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ORIGINAL ARTICLE

Probiotics administration following sleeve gastrectomy surgery: a randomized double-blind trial

S Sherf-Dagan[1,2,3,13], S Zelber-Sagi[1,4,13], G Zilberman-Schapira[5], M Webb[1], A Buch[1,3], A Keidar[2,6], A Raziel[2], N Sakran[2,7], D Goitein[2,3,8], N Goldenberg[2], JA Mahdi[5,9], M Pevsner-Fischer[5], N Zmora[5,10,11], M Dori-Bachash[5], E Segal[12], E Elinav[5] and O Shibolet[1,3]

BACKGROUND: Probiotics are commonly used after bariatric surgery; however, uncertainty remains regarding their efficacy. Our aim was to compare the effect of probiotics vs placebo on hepatic, inflammatory and clinical outcomes following laparoscopic sleeve gastrectomy (LSG).

METHODS: This randomized, double-blind, placebo-controlled, trial of 6-month treatment with probiotics (Bio-25; Supherb) vs placebo and 6 months of additional follow-up was conducted among 100 morbidly obese nonalcoholic fatty liver disease (NAFLD) patients who underwent LSG surgery. The primary outcome was a reduction in liver fat content, measured by abdominal ultrasound, and secondary outcomes were improvement of fibrosis, measured by shear-wave elastography, metabolic and inflammatory parameters, anthropometrics and quality of life (QOL). Fecal samples were collected and analyzed for microbial composition.

RESULTS: One hundred patients (60% women, mean age of 41.9 ± 9.8 years and body mass index of 42.3 ± 4.7 kg m^{-2}) were randomized, 80% attended the 6-month visit and 77% completed the 12-month follow-up. Fat content and NAFLD remission rate were similarly reduced in the probiotics and placebo groups at 6 months postsurgery (-0.9 ± 0.5 vs -0.7 ± 0.4 score; $P = 0.059$ and 52.5 vs 40%; $P = 0.262$, respectively) and at 12 months postsurgery. Fibrosis, liver-enzymes, C-reactive protein (CRP), leptin and cytokeratin-18 levels were significantly reduced and QOL significantly improved within groups ($P \leqslant 0.014$ for all), but not between groups ($P \geqslant 0.173$ for all) at 6 and 12 months postsurgery. Within-sample microbiota diversity (alpha-diversity) increased at 6-month postsurgery compared with baseline in both study arms ($P \leqslant 0.008$) and decreased again at 12 months postsurgery compared with 6 months postsurgery ($P \leqslant 0.004$) but did not reach baseline values.

CONCLUSIONS: Probiotics administration does not improve hepatic, inflammatory and clinical outcomes 6- and 12 months post-LSG.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome and presently represents the leading cause of global liver disease.[1] Because of the established role of obesity in the pathophysiology of NAFLD and its progression, weight reduction remains the primary target for intervention.[1] Bariatric surgery (BS) is markedly superior to conservative treatment in reducing weight and alleviating obesity-related illnesses, including NAFLD.[2] Laparoscopic sleeve gastrectomy (LSG) is an accepted primary procedure for morbid obesity because of its simplicity and effectiveness.[3] The underlying mechanisms of the beneficial effects of BS are complex and include changes in diet and behavior, gastrointestinal anatomy and motility,[4] intestinal hormones, bile acid flow and the gut microbiome.[5] At present, only few studies involving a small number of participants and spanning over a short duration have characterized the post-BS gut microbiota changes in humans.[5–12] One potential gut microbiota-modifying approach consists of administration of live commensal preparations, or 'probiotics', which are considered a safe therapy because the microorganisms they contain are natural residents of the human microbiota.[13] Probiotics as a treatment for NAFLD are an area of active clinical research,[14] with some protective effect suggested by preliminary human studies.[15–17] Furthermore, only a few small sample short-term trials have studied the impact of probiotics supplementation following BS.[13,18,19] Despite the lack of sufficient evidence, probiotics are commonly prescribed following LSG. The aim of our study was to evaluate the effect of a 6-month probiotics supplementation vs placebo on hepatic, metabolic and inflammatory parameters and microbiota composition in 100 morbidly obese NAFLD patients undergoing LSG surgery.

[1]Department of Gastroenterology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]Assuta Medical Center, Tel Aviv, Israel; [3]Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; [4]School of Public Health, Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, Israel; [5]Immunology Department, Weizmann Institute of Science, Rehovot, Israel; [6]Department of General Surgery, Assuta Ashdod Public Hospital, Affiliated to the Ben-Gurion University, Beer-Sheba, Israel; [7]Department of Surgery A, Emek Medical Center, Afula, Israel; [8]Department of Surgery C, Sheba Medical Center, Tel Hashomer, Israel; [9]Department of Microbiology Immunology and Genetics, Ben-Gurion University, Beer-Sheba, Israel; [10]Research Center for Digestive Tract and Liver Diseases, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; [11]Internal Medicine Department, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel and [12]Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel. Correspondence: Dr S Sherf-Dagan, Department of Gastroenterology, Tel Aviv Medical Center, 6 Weizman Street, Tel-Aviv 6423906, Israel.
E-mail: shirisherf@gmail.com
[13]These authors contributed equally to this work.
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MATERIALS AND METHODS

Patient eligibility and enrollment

A randomized double-blind, placebo-controlled, clinical trial included 6-month treatment with probiotics (Bio-25, Supherb, Israel) vs identical-looking placebo and 6 months of additional follow-up. One hundred NAFLD patients who underwent LSG surgery at the Assuta Medical Centers were recruited during February 2014 to January 2015 through the BS clinic. Patients were randomized into two arms (probiotics vs placebo) using the sequentially numbered, opaque-sealed envelope method, with stratification by gender and permuted blocks for every four patients according to Doig and Simpson.[20] The probiotics supplementation consisted of 11 different species of patented probiotics bacteria and >25 billion active bacteria in each capsule (Supplementary Appendix 1). Patients received 2 capsules per day, thus 50 billion bacteria or placebo. We considered compliance to treatment ('per-protocol') as taking 80% of the planned amount of capsules.[21]

Inclusion criteria were age between 18 and 65 years, body mass index (BMI) >40 kg m^{-2} or BMI >35 kg m^{-2} with co-morbidities, approval of the Assuta Medical Center committee to undergo BS and ultrasound (US)-diagnosed NAFLD. The major exclusion criteria included infection with hepatotrophic viruses (hepatitis B and hepatitis C viruses), fatty liver suspected to be secondary to hepatotoxic drugs, excessive alcohol consumption ($\geqslant 30$ g day^{-1} in men or $\geqslant 20$ g day^{-1} in women), use of antibiotics or probiotics in the past 3 months or use of antibiotics for >10 days during the study and previous BS. Diabetic patients who were treated with antidiabetic medications, other than exclusive treatment with Metformin at a stable dose for at least 6 months, were also excluded.

Medical history was obtained from the patients' medical records and by a structured interview.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committees of the Assuta Medical Centers and the Tel-Aviv Medical Center. Informed consent was obtained from each patient included in the study. The study was preregistered in the NIH registration website (TRIAL no. NCT01922830).

The study methods are in accordance with the 2010 Consolidated Standards of Reporting Trials statement guidelines for reporting randomized trials.[22] The detailed study protocol is published as Supplementary Material to this article.

Baseline and follow-up evaluations

Baseline and follow-up evaluations were performed at the Tel-Aviv Medical Center at baseline (M0, before surgery), 3 (M3), 6 (M6) and 12 (M12) months postsurgery. Both study arms received an identical standard BS medical care during the study period. Once every 2 weeks, follow-up phone calls were performed to all patients in order to monitor adverse event (AE) and to ensure compliance to treatment.

The patients filled out a demographic details questionnaire[23] at baseline, a structured questionnaire on weekly hours spent doing physical activity in the past 3 months and a short validated questionnaire for quality of life (QOL) assessment, the SF-12.[24] The SF-12 is composed of a physical component score (PCS) and a mental component score (MCS).

Primary outcome

Fatty liver by US. Fatty liver and fatty liver remission was diagnosed by abdominal US using standardized criteria.[25] In all participants, US was performed with the same equipment (Preirius scanner, Hitachi Medical Corporation, Tokyo, Japan) and by the same experienced radiologist as previously described.[26] The ratio between the median brightness level of the liver and the right kidney cortex was calculated to determine the Hepato-Renal Index (HRI) score. The HRI score, an objective operator-independent examination, has been validated against liver biopsy as previously described, with cutoff of $\geqslant 1.5$ for the prediction of steatosis $>5\%$.[27]

At M6, there was an excellent agreement between the diagnosis of fatty liver by abdominal US using the standardized criteria and diagnosis by the HRI score cutoff of $\geqslant 1.5$ as reflected by a kappa of 0.75.[28] Furthermore, liver fat percentage measured by magnetic resonance imaging with the 'in-phase and out-of-phase' method at M0 in a subsample of 18 patients was significantly correlated by Pearson correlation with liver fat content measured by HRI score ($r=0.584$, $P=0.011$). In addition, US images of gallbladder stones were recorded. Abdominal US was performed at M0, M6 and M12.

Secondary outcomes

Liver stiffness by shear wave elastography. Liver stiffness was measured by advanced shear wave elastography (Supersonic Aixplorer, Aix-en-Provence, France) at M0, M6 and M12. Shear waves was created in the liver tissue by an acoustic radiation force generated by focalized US and recorded.[29] The final data were displayed as a continuous variable in the units of kPa.

Anthropometric measurements. Weight and height were measured on a digital medical scale and BMI was calculated. Additionally, waist circumference was measured twice at the level of the umbilicus according to a uniform protocol.

Weight and waist circumference were measured at all time points. Percentages of excess weight loss (%EWL) was calculated as follows: ((preoperation weight − postoperation weight)/(preoperation weight − ideal weight)) × 100. Ideal body weight was considered as the weight for BMI 25 kg m^{-2}.[30]

Biochemical parameters. All blood samples were drawn following 12-hour fast and analyzed by uniform laboratory methods. These included: lipids profile, C-reactive protein, glucose, hemoglobin A1c, liver enzymes, insulin, ferritin, and blood count. Homeostasis Model Assessment was used as a surrogate marker of insulin resistance.[31]

Frozen serum samples from all participants at M0 and M6 were stored at − 80 °C until analyses were conducted for leptin and adiponectin (Human Leptin and Human Adiponectin, DuoSet ELISA, R&D Systems, Inc., Minneapolis, MN, USA), hepatocyte apoptosis marker cytokeratin-18 (M30 Apoptosense ELISA, PEVIVA, Nacka, Stockholm, Sweden), inflammatory factors (tumor necrosis factor α, interleukin-6, interleukin-10) (Human High Sensitivity Cytokine Base Kit A, Magnetic Luminex, R&D Systems Inc.) and bile acid (Total Bile Acids Assay Kit, Diazyme Laboratories, Poway, CA, USA).

Microbiota composition. Stool samples were self-collected in sterile tubes that were given to the participants in advance at M0, M6 and M12 and stored at − 80 °C until processed and analyzed. DNA was isolated using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). For 16S amplicon pyrosequencing, PCR amplification was performed spanning the V4 region using the primers 515F/806R of the 16S rRNA gene and subsequently sequenced using 2 × 250 bp paired-end sequencing (Illumina MiSeq, San Diego, CA, USA).[32] Custom primers were added to the Illumina MiSeq Kit resulting in 253 bp fragment sequenced following paired end joining:

Read1: 5′-TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3′;

Read2: 5′-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3′; and

Index sequence primer: 5′-ATTAGAWACCCBDGTAGTCCGGCTGACTGAC TATTAGAA-3′.

The collected data were analyzed using the QIIME pipeline. Sequences sharing 97% nucleotide sequence identity in the 16S region were binned into operational taxonomic units (97% ID OTUs). Each OTU was assigned a taxonomical classification by applying the Uclust algorithm against the Greengenes database, and an OTU table was created.

Following the pipeline, we filtered and normalized the data (only for the taxa summary analysis) by applying a bottom threshold of 0.002 relative abundance to all values under detection level (0–0.002) and only taxa that appeared in at least 20% of all samples were maintained.

For beta-diversity, unweighted UniFrac measurements were plotted according to the two principal coordinates based on samples with >9000 reads per sample. For alpha diversity calculations, a rarefaction to 7824 reads per sample with 10 repetitions was applied.

The data presented describe the microbial composition at the phyla and genus levels, as well as alpha and beta-diversity at M0, M6 and M12 across study arms.

Statistical methods. Statistical analyses were performed using the SPSS software version 23 (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as means ± s.d. and dichotomous/categorical variables as proportions. Normality distribution of continuous variables was tested by Kolmogorov–Smirnov test. If normality was rejected, nonparametric tests were used. To test differences in continuous variables between two groups, independent-samples *t*-test, Mann–Whitney test or Monte Carlo permutations test (to compare the alpha diversities) were performed. For comparison of dichotomous or categorical variables, Pearson Chi-Square test or Fisher's Exact test was performed. To compare continuous variables between two time points, paired-samples *t*-test was performed or sign-rank test when needed, and for dichotomous variables, McNemar test was

performed. Mixed model for repeated measures was performed to test differences in continuous variables between the treatment groups at several time points. Generalized estimating equations logistic regression (GENMOD procedure) was used to test differences in binary parameters between the treatment groups at several time points. $P < 0.05$ was considered statistically significant for all analyses. Benjamini–Hochberg false discovery rate was used in order to control for multiple testing when needed.

The sample size was calculated with 80% power and 5% two-sided significance level to detect between-group difference in HRI of 0.55 with an s.d. of 0.75.[26] A sample size of 38 patients per group was calculated. Additionally about 20% patients were recruited taking into consideration attrition or protocol violation, yielding a sample size of 100 patients. The study end points were analyzed according to the intention-to-treat principle.

RESULTS

Flow of trial participants and comparison between arms

One hundred NAFLD patients (60% women) scheduled to undergo LSG surgery were randomized to receive probiotics ($n = 50$) or identical-looking placebo ($n = 50$). Eighty patients (80%) attended the 6-month visit and 77 participants (77%) completed the 12-month follow-up period. Six patients were excluded owing to serious AE and 17 additional patients withdrew from the study. The same dropout rate occurred in both study arms. A flow chart of trial participation is described in Figure 1. Patients who completed participation in the study were older and more educated than patients who stopped their participation in the study for any reason (43.1 ± 9.3 vs 37.5 ± 10.4 years; $P = 0.015$ and 66.2 vs 39.1% holding academic degree; $P = 0.032$, respectively). A high adherence to the protocol was reported in both study arms; 92.5% of the probiotics group vs 87.5% of the placebo group took the supplements 'per-protocol', with no difference between arms ($P = 0.712$).

All baseline characteristics were similar in both study arms, including: age (41.9 ± 9.0 vs 41.8 ± 10.6 years), preoperative BMI (42.1 ± 5.0 vs 42.5 ± 4.4 kg m^{-2}) and diagnosis of diabetes (14 vs 12%) for the probiotics and placebo groups, respectively (Table 1).

The most widely used antidiabetic medication, Metformin, was previously associated with microbiome alterations.[33] In our study, only 6 patients reported taking Metformin presurgery (three patients in each study arm); however, no patients reported on taking it at M6 or M12. Thus its impact on our microbiota results was expected to be minor.

There were no significant differences between arms in the number of follow-up meetings with a dietitian, hours invested in physical activity, macronutrients intake and supplement use.

Primary outcome

Liver fat content measured by HRI scores was decreased at M6, as compared with M0 in both study-arms, without a significant difference between arms (−0.9 ± 0.5 vs − 0.7 ± 0.4 score for the probiotics and placebo groups, respectively; $P = 0.059$). Significant steatosis remission rate according to standard US criteria at M6 was achieved in both arms, without a significant difference between arms (52.5 vs 40% for the probiotics and placebo groups, respectively; $P = 0.262$). Similarly, no differences between arms were noted in the primary outcome at M12 (Table 2). HRI score changes and steatosis remission rates along the yearly follow-up are depicted in Figures 2a and b, respectively.

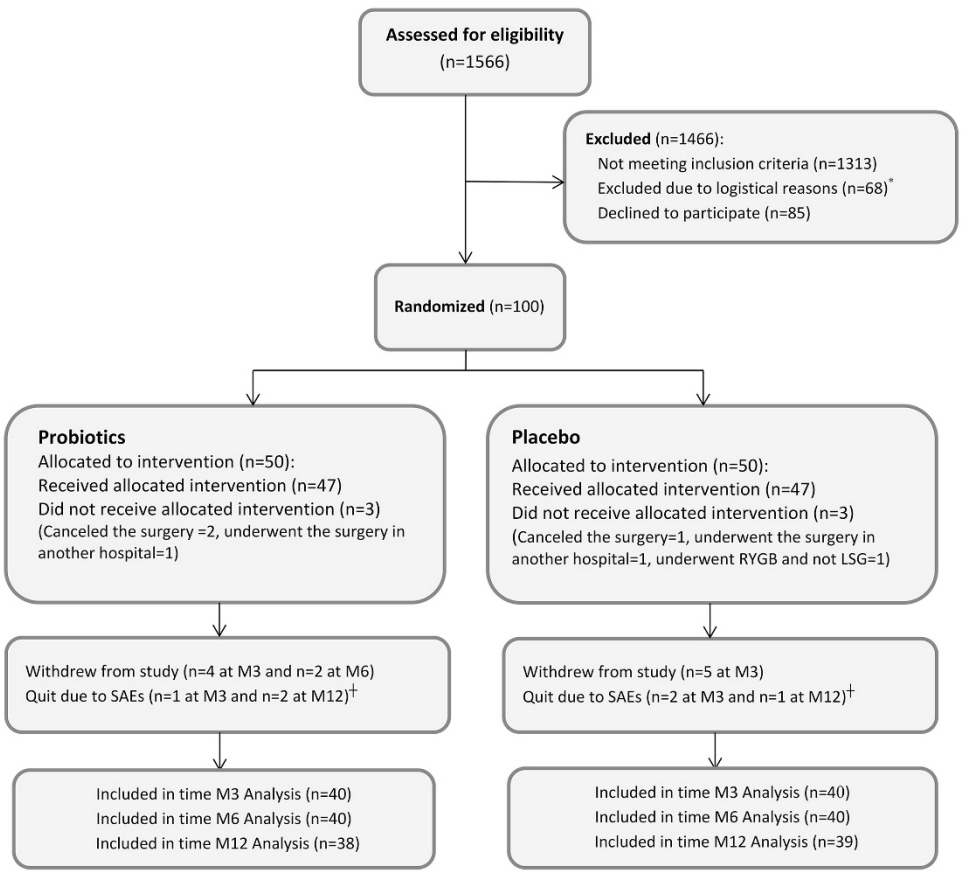

Figure 1. Flow chart of the study population. SAE, serious AE, M3, 3 months postsurgery, M6, 6 months postsurgery, M12, 12 months postsurgery. *Logistical constraints: 4 were unreachable on the phone, 2 were soldiers and their participation was not approved by the army, and 46 were excluded due unavailability to complete baseline tests prior to the operation. +Unrelated to participation in the research.

Secondary outcomes

Hepatic, inflammatory and biochemical parameters. Liver stiffness was significantly improved at M6, as compared with M0 in both study arms, but without a significant difference between arms (−1.4 ± 1.8 vs − 1.5 ± 2.8 kPa for the probiotics and placebo groups, respectively; $P = 0.888$).

Liver enzymes, cytokeratin-18, C-reactive protein and leptin were significantly decreased at M6, as compared with M0 in both study arms ($P \leqslant 0.004$ for all) but not between arms ($P \geqslant 0.173$ for all). No differences between arms were noted at 12 months of follow-up (Table 2). An opposite trend in serum bile acid levels at M6, as compared with M0 were shown between study arms (0.3 ± 2.6 and − 0.4 ± 3.5 μmol l^{-1} for the probiotics and placebo groups, respectively, $P = 0.273$) (Table 2). Change in alanine aminotransferase levels along the yearly follow-up and changes in cytokeratin-18, leptin and bile acids along the intervention period are depicted in Figures 3a–d, respectively.

Biochemical metabolic parameters glucose, hemoglobin A1c, Homeostasis Model Assessment, total cholesterol and triglycerides were significantly improved at M6 in both study arms but not between arms (Table 2).

Anthropometric parameters. BMI and waist circumference were significantly improved at M6 in both study arms but not between arms (−10.2 ± 2.6 vs − 10.7 ± 2.5 kg m^{-2}; $P = 0.469$ and − 24.1 ± 6.4 vs − 24.5 ± 6.7 cm; $P = 0.821$, for the probiotics and placebo groups, respectively). EWL percentage at M6 was 61.2 vs 64.0% for the probiotics and placebo groups, respectively, $P = 0.540$. No differences in anthropometric measurements were noted between arms at M12 (Table 2). Change in %EWL along the yearly follow-up is presented in Figure 3e.

Quality of life. QOL as assessed by both PCS and MCS of the SF-12 was similarly and significantly improved at M6 in both study arms ($P = 0.255$ for QOL-PCS score and $P = 0.281$ for QOL-MCS score; Table 2).

Microbiota composition. This analysis was available for 73 participants at M0 and M6 (2 samples were not provided by the participants and 5 samples were excluded owing to low reads count < 9000). At M12, this analysis was available for 65 participants. The changes in the relative abundance of main phyla and genus along the yearly follow-up are presented in Figure 4a and Supplementary Appendix Table 2, respectively. Overall, no significant difference was found for the microbiota composition between the study arms, except at M6 for *Proteobacteria* (mainly the *Gammaproteobacteria* class), *Actinobacteria* (mainly the *Coriobacteriia* class) and *Collinsella* genus, which were higher in the probiotics group compared with the placebo group ($P = 0.034$, $P = 0.019$ and $P = 0.003$, respectively) (Figure 4a and Supplementary Appendix Table 2). The within-sample diversity (alpha-diversity) increased at M6 compared with M0 in both study arms ($P \leqslant 0.008$) and decreased again at M12 compared with M6 ($P \leqslant 0.004$), however not returning to the baseline values (Figure 4b). The between-sample diversity (beta-diversity) is presented in Figure 4c.

Safety. Serious AEs and common AEs by study arm during the 6-month intervention period are presented in Supplementary Appendix Table 3. None of the serious AEs was considered to be related to the intervention. There was no significant difference between the study arms in the occurrence of any AEs ($P \geqslant 0.310$).

Table 1. Baseline characteristics of the study participants who ended the 6-month treatment

Parameter[a]	*Probiotics* (n = *40*)	*Placebo* (n = *40*)
Demographic factors		
Female sex (%)	60	55
Age (years)	42.1 ± 9.0	44.2 ± 9.4
Marital status (% married)	70	77.5
Children (no.)	1.9 ± 1.3	2.2 ± 1.3
Education (% holding academic degree)	65	65
Religious (% secular)	77.5	80
Anthropometric factors		
Weight (kg)	121.6 ± 18.6	120.5 ± 19.3
Height (m)	1.70 ± 0.09	1.69 ± 0.10
BMI (kg m^{-2})	42.1 ± 5.0	42.1 ± 4.7
WC (cm)	125.7 ± 12.2	124.4 ± 12.5
Liver status		
HRI	2.3 ± 0.5	2.1 ± 0.4
Fibrosis (kPa)	7.9 ± 1.8	8.1 ± 2.1
Metabolic factors		
Defecation per day (no.)	2.2 ± 1.3	1.6 ± 0.8*
Type 2 diabetes (%)	12.5	12.5
Hypertension (%)	27.5	20
Sleep apnea (%)	7.5	15
Health behavior		
Current smoker (%)	12.5	2.5
Hours of training per week	1.1 ± 2.0	0.6 ± 1.2
Quality of life		
Score QOL-PCS	40.9 ± 10.2	41.2 ± 9.6
Score QOL-MCS	49.2 ± 11.5	48.2 ± 9.9
Serum tests		
TC (mg dl^{-1})	186.1 ± 35.6	189.8 ± 25.7
LDL (mg dl^{-1})	110.6 ± 29.7	112.8 ± 22.6
HDL (mg dl^{-1})	46.6 ± 15.4	45.7 ± 13.6
Triglycerides (mg dl^{-1})	155.8 ± 98.2	156.7 ± 69.3
Glucose (mg dl^{-1})	92.3 ± 31.1	91.2 ± 10.9
HOMA	6.5 ± 5.6	5.6 ± ± 3.0
HbA1C (%)	5.8 ± 1.0	5.8 ± 0.5
ALT (U l^{-1})	36.5 ± 17.0	36.6 ± 21.0
AST (U l^{-1})	27.3 ± 9.4	26.6 ± 9.9
GGT (U l^{-1})	36.7 ± 28.2	36.5 ± 20.9
Ferritin (ng ml^{-1})	111.3 ± 91.0	116.0 ± 120.7
CRP (mg l^{-1})	11.5 ± 8.9	12.3 ± 11.8
TNF-α (pg ml^{-1})	6.1 ± 2.5	6.1 ± 2.7
IL6 (pg ml^{-1})	2.2 ± 3.9	1.8 ± 1.2
IL10 (pg ml^{-1})	0.2 ± 0.2	0.3 ± 0.5
Leptin (ng ml^{-1})	693.7 ± 287.9	697.0 ± 304.6
Adiponectin (μg ml^{-1})	4,157.7 ± 835.2	4,207.0 ± 829.3
CK-18 (U l^{-1})	190.2 ± 134.4	156.3 ± 91.5
Bile acids (μmol l^{-1})	2.9 ± 1.7	3.5 ± 3.9
Hb (g dl^{-1})	13.9 ± 1.3	13.9 ± 1.5

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK-18, cytokeratin-18; CRP, C-reactive protein; GGT, gamma glutamyltransferase; Hb, hemoglobin; HbA1C, hemoglobin A1c; HDL, high-density lipoprotein; HOMA, Homeostasis Model Assessment; HRI, Hepato-Renal Index; IL6, interleukin 6; LDL, low-density lipoprotein; MCS, mental component summary; PCS, physical component summary, QOL, quality of life; TC, total cholesterol; TNF-α, tumor necrosis factor α; WC, waist circumference. *$P = 0.026$. [a]Values are expressed as mean ± s.d., unless otherwise stated.

DISCUSSION

Administration of probiotics is common following BS, despite a lack of evidence for its efficacy. In this novel study, probiotics administration did not improve hepatic, inflammatory and clinical outcomes at 6 and 12 months post-LSG. To the best of our knowledge, only three studies have been published to date on the impact of supplementation with probiotics following BS, all

Table 2. Comparison of change in primary and secondary outcomes between the probiotics vs placebo groups following 3 and 6 months of treatment and 6 months of additional follow-up

Outcome variable	*Baseline mean (s.d.) (n = 40)*	*Probiotics*			*Baseline mean (s.d.) (n = 40)*	*Placebo*			P-*value for between-group changes*		
		Change from baseline, mean ± s.d.				*Change from baseline, mean ± s.d.*					
		M3 (n = 40)	*M6 (n = 40)*	*M12 (n = 38)*		*M3 (n = 40)*	*M6 (n = 40)*	*M12 (n = 39)*	*M3*	*M6*	*M12*
HRI	2.3 ± 0.5	—	− 0.9 ± 0.5**	− 1.0 ± 0.5**	2.1 ± 0.4	—	− 0.7 ± 0.4**	− 0.9 ± 0.4**	—	0.059	0.194
NAFLD remission (%)	—	—	52.5**	73.0**	—	—	40.0**	73.7**	—	0.262	0.945
Fibrosis (kPa)	7.9 ± 1.8	—	− 1.4 ± 1.8**	− 1.1 ± 2.1*	8.1 ± 2.1	—	− 1.5 ± 2.8**	− 1.5 ± 2.6**	—	0.888	0.401
BMI (kg m^{-2})	42.1 ± 5.0	− 7.2 ± 1.7**	− 10.2 ± 2.6**	− 12.8 ± 4.1**	42.1 ± 4.7	− 7.5 ± 1.6**	− 10.7 ± 2.5**	− 13.1 ± 4.0**	0.662	0.469	0.623
EWL (%)	—	43.7 ± 10.2	61.2 ± 14.1*	75.1 ± 17.7**	—	45.7 ± 11.0	64.0 ± 12.4**	79.3 ± 18.3**	0.693	0.540	0.280
WC (cm)	125.7 ± 12.2	− 17.0 ± 4.7**	− 24.1 ± 6.4**	− 29.6 ± 9.8**	124.4 ± 12.5	− 16.8 ± 5.3**	− 24.5 ± 6.7**	− 29.7 ± 8.1**	0.902	0.821	0.974
ALT (U l^{-1})	36.5 ± 17.0	− 13.2 ± 14.2**	− 16.0 ± 17.6**	− 18.7 ± 15.0**	36.6 ± 21.0	− 11.6 ± 18.8**	− 16.0 ± 21.3**	− 18.1 ± 20.6**	0.699	0.990	0.878
AST (U l^{-1})	27.3 ± 9.4	− 5.1 ± 8.8*	− 7.1 ± 9.8**	− 7.0 ± 8.5**	26.6 ± 9.9	− 2.8 ± 9.8	− 4.8 ± 12.2*	− 5.2 ± 12.9*	0.312	0.321	0.451
GGT (U l^{-1})	36.7 ± 28.2	− 15.7 ± 22.0**	− 14.8 ± 23.9**	− 12.8 ± 12.7**	36.5 ± 20.9	− 16.1 ± 13.7**	− 10.6 ± 30.2*	− 13.2 ± 27.4**	0.937	0.394	0.939
Glucose (mg dl^{-1})	92.3 ± 31.1	− 12.7 ± 29.8**	− 13.4 ± 29.9**	− 11.3 ± 11.4**	91.2 ± 10.9	− 9.8 ± 11.4*	− 12.1 ± 11.2**	− 12.7 ± 9.9**	0.516	0.768	0.749
HbA1C (%)	5.8 ± 1.0	− 0.4 ± 0.8**	− 0.4 ± 0.9**	− 0.3 ± 0.4*	5.8 ± 0.5	− 0.3 ± 0.4*	− 0.3 ± 0.4**	− 0.3 ± 0.3*	0.435	0.592	0.985
HOMA	6.5 ± 5.6	− 4.5 ± 5.2**	− 4.6 ± 5.3**	− 4.5 ± 3.7**	5.6 ± 3.0	− 3.6 ± 2.7**	− 4.0 ± 2.7**	− 4.2 ± 2.4**	0.304	0.482	0.738
TC (mg dl^{-1})	186.1 ± 35.6	− 15.4 ± 26.3**	− 14.8 ± 26.3**	− 12.5 ± 23.0*	189.8 ± 25.7	− 15.5 ± 26.6**	− 14.1 ± 25.0**	− 11.5 ± 25.5*	0.977	0.901	0.859
LDL (mg dl^{-1})	110.6 ± 29.7	− 4.4 ± 22.7	− 6.5 ± 25.8	− 9.0 ± 21.3*	112.8 ± 22.6	− 3.8 ± 22.3	− 3.7 ± 20.3	− 9.0 ± 22.6*	0.911	0.575	0.996
HDL (mg dl^{-1})	46.6 ± 15.4	− 3.2 ± 8.5*	0.8 ± 7.8	8.1 ± 8.8**	45.7 ± 13.6	− 5.0 ± 8.8*	− 1.0 ± 12.7	9.1 ± 11.7**	0.436	0.408	0.639
Triglycerides (mg dl^{-1})	155.8 ± 98.2	− 46.6 ± 68.7**	− 54.7 ± 83.0**	− 65.6 ± 85.1**	156.7 ± 69.3	− 33.6 ± 59.9*	− 46.9 ± 63.6**	− 58.1 ± 64.9**	0.413	0.623	0.646
Hb (g dl^{-1})	13.9 ± 1.3	− 0.3 ± 0.7*	− 0.1 ± 0.6	− 0.2 ± 0.6	13.9 ± 1.5	− 0.1 ± 0.8	0.2 ± 0.8*	− 0.0 ± 0.8	0.306	0.394	0.270
Ferritin (ng dl^{-1})	111.3 ± 91.0	10.8 ± 51.4	22.2 ± 121.5	3.7 ± 48.7	116.0 ± 120.7	24.0 ± 73.2	16.8 ± 75.9	− 6.6 ± 68.2	0.443	0.754	0.557
CRP (mg l^{-1})	11.5 ± 8.9	− 2.7 ± 4.9*	− 4.3 ± 5.5**	− 6.9 ± 7.8**	12.3 ± 11.8	− 5.6 ± 7.3**	− 6.7 ± 10.2**	− 10.1 ± 9.7**	0.099	0.173	0.072
TNF-α (pg ml^{-1})	6.1 ± 2.5	—	2.3 ± 3.9**	—	6.1 ± 2.7	—	3.3 ± 5.0**	—	—	0.346	—
IL6 (pg ml^{-1})	2.3 ± 3.9	—	0.4 ± 3.2	—	1.8 ± 1.1	—	− 0.1 ± 1.4	—	—	0.346	—
IL10 (pg ml^{-1})	0.3 ± 0.2	—	0.1 ± 0.3**	—	0.3 ± 0.5	—	0.0 ± 0.5**	—	—	0.950	—
Leptin (ng ml^{-1})	693.7 ± 287.9	—	− 352.6 ± 241.9**	—	697.0 ± 304.6	—	− 411.1 ± 276.2**	—	—	0.316	—
Adiponectin (μg ml^{-1})	4157.7 ± 835.2	—	1105.1 ± 1081.8**	—	4207.0 ± 829.3	—	1504.1 ± 1048.8**	—	—	0.098	—
CK-18 (U l^{-1})	190.2 ± 134.4	—	− 121.1 ± 121.5**	—	156.3 ± 91.5	—	− 120.7 ± 103.1**	—	—	0.788	—
Bile acids (μmol l^{-1})	2.9 ± 1.7	—	0.3 ± 2.6	—	3.5 ± 3.9	—	− 0.4 ± 3.5	—	—	0.273	—
Defecation per day (no.)	2.2 ± 1.3	− 1.2 ± 1.1**	− 1.1 ± 1.2**	− 0.9 ± 1.3**	1.6 ± 0.8	− 0.7 ± 0.8**	− 0.8 ± 0.7**	− 0.6 ± 0.6**	**0.019**	0.163	0.224
Score QOL-PCS	40.9 ± 10.2	10.7 ± 10.6**	12.5 ± 10.1**	12.9 ± 9.7**	41.2 ± 9.6	8.9 ± 6.9**	10.1 ± 8.1**	12.1 ± 9.6**	0.400	0.255	0.704
Score QOL-MCS	49.2 ± 11.5	3.2 ± 9.5*	3.6 ± 10.5*	3.0 ± 9.8*	48.2 ± 9.9	4.6 ± 8.4*	5.9 ± 8.2**	2.6 ±9.4	0.489	0.281	0.856

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK-18, cytokeratin-18; CRP, C-reactive protein; EWL, excess weight loss; GGT, gamma glutamyltransferase; Hb, hemoglobin; HbA1C, hemoglobin A1c; HDL, high-density lipoprotein; HOMA, Homeostasis Model Assessment; HRI, Hepato-Renal Index; IL6, interleukin 6; LDL, low-density lipoprotein; M3, 3 months postsurgery; M6, 6 months postsurgery; M12, 12 months postsurgery; MCS, mental component summary; NAFLD, nonalcoholic fatty liver disease; PCS, physical component summary, QOL, quality of life; TC, total cholesterol; TNF-α, tumor necrosis factor α; WC, waist circumference. *$P < 0.05$, **$P < 0.001$ for within-group changes.

among Roux-en-Y gastric bypass (RYGB) patients with a relatively small sample size, a short-term follow-up and without hepatic outcomes or evaluation of microbiota composition.[13,18,19] In a randomized controlled trial of 6-month treatment with probiotics among 40 RYGB patients, a greater %EWL, reduction in bacterial overgrowth and an increase in vitamin B12 levels were achieved in the probiotics group.[13] Similarly, in a randomized controlled trial of 14 days, intervention with probiotics among 60 RYGB patients was shown to improve postoperative gastrointestinal tract episodes, such as abdominal pain, bloating, belching and QOL.[19] In a pilot randomized controlled trial of 15 days comparing prebiotics (fructo-oligosaccharide) vs symbiotic (fructo-oligosaccharide+probiotics) vs placebo among 9 RYGB patients, only supplementation with prebiotics increased weight loss, whereas both prebiotics and synbiotics were not able to promote significant changes in inflammatory markers.[18]

Only a few studies included an assessment of gut microbiota alterations following BS, mostly performed in RYGB patients,[5–10,12] demonstrating a reduction in the *Firmicutes*:*Bacteroides* ratio[6,7,10] and an increase in *Proteobacteria* phylum.[5–9,11] Overall, at M6 the microbiota composition analysis indicated a clear significant changes consisting of a great reduction is the variability between samples and an increase in variability within samples. At M6, the ratio of *Firmicutes/Bacteroidetes* phyla in both arms was increased. This result is in contrary to most previous studies on RYGB patients[6–9] and to two small studies that have been published on LSG patients.[10,12] Similar to previous studies, we demonstrated an increase in the *Proteobacteria* phylum, mostly in the *Gammaproteobacteria* class, in both arms.[6–9] Two new phyla appeared at M6 in this study: *Verrucomicrobia* as described previously[6] and *Actinobacteria* (mainly, *Bifidobacterium* genus), which decreased in previous studies.[6,7,9,12,34] Additionally, our results showed an increase in gut microbiota richness at M6, similar to previous studies on RYGB patients[9,12] and in contrary to another study on LSG patients.[12] Despite the dramatic changes that appeared at M6 and contrary to previous study on RYGB patients,[11] at M12, these changes were almost abolished both in terms of composition and

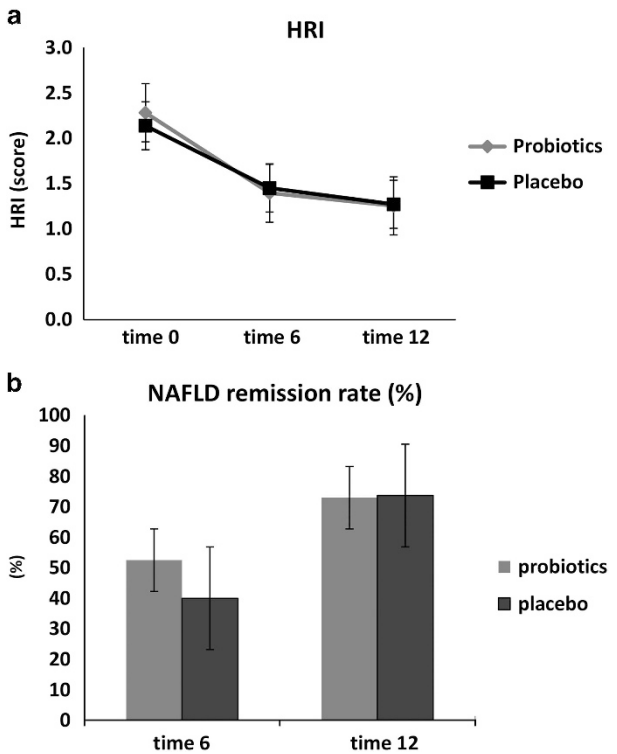

Figure 2. Change from baseline in the primary outcome: HRI (**a**) and complete remission of NAFLD on US (**b**). **n* = 40 at M0 and at M6 for both arms and *n* = 38 for the probiotics arm and *n* = 39 for the placebo arm at M12.

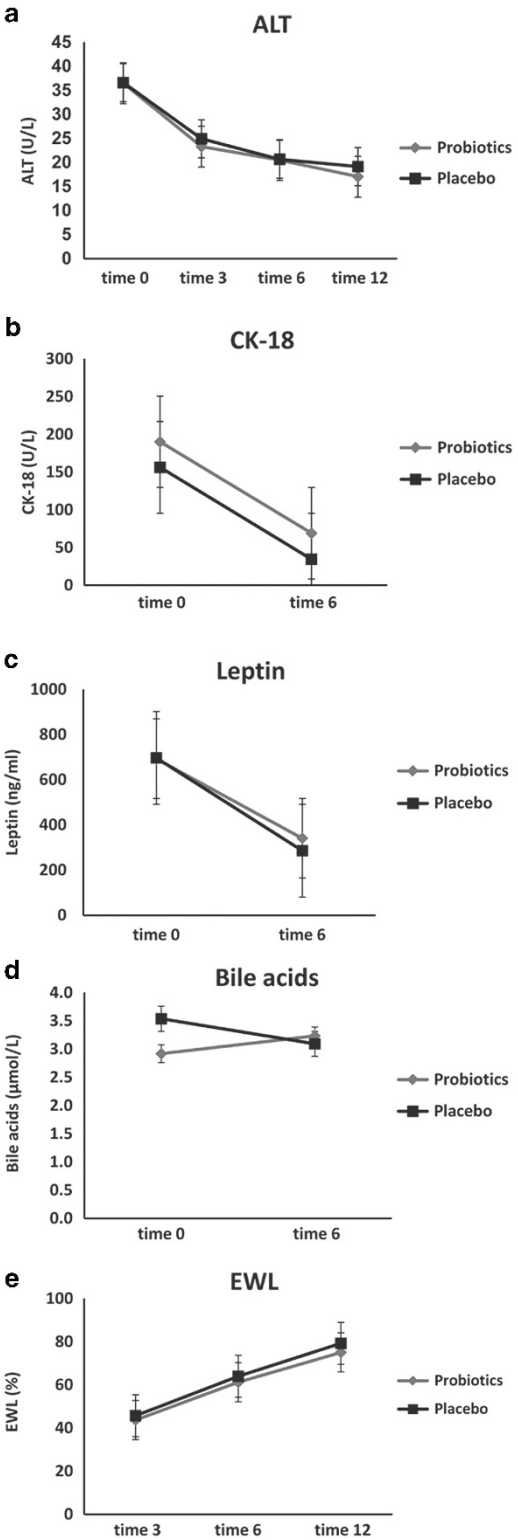

Figure 3. Change from baseline in main secondary outcomes: alanine aminotransferase (ALT) (**a**), cytokeratin-18 (CK-18) (**b**), leptin (**c**), bile acids (**d**) and %EWL (**e**). **n* = 40 at M0 and at M6 for both arms and *n* = 38 for the probiotics arm and *n* = 39 for the placebo arm at M12.

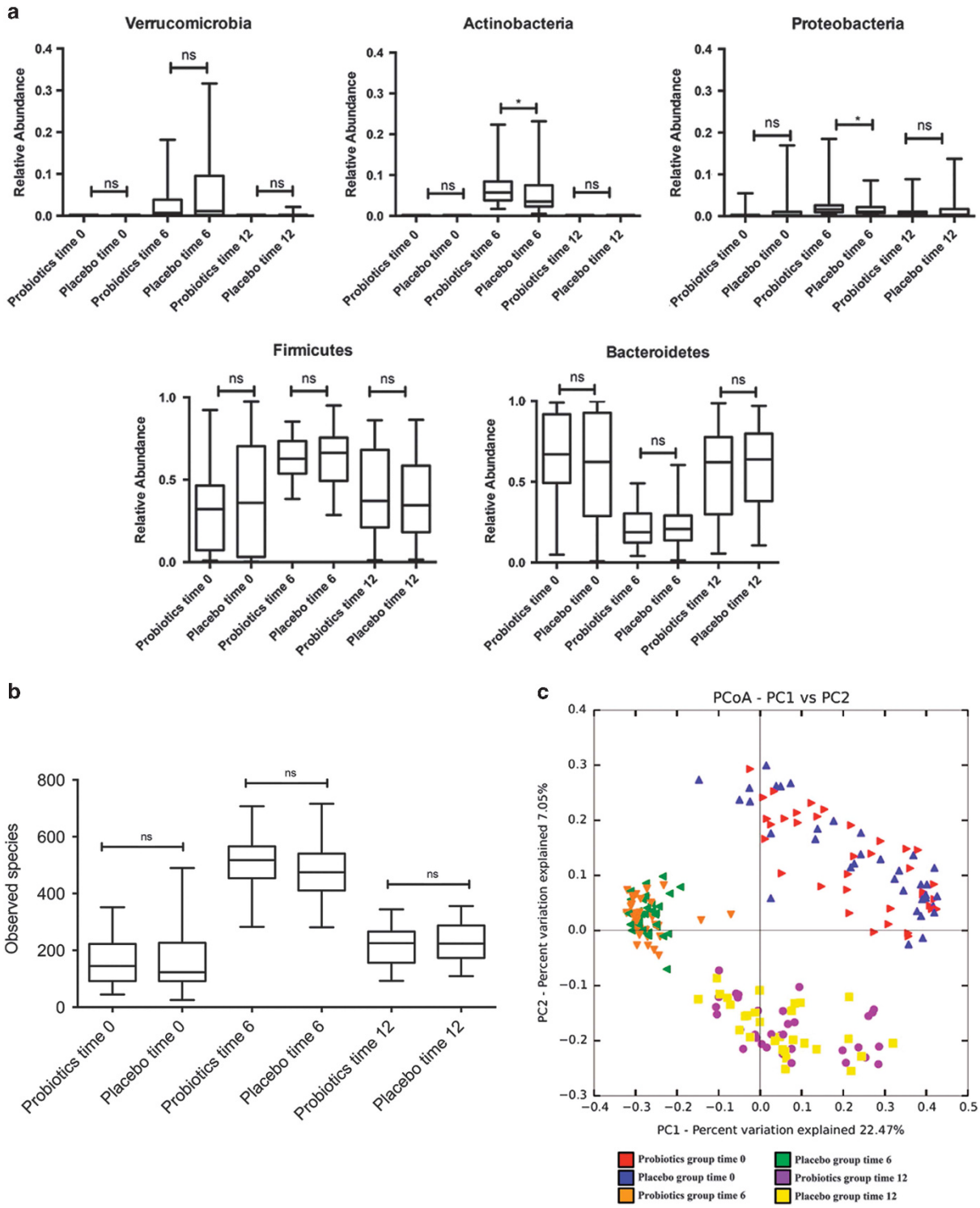

Figure 4. Gut microbiota changes at 6 and 12 months postsurgery; composition differences between study arms at the phyla level (**a**), alpha diversity (**b**) § and beta diversity (**c**). (**a**–**c**) $n = 37$ for the probiotics arm and $n = 36$ for the placebo arm at M0 and at M6, $n = 35$ for the probiotics arm and $n = 30$ for the placebo arm at M12. §, False discovery rate correction was used. NS, not significant.

diversity, so that eventually the composition was more similar to baseline. The potential effects of dietary changes following LSG should also be taken under consideration when trying to understand the underlying causes for the microbiota change over follow-up. Importantly, in our study no differences were noted in food intake between the study groups.

Recent evidence suggests that modulation of the gut microbiota may represent a new way to treat or prevent a variety of liver diseases, including NAFLD.[35] A meta-analysis of clinical trials pertaining to the effects of probiotics in NAFLD was recently published, concluding that probiotics therapy reduced serum liver enzyme levels, total cholesterol and tumor necrosis factor α and

improved insulin resistance.[35] However, we did not find any difference between group in either of these parameters, perhaps because of the major effect of BS on metabolic and inflammatory status leaving a little or no place for an additive effect of probiotics.

We observed opposite trends for serum bile acid changes at M6, with a trend toward elevation in the probiotics group and a trend toward reduction in the placebo group. The results regarding bile acids following BS are conflicting, demonstrating increased, decreased or unchanged serum bile acid levels postsurgery.[5] The cause and effect of changes in bile acid flow and change in gut bacteria remains to be determined.

There are two major possible explanations for the negative results in this trial. First, LSG induced a major impact on anthropometric, hepatic, inflammatory and metabolic parameters, including on the microbiota composition, and therefore, it is possible that probiotics treatment does not bring added benefit beyond the influence of the surgery itself. Second, LSG provokes rapid gastric emptying, accelerates intestinal transit[4,10] and decreases acid production.[36] These conditions may influence the passage and survival of probiotic bacteria through the gastrointestinal tract post-LSG.[37]

Our study has several limitations. The major one stems from the lack of repeated liver biopsies to confirm steatosis remission and enable evaluation of nonalcoholic steatohepatitis. However, HRI has been validated against liver biopsy[27] and proton magnetic resonance spectroscopy as a reference standard.[38] A second limitation is the lack of short-term assessment of the microbiota composition. However, all metabolic parameters that were obtained at M3 did not indicate any differences between the study arms. A third limitation is that the microbiota analysis included 10 subjects who used antibiotics for < 10 days during the yearly follow-up and oral antibiotics use can dramatically affect the gut microbial figuration.[14] However, 60% of them used it following the end of the intervention period and they were equally distributed across arms.

The strengths of our study are its randomized design, the relatively large sample size and the high adherence to the protocol.

In conclusion, probiotics administration does not improve hepatic, inflammatory and clinical outcomes at 6 and 12 months following LSG. Therefore, recommendation of their routine post-LSG usage cannot be justified.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

SS-D, SZ-S and OS designed and performed the study, analyzed the data and wrote the paper. GZ-S, EE, ES, JAM, MP-F, NZ and MD-B carried out the microbiota analysis. MW performed the US examinations and NG assisted in MRI decoding. AB, AK, AR, DG and NS assisted in data collection. All authors critically reviewed the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work and read and approved the final manuscript.

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