ORIGINAL CONTRIBUTIONS





Effects of Milkfat on the Gut Microbiome of Patients After Bariatric Surgery, a Pilot Study

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Abstract

Background The efficacy of bariatric surgery may be in part attributed to altered metabolism *via* new gut microbiome. Milkfat may promote the growth of microbes that are beneficial in long-term weight loss. Understanding the specific gut microbiome changes after surgery and their relationship to milkfat consumption may yield important strategies for managing obesity after bariatric procedures.

Methods In this pilot study, stool samples were collected from nine patients before and at the time of surgery, and at 1, 3, and 6 months post-surgery. At each time-point, dairy consumption was determined from dietary surveys. 16 s rRNA gene sequencing was performed followed by alpha diversity analysis. Comparisons of relative abundances of microbial taxa and analyses of fatty acids changes were performed.

Results Bariatric surgery led to enrichment of (i) *Roseburia*, associated with weight loss and (ii) *Christensenellaceae*, inversely related to body mass index. High milk-fat consumption correlated with enrichment of *Blautia*, inversely associated with visceral fat accumulation. *Faecalibacterium*, possibly associated with obesity, increased in patients with low milk-fat consumption. Butter was associated with decreased alpha diversity in all subjects (p-value = 0.038) and the frequency of its use was associated with decreased alpha diversity in patients (correlation = -0.68, p-value = 0.042). Low-milk-fat consumers showed higher concentration of saturated fatty acids.

Conclusions Our results suggest that incorporating dairy products in post-bariatric-surgery dietary plans may help cultivate a gut microbiome that is effective in regulating fat storage as well as digesting beneficial metabolites. These observations will be helpful for the management of obesity in general population as well.

Keywords Bariatric surgery · Gut microbiome · Weight loss · Milkfat · Metabolome

Key Points

- Post-bariatric surgery enrichment of (i) *Roseburia*, associated with weight loss and (ii) *Christensenellaceae*, inversely related to body mass index.
- High milk-fat consumption correlated with enrichment of *Blautia*, inversely associated with visceral fat accumulation.
- Low-milk-fat consumers showed higher concentration of saturated fatty acids.
- Incorporating dairy products in post-bariatric-surgery dietary plans may be helpful for the management of obesity.
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Introduction

Prevalence of obesity has been rapidly increasing in the United States as well as other countries in the last 20 years, with 48.2% of adults now classified as either overweight or obese [1]. Bariatric surgery has the highest effect for substantial weight loss compared to behavioral modifications. However, the recurrence of weight gain has been shown to be as high as 50% in patients who underwent a gastric

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bypass [2]. As such, extensive research and public health initiatives have been aimed to identify new strategies in preventing post-operative recurrence. The altered gastrointestinal anatomy and altered absorptive processes following surgery may positively influence the gut microbiome, which is significantly involved in fat and energy regulation [3, 4].

Milkfat likely reduces gut dysbiosis and facilitates many of the essential metabolic processes associated with weight loss [5]. While support for dairy consumption in controlling weight has been controversial, there is a paucity of research on the effects of milkfat and sustained weight loss via changes in gut microbiome [6]. We hypothesized that dairy consumption may have an important role in postoperative outcomes. With the rates of performed bariatric surgical procedures increasing each year, understanding the specific gut flora changes before and after surgery and its relationship to milkfat consumption may yield important strategies for managing sustained weight-loss after bariatric surgery. Herein, we profile microbiome changes in patients who underwent bariatric surgery and had varying degrees of milkfat consumption. We also analyzed patient samples to see if bariatric surgery and more specifically consumption of high milk-fat, leads to changes in saturated, monounsaturated and polyunsaturated fatty acids. Our results will also be useful for the management of obesity in general population.

Materials and Methods

Study Design

Patients who were at least 18 years of age and scheduled for bariatric surgery at Cooper University hospital, Camden, New Jersey, from April to December 2019 were recruited. Surveys about demographics, medical history including antibiotic use, and baseline milkfat consumption were collected at the time of recruitment. Patients were asked to provide stool samples and dairy intake surveys (Supplemental file) 2 weeks before surgery, at time of the surgery, and at 1, 3, and 6 months postop. Baseline patient characteristics at enrollment 3–6 weeks prior to surgery are shown in Table 1. Of the 9 patients studied, 1 was diabetic and 3 were pre-diabetic. Of these 4 patients, 1 patient was receiving treatment for impaired glucose tolerance (BS139—prediabetic) prior to surgery and treatment was discontinued following surgery. The remaining patients were not on any diabetes medications.

Sample Collection

Patients were given detailed instructions about sample collection and were provided with a kit that included all

the necessary materials such as collection tube, gloves, toilet hat, labels, zip lock bags etc. Stool samples were collected at home in Stool Nucleic Acid Collection and Preservation Tubes (Norgen, Ontario, Canada). The stabilizer allows easy storage and transport of samples to laboratory. Upon receipt, samples were stored at - 80 °C for long term storage and subsequent analysis.

16S rRNA Gene Sequencing

Total genomic bacterial DNA was extracted from fecal samples using the Qiagen DNeasy PowerSoil HTP Kit (Qiagen, California, USA). For high throughput sequencing, 515F–806R Golay barcodes were used to target the hypervariable V4 region of the 16S rRNA gene, which is highly conserved and ideal for gut microbiome analysis. Marker genes in isolated DNA were PCR-amplified using GoTaq Master Mix (Promega, Wisconsin, USA) [7]. 16S rRNA gene sequencing was performed on normalized pooled amplicons using the Illumina MiSeq System using a MiSeq reagent kit v2.

Analysis of Data from 16S rRNA Gene Sequencing

Analysis was performed using Python-based packages (Python 3.6.12), Quantitative Insights Into Microbial Ecology (QIIME) 2 2020.11, and Linear Discriminant Analysis using LEfSe10 [8, 9]. Sequences were de-multiplexed, then filtered and clustered into sub-operational taxonomic units (sOTUs) using QIIME 2 DADA2 [10]. Phylogenetic tree was created using SATé-enabled phylogenetic placement (SEPP) via Qiime 2 [11]. Taxonomy was assigned using a naïve-Bayes classifier based on the latest SILVA 16S rRNA gene database (March 2021) via the QIIME 2 interface. Additional Python packages (SciPy.stats, Scikit-bio) were used for statistical tests on QIIME 2-generated data. Inter-class comparisons were made based on attributes of our population. Alpha analyses were performed in QIIME 2, rarefied to an even sampling depth of 22,400 [12].

Alpha diversity was assessed by evenness analysis for factors, time-points, age, number of people at home, initial BMI, frequency of smoking, drinks/week, dairy/week (milk, yogurt, cottage cheese, sour cream, kefir, cheese, butter and margarine, ice cream and similar foods, using butter for cooking, other milk products and total milk consumption). To identify variables on which later analyses could focus, differences in alpha diversity were assessed by Kruskal–Wallis non-parametric rank test for categorical variables for Spearman for continuous or discrete variables across the dataset. To address non-independence of samples, we identified the variables that were most strongly associated with alpha diversity from the exploratory analysis and Table 1Baseline patientcharacteristics at enrollment3-6 weeks prior to surgery

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Patient	Age	Sex	Ethnicity	BMI (kg/m ²)	Diabetes	HbA1C	Smoking	Current alcohol use
BS124	30	М	White	55.67	Prediabetic	5.7	No	No
BS131	62	М	White	45.71	Nondiabetic	5.6	No	Yes
BS135	54	F	White	48.81	Nondiabetic	5.6	No	Yes
BS138	31	F	Other	36.94	Nondiabetic	5.3	No	Yes
BS139	41	F	Other	54.03	Prediabetic	6.1	Yes	No
BS140	42	М	Other	42.91	Nondiabetic	5.5	Yes	Yes
BS142	49	F	Black or AA	42.91	Nondiabetic	5.6	No	Yes
BS143	66	М	White	36.58	Prediabetic	5.9	No	No
BS145	61	F	White	45.91	Diabetic	7.3	No	No

AA African American

then performed more conservative tests that took non-independence into account. For categorical variables that did not change across time-points within a participant (such as use of butter while cooking during the study), a Kruskal-Wallis test was performed on the average alpha diversity values for each participant. Likewise, for discrete and continuous variables that did not change over time, Spearman correlation tests were performed on each participant's average alpha diversity value. For discrete and continuous variables that changed within each participant, such as time-point, a repeated measures correlation test was performed on the ranks of Faith's phylogenetic diversity, since assumptions of normality were not met. Kruskal-Wallis and Spearman correlation tests were performed via the Python package SciPy. stats, and the repeated measures correlation was performed via the package Pingouin.

Comparisons of relative abundances of microbial taxa between groups were performed using Linear Discriminant Analysis (LDA) Effect Size (LEfSe) with the Huttenhower group online interface [13]. Taxa with an LDA value of 2.0 or greater and a two-tailed p value < 0.05 with Kruskal–Wallis and pairwise Wilcoxon analyses were considered significantly enriched. Volatility plot was created in Qiime2 via longitudinal plug in.

Non-targeted fatty acid analysis

Analysis of fecal samples was performed using gas chromatography–mass spectrometry (GC–MS) by Metabolon, Inc. (Durham, NC, USA). Metabolon TAM112-002 measures the total fecal content of 30 fatty acids after conversion into their corresponding fatty acid methyl esters (FAMEs). The measured concentrations are provided in weight corrected μ g/g of sample. Values below the lower limit of quantification were treated as a concentration of 0.001 μ g/mL. In this method, homogenized stool samples were weighed out in 100 mg aliquots in test tubes. Liquid/liquid extraction was performed to extract fatty acids and remove the nucleic acid preservative. A 250 µL aliquot of each extract was transferred to a clean analysis tube. The solvent was removed by evaporation under a stream of nitrogen. Internal standard solution was added to the dried sample extracts, quality controls (QCs), and calibration standards. The solvent was again removed by evaporation under a stream of nitrogen. The dried samples and QCs were subjected to methylation/ transmethylation with methanol/sulfuric acid, resulting in the formation of the corresponding methyl esters (FAME) of free fatty acids and conjugated fatty acids. The reaction mixture was neutralized and extracted with hexanes. An aliquot of the hexanes layer was injected onto a 7890A/5975C GC/MS system (Agilent Technologies, Santa Clara, CA, USA). Mass spectrometric analysis was performed in the single ion monitoring (SIM) positive mode with electron ionization. Quantitation was performed using both linear and quadratic regression analysis generated from fortified calibration standards prepared immediately prior to each run. Raw data were collected and processed using Agilent MassHunter GC/MS Acquisition B.07.04.2260 and Agilent MassHunter Workstation Software Quantitative Analysis for GC/MS B.09.00/ Build 9.0.647.0. Analysis of data based on T-test/ANOVA and creation of a hierarchical clustering heat map was performed using MetaboAnalyst 5.0.

Calculation of Milkfat Units

Milkfat consumption was calculated for each subject at each time-point and in total in milkfat units (MFU) using the formula: Σ [(milkfat % of product)(frequency of product consumed)]. The formula was based on the type of dairy product, the specific milkfat percentage of the product, and frequency of consumption. The specific milkfat percentage of each product was obtained from the U.S. Food and Drug Administration's website. A cutoff of 250 MFU was used to separate low and high milkfat consumers. Our calculation of

Table 2	Laboratory	work-up	of	patients	before	and	after	surgery
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Patient	MF [#]	Time-point	Weight	Wt change*	BMI	HbA1C	SBP	DBP	AST	ALT	AP	Bilirubin
BS124	Low	At surgery	334	-	55.67	5.7	151	92	38	94	58	0.4
		6 months post op	232	-	34.3	4.8	130	75	16	15	59	0.7
		1 year post op	186	-44.31%	27.47	4.5	120	80	19	26	57	0.6
BS131	High	At Surgery	328	-	45.7	5.6	126	76	19	30	65	0.3
		6 months post op	238	-	33.2	5.5	120	82	-	-	-	-
		1 year post op	245	-25.30%	34.2	5.4	131	88	28	42	69	0.3
BS135	Low	At surgery	293	-	48.8	5.6	118	80	34	70	71	0.9
		6 months post op	241	-	40.1	5.6	104	76	19	25	71	0.7
		1 year post op	236	- 19.45%	39.3	5.2	117	70	21	24	75	0.7
BS138	Low	At surgery	223	-	36.94	5.3	110	54	18	15	44	0.3
		6 months post op	160	-	25.82	5.1	113	54	16	15	35	0.5
		1 year post op	145	-34.98%	23.4	5.4	107	62	20	18	33	0.3
BS139	Low	At surgery	305	-	54.03	6.1	118	55	15	22	69	0.3
		6 months post op	251	-	43.4	-	115	82	-	-	-	-
		1 year post op	245	- 19.67%	42.2	-	134	82	-	-	-	-
BS140	High	At surgery	315	-	42.9	5.5	131	78	20	27	62	0.3
		6 months post op	232	-	32.4	5.3	92	66	16	13	65	0.4
		1 year post op	225	-28.57%	30.7	5.2	123	77	20	18	77	0.2
BS142	Low	At surgery	242	-	42.9	5.6	129	68	20	21	76	0.2
		6 months post op	184	-	32.6	5.5	110	70	15	17	79	0.4
		1 year post op	180	-25.62%	31.9	5.3	182	87	17	15	74	0.5
BS143	Low	At Surgery	269	-	36.58	5.9	143	70	37	34	84	0.6
		6 months post op	201.4	-	27.3	5.3	98	78	29	20	81	0.6
		1 year post op	203	-24.54%	27.5	5.4	124	60	25	23	87	0.8
BS145	High	At surgery	337	-	45.9	7.3	124	78	22	24	64	0.8
		6 months post op	250	-	36.9	-	122	72	-	-	-	-
		1 year post op	235	- 30.27%	34.7	5.3	134	78	18	18	94	0.8

[#]Milkfat consumption

Weight (lb)

*Percent change in Weight

BMI (kg/m2)

SBP systolic blood pressure (mmHg)

DBP diastolic blood pressure (mmHg)

AST aspartate transaminase (units/L)

ALT alanine transaminase (units/L)

AP alkaline phosphatase (IU/L)

Bilirubin, total (mg/dL)

the milkfat units thus takes into account the different percentages of milkfat in various dairy products (fat-free, skimmed etc.) as well as the frequency of consumption per week.

Results

Gut Microbiome Changes with Respect to Bariatric Surgery and Low/High Milkfat Consumption

The median age of the study cohort (n=9) was 45.5 years old (range: 30–66). Four patients were male. Five patients

identified as White/Non-Hispanic, one as African American, and three as Other. Six patients (BS124, BS135, BS138, BS139, BS142, BS143) were low milkfat consumers and three (BS131, BS140, and BS145) were high milkfat consumers. Table 2 shows clinical markers at the time of surgery, 6 months after surgery, and one year after surgery. These clinical markers include changes in weight, BMI, Hemoglobin A1C, systolic blood pressure, diastolic blood pressure, AST, ALT, alkaline phosphatase, and total bilirubin. The average percent change in weight 1-year after surgery for low milkfat consumers (n=6) was 28.1% while high milkfat consumers (n=3) demonstrated an average





Fig. 1 Linear discriminant analysis effect size (LEfSe). LEfSe scores via Kruskal–Wallis and Wilcoxon tests with two-tailed $\alpha = 0.05$, for taxa that were differentially distributed in (**A**) bariatric patients before (n=9) versus after (n=9) surgery (6 months) and (**B**) bariatric patients that had low (n=6) versus high (n=3) milkfat intake. Negative values represent taxa that were abundant before surgery, whereas

positive values represent taxa that were abundant after surgery (\mathbf{A}) . Negative values represent taxa that were abundant in low milkfat intake group, whereas positive values represent taxa that were abundant in high milkfat intake group (\mathbf{B}) . Phyla corresponding to different colors are noted in the respective panels

percent change of 28.05%. The median difference in BMI was 11.6 kg/m² for low milkfat consumers and 11.5 kg/m² for high milkfat consumers. The HbA1C status at surgery and 1 year later did not differ between the two groups.

Sequencing analysis and subsequent linear discriminant analysis effect size showed an overall post-surgery enrichment of *Roseburia* and *Christensenellaceae* (Fig. 1A). Similar analysis showed that the genus *Lachnospira* was abundant in low milkfat consumers. The genera *Lachnospiraceae*, *Candidatus*, *Sellimonas*, *Faecalitalea*, and *Blautia* along with the species *Bacteroides* were all enriched in high milkfat consumers (Fig. 1B). Additionally, there was an overall increase in the *Bacteroides* species in all high milkfat consumers. Volatility plot (Fig. 2) shows how taxa changed over time. *Faecalibacterium* significantly increased more than any other bacteria after bariatric surgery in patients 135, 139 and 142, over the duration of the study. Interestingly, as mentioned above, these three patients were low milkfat consumers.





Fig. 3 Alpha diversity assessment by (A) the frequency of use of butter for cooking by evenness-vector-correlation analysis and (B) the usage of butter by evenness-vector-significance analysis

Out of the dairy products tested, the frequency of use of butter for cooking was associated with decreased alpha diversity in patients (correlation = -0.68, *p*-value = 0.042) (Fig. 3A). Usage of butter was associated with decreased alpha diversity in patients (*p*-value = 0.038) (Fig. 3B).

Fecal Fatty Acid Analysis

To evaluate the influence of intake of dairy fat on fatty acid composition of the patients who underwent bariatric surgery, we performed non-targeted metabolomics analysis of a panel of 30 fatty acids including long chain fatty acids (LCFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Analysis of data based on T-test/ANOVA was performed using MetaboAnalyst 5.0. Hierarchical clustering heatmap presented in Fig. 4 provides visualization of the metabolome data. The heat map shows that myristic acid (SFA: saturated fatty acid) and/or myristoleic acid (MUFA) was observed in high amounts in patients BS124, BS135, BS138 and BS139. Patient BS124 also showed high amount of cis-13-16-docosadienoic acid, cis-11,14-eicosadienoic acid (20:2n6) and *cis*-11-eicosaenoic acid (20:1n9) and high amount of two more SFAs: arachidic and stearic acid. On the other hand, omega-6 PUFAs such as linoleic acid, gamma-linolenic acid and osbond acid were in high amount in patients BS124, BS131, BS139 and BS145. High concentration of an omega-3-PUFA, alpha-linolenic acid, was observed in patient BS131.

Discussion

Obesity and type 2 diabetes are diseases that need a combined treatment such as bariatric/metabolic surgery[14]. In this study, we assessed the association milkfat may have with microbiome changes. It is important to note that other dietary components and lifestyle changes likely contributed to differences observed in the microbiome. Additionally, hormonal changes such as differences in the leptin-adiponectin ratio axis and serum amyloid A concentrations may contribute to the changes in microbiome profiles [15, 16]. Our pilot study however, introduces new insight on the association of milkfat with postoperative bariatric microbiome profiles.

The sample size of our pilot study although small was comparable to other studies focused on post-bariatric surgery changes in human gut microbiome. For example, one study included before and 3 months post-bariatric surgery samples from total of six patients [17], while another study included eight gastric surgery patients and four medical weight loss patients [18]. The small sample size of our study is a limitation, but we did find several important observations that will help further direct research on this topic. The post-bariatric surgery increase seen in our study for Roseburia, Christensenellaceae, and Blautia is encouraging. Roseburia is a bacterium associated with weight loss and diabetes remission [19]. Christensenellaceae is inversely related to host body mass index [20]. The genus *Blautia* was observed as the only gut microbe that was inversely associated with visceral fat, independent of sex in a weight loss study in Japan [21]. Blautia is proposed to decrease obesity by producing butyric acid and acetic acid, which regulate G-coupled receptors (GPR) 41 and 43. While the function of these receptors is debated, studies on knockout mice implicate GPR41 and GPR43 in obesity [22]. Additionally, we observed an overall increase in the Bacteroides species in all high milkfat consumers. An overall increase in Bacteroides is negatively associated with obesity and promotes health and long-term weight loss [23, 24]. This suggests that the gut microbiome changes observed in our study in bariatric surgery patients with higher milkfat consumption are beneficial. Although high milkfat was seen to have a beneficial association, we observed reduced gut microbiome diversity when butter was used. Reduced diversity of gut microbiome is associated with obesity and diseases such as inflammatory bowel disease, cardiovascular disease, and asthma. Our results thus suggest that the use of butter did not have a beneficial association. Unlike most other dairy products, butter does not contain any probiotics, which may explain the lack of diversity associated with its use. Thus, this dairy product may not be associated with promoting a healthy



Fig. 4 Hierarchical clustering heatmap of fecal fatty acids. Analysis is based on T-test/ANOVA. Each colored cell on the map corresponds to a concentration value in the data table, with patient samples in columns and analyzed fatty acids in rows. Class values (0–4) correspond to the five time-points at which samples were collected (2 weeks before surgery, at the time of the surgery, and at 1, 3, and 6 months postop, respectively). A diverging color palette is used with hierarchy of colors from the dark brown (highest values) to dark blue (lowest values). Fatty acids analyzed: myristic acid (14:0), pentadecanoic acid (15:0), palmitic acid (20:0), behenic acid (22:0), lignoceric

microbiome even though it is generally associated with high milkfat content.

We observed that *Faecalibacterium* significantly increased more than any other bacteria after bariatric surgery in patients 135, 139 and 142, over the duration of the study. These were low-milk-fat consumers. A study showed that bariatric surgery leads to an increase in *F. prausnitzii* [18]. The authors discussed this as a beneficial bacterium. In contrast, another study reported significantly higher levels of *F. prausnitzii* in the obese compared with the non-obese participants [25]. The role of *Faecalibacterium* in obesity thus remains to be fully elucidated. It was suggested that, these discrepancies emphasize that population characteristics, age and diets should be taken into consideration before drawing conclusion regarding the role of this bacterium in obesity [26].

acid (24:0), myristoleic acid (14:1n5), palmitoleic acid (16:1n7), vaccenic acid (18:1n7), oleic acid (18:1n9), *cis*-11-eicosaenoic acid (20:1n9), erucic acid (22:1n9), nervonic acid (24:1n9), mead acid (20:3n9), linoleic acid (18:2n6), gamma-linolenic acid (18:3n6), dihomo-gamma-linolenic acid (20:3n6), arachidonic acid (20:4n6), adrenic acid (22:4n6), osbond acid (22:5n6), *cis*-11,14-eicosadienoic acid (20:2n6), *cis*-13–16-docosadienoic acid (22:2n6), alpha-linolenic acid (18:3n3), stearidonic acid (18:4n3), eicosatetraenoic acid (ETA) (20:4n3), eicosapentaenoic acid (EPA) (20:5n3), docosapentaenoic acid (22:5n3)

Role of short chain fatty acids in health and disease is still controversial. One study showed that bariatric surgery can lead to reduction in short chain fatty acids such as acetate, butyrate and propionate in patient fecal samples [27]. These are perceived to be linked to obesity. Another study reported that whole milk supplementation can alter the intestinal gut microbiome composition, but does not lead to significant changes to fecal short chain fatty acids [28]. A study using rat model showed that incorporation of dairy lipids increased tissue levels of long chain omega-3-fatty acids [29]. In the present study, we thus explored if bariatric surgery and more specifically consumption of high milk-fat, leads to changes in the fecal patterns of SFA, MFA and PUFA. It is interesting to note that the three patients BS124, BS138, and BS139 showing higher concentration of saturated fatty acids such as myristic acid were low-milk-fat consumers. On the other hand, BS131, a high-milk-fat consumer, showed very high concentration of beneficial omega-3-PUFA. Direct relevance of these observations with respect to dairy intake remains to be seen.

It would have been informative to carry out gut microbiome analysis of samples and collection of dietary surveys one year post-surgery as well. However, our area was one of the hardest hit areas by the COVID-19 pandemic. One year post-surgery time-points of all the patients who participated in this study were after the onset of pandemic in our area. Pandemic led to severe restrictions on research-related interactions with patients and restrictions on non-emergency in-person patient visits, which in turn posed challenges for collection of one year post-surgery samples and surveys. This will be considered for future studies. Corroborating clinical data with milkfat usage did not show clear clinical relationships in the course of one year; however, this should be interpreted with caution due limiting factors such as (i) small sample size of the pilot study, (ii) lack of information if patients had continued these dietary practices for one year, (iii) lack of information about compounding factors such as stress and/or changes if any, imposed on diet and other lifestyle aspects due to the pandemic.

Conclusion

While bariatric surgery is considered an efficient intervention to achieve major and rapid weight reduction, a high percentage of patients experience weight relapse post-operatively. Dietary interventions aimed at manipulating microbiome composition may complement the long-term efficacy of bariatric procedures. The findings in the present study suggest that incorporating dairy products in post-operative dietary plans may help cultivate a gut microbiome that is effective in regulating fat storage as well as digesting beneficial metabolites.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11695-021-05805-z.

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Declarations

Ethics Approval The study was performed 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. This study received approval by Cooper Health System Institutional Review Board (IRB) (17-120EX) and all the steps including informed consent were carried out as per the standards set by the IRB. **Informed Consent** Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare no competing interests.

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