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ORIGINAL CONTRIBUTIONS

The Effects of Probiotic Supplements on Blood Markers of Endotoxin and Lipid Peroxidation in Patients Undergoing Gastric Bypass Surgery; a Randomized, Double-Blind, Placebo-Controlled, Clinical Trial with 13 Months Follow-Up

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Abstract

Background The effect of probiotic supplements among subjects undergoing bariatric surgery indicates conflicting results. Moreover, whether these effects remain after ceasing the treatment remained to be elucidated. This study was conducted to assess the effect of probiotic supplements on blood markers of endotoxin (lipopolysaccharides-binding protein: LBP), inflammation and lipid peroxidation (malondialdehyde: MDA) in patients with morbid obesity undergoing the one-anastomosis gastric bypass (OAGB).

Methods This study is a placebo-controlled, double-blind, and randomized clinical trial and 9 months of additional follow-up. Forty-six morbid obese patients undergoing OAGB were randomized to 4 months of probiotic or placebo supplements. Anthropometric indices and blood concentration of LBP, inflammatory markers, MDA, vitamin D3, and B_{12} were measured at 0, 4, and 13 months of study.

Results Probiotic supplements could improve serum LBP ($P = 0.039$), TNF-α ($P = 0.005$), vitamin B_{12} ($P = 0.03$), vitamin D3 ($P = 0.001$), and weight loss ($P = 0.01$) at month 4 in comparison to placebo; however, only serum MDA concentrations decreased significantly in the probiotic group compared with those in the placebo group ($P = 0.013$) at the end of follow-up period.

Discussion It was observed that 4 months probiotic supplementation compared with placebo prohibited an elevation in the LBP levels and improved serum TNF-α and 25-OH vitamin D3 concentrations and weight loss in patients undergoing the OAGB surgery. However, these effects did not persist 9 months after the cessation of the treatment. Further investigations are required to find how long supplementation and which dosage of it can benefit body status for the long-term.

Trial Registration This study has been registered at Clinicaltrial.gov with registration number NCT02708589.

Keywords Probiotics · Obesity · Endotoxin · Bariatric surgery · Gastric surgery

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Introduction

Obesity is a worldwide epidemic that increases the risk of chronic diseases, such as cardiovascular disease, type 2 diabetes (T2D), cancers, and osteoarthritis [1]. These risks are associated with low-grade inflammatory state and increased oxidative stress, which are characteristics of obesity [2].

Although several potential sources have been proposed for this inflammation, the alteration in gut microbiome plays a pivotal role in initiation and maintenance of obesity-related inflammation [3]. Innate immunity can be triggered by gut microbial metabolites, especially lipopolysaccharides (LPS), which are glycolipids in the outer membrane of Gram-negative bacteria via interaction with immune receptors and proteins, including the LPS-binding protein (LBP), cluster of differentiation 14 (CD14), myeloid differentiation protein-2 (MD2), and Toll-like receptor 4 (TLR4), ultimately activating nuclear factor-kB (NF-κB) and pro-inflammatory cytokines [3]. LBP, which binds to LPS and presents it to CD14, is a necessary part of the LPS-induced inflammatory response [3]. Taking into account the technical difficulties for quantifying LPS in serum/plasma, their short half-life [4], and increased LBP levels in response to LPS [5], the measurement of LBP is considered to reflect the circulating LPS status. Several studies suggest that obesity is associated with increased LPS and LPB levels. Furthermore, a chronic low-level raise of LPS (endotoxemia) may be involved in obesity-related metabolic disorders [6].

Bariatric surgery (BS) is well approved as an effective treatment for morbid obesity to induce substantial long-term weight loss [7]. Laparoscopic one-anastomosis gastric bypass (OAGB), which is being promoted as a quick and efficient procedure in comparison with the standard Roux-en-Y gastric bypass (RYGB) [8], acts by a combination of restriction and malabsorption of food intake and change in gut hormones involved in appetite control [8]. Despite the advantages of BS, abdominal symptoms and nutritional deficiencies are the side effects of these procedures [9]. Moreover, notwithstanding the noticeable weight loss in the early months after BS, serum LBP levels and some other inflammatory factors remain elevated [10–12], revealing that inflammatory responses still continue. In addition, some evidence reported that oxidative stress markers may not reduce significantly during this time [13, 14]. Bacterial overgrowth, alteration of the intestinal microbial composition, and increased intestinal permeability resulting from the anatomical and physiological modifications of the gastrointestinal tract may contribute to ongoing inflammation [15–17].

Given the growing evidence supporting the involvement of gut microbiota in the inflammation process and the possibility of being affected by BS, it is possible that the use of modulators of gut microbiota, such as probiotics, should be beneficial in patients undergoing this surgery. Extensive evidence supports the potential modulating effects of probiotic supplements, especially containing *Bifidobacterium* and *Lactobacillus* species, on endotoxin [18, 19], inflammatory and oxidative stress status [20, 21], and weight loss [22]. Moreover, the status of certain micronutrients, such as vitamin D3 and vitamin B12, may be affected by the consumption of probiotics [23, 24].

To date, the effect of probiotic supplements among subjects with morbid obesity undergoing BS has been assessed in few studies, indicating conflicting results [16, 25, 26]. Recently, we have shown that probiotic supplements improved inflammation, weight loss, and vitamin D3 status in patients undergoing OAGB [27]. However, the effects of probiotic supplements after ceasing the treatment remained to be elucidated. Patients' follow-up after the intervention period will provide valuable findings for clinical practice. Thus, the present study was designed to examine the effects of probiotic supplements on serum LBP and malondialdehyde (MDA) levels as a lipid peroxidation marker in patients with morbid obesity undergoing the OAGB surgery and monitor the blood markers of endotoxin (LBP), inflammatory factors, such as tumor necrotizing factor (TNF_α), interlukin-6 (IL-6), and high sensitive C-reactive protein (hs-CRP), lipid peroxidation (MDA), anthropometric indices, and serum levels of 25-hydroxy vitamin D3 and vitamin B_{12} 9 months after the completion of the probiotics supplementation.

Methods and Materials

This was a placebo-controlled, double-blind, randomized clinical trial, and 9 months of additional follow-up. Patients were recruited from Hazrat Rasul Hospital in Tehran, Iran, from May 2015 to March 2017. Subjects who were 18–60 years old, candidates for the laparoscopic OAGB surgery in the next month, morbid obese (BMI ≥ 40 kg/m^2 or 40 > BMI > 35 kg/m^2 with comorbidities), and no evidence of chronic gastrointestinal, liver, and kidney disorders, were recruited. Participants who took antibiotics, probiotic supplements, foods fortified with probiotics and/or immunosuppressive treatment, and insulin within 4 weeks before the start of the study and during the study were excluded from the study. Furthermore, subjects were excluded if they were pregnant. The estimated sample size for each group was 23 patients with regarding a power $(1-\beta)$ of 90% and $\alpha = 0.05$ to detect a difference of 10 kg/m^2 in BMI measure with a standard deviation of 10 kg/m^2, attained from Woodard et al.'s study [16]. Trial was registered at Clinicaltrial.gov (NCT02708589). The ethical committee of Shahid Beheshti University of Medical Sciences approved the study protocol (1394215/787), and written informed consent was obtained from all the participants who were included in the trial.

Surgical Method

Gastric-bypass surgery by OAGB included the creation of a long sleeved gastric tube along the lesser curvature side with a Billroth type II loop gastro-jejunostomy with a 180–200 cm or longer afferent limb [8].

Randomization and Treatment

A total of 46 patients were recruited into the study, and after stratifying (1:1) into two groups based on their type 2 diabetes (T2D) status (with or without T2D), they were randomly allocated to receive either the probiotic supplement ($n = 23$) or the placebo supplement ($n = 23$) for 4 months (from a month before surgery to 3 months after the surgery). Randomization sequence was computer-generated by a statistician in blocks of four patients and stratified based on their T2D status (with or without T2D). Patients were allocated to randomization code letters (A or B) in chronological order. Information about the treatment each participant was placed in a sealed envelope, which was not opened until the investigation was completed. Patients were instructed to take one supplement capsule each day and to refrigerate the unused capsules. The patients were requested not to consume the probiotic supplements on the day of surgery until hospital discharge (about 2 days). Each probiotic capsule (ZistTakhmir, Co., Tehran, Iran) contained seven species of probiotic bacteria (*Lactobacillus casei* (3.5 × 109 CFU/g), *Lactobacillus rhamnosus* (7.5 × 108 CFU/g), *Streptococcus thermophiles* (1 × 108 CFU/g), *Bifidobacterium breve* (1 × 1010 CFU/g), *Lactobacillus acidophilus* (1 × 109 CFU/g), *Bifidobacteriumlongum* (3.5 × 109 CFU/g), and *Lactobacillus bulgaricus* (1 × 108 CFU/g)) and 38.5-mg fructo-oligosaccharide. Placebo capsules contained the same amount of maltodextrin. The surgeon, medical staff related to the care of the patient, the research staff, and patients were all blinded to the treatment assignment.

Follow-up Assessments and Compliance

Follow-up assessments were performed at the months 0 (first visit), 1, 2, 3, 4, and 13 of the study. Adherence to protocol of the study and adverse effects was ascertained at each visit. Compliance with consumption of capsules in the intervention period was determined by supplement count at each visit and weekly telephone call. A loss of more than 10% of the supplements was regarded as incompliance, which resulted in exclusion from the study. Both groups were recommended to adhere dietary and physical activity advices based on clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults [28] and clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the BS patients [29]. Furthermore, all patients adhered to the protocol of the medical center. Adherence to this protocol was also assessed during follow-up visits. If participants did not follow the protocol, they were excluded from the study. Assessment of clinical, paraclinical, and dietary intakes was carried out at the baseline and month 4 (3 months after the surgery) and month 13 of the study (12 months after the surgery).

Clinical, Paraclinical, and Dietary Intake Assessment

Anthropometric measurements including weight, height, and waist circumferences; paraclinical parameters including the serum levels of LBP and interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), high sensitivity C-reactive protein (hs-CRP), MDA, 25-hydroxy vitamin D3, and vitamin B12; and dietary intakes were measured at the baseline, month 4 (3 months after the surgery), and month13 of the study (12 months after the surgery). Percentage of the excess weight loss (% EWL) was determined 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^2 [30]. All the biochemical parameters were assessed in the same laboratory by standard commercial methodologies. Fasting LBP and inflammatory markers including IL-6, tumor necrosis factor-alpha TNF-α, and hs-CRP concentrations were determined using the enzyme-linked immunosorbent assay (ELISA) (ZellBio, Ulm, Germany, for LBP and hs-CRP; Diaclone, Besancon, France, for TNF-α and IL-6), and the serum levels of MDA were assayed using a commercial chemical colorimetrical assay kit (MDA assay kit; ZellBio GmbH, Ulm, Germany). Serum concentrations of 25-hydroxy vitamin D3 (Diagnostics Biochem Canada Inc., Ontario, Canada) and vitamin B12 (Zell Bio, Ulm, Germany) were measured by ELISA method according to the manufacturers' protocols. In addition, the dietary intakes of participants were assessed by a 3-day food recall (two weekdays and one weekend) in months 0, 4, and 13; after that, all the record data were verified by a dietitian. Dietary intakes were analyzed using Nutritionist V (First Databank, Hearst Corp, San Bruno, CA, USA).

Primary and Secondary Outcomes

The primary outcome of the study was a significant reduction in serum LBP levels and inflammatory factors measured on months 4 and 13 from the start of treatment. Secondary outcome measures were circulating MDA levels, anthropometric variables, and concentrations of 25-hydroxyvitamin D3 (25-OH vitamin D3) and vitamin B12 in serum at the months 4 and 13.

Statistical Analyses

To detect normality of data distribution, the Kolmogorov–Smirnov test was used. To compare variables within each group, the analysis of variance for repeated measures and the Bonferroni post hoc test were used. Student's *t* test was performed to find differences between groups. 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 performed using SPSS for Windows (version 19; SPSS Inc., Chicago, IL). *P* value < 0.05 was considered statistically significant.

Results

From 46 eligible patients, 45 (97.82%) completed the 4-month probiotic intervention and 38 (82.61%) accomplished the 13-month follow-up period. Among the patients in the placebo group, four patients were excluded (postponed date of surgery, $n = 1$; pregnancy, $n = 1$; withdraw from study, $n = 2$). Four individuals in the probiotic group were excluded (pregnancy, $n = 1$; moving to another city, $n = 1$; withdraw from study, $n = 2$) (Fig. 1). Dropout rate was similar in both groups, and all enrolled participants were included in the analysis of outcomes. None of the patients reported any serious adverse effects during consumption of probiotic supplements. As shown in Table 1, all baseline characteristics of the participants in both groups were similar except the serum 25-OH vitamin D3 levels, which was significantly greater in the probiotic group compared to those in the placebo group (83.49 ± 25.16 vs. 52.20 ± 24.30, $P < 0.001$), and vitamin D3 supplement use at the baseline, which was significantly higher in the placebo group compared to that in the probiotic group ($P = 0.026$).

Primary Outcome

At the beginning of the study, no significant differences were observed between two groups in LBP and inflammatory cytokines except for hs-CRP levels, which tended to be higher in the placebo group compared to those in the probiotic group ($P = 0.050$). Within group comparisons of LBP indicated that the probiotic supplements in the intervention group prohibited an augmentation in LBP, whereas in the placebo group a significant rise in LBP was observed at month 4. So, a significant difference between-groups was noted at month 4 (0.06; 95% CI − 3.54 to 3.67 in the probiotic group vs.5.69; 95% CI 1.88 to 9.50 in the placebo group, $P = 0.039$); although after adjusting for confounder variables, it was disappeared. The

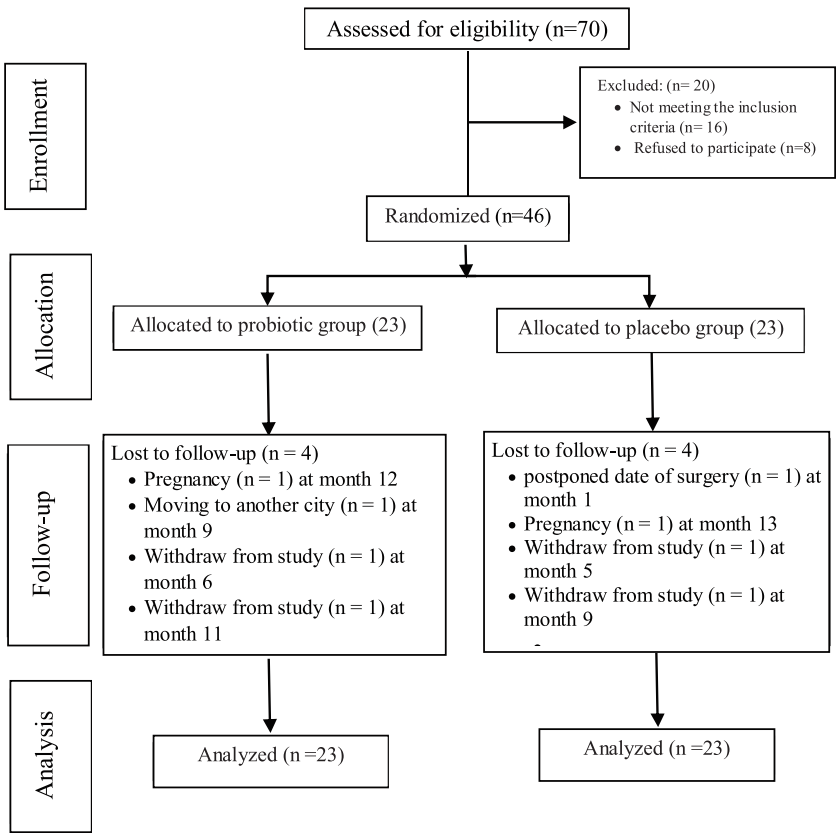

Fig. 1 The study consort flowchart

Table 1 Baseline characteristics of patients in the probiotic group and the placebo group

	Probiotic group ($n = 23$)	Placebo group ($n = 23$)	P value[a]
Age (years)[*]	32.35 ± 6.88	36.95 ± 11.00	0.103
Current smokers[#]	2(8.7)	0 (0)	0.157
Marital status (married)[#]	14 (63.6)	17 (77.3)	0.599
Type 2 diabetes mellitus[#]	3(13.0)	3(13.6)	0.953
Hypertension[#]	6(26.1)	8(36.4)	0.457
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
Weight (kg)[*]	120.04 ± 15.10	119.34 ± 15.83	0.880
BMI (kg/m^2)[*]	44.59 ± 4.30	44.95 ± 4.52	0.792
WC (cm)[*]	123.91 ± 11.50	121.09 ± 11.18	0.409
Total energy (kcal)[*]	1757.63 ± 123.68	1583.37 ± 155.96	0.396
Dietary carbohydrate (g/day)[*]	255.62 ± 76.21	230.71 ± 102.41	0.432
Dietary protein (g/day)[*]	70.63 ± 15.56	61.83 ± 28.67	0.283
Dietary 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
25-OH vitamin D (ng/mL)[*]	83.49 ± 25.16	52.20 ± 24.30	< 0.001
Vitamin B12 (pmol/L)[*]	201.52 ± 29.61	205.83 ± 30.73	0.635
Intake of supplement at the baseline			
Multivitamin-mineral[#]	2(8.7)	2(9.1)	0.963
Vitamin D supplementation[#]	2(8.7)	8(36.4)	0.026
Medications were used at the baseline			
Antihypertensive drugs[#]	2 (8.7)	4 (18.2)	0.349
Oral hypoglycemic agents[#]	3 (13.0)	3 (13.6)	0.673
Proton pump inhibitors[#]	0 (0.0)	2 (9.1)	0.139
Levothyroxine[#]	2 (8.7)	6 (27.3)	0.103
Intake of supplement at the month 13			
Multivitamin-mineral[#]	16 (69.6)	16 (72.7)	0.815
Vitamin D supplementation[#]	9 (45)	5 (26.3)	0.224
Vitamin B12 supplementation[#]	7 (35.0)	7 (38.9)	0.804

[*] Values are the mean ± SD

[#] Values are n (%)

[a] Independent t test for quantitative variables and χ^2 test for qualitative variables

serum LBP levels were similarly unchanged compared to the baseline at month 13 in both groups. Moreover, a significant improvement in serum TNF-α levels was seen in the probiotic group in comparison with the baseline values (− 7.36; 95% CI − 11.86 to 2.86, $P = 0.01$) and the placebo group ($P = 0.001$) at the end of intervention period. Finally, all of the inflammatory markers significantly decreased at the end of follow-up period, as compared to the baseline in all patients, without a significant difference between groups (Table 2).

Secondary Outcomes

At the study baseline, no significant differences were observed between the two groups regarding to MDA levels. Within-group and between-group differences in levels of MDA did not significantly differ in both groups at the end of treatment period. The circulating MDA concentrations decreased significantly in the probiotic group compared with those in the baseline ($P = 0.031$) and the placebo group ($P = 0.013$) at the end of follow-up period (Table 2).

As indicated in Fig. 2, within-group differences revealed a significant reduction in the anthropometric measures (weight, BMI, and waist circumference) in both groups at month 4 and month 13 of study in comparison to the baseline. The mean changes in weight, % EWL, and BMI were significantly higher in the probiotic group compared to those in the placebo group ($P = 0.026$, $P = 0.014$, and $P = 0.027$, respectively) just at month 4 of study, and no significant differences in these

Table 2 Serum LBP, inflammatory factors and MDA concentrations of patients in the probiotic group and the placebo group over the study period

	Probiotic group	Placebo group	P^b	P^c	P^d
LBP (μg/mL)					
Baseline (mean ± SD)	39.86 ± 10.39	36.04 ± 11.69	0.253		
4 months (mean ± SD)	39.53 ± 10.04	42.01 ± 9.75 [a]			
13 months (mean ± SD)	39.60 ± 12.07	38.42 ± 5.29			
Change 4 months from baseline (95% CI)	0.06 (− 3.54, 3.67)	5.69 (1.88, 9.50)		0.039	0.493
Change13 months from baseline (95% CI)	− 0.68 (− 6.10, 4.74)	2.03 (− 3.09, 7.15)		0.473	0.500
TNF-a (pg/mL)					
Baseline (mean ± SD)	28.18 ± 12.94	24.89 ± 13.41	0.418		
4 months (mean ± SD)	21.33 ± 7.42[a]	29.09 ± 20.17			
13 months (mean ± SD)	15.05 ± 4.72[a]	17.93 ± 6.18[a]			
Change 4 months from baseline (95% CI)	− 7.36 (− 11.86, 2.86)	2.30 (− 2.30, 6.91)		0.005	0.001
Change13 months from baseline (95% CI)	− 12.91 (− 18.84, − 6.97)	− 5.15 (− 11.24, 0.94)		0.079	0.138
lL-6 (pg/mL)					
Baseline (mean ± SD)	10.89 ± 5.89	11.09 ± 3.51	0.562		
4 months (mean ± SD)	8.15 ± 2.05[a]	9.90 ± 5.24			
13 months (mean ± SD)	7.38 ± 0.55[a]	9.34 ± 1.71[a]			
Change 4 months from baseline (95% CI)	− 2.78(− 4.86, − 0.70)	− 1.14(− 3.32, 1.04)		0.285	0.905
Change13 months from baseline (95% CI)	− 2.53(− 3.93, − 1.12)	− 1.87(− 3.24, − 0.51)		0.509	0.457
hs-CRP (ng/mL)					
Baseline (mean ± SD)	8098.411 ± 1364.49	8822.08 ± 975.388	0.05		
4 months (mean ± SD)	5715.96 ± 2715.49 [a]	6595.17 ± 2634.45[a]			
13 months (mean ± SD)	1677.75 ± 1880.08 [a]	2483.08 ± 3074.33[a]			
Change 4 months from baseline (95% CI)	− 2470.66 (− 3547.13, − 1394.19)	− 2134.70 (− 3236.14, − 1033.24)		0.667	0.811
Change13 months from baseline (95% CI)	− 6418.91 (− 7539.01, − 5298.80)	− 6245.11 (− 7365.22, − 5125.01)		0.829	0.863
MDA (μmol/L)					
Baseline (mean ± SD)	5.48 ± 0.94	5.07 ± 1.47	0.670		
4 months (mean ± SD)	5.35 ± 0.73	5.21 ± 1.50			
13 months (mean ± SD)	4.22 ± 1.53[a]	4.94 ± 1.67			
Change 4 months from baseline (95% CI)	− 0.19 (− 0.79, 0.41)	0.10 (− 0.51, 0.71)		0.510	0.269
Change13 months from baseline (95% CI)	− 1.46 (− 2.37, − 0.54)	0.06 (− 0.85, 0.98)		0.025	0.013

LBP, lipopolysaccharide binding protein; TNF-a, tumor necrosis factor-alpha; Il-6, interleukin 6; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde

[a] $P < 0.05$, different from the baseline, based on linear mixed model for repeated measures tests and the Bonferroni post hoc test

[b] Between-group at the baseline, based on independent *t* test

[c] Based on an ANCOVA and controlling for age

[d] Based on an ANCOVA and controlling for age and the mean changes in weight and energy intake from the baseline

parameters were found between the two groups at month 13. According to 3-day dietary records, the dietary components were significantly different within-group at month 4 and month 13 compared to that in the initiation of study, while these changes did not differ significantly between groups at the 4thmonth of intervention and at the end of the study (Fig. 3).

For the serum 25-OH vitamin D3 levels, an opposite trend was seen during the study period: at the end of 4th month, a significant increase was observed in both groups ($P < 0.001$), while a reduction was found at the end of follow-up period. Furthermore, the elevation at month 4 and the attenuation at month 13 were significantly greater in the probiotic group ($P = 0.001$, and $P < 0.001$, respectively). Within-group differences showed a marginal significant reduction in circulating vitamin B12 concentrations in the placebo group ($P = 0.050$) at month 4 and no significant difference in the probiotic groups throughout the study; between-group differences revealed a trend toward significant difference ($P = 0.078$) at month 4 of study (Fig. 4).

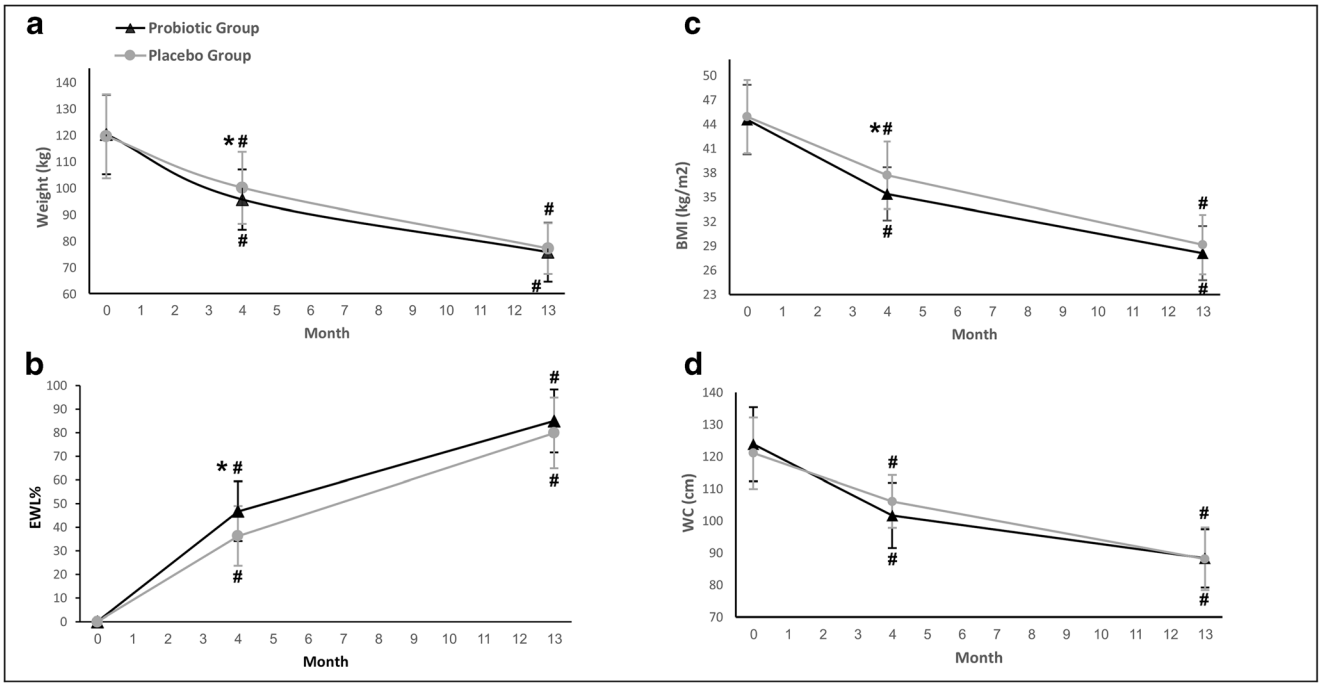

Fig. 2 Anthropometric measurements of patients in the probiotic group and the placebo group over the study period; weight (**a**); EWL (%), percentage excess weight loss (**b**); BMI, body mass index (**c**); WC, waist circumference (**d**). Values are shown as the mean ± SD. $^{\#}P < 0.05$, different from the baseline, based on linear mixed model for repeated measures tests and the Bonferroni post hoc test. $^{*}P < 0.05$, vs the placebo group, based on independent *t* test

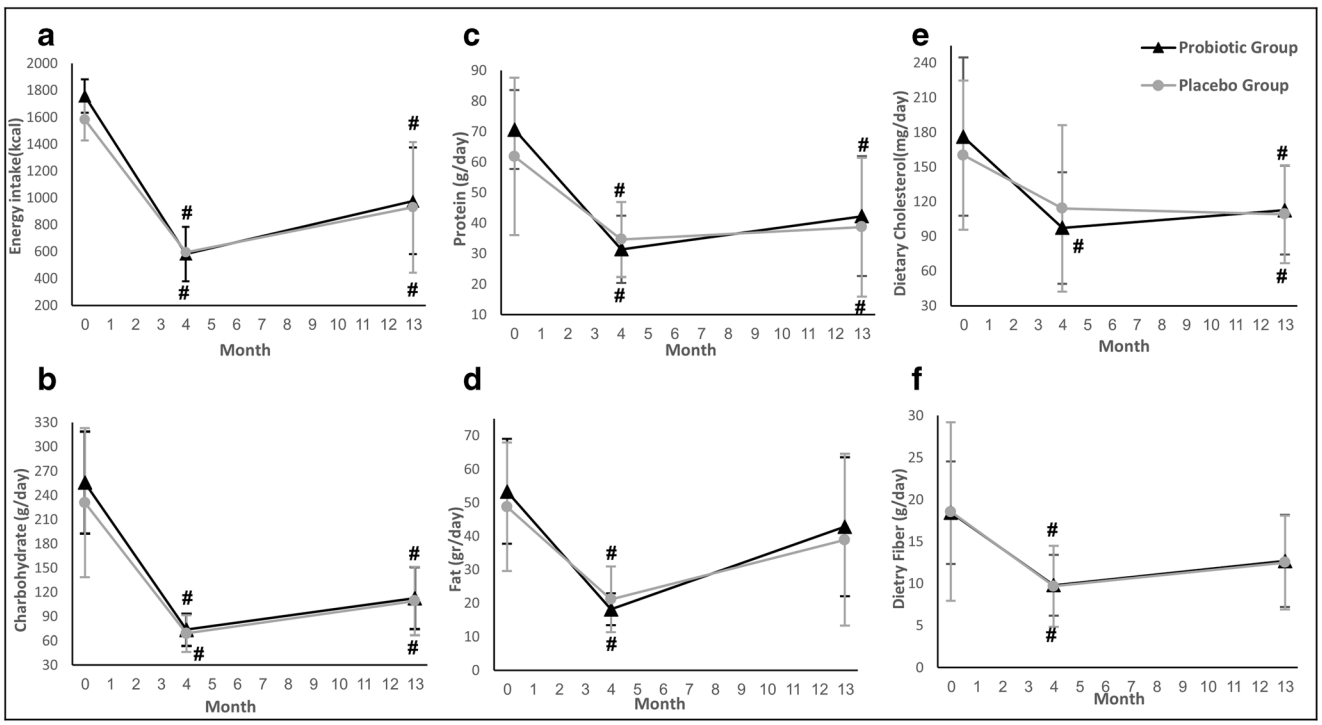

Fig. 3 Energy and some of the other food ingredients intakes of patients in the probiotic group and the placebo group over the study period; energy intake (**a**), carbohydrate (**b**), protein (**c**), fat (**d**), dietary cholesterol (**e**), and dietary fiber (**f**). Values are shown as the mean ± SD. $^{\#}P < 0.05$, different from the baseline, based on linear mixed model for repeated measures tests and the Bonferroni post hoc test

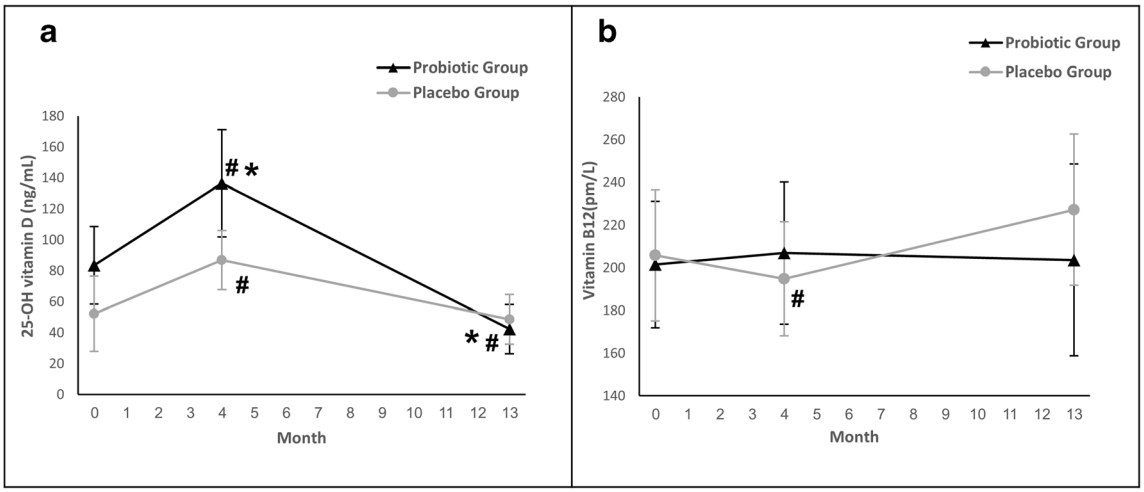

Fig. 4 Serum 25-OH vitamin D3 and vitamin B12 concentrations of patients in the probiotic group and the placebo group over the study period; serum 25-OH vitamin D3 levels (**a**), serum vitamin B12 levels (**b**). Values are shown as the mean ± SD. $^{\#}P < 0.05$, different from the baseline, based on linear mixed model for repeated measures tests and the Bonferroni post hoc test. $^{*}P < 0.05$, vs the placebo group, based on an ANCOVA and controlling for the baseline measure

Discussion

Our findings indicate that 4-month consumption of probiotic supplements prohibited an elevation in the LBP levels and improved serum TNF-α and 25-OH vitamin D3 concentrations and weight loss compared to placebo consumption. However, most of the effects of probiotics that were observed at 4th month of supplementation were diminished 9 months after cessation of them. Only MDA, as a marker of oxidative stress status, remained significantly lower in the probiotic group compared to those in the placebo group.

Previous studies indicated that serum LBP or LPS levels do not improve in obese patients after BS; even though they may elevate during first months after RYGB, owing to the metabolic stress induced by surgical stress and significant weight loss [10, 11]. In the present study, an elevation in LBP 3 months after OAGB was suppressed by probiotic supplements. This was accompanied by declining in serum levels of TNF-a, suggesting the probability that the probiotic treatment diminished metabolic endotoxemia and related down-stream inflammatory mediators. However, when the impact on LBP was adjusted for weight loss, this effect disappeared. This might be explained by the effects of probiotics on weight loss improvement.

The evidence suggests that gut microbiota manipulation by probiotics is effective in attenuating increased LPS, LBP, and inflammatory factors induced by high fat diet or dysbiosis in several rodent models and patients with inflammatory conditions [18, 19, 31]. To our knowledge, only limited clinical trials evaluated the impact of probiotics in obese patients following BS. The results of previous studies on inflammatory markers are controversial [16, 25, 26]. This discrepancy may be due to variations in the duration of supplementation, dissimilar dosages and strains of probiotic bacteria, as well as different procedures of surgery. Additionally, one clear and potentially important difference between this study and some previous studies was that they administrated probiotics following surgery; however, in the current study, initiation of the treatment was pre-operation. Decrease in secretion of gastric acid and constraint of anaerobic organisms caused by anatomical changes in gastric bypass may contribute in diminishment of the growth and survival of *Bifidobacteria* and *Lactobacilli* [26, 32].

Beneficial influences of probiotics on the blood endotoxin markers and pro-inflammatory cytokines could be resulted from alterations in composition of gut microbiota, leading to a diminution in intestinal endotoxin, modulated intestinal barrier function, attenuation of the LPS-induced NF-κB activation, and other immunomodulatory properties [33, 34].

At the end of follow-up period (1 year after the surgery), in contrast to previous studies [10, 35, 36], mainly conducted on RYGB, no changes were observed in the serum LBP levels. Additionally, the results showed a considerable reduction in all the inflammatory factors among all the patients in comparison with pre-operative findings, which is in agreement with other studies [12, 37, 38]. The exact mechanism of the persistent increase of LBP after the surgery is unclear; however, increased intestinal permeability [15], bacterial overgrowth [16, 39], a decline in *Firmicutes* and *Bacteroidetes* (belonging to gram-positive bacteria), and an enhancement of *Proteobacteria* (belonging to gram-negative bacteria), which were reported by several studies [17, 32], might contribute to this finding.

In contrast to the previously reported beneficial effects of probiotics on serum MDA [20], we observed the serum levels of MDA were unchanged and not influenced by probiotic administration at the end of intervention period. Given that the impact of probiotics on oxidative stress status is considerably strain-dependent [40], the difference in findings may be due to the different strains of bacteria that were employed. Furthermore, dissimilar examined populations might be attributed to this result. It seems that in early months after surgery, rapid substantial weight loss following BS and the consequent immense load of fatty acids in the liver prevented from decreasing lipid peroxidation [41]. Finally, similar to previous studies in BS [38, 42], an attenuation in MDA concentrations was observed 1 year after BS, although this was significant only in the probiotic group. This might be explained by the effects of probiotics on attenuation of LBP, leading to less inflammation and oxidative stress.

As previously reported [27], the weight loss in patients undergoing BS can be accentuated by administration of probiotics; such finding has also been achieved with the consumption of *Lactobacillus* species among individuals undergoing RYGB [16] and differed from those reported by Sherf-Dagan et al., who found that weight loss did not vary significantly during probiotics (*Lactobacillus* and *Bifidobacterium* species) supplementation for a 12-month interval (a 6-month period of treatment and a 6-month follow-up), although it was conducted on patients following sleeve gastrectomy [25]. This inconsistency might be related to the differences in the designs of the studies. Nine months after the cessation of probiotics, in spite of a significant within-group improvement in body weight and central obesity, changes in anthropometric indices were similar between the two groups. This result suggests that probiotics, whenever administrated, can induce beneficial effects only on body weight in patients undergoing BS and are unable to lead long-stand advantageous effects for these patients. Therefore, it seems that a long period of supplementation might lead to further advantages.

In the present study, even though weight loss was not significantly different between the two groups at the end of the study, it was numerically higher in the probiotic group compared to that in the placebo group, which potentially contributed to outcomes of the surgery, such as greater decline in TNF-a and MDA levels. The promoting impact of probiotics on weight loss could be attributed to changes in the gut microbial taxa, which is associated with decline in energy harvest from diet and influence gut satiety hormones [43].

Since changes in the measured parameters of the present study might be affected by dietary changes after surgery, we assessed dietary intakes of participants; however, no differences were observed in the dietary components between the study groups; we adjusted their effects in all analysis.

In the current study, a temporary rise in serum vitamin D3 levels was found in the initial months following MGB, followed by a declining trend in the concentrations from the third to twelfth month postoperative. Similar findings have also been reported in earlier studies [44]. This dual trend in serum vitamin D3 after the surgery may be explained by the increased bioavailability of vitamin D3 after releasing from adiposity tissues in early months after surgery and malabsorption of vitamin D3 derived from the manipulated gastrointestine in long term after surgery [45].

Moreover, our study demonstrated that consumption of the probiotic supplements was able to prevent from a slight decline in B12 serum levels, which is in agreement with the findings of Woodard et al. [16]. Additionally, the vitamin B12 status of all participants was normal 12 months after MGB. Similar finding has been reported by previous studies [46]. These findings are probably due to noticeable vitamin B12 body storage (about 2000 μg) in comparison with the small daily requirements (2 μg/day). Consequently, vitamin B12 deficiency can be expected two or more years after gastric bypass surgery [47].

The main limitation of the current study was that it did not assess alterations in the gut microbiota; thus, it remained unknown what specific taxa of bacteria were modified by the probiotic supplements and OAGB surgery. Furthermore, the findings of this study may not be generalized to men, since all the participants of this study were unintentionally female.

In conclusion, it was observed that 4-month probiotic supplementation compared with placebo prohibited an elevation in the LBP levels and improved serum TNF-α and vitamin D3 concentrations and weight loss in patients undergoing the OAGB surgery. However, these effects did not persist 9 months after the cessation of the treatment. Further investigations are required to find how long supplementation and which dosage of it can benefit body status for.

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Author Contribution Z M and A H conceptualized and designed the study and wrote the manuscript; Z M and Z K collected data; A P, A K, and P M provided the study administration works. A H interpreted the data, provided professional comments, and critically revised the manuscript for intellectual content and data accuracy.

All authors had access to the study data and reviewed and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study. All patients signed the informed consent form.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Institutional Review Board Statement The study was approved by the Ethics Committee of the National Nutrition and Food Technology Research Institute, Tehran, Iran.

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