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ORIGINAL CONTRIBUTION

Lactobacillus GG is associated with mucin genes expressions in type 2 diabetes mellitus: a randomized, placebo-controlled trial

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Abstract

Purpose Recent studies indicate that dysbiosis of gut microbiota and low-grade inflammation are important pathogenic determinants of type two diabetes mellitus (T2DM). The aim of this study is to investigate the effects of Lactobacillus GG on glycemic control, lipid profile, inflammatory parameters, and some gene expression levels in individuals with T2DM.

Methods In a randomized, placebo-controlled trial, 34 women, aged 30–60 years with T2DM consumed daily probiotics or placebo for 8 weeks. The probiotic group consumed 10×10^9 Cfu/day *Lactobacillus rhamnosus* GG ATCC 53,103 (LGG), approved by the TR Ministry of Food, Agriculture, and Livestock. Anthropometric measurements, food diary, fasting blood, and fecal samples were taken at baseline and post-treatment.

Results Fasting blood glucose was significantly decreased in probiotic ($p = 0.049$) and placebo ($p = 0.028$), but there was no difference between the groups. In the probiotic group, no significant difference was observed in HbA1c, fructosamine, lipid profile, and inflammatory variables compared to baseline. In this group, with LGG supplementation, mucin 2 and 3A (MUC2 and MUC3A) gene expressions increased more than ninefolds ($p = 0.046$ and $p = 0.008$, respectively) at post-treatment. Meanwhile, there was no significant change in any of the gene expressions in the placebo group. There was no significant difference in energy, protein, dietary fiber, and cholesterol intakes between placebo and probiotic groups during the study. However, daily fat intake ($p = 0.003$), body weight ($p = 0.014$), and body fat ($p = 0.015$) in the probiotic group were significantly decreased.

Conclusion In this study, the effects of a single probiotic strain were investigated for 8 weeks. At the end of the study, although there was no finding that clearly reflected on the glycemic parameters of T2DM, its beneficial effects on the expression of mucin genes, which are responsible for weight loss and protection of intestinal barrier functions, cannot be denied. Further studies are needed to reveal the importance of these findings.

Clinical trial registration ID: NCT05066152, October 4, 2021 retrospectively registered in ClinicalTrials.gov PRS web site.

Keywords Type 2 diabetes mellitus · *Lactobacillus GG* · Probiotic · Mucin · Gene expression

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Introduction

Diabetes is a chronic, metabolic disease characterized by elevated blood glucose levels which may result from either insufficient insulin secretion, resistance to insulin action or both. International Diabetes Federation estimated that about 537 million people (aged 20–79 years) around the world suffered from diabetes in 2021 [1]. The prevalence of this condition among the Turkish adult population was 13.7% in 2010 [2].

Larsen N. et al. reported that, in 2010, T2DM is associated with compositional changes in the intestinal microbiota (IM) mostly apparent at phylum [3]. The human gut microbiome is comprised of four main bacterial phyla, Firmicutes, Proteobacteria, Actinobacteria (Gram-positive bacteria), and Bacteroidetes (Gram-negative bacteria) [4]. Overgrowth of Gram-negative bacteria increases the amount of enterotoxin-like bacterial lipopolysaccharides (LPS) in the circulation, causing chronic endotoxemia, increased intestinal permeability, and consequently subclinical inflammation leading to insulin resistance [5]. Toll-like receptors (TLRs; especially TLR4) are another important mechanism in the formation of insulin resistance [6]. TLRs are capable to affect both the innate immune system, providing the first-line host defense and also stimulate adaptive immunity, once they induce the secretion of inflammatory cytokines. Bacterial LPS produces inflammation in adipocytes through the activation of TLR-4 signaling [5, 6]. TLR2 functions as a ligand of bacterial products such as peptidoglycan, lipoteichoic acid, and lipoprotein [7]. Ensuring intestinal epithelial integrity and increasing its permeability, regulating immunity, and reducing TLR signals are accepted as indicators of the hypoglycemic effects of probiotics [8].

Another component that affects intestinal health is the gut mucous layer. The composition of gut microbiota is an important factor contributing to the regulation of intestinal mucous barrier function. Mucin glycoproteins (mucins) are one of the main elements of the epithelial mucous barrier [9]. The major mucin released from goblet cells which are mostly scattered between the intestinal epithelia is MUC2 and its gene is located on chromosome 11p15. Once it is secreted into the lumen of the large intestine, it polymerizes in a gel where it makes up 80% of the weight of oligosaccharide side chains added as post-translational modifications to mucin proteins, with small amounts of related mucin proteins. This gel provides an insoluble mucosal barrier that serves to protect the intestinal epithelium [10, 11]. MUC3A, a main member of the mucin family, is commonly expressed on the surface of intestinal and other epithelial cells. MUC3A, one of the human intestinal mucin genes is a membrane-bound mucin processed into a mucin cluster by 17 amino acid tandem repeats located on chromosome 7q22 [11, 12].

Probiotics are live organisms when administered in adequate amounts, confer a health benefit to the host [13]. *Lactobacillus rhamnosus GG* (or *Lactobacillus GG*: LGG) is a widely used and safe probiotic microorganism [14]. Studies have shown that LGG prevents diarrhea and atopic dermatitis, provides antitumor activity, improves immune regulation, and lowers serum cholesterol levels [15]. There are limited data about the effects of LGG on the glycemic control in diabetic animal models; however, human studies are even more scarce. It has been shown that a diet supplemented with LGG, resulted in a significant improvement of glucose tolerance and decreased glycosylated hemoglobin (HbA1c) in neonatal streptozotocin-induced diabetic rat model [16]. Another study in the KK-Ay mice model indicated that oral administration of LGG decreased fasting blood glucose (FBG) and HbA1c, as well as reduced the glycemic excursion at 30 min of the oral glucose tolerance test (OGTT) [17].

While establishing our hypothesis, we thought that there might be interactions between the genes involved in the regulatory pathways of the immune system and glucose metabolism [18]. Accordingly, we hypothesized that probiotics could affect the gut microbiota through the altered expression of certain genes (TLR2, TLR4, MUC2, and MUC3A) to improve glycemic control, lipid profile, and inflammatory responses in T2DM [19, 20]. There are numerous studies investigating the effects of probiotic use on insulin sensitivity, glycemic control, lipid profile, and inflammatory parameters in T2DM [21–24]. However, the researchers mostly studied the effects of different probiotic strains or the cocktails of probiotics and prebiotics. There is no study in the current literature evaluating the effect of using a single probiotic (in this case LGG) in conjunction with the interaction of genes on metabolic control in T2DM. Therefore, we aimed to investigate the effects of LGG on glycemic control, lipid profile, inflammatory parameters, along with the possible interactions of certain gene expressions in patients with T2DM.

Materials and methods

Participants

This placebo-controlled randomized clinical trial was carried out in a tertiary care diabetes outpatient clinic from February 2016 to July 2017. The study included 38 women with T2DM, aged 30–60 years. All volunteers were on oral anti-diabetic medications. The sample size and power analysis required for the research were calculated by G power V.3.1.9.7 program. We used an appropriate formula to

estimate the required sample size where the type 1 (α) and type 2 errors (β) were considered, respectively, 0.05 and 0.20 (power = 80%). In addition, the HOMA-IR score was defined as the key variable. We considered 2.0 as the meaningful difference in mean HOMA-IR scores between the two groups. Therefore, the required sample size was estimated to be 19 subjects in each grup [25–27].

Current smokers and heavy alcohol drinkers; those having an inflammatory bowel or an autoimmune disease or a severe immunodeficiency state; those who use anti-epileptics, dietary supplements, incretin enhancers (dipeptidyl peptidase 4, DPP-4 inhibitors, or glucagon-like peptide 1 receptor agonists, GLP-1RAs), or insulins; those who used systemic antibiotics within 6 weeks, or probiotic supplements within 3 months before inclusion; and women presently breast feeding or pregnant were excluded from the study.

The study was conducted in accordance with the rules of the Declaration of Helsinki in the Biomedical Research which revised in 2013, and also followed the European Good Clinical Practice Guidelines. It was approved by the Local Ethics Committee of the Istanbul Medipol University (Number: 358) and was registered on the ClinicalTrials.gov PRS website (ID: NCT05066152). A written informed consent was obtained from all subjects participated in the study.

Study design and characteristics of supplements

Subjects were randomized into two groups to receive either probiotic supplements (liquid form) or a placebo for 8 weeks. One probiotic drop contained a formulation of 1×10^9 Cfu *Lactobacillus rhamnosus GG* (LGG; ATCC 53,103), corn oil, and an emulsifier. Patients in the intervention group received 10 probiotic drops (1×10^{10}Cfu LGG) once daily at breakfast. Patients in the placebo group received 10 drops of corn oil having the same fatty acid profile with the probiotic drop. The volunteers were followed-up by telephone visits once a week to check their probiotic or placebo use.

Fasting blood and stool samples were taken at baseline and post-treatment to measure glycemic parameters, lipid profile, biomarkers of inflammation, and investigate gene expression. Blood samples were analyzed for FBG, fructosamine (FRMN), insulin, and lipid profile (total and high-density lipoprotein cholesterol, HDL-C; triglycerides), high sensitivity C-reactive protein (hs-CRP) by enzymatic colorimetric method (Roche Cobas 8000 (c702) Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) level was estimated by calculating from Friedewald formula in patients without hypertriglyceridemia [28]. HbA1c was measured with ion exchange high-performance liquid chromatography (Bio-rad Variant ll Turbo, Japan). IL-6 was measured by ELISA kit (DIAsource IL-6 EASIA Kit). The homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were used as indicators of insulin resistance and insulin sensitivity, and calculated using the following equations:

$$\text{HOMA - IR} = \text{Fasting plasma glucose (mg/dL)} \times \text{Fasting plasma insulin (}\mu\text{U/mL)}/405$$

$$\text{QUICKI} = 1/[\log(\text{fasting plasma insulin (}\mu\text{U/mL)} + \log(\text{fasting blood glucose (mg/dL)}]$$

[27, 29].

At baseline and post-treatment of the study, participants collected their stool samples in sterile containers at the hospital. Stool samples were stored immediately after defecation at − 80 °C until RNA isolation. Stool samples were thawed on ice and transferred to bead tubes for homogenization and 600 µL of lysis buffer was added for every 100 µg sample. Tubes were homogenized for 1 min at 6500 rpm. The tubes removed from the homogenizer were kept in cold block for 3–5 min. A 600 µL homogenate was transferred to new Eppendorf tubes. Then 600 µL of 70% ethanol was added and vortexed. Of this mixture, 700 µL was taken and transferred to the spin columns and spun at 13,000 rpm for 15 s. Collection tubes were replaced. The remaining homogenate in the previous step was transferred to the filter tube. It was spun at 13,000 rpm for 15 s. The collection tubes were changed and 700 µL Wash Buffer-I was added. It was centrifuged at 13,000 rpm for 15 s. The collection tubes were changed and 500 µL Wash Buffer-II was added. It was spun at 13,000 rpm for 15 s. The same process was repeated once more. Collection tubes were discarded. The filters were placed in Eppendorf tubes and the RNA was dissolved by adding 30 µL of RNase Free Water. After waiting for 1–2 min, RNA was precipitated by centrifuging at 13,000 rpm for 2 min. The concentration of total RNA samples obtained was measured by NanoDrop2000 spectrophotometer. Samples with 260 nm/230 nm ratio in the range of 2.0 ± 0.2 were included in the study. cDNA synthesis was performed. cDNA control PCR and melting curve analysis were used to determine the suitability of cDNA samples. The expression levels of TLR2, TLR4, MUC2, and MUC3A genes were determined by a quantitative rt-PCR method. β-Actin was used as the housekeeping gene, and all experiments were repeated three times for the reliability of the study. Roche LightCycler 480II Real-Time PCR device was used for determination of gene expression levels and BioRad T100 conventional PCR device for cDNA synthesis. A concentration of 0.5 µL was considered adequate as measured by the NanoDrop 2000 spectrophotometer. The relative gene expression (fold change) was measured with the comparative CT method using β-actin as the housekeeping gene and the $2^{-\Delta\Delta ct}$ formula.

All anthropometric measurements were performed by a dietitian in a fasting state taken at baseline and

post-treatment. Body weight and body composition were assessed by a bioelectrical impedance analysis device (Tanita BC-420 MA). Body mass index (BMI) was calculated as body weight (kg) divided by squared of the height (m^2). Waist circumference was measured from the midway between lowest rib and iliac crest, and hip circumference should be measured around the widest portion of the buttocks, with the non-stretchable tape parallel to the floor. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference to the hip circumference [30].

Dietary intake was evaluated by a 3-day food record at 4th and 8th weeks. First, patients were trained with detailed oral session and also a written instruction was given regarding the completion of food record, consisting of 2 weekdays and 1 weekend day. To determine the amounts of consumed foods correctly, information was given about measuring cups such as water glass, tea glass, teaspoon, tablespoon, serving spoon, bowl, etc. Dietary intake was assessed using a food composition database of BeBiS version 8.2; the program was adjusted for specific Turkish foods [31]. Volunteers were reminded for not to do any non-routine exercise at each telephone visit.

Statistical analysis

All analysis were performed using the Statistical Package for Social Sciences version 21.0 (SPSS-21) program. Descriptive statistics were given as mean, standard deviation (SD), and median (minimum to maximum) for continuous measures. Categorical variables were expressed as case numbers and percentage values. The Shapiro–Wilk tests were used to determine whether the distribution of continuous measures was normal. Student's t test and Mann–Whitney U test were used for the two groups' comparisons according to whether the variables showed normal distribution. Comparisons of changes in groups within themselves (baseline and post-treatment) were made using the t test if the variances in the cohort were normal, and the Wilcoxon test if not normal. The web-based RT^2 Profiler Polymerase Chain Reaction (PCR) Array Data Analysis program was used to determine the change of ΔCt values obtained from the reverse transcriptase PCR (rt-PCR) gene expression study (baseline and post-treatment). $p < 0.05$ was considered statistically significant.

Results

Among individuals in the placebo group, two patients (due to antibiotic treatment, $n = 1$; dietary supplement, $n = 1$) and in the probiotic group, two patients (unwilling to continue, $n = 2$) were excluded. Finally, 34 participants (placebo group, $n = 17$; probiotic group, $n = 17$) completed the trial (Fig. 1). There were no significant differences between the probiotic and placebo groups in terms of mean age, body weight, height, BMI, waist circumference, hip circumference, WHR, and diabetes duration at baseline (Table 1).

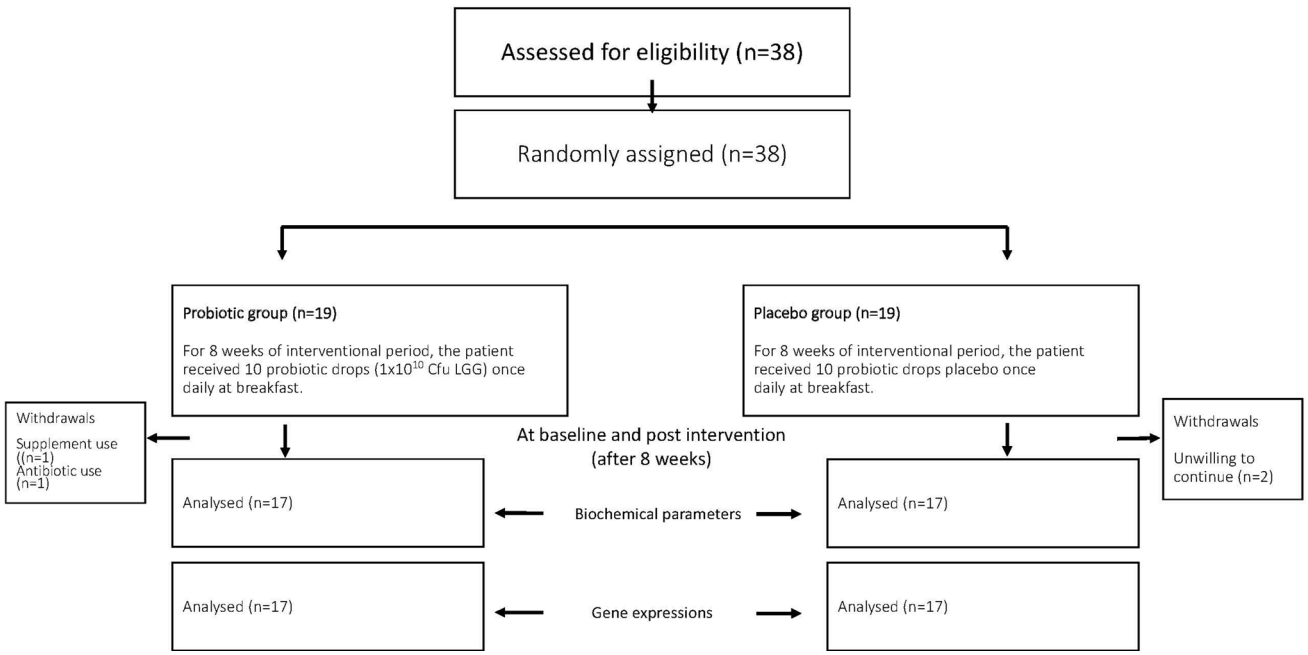

Fig. 1 Summary of patient flow

Table 1 Comparison of clinical parameters of probiotic and placebo groups at the baseline and post-treatment

	Probiotic ($n=17$)			Placebo ($n=17$)			p value between groups	
	Baseline	Post-treatment	p value (within group)	Baseline	Post-treatment	p value (within group)	Baseline	Post-treatment
Age (year)	45.0 (30.0–54.0)	–	–	48.0 (33.0–60.0)	–	–	0.10	–
Diabetes duration (year)	5.0 (1.0–15.0)	–	–	6.0 (1.0–10.0)	–	–	0.70	–
Weight (kg)	88.7 (62.6–110.5)	85.1 (59.7–108.1)	0.01*	74.5 (60.8–113.1)	73.5 (63.3–112.1)	0.12	0.15	0.17
BMI (kg/m^2)	33.5 (25.1–48.2)	32.5 (23.9–48.5)	0.01*	31.1 (24.7–44.0)	31.4 (25.0–44.3)	0.14	0.47	0.48
WHR	0.89 (0.71–1.00)	0.89 (0.71–1.00)	0.09	0.92 (0.78–1.10)	0.93 (0.83–1.11)	0.73	0.11	0.08
Body fat (%)	41.3 (24.2–49.4)	39.9 (23.6–48.8)	0.01*	41.2 (34.3–51.4)	39.9 (22.5–50.9)	0.55	0.63	0.63
Body fat mass (kg)	36.8 (18.1–54.6)	35.3 (15.0–52.8)	0.001*	31.3 (21.1–58.1)	28.9 (14.3–57.2)	0.11	0.61	0.51
Lean body mass (kg)	52.1 (43.8–56.9)	51.4 (44.7–57.5)	0.11	45.5 (39.3–56.7)	45.7 (33.6–57.0)	0.87	0.02*	0.02*
Muscle mass (kg)	49.5 (41.6–54.0)	48.8 (42.4–54.6)	0.17	43.2 (37.3–53.8)	44.5 (38.5–53.0)	0.95	0.02*	0.02*
Total body water (kg)	37.5 (30.4–41.2)	36.9 (31.0–41.7)	0.14	31.9 (27.0–41.0)	32.1 (27.9–42.0)	0.75	0.02*	0.04*
Total body water (%)	42.7 (37.1–93.2)	42.3 (37.5–53.9)	0.43	41.9 (35.7–46.0)	42.5 (36.1–55.4)	0.56	0.78	0.81
Bone mass (kg)	2.6 (2.2–2.9)	2.6 (2.3–2.9)	0.56	2.3 (2.0–2.9)	2.3 (2.1–2.9)	0.48	0.02*	0.04*
Basal metabolic rate (kcal)	1604.0 (1305.0–1777.0)	1582.0 (1329.0–1775.0)	0.06	1376.0 (1198.0–1755.0)	1388.0 (1226.0–1758.0)	0.97	0.02*	0.04*

Data are presented as median (min–max)

Min–max minimum–maximum, *WHR* waist to hip ratio, *BMI* body mass index

*Significant difference ($p<0.05$)

Anthropometric measurements and body composition

Anthropometric measurements of study participants at baseline and post-treatment are presented in Table 1. Compared to baseline, body weight ($p=0.01$) and body fat (mass: $p=0.01$, percentage: $p=0.001$) decreased significantly in the probiotic group, while numerical decreases only were observed in the placebo group. On the other hand, no significant changes were observed in WHR, lean body mass, total body water, and bone mass in both groups. A decreased trend was detected in basal metabolic rate only in the probiotic group ($p=0.06$). Compared to the placebo group, patients in the probiotic group had significantly higher lean body mass, muscle mass, total body water (kg), bone mass, and BMR at baseline and all differences did not change after the treatment.

Biochemical parameters

The biochemical parameters of patients receiving probiotic and placebo at the baseline and post-treatment are given in Table 2. FBG decreased in the probiotic ($p=0.04$) and placebo ($p=0.02$) groups. Although HbA1c decreased numerically in both groups, no significant change was detected, while FRMN decreased significantly compared to baseline only in the placebo group ($p=0.02$). HOMA-IR decreased and QUICKI increased in both groups, however, changes from baseline were significant only in the placebo group ($p=0.01$ and $p=0.004$, respectively). LDL-C decreased in both groups, the change from baseline remained at the trend level ($p=0.07$) in the probiotic group but was significant in the placebo group ($p=0.01$). The mean values of inflammation parameters (hs-CRP and IL-6) were found within the reference intervals. No significant changes were observed in inflammatory variables in either group. Comparison of all laboratory parameters of two groups at baseline and post-treatment was not significantly different.

The mean daily intakes of energy, macronutrient, dietary fiber, and cholesterol in both groups at 4th week and post-treatment (8th week) are shown in Table 3. Daily carbohydrate consumption (amount: $p=0.03$, percent: $p=0.001$) increased in the probiotic group, whereas daily fat consumption (amount: $p=0.003$, percentage: $p=0.009$) decreased. Although there was no significant difference in fat intake between the two groups at baseline, the post-treatment

Table 2 Comparison of biochemical parameters of probiotic and placebo groups at the baseline and post-treatment

	Probiotic ($n=17$)			Placebo ($n=17$)			p value between groups	
	Baseline	Post-treatment	p value (within group)	Baseline	Post-treatment	p value (within group)	Baseline	Post-treatment
Glycemic parameters								
FBG[a] (mg/dL)	113.0 (92.0–210.0)	104.0 (87.0–188.0)	0.04*	134.5 (92.0–282.0)	118.5 (88.0–268.0)	0.02*	0.17	0.29
FRMN (μmol/L)	2.7 (2.4–4.0)	2.81 (2.4–3.9)	0.90	3.1 (2.6–6.1)	2.9 (2.3–5.6)	0.02*	0.23	0.75
HbA1c (%)	6.4 (5.4–10.1)	6.4 (5.3–9.3)	0.20	6.6 (5.4–13.3)	6.5 (5.5–12.1)	0.10	0.58	0.55
HOMA-IR	4.1 (1.1–10.6)	3.7 (0.8–9.4)	0.06	4.4 (1.3–10.7)	3.4 (0.3–6.8)	0.001*	0.48	0.33
QUICKI	0.3 (0.3–0.4)	0.3 (0.3–0.4)	0.13	0.3 (0.3–3.9)	0.3 (0.3–0.4)	0.75	0.60	0.94
Lipid profile								
HDL-C (mg/dL)	45.0 (33.0–65.0)	42.5 (35.0–56.0)	0.77	46.0 (34.0–68.0)	43.0 (35.0–71.0)	0.34	0.51	0.86
LDL-C (mg/dL)	130.5 (77.0–182.0)	117.5 (59.0–169.0)	0.07	150.0 (94.0–252.0)	119.0 (80.0–193.0)	0.01*	0.47	1.00
Triglycerides (mg/dL)	138.6 (85.2–256.4)	139.3 (65.5–278.4)	0.83	132.6 (57.0–244.7)	163.8 (51.0–214.7)	0.64	0.68	0.69
Inflammatory parameters								
hs-CRP (mg/L)	3.4 (0.3–24.3)	3.4 (0.4–24.3)	0.32	6.0 (1.3–12.9)	5.5 (1.0–12.8)	0.88	0.45	0.89
IL-6 (pg/mL)	7.7 (0.6–18.6)	7.7 (1.8–27.4)	0.33	10.6 (4.7–42.1)	10.6 (4.1–30.3)	0.42	0.07	0.32

Data are presented as median (min–max)

Min–max, minimum–maximum, *FBG* fasting blood glucose, *FRMN* fructosamine, *HOMA-IR* homoeostasis model assessment of insulin resistance, *QUICKI* quantitative insulin sensitivity check index, *HDL-C/LDL-C* high-density/low-density lipoprotein

*Significant difference ($p<0.05$)

[a]By Mann–Whitney U test for significant changes at baseline and post-treatment FBG value in probiotic and placebo groups. $p=0.709$

difference was significant ($p=0.04$). There was no change in daily energy, protein, dietary fiber, and cholesterol consumptions in both groups compared to baseline.

Gene expressions

Table 4 presents the change in gene expressions of probiotic and placebo groups from baseline to post-treatment. In the probiotic group, after 8 weeks of treatment, MUC2 and MUC3A expressions increased more than nine times ($p=0.04$ and $p=0.008$, respectively). In addition, numerical increases were observed in TLR2 and TLR4 expressions with no statistical significance. Meanwhile, there was no significant change in any of the gene expressions in the placebo group.

Discussion

To the best of our knowledge, this study is the first to assess the impact of LGG supplementation on glycemic and lipid, inflammatory markers, and interaction with gene expression levels (MUC2, MUC3A, TLR2, and TLR4) in patients with T2DM. It was found that taking probiotic supplements for 8 weeks in women with T2DM had beneficial effects on mucin gene expressions and weight loss, but had no influence on other glycemic parameters, lipid profile, and inflammatory markers.

Our study demonstrated that, compared with placebo, 8 weeks of LGG supplementation in patients with T2DM increased the expression of MUC2 and MUC3A genes, which are part of the intestinal mucous barrier, by more than ninefold. Limited in vitro studies showed that several *Lactobacillus* species increased mucin gene expressions in human intestinal cell lines. VSL3 probiotic supplement, which contains some *Lactobacillus* species, raised the expressions of MUC2, MUC3, and MUC5AC in HT29 cells [32]. In another study, it was signified that *Lactobacillus plantarum* 299v and *LGG* inhibited the adherence of attaching pathogenic *Escherichia coli* to HT-29 intestinal epithelial cells [33]. In an experimental study, VSL3 probiotic supplementation for 14 days did not alter mucin expression or mucous layer thickness in mice [34]. Inversely, rats given VSL3 probiotic at a similar daily dose for 7 days showed a 60-fold increase in MUC2 expression [35]. These studies notably implied that mucous production may be increased by

Table 3 Comparison of mean daily intakes of energy, macronutrients, dietary fiber, and cholesterol in probiotic and placebo groups at 4th week and post-treatment (8th week)

	Probiotic ($n=17$)			Placebo ($n=17$)			p value between groups	
	4th week	Post-treatment	p value (within group)	4th week	Post-treatment	p value (within group)	4th week	Post-treatment
Energy (kcal/day)	1126.8 (536.2–1972.4)	977.4 (539.1–2059.6)	0.65	1450.0 (950.5–1829.6)	1353.7 (673.5–1880.6)	0.10	0.008*	0.14
Carbohydrate (g/day)	101.3 (36.5–209.0)	148.3 (46.2–251.7)	0.03*	147.6 (72.4–259.7)	135.7 (51.3–228.5)	0.43	0.02*	0.65
Carbohydrate (%)	39.0 (28.0–49.0)	44.0 (35.0–65.0)	0.001*	42.0 (21.0–58.0)	42.0 (31.0–55.0)	0.97	0.27	0.73
Protein (g/day)	47.8 (22.5–99.3)	43.7 (25.5–100.7)	0.79	54.7 (23.1–87.8)	55.7 (27.0–83.2)	0.64	0.35	0.58
Protein (%)	18.0 (11.0–31.0)	18.0 (11.0–25.0)	0.73	15.0 (8.0–24.0)	16.5 (12.0–26.0)	0.31	0.89	0.58
Fat (g/day)	54.0 (28.4–85.8)	36.1 (24.8–70.7)	0.003*	63.1 (51.0–100.4)	61.4 (33.3–70.3)	0.23	0.04*	0.01*
Fat (%)	42.0 (32.0–54.0)	36.0 (24.0–46.0)	0.009*	40.5 (27.0–64.0)	42.5 (30.0–55.0)	0.49	0.45	0.04*
Dietary fiber (g/day)	15.3 (4.7–32.5)	13.5 (7.3–34.2)	0.43	21.1 (10.7–48.6)	21.9 (6.2–48.6)	0.91	0.005*	0.13
Cholesterol (mg/day)	220.7 (65.3–375.3)	184.9 (47.4–306.2)	0.75	179.4 (92.9–539.6)	174.6 (95.0–368.6)	0.46	0.91	0.51

Data are presented as median (min–max). Min–max, minimum–maximum

*Significant difference ($p<0.05$)

Table 4 Gene expressions of probiotic and placebo groups at post-treatment

	Probiotic		Placebo	
	Fold change (95% CIs)	p value	Fold change (95% CIs)	p value
MUC2	9.2 (1.3–17.1)	0.04*	1.0 (0.3–1.7)	0.86
MUC3A	9.3 (0.5–18.2)	0.008*	0.8 (0.3–1.4)	0.70
TLR2	5.80 (0.0–14.2)	0.25	1.3 (0.5–2.2)	0.35
TLR4	1.7 (0.7–2.7)	0.15	1.0 (0.4–1.6)	0.89

CIs confidence intervals, *MUC2* mucin 2 gene, *MUC3A* mucin 3A gene, *TLR2* toll-like receptor 2, *TLR4* toll-like receptor 4

*Significant difference ($p < 0.05$)

probiotics in vivo. Nevertheless, before reaching to a definite conclusion, further studies are warranted in humans.

Earlier studies mostly in humans reported that no effects of probiotics were observed on BMI and body fat mass in patients with T2DM [24, 36–39]. On contrary, one study reported that after 24 weeks of *Lactobacillus rhamnosus* CGMCC1.3724 supplementation, significant loss in body weight and fat mass in women with obesity without diabetes was observed [40]. Similar findings were reported in an experimental study as consumption of 1×10^8Cfu LGG for 13 weeks in high-fat-fed mice reduced the adiposity and led to limited weight gain [41]. In our study, nutritional counseling was given to all patients in a general manner considering healthy eating habits, and no specific medical nutrition therapy was planned. In the probiotic group, carbohydrate intake decreased and fat intake increased, but there was no change in total energy intake. The significant weight loss observed in the probiotic group may still be due to possible changes in physical activity level, even though we did not make any recommendations to patients to encourage their physical activity during the study.

Our findings have shown that probiotic supplementation for 8 weeks in patients with T2DM tends to decrease FBG, insulin, FRMN, HbA1c, and HOMA-IR and increase QUICKI index; however, the changes were not statistically significant. In line with our study, Mazloom et al. did not observe any significant influence on FBG and insulin levels in individuals who had received a probiotic capsule containing 4 *Lactobacillus* strains twice daily, for 6 weeks [22]. Few studies have reported the beneficial effects of synbiotics and

high-dose probiotics on insulin metabolism. In a study by Asemi et al., consumption of synbiotics containing several strains of *Lactobacilli*, *Bifidobacterium*, and *Streptococcus thermophilus*, and prebiotics for 8 weeks in patients with T2DM prevented rise in FBG compared with placebo [25]. A short-term (30 days) study with limited ($n = 20$) participants with T2DM revealed that consumption of a synbiotic shake considerably decreased FBG levels [23]. In another study by Asemi et al., consumption of synbiotic supplementation for 6 weeks decreased insulin concentrations without changing the FBG and HbA1c in patients with T2DM [42]. Similar results were observed by Firouzi et al., who reported a significant reduction in insulin levels with the administration of high-dose combined probiotic supplementation for 12 weeks in patients with T2DM [38]. Additionally, in studies where probiotic supplements were provided with fermented milk and dairy products, significant reductions in FBG and HbA1c levels were reported [37, 43].

Although many studies demonstrated positive effects of prebiotics and probiotics on blood lipid profile, others reported conflicting results. In the present study, serum total cholesterol levels markedly improved in the probiotic and placebo groups, but no difference between the groups. The mean dietary cholesterol intake decreased from < 220 mg at baseline to < 200 mg per day at the end of the study and corresponds with the current recommendations. LDL-C levels were also reduced in both groups, the difference was significant only in the placebo group. No change was observed in triglycerides and HDL-C levels in both groups. In line with our findings, Mazloom et al., found that consumption of a probiotic capsule containing 4 *Lactobacillus* strains in patients with T2DM for 6 weeks resulted in no change in total cholesterol or LDL-C levels [22]. Asemi et al., demonstrated that the consumption of synbiotics for 8 weeks in patients with T2DM had no effect on serum HDL-C and LDL-C concentrations [25]. However, consumption of 200 mL/day of a synbiotic shake resulted in increased serum HDL-C in elderly individuals with T2DM [23]. Additionally, in studies that used a fermented milk product with probiotics, a marked effect on lipid profile was reported [24, 44]. The inconsistency of our findings with other studies could be explained by the difference in the studied populations as well as the probiotic strains and dosages.

It is well-known that low-grade inflammation plays an important role in the pathogenesis of T2DM; accordingly, inflammatory markers are found at higher levels in patients with T2DM than in healthy individuals. We also evaluated hs-CRP and IL-6 levels in our study. Blood levels of hs-CRP were slightly high at baseline and post-treatment in both groups; however, there were no remarkable difference between hs-CRP and IL-6 levels in the probiotic and placebo groups. This is in line with other studies which found no significant improvement in hs-CRP and IL-6 after probiotic supplementation [21, 22, 45]. Although, few studies have shown that synbiotic supplementation has a positive effect on hs-CRP [25, 42, 46]. Overall, the impact of probiotics supplementation on hs-CRP remains controversial.

Ahmad et al., reported that TLR2 and TLR4 and their adapter proteins were overexpressed in peripheral blood mononuclear cells from subjects with obesity, which correlated with increased expression of TNF-α and IL-6 [47]. Dasu et al., have shown that TLR2 and TLR4 expression and their ligands, signaling, and functional activation are increased in recently diagnosed T2DM. These associations explained a potential pathophysiological link between obesity and inflammation leading to insulin resistance [48]. To our knowledge, no clinical study was found in the literature in which TLR2 and TLR4 activities were evaluated after probiotic treatment in patients with T2DM. In this study, the expression of TLR4 was not different in post-treatment with LGG; meanwhile, TLR2 expression increased in the probiotic group but not statistically significant.

The lack of microbiota analysis due to the limited budget constitutes one of the main limitations of our study. Another limitation was the small sample size. In addition, there were different values (lean body mass, muscle mass, total body water, bone mass, and basal metabolic rate) between the two groups at the baseline, even though we adjusted and analyzed them at the end of the study. Moreover, our study has a relatively short duration of treatment and a single probiotic strain and low dosages were used. Longer term interventions with multispecies probiotic supplements could result in greater changes.

Conclusion

In conclusion, MUC2 and MUC3A expressions were significantly increased in this study in the probiotic group. The main innovation of this study is the beneficial effects of Lactobacillus GG on the expression of mucin genes responsible for weight loss and maintenance of intestinal barrier functions. However, before generalizing the results of our study, it needs to be confirmed by interventions in which multiple probiotic strains are used in larger numbers of patients and followed for a longer period.

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writing—review and editing. FA: supervision, project administration, writing—review and editing.

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Data availability The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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