutritional education class that focused on basics of healthy diet.

Intervention

In the treatment group, patients received a probiotic capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* (2 ×

10^9 CFU/g each) for 6 weeks. Probiotic supplements and placebos (corn starch) were produced by LactoCare®, Zisttakhmir Company (Tehran, Iran), that is approved by the Food and Drug Administration.

Assessment of Anthropometric Measures

A trained staff at the clinic took anthropometric measurements at baseline and after the 12-week intervention. Body weight was quantified after an overnight fast using a digital scale (Seca, Hamburg, Germany).

Assessment of Outcomes

In the current study, we considered gene expression of PPAR-γ as the primary outcome and other metabolic and genetic profiles considered as secondary outcomes. Twenty-milliliter fasting blood samples were collected at baseline and 6 weeks after the intervention at Kashan reference laboratory. Then, the samples were stored at − 80 °C before analysis. Serum insulin concentrations were quantified by the use of an ELISA kit (DiaMetra, Milano, Italy) with inter- and intra-assay coefficient variances (CVs) below 5%. HOMA-IR and the quantitative insulin sensitivity check index (QUICKI) were determined according to the standard formula [15]. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to determine fasting plasma glucose (FPG) and lipid profiles with inter- and intra-assay CVs below 5%. Serum hs-CRP concentrations were determined by commercial ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs below 7%. The plasma nitric oxide (NO) was determined using Griess method [16], total antioxidant capacity (TAC) by the method of ferric reducing antioxidant power developed by Benzie and Strain [17], total glutathione (GSH) using the method of Beutler et al. [18], and malondialdehyde (MDA) concentrations were determined by the thiobarbituric acid reactive substance spectrophotometric test [19] with inter- and intra-assay CVs below 5%.

Isolation of Lymphocyte, RNA Extraction, and cDNA Synthesis

Lymphocytes were isolated using 50% Percoll solution (Sigma-Aldrich, Dorset, UK) gradient by centrifugation for 20 min and 3000 rpm at 4 °C [20]. Total RNA was extracted based on acid guanidinium-phenol-chloroform procedure using RNX™-plus reagent (Cinnacolon, Tehran, Iran) according to the manufacturer's instructions. RNA was treated with DNAse I (Fermentas, Lithuania) for the elimination of any genomic DNA contamination. Three micrograms of total RNA was used for cDNA synthesis with random hexamer and oligo (dT) 18 primers through RevertAid™ Reverse Transcriptase (Fermantas, Canada) in total 20 μL reaction mixture [20].

Real-Time PCR Analysis

Appropriate primers for PPAR-γ, low-density lipoprotein receptor (LDLR), interleukin-1 (IL-1), IL-8, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), and glyceraldehyde-3 phosphate dehydrogenase were designed (Table 1). Quantitative real-time PCR was performed by the LightCycler® 96 sequence detection systems (Roche Diagnostics, Rotkreuz, Switzerland) using 4 μL of 5× EvaGreen I Master Mix (Salise Biodyne, Japan), 10 ng cDNA, and 200 nM of each forward and reverse primers in a final volume of 20 μL.

Sample Size

In this study, we used a randomized clinical trial sample size calculation formula where type one (α) and type two errors (β) were 0.05 and 0.20 (power = 80%), respectively. According to the previous trial [21], we used 0.15 as the SD and 0.13 as the fold change in mean (d) of PPAR-γ as a primary outcome. Based on the formula, we needed 20 subjects in each group; after allowing for five dropouts in each group, the final sample size was 25 persons in each group (Table 2).

Randomization

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

Statistical Methods

The Kolmogorov-Smirnov test was done to determine the normality of data. To detect the differences in anthropometric parameters, dietary intakes and gene expression related to insulin, lipid, and inflammation between treatment groups, we used the independent-samples t test. Multiple linear regression models were used to assess treatment effects on study outcomes after adjusting for confounding variables, including age and BMI. The effect sizes were presented as the mean differences with 95% confidence intervals. P values < 0.05 were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL, USA).

Results

Among individuals in the probiotic group, one person due to hospitalization was excluded (Fig. 1). The exclusion in the

Table 1 Specific primers used for real-time quantitative PCR

Gene	Primer	Product size (bp)	Annealing temperature (C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG R: TCTTCCTCTTGTGCTCTTGCTGG	126	61.3
PPAR-γ	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGGCTCAG	210	54
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57
IL-1	F: GCTTCTCTCTGGTCCTTGG R: AGGGCAGGGTAGAGAAGAG	174	56
IL-8	F: GCAGAGGGTTGTGGAGAAGT R: ACCCTACAACAGACCCACAC	150	56
TNF-α	F: GTCAACCTCCTCTCTGCCAT R: CCAAAGTAGACCTGCCCAGA	188	52
TGF-β	F: TTGAGACTTTTCCGTTGCCG R: CGAGGTCTGGGGAAAAGTCT	227	56
VEGF	F: CTTCTGAGTTGCCCAGGAGA R: CTCACACACACACAACCAGG	216	54

GAPDH glyceraldehyde-3-phosphate dehydrogenase, *IL-1* interleukin-1, *IL-8* interleukin-8, *LDLR* low-density lipoprotein receptor, *PPAR-γ* peroxisome proliferator-activated receptor gamma, *TNF-α* tumor necrosis factor alpha, *TGF-β* transforming growth factor beta, *VEGF* vascular endothelial growth factor

placebo group was also one participant due to placenta abruption. Finally, fourth-eight participants (24 in each group) completed the trial. The compliance rate in our study was high; participants reported that more than 90% of probiotic and placebo capsules were consumed during the course of the trial. No side effects were reported following the intake of probiotic in patients with GDM throughout the study.

Mean age, height, weight, and BMI at baseline as well as mean weight and BMI after intervention were not statistically different between the two groups (Table 2).

Based on the 3-day dietary records obtained throughout the trial, we found no significant change in dietary macro- and micro-nutrient intakes (data not shown).

After the 6-week intervention, probiotic supplementation significantly decreased FPG (*β*, −3.43 mg/dL; 95% CI, −6.48, −0.38; $P = 0.02$), serum insulin levels (*β*, −2.29 μIU/mL; 95% CI, −3.60, −0.99; $P = 0.001$), and HOMA-IR (*β* −0.67; 95% CI, −1.05, −0.29; $P = 0.001$) and significantly increased QUICKI (*β*, 0.009; 95% CI, 0.004, 0.01; $P = 0.001$) compared with the placebo (Table 3). Additionally, consuming probiotic significantly decreased triglycerides (*β*, −19.17 mg/dL; 95% CI, −35.86, −2.48; $P = 0.02$), VLDL-cholesterol (*β*, −3.83 mg/dL; 95% CI, −7.17, −0.49; $P = 0.02$), and total-/HDL-cholesterol ratio (*β*, −0.51; 95% CI, −0.86, −0.15; $P = 0.006$) and significantly increased HDL-cholesterol levels (*β*, 3.61; 95% CI, 0.31, 6.91; $P = 0.03$) compared with the placebo. Finally, probiotic administration led to a significant reduction in plasma MDA (*β*, −0.63 μmol/L; 95% CI, −0.80, −0.47; $P < 0.001$) and a significant elevation in plasma NO (*β*, 2.00 μmol/L; 95% CI, 0.34, 3.65; $P = 0.01$) and TAC (*β*, 49.99 mmol/L; 95% CI, 9.20, 90.79; $P = 0.01$) was observed compared with the placebo. Probiotic intake did not change other metabolic parameters.

Table 2 General characteristics of study participants

	Placebo group ($n = 24$)	Probiotic group ($n = 24$)	P[a]
Age (year)	29.0 ± 4.2	28.8 ± 4.3	0.81
Height (cm)	161.2 ± 3.7	161.5 ± 3.1	0.76
Weight at study baseline (kg)	68.9 ± 7.3	68.0 ± 5.0	0.63
Weight at end-of-trial (kg)	70.8 ± 7.3	70.1 ± 5.2	0.67
Weight change (kg)	1.9 ± 0.5	2.1 ± 0.5	0.41
BMI at study baseline (kg/m^2)	26.5 ± 2.7	26.1 ± 2.2	0.57
BMI at end-of-trial (kg/m^2)	27.3 ± 2.7	26.9 ± 2.3	0.62
BMI change (kg/m^2)	0.7 ± 0.2	0.8 ± 0.3	0.47

Data are means ± SDs

[a] Obtained from independent *t* test

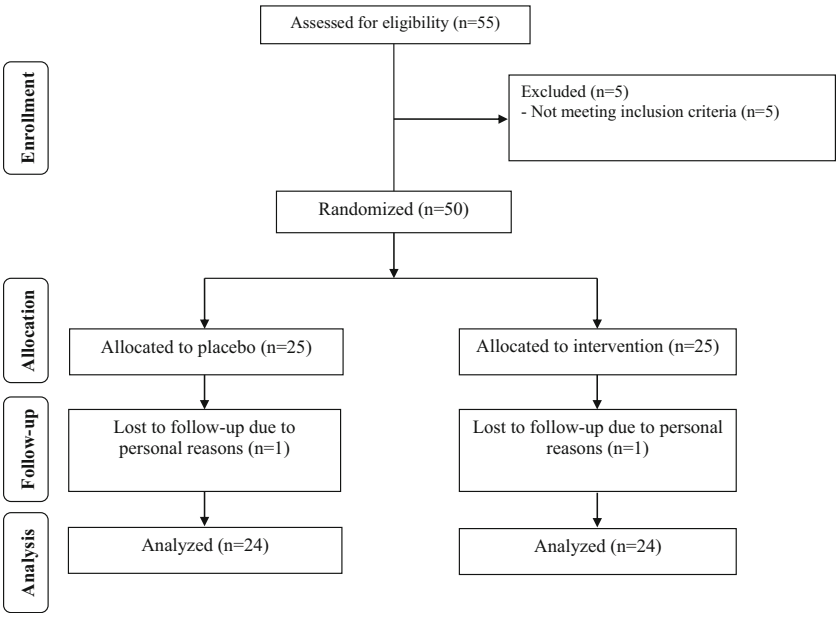

Fig. 1 Summary of patient flow diagram

Probiotic intake upregulated PPAR-γ ($P = 0.01$), TGF-β ($P = 0.002$), and VEGF ($P = 0.006$) and downregulated gene expression of TNF-α ($P = 0.03$) in peripheral blood mononuclear cells of subjects with GDM (Figs. 2 and 3). Probiotic supplementation did not affect gene expression of LDLR, IL-1, and IL-8.

Table 3 Metabolic profiles, biomarkers of inflammation, and oxidative stress at baseline and after the 6-week intervention in patients with gestational diabetes mellitus that received either probiotic supplements or placebo

Variables	Placebo group ($n = 24$)		Probiotic group ($n = 24$)		Difference in outcome measures between probiotic and placebo treatment groups[a]	
	Baseline	Week 6	Baseline	Week 6	β (95% CI)	P^b
FPG (mg/dL)	90.3 ± 6.9	91.3 ± 8.7	92.2 ± 11.2	89.2 ± 8.9	− 3.43 (− 6.48, − 0.38)	0.02
Insulin (μIU/mL)	11.8 ± 2.3	12.7 ± 3.8	12.0 ± 2.3	10.5 ± 2.3	− 2.29 (− 3.60, − 0.99)	0.001
HOMA-IR	2.6 ± 0.5	2.9 ± 1.1	2.7 ± 0.6	2.3 ± 0.5	− 0.67 (− 1.05, − 0.29)	0.001
QUICKI	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.33 ± 0.009	0.009 (0.004, 0.01)	0.001
Triglycerides (mg/dL)	191.1 ± 66.6	208.2 ± 69.4	199.3 ± 51.8	196.5 ± 43.5	−19.17 (− 35.86, − 2.48)	0.02
VLDL-cholesterol (mg/dL)	38.2 ± 13.3	41.6 ± 13.9	39.9 ± 10.4	39.3 ± 8.7	− 3.83 (− 7.17, − 0.49)	0.02
Total cholesterol (mg/dL)	210.0 ± 40.0	214.0 ± 43.1	204.2 ± 41.9	201.7 ± 38.8	− 6.87 (− 16.05, 2.32)	0.13
LDL-cholesterol (mg/dL)	117.6 ± 30.1	120.7 ± 36.1	112.1 ± 38.1	108.2 ± 35.5	− 7.23 (− 17.06, 2.60)	0.14
HDL-cholesterol (mg/dL)	54.2 ± 6.0	51.7 ± 5.7	52.3 ± 11.3	54.2 ± 9.3	3.61 (0.31, 6.91)	0.03
Total-/HDL-cholesterol ratio	3.9 ± 0.8	4.2 ± 1.0	4.0 ± 1.0	3.8 ± 0.8	− 0.51 (− 0.86, 0.15)	0.006
NO (μmol/L)	30.7 ± 3.2	30.1 ± 3.0	30.9 ± 4.0	32.1 ± 4.7	2.00 (0.34, 3.65)	0.01
TAC (mmol/L)	716.4 ± 128.2	712.4 ± 113.1	710.6 ± 92.7	758.3 ± 93.7	49.99 (9.20, 90.79)	0.01
GSH (μmol/L)	584.9 ± 96.1	568.3 ± 98.3	627.1 ± 128.5	609.4 ± 98.4	35.71 (− 23.74, 95.16)	0.23
MDA (μmol/L)	3.0 ± 0.8	3.2 ± 0.7	2.8 ± 0.2	2.4 ± 0.2	− 0.63 (− 0.80, − 0.47)	< 0.001

Data are mean ± SDs

FPG fasting plasma glucose, *GSH* total glutathione, *HOMA-IR* homeostasis model of assessment-insulin resistance, *HDL-cholesterol* high-density lipoprotein-cholesterol, *LDL-cholesterol* low-density lipoprotein-cholesterol, *MDA* malondialdehyde, *NO* nitric oxide, *QUICKI* quantitative insulin sensitivity check index, *VLDL-cholesterol* very low-density lipoprotein-cholesterol, *TAC* total antioxidant capacity

[a] "Outcome measures" refers to the change in values of measures of interest between baseline and week 6. β [difference in the mean outcomes measures between treatment groups (probiotic group = 1 and placebo group = 0)]

[b] Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age, and baseline BMI)

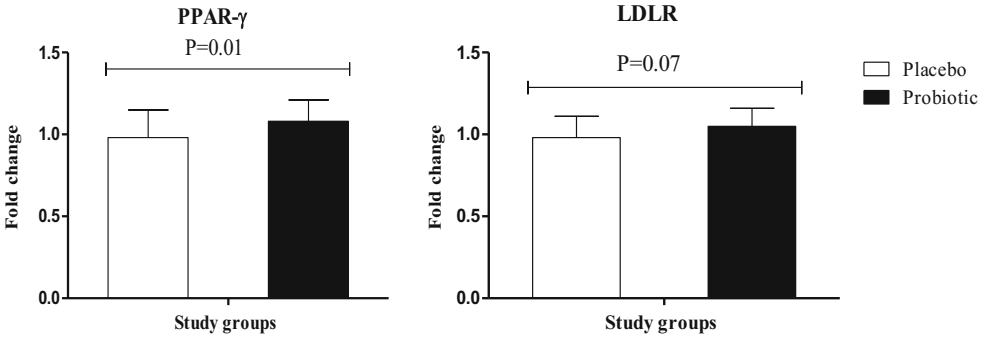

Fig. 2 Fold change (means ± SDs) in gene expression levels of PPAR-γ and LDLR in women with GDM who were received probiotic supplements and placebo. *P* value was obtained from independent *t* test. *N* = 24 in each group.

Discussion

In the current study, we investigated the effects of probiotic supplementation on genetic and metabolic profiles in subjects with GDM. We found that taking probiotic for 6 weeks by patients with GDM had beneficial effects on gene expression related to insulin and inflammation, glycemic control, few lipid profiles, inflammatory markers, and oxidative stress.

Effects on Glycemic Control and Lipid Profiles

We found that taking probiotic supplements for 6 weeks by patients with GDM led to a significant increase in gene expression of PPAR-γ, QUICKI, and HDL-cholesterol levels and a significant reduction in FPG, insulin, HOMA-IR, triglycerides, VLDL-cholesterol, total-/HDL-cholesterol but did not affect gene expression of LDLR and other lipid profiles. However, data on probiotic effects on gene expression related to insulin and lipid metabolism are scarce; several studies have documented the beneficial effects of probiotic on glycemic control and lipid profiles. We have previously shown that taking probiotic supplements by patients with Parkinson's disease for 12 weeks significantly increased gene expression of PPAR-γ but did not affect gene expression of LDLR [22]. In another study, *Enterococcus faecium* upregulated the mRNA expression of PPAR-γ in the spleen 3 and 7 days post-infection in *Escherichia coli* O78-challenged broiler chickens [23]. Moreover, *Lactobacillus reuteri* I5007 could improve the gut health of neonatal piglets through the increase in colonic butyric acid concentration and the upregulation of butyric acid, PPAR-γ [24]. Also, probiotic *L. casei* significantly reduced pro-inflammatory cytokines and hepatic inflammation through modulating the toll-like receptor-mitogen-activated protein kinase-PPAR-γ signaling pathways and intestinal microbiota [25]. PPAR-γ plays key functions in the regulation of metabolism, including regulating insulin sensitivity, mitochondrial biogenesis, carbohydrate and lipid homoeostasis, and trophoblast differentiation [26, 27]. Kuzmicki et al. [28] demonstrated lower gene expression levels of PPAR-γ in women with GDM rather than those with normal glucose tolerance. In another study, polymorphisms in PPAR-γ were highly correlated with GDM occurrence in pregnant women [29]. Therefore, probiotics due to their beneficial actions on PPAR-γ may be useful to control metabolic profiles in women with GDM. In accordance with our study, results of a meta-analysis conducted in women with GDM demonstrated that probiotics

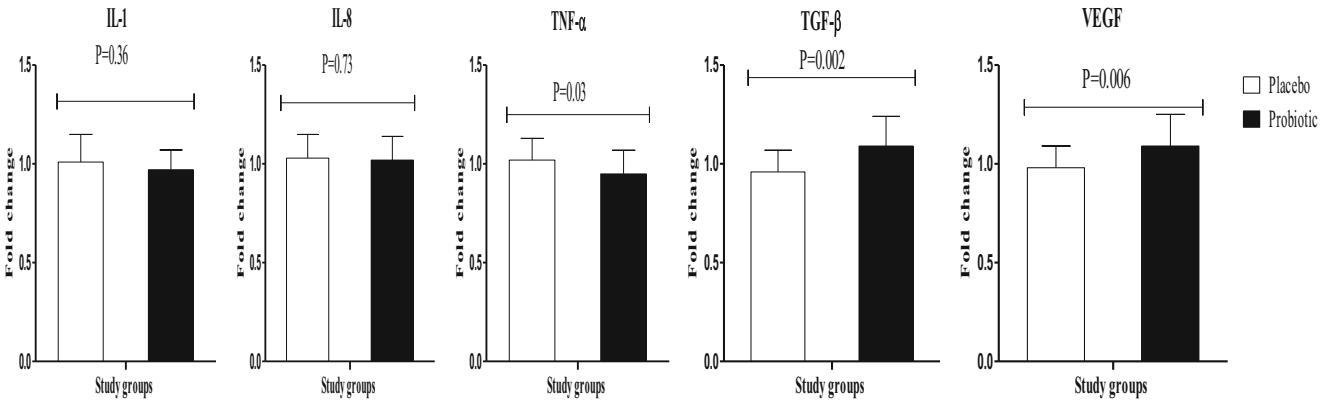

Fig. 3 Change (means ± SDs) in gene expression levels of IL-1, IL-8, TNF-α, TGF-β, and VEGF in women with GDM who received probiotic supplements and placebo. *P* value was obtained from independent *t* test. *N* = 24 in each group. GDM, gestational diabetes mellitus; IL-1, interleukin-1; IL-8, interleukin-8; LDLR, oxidized low-density lipoprotein; PBMC, peripheral blood mononuclear cells; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor

improved glycemic control, triglycerides, and VLDL-cholesterol levels [30]. In addition, in another meta-analysis conducted by Taylor et al. [10], it was seen that probiotic supplementation in women with GDM was associated with a considerable decrease in HOMA-IR. Dolatkhah et al. [8] observed that taking probiotic supplements containing four strains *L. acidophilus* LA-5, *B.* bifidum BB-12, *Streptococcus thermophilus* STY-31, and *Lactobacillus delbrueckii bulgaricus* LBY-27 by pregnant women for 6 weeks significantly decreased insulin resistance. These results reinforce the findings of Hyronimus et al. [31] who recommend probiotic administration for at least 3 months. Insulin resistance in women with GDM results in inflammation, resulting in increased inflammatory markers, such as C-reactive protein and fibrinogen [32]. On the other hand, the influence of gut microbiome diversity improvement during pregnancy has been evaluated. Pregnancies, type of delivery, gestation period (term/preterm), and use of antiobiotics during pregnancy are all correlated with the human microbial community composition [33]. Furthermore, the use of probiotics might help to regulate the microbiota to promote beneficial metabolic activity, produce favorable metabolites, and regulate the diversity of gut microbiota [34]. The beneficial effects of probiotics on glycemic control and lipid profiles may be due to the increased production of SCFA that increases GLP-1 secretion [35], modulating the expression of lipogenic and glucogenic gene, including PPAR-γ, glucose transporter type 4, and glucose-6-phoshatase [36], and decreasing toll-like receptor activity, which in turn reduces muscle insulin resistance [37].

Effects on Biomarkers of Inflammation and Oxidative Stress

The present data showed that the consumption of probiotic by patients with GDM significantly reduced gene expression of TNF-α and significantly increased gene expression of TGF-β and VEGF. In addition, probiotic supplementation to patients with GDM caused a significant increase in NO and TAC, and a significant reduction of MDA levels, but did not affect gene expression of IL-1 and IL-8 and GSH levels. We have previously shown that taking probiotic containing *L. acidophilus*, *L. casei*, and *B. bifidum* (2×10^9 CFU/g each) by women with GDM for 6 weeks had beneficial effects on serum hs-CRP, plasma TAC, and MDA levels. In another study, supplementation with *Lactobacillus paracasei* and *L. reuteri* in an animal model decreased gene expressions of hepatic IL-1β, IL-6, and TNF-α through inhibiting the mitogen-activated protein kinase and nuclear factor κB signaling pathways [38]. In addition, the administration of *L. casei* and *Enterococcus faecalis* to an animal model significantly reduced gene expression of TNF-α and significantly increased gene expression of TGF-β in the jejunum [39]. Probiotic administration for 12 weeks to patients with multiple sclerosis significantly decreased gene expression of IL-8 and TNF-α but did not influence gene expression of IL-1 [21]. Furthermore, newborns receiving *L. reuteri* DSM 17938 for 1 month had a significant decrease in fecal pro-inflammatory cytokines, IL-17, IL-8, and TNF-α and a significant increase in the fecal anti-inflammatory cytokine IL-10 [40]. Also, *Lactobacillus rhamnosus* GG significantly attenuated pathogen-induced TNF-α mRNA expression in the human fetal gut [41]. However, in a meta-analysis, probiotic supplementation to patients with rheumatoid arthritis did not affect inflammatory parameters (TNF-α, IL-1β, IL-6, IL-10, and IL-12) and oxidative stress indices (TAC and MDA) [42]. Increased inflammatory markers can increase the incidence of maternal CVD in later life [43]. In addition, oxidative stress and related toxic products can damage biological molecules, which in turn enhance the susceptibility of offspring to chronic disease [44, 45]. Therefore, probiotics due to their anti-inflammatory and antioxidative actions may be useful to reduce complications related to metabolic disturbances in women with GDM. The upregulation of gene expression of interleukin-18 by SCFA [46] and increased production of methylketone family in the gut following the supplementation of probiotic [47] might explain its anti-inflammatory and antioxidant effects. Unfortunately, we did not evaluate SCFA levels in the current study as a mechanism for the observed effects but this might be explored in future studies.

This study had few limitations. In the current study, we did not measure fecal bacteria loads before and after probiotic supplementation. Due to funding limitations, we could not assess gene expression related to oxidative stress.

Conclusions

Overall, probiotic supplementation for 6 weeks to patients with GDM had beneficial effects on gene expression related to insulin and inflammation, glycemic control, few lipid profiles, inflammatory markers, and oxidative stress. Our findings suggest that probiotic supplementation may be relevant valuable therapeutic agent for patients with GDM. Further research is recommended to confirm the beneficial effects of probiotic supplementation in other populations.

Acknowledgements The authors would like to thank the staff of Naghavi Clinic (Kashan, Iran) for their assistance in this project. We are grateful to thank LactoCare®, Zisttakhmir Company, in Tehran that provided probiotic capsules for the present study.

Author Contributions ZA: conception, design, and statistical analysis, drafting of the manuscript, and supervised the study.

MB, AK, EA, FB, AM, RS, and MS: data collection and manuscript drafting.

Funding This study was supported by the research grant provided by the Research Deputy of Kashan University of Medical Sciences (KAUMS).

Compliance with Ethical Standards

Competing Interests The authors declare no conflict of interest.

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Clinical trial registration number

http://www.irct.ir: IRCT20171010036697N1