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Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Objective: We are aware of no study examining the effects of probiotic supplementation on symptoms of depression, metabolic profiles, serum high-sensitivity C-reactive protein (hs-CRP), and biomarkers of oxidative stress in patients with major depressive disorder (MDD). The present study was designed to determine the effects of probiotic intake on symptoms of depression and metabolic status in patients with MDD.

Methods: This randomized, double-blind, placebo-controlled clinical trial included 40 patients with a diagnosis of MDD based on DSM-IV criteria whose age ranged between 20 and 55 y. Patients were randomly allocated into two groups to receive either probiotic supplements (n = 20) or placebo (n = 20) for 8 wk. Probiotic capsule consisted of three viable and freeze-dried strains: *Lactobacillus acidophilus* (2×10^9 CFU/g), *Lactobacillus casei* (2×10^9 CFU/g), and *Bifidobacterium bifidum* (2×10^9 CFU/g). Fasting blood samples were taken at the beginning and end of the trial to quantify the relevant variables. All participants provided three dietary records (two weekdays and one weekend) and three physical activity records during the intervention.

Results: Dietary intake of study participants was not significantly different between the two groups. After 8 wk of intervention, patients who received probiotic supplements had significantly decreased Beck Depression Inventory total scores (-5.7 ± 6.4 vs. -1.5 ± 4.8, $P = 0.001$) compared with the placebo. In addition, significant decreases in serum insulin levels (-2.3 ± 4.1 vs. 2.6 ± 9.3 μIU/mL, $P = 0.03$), homeostasis model assessment of insulin resistance (-0.6 ± 1.2 vs. 0.6 ± 2.1, $P = 0.03$), and serum hs-CRP concentrations (-1138.7 ± 2274.9 vs. 188.4 ± 1455.5 ng/mL, $P = 0.03$) were observed after the probiotic supplementation compared with the placebo. Additionally, taking probiotics resulted in a significant rise in plasma total glutathione levels (1.8 ± 83.1 vs. -106.8 ± 190.7 μmol/L, $P = 0.02$) compared with the placebo. We did not find any significant change in fasting plasma glucose, homeostatic model assessment of beta cell function, quantitative insulin sensitivity check index, lipid profiles, and total antioxidant capacity levels.

Conclusions: Probiotic administration in patients with MDD for 8 wk had beneficial effects on Beck Depression Inventory, insulin, homeostasis model assessment of insulin resistance, hs-CRP concentrations, and glutathione concentrations, but did not influence fasting plasma glucose, homeostatic model assessment of beta cell function, quantitative insulin sensitivity check index, lipid profiles, and total antioxidant capacity levels.

Introduction

Major depressive disorder (MDD) is a complex and multifactorial disorder that involves marked disabilities in global functioning, anorexia, and severe medical comorbidities [1]. It affects around 20% of the population at some point during their lifetime [2]. Previous studies have shown a link between metabolic profiles, biomarkers of inflammation, oxidative stress, and MDD [1,3,4]. Depression or depressive episodes may affect cortisol dysregulation, which might in turn result in the development of insulin resistance in patients with depression [5]. In addition, recent studies have reported that decreased antioxidant levels, especially of glutathione (GSH), are associated with increased anhedonia severity, which subsequently might lead to involvement of neuroinflammation and oxidative stress in patients with MDD [6].

Probiotics are proposed to have a range of health benefits. Their beneficial impacts on a wide range of symptoms have been examined, including relief of irritable-bowel syndrome and inflammatory bowel disease, as well as the amelioration of lactose intolerance, and the prevention of bowel cancer [7,8]. Moreover, emerging research has reported that the microflora of the intestines may affect the immune system and functioning beyond the gut [9]. Probiotics might have favorable effects on mood and psychological problems [10]. In a study by Mohammadi et al. [11], consumption of probiotic yogurt or a multispecies probiotic capsule for 6 wk had beneficial effects on mental health parameters in petrochemical workers. Other studies have also reported the favorable effects of probiotic administration in healthy subjects [12] and patients with chronic fatigue syndrome [13]. In a study by Benton et al. [14], consumption of probiotic yogurt improved the mood of those whose mood was initially poor. In addition, improved metabolic status, biomarkers of inflammation, and oxidative stress were observed after a 2-mo supplementation with probiotics in pregnant women and patients with type 2 diabetes mellitus [15,16]. However, probiotic supplementation containing *Lactobacillus rhamnosus* strain GG and *Bifidobacterium* had no beneficial effects in people with schizophrenia after 14 wk [17].

Probiotics may result in reduced depressive symptoms, as well as improved metabolic status, biomarkers of inflammation, and oxidative stress, through their effect on neuronal circuits and the central nervous system mediated by the microbiota-gut-brain axis [18] and through affecting gene expression [19]. In addition, experimental studies in the animal model of depression have demonstrated that the oral administration of a probiotic can increase plasma tryptophan concentrations, decrease serotonin metabolite concentrations in the frontal cortex, and dopamine metabolite concentrations in the amygdaloid cortex [20]. However, whether probiotics have direct benefits on depressive symptoms and metabolic status in patients with MDD has to date not been assessed. The present study was therefore conducted to assess the favorable effects of probiotic supplementation on symptoms of depression, parameters of glucose homeostasis, lipid concentrations, biomarkers of inflammation, and oxidative stress in patients with MDD.

Materials and methods

Participants

Forty patients with MDD whose age ranged between 20 and 55 y were recruited for this randomized, double-blind, placebo-controlled trial from July 2014 to September 2014. To determine the sample size, we applied a randomized clinical trial sample size formula considering type I (α) and type II errors (β) of 0.05 and 0.20 (power = 80%), respectively. On the basis of a previous study [11], we used a standard deviation (SD) of 18.5 and a difference in mean (d) of 18, considering depression anxiety and stress scale as the key variable. This calculation indicated a total of 17 patients for each group. However, we recruited 40 patients with MDD in total (20 patients for each group) to compensate for the probable loss to follow-up. Patients with a diagnosis of MDD based on DSM-IV criteria and with a score of $\geq$15 on the 17-item Hamilton Depression Rating Scale were included in the study, and they were referred from Kargarneghad Hospital, Kashan University of Medical Sciences (KUMS), Kashan, Iran. Exclusion criteria were age <20 y or >55 y; a history of coronary infarction, angina pectoris, pregnancy or lactation, or substance abuse; and taking dietary supplements or probiotic supplements during the previous 2 mo. All procedures were followed according to the ethics standards of the responsible committee on human experimentation (institutional and national) and to the Declaration of Helsinki. In addition, the ethics committee of KUMS approved the study. All patients provided written informed consent. This study was registered in the Iranian website (www.irct.ir) for registration of clinical trials (IRCT Code: IRCT2014060717993 N1).

Study design

In the present study, patients were randomly allocated into two groups to receive either probiotic supplements (17 women and 3 men: n = 20) or placebo (17 women and 3 men: n = 20) for 8 wk. Patients in the probiotic group received daily one probiotic capsule containing *Lactobacillus acidophilus* (2×10^9 CFU/g), *Lactobacillus casei* (2×10^9 CFU/g), and *Bifidobacterium bifidum* (2×10^9 CFU/g). It would be more appropriate if the strains used in probiotic supplements for human consumption were derived from the human intestinal tract, well characterized, able to outlive the rigors of the digestive tract and possibly colonize, biologically active against the target, and stable and amenable to commercial production and distribution [21]. Because of the lack of evidence about the appropriate dosage of probiotics for patients with MMD, we used the above-mentioned doses based on a few previous studies in healthy subjects [11,14]. Subjects in the placebo group received the placebo that contained starch but no bacteria. The appearance of the placebo was indistinguishable in color, shape, size, packaging, smell, and taste from that of the probiotic supplement. All capsules were provided by Tak Gen Zist Pharmaceutical Company (Tehran, Iran) and were approved by the Food and Drug Administration of Iran. Random assignment was performed by the use of computer-generated random numbers. Randomization and allocation were concealed from the researchers and participants until the main analyses were completed. The randomized allocation sequencing, enrolling patients, and allocating participants to interventions were done by a trained nutritionist at a psychiatry clinic. At the beginning of the study, patients were requested not to change their routine physical activity or usual dietary intakes throughout the study; not to consume any supplements other than the one provided to them by the investigators; and not to take, during the 8-wk intervention, any medications that might affect findings. Compliance to probiotic and placebo capsules was monitored by asking participants to return the medication containers. All participants provided three dietary records (two weekdays and one weekend) and three physical activity records to ensure that they maintained their usual diet and physical activity during the intervention. Both dietary and physical activity records were taken at weeks 2, 4, and 6 of the intervention. To obtain nutrient intakes of participants based on the three-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods.

Anthropometric assessment

Body weight and height were determined in an overnight fasting state, with minimal clothing and without shoes, by the use of a digital scale (Seca, Hamburg, Germany) by a trained nutritionist at a psychiatry clinic at the beginning of the study and at the end point. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared.

Outcomes

In this study, the primary outcome was Beck Depression Inventory (BDI) score. Depressed mood was judged with BDI at the beginning and end of the study. BDI is a self-compiled questionnaire of 21 items in multiple-choice format [22]. On each item there are four statements, and the subjects were instructed to choose the one that best described their situation during the last 2 wk. The declarations are given the scores 0, 1, 2, and 3, with 0 for the normal or least depressive statement and 3 for the most depressive statement. We calculated the total BDI score by adding together the scores of each item. Secondary outcomes were fasting plasma glucose (FPG), markers of insulin metabolism, lipid concentrations, serum high-sensitivity C-reactive protein (hs-CRP), and biomarkers of oxidative stress, including total antioxidant capacity (TAC) and GSH levels. Fasting blood samples (10 mL) were obtained at the baseline and study endpoint at the Kashan reference laboratory in an early morning after an overnight 12-h fast. Blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate serum. Then, the samples were stored at $-80°C$ until analysis at the KUMS reference laboratory. To determine the concentrations of FPG, triacylglycerol, total cholesterol, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and high density lipoprotein cholesterol, we used commercial kits (Pars Azmun, Tehran, Iran). All inter- and intraassay coefficient of variations (CVs) for FPG and lipid profile measurements were less than 5%. To determine serum insulin and hs-CRP concentrations, we used ELISA kits (Monobind, Lake Forest, CA, USA, and LDN, Nordhorn, Germany, respectively). The homeostasis model of assessment of insulin resistance (HOMA-IR), homeostasis model of assessment of β-cell function (HOMA-B), and quantitative insulin sensitivity check index (QUICKI) were determined based on suggested formulas [23]. Plasma TAC was quantified by using the FRAP method modified by Benzie and Strain [24] and GSH by the method modified by Beutler et al. [25].

Statistical analysis

To determine the normal distribution of variables, we used the Kolmogorov-Smirnov test. The analyses were conducted based on the intention-to-treat approach. Missing values were handled based on the last-observation-carried-forward method. To detect differences in general characteristics and dietary intakes between the two groups, we used the independent samples Student's *t* test. To determine the effects of probiotic administration on markers of insulin metabolism, lipid concentrations, serum hs-CRP, and biomarkers of oxidative stress, we used one-way repeated measures analysis of variance. Within-group comparisons (endpoint vs. baseline) were done based on the paired-samples *t* test. To control for several confounders, we applied analysis of covariance in which the confounding effects of these variables were taken into account. *P* value <0.05 was considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science, version 17 (SPSS Inc., Chicago, IL, USA).

Results

In the probiotic group, three patients withdrew because of personal reasons (n = 3). In the placebo group, two patients withdrew because of personal reasons (n = 2). Finally, 35 persons (probiotic group, n = 17; placebo group, n = 18) completed the trial (Fig. 1). However, as the analysis was done based on the intention-to-treat approach, all 40 patients with MDD were included in the final analysis. In total, the rate of compliance in the present study was high, as more than 90% of capsules were taken throughout the study in both groups.

Baseline and end-of-trial means of weight and BMI were not significantly different between the probiotic and placebo groups (Table 1).

No significant difference was observed between the two groups in terms of dietary intakes of energy, carbohydrates, protein, fat, saturated fatty acid, polyunsaturated fatty acid,
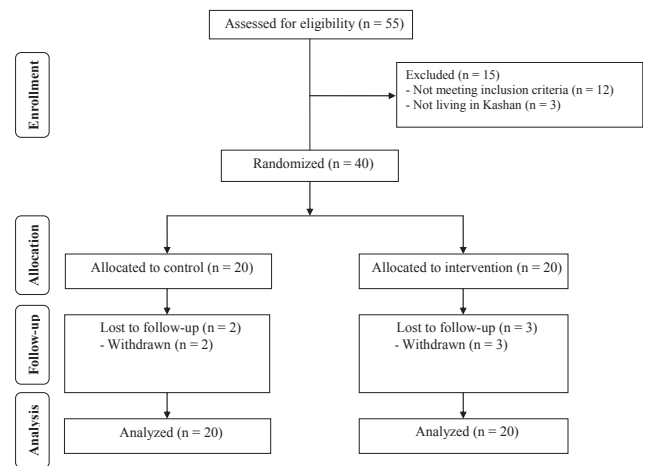

Fig. 1. Summary of patient flow diagram.

monounsaturated fatty acid, cholesterol, dietary fiber, magnesium, manganese, and zinc, whose details were obtained from the three-day dietary records throughout the intervention (Table 2).

After 8 wk of intervention, patients who received probiotic supplements had significantly decreased BDI total score ($-5.7 ± 6.4$ vs. $-1.5 ± 4.8$, P = 0.001) compared with the placebo (Fig. 2). In addition, significant decreases in serum insulin levels ($-2.3 ± 4.1$ vs. $2.6 ± 9.3$ μIU/mL, P = 0.03), HOMA-IR ($-0.6 ± 1.2$ vs. $0.6 ± 2.1$, P = 0.03), and hs-CRP concentrations ($-1138.7 ± 2274.9$ vs. $188.4 ± 1455.5$ ng/mL, P = 0.03) were observed after the supplementation with probiotics compared with the placebo. Additionally, taking probiotics resulted in a significant rise in plasma GSH levels ($1.8 ± 83.1$ vs. $-106.8 ± 190.7$ μmol/L, P = 0.02) compared with the placebo.

A trend toward a significant decrease in HOMA-B ($-7.1 ± 13.7$ vs. $9.8 ± 37.4$, P = 0.06) and an increase in QUICKI score ($0.009 ± 0.01$ vs. $-0.003 ± 0.02$, P = 0.07) was observed after probiotic supplementation (Table 3). We did not find any significant change in FPG, HOMA-B, QUICKI, lipid profiles, and TAC levels after supplementation.

Baseline levels of FPG were significantly different between the two groups. Therefore, we controlled the analyses for the baseline levels, age, and baseline BMI. However, after this adjustment no significant changes in our findings occurred, except for BDI score (P = 0.05) and serum insulin levels (P = 0.05) (Table 4).

Table 1
General characteristics of the study participants

Characteristics	Placebo group (n = 20)	Probiotic group (n = 20)	$P*$
Age (y)	36.2 ± 8.2	38.3 ± 12.1	0.52
Height (cm)	160.9 ± 4.9	163.3 ± 9.5	0.31
Weight at study baseline (kg)	68.0 ± 11.5	72.6 ± 11.3	0.21
Weight at end of trial (kg)	68.7 ± 10.5	72.5 ± 11.1	0.28
Weight change (kg)	0.7 ± 2.7	$-0.1 ± 1.6$	0.26
BMI at study baseline (kg/m²)	26.3 ± 4.1	27.6 ± 6.0	0.42
BMI at end of trial (kg/m²)	26.5 ± 3.9	27.5 ± 5.9	0.53
BMI change (kg/m²)	0.2 ± 1.0	$-0.1 ± 0.6$	0.23

BMI, body mass index
Data are means ± SDs
* Obtained from independent *t* test.

Table 2
Dietary intakes of study participants throughout the study

Intake	Placebo group (n = 20)	Probiotic group (n = 20)	P*
Energy (kcal/d)	2222 ± 97	2268 ± 112	0.17
Carbohydrates (g/d)	305.9 ± 46.2	318.2 ± 38.4	0.36
Protein (g/d)	85.2 ± 25.9	80.9 ± 9.4	0.48
Fat (g/d)	75.9 ± 17.5	78.1 ± 14.3	0.66
SFA (g/d)	22.2 ± 7.1	23.6 ± 6.9	0.52
PUFA (g/d)	25.6 ± 4.5	26.1 ± 5.9	0.77
MUFA (g/d)	20.3 ± 7.1	19.8 ± 5.0	0.79
Cholesterol (mg/d)	240.2 ± 185.5	202.2 ± 119.5	0.44
TDF (g/d)	16.9 ± 3.5	16.8 ± 4.4	0.95
Magnesium (mg/d)	249.1 ± 38.6	256.3 ± 40.9	0.58
Manganese (mg/d)	1.9 ± 0.8	2.0 ± 0.7	0.70
Zinc (mg/d)	8.9 ± 2.9	9.4 ± 2.8	0.51

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber
Data are means ± SDs
 * Obtained from independent *t* test.

Discussion

In the present study, we examined the beneficial effects of probiotic administration on BDI score, markers of insulin metabolism, lipid profiles, hs-CRP, and biomarkers of oxidative stress in patients with MDD. The main findings were that probiotic supplementation improved the BDI score and insulin function, and decreased oxidative stress in patients with MDD. To the best of our knowledge, this study is the first that reports the effect of probiotic administration on symptoms of depression, metabolic status, biomarkers of inflammation, and oxidative stress in patients with MDD.

Patients with MDD are predisposed to some complications, including morbidity, mortality [26], increased risk of cardiovascular disease (CVD), dyslipidemia, and impaired insulin function [27]. Our study found that, in patients with MDD, taking probiotic supplements for 8 wk resulted in a significant decrease in BDI score compared with the placebo group. However, few studies have assessed the effects of probiotic supplementation on symptoms of depression. In a study by Rao et al. [13], a significant decrease in anxiety symptoms was observed in those taking the probiotics compared with the control subjects;

however, the authors failed to find any significant effect on BDI score. In addition, supplementation with probiotic sachet containing two strains of *Lactobacillus helveticus* and *Bifidobacterium longum* (3×10^{12} CFU/1.5 g sachet), for 30 d in healthy persons resulted in a significant improvement in mental health [12]. However, no significant improvement was observed after the supplementation of *L. rhamnosus* and *Bifidobacterium animalis* for 14 wk in schizophrenia patients [17]. The accurate mechanism of probiotics in the brain and their effects on depression are not completely understood. The administration of probiotics might result in improved symptoms of depression through increased plasma tryptophan levels, decreased serotonin metabolite concentrations in the frontal cortex, and decreased dopamine metabolite concentrations in the amygdaloid cortex [20]. Various factors, including host physiology, immunology, diet, antibiotic use, and enteric infection, can affect the gut microbiota composition and its activity. Probiotic bacteria through fermenting dietary ingredients might lead to specific changes in the composition and/or activity of the gastrointestinal microbiota, which might result in improved peripheral (gastrointestinal) and central (psychological) symptoms [28]. Probiotics may influence both the enteric nervous system and the central nervous system in addition to their effects on the mucosal immune system by modifying the gastrointestinal tract microbiome [28]. In addition, a few studies have found that probiotics might improve carbohydrate malabsorption [29], which is associated with both the early signs of depression [30] and reduced tryptophan levels [31].

We found that probiotic supplementation for 8 wk in patients with MDD led to significant decreases in serum insulin concentrations and HOMA-IR compared with the placebo group, but it did not affect FPG, HOMA-B, QUICKI, and lipid profiles. Firouzi et al. [32] conducted a review on this subject and found that 16 out of 17 studies in animals and 3 out of 4 studies in humans had reported significant improvements in at least one glucose homeostasis-related parameter [32], which is in agreement with our study. In addition, in a study by Ejtahed et al. [33], consumption of probiotic yogurt containing *L. acidophilus* and *Bifidobacterium lactis* for 6 wk did not affect lipid profiles in patients with type 2 diabetes mellitus. Some investigators did not observe any beneficial effects of probiotic supplementation on markers of insulin metabolism. For instance, supplementation with the
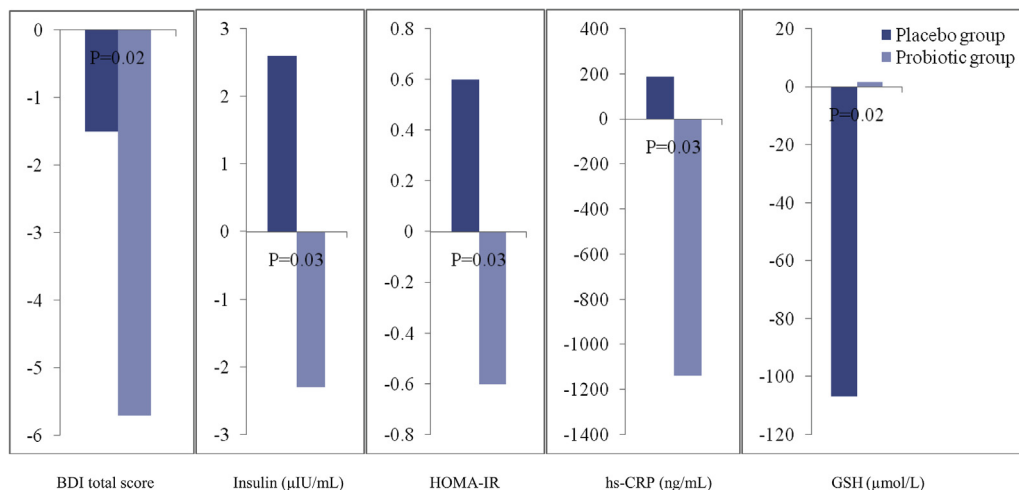
Fig. 2. Changes in means ± standard deviations of BDI score and metabolic status after 8 wk of intervention. BDI, Beck Depression Inventory; GSH, total glutathione; HOMA-IR, homeostasis model of assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

Table 3
Metabolic status at baseline and after the intervention

Variables	Placebo group (n = 20)				Probiotic group (n = 20)				$P^{\dagger}$
	Baseline	End of trial	Change	$P*$	Baseline	End of trial	Change	$P*$	
FPG (mg/dL)	89.4 ± 7.8	89.3 ± 7.6	−0.1 ± 7.0	0.94	102.3 ± 17.7	99.7 ± 17.4	−2.6 ± 9.2	0.22	0.34
HOMA-B	37.6 ± 15.2	47.4 ± 41.6	9.8 ± 37.4	0.25	29.8 ± 19.0	22.7 ± 11.9	−7.1 ± 13.7	0.03	0.06
QUICKI	0.34 ± 0.02	0.34 ± 0.03	−0.003 ± 0.02	0.57	0.33 ± 0.02	0.34 ± 0.01	0.009 ± 0.01	0.03	0.07
Triacylglycerols (mg/dL)	105.0 ± 42.8	111.3 ± 40.0	6.3 ± 27.1	0.31	126.1 ± 69.3	134.7 ± 68.3	8.6 ± 29.7	0.21	0.80
VLDL cholesterol (mg/dL)	21.0 ± 8.6	22.2 ± 8.0	1.2 ± 5.4	0.31	25.2 ± 13.9	26.9 ± 13.7	1.7 ± 5.9	0.21	0.80
Total cholesterol (mg/dL)	184.1 ± 30.3	179.6 ± 31.0	−4.5 ± 20.9	0.34	174.0 ± 34.4	172.5 ± 33.9	−1.5 ± 21.5	0.75	0.65
LDL cholesterol (mg/dL)	110.9 ± 26.8	105.3 ± 27.9	−5.6 ± 17.4	0.16	100.9 ± 30.4	93.1 ± 30.1	−7.8 ± 22.4	0.13	0.74
HDL cholesterol (mg/dL)	52.2 ± 12.4	52.1 ± 9.4	−0.1 ± 7.6	0.95	47.9 ± 11.6	52.4 ± 11.3	4.5 ± 10.1	0.05	0.10
TAC (mmol/L)	865.9 ± 159.0	851.6 ± 155.6	−14.3 ± 137.2	0.64	894.9 ± 135.3	877.5 ± 87.1	−17.4 ± 109.9	0.48	0.93

FPG, fasting plasma glucose; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment of beta cell function; LDL, low-density lipoprotein; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity; VLDL, very low-density lipoprotein

Data are means ± SDs

* Obtained from paired-samples t tests.
$\dagger$ Obtained from repeated measures ANOVA test.

probiotic strain of *L. casei* Shirota for 12 wk did not improve insulin sensitivity and β-cell function in subjects with metabolic syndrome [34]. The mechanism by which probiotic intake might improve markers of insulin metabolism may be attributed to an increase in hepatic natural killer T-cell number and a reduction in inflammatory signaling [35]. Moreover, conjugated linoleic acid is produced by some species of *Lactobacillus*, including *acidophillus*, *plantarum*, *paracasei*, and *casei*, and it might up regulate adiponectin, down regulate inflammation, and block suppression of glucose transporter type 4 [36]. The different findings might be explained by different study designs, different dosages of probiotics used, and different participants of the study.

Findings from the present study revealed that taking supplemental probiotics resulted in decreased serum hs-CRP levels in patients with MDD. Supporting our study, Zarrati et al. [37] reported that consumption of probiotic yogurt containing *L. acidophilus*, *B. animalis*, and *L. casei* for 8 wk resulted in a significant decrease in hs-CRP levels in overweight and obese individuals. In addition, a significant decrease in hs-CRP levels was observed

after the administration of probiotic yogurt in pregnant women for 9 wk [38] and patients with established rheumatoid arthritis [39]. However, an 8-wk multispecies probiotic supplementation did not influence CRP levels in patients with polycystic ovary syndrome [40]. hs-CRP, as a marker of systemic inflammation, is an important independent predictor of risk of future myocardial infarction, stroke, and peripheral arterial disease [41]. The anti-inflammatory effects of probiotics might be explained by the production of short-chain fatty acids in the colon [42] and by the decreased expression of interleukin-6 [43].

The present study found that patients who received probiotic supplements had significantly increased plasma GSH levels compared with the placebo group, but did not find any effect on TAC levels. Our findings were in accordance with those reported by other researchers, showing increased GSH levels in patients with type 2 diabetes mellitus after probiotic intake for 8 wk [15, 44]. Furthermore, a significant increase in GSH concentrations was observed after intake of *Lactobacillus plantarum* for 14 d in rats. However, our previous study in pregnant women revealed that consumption of probiotic yogurt containing two strains of *L. acidophilus* and *B. animalis* for 9 wk did not influence plasma GSH levels compared with the conventional yogurt [16]. The accurate mechanisms by which intake of probiotic supplements might affect biomarkers of oxidative stress are unknown. The beneficial effects of probiotics on GSH might be explained by the enhanced glutamate-cysteine-ligase activity, increased mRNA expression of glutamate-cysteine-ligase subunits, and increased synthesis of GSH [45].

Some limitations of the present study need to be considered. We were not able to analyze the effect of probiotic supplementation on other biomarkers of inflammation and oxidative stress. Another limitation of the study was the duration of intervention. We were unable to administer probiotic supplements for more than 8 wk. Long-term interventions would be required to confirm the beneficial effects on lipid profiles. In addition, we do not know which strain in the probiotic supplements caused the treatment effect observed in our study. Therefore, further studies are needed that employ each of the strains used in the present study individually to evaluate their beneficial effects on symptoms of depression and metabolic status in patients with MDD. In the present study, only one depression variable was used to estimate sample size because the largest sample size was obtained when we used this variable. Therefore, the sample size obtained based on this variable was covering the required sample size for all other variables. The study power was 80%. Despite this, we agree that large-scale trials would be needed to confirm our findings.

Table 4
Adjusted changes in metabolic variables in patients with MDD who received either probiotic supplements or placebo

Variables	Placebo group (n = 20)	Probiotic group (n = 20)	$P*$
BDI total score	−1.8 ± 1.2	−5.3 ± 1.2	0.05
FPG (mg/dL)	−1.5 ± 1.7	−1.2 ± 1.7	0.92
Insulin (μIU/mL)	2.5 ± 1.6	−2.2 ± 1.6	0.05
HOMA-IR	5.4 ± 0.4	−0.6 ± 0.4	0.04
HOMA-B	9.9 ± 6.5	−7.2 ± 6.6	0.08
QUICKI	−0.001 ± 0.005	0.007 ± 0.005	0.17
Triacylglycerols (mg/dL)	5.8 ± 5.9	9.1 ± 5.9	0.69
VLDL cholesterol (mg/dL)	1.2 ± 1.2	1.8 ± 1.2	0.69
Total cholesterol (mg/dL)	−2.8 ± 4.6	−3.2 ± 4.6	0.96
LDL cholesterol (mg/dL)	−4.5 ± 4.4	−8.9 ± 4.4	0.49
HDL cholesterol (mg/dL)	1.0 ± 1.7	3.4 ± 1.7	0.32
hs-CRP (ng/mL)	188.5 ± 378.2	−1138.9 ± 378.2	0.01
TAC (mmol/L)	−21.1 ± 22.9	−10.6 ± 22.9	0.75
GSH (μmol/L)	−101.6 ± 33.2	−3.4 ± 33.2	0.04

BDI, Beck Depression Inventory; FPG, fasting plasma glucose; GSH, total glutathione; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment of beta cell function; HOMA-IR, homeostasis model of assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MDD, major depression disorder; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity; VLDL, very low-density lipoprotein cholesterol

All values are means ± SEs. Values are adjusted for baseline values, age, and baseline body mass index

* Obtained from analysis of covariance test.

Taken together, probiotic administration in patients with MDD for 8 wk had beneficial effects on BDI, insulin, HOMA-IR, hs-CRP, and GSH levels, but did not influence FPG, HOMA-B, QUICKI, lipid profiles, and TAC levels.

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