Impact of bariatric surgery on gut microbiota composition in obese patients compared to healthy controls

Nima Mohammadzadeh[1], Shabnam Razavi[2,3] and Gholamhossein Ebrahimipour[1*]

Abstract

Bariatric surgery is vital for sustainable weight loss and metabolic improvement in obese individuals, but its effects on gut microbiota and their role in these benefits require further investigation. Investigate the temporal changes in gut microbiota in obese patients undergoing bariatric surgery (gastric sleeve gastrectomy or Roux-en-Y Gastric Bypass (RYGB)) compared to healthy controls, aiming to understand their role in weight loss and metabolic health improvement. A case-control study included 30 obese patients aged 65–95 undergoing bariatric surgery, and 18 matched healthy controls. Selection criteria were based on age, race, BMI, history of antibiotics, probiotics, and prebiotics usage. Stool samples were collected at baseline, three months, and six months post-surgery for DNA extraction and quantitative real-time PCR analysis to assess gut microbiota changes. Physical activity and dietary intake were evaluated using standardized questionnaires. Statistical analyses were performed using R. Post-surgery, patients showed significant reductions in weight and BMI, with changes in dietary habits and physical activity. Quantitative real-time PCR analysis revealed substantial alterations in bacterial groups such as *Bacteroides* and *Fusobacterium*. However, some groups showed no significant changes, indicating a complex interaction between gut microbiota and bariatric surgery. Notable correlations were found between body weight, BMI, and specific bacterial groups like the *C. cluster* IV and *Lactobacillus*, particularly in RYGB patients. Bariatric surgery significantly alters gut microbiota, aiding weight loss and metabolic regulation in obese patients. Understanding these changes is crucial for developing effective obesity management strategies, requiring further research to optimize outcomes.

Keywords Intestinal microbiota, Obesity, Bariatric surgery, Mini gastric bypass, Real-time PCR

*Correspondence:
Gholamhossein Ebrahimipour
g-ebrahimi@sbu.ac.ir
[1]Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3]Microbial Biotechnology Research Center, Iran University of Medical Sciences, Tehran, Iran

Introduction

Bariatric surgery has emerged as a pivotal intervention for achieving sustainable weight loss and improving metabolic health in obese individuals (Nguyen and Varela 2017). Unlike traditional methods such as diet and exercise, which often fail to produce long-term results, bariatric surgery results in significant and sustained weight loss, as well as a reduction in obesity-related comorbidities and overall mortality (Wiggins et al. 2020). This surgical approach reduces dietary intake and modifies metabolic control, hunger-satiety mechanisms, and the gut microbiome, contributing to its effectiveness (Farias et al. 2017).

Numerous studies have demonstrated the effectiveness of bariatric surgery in promoting long-term weight loss and improving metabolic outcomes. For instance, a comprehensive review by Hua (2022) highlighted that bariatric surgery is a valuable tool that brings additional benefits, including morbidity protection and life span extension (Hua et al. 2022). Similarly, a study by Aderinto et al. emphasized the impact of preoperative weight loss on postoperative outcomes, suggesting that patients who lose weight before surgery tend to have better results (Aderinto et al. 2023). Despite these benefits, some patients do not achieve the expected weight loss, indicating the need for further investigation into the factors influencing these outcomes.

A particularly novel aspect of bariatric surgery is its impact on the gut microbiota. Studies have shown that these surgeries induce significant and lasting changes in the composition and function of the intestinal microbiome, which are associated with improved metabolic outcomes and fat mass regulation. Tremaroli and Karlsson (2015) demonstrated that bariatric surgery leads to decreased *Firmicutes* and increased Bacteroidetes, which are linked to reduced obesity and improved metabolic health (Tremaroli et al. 2015). Further evidence from systematic reviews supports that these microbial shifts directly affect the metabolic improvements observed post-surgery.

Given the critical role of the gut microbiota in mediating the benefits of bariatric surgery, a molecular assessment of its changes before and after surgery is essential. Recent studies have indicated that gut microbiota alterations may persist for years after surgery, highlighting the long-term impact of these procedures on the intestinal microbiome. For example, Guevara et al. reported significant modifications in gut microbiota composition and function, including decreased metabolic endotoxemia, contributing to weight loss and metabolic improvements (Guevara-Cruz et al. 2019). However, the specific mechanisms through which these microbial changes affect weight loss and metabolic outcomes remain unclear.

This study investigates the molecular changes in gut microbiota associated with bariatric surgery to understand better the procedure's effects on metabolic health and weight regulation. By evaluating the gut microbiome in obese patients before and after surgery compared to healthy individuals, the research aims to identify specific microbial alterations and their implications. The findings could enhance bariatric surgery outcomes and inform more effective obesity management strategies. Addressing a critical gap in understanding microbial changes post-surgery, the study's molecular approach seeks to provide a deeper, comprehensive insight, potentially leading to personalized obesity treatments and improved patient outcomes.

Materials and methods
Study design and participant selection
This case-control study aimed to investigate temporal changes in gut microbiota composition among obese patients undergoing bariatric surgery (gastric sleeve gastrectomy or Roux-en-Y Gastric Bypass) compared to demographically matched healthy controls. The study was investigated and selected based on predefined inclusion/exclusion criteria.

Data extraction center characteristics
Participants were recruited from the Low-Invasive Surgery Research Center in Tehran, Iran. This center is a governmental and referral institution specializing in minimally invasive surgical procedures. The center's status as a referral facility ensures that it attracts a diverse and comprehensive patient population, making it an ideal setting for this study.

Sampling method
The study employed convenience sampling to select participants. Details on the sampling method are crucial to understanding the representativeness of the study sample.

Recruitment of patients
Individuals with obesity were recruited based on the selection criteria mentioned above. Recruitment spanned from November 2016 to July 2018. Seventeen obese patients underwent mini-gastric bypass surgery, 13 underwent post-classic bipolar surgery, and 18 healthy controls were enrolled.

Inclusion and exclusion criteria
The inclusion criteria required participants to be adults with a BMI indicative of obesity and scheduled for bariatric surgery. Exclusion criteria included a history of severe gastrointestinal disorders, recent use of antibiotics, probiotics, or prebiotics that could alter gut

microbiota composition, pregnancy, and any previous bariatric surgery.

Study population and sample size calculation

Based on these criteria, out of 400 subjects initially considered, 66 subjects were included in the study: 48 obese patients and 18 healthy controls. The exclusion criteria led to the exclusion of 334 potential participants who either did not meet the inclusion criteria or fulfilled one or more exclusion criteria.

Sample collection

Stool samples were collected at three-time points: baseline (pre-surgery), three months, and six months post-surgery for subsequent molecular analysis (Karami et al. 2021). This longitudinal approach allows for a detailed examination of the temporal changes in gut microbiota composition following bariatric surgery.

Assessment of physical activity and dietary intake

The evaluation of physical activity among patients and healthy subjects employed the International Physical Activity Questionnaire (IPAQ), while dietary intake was recorded using a Food Frequency Questionnaire (FFQ). Body mass index (BMI) for both obese and healthy subjects was determined using the formula BMI=weight (kg)/height (m^2).

DNA extraction and quantification

Following standard protocols, fecal specimens (200 µl) were processed using a QIAamp® DNA Stool mini kit (Qiagen Retsch GmbH, Hannover, Germany). DNA quantity and quality were assessed using a Thermo Scientific™ NanoDrop™ One, measuring optical density (OD) at 230 nm, 260 nm, and 280 nm. DNA integrity was verified through agarose gel electrophoresis.

Primer and probe design

Bacterial 16 S rRNA sequences were retrieved from the SILVA High-Quality Ribosomal RNA database and converted into 16 S rDNA. Primers and TaqMan probes for amplification were designed using various tools, including NCBI-primer blast, oligo7 software, Probebase, IDT, EMBL-EBI, and AlleleID software. The characteristics of the designed primers and probes are detailed in Table 1. Before Real-Time PCR, a gradient-PCR method was employed to ensure primer specificity and optimal annealing temperature.

Quantitative real-time PCR analysis

The specificity of designed primers for selected bacterial phyla was confirmed via conventional species. Standard curves for all 16 S rDNA-specific primers and probes were prepared, and qPCR efficiency was calculated. Real-time PCR analysis was conducted using a Rotor-Gene

Table 1 Primer and prob sequences for 16 S rRNA gene-targets

Target Phylum	Primers	Sequence (5'–3')	Product size (bp)	Reference
Lactobacillus group	Forward	GTCTGATGTGAAAGCCYTCG	204	
	Reverse	CCAGGGTATCTAATCCTGTTYG		Sedighi et al. (2017)
	Probe	YCACCGCTACACATGRAGTTCCACT		
Bifidobacterium group	Forward	GGTTAACTCGGAGGAAGG	85	
	Reverse	GTACCGGCCATTGTAGCA		Sedighi et al. (2017)
	Probe	CGTCAGATCATCATGCCCCTTACG		
Fusobacterium group	Forward	GTATGTCRCAAGCGTTATCC	100	
	Reverse	AACGCAATACRGAGTTGAGC		Mohammadzadeh et al. (2019)
	Probe	CCTAGACGCGCTTTACGCCCAAT		
Prevotella group	Forward	CGAACAGGATTAGATACCC	134	
	Reverse	CTTTGAGTTTCACCGTTG		Sedighi et al. (2017)
	Probe	AAACGATGGATGCCCGC		
Faecalibacterium prausnit	Forward	ATAATGACGGTACTCAACAAGGA	171	
	Reverse	ACAGTTTTGAAAGCAGTTTATGG		Navab-Moghadam et al. (2017)
	Probe	ACTTCCAACTTGTCTTCCCGCCTG		
B. fragilis	Forward	CGAGGGGCATCAGGAAGAA	136	
	Reverse	CGGAATCATTATGCTATCGGGTA		Navab-Moghadam et al. (2017)
	Probe	CTTGCTTTCTTTGCTGGCGACCG		
B. longum	Forward	GTGGCTTCGACGGGTAG	200	
	Reverse	ACGGGTAAACTCACTCTCG		Navab-Moghadam et al. (2017)
	Probe	TTGCTCCCCGATAAAAGAGGTTTACA		
C. cluster IV	Forward			
	Reverse			
	Probe			

References in parentheses correspond to the source references for primer sequences

Q real-time PCR cycler (Qiagen Corbett, Hilden, Germany). Gut microbial quantitative alterations were assessed for specific bacterial groups. Nine serial dilutions of a known DNA concentration were used for each detection, and triplicate samples were analysed for mean value determination.

Calculation of quantitative expression data
The quantitative expression data from real-time PCR were calculated using the 2^(-ΔΔCt) method. In this method, ΔCt represents the difference in threshold cycles for the target and reference genes, and ΔΔCt represents the difference between the ΔCt of the samples and the control. This method allows for the relative quantification of gene expression levels.

Statistical analysis and visualization
Statistical analysis was conducted using the R programming language, leveraging the ggstatplot and gtsummary packages for comprehensive data exploration and visualization. The analysis involved independent samples t-test or Mann Whitney U test based on normality distribution. The chi-square (χ^2) association test was employed to compare characteristics between study groups. The mean±SD represented the data, and Pearson correlation assessed linear relationships between variables. Significance was determined at $P<0.05$. The results of quantitative real-time PCR were visually presented through Box and Whisker charts generated using the ggstatplot package in R.

Results
Subject characteristics
We studied stool samples from 48 subjects in 3 groups: 18 normal-weight and 30 obese, including 17 Roux-en-Y Gastric Bypass and 13 SG subjects. Participant age was similar in the three groups (average weight, 50.1±7.56 years; RGYB obese, 57.3±7.62 years; SG obese, 55.1±8.23 years). The characteristics of the participants in this study, including age, weight, and BMI, are presented in Table 2.

Temporal changes in weight and BMI
The figures in Fig. 1 show the changes in weight and BMI for patients undergoing Sleeve Gastrectomy (SG) and Roux-en-Y Gastric Bypass (RYGB) in pre-surgery, three months post-surgery, and six months post-surgery.

Table 2 Characteristics of the participants in this study

Characteristics	Control (mean ± SD)	RGYB (mean ± SD)	SG (mean ± SD)
Age (year)	54.1 ± 7.56	55.1 ± 8.23	57.3 ± 7.62
Weight (kg)	65.72 ± 6.75	122.8 ± 14.18	117.66 ± 16.61
BMI (kg/m2)	24.43 ± 2.98	45.3 ± 3.72	43.6 ± 3.55

For SG, weight decreased from 117.66 kg to 95.29 kg at three months and 87.21 kg at six months ($P<0.001$), with BMI dropping from 43.62 to 35.23 at three months and 32.18 at six months ($P<0.001$). The RYGB group showed similar trends. Combined data for both surgeries showed weight decreasing from 120.49 kg to 98.60 kg at three months and 90.60 kg at six months ($P<0.001$), with BMI dropping from 44.61 to 36.40 at three months and 33.41 at six months ($P<0.001$). These findings confirm the efficacy of bariatric surgery in significantly reducing weight and BMI at both 3- and 6-months post-surgery.

### Food frequency questionnaire (FFQ) results
For the Sleeve Gastrectomy group, the reduction in fast food consumption from an average of 4 times per week to 1 time per week was statistically significant ($P=0.002$). Similarly, the decrease in sugary drink consumption from 10 to 2 servings per week was statistically significant ($P=0.004$). Furthermore, the increase in vegetable intake from 7 servings per week to 14 servings per week and fruit intake from 5 servings to 10 servings per week were statistically significant ($P=0.001$). In terms of physical activity, the increase in moderate physical activity from an average of 90 min per week to 150 min per week was statistically significant ($P=0.008$), as was the increase in vigorous physical activity from 20 min per week to 50 min per week ($P=0.012$) (Table 3) (Fig. 2).

International physical activity questionnaire (IPAQ)
Similarly, for the RYGB group, the reduction in processed meat consumption from 5 times per week to one time per week was statistically significant ($P=0.003$). The increase in whole grain intake from three to nine servings per week was statistically significant ($P=0.005$). However, the decline in dairy product consumption from seven to five servings per week was not statistically significant ($P=0.214$). In terms of physical activity, the substantial increase in vigorous physical activity from 30 min per week to 120 min per week was statistically significant ($P=0.001$), as was the increase in moderate physical activity from 60 min per week to 180 min per week ($P=0.007$). The increase in walking activities from 100 to 200 min per week was also statistically significant ($P=0.009$) (Table 3).

Real-time qPCR analysis of gut microbiota
Table 4 shows the results from the real-time quantitative polymerase chain reaction (qPCR) analysis of bacterial groups in fecal samples, comparing pre-intervention and post-intervention samples. Significant changes include a decrease in Bacteroides (9.62±0.59 to 9.07±0.77; $P=0.003$) and increases in *Fusobacterium* (5.25±1.32 to 6.68±1.31; $P<0.001$) and Proteobacteria (7.10±1.37 to 7.83±1.09; $P=0.023$). No significant changes were found
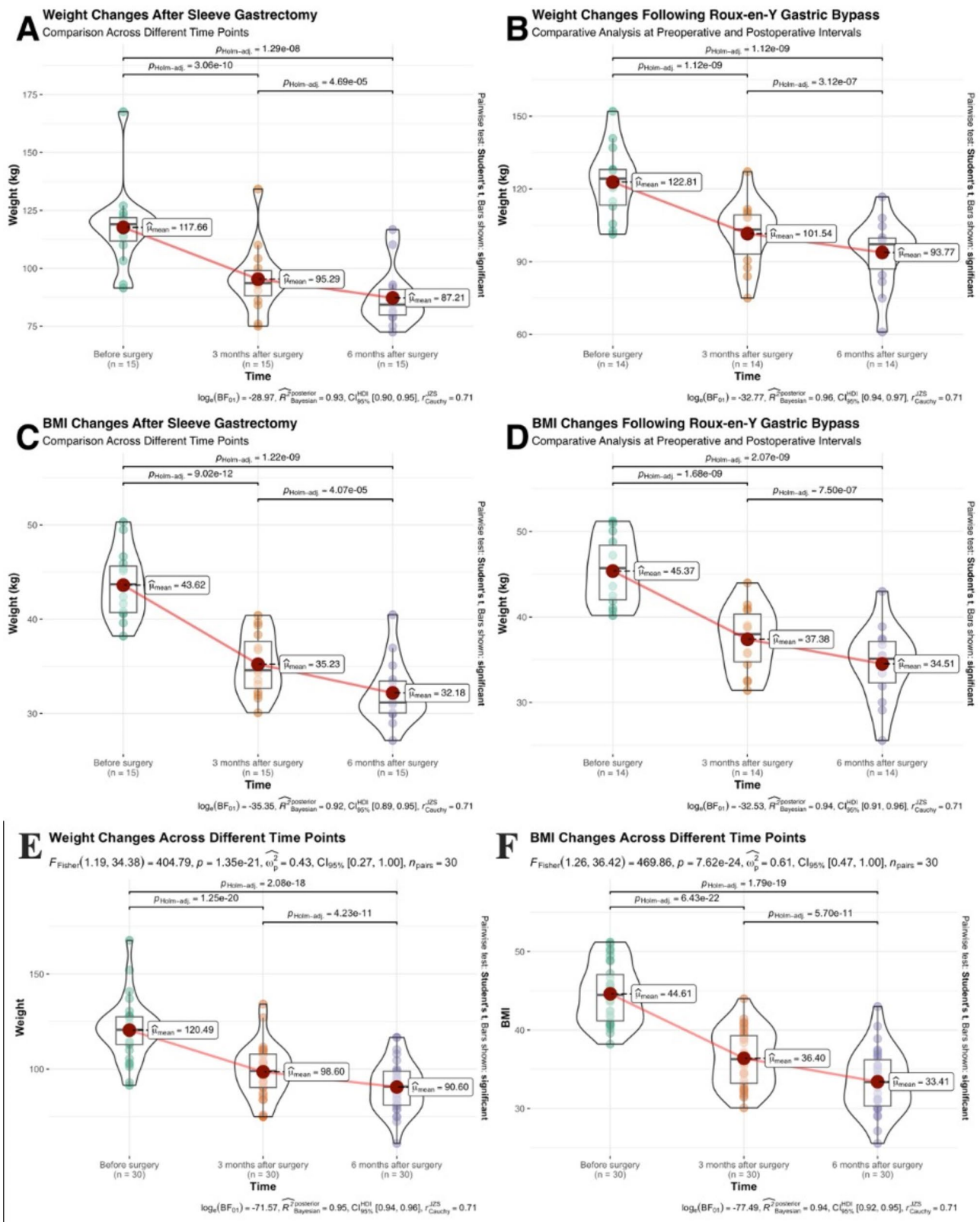
Fig. 1 Comparative analysis of weight and BMI in patients undergoing bariatric surgery. **A** Weight trends at three time points in patients undergoing sleeve gastrectomy. **B** Weight trends at three time points in patients undergoing RYGB surgery. **C** BMI trends at three time points in patients undergoing sleeve gastrectomy. **D** BMI trends at three time points in patients undergoing RYGB surgery. **E** Comparative weight trends at three time points in patients undergoing both surgery types. **F** Comparative BMI trends at three time points in patients undergoing both surgery types

Table 3 Comparative analysis of dietary and physical activity changes in sleeve gastrectomy and RYGB patients before and after surgery

Variables	Pre-surgery (Mean±SD)	Post-surgery (Mean±SD)
Sleeve Gastrectomy Group		
Dietary Habits		
Fast Food Consumption	4±1.5 times/week	1±0.5 times/week
Sugary Drinks	10±2.5 servings/week	2±0.8 servings/week
Vegetable Intake	7±1.2 servings/week	14±2.0 servings/week
Fruit Intake	5±1.0 servings/week	10±1.5 servings/week
Physical Activity Levels		
Moderate Activity	90±15.0 min/week	150±20.0 min/week
Vigorous Activity	20±5.0 min/week	50±10.0 min/week
RYGB Group		
Dietary Habits		
Processed Meat Consumption	5±1.0 times/week	1±0.5 times/week
Whole Grain Intake	3±0.5 servings/week	9±1.5 servings/week
Dairy Product Intake	7±1.0 servings/week	5±0.8 servings/week
Physical Activity Levels		
Vigorous Activity	30±7.0 min/week	120±15.0 min/week
Moderate Activity	60±10.0 min/week	180±25.0 min/week
Walking Activity	100±12.0 min/week	200±20.0 min/week

This table illustrates the changes in dietary habits and physical activity levels for patients undergoing Sleeve Gastrectomy and RYGB surgery. The data, presented as mean±standard deviation, compares pre-surgery and post-surgery behaviors, highlighting significant shifts in lifestyle choices such as food consumption and exercise frequency

in *B. fragilis*, *Firmicutes*, *B. longum*, *Bifidobacteria*, C. cluster IV, *Lactobacillus*, and *Prevotella*. These findings highlight the specific bacterial groups affected by the intervention, providing insights into its impact on gut microbiota composition and potential implications for host health.

Associations between body weight, BMI, and gut microbiota composition pre- and post-bariatric surgery

An investigation analysed the association between body weight, BMI, and bacterial counts in patients undergoing Sleeve Gastrectomy (SG) and Roux-en-Y Gastric Bypass (RYGB) at baseline, three months, and six months post-operation. Various bacterial species were studied. In the SG group, BMI negatively correlated with C. cluster IV at three months ($P=0.035$) and six months ($P=0.012$) and positively correlated with *Lactobacillus* at three months ($P=0.020$) and six months ($P=0.036$). In the RYGB group, weight negatively correlated with *Firmicutes* at baseline ($P=0.219$) but not post-surgery and positively correlated with *Fusobacterium* at six months ($P=0.071$). C. cluster IV negatively correlated with weight at three months ($P=0.357$) and six months ($P=0.237$), while *Lactobacillus* positively correlated with weight at six months

post-RYGB ($P=0.020$ for three months and $P=0.036$ for six months). No significant correlation with *Prevotella* was detected in the RYGB group. These findings suggest that while most bacterial species do not significantly correlate with weight or BMI changes post-bariatric surgery, specific groups like C. cluster IV and *Lactobacillus* may be influenced, especially in the RYGB group. Further research is needed to explore these interactions.

Analysis of bacterial population changes post-bariatric surgery

Table 5 presents a comparative analysis of gut microbiota in obese subjects before and after two bariatric surgeries: Roux-en-Y Gastric Bypass (RYGB) and Sleeve Gastrectomy (SG). The study highlights significant changes in bacterial populations due to these surgeries.

Bacteroides, linked to high-fat diets, significantly decreased post-surgery in RYGB ($P=0.036$) and SG ($P=0.038$), suggesting a shift towards a gut environment less conducive to fat digestion and absorption, contributing to weight loss. *Fusobacterium*, associated with inflammation, significantly increased after both RYGB ($P=0.004$) and SG ($P<0.001$), warranting further investigation into its role in post-surgery inflammation.

The *Lactobacillus* group, known for beneficial gut health effects, decreased significantly only after RYGB ($P=0.002$), raising questions about RYGB's differential impact on gut protective mechanisms compared to SG, where the change was not significant ($P=0.107$).

No significant changes were found in the counts of *B. fragilis*, *Firmicutes*, *B. longum*, *Bifidobacteria*, C. cluster IV, and *Prevotella*, indicating their resilience to metabolic shifts post-surgery or a less influential role in weight loss.

Proteobacteria, including various pathogens, increased significantly overall ($P=0.023$), with a significant rise in RYGB ($P=0.005$) but not in SG ($P=0.575$), suggesting distinct microbial habitat changes induced by each surgery type.

Discussion

This study provides valuable insights into the impact of bariatric surgery on gut microbiota composition and its potential implications for weight management and metabolic health in obese patients.

The composition of the gut microbiota changes over time and significantly influences various human diseases, including obesity (Lu and Ni 2015). Dysbiosis of the gut microbiota, characterized by an increase in the *Firmicutes* to Bacteroidetes ratio, has been associated with obesity (Mitev and Taleski 2019). However, further research is needed to determine significant differences in gut microbiota characteristics between patients with subcutaneous and visceral obesity (Liu et al. 2021).
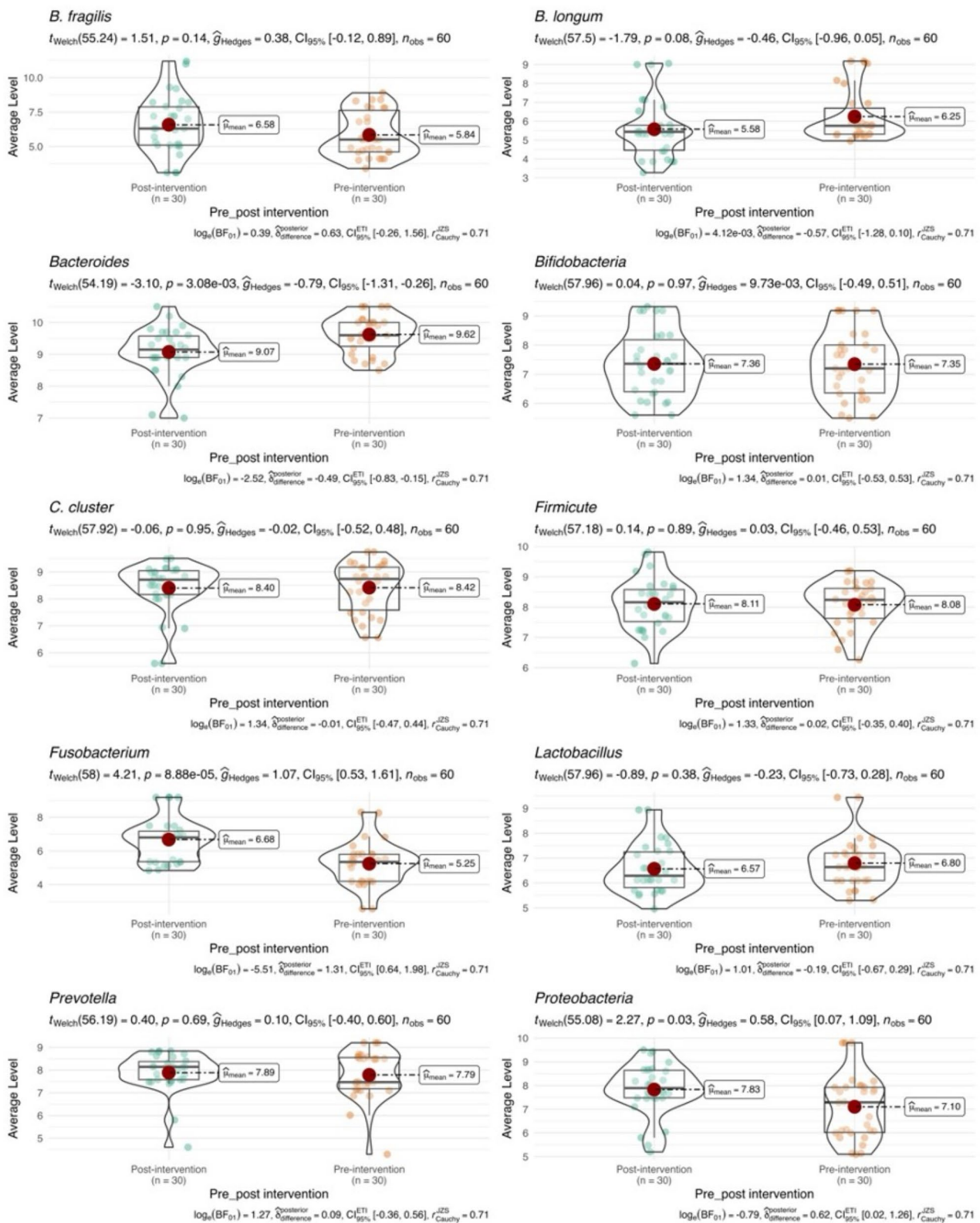
Fig. 2 Comparison of logarithmic fecal PCR target genetic amplicon copy numbers pre- and post-bariatric surgery. Figure depicts the mean logarithmic values of fecal PCR target genetic amplicon copy numbers per gram of feces, as analyzed through real-time quantitative polymerase chain reaction (qPCR). This comparison highlights the changes in bacterial group populations in fecal samples of patients before and after bariatric surgery interventions, providing insights into the gut microbiota's response to surgical procedures (Fig. 3)

Bacterial phylum	RYGB						Sleeve					
	Weight Correlation (Sig.)			BMI Correlation (Sig.)			Weight Correlation (Sig.)			BMI Correlation (Sig.)		
	Bassline	3 months	6 months	Bassline	3 months	6 months	Bassline	3 months	6 months	Bassline	3 months	6 months
B. fragilis	0.06 (0.82)	-0.079 (0.762)	-0.21 (0.418)	-0.178 (0.495)	-0.047 (0.856)	-0.186 (0.475)	0.045 (0.884)	0.05 (0.871)	0.059 (0.847)	0.017 (0.956)	-0.394 (0.183)	-0.307 (0.308)
Bacteroides	0.11 (0.674)	-0.038 (0.885)	-0.062 (0.812)	0.003 (0.991)	-0.132 (0.614)	-0.137 (0.6)	-0.23 (0.45)	0.066 (0.829)	0.068 (0.825)	0.166 (0.587)	-0.165 (0.59)	-0.124 (0.687)
Firmicute	-0.314 (0.219)	-0.06 (0.82)	-0.021 (0.935)	-0.351 (0.168)	-0.153 (0.557)	-0.088 (0.737)	0.237 (0.435)	0.066 (0.831)	0.074 (0.81)	0.166 (0.587)	-0.132 (0.668)	-0.781
B. longum	0.138 (0.597)	-0.016 (0.953)	0.015 (0.956)	0.431 (0.084)	-0.061 (0.815)	-0.014 (0.957)	-0.388 (0.19)	0.163 (0.594)	0.135 (0.66)	-0.221 (0.469)	0.17 (0.578)	0.13 (0.671)
Bifidobacteria	0.232 (0.371)	-0.035 (0.893)	-0.211 (0.416)	0.089 (0.735)	-0.317 (0.216)	-0.443 (0.075)	0.161 (0.599)	-0.108 (0.726)	-0.018 (0.953)	0.12 (0.696)	-0.125 (0.684)	0.002 (0.995)
Fusobacterium	-0.001 (0.996)	-0.042 (0.874)	-0.021 (0.935)	0.085 (0.746)	-0.038 (0.884)	-0.005 (0.984)	0.291 (0.334)	0.172 (0.574)	0.144 (0.64)	0.516 (0.071)	0.174 (0.571)	0.133 (0.664)
C. cluster IV	0.051 (0.847)	-0.238 (0.357)	-0.303 (0.237)	0.337 (0.186)	-0.15 (0.567)	-0.179 (0.492)	0.081 (0.791)	-0.322 (0.284)	-0.405 (0.17)	0.415 (0.158)	-0.587* (0.035)	-0.671* (0.012)
Proteobacteria	-0.003 (0.99)	-0.021 (0.937)	-0.176 (0.498)	0.109 (0.677)	-0.008 (0.977)	-0.178 (0.493)	0.122 (0.692)	-0.13 (0.672)	-0.075 (0.807)	-0.382 (0.197)	-0.232 (0.446)	-0.13 (0.671)
Lactobacillus	0.053 (0.839)	0.215 (0.408)	0.184 (0.48)	0.411 (0.101)	0.004 (0.988)	-0.085 (0.747)	-0.168 (0.584)	0.635* (0.02)	0.585* (0.036)	-0.148 (0.63)	0.271 (0.37)	0.258 (0.395)
Prevotella	0.015 (0.954)	-0.052 (0.843)	-0.016 (0.952)	0.175 (0.502)	0.097 (0.711)	0.138 (0.597)	0.418 (0.156)	0.059 (0.849)	0.115 (0.708)	0.202 (0.509)	-0.437 (0.135)	-0.283 (0.349)

Fig. 3 Correlation between weight and BMI of patients in RYGD and sleeve surgery group. Figure displays the correlation between gut bacterial phyla frequencies and weight and BMI in patients undergoing RYGB and Sleeve Gastrectomy. Each cell presents the Pearson correlation coefficient and its significance (Sig.), with red indicating a more positive correlation and blue showing a more negative correlation between the bacterial frequencies and the patients' weight or BMI at baseline, three months, and six months post-surgery. Statistically significant correlations are marked with an asterisk (*)

Table 4 Differential abundance of fecal bacterial groups pre- and Post-intervention as analyzed by qPCR

Bacteria	Pre-Intervention (Mean ± SD)	Post-Intervention (Mean ± SD)	P-Value
B. fragilis	5.84 ± 1.66	6.58 ± 2.08	0.0829
Bacteroides	9.62 ± 0.59	9.07 ± 0.77	0.0034
Firmicute	8.08 ± 0.74	8.11 ± 0.84	0.887
B. longum	6.25 ± 1.38	5.58 ± 1.52	0.0956
Bifidobacteria	7.35 ± 1.17	7.36 ± 1.13	0.9719
Fusobacterium	5.25 ± 1.32	6.68 ± 1.31	< 0.001
C. cluster	8.42 ± 0.94	8.40 ± 0.98	0.9189
Proteobacteria	7.10 ± 1.37	7.83 ± 1.09	0.0235
Lactobacillus	6.80 ± 1.01	6.57 ± 0.98	0.3503
Prevotella	7.79 ± 1.06	7.89 ± 0.88	0.5706

Table presents a comparative analysis of bacterial group abundance in fecal samples before and after a clinical intervention, as determined by quantitative real-time PCR (qPCR). Data are expressed as mean logarithmic values ± standard deviation (SD) of the genetic amplicon copy numbers per gram of feces. Statistically significant differences, denoted by a p-value less than 0.05, were identified for *Bacteroides*, *Fusobacterium*, and *Proteobacteria*, indicating alterations in gut microbiota composition post-intervention

Studies using germ-free animals and fecal microbiota transplantation (FMT) experiments have confirmed the critical role of gut microbiota dysbiosis in obesity development Field (Zhou et al. 2023).

Evidence supports the causal role of gut microbiota in obesity, with the gut microbiota undergoing dynamic changes during behavioral weight loss interventions (Stanislawski et al. 2021). Molecular approaches such as PCR-DGGE and real-time PCR have provided valuable insights into the association between gut microbiota and liver diseases in patients with liver cirrhosis (Liu et al. 2012).

Longitudinal studies have provided valuable insights into the dynamics of gut microbiota under both healthy and disease conditions, emphasizing the importance of understanding the temporal changes in gut microbiota (Gnanasekaran et al. 2023).

Our findings indicate significant changes in weight and body mass index (BMI), as well as notable shifts in dietary habits and physical activity following Roux-en-Y Gastric Bypass (RYGB) and Sleeve Gastrectomy (SG). Both surgical approaches led to substantial reductions in weight and BMI, corroborating previous research that highlights the effectiveness of bariatric surgery as a treatment for obesity. Patients in the SG group experienced a dramatic decrease in weight from an average of 117.66 kg to 87.21 kg. These findings align with existing literature, reinforcing the understanding that bariatric surgery is a viable intervention for achieving significant weight loss and improving obesity-related health outcomes.

The study also observed significant improvements in dietary habits among participants post-surgery. Reduced fast food, sugary drink consumption, and increased vegetable and fruit intake are critical shifts towards healthier

Table 5 Changes in bacterial frequency in patients undergoing RYGB and Sleeve Gastrectomy

Bacteria	Overall (Mean ± SD)			RYGB (Mean ± SD)			Sleeve		
	Pre	Post	P	Pre	Post	P	Pre	Post	P
B. fragilis	5.84 ± 1.66	6.58 ± 2.08	0.082	5.49 ± 1.47	6.24 ± 2.41	0.244	6.15 ± 1.79	6.88 ± 1.76	0.221
B. longum	6.25 ± 1.38	5.58 ± 1.52	0.095	6.96 ± 1.73	5.54 ± 1.26	0.035	5.62 ± 0.45	5.61 ± 1.75	0.968
Bacteroides	9.62 ± 0.59	9.07 ± 0.77	0.003	9.59 ± 0.55	8.86 ± 0.98	0.036	9.65 ± 0.64	9.26 ± 0.50	0.038
Bifidobacteria	7.35 ± 1.17	7.36 ± 1.13	0.972	7.35 ± 1.06	7.21 ± 1.10	0.758	7.34 ± 1.28	7.48 ± 1.18	0.754
C. cluster	8.42 ± 0.94	8.40 ± 0.98	0.919	8.57 ± 0.99	8.32 ± 1.24	0.293	8.28 ± 0.91	8.48 ± 0.70	0.319
Firmicute	8.08 ± 0.74	8.11 ± 0.84	0.887	8.14 ± 0.65	7.94 ± 0.94	0.560	8.02 ± 0.83	8.25 ± 0.73	0.308
Fusobacterium	5.25 ± 1.32	6.68 ± 1.31	< 0.001	4.91 ± 1.48	6.32 ± 1.22	0.004	5.54 ± 1.14	6.99 ± 1.34	< 0.001
Lactobacillus	6.80 ± 1.01	6.57 ± 0.98	0.350	7.43 ± 1.02	6.40 ± 0.68	0.002	6.24 ± 0.59	6.72 ± 1.19	0.107
Prevotella	7.79 ± 1.06	7.89 ± 0.88	0.571	7.75 ± 0.81	7.76 ± 1.01	0.983	7.82 ± 1.26	8.00 ± 0.76	0.449
Proteobacteria	7.10 ± 1.37	7.83 ± 1.09	0.023	6.71 ± 1.36	6.92 ± 1.53	0.005	7.44 ± 1.33	8.00 ± 1.55	0.575

This table compares various bacterial frequencies in patients before and after undergoing RYGB and Sleeve Gastrectomy surgeries. The data, expressed as mean ± standard deviation, include pre-surgery and post-surgery values and P-values to indicate statistical significance. Key bacterial strains such as *B. fragilis*, *B. longum*, *Bacteroides*, and *Fusobacterium* are analyzed to understand the alterations in gut microbiota resulting from these bariatric procedures

eating patterns. Such changes are essential not only for weight loss but also for fostering long-term metabolic health. Similarly, the enhancement in physical activity levels reported by both groups further emphasizes the importance of lifestyle modifications in conjunction with surgical interventions. Increased physical activity is vital for weight loss and overall health post-surgery.

The investigation of the association between body weight, BMI, and bacterial counts in patients undergoing bariatric surgery revealed intriguing insights into the dynamics of gut microbiota composition pre-and post-surgery (Mika et al. 2022). The analysis encompassed a diverse range of bacterial species, including *Bacteroides fragilis, Bacteroides, Firmicutes, Bifidobacterium longum, Bifidobacteria, Fusobacterium, Clostridium* cluster, *Proteobacteria, Lactobacillus,* and *Prevotella* (Ghosh and Pramanik 2021).

While most bacterial species did not significantly correlate with weight or BMI changes post-bariatric surgery, these factors may influence specific bacterial groups such as C. cluster IV and *Lactobacillus*, particularly in the RYGB group. Notably, a significant negative correlation was observed between the frequency of C. cluster IV and weight at 3 and 6 months post-RYGB surgery, suggesting a potential influence of surgical intervention on this bacterial population. A positive correlation was also present between *Lactobacillus* frequency and BMI at 3- and 6-months post-surgery. However, for *Prevotella*, no significant correlation with weight or BMI was detected in the RYGB group at any of the studied time points, highlighting the variability in the bacterial response to weight and BMI changes post-bariatric surgery.

The comparative analysis of the gut microbiota in obese subjects before and after RYGB and SG procedures revealed significant alterations in bacterial populations, shedding light on the microbiome's dynamic response to anatomical and physiological changes induced by these interventions. The observed decrease in the abundance

of Bacteroides following both RYGB and SG suggests a potential shift towards a gut environment less conducive to fat digestion and absorption post-surgery, which may contribute to the weight loss observed in these patients. Conversely, the significant increase in *Fusobacterium* after both RYGB and SG raises questions about the clinical implications of this rise, particularly considering the complex role of inflammation in obesity and metabolic health. The study highlights significant changes in gut microbiota following Roux-en-Y Gastric Bypass (RYGB) and Sleeve Gastrectomy (SG). *Lactobacillus* decreased significantly only after RYGB, indicating a unique impact on gut protection. Bacteria such as *B. fragilis, Firmicutes, B. longum, Bifidobacteria, C. cluster* IV, and *Prevotella* showed no significant changes, suggesting resilience to metabolic shifts. Proteobacteria, including various pathogens, increased overall, particularly after RYGB, indicating distinct microbial changes with each surgery type. These findings underscore the complex impact of bariatric surgery on gut microbiota, highlighting the need for further research to understand the specific mechanisms involved and optimize post-surgery outcomes.

Abbreviations
RYGB	Roux-en-Y Gastric Bypass
IPAQ	Physical Activity Questionnaire
FFQ	Food Frequency Questionnaire
BMI	Body mass index
(OD	Optical density
qPCR	Quantitative polymerase chain reaction

Authors' contributions
Nima Mohammadzadeh: Conceptualization, methodology, data collection, data analysis, manuscript writing, and project administration. Shabnam Razavi: Methodology, data analysis, and manuscript editing. Gholamhossein Ebrahimipour: Supervision, funding acquisition, and manuscript revision. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate
This study was approved by the Iran University of Medical Science, Tehran, Iran (project no: IR.IUMS.REC.1397.1072). Before enrollment, all participants were given written informed consent. They were also informed about the study's aims, procedures, potential risks, and benefits and their right to withdraw from the study at any time without consequence.

Competing interests
None of the group members reported conflicts of interest, so they did not influence the guideline process or the development of recommendations.

References

Aderinto N, Olatunji G, Kokori E, Olaniyi P, Isarinade T, Yusuf IA (2023) Recent advances in bariatric surgery: a narrative review of weight loss procedures. Ann Med Surg (Lond) 85(12):6091–6104

Farias G, Netto BDM, Bettini SC, Dâmaso AR, de Freitas ACT (2017) Neuroendocrine regulation of energy balance: implications on the development and surgical treatment of obesity. Nutr Health 23(3):131–146

Ghosh S, Pramanik S (2021) Structural diversity, functional aspects and future therapeutic applications of human gut microbiome. Arch Microbiol 203(9):5281–5308

Gnanasekaran T, Sarathi A, Fang Q, Azarm A, Assis Geraldo J, Nigro E, Arumugam M (2023) Quantitative differences in synthetic gut microbial inoculums do not affect the final stabilized in vitro community compositions. Msystems 8(4):e01249–e01222

Guevara-Cruz M, Flores-López AG, Aguilar-López M, Sánchez-Tapia M, Medina-Vera I, Díaz D, Tovar AR, Torres N (2019) Improvement of lipoprotein profile and metabolic endotoxemia by a lifestyle intervention that modifies the gut microbiota in subjects with metabolic syndrome. J Am Heart Assoc 8(17):e012401

Hua Y, Lou Y-X, Li C, Sun J-Y, Sun W, Kong X-Q (2022) Clinical outcomes of bariatric surgery—updated evidence. Obes Res Clin Pract 16(1):1–9

Karami R, Kermansaravi M, Pishgahroudsari M, Talebi M, Mohammadzadeh N, Pazouki A (2021) Changes in gut microbial flora after Roux-en-Y gastric bypass and sleeve gastrectomy and their effects on post-operative weight loss. Updates Surg 73(4):1493–1499. https://doi.org/10.1007/s13304-020-00900-9

Liu J, Wu D, Ahmed A, Li X, Ma Y, Tang L, Mo D, Ma Y, Xin Y (2012) Comparison of the gut microbe profiles and numbers between patients with liver cirrhosis and healthy individuals. Curr Microbiol 65:7–13

Liu B-N, Liu X-T, Liang Z-H, Wang J-H (2021) Gut microbiota in obesity. World J Gastroenterol 27(25):3837

Lu C-Y, Ni Y-H (2015) Gut microbiota and the development of pediatric diseases. J Gastroenterol 50:720–726

Mika A, Janczy A, Waleron K, Szymanski M, Kaska L, Sledzinski T (2022) The impact of the interplay of the intestinal microbiome and diet on the metabolomic and health outcomes of bariatric surgery. Obes Rev 23(8):e13455

Mitev K, Taleski V (2019) Association between the gut microbiota and obesity. Open Access Macedonian J Med Sci 7(12):2050

Mohammadzadeh N, Kalani BS, Bolori S, Azadegan A, Gholami A, Mohammadzadeh R, Masjedian Jazi F (2019) Identification of an intestinal microbiota signature associated with hospitalized patients with diarrhea. Acta Microbiol Immunol Hung 66(2):189–202

Navab-Moghadam F, Sedighi M, Khamseh ME, Alaei-Shahmiri F, Talebi M, Razavi S, Amirmozafari N (2017) The association of type II diabetes with gut microbiota composition. Microb Pathog 110:630–636

Nguyen NT, Varela JE (2017) Bariatric surgery for obesity and metabolic disorders: state of the art. Nat Rev Gastroenterol Hepatol 14(3):160–169

Sedighi M, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, Amirmozafari N (2017) Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. Microb Pathog 111:362–369

Stanislawski MA, Frank DN, Borengasser SJ, Ostendorf DM, Ir D, Jambal P, Bing K, Wayland L, Siebert JC, Bessesen DH (2021) The gut microbiota during a behavioral weight loss intervention. Nutrients 13(9):3248

Tremaroli V, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T, Fändriks L, le Roux CW, Nielsen J, Bäckhed F (2015) Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. Cell Metabol 22(2):228–238

Wiggins T, Guidozzi N, Welbourn R, Ahmed AR, Markar SR (2020) Association of bariatric surgery with all-cause mortality and incidence of obesity-related disease at a population level: a systematic review and meta-analysis. PLoS Med 17(7):e1003206

Zhou M, Johnston LJ, Wu C, Ma X (2023) Gut microbiota and its metabolites: bridge of dietary nutrients and obesity-related diseases. Crit Rev Food Sci Nutr 63(18):3236–3253

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