



# Probiotic Supplementation in Morbid Obese Patients Undergoing One Anastomosis Gastric Bypass-Mini Gastric Bypass (OAGB-MGB) Surgery: a Randomized, Double-Blind, Placebo-Controlled, Clinical Trial

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## Abstract

**Background** Bariatric surgery is known as one of the most effective treatments for sustainable weight loss; however, it may be associated with some complications. This study was designed to examine the effects of probiotic supplementation on some morbidities related to this surgery.

**Methods** This was a placebo-controlled, double-blind, randomized clinical trial on morbid obese patients referred for One Anastomosis Gastric Bypass- Mini Gastric Bypass (OAGB-MGB) surgery to a tertiary referral center. Patients were assigned to receive a probiotic supplement (Famifact®) or placebo from 4 weeks prior to surgery to 12 weeks after surgery. Anthropometric, biochemical, and inflammatory indices were evaluated at the beginning and the end of the study.

**Results** At the end of study, significant improvements in some serum inflammatory markers, vitamin D status, and anthropometric measurements were observed ( $p < 0.05$ ), which were significantly more in probiotic group rather than placebo group ( $p < 0.05$ ). Moreover, significant improvements in glycemic indices and lipid profile were observed in both groups; however, these changes were not significantly different between the groups. There was no significant difference in serum levels of vitamin B<sub>12</sub>, folate, and homocysteine between groups at week 16 of the study.

**Discussion** Our results indicate that probiotic supplementation promotes inflammatory markers, body weight loss, and status of vitamin D in patients undergoing OAGB-MGB bypass. Whether these findings will sustain in longer treatment duration remained to be elucidated in future studies.

**Trial Registration** This study has been registered at [Clinicaltrials.gov](https://clinicaltrials.gov) with registration number NCT02708589.

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**Keywords** Probiotics · Gastric surgery · Obesity · OAGB-MGB · One anastomosis gastric bypass- mini gastric bypass surgery · Morbidity · Clinical trial

## Introduction

Obesity is an important risk factor for cardiovascular and kidney diseases, diabetes, some cancers, and musculoskeletal disorders [1]. It is generally accepted that obesity and excess body fat are characterized by a low-grade inflammatory condition and incremented serum levels of pro-inflammatory factors, which may have contributed to the development of comorbidities [2].

Bariatric surgery has been recognized as one of the most effective treatments for sustainable weight loss. Roux-en-Y gastric bypass (RYGB) and one anastomosis gastric bypass-mini gastric bypass (OAGB-MGB) are common bariatric surgeries [3, 4]. RYGB is a combination of both restriction and malabsorption. The creation of a small stomach pouch leads to early satiety combined with the bypassing of the stomach, duodenum, and up to 200 cm of jejunum leading to malabsorption. MGB is being promoted as a quick and effective alternative to the standard RYGB, which works both by restricting food intake at any one time and by altering gut hormones involved in appetite control [5]. The surgery includes primarily of a long linear lesser-curvature gastric tube with a terminolateral gastroenterostomy 180–200 cm distal to the duodenojejunal junction (ligament of Treitz) [6]. Although these surgeries are successful in management of obesity and its related morbidities, they are known as invasive methods compared with lifestyle modification, and as might be expected, it may bring about several complications such as abdominal symptoms and nutritional deficiencies [7]. Furthermore, some evidence indicate that inflammatory factors did not modulate during the early months after bariatric surgery [8–10]. This is possibly due to several factors such as decreased intestinal integrity by physical damage of the intestinal mucosa as well as changes in gut microbiota, which may induce bacterial translocation and active inflammatory responses [11].

Increasing evidence in recent decades suggest that gut microbiome is linked with obesity, weight loss, and inflammation [12–20]. Alteration in the microbiome's abundance and community structure (increased Firmicutes and reduced Bacteroidetes) has been found in obese people [21]. Moreover, gut microbiome can be affected by anatomical and physiological modifications of the gastrointestinal tract. Overgrowth of bacteria has been reported after gastric bypass [22, 23]. It is possible that gut microbial metabolites such as lipopolysaccharide (LPS) influence secretion of pro-inflammatory factors by immune cells [24].

Several studies have shown that probiotic supplementation is a possible strategy for gut microbiota manipulation and is associated with reduced concentrations of LPS, ameliorated

inflammatory state, as well as improved anthropometric indices [25–27].

Furthermore, as reported in the literature, particular species of *Lactobacillus* and *Bifidobacterium* have the ability to produce some vitamins such as folates and vitamin B<sub>12</sub> and may influence vitamin D status [28, 29]. Considering that after bariatric surgery the deficiency of these micronutrients may increase or happen de novo, in spite of taking multivitamin-mineral supplement [30], probiotics may provide some advantages for these patients.

Until now, there is limited evidence on the effect of probiotic supplements on the immune, anthropometric and micronutrient status after gastric bypass surgery with controversial results [22, 31, 32]. Therefore, the present study was designed to examine the effect of probiotic supplementation on inflammatory factors, anthropometric indices, and serum levels of vitamin B<sub>12</sub>, folate, homocysteine, and 25-hydroxy vitamin D<sub>3</sub> in morbid obese patients undergoing OAGB-MGB surgery.

## Methods

### Participants

This was a placebo-controlled, double-blind, randomized clinical trial and was conducted on individuals who were referred for OAGB-MGB surgery to Hazrat Rasul Hospital in Tehran, Iran, from May 2015 to March 2016. Women aged 18–60 years old meeting the following inclusion criteria were enrolled: candidates for the laparoscopic OAGB-MGB surgery in the next month, morbid obesity (BMI  $\geq 40$  kg/m<sup>2</sup> or 40 > BMI > 35 kg/m<sup>2</sup> with comorbidities), no evidence of chronic disorders in the gastrointestinal, liver, and kidney, and the absence of pregnancy or lactation in women. Patients were excluded if they took antibiotics, probiotic supplements, foods fortified with probiotics and/or immunosuppressive or corticosteroid treatment, non-steroidal antiinflammatory drugs, and insulin within 4 weeks before the start of the study and during the study. Yogurt consumption was permitted for all participants, because the extent of probiotics in yogurt is trivial in comparison with our supplement. Trial was registered at [Clinicaltrial.gov](http://Clinicaltrial.gov) (NCT02708589). The ethical committee of Shahid Beheshti University of Medical Sciences approved the study protocol (1394215/787), and informed consent was obtained from all the participants who were included in the trial.

## Randomization and Treatment

Patients who fulfilled all eligibility criteria were stratified (1:1) into two groups according to their diabetes status (with or without diabetes) and allocated according to a randomized number table to receive either the probiotic supplements or the identical-appearing placebo supplements (maltodextrin) once daily from 4 weeks before surgery to 12 weeks after surgery, in addition to their prescribed medications. The surgeon and medical staff related to the care of the patient, the research staff, and patients were all blinded to the treatment assignment. Each probiotic supplement (Zist Takhmir, Co, Tehran, Iran) contained seven species of probiotic bacteria (*Lactobacillus casei* ( $3.5 \times 10^9$  CFU/g), *Lactobacillus rhamnosus* ( $7.5 \times 10^8$  CFU/g), *Streptococcus thermophilus* ( $1 \times 10^8$  CFU/g), *Bifidobacterium breve* ( $1 \times 10^{10}$  CFU/g), *Lactobacillus acidophilus* ( $1 \times 10^9$  CFU/g), *Bifidobacterium longum* ( $3.5 \times 10^9$  CFU/g), and *Lactobacillus bulgaricus* ( $1 \times 10^8$  CFU/g)) and 38.5 mg fructo-oligosaccharide, and placebo supplements contained the same amount of maltodextrin.

## Follow-up Assessments

Patients were visited at the first visit (week 0) and at weeks 4, 8, and 12. Both groups were advised to follow dietary and physical activity recommendations according to the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults [33] and clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patients [34]. All patients adhered to the protocol of the medical center, including taking a daily multivitamin and mineral supplement, 40 mg pantoprazole (antacid), and ursodeoxycholic acid (for prevention of biliary stone formation during weight loss). Furthermore, beginning at 2 months postoperatively, intramuscular vitamin B<sub>12</sub> 1000 µg was administered every month. Also, according to measured values of nutrients, other oral supplements were prescribed as needed. Adherence to study medications was determined by supplement count. A loss of more than 10% of the supplements was regarded as noncompliance, which resulted in exclusion from the study. Assessment of clinical, paraclinical, and dietary intakes was performed at baseline and week 16 (12 weeks after surgery).

## Clinical, Paraclinical, and Dietary Intake Assessment

Anthropometric measurements, including weight, height, waist, and hip circumferences of all participants, were measured at baseline and after 16 weeks by the same investigator. Percentage of the excess weight loss (% EWL) was calculated by the following formula: (preoperative weight – current weight) / (preoperative weight – ideal weight) × 100, while the ideal weight was

considered as the weight of participant with assumed BMI of 25 kg/m<sup>2</sup> [35]. Body mass index (BMI) was calculated as body weight (kg)/height squared (m<sup>2</sup>). Waist-to-hip ratio (WHR) was calculated according to the WHO recommendation [36].

Fasting blood samples were collected at baseline and week 16. All the biochemical parameters were assessed in the same laboratory using standard commercial methodologies. The serum levels of inflammatory factors including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C-reactive protein (hs-CRP) were determined using the enzyme-linked immunosorbent assay (ELISA) (Diaclone, Besancon, France, for TNF-α and IL-6; ZellBio, Ulm, Germany for hs-CRP).

Fasting glucose concentrations were measured using colorimetric enzymatic method (Pars Azmun, Tehran, Iran). Fasting insulin concentrations were measured using ELISA (Mercodia, Uppsala, Sweden). Insulin resistance was calculated by the homeostatic model assessment of insulin resistance (HOMA-IR) using the following equation:  $HOMA = [\text{fasting blood glucose (mg/dL)} \times 3 \text{ fasting insulin (mU/L)}] / 405$  [37]. Insulin sensitivity was measured using the quantitative insulin sensitivity check index (QUICKI) according to the following formula:  $1 / (\log \text{ fasting insulin (}\mu\text{U/L)} + \log \text{ fasting glucose (mg/dL)})$  [38]. Plasma total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured by colorimetric enzymatic method (Pars Azmun, Tehran, Iran). Serum concentration of vitamin B<sub>12</sub>, folate, homocysteine (hCys) (Zell Bio, Ulm, Germany), and 25-hydroxy vitamin D<sub>3</sub> (Diagnostics Biochem Canada Inc., Ontario, Canada) were determined using ELISA method according to the manufacturers' protocols.

All the participants completed a 3-day food recall (two weekdays and one weekend) at the beginning and at the end of the study, after that all the record data were verified by a dietician. Dietary intakes were analyzed using Nutritionist V (First Databank, Hearst Corp, San Bruno, CA, USA). Other information including age, smoking habits, alcohol consumption, current use of medications, and medical history were evaluated using questionnaires.

## Primary and Secondary Outcomes

The primary outcome measure was a significant reduction in inflammatory factor concentrations in serum. Secondary outcome measures were anthropometric variables, glycemic indices, lipid profile, and concentrations of vitamin B<sub>12</sub>, folate, homocysteine, and 25-hydroxyvitamin D<sub>3</sub> (25-OH Vitamin D<sub>3</sub>) in serum.

## Statistical Analysis

To define normality of data distribution, the Kolmogorov–Smirnov test was used and log transformation was used if required. Baseline characteristics, biochemical parameters, and dietary intakes were analyzed using *t* test for continuous variables and chi-squared for categorical variables. To compare variables within and between groups, paired *t* test and Student's *t* test were used, respectively. To remove the effects of confounding factors, either in the beginning or during the study, the analysis of covariance test (ANCOVA) was used. The data were analyzed according to the intention-to-treat principle. All the statistical analyses were done using SPSS for Windows (version 19; SPSS Inc., Chicago, IL). *P* value < 0.05 was considered statistically significant.

## Results

### Characteristics of the Participants

From 46 eligible patients, 45 (97.82%) completed 16-week study intervention. One subject from the control group did not accomplish the study for postponed date of surgery (Fig. 1). All enrolled participants were included in the analysis of outcomes. The baseline demographic, clinical, and paraclinical data of the participants are presented in Table 1. Except for significantly higher serum levels of 25-OH Vitamin

D<sub>3</sub> in the probiotic group ( $83.49 \pm 25.16$ ) compared with the placebo group ( $52.20 \pm 24.30$ ), there were no significant differences between two groups at the baseline. Serum levels of hs-CRP tended to be higher in the placebo group than in the probiotic group ( $p = 0.050$ ). Table 2 shows the dietary intakes of two groups at baseline and after 16 weeks of study. The dietary components were significantly different within-group during the study period. However, these changes did not differ significantly between groups.

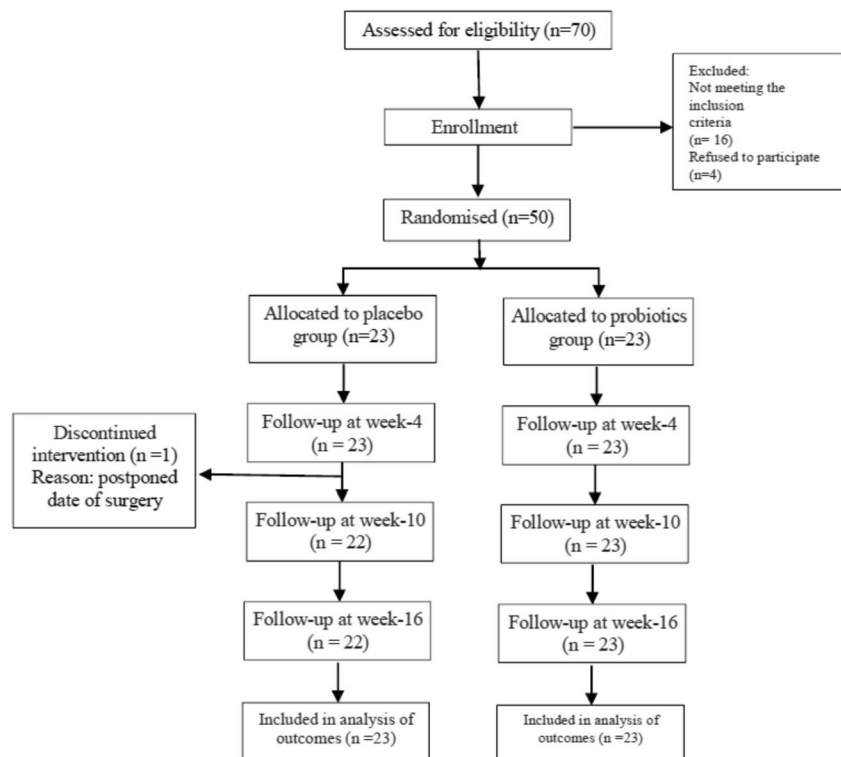
### Primary Outcome

At the end of the treatment, significant improvements in serum inflammatory concentrations were detected in probiotic group in comparison to the baseline values ( $p < 0.05$ ); however, only TNF- $\alpha$  reduction was significantly more in probiotic group in comparison to placebo groups ( $p = 0.030$ ). Moreover, a significant decrease in hs-CRP was found in both groups (both  $p \leq 0.001$ ) (Table 3).

### Secondary Outcomes

The anthropometric parameters (weight, BMI, and waist circumference) decreased significantly within both groups. Furthermore, weight, % EWL, and BMI decreased in the probiotic group significantly more than placebo group ( $p = 0.026$ ,  $p = 0.014$ , and  $p = 0.027$ , respectively) and waist circumference

**Fig. 1** The study consort flowchart



**Table 1** Baseline characteristics, biochemical parameters, and dietary intakes of study participants at baseline

	Probiotic group ( <i>n</i> = 23)	Placebo group ( <i>n</i> = 23)	<i>P</i> value <sup>a</sup>
Age (years)	32.35 ± 6.88	36.95 ± 11.00	0.103
Weight (kg)	120.04 ± 15.10	119.34 ± 15.83	0.880
BMI (kg/m <sup>2</sup> )	44.59 ± 4.30	44.95 ± 4.52	0.792
WC (cm)	123.91 ± 11.50	121.09 ± 11.18	0.409
WHR	0.87 ± 0.08	0.86 ± 0.06	0.506
Systolic blood pressure (mmHg)	116.00 ± 7.54	122.50 ± 14.82	0.09
Diastolic blood pressure (mmHg)	80.50 ± 3.95	81.25 ± 12.96	0.807
Current smokers	2 (8.7)	0 (0)	0.157
Diabetes type 2	3 (13.0)	3 (13.6)	0.953
Hypertension	6 (26.1)	8 (36.4)	0.457
Vitamin D supplementation	2 (8.7)	8 (36.4)	0.026
Serum biochemistry tests			
Inflammatory factors			
Il-6 (pg/mL) <sup>b</sup>	10.89 ± 5.89	11.09 ± 3.51	0.562
TNF-α (pg/mL)	28.18 ± 12.94	24.89 ± 13.41	0.418
hs-CRP (ng/mL) <sup>b</sup>	8098.411 ± 1364.49	8822.08 ± 975.388	0.05
FBS (mg/dL)	102.34 ± 35.46	98.96 ± 15.35	0.904
Insulin (mU/L)	17.21 ± 10.56	16.87 ± 8.99	0.841
HOMA-IR	12.53 ± 7.53	12.35 ± 6.52	0.939
QUICKI	0.31 ± 0.03	0.32 ± 0.03	0.85
Total cholesterol (mg/dL)	171.87 ± 30.80	166.40 ± 25.35	0.520
LDL (mg/dL)	92.45 ± 33.42	87.85 ± 27.96	0.620
HDL (mg/dL)	47.00 ± 12.00	47.87 ± 10.56	0.799
Triglycerides (mg/dL)	162.10 ± 69.89	153.42 ± 59.35	0.656
Folate (nmol/L) <sup>b</sup>	9.78 ± 2.85	10.22 ± 2.24	0.431
Vitamin B <sub>12</sub> (pmol/L)	201.52 ± 29.61	205.83 ± 30.73	0.635
Homocysteine (μmol/L)	9.01 ± 8.19	8.60 ± 11.25	0.292
25-OH-vitamin D (ng/mL)	83.49 ± 25.16	52.20 ± 24.30	< 0.001
Total energy (kcal)	1757.63 ± 123.68	1583.37 ± 155.96	0.396
Carbohydrate (g/day)	255.62 ± 76.21	230.71 ± 102.41	0.432
Protein (g/day)	70.63 ± 15.56	61.83 ± 28.67	0.283
Fat (g/day)	53.34 ± 18.94	48.77 ± 21.30	0.516
Dietary cholesterol (mg/day)	176.14 ± 82.76	160.09 ± 76.11	0.572
Dietary fiber (g/day)	18.44 ± 7.40	18.55 ± 11.80	0.975

Values are the mean ± SD or *n* (%)

*BMI* body mass index, *WC* waist circumference, *WHR* waist-to-hip ratio, *Il-6* interleukin 6, *TNF-α* tumor necrosis factor-alpha, *hs-CRP* high-sensitivity C-reactive protein, *FBS* fasting blood sugar, *HOMA-IR* homeostasis model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *LDL* low-density lipoprotein cholesterol, *HDL* high-density lipoprotein cholesterol

<sup>a</sup> Independent *t* test for quantitative variables and  $\chi^2$  test for qualitative variables

<sup>b</sup> Log-transformed data

reduction tended to be significantly more in the probiotic group in comparison to placebo group ( $p = 0.053$ ) (Table 4).

As it is shown in Table 5, significant improvements in FBS and insulin concentrations, HOMA-IR, and QUICKI were observed in both groups; however, modulation of glycemic indices was not significantly different between the two groups. Compared with the baseline, the concentrations of total cholesterol and triglycerides were significantly decreased in both

groups; however, the difference between groups was not significant. Fasting serum LDL concentration decreased significantly in the probiotic group from baseline ( $p < 0.046$ ). However, this reduction was not significant compared with placebo group. Also, there were no significant differences found for within- or between-group plasma concentrations of HDL in this study.

Table 6 shows that serum vitamin B<sub>12</sub> concentrations increased slightly during study period in the probiotic group,

**Table 2** Energy and some of the other food ingredients intakes of participants at the baseline and after 16 weeks

	Probiotic group	Placebo group	$p^b$
<b>Total energy (kcal)</b>			
Baseline (mean $\pm$ SD)	1757.63 $\pm$ 123.68	1583.37 $\pm$ 155.96	
16 weeks (mean $\pm$ SD)	581.51 $\pm$ 200.90	592.37 $\pm$ 194.47	
$p^a$	< 0.001	< 0.001	
Change (95% CI)	-1233.27 (-1615.80, -850.74)	-1078.62 (-1420.76, -736.47)	0.540
<b>Carbohydrate (g/day)</b>			
Baseline (mean $\pm$ SD)	255.62 $\pm$ 76.21	230.71 $\pm$ 102.41	
16 weeks (mean $\pm$ SD)	73.49 $\pm$ 28.14	68.85 $\pm$ 24.54	
$p^a$	< 0.001	< 0.001	
Change (95% CI)	-188.02 (-246.28, -129.75)	-175.21 (-227.32, -123.09)	0.739
<b>Protein (g/day)</b>			
Baseline (mean $\pm$ SD)	70.63 $\pm$ 15.56	61.83 $\pm$ 28.67	
16 weeks (mean $\pm$ SD)	31.37 $\pm$ 15.59	34.60 $\pm$ 13.21	
$p^a$	< 0.001	0.004	
Change (95% CI)	-41.28 (-58.19, -24.38)	-29.21 (-44.33, -14.10)	0.284
<b>Fat (g/day)</b>			
Baseline (mean $\pm$ SD)	53.34 $\pm$ 18.94	48.77 $\pm$ 21.30	
16 weeks (mean $\pm$ SD)	18.10 $\pm$ 6.74	21.16 $\pm$ 10.61	
$p^a$	< 0.001	< 0.001	
Change (95% CI)	-37.02 (-50.64, -23.41)	-31.10 (-43.28, -18.92)	0.511
<b>Dietary cholesterol (mg/day)</b>			
Baseline (mean $\pm$ SD)	176.14 $\pm$ 82.76	160.09 $\pm$ 76.11	
16 weeks (mean $\pm$ SD)	97.24 $\pm$ 68.08	114.10 $\pm$ 77.80	
$p^a$	0.018	0.114	
Change (95% CI)	-87.30 (-156.37, -18.23)	-57.68 (-124.05, -8.68)	0.529
<b>Dietary fiber (g/day)</b>			
Baseline (mean $\pm$ SD)	18.44 $\pm$ 7.40	18.55 $\pm$ 11.80	
16 weeks (mean $\pm$ SD)	9.79 $\pm$ 5.13	9.66 $\pm$ 5.23	
$p^a$	0.002	0.008	
Change (95% CI)	-7.95 (-14.52, -1.38)	-10.58 (-16.45, -4.70)	0.545

<sup>a</sup> Based on paired Student's *t* tests<sup>b</sup> Based on independent *t* test

while it decreased marginally significantly in the placebo group ( $p = 0.050$ ). Both probiotic and placebo groups showed a significant decrease in concentrations of serum folate ( $p < 0.001$ ) and a significant increase in serum homocysteine ( $p < 0.005$ ), during the study period; however, these changes were not significantly different between two groups. Serum 25-OH Vitamin D<sub>3</sub> levels were increased in both groups ( $p < 0.001$ ); however, this elevation was significantly greater in the probiotic group ( $p = 0.019$ ). During the study interventions, none of the participants reported any severe adverse effects.

## Discussion

The results of the present study showed that probiotic supplementation in morbid obese patients undergoing OAGB-MGB

surgery improved pro-inflammatory biomarker (TNF- $\alpha$ ), weight loss, and status of vitamin D<sub>3</sub>. Furthermore, there was a significant decline in serum folate and an increase in serum homocysteine in all of the patients at the end of the investigation.

It is common knowledge that manipulating the gut microbiota with probiotics, particularly *Lactobacillus* and *Bifidobacterium* species, has an important role in the modulation of inflammatory status [25, 39]. Limited studies have assessed the effect of probiotic supplementation on inflammatory factors in patients undergoing bariatric surgery [22, 31, 40], which have not found any significant effects. Small sample size, short duration of treatment, different species of probiotic bacteria, and dissimilar procedure of surgery could have contributed to these results. The anatomic alterations made by gastric bypass induce restriction of anaerobic organisms and reduction in secretion of gastric acid, which can be a deterrent to the growth of *Bifidobacteria* and *Lactobacilli*

**Table 3** Inflammatory factor concentrations of participants at the baseline and after 16 weeks

	Probiotic group	Placebo group	<i>p</i> <sup>b</sup>
IL-6 (pg/mL)			
Baseline (mean ± SD)	10.89 ± 5.89	11.09 ± 3.51	
16 weeks (mean ± SD)	8.15 ± 2.05	9.90 ± 5.24	
<i>p</i> <sup>a</sup>	0.026	0.187	
Change (95% CI)	− 3.41 (− 5.11, − 1.70)	− 2.74 (− 4.21, − 1.27)	0.563
TNF-α (pg/mL)			
Baseline (mean ± SD)	28.18 ± 12.94	24.89 ± 13.41	
16 weeks (mean ± SD)	21.33 ± 7.42	29.09 ± 20.17	
<i>p</i> <sup>a</sup>	0.01	0.371	
Change (95% CI)	− 6.18 (− 12.69, 0.32)	4.04 (− 1.18, 9.26)	0.03
hs-CRP (ng/mL) <sup>c</sup>			
Baseline (mean ± SD)	8098.411 ± 1364.49	8822.08 ± 975.388	
16 weeks (mean ± SD)	5715.96 ± 2715.49	6595.17 ± 2634.45	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	− 2651.81 (− 4491.65, − 811.97)	− 3115.72 (− 4632.77, − 1598.66)	0.707

Values are the mean ± SD

*IL-6* interleukin 6, *TNF-α* tumor necrosis factor-alpha, *hs-CRP* high-sensitivity C-reactive protein

<sup>a</sup> Based on paired Student's *t* tests

<sup>b</sup> Based on an ANCOVA and controlling for the baseline BMI, waist circumference, and mean changes in energy intake

**Table 4** Anthropometric measurements of participants at the baseline and after 16 weeks

	Probiotic group	Placebo group	<i>p</i> <sup>b</sup>
Weight (kg)			
Baseline (mean ± SD)	120.04 ± 15.10	119.34 ± 15.83	
16 weeks (mean ± SD)	95.41 ± 11.38	99.87 ± 13.57	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	− 24.28 (− 27.60, − 20.95)	− 18.95 (− 22.19, − 15.71)	0.026
EWL (%)			
Baseline (mean ± SD)	NA	NA	
16 weeks (mean ± SD)	46.82 ± 12.69	36.34 ± 12.66	
<i>p</i> <sup>a</sup>	—	—	
Change (95% CI)	46.82 (40.93, 52.71)	36.34 (30.60, 42.08)	0.014
BMI (kg/m <sup>2</sup> )			
Baseline (mean ± SD)	44.59 ± 4.30	44.95 ± 4.52	
16 weeks (mean ± SD)	35.42 ± 3.26	37.72 ± 4.16	
<i>p</i> <sup>a</sup>	0.001	< 0.001	
Change (95% CI)	− 9.04 (− 10.25, − 7.82)	− 7.11 (− 8.29, − 5.92)	0.027
WC (cm)			
Baseline (mean ± SD)	123.91 ± 11.50	121.09 ± 11.18	
16 weeks (mean ± SD)	101.75 ± 10.18	106.06 ± 8.23	
<i>p</i> <sup>a</sup>	< 0.001	0.001	
Change (95% CI)	− 20.37 (− 24.38, − 16.37)	− 14.88 (− 18.77, − 10.10)	0.053

*EWL (%)* percentage excess weight loss, *BMI* body mass index, *WC* waist circumference

<sup>a</sup> Based on paired Student's *t* tests

<sup>b</sup> Based on independent *t* test

**Table 5** Glycemic parameters and lipid profile of participants at the baseline and after 16 weeks

	Probiotic group	Placebo group	<i>p</i> <sup>b</sup>
<b>FBS (mg/dL)</b>			
Baseline (mean ± SD)	102.34 ± 35.46	98.96 ± 15.35	
16 weeks (mean ± SD)	86.33 ± 10.93	84.27 ± 7.47	
<i>p</i> <sup>a</sup>	0.004	< 0.001	
Change (95% CI)	− 11.16 (− 18.48, − 3.85)	− 13.43 (− 20.54, − 6.32)	0.671
<b>Insulin (mU/L)</b>			
Baseline (mean ± SD)	17.21 ± 10.56	16.87 ± 8.99	
16 weeks (mean ± SD)	7.10 ± 3.09	6.25 ± 3.78	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	− 7.07 (− 13.40, − 0.74)	− 9.88 (− 15.10, − 4.66)	0.509
<b>HOMA-IR</b>			
Baseline (mean ± SD)	12.53 ± 7.53	12.35 ± 6.52	
16 weeks (mean ± SD)	4.52 ± 2.10	3.90 ± 2.43	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	− 5.68 (− 9.84, − 1.53)	− 7.34 (− 10.77, − 3.92)	0.552
<b>QUICKI</b>			
Baseline (mean ± SD)	0.31 ± 0.03	0.32 ± 0.03	
16 weeks (mean ± SD)	0.36 ± 0.02	0.37 ± 0.03	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	0.04 (0.01, 0.07)	0.05 (0.03, 0.08)	0.642
<b>Total cholesterol (mg/dL)</b>			
Baseline (mean ± SD)	171.87 ± 30.80	166.40 ± 25.35	
16 weeks (mean ± SD)	152.63 ± 18.89	153.05 ± 19.47	
<i>p</i> <sup>a</sup>	0.004	0.002	
Change (95% CI)	− 21.44 (− 33.85, − 9.03)	− 11.95 (− 24.01, 0.10)	0.298
<b>LDL (mg/dL)</b>			
Baseline (mean ± SD)	92.45 ± 33.42	87.85 ± 27.96	
16 weeks (mean ± SD)	81.42 ± 24.34	82.38 ± 19.09	
<i>p</i> <sup>a</sup>	0.046	0.266	
Change (95% CI)	− 12.30 (− 24.66, 0.07)	− 4.91 (− 16.93, 7.11)	0.415
<b>HDL (mg/dL)</b>			
Baseline (mean ± SD)	47.00 ± 12.00	47.00 ± 12.00	
16 weeks (mean ± SD)	48.85 ± 11.64		
<i>p</i> <sup>a</sup>	0.148	0.664	
Change (95% CI)	1.79 (− 1.03, 4.61)	− 0.14 (− 2.60, 2.87)	0.423
<b>Triglycerides (mg/dL)</b>			
Baseline (mean ± SD)	162.10 ± 69.89	153.42 ± 59.35	
16 weeks (mean ± SD)	117.28 ± 41.80	116.50 ± 47.46	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	− 50.24 (− 72.40, − 28.07)	− 33.79 (− 55.33, − 12.25)	0.313

*FBS* fasting blood sugar, *HOMA-IR* homeostasis model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *LDL* low-density lipoprotein cholesterol, *HDL* high-density lipoprotein cholesterol

<sup>a</sup> Based on paired Student's *t* tests

<sup>b</sup> Based on an ANCOVA and controlling for the baseline BMI, waist circumference, and mean changes in energy intake

[40]. In contrast to previous studies, the initial treatment was pre-operation in our study. Thus, initiation of probiotic use in pre-operation may associate with a better niche for the development

of these bacteria. These results have also been shown in the previous studies, in that probiotics reduced the levels of IL-6 and TNF- $\alpha$  in patients with inflammatory conditions [25, 41].



**Table 6** Serum vitamin B<sub>12</sub>, folate, homocysteine, and 25-OH Vitamin D concentrations of participants at the baseline and after 16 weeks

	Probiotic group	Placebo group	<i>p</i> <sup>b</sup>
Vitamin B <sub>12</sub> (pmol/L)			
Baseline (mean ± SD)	201.52 ± 29.61	205.83 ± 30.73	
16 weeks (mean ± SD)	206.93 ± 33.29	194.86 ± 26.77	
<i>p</i> <sup>a</sup>	0.465	0.05	
Change (95% CI)	5.41 (−7.36, 18.18)	−10.96 (−24.02, 2.10)	0.078
Folate (nmol/L)			
Baseline (mean ± SD)	9.78 ± 2.85	10.22 ± 2.24	
16 weeks (mean ± SD)	8.71 ± 2.82	8.52 ± 2.92	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	−1.07 (−2.17, 0.05)	−1.70 (−2.82, −0.58)	0.423
Homocysteine (μmol/L)			
Baseline (mean ± SD)	9.01 ± 8.19	8.60 ± 11.25	
16 weeks (mean ± SD)	10.17 ± 9.46	10.88 ± 15.43	
<i>p</i> <sup>a</sup>	0.006	0.040	
Change (95% CI)	0.65 (−0.56, 1.86)	1.21 (−0.5, 2.48)	0.518
25-OH Vitamin D (ng/mL)			
Baseline (mean ± SD)	83.49 ± 25.16	52.20 ± 24.30	
16 weeks (mean ± SD)	136.60 ± 34.66	86.86 ± 19.04	
<i>p</i> <sup>a</sup>	< 0.001	< 0.001	
Change (95% CI)	53.10 (42.46, 63.74)	34.66 (23.78, 45.54)	0.019

<sup>a</sup> Based on paired Student's *t* tests<sup>b</sup> Based on independent *t* test

In the present study, the diminution of hs-CRP appeared in the probiotic group and is consistent with decline in serum levels of pro-inflammatory cytokines; however, this did not reach statistical significance between groups. Previous studies have reported that there are some controversies in the effect of various microbial species on CRP levels [22, 41–43]. Thus, these findings suggest that not all commensal microbes can modulate concentrations of CRP.

Although the specific mechanisms by which the host immune response can be affected by the probiotics remained to be fully clarified, several lines of evidence have suggested that this may be due to several factors such as prevention of bacterial translocation by stabilizing the intestinal mucosal integrity, competition with bacterial pathogens for binding sites on intestinal epithelial cells, augmentation of immunologic gut through increase in secretory intestinal immunoglobulin A, regulating of T-lymphocyte proliferation, and some signaling pathways for instance diminution of nuclear activating factor kappa B (NF-κB) activation [26, 44].

The results of this study showed a significant reduction in postoperative body weight, percentage of excess weight loss, BMI, and abdominal obesity after 16 weeks of probiotic supplementation, which is in line with previous studies [27]. In addition, another study has shown a similar weight loss in patients after RYGB who were supplemented with probiotics (*Lactobacillus* species) for 6 months [22], although it was not blinded and had

no placebo group. In spite of this, a number of studies have revealed that probiotics had no effect on weight loss and body fat [40, 45]. This discrepancy may be due to the heterogeneity among studies. Previous studies did not consider the effects of energy intake differences in intervention groups. In this study, although the baseline energy intakes of two groups were not statistically significant, it was numerically somewhat more in probiotic group in comparison to placebo group, which potentially resulted in a greater calorie deficit postoperatively, which may be involved in higher weight loss of this group.

Some possible explanations for the effects of probiotics on body weight and body fat distribution are modification in gut microbiome composition and gastrointestinal appetite hormones [46]. These changes can modify the energy harvested from diet and energy hemostasis of the host.

The present study also showed probiotic supplementation could not find any significant change in fasting glucose, insulin indices, and lipid profile. Some inconsistent findings were observed about the effects of probiotics on glycemic and lipid profile in previous studies. Evidence suggested probiotics may have induced less improvement on glucose or lipid control in the subjects who did not have poor glycemic control or abnormal serum lipid levels in comparison with diabetics or hyperlipidemic subjects [47, 48].

Another finding of the present study is that diminution of serum vitamin B<sub>12</sub> has been prevented by probiotics; however,

folate and homocysteine concentrations were not affected by probiotic supplementation, which is similar to what Woodard et al. [22] have reported. However, several studies have shown that supplementation with selected probiotic bacteria leads to gut bacterial composition changes, which is associated with an improvement in vitamin B<sub>12</sub> and folate levels and decrease in homocysteine [28, 49]. These findings may be due to the fact that in contrast to the capability of the most species of *Lactobacillus* and *Bifidobacterium* to produce vitamin B<sub>12</sub>, the ability of microbes to generate or use folate differs noticeably according to the species/strains-dependent trait [28, 50]. Therefore, it seems that probiotic strains have been used in the present study might not be appropriate to increase folate concentrations.

Deficiencies in serum vitamin B<sub>12</sub> and folate may enhance homocysteine concentrations, which is a strong independent factor for cardiovascular disorders [51] and recognized as a risk factor for neural tube defects, prematurity, and preeclampsia, when females become pregnant after OAGB-MGB surgery [52]. Further studies are needed to clarify the long-term consequences of hyperhomocysteinemia in patients after gastric bypass surgery and probable therapeutic options.

In the present study, an improvement in vitamin D status was observed in all of the patients at the end of the study, which was consistent with other studies on these patients [53, 54], which have shown that in the early months after bariatric surgery, 25-OH-vitamin D<sub>3</sub> serum concentrations enhanced. On the other hand, a deterioration in vitamin D<sub>3</sub> status was reported in the later months and years postoperative [54, 55]. Furthermore, our results showed that probiotic consumption could increase levels of serum 25(OH)D<sub>3</sub> significantly compared with placebo. The similar finding was reported by Jones et al. [29]. Although the mechanism of action is not well-known, it seems possible that this might be due to enhanced production of intestinal lactic acid, synthesis of 7-dehydrocholesterol, and high expression and activity of vitamin D receptors [29, 56].

This study has some strengths including design of the study as a randomized, double-blind, placebo-controlled clinical trial, starting the supplementation 1 month before the surgery, and assessment of participants dietary intakes and adjusting for its effects.

The present study had some limitations. The modifications in the gut microbiome were not analyzed. So, it remains unclear what changes in microbiome were made by probiotic administration in these patients. In addition, all of our participants were accidentally female; therefore, the findings might not be generalized to men.

In conclusion, this randomized, double-blind, placebo-controlled trial showed that probiotic administration improves inflammatory markers, body weight loss, and status of vitamins B<sub>12</sub> and D in patients undergoing OAGB-MGB.

Whether these findings will sustain in a longer treatment duration remained to be elucidated in future studies.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest. The study was financially supported by Endocrine Research Institute of SBMU with grant number 1394215/787.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

**Ethical Approval** All procedures performed in this study were approved by the institutional research committee and in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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