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Original article

Impact of laparoscopic Roux-en-Y gastric bypass and sleeve gastrectomy on gut microbiota: a metagenomic comparative analysis

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Abstract

Background: Bariatric surgery is an effective therapeutic procedure for morbidly obese patients. The 2 most common interventions are sleeve gastrectomy (SG) and laparoscopic Roux-en-Y gastric bypass (LRYGB).

Objectives: The aim of this study was to compare microbiome long-term microbiome after SG and LRYGB surgery in obese patients.

Setting: University Hospital, France; University Hospital, United States; and University Hospital, Switzerland.

Methods: Eighty-nine and 108 patients who underwent SG and LRYGB, respectively, were recruited. Stools were collected before and 6 months after surgery. Microbial DNA was analyzed with shotgun metagenomic sequencing (SOLiD 5500 xl Wildfire). MSPminer, a novel innovative tool to characterize new in silico biological entities, was used to identify 715 Metagenomic Species Pan-genome. One hundred forty-eight functional modules were analyzed using GOmixer and KEGG database.

Results: Both interventions resulted in a similar increase of Shannon's diversity index and gene richness of gut microbiota, in parallel with weight loss, but the changes of microbial composition were different. LRYGB led to higher relative abundance of aero-tolerant bacteria, such as *Escherichia coli* and buccal species, such as *Streptococcus* and *Veillonella* spp. In contrast, anaerobes, such as *Clostridium*, were more abundant after SG, suggesting better conservation of anaerobic conditions in the gut. Enrichment of *Akkermansia muciniphila* was also observed after both surgeries. Function-level changes included higher potential for bacterial use of supplements, such as vitamin B12, B1, and iron upon LRYGB.

Conclusion: Microbiota changes after bariatric surgery depend on the nature of the intervention. LRYGB induces greater taxonomic and functional changes in gut microbiota than SG. Possible long-term health consequences of these alterations remain to be established. (Surg Obes Relat Dis 2020;16: 852–862.)

Key words: Laparoscopic Roux-en-Y gastric bypass; Sleeve gastrectomy; Microbiome; Metagenomics; Bariatric surgery

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The last decades have seen a dramatic increase in obesity rates worldwide [1]. Bariatric surgery is currently the most effective strategy for morbidly obese patients with a sustainable weight loss and a success rate at 5 years >66% [2]. The 2 main surgical procedures are laparoscopic Roux-en-Y gastric bypass (LRYGB) and sleeve gastrectomy (SG) [3].

A recent report indicates no difference regarding the excessive body mass index loss, quality of life, improvement of co-morbidities, and fatal consequences over a period of 5 years between LRYGB and SG even if LRYGB was more efficient to treat gastroesophageal reflux disease and dyslipidemia [4].

Several studies support the hypothesis that gut microbiota plays a key role in obesity [5–8]. Energy metabolism, one of the major roles of gut microbiome, occurs through by-products, such as short-chain fatty acids, that result from microbial fermentation of carbohydrate fibers in the gut [9]. Individuals with lower gene richness are more prone to insulin resistance and dyslipidemia [8]. *Akkermansia muciniphila,* has been consistently reported to be negatively associated to obesity and insulin sensitivity and its ability to degrade mucin can improve metabolic health during dietary interventions [10–12]. To investigate the impact of bariatric surgeries on gut microbiota, patients who have undergone either LRYGB or SG were compared with their preoperative (baseline) microbiota [13–19]. *A. muciniphila* and *Escherichia coli* were both reported to be highly increased after LRYGB [13,14]. Liou et al. [14], transplanted germ-free mice with stool from LRYGB-treated mice and observed a reduction of fat mass, suggesting the gut composition induced by surgery has a direct effect on weight loss and host metabolism [20,21].

Metagenomics shotgun sequencing surpasses the limitations and biases of 16 S gene amplicon sequencing by providing higher resolution taxonomic and functional profiling [22]. To our knowledge, only a few studies used a shotgun sequencing–based approach to characterize the microbiome modulation after LRYGB [13,19,20,23–25] and SG [19,25–27]. However, previous reports [13,19,20,23,24,26] were limited by low number of patients, varying from 6 to 23, leading to a lack of statistical power. No direct comparison between the 2 interventions involving large cohorts and their effect on the gut microbiome has been published.

Here, we present the largest metagenomics study to date investigating the effects of LRYGB and SG on gut microbiome based on preoperative and 6-month postoperative stools. This international multicenter study is based on 89 and 108 obese patients who underwent LRYGB and SG, respectively. Our analysis depends on a recent in silico method that discovers and quantifies microbial species, including those previously unknown [28]. The goal was to understand taxonomic and functional changes that the 2 surgery types induce in the gut microbiome and also the differences between both interventions.

Methods

Study population

In total, 275 patients diagnosed with obesity scheduled for LRYGB or SG have been recruited in 4 clinical centers in the following 3 countries: France, Switzerland, and the United States. For the present investigation, inclusion criteria were age ≥18 years and body mass index ≥35. Exclusion criteria were use of antibiotics and bowel cleansing for colonoscopy during the last 2 months before fecal sampling. The study was approved by the Ethical Committee of Basel in Switzerland (reference 272/05), the French Ethical Committee "Comité de Protection des Personnes" Ile de France VI (reference 2604-2012), and the Geisinger institutional review board in the United States (2004-0255).

Data collection

Fecal samples were self-collected 1 month before and 6 months after the surgery and stored essentially as described by the International Human Microbiome Standards consortium standard operating protocol 5 [29]. The medical records of participants were reviewed for demographic data, co-morbidities, and medications. In total, 531 fecal samples were collected before and 6 months after surgery from 275 patients. Patients with only 1 collected stool sample were excluded. Consequently, 197 patients were analyzed with their respective 197 preoperative and 197 postoperative stools that were collected and sequenced.

DNA extraction and sequencing

Fecal DNA extraction was carried out according to the International Human Microbiome Standards consortium standard operating protocol 7 [30]. Shotgun metagenomic sequencing was performed using SOLiD 5500 xl Wildfire sequencing system (Life Technologies [Life Technologies, Carlsbad, CA, USA] then ThermoFisher [ThermoFisher, Waltham, MA, USA]). Five hundred thirty-one samples were sequenced yielding an average of 81.782 million (±33.261 million) 35 base-long single reads.

Reads mapping

Reads were cleaned using an in-house procedure included in METEOR software [31] to remove (1) those containing resilient sequencing adapters/barcodes, and (2) those with average quality <20. Cleaned reads were subsequently filtered from human and other possible food contaminant DNA (using Human genome RCh37-p10, *Bos taurus* and *Arabidopsis thaliana*) using Bowtie 1 [32] (2 mismatches permitted) included in METEOR software. The gene abundance profiling was based on the integrated catalogue of reference genes in the human gut microbiome [33]. Filtered high-quality reads were mapped to the 9.9 M gene catalogue

using Bowtie 1 (3 mismatches permitted) included in METEOR software. Using METEOR, the gene abundance profiling table was generated using a 2-step procedure. First, the unique mapped reads (reads mapped to a unique gene in the catalogue) were attributed to their corresponding genes. Second, the shared reads (reads that mapped with the same alignment score to multiple genes in the catalogue) were attributed according to the ratio of their unique mapping counts. The counts were then normalized according to the reads per kilobase of exon model per million mapped reads strategy (normalization by the gene size and the number of total mapped reads reported in frequency) to give the final gene relative abundance profile table.

Taxonomic annotation

The genes were annotated using BLASTN alignment method against KEGG and RefSeq genomic databases [34,35]. The gene annotation method was adapted from Li et al [33]. Only the hits with a minimum of 80% of query sequence length and 65% nucleotide identity were considered in the annotation process. The similarity thresholds for the phylum, genus, and species taxonomic ranges were 65%, 80%, and 95%, respectively. Genes with multiple hits deprived of any consensus (a consensus was defined as 10% of hits having the same annotation) for their taxonomic associations were annotated at a higher taxonomic range until a consensus was established.

Functional annotation

Translated genes were aligned using BLASP version 2.6.0 against KEGG Release 81.0 (April 2017). Hits with a bitscore inferior to 60 or with an e-value superior to .01 were discarded. Each gene was finally assigned to the functional group (KEGG Ortholog or Enzyme Commission) associated with the most significant hit.

Metagenomic species Pan-genomes processing

The integrated reference catalogue of the human gut microbiome [33] was organized into 1696 Metagenomic Species Pan-genomes (MSPs) with MSPminer [28] by grouping coabundant genes across 1267 stool samples coming from cohorts distinct of the one used in this study. For each MSP, the median vector of the square-root transformed counts of its core genes was computed by using the 531 samples of this study. MSPs detected in <5 samples were discarded. Then, the relationship between this median vector and the core genes was assessed with the concordance correlation coefficient by Lin [36]. Finally, the abundance of the MSP was estimated from its 30 core genes with the highest concordance.

Relative abundance estimation and feature selection

Of 531 samples, 394 from 197 patients with 2 timepoints were used for further analysis. The relative abundances at phylum level, based on the National Center for Biotechnology Information taxonomy, were computed by summing the relative abundances of all the genes belonging to the same phylum. The relative abundances of functional modules, which were based on the KEGG annotation, were computed by summing the relative abundances of all the genes belonging to the KEGG Orthologous (KO) groups. Abundances of 130 modules from GOmixer [37] were calculated by summing the abundances of each KO that belonged to the same module. This set of metabolic modules was selected because it was manually curated based on rigorous literature specific to gut bacterial functions. To increase important functional units that were lacking, 20 modules from KEGG were added to the GOmixer modules (Supplementary Table 1). The relative abundances of MSP were calculated by estimating the median of the 30 top genes belonging to its core genes. Microbial features that were present in $<70\%$ of all the samples were removed. After filtering, statistical analysis was performed with the abundances of 15 phyla, 302 MSP, and 5348 KO groups. The values were log10 transformed, to approach normal distributions; a pseudo count equal to the lowest relative abundance value in the cohort was added to all relative abundances, to deal with zeros.

Gene richness and taxonomic diversity

The gene richness is a measure of how many unique genes are present in a sample. It was computed from the raw abundance genes table after downsizing based on 5 million–simulated sequencing depth (5 million reads are randomly selected from the original pool of reads) and then computing the mean number of unique genes over 30 repeats (https://github.com/fplaza/CountMatrixDownsizer). To compare with the original work introducing the concept of gene richness with a downsizing at 11 M of reads [8], it was then recalculated using an in-house predictive model (linear regression). The Shannon index was computed to assess taxonomic diversity species level [38].

Statistical test analysis

All statistical analyses were performed with R, version 3.3.2 (The R Project for Statistical Computing, Vienna, Austria). Clinical variables were summarized as medians with interquartile ranges or as frequencies with percentages. The Fisher's exact test was used to compare categoric variables between patients who underwent SG and LRYGB surgery. Adonis tests were used from the R package vegan for differential analysis according to groups [39]. Dissimilarity matrices were calculated using Bray-Curtis dissimilarity on relative abundance values of MSP.

Statistical analysis to assess the effect of surgery on microbiota

First, we performed 2 independent analyses for LRYGB and for SG. To test the normality assumption of

metagenomic variables, all 302 MSPs and 150 modules distributions were tested with Shapiro-Wilk test. Normality was rejected for all of these metagenomic features (Shapiro-Wilk test, $P < 2 \times 10^{-11}$). Microbiome data are also known to be sparse and overdispersed [40]. Wilcoxon signed-rank test was used because it does not require the assumption of normal distribution. Because this is a prospective study and the samples are not independent, 2-sided Wilcoxon signed-rank test was used. Multiple testing was controlled by Benjamini-Hochberg false-discovery rate [41]. To ensure the MSP were significant, an additional filter based on the median fold change of relative abundance was applied. The median log2 fold change (FC_log2) is the ratio of the median of this feature in all patients after surgery divided by the median of this feature in all patients before the gastric surgery.

$$FC_{log2} = log2\left(\frac{median\ Postoperative\ stool}{median\ Preoperative\ stools}\right)$$

To summarize, a P value $< .05$ and a (2-fold) FC ≥ 1 or FC ≤ -1 were considered statistically significant.

Statistical analysis to compare the 2 types of surgery

To evaluate the change of microbiome composition induced by the gastric surgery, the relative abundances from the postoperative stool were normalized by dividing them with relative abundances from preoperative stools for each patient and then log2 transformed. The abundance ratios were then used to compare the surgery types by performing Wilcoxon rank-sum test on the microbial features that were significant at least in 1 surgery group. Multiple testing was controlled by Benjamini-Hochberg.

Correlation between functional modules and MSPs

We performed 2 independent analyses for LRYGB and for SG groups. In this context, Spearman correlation coefficients were calculated for every MSP that was found significantly impacted by gastric surgery and 150 functional modules. Relative abundances before and after surgery were used for the correlation and only correlation coefficients superior to .7 were retained for subsequent analysis.

Results

Demographic description

One hundred ninety-seven patients have been included in 3 different countries (France, the United States, and Switzerland) before they underwent bariatric surgery (89 LRYGB and 108 SG). Of these, 86 patients were included by 2 French clinical centers, 73 patients from the United States, and 38 patients from Switzerland. We checked if the presurgery characteristics of the cohort were homogeneous depending on the type of surgery performed (Supplementary Table 2). Significant differences were found only for the country of origin (Fisher exact test, $P < .001$) and diabetes prevalence (Fisher exact test, $P < .05$). Important decrease of body mass index was important after both surgical procedures (Supplementary Tables 3 and 4).

Analysis of gut microbiota before surgery

Because the surgery type was confounded with the country of origin, we first analyzed the differences in microbial gut composition at MSP level between the patients before surgery, using a principal component analysis of log-transformed relative abundances of MSPs (Fig. 1). Patients from Europe (France and Switzerland) were clearly separated from the U.S. cohort (Fig. 1A, Adonis P value = .001) and by the type of surgery performed (Fig. 1B, Adonis $P = .001$). However, in the Swiss cohort, no difference (Fig. 1C, Adonis $P = .074$) was observed between the patients who underwent LRYGB (n = 20) and SG (n = 18). To overcome this limitation, Wilcoxon rank-sum test on ratio was used to normalize the microbiome profile at baseline to reduce variability induced by country of origin.

Surgery effects on gut microbial diversity and phylum-level composition

We estimated Shannon microbial diversity and gene richness in each sample. Compared with baseline, the Shannon index (Fig. 2) was significantly increased by surgery after 6 months (Wilcoxon signed-rank test, LRYGB: $P = 7.5 \times 10^{-6}$ and SG: $P = 7.3 \times 10^{-11}$). Similarly, gene richness was increased very significantly ($\sim$15%) by both procedures (LRYGB: $P = 3.8 \times 10^{-5}$, SG: $P = 5.9 \times 10^{-10}$). As expected, the following 5 phyla were the most abundant in human intestinal gut: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* (Supplementary Fig. 1). Before surgery, phylum composition was comparable between the 2 groups. *Proteobacteria* was significantly increased by LRYGB (Wilcoxon signed-rank test, $P = 1.9 \times 10^{-12}$) and SG (Wilcoxon signed-rank test, $P = 5.4 \times 10^{-6}$). *Verrucomicrobia* was enriched by LRYGB (Wilcoxon signed-rank test, $P = 2.1 \times 10^{-10}$) and SG (Wilcoxon signed-rank test, $P = 3.0 \times 10^{-10}$). *Firmicutes* showed a slight decrease after both surgeries.

Surgery effects on microbiome species composition

Fifty-one MSP were significantly impacted by LRYGB ($P < .05$ and FC_log2 ≥ 1 or FC_log2 ≤ -1, Wilcoxon signed-rank tests) and 48 after SG (Figs. 3A and 4A; detailed view in Supplementary Figs. 2 and 3, numeric values in Supplementary Tables 5 and 6). Of these, 20 were common to both procedures and were overwhelmingly (18 of 20) enriched upon surgery. Notably, the beneficial *Verrucomicrobia A. muciniphila* was enriched (LRYGB: FC_log2 = 1.61,

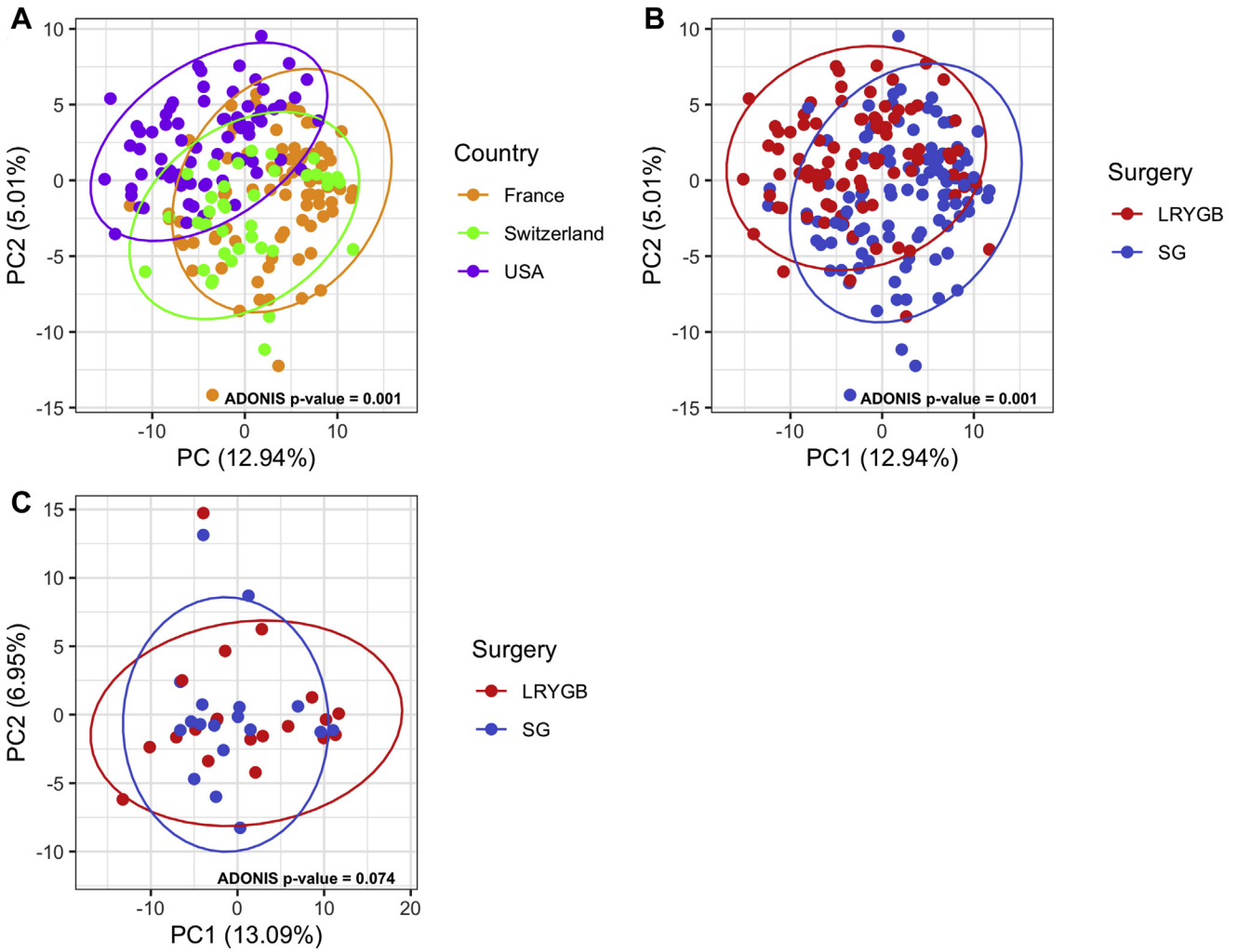

Fig. 1. Principal component analysis based on log transformed Metagenomic Species Pan-genome abundances. Adonis tests were performed to assess the variance in preoperative microbiota profiles. Associated *P* values are shown for each analysis and a *P* value $< .05$ was considered as significant. Separation between patients from the United States and Europe before surgery (A). Separation of patients who underwent laparoscopic Roux-en-Y gastric bypass (LRYGB) and sleeve gastrectomy (SG) before surgery (B). No separation for Swiss patients before surgery (C).

SG: FC_log2 = 1.82), but also the potentially proinflammatory *Proteobacteria* like *E. coli* (LRYGB: FC_log2 = 5.22, SG: FC_log2 = 1.04), *Klebsiella pneumoniae* (LRYGB: FC_log2 = 4.03, SG: FC_log2 = 2.09), and *Haemophilus parainfluenzae* (LRYGB: FC_log2 = 1.61, SG: FC_log2 = 1.4). *Faecalibacterium prausnitzii* was less abundant only after LRYGB (FC_log2 = −1.43). Five oral species were enriched by both procedures, of which most strongly *Veillonela parvula* (LRYGB: FC_log2 = 2.65, SG: FC_log2 = 1.92) and *Streptococcus salivarius* (LRYGB: FC_log2 = 4.07, SG: FC_log2 = 1.98) but also *Streptococcus gordonii*, *Streptococcus mutans*, and *Streptococcus parasanguinis*. Additional 6 oral species *(Fusobacteria nucleatum*, *Streptococcus anginosus*, *Streptococcus oralis*, *Streptococcus vestibularis*, *Veillonela atypica*, and *Veillonela sp oral*) were enriched by LRYGB but not SG. Interestingly, 2 oral *Bifidobacteria* were depleted by interventions, 1 by LRYGB (*Bifidobacteria bifidum*) and 1 by SG (*Bifidobacteria dentium*). Four MSPs with no species-level annotation were enriched by both surgery procedures (msp_0868, msp_0344, msp_0582, and msp_0355).

Of 78 MSPs impacted by LRYGB and/or SG 24 had abundance ratios significantly different between LRYGB and SG groups ($P < .05$, Wilcoxon rank-sum tests; Fig. 5A; Supplementary Table 7). Two *Proteobacteria (E. coli* and *K. pneumonia*), and 7 oral MSPs (2 *S. oralis*, *S. parasanguinis*, *S. salivarius*, *V. atypica*, *V. parvula*, and *V. sp. oral*) were more enriched by LRYGB than SG surgery. One oral species, *B. bifidum*, was more depleted. Two *Roseburia* (*R. faecis* and *R. hominis*) were also more enriched by LRYGB, as was *E. faecalis.* The 5 MSPs more enriched by SG and annotated at the species level (*A. hadrus*, *C. sp KLE*, *F. plautii*, *O. sp. KLE*, and *R. gnavus*) all belonged to *Firmicutes* order *Clostridiales*.

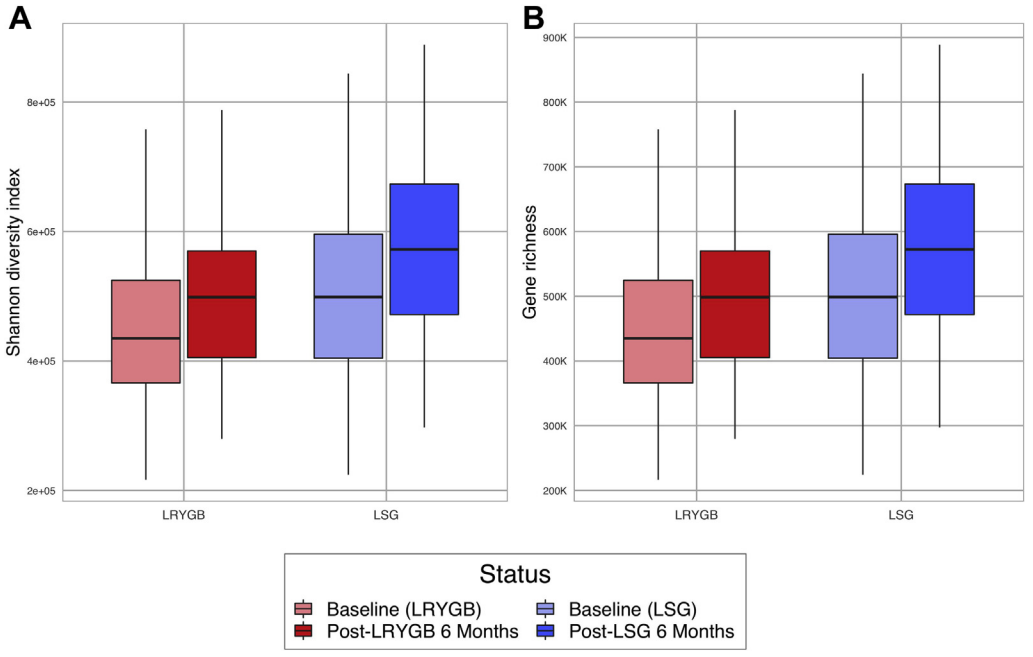

Fig. 2. Shannon microbial diversity and gene richness. Compared with baseline, the Shannon index (left side) was significantly increased by surgery after 6 months (Wilcoxon signed-rank test, laparoscopic Roux-en-Y gastric bypass [LRYGB]: $P = 7.5 \times 10^{-6}$ and sleeve gastrectomy [SG]: $P = 7.3 \times 10^{-11}$). Gene richness (right side) was also significantly higher 6 months after LRYGB (Wilcoxon signed-rank test, LRYGB: $P = 3.8 \times 10^{-5}$ and after SG: $P = 5.9 \times 10^{-10}$).

Effect of LRYGB and SG on microbiome functions

Thirteen functional modules were significantly more abundant after LRYGB and 6 after SG; 5 were common to both surgical interventions (Figs. 3B and 4B, Supplementary Tables 8 and 9, Supplementary Figs. 4 and 5). Five common modules were ABC transporters implied

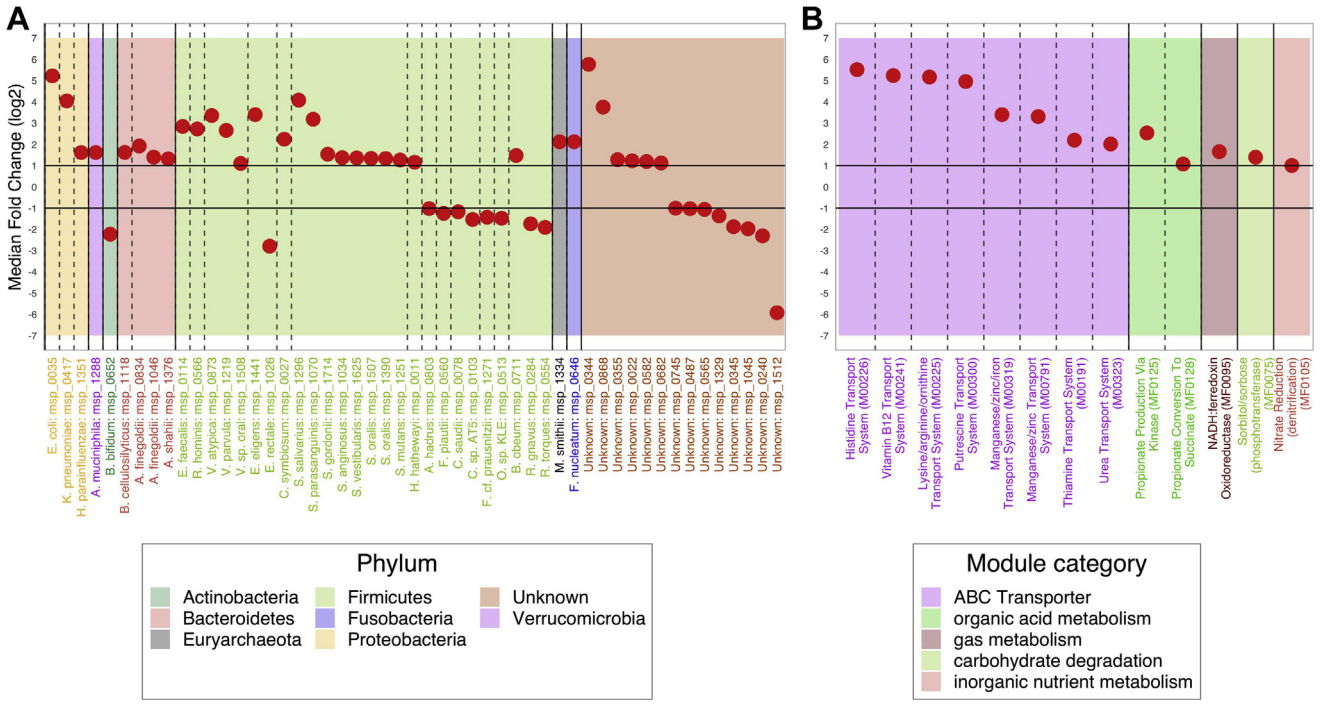

Fig. 3. Microbial changes (MSP) after LRYGB. Median fold changes (Log2) of relative abundances (postsurgery/baseline) for 51 MSP that were significantly impacted by laparoscopic Roux-en-Y gastric bypass compared with baseline. MSP were regrouped by phylum and ordered according their fold change within the phylum (A). Eleven modules were significantly increased after laparoscopic Roux-en-Y gastric bypass and were regrouped by functional category (B).

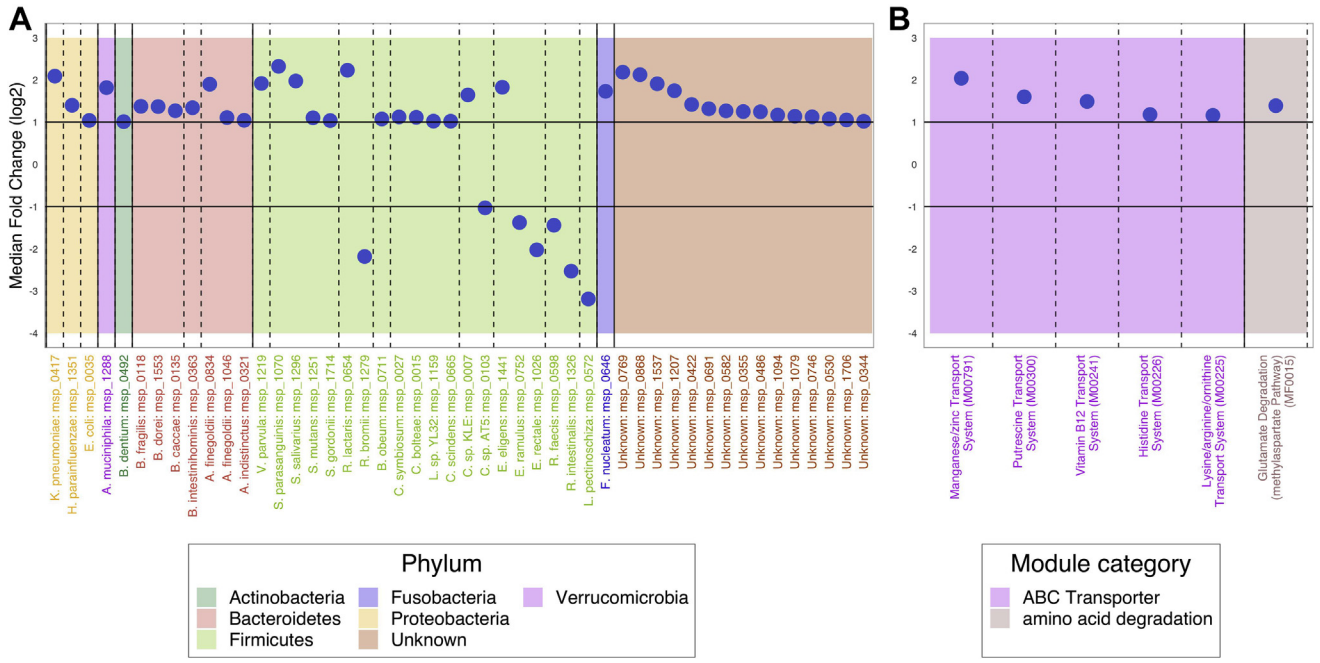

Fig. 4. Microbial changes (MSP) after sleeve gastrectomy. Median fold changes (Log2) of relative abundances (postsurgery/baseline) for 49 MSP that were significantly impacted by laparoscopic Roux-en-Y gastric bypass compared with baseline. MSP were regrouped by phylum and ordered according their fold change within the phylum (A). Four modules were significantly increased after laparoscopic Roux-en-Y gastric bypass and were regrouped by functional category (B).

in vitamin B12 (LRYGB: FC_log2 = 5.23, SG: FC_log2 = 1.49), histidine (LRYGB: FC_log2 = 5.51, SG: FC_log2 = 1.18), lysine/arginine (LRYGB: FC_log2 = 5.16, SG: FC_log2 = 1.16), putrescin (LRYGB: FC_log2 = 4.95, SG: FC_log2 = 1.6), and manganese/zinc (LRYGB: FC_log2 = 3.30, SG: FC_log2 = 2.04) transport. Another manganese/zinc transport system was increased only after LYRGB (M00319; FC_log2 = 3.39), as were Thiamine and Urea transport systems. Glutamate degradation module (FC_log2 = 1.39) was more abundant after SG while significant increase of nitrate reduction (FC_log2 = 1.00) and propionate production (FC_log2 = 2.53) modules were enriched after LRYGB.

Of 14 functional modules affected by LRYGB and/or SG 13 were found to be significantly different between LRYGB and SG groups ($P < .05$, Wilcoxon rank-sum tests; Fig. 5B, Supplementary Table 10). Notably, 8 functional modules involved in ABC transporters were more enriched in patients after LRYGB compared with the SG surgery (histidine and lysine, putrescin, vitamin B12, manganese/zinc, urea, and thiamine transport system). Modules involved in nitrate respiration and propionate production via kinase were more increased by LRYGB.

Correlations of functional modules and MSPs

Spearman correlations between relative abundances of 78 MSP and 150 modules were computed independently for 2 groups of patients, LRYGB and SG. Regarding the former, 8 modules were correlated to a set of 5 MSP annotated to *V. parvula*, *S. vestibularis*, *S. salivarius*, and *S. parasanguinis*, and *E. coli* (Spearman's rho $\geq .7$, Supplementary Table 11).

Discussion

Microbiome composition is differentially altered by LRYGB and SG

Physiologic and anatomic changes of the gastrointestinal tract after bariatric surgery modify gut motility, gastric acid secretion, bile acid processing, and gut hormone secretion [42]. Using whole metagenome shotgun sequencing with the largest cohort of patients to date, we confirmed gut microbiota was strongly modulated after SG and LRYGB with notable similarities and differences. Gene richness was increased for most of the patients after both surgical interventions. However, the most important divergence was the extent of the increase of *Proteobacteria* species. *E. coli* and *K. pneumoniae* were both increased by surgery but the increase was significantly stronger after LRYGB confirming results from other studies [13,23]. Increase of *E. coli* may reflect host and gut adaptation to maximize energy harvest in starvation-like conditions after bariatric surgery [16]. *A. muciniphila*, known to be negatively correlated to inflammation, was increased in patients after SG or LRYGB in similar proportion in our study confirming results from other studies [13,18,23]. This species has been found to reverse obesity and increase mucus layer thickness

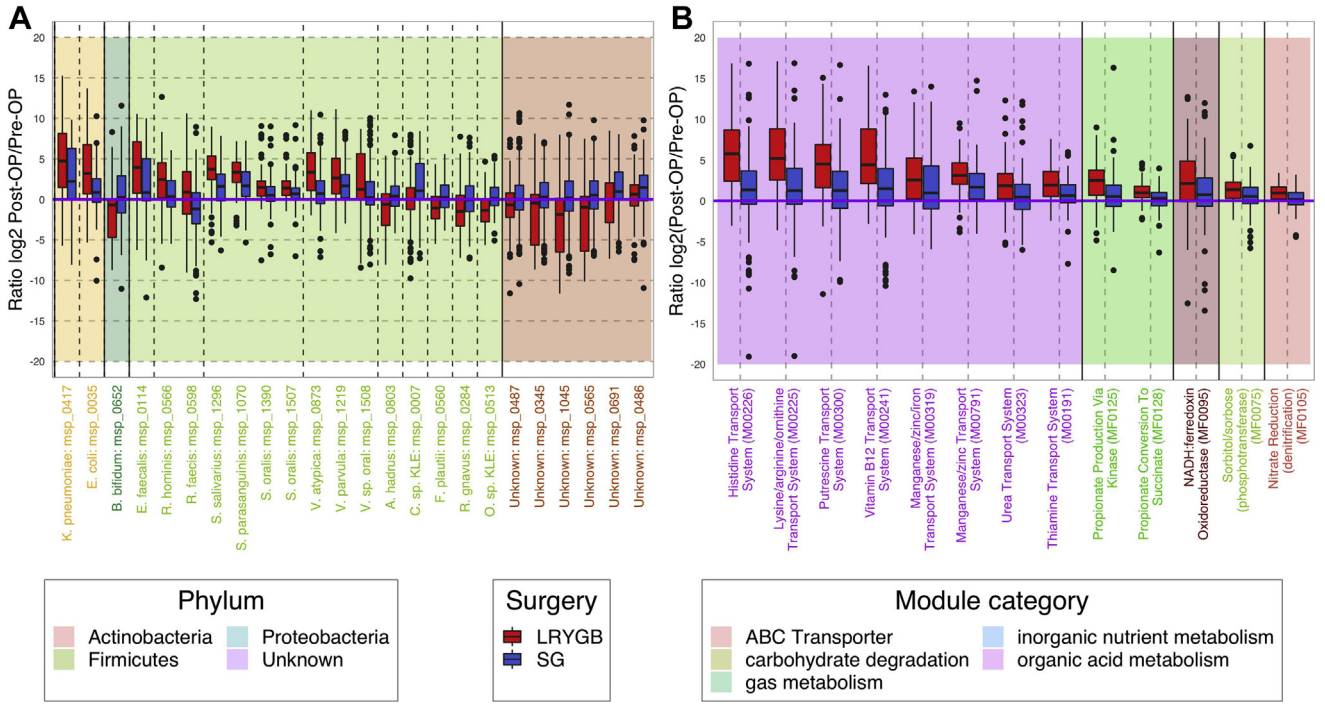

Fig. 5. Differences of changes induced by laparoscopic Roux-en-Y gastric bypass (LRYGB) and sleeve gastrectomy (SG) at microbial changes level. Log2 transformed relative abundance ratios (postsurgery/baseline) of 24 microbial changes that were significantly different regarding the surgery type (A). Log2 transformed relative abundance ratios (postsurgery/baseline) of 11 modules that were significantly different regarding the surgery type (B).

in mice fed a high-fat diet [11]. Our study allows us to make the assumption that LRYGB has a higher impact on microbiome than SG in contradiction with a recent study reporting both interventions appeared to have the same impact on gut [43].

In contrast, the number of MSP being negatively affected by bariatric surgery was lower. *F. prausnitzii*, which is a butyrate-producer, decreased 6 months after surgery in LRYGB while SG had no effect on its presence in feces. The decrease of *F. prausnitzii* in LYRGB patients was reported in 2 previous studies as well [13,18,23]. Similarly, *R. gnavus* and *R. torques* were also reportedly decreased in LRYGB. This species is well known to produce transcialidase to degrade mucin [44] and to be associated with inflammatory bowel diseases and metabolic disorders [8]. Some MSPs not annotated at species-level were also detected to be affected by both interventions. These observations were possible because MSPminer does not rely on reference genomes and can reveal new biological entities of interest.

LRYGB promotes aerotolerant colonization more than SG

We observed that LRYGB led to a higher increase of oral colonizers (*Veillonela* and *Streptococcus* genus) than SG. Possibly, less exposure to the acidic stomach compartment favors access of oral bacteria to the gut. However, facilitated access appears to be insufficient for implementation in the gut and other factors may be needed, as oral *Bifidobacteria* were depleted after surgery. Moreover, bypassing the duodenum might introduce some oxygen to the gastrointestinal tract [45], which is usually anaerobic, inhibiting growth of obligate anaerobes, such as the *Clostridium* genus, and promote domination of aerobes [46]. According to our results, *Clostridium* species were negatively impacted by LRYGB while they were enriched after SG suggesting the gut is still largely in anaerobic after SG. Along the same lines, a higher relative abundance of ferredoxin oxidoreductase, which is usually associated with aerobic respiration, was observed after LRYGB relative to SG. Evidence of oxidative stress was also reported with enrichment of functional modules involved in glutathione metabolism in LRYGB patients in concordance with others studies [13,20].

Nitrates respiration may favor E. coli *expansion after LRYGB*

After LRYGB, signs of nitrate reduction as an alternative form of respiration were observed. Interestingly, it has been recently observed that nitrate can boost growth of *E. coli* to outcompete species that rely on fermentation only [47,48]. LRYGB led also to enrichment of a functional module involved in urea transport system. After LRYGB, we also observed an increased potential for trimethylamine oxidized (TMAO) utilization via pathways found in Proteobacteria (torYZ and torC, Supplementary Table 12). These results

are in agreement with previous observations showing higher level of plasma circulating plasma TMAO levels in patients after LRYGB compared with SG. A retroconversion model of trimethylamine produced in gut by *Enterobacteriaceae* from TMAO reduction by gut bacteria has been reported [49].

Microbial transportation of supplements is stimulated by bariatric surgery

The strong increase of ABC transporters, especially Vitamin B12, B1, and manganese/iron transport systems was more pronounced in LRYGB, confirming previous findings [13]. After surgery, multivitamin, iron, and calcium supplements are provided to compensate for deficiencies caused by food intake reduction and malabsorption [24]. Of note, in supplement tablets, vitamin B12, not bound to protein, is subsequently available to bacteria in the intestine. High levels of transport potential of vitamin B12, B1, and iron, particularly in LRYGB, suggest opportunist use of these nutrients by microbes. Transport of vitamin B12 was highly correlated with *E. coli* while iron transporter was associated with *S. salivarius* and *V. parvula* (Supplementary Table 11); these functions may have facilitated enrichment of the cognate species, acting synergistically with the decrease of exposure to acidic stomach compartment.

Short chain fatty acids are altered by LRYGB and may impact weight loss via a glucagon-like peptide-1–dependent mechanism

Short-chain fatty acids, metabolites formed by gut microbiota from carbohydrate substrates, can have several beneficial effects on the host. Butyrate and propionate were reported to be associated with weight loss and to have protective properties against diet-induced obesity in mice [50]. More precisely, the study indicated butyrate and propionate impact on gut hormones (by stimulating glucagon-like peptide-1) and food intake reduction. In parallel, postprandial glucagon-like peptide-1 was observed to be significantly increased after LRYGB [51]. Abundance of propionate production module was higher after LRYGB and was also correlated with *E. coli* (Spearman's rho = .76). In concordance to this observation, Ilhan et al. [24] has reported an increase of propionate after LRYGB. Visceral and liver fat were also reduced after propionate delivery in humans [52]. General glycemic improvement was more pronounced after LRYGB and could be related to higher gut propionate production. Breton et al. [53] identified *ClpB*, a bacterial protein produced by *E. coli*, as an antigen-mimetic of a neuropeptide involved in the satietogenic system by stimulating GLP-1. The gene coding *ClpB* was found to be increased by 100 after LRYGB ($P < .05$, Wilcoxon signed-rank test; not shown), in parallel with the increase of *E. coli* that encodes it. Taken together, the data from literature and our results support that *E. coli* may potentially influence both host appetite and metabolism via a complex activation cascade.

Conclusions

A direct comparison of microbiome changes after SG and LRYGB procedures, assessed via large cohorts and shotgun metagenomic indicated a profound modification of bacterial gut composition 6 months after bariatric surgery in parallel with weight loss. Similar taxonomic and functional changes were observed after both intervention (*Proteobacteria*, oral colonizers, nitrate, and TMAO oxidation and vitamin B12 use increase) but less important after SG. The main limitations of our study are the lack of measure of metabolites as short-chain fatty acids to validate what we observed at KO level. Long-terms observations from surgery, possibly 3 to 5 years, are necessary to highlight the clinical relevance of these findings. We infer from these data that LRYGB had greater impact on microbiome composition than SG. This could be considered when advising the patient on the type of bariatric surgery or postoperative diet.

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Disclosures

The authors have no commercial associations that might be a conflict of interest in relation to this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.soard.2020.03.014.

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