Gut Microbiota Dysbiosis in Human Obesity: Impact of Bariatric Surgery

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Title page

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Abstract 235

Purpose of the review: We herein summarize what is currently described in terms of Gut microbiota (GM) dysbiosis modification post-Bariatric surgery (BS) and their link with BS-induced clinical improvement. Noteworthy, we discuss how the major inter-individual variability in terms of GM changes could impact the variable clinical improvements seen in patients.

Recent findings: The persisting increase in severe obesity prevalence has led to the subsequent burst in BS number. Indeed, it is to date the best treatment option to induce major and sustainable weight loss and metabolic improvement in these patients. During obesity, the gut microbiota (GM) displays distinctive features such as low microbial gene richness and compositional and functional alterations (termed dysbiosis) which have been associated with low-grade inflammation, increased body weight and fat mass, as well as type-2 diabetes. Interestingly, GM changes post-BS is currently being proposed as one the many mechanism explaining BS beneficial clinical outcomes.

Summary: BS enables partial rescue of GM dysbiosis observed during obesity. Some of the GM characteristics modified post-BS (composition in terms of bacteria or functions) are linked to BS beneficial outcomes such as weight loss or metabolic improvements. Nevertheless the changes in GM post-BS display major variability from one patient to the other. Thus further large sample size studies associated with GM transfer studies in animals are still needed to completely decipher the role of GM in the clinical improvements observed post-surgery.

Keywords

Bariatric surgery; gut microbiota; metagenomics; richness; obesity; metabolism; Akkermansia muciniphila; Faecalibacterium prausnitzii; microbial gene richness; type-2 diabetes; Roux-en-Y gastric bypass; sleeve gastrectomy; adjustable gastric banding; Roseburia intestinalis; proteobacteria; gammaproteobacteria; firmicutes; Bacteroidetes; bmi; hba1c; remission; illumina

Introduction

The gut microbiota (GM) colonizes the digestive tract at birth[1,2] with bacterial compositional changes and diversification until 2 years of age. Although a series of endogenous and exogenous factors (such as diet, drugs and diseases) can impact its composition, the GM is generally stable throughout adolescence and adulthood until individuals reach 70-75 years old[3,4]. The digestive tract harbors 10^{14} microorganisms (at least in the colon) which remain mostly unidentified[5]. In humans and rodents, the GM is segmented into two main phyla: Bacteroidetes and Firmicutes[6]. New culture-independent "omics" technologies, mainly metagenomics and metabolomics[7–9], have provided major insights into GM composition and functions in both health and diseases[10].

During obesity, a common and frequent fecal microbiota characteristic is reduced microbial gene richness (MGR) and diversity. Low MGR has been observed in obese mice[11] and humans[12,13] and is more prevalent in populations with a high incidence of obesity[4]. Low MGR is defined using shotgun analysis and is represented by the total number of non-redundant microbial genes below the threshold of 480 000 genes[12,13] and is associated with increased BMI, low grade inflammation and insulin resistance[12,13]. As such, low MGR can be

found in up to 40% of overweight/moderately obese patients. Recently, we have shown that the most extreme forms of obesity (i.e. severe obesity) are characterized by a very high prevalence (75% of the patients) of low MGR [8]. Beyond corpulence, this decreased MGR is further associated with adverse adipose tissue repartition (i.e. increased trunk-fat mass), Type 2 diabetes (T2D), and hypertension and its severity as witnessed by increased polypharmacy[8]. However, dietary habits are critical in modulating MGR and gut bacterial diversity. Indeed, European children who consume half the fiber intake of their African counterparts display a lower bacterial diversity[14] compared to the African children. Furthermore, high MGR[15] is also observed in moderately obese individuals following a healthy diet. Interestingly, in weight loss intervention programs, obese patients who follow a restrictive diet yet with adequate nutrition display higher gut bacterial diversity as compared to those with self-prescribed dietary restriction and inadequate nutrition[16].

In addition to reduced bacterial richness, the GM undergoes profound compositional and functional changes during obesity. A pioneering study published in 2005 found that the ratio of *Bacteroidetes* to *Firmicutes* (the two most common phyla within the GM) is decreased in genetically obese mice (ob/ob) as compared their heterozygous or wild-type littermates[17,18]. Although this finding was confirmed in humans shortly afterwards[19,20], since then, several studies have found diverging results[21] with the current literature suggesting that this biomarker is probably not universal in obesity. In addition to phylogenetic changes, the GM of obese animals extract more energy from fermentation than that of lean animals[19], and this feature is (at least partially) transmissible via Fecal Microbiota Transfer (FMT), into germ-free animals[19,22]. FMT from obese individuals into germ-free mice also induces susceptibly to weight gain in germ-free mice when compared to mice transferred with GM from lean donors[23]. Several studies using metagenomic sequencing further assessed GM functional differences between obese and lean controls as well as in individuals with high vs. low

MGR[12,13]. These studies reported that subjects with obesity and low MGR harbored less butyrate-producing bacteria, reduced hydrogen and methane production, increased potential to degrade intestinal mucus, and increased oxidative stress management potential[12].

Overall, these studies demonstrate obesity is associated with major GM dysbiosis, which further worsens with increasing BMI and disease aggravation[8]. Whether, this dysbiosis can be reversed upon weight loss has been evaluated using various means, including bariatric surgery (BS), which is the focus of the present review. We here summarize the GM compositional changes after several BS techniques and their link with clinical outcomes. We also discuss the factors potentially involved in major differences and variability observed across studies.

Bariatric surgeries techniques and outcomes

Bariatric surgery is classically recommended for individuals with BMI ≥40kg/m² or ≥35kg/m² with associated comorbidities[24]. All BS procedures (adjustable gastric banding (AGB), sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB)) consist of a reduction of gastric volume by creating a gastric pouch of roughly 30 milliliters, which drastically reduces food intake[25,26]. Depending on the surgical technique used (with the exception of AGB), there are also further modifications of the intestinal tract, which have potential consequences on GM composition. For instance, SG induces modifications of pH and gut hormones secretion profiles, whereas, RYGB a degree of adds malabsorption and bile flow diversion (via the exclusion of the duodenum and the proximal jejunum from the intestinal tract), as well as modifications of food taste and macronutrient intake[27]. These mechanisms have been collectively summarized as the BRAVE effect[28] of BS. The gut architecture and digestive ecology is thus deeply modified following BS and leads to a significant pressure on the gut microbial ecosystem (as reviewed in length[5]). To date, BS is

an efficient therapeutic option to induce rapid and significant weight loss[29] over time with a variable degree of weight loss maintenance[29]. Because of the progression of severe obesity worldwide, the number of BS intervention has progressed in parallel, reaching a 3-fold increase the past 10 years[30]. However, weight loss outcomes display major inter-individual variability. While some patients are considered as good responders[31,32] (i.e. they lose a large amount of weight and further stabilize this weight loss during follow-up), others lose less weight during the first year[31,32] or regain weight at mid-term[33]. While several clinical or biological factors including Type 2 diabetes[34], surgery conversion[35], and adipose tissue fibrosis[31,32,36] are involved in the variability of individuals' responses, it is suggested that differential changes within the gut microbiota could also contribute to the inter-individual variability observed for post-bariatric surgery outcomes.

Concomitantly to weight loss, patients undergo drastic improvements of their metabolic conditions post-BS[37], due both to weight loss itself but also to other weight-independent mechanisms extensively described elsewhere[38]. In this context, a growing amount of literature suggests that GM modifications could be associated with or eventually explain BS-induced metabolic and inflammatory improvements as previously reviewed[39]. Indeed, strong evidences have emerged from FMT studies using either mice[40,41] or human[42] donors and germ-free mice recipients, which have shown that the modified GM post-BS is able to induce moderate weight loss upon FMT when compared to FMT in sham operated animals or non-operated subjects. However, the precise mechanisms involved in the GM-mediated improvements post-BS remains scarce.

Bariatric surgery and gut microbiota modulation

Microbial Richness

Bariatric surgery has been shown to increase gut bacterial richness and diversity in different studies with various sequencing techniques (**Table 1**). Using 16S rRNA pyrosequencing, we previously demonstrated a significant increase in diversity from baseline to 3 months which further remains stable at 6 months post-RYGB. This observation was further confirmed for up to one year post-RYGB[43] using Illumina shotgun sequencing. Recently, Palleja *et al.*, have confirmed this increase in diversity using the same method, yet due to a limited number of patients, it did not reach significance[44]. Furthermore, we confirmed and reinforced this observation showing a significant increase in gut microbial richness (as estimated by bacterial gene count via SOLiD shotgun sequencing) only one year post-BS both after RYGB and AGB[8]. Most interestingly, in another group of patients followed up to 5 years post-RYGB, we observed that the significant increase in MGR obtained at one year remains stable thereafter[8]. Most importantly, BS is not able to completely reverse the initial obesity-associated decrease in MGR, although patients exhibit major weight reductions and metabolic and inflammatory improvements[8,45]. Since severely obese patients present with very low MGR at baseline, BS is not sufficient enough to enable a switch from low to high MGR[8]. Whereas partial, the reason why the bacterial gene richness is improved is not fully understood and could originate from many factors besides gut anatomy modification and could include improvements in metabolism and inflammation, in body composition, and weight loss[8]. Some bacterial genus changes such as *Eubacterium spp*, *Ruminococcaceae spp* and *Faecalibaceterium spp*, are associated with the amelioration of metabolic factors, including HbA1c. Moreover, the healthy diet recommended post-BS[24,46] might also play a role in increasing MGR, as proposed by Griffin *et al.*,[16].

The findings discussed above are reported after AGB and RYGB. However, SG is becoming the most preferred and performed BS intervention worldwide[30], and studies have started assessing gut microbiota modulation post-SG compared to other BS techniques. A

recent murine study demonstrated that both SG and RYGB similarly increase diversity as assessed by 16S-pyrosequencing[47]. This significant increase in diversity was confirmed in humans 3 months post-SG[48], using shot gun sequencing; however, diverging results are also reported. Although Murphy *et al.* observed a significant increase in MGR post-RYGB, no difference was observed post-SG[43]. More powered studies, with a higher number of patients and including follow-ups, are needed to further assess the effect of BS surgery techniques on gut bacterial richness and diversity and to relate the observed changes with lifestyle and clinical improvements.

Post-BS evolution of gut microbiota composition

Bariatric surgery modifies GM composition in the short-[49–51], mid- [44,48,52] and long-term, up to 9 years[8,42]. These bacterial compositional changes have been extensively reviewed in the literature [53–56]**.** Interestingly, several bacterial and metabolic signatures have been consistently described, and are described here in **Table 1**, whereas some bacterial changes have been further associated with clinical parameters, as illustrated in **Table 2**. Both bacterial changes and their association with clinical parameters are summarized in the Figure 1.

Gammaproteobacteria[39] represents the class that has been the most consistently described as increased post-BS in animals as well as in both obese and obese diabetic patients[44,50,52,57]. In some studies, this increase is associated with the amount of weight loss[58]. In our previous study using 16S rRNA pyrosequencing, we observed increased *Escherichia coli*, which is within the *Proteobacteria* phylum, parallels the decrease in leptin post-BS[49]. Intriguingly, indirect data regarding the mechanism of action of metformin suggest that this increase in *Gammaproteobacteria* could be involved in the post-BS metabolic improvements[59]. Furthermore, disrupting the GM of rodents with a cocktail of large spectrum

antibiotics induces a major increase in *Proteobacteria*, which is associated a beneficial phenotype of decreased systemic inflammation and improved glucose homeostasis[60]. Finally, an increase in *Proteobacteria*, including *Escherichia coli*, has also been reported in rodents or in drug naïve T2D humans after metformin treatment inducing improved glucose homeostasis, which further suggests that *Proteobacteria* could be involved in metabolic improvements[61]. However, this beneficial increase of *Gammaproteobacteria* could be seen as a paradox since an elevation of *Proteobacteria* and *Enterobacteria* is generally seen as deleterious in many intestinal diseases, such as inflammatory bowel diseases and colon cancer [62]. The precise mechanisms of this apparent paradox need to be deciphered. Indeed, it is known that *Proteobacteria* are gram negative bacteria that express lipopolysaccharide (LPS) in their membrane. Since LPS is one of the main drivers of metabolic endotoxemia[63], one could argue whether increasing *Proteobacteria* should really translate into real clinical benefits. Interestingly, although increased LPS synthesis within the GM has been observed post-BS[42], it is not associated with exacerbated systemic inflammation. This rather suggests that BS might be associated with decreased LPS translocation within the intestine into the systemic circulation, via a potential decreased intestinal permeability post-BS. Murine data have observed that RYGB improves tight-junction integrity and in-vivo intestinal permeability while reducing metabolic endotoxemia and systemic inflammation[64]. Yet, such observations in mice following BS remain to be confirmed in humans.

Akkermansia muciniphila has been shown to have an important impact both on improved glucose homeostasis and weight loss as well as on the gut epithelium health in obese mice treated with prebiotics or after oral administration of the live bacteria[51,65–67]. *Akkermansia muciniphila* also is associated with insulin sensitivity in mice[65] and humans[66]. Indeed, obese individuals with increased *A. muciniphila* have improved metabolic condition[66]. Studies on small number of patients have also shown that *A. muciniphila* increases post-BS[44,50,51,68], yet

whether it relates to improved glucose homeostasis needs further validation. In an unpublished observation from our group, we did not observe an association between *A. muciniphila* increase post-BS and glucose metabolism improvement (Dao *et al.*, unpublished).

Impact of different bariatric surgery techniques

Although SG and RYGB display relatively similar clinical outcomes[69], the gut architecture modification significantly differs between the two procedures, possibly inducing differential GM modulations. Therefore, some, yet still scarce, studies have assessed GM changes after both interventions, after either SG or very low-calorie diet (VLCD), or finally, solely post-SG to assess SG specific effects.

SG induces specific and distinct GM shifts as seen in a small study comparing VLCD and SG effects on gut microbiota[70]. *Bacteroides vulgatus*, a bacteria found increased in severe obesity and positively correlated with HbA1c[8], is reduced significantly post-SG, whereas it is not significantly affected by either post-AGB or RYGB[8]. Furthermore, SG also increases *Faecalibacterium prausnitzii*[70], another bacterium found decreased in severely obese individuals with T2D and which increases post-RYGB[49]. Based on these observations, it is tempting to speculate that the change in these bacteria could be involved in glucose improvement observed post-SG, however this has not been clearly described. In another study with small sample size comparing SG and RYGB, Murphy *et al.*, observed that although SG was associated with functional changes in GM, they were fewer than those observed post-RYGB[43]. Furthermore, whereas both surgery types induce similar clinical improvement and diet intakes, gut microbiota modifications involve distinct pathways according to the surgical technique[43]. In particular, they observed an increased amino acids

biosynthesis capacity post-SG[43] a mechanism that could be linked to the improvement of glucose control.

A recent human study, including a larger number of individuals undergoing SG, demonstrated a rapid shift of microbial functions 3 months post-SG[48], becoming similar to that of healthy lean controls. Moreover, functions involved in carbohydrate fermentation, citrate cycle, glycosaminoglycan degradation and LPS synthesis pathway rapidly decreased in these individuals. Most interestingly, *Bacteroides thetaiotaomicron*, which was found to be decreased in obesity, increased 3 months post-SG and this increase was found to be associated with the decrease in BMI[48]. In this study, *A. muciniphila*, also significantly increased post-SG, a finding concordant with previous data obtained post-RYGB[51]. This study combining metagenomics and metabolomics exploration thus provides a potential link between these GM changes and metabolic improvement post-SG.

Inter-individual microbial modulation

Even though significant shifts in gut microbiome composition and functions are reported in BS cohorts, the reported GM signatures show a major inter-individual variability amongst subjects post-BS that merits consideration. These individual profiles are nevertheless difficult to grasp in published studies as individual data are scarcely presented.

Gut microbial diversity and richness inter-individual variability is observed both pre- and post-BS[8]. For example, we have reported that the mean baseline MGR is higher in patients who undergo AGB as compared to RYGB, which is likely due to less severe obesity-related comorbidities at baseline in AGB subjects. However, the baseline variance for MGR in both groups is large with the GM of patients undergoing AGB having between 300k and 600k genes, while the GM of patients in the RYGB group ranging between 125k to 550k genes. Currently, the underlying individual factors explaining this variability are unknown.

Moreover, whether we can exploit this inter-individual variability in order to find predictive biomarkers of BS-induced weight loss merit consideration and needs larger scale studies. Similarly, although the mean MGR significantly increases post-BS, the individual variability remains relatively high, yet lower than that observed at baseline. One could hypothesize that this MGR variability could be due to subjects' lifestyle (including food patterns) and clinical condition before and after BS. However, it could also be related to differential clinical developments post-BS, including the amount of weight loss and the amplitude of metabolic improvements, and this needs to be examined in dedicated prospective studies.

To date, only one study examined individual relative abundance of GM composition. This study explored three healthy controls as well as in three unpaired obese patients and three patients who underwent RYGB, albeit with variable follow-up duration[51]. The relative abundance of most bacterial classes was found to be highly variable not only between groups of patients, but also between patients within the same group; *Proteobacteria* and *Clostridia* were the most variable in the GM of obese and RYGB-operated patients, while *Verrucomicrobia* and *Bacteroidetes* were the most variable in the healthy controls [51]. We and others[8,71] have also recently reported this large inter-individual variability in GM modulation post-BS.

Collectively, the literature thus confirms that bariatric surgery modifies GM composition and function, yet differentially from one individual to the other. This could be related to variable clinical outcomes, which is largely described in bariatric cohorts[32,33,37]. Yet, it could also be due to several biases and/or confounding factors discussed below.

Discussion

Although some GM signatures observed post-BS are replicated across studies (as discussed above), this is not always the case as some studies display controversial results.

This variability in these findings might originate from the different DNA extraction and sequencing techniques used (DGGE[72], qPCR[49], 16S rRNA pyrosequencing[52], shotgun metagenomics (SOLiD [8,70] or Illumina[42–44,48,50,73], see Table 1) across studies, the different bariatric procedures, or different time points of stool collection post-BS (either short-[49,50,52], mid-[8,44,48] or long-term[8,42] follow-up) where clinical outcomes also differ. Moreover, cohort ethnicity might also play a role and is, in general, not taken into account in these studies. Ethnicity has been shown to influence GM composition[74], and study location (Europe[8], Asia[48], or Oceania[43]) could underlie the different BS-induced GM modulations due to different genetic backgrounds and lifestyles. As such, dietary intake[15,75] is critical in explaining variability in the modulation of GM composition, which also differs from one country to another but also between baseline and post-surgery follow-up[25,26]. For example, diet drastically changes post-BS, especially fiber intake[25], which is known to have a critical impact on GM composition and function[76]. In a previous study, we observed associations between some bacterial changes and improvements in corpulence, metabolic or inflammatory markers, yet half of these associations are strongly dependent on food intake[49]. Dietary patterns also differ from one individual to another post-BS[25,26,46] and dietary recommendations between clinical centers may differ as well[51]. It is thus necessary to better examine the link between post-BS dietary intake and lifestyle changes (such as physical activity) and gut microbiota modulation to explain the reported variability in GM composition.

Indeed, even though individuals can share broad GM resemblances, as seen with the enterotypes[77], a myriad of environmental factors play a role in this high inter-individual variability[76,78], including lifestyle factors but also medications. In the context of BS, patients are frequently heavily treated for a large set of obesity-associated comorbidities including T2D and dyslipidemia before the intervention [37]. These therapies, such as metformin (the first

line of treatment for T2D) or statins, can have profound effects on the GM composition[7,59,79,80]. Since BS induces major metabolic improvement, some, but not all patients, can stop drugs originally taken at baseline, in particular glucose-lowering agents including metformin[81,82]. Thus, these changes in drug intake, variable from one patient to another, could be involved in the major GM changes seen across individuals.

Finally, although BS induces drastic changes in GM richness and composition[8,40–43,49] some of which are maintained in the longer-term[42], BS does not rescue the GM dysbiosis seen in severe obesity[8]. While showing some improvement, gut microbial richness remains under the cut-off for low diversity[12,13]. In studies comparing BS individuals before and after surgery and lean controls, the GM profile at the phylum level does not reach that of lean individuals[49,51]. It is important to examine whether this partial correction of GM dysbiosis post-BS could be involved in weight regain or the reoccurrence of obesity related comorbidities in some patients[33,37], which is also associated with a switch towards a less healthy diet and a more sedentary lifestyle. A recent mouse study demonstrated that weight cycling induces GM modulations but with a persistent dysbiotic signature after the first initial weight loss. Most importantly, this dysbiotic GM is associated with increased weight gain when compared to high-fat diet fed mice who never were subjected to the weight loss intervention[83]. Therefore, one could hypothesize that although BS improves GM composition and function, it does not normalize it and this could be linked to adverse clinical outcomes in the long-term, including weight regain and metabolic deterioration[33].

Conclusion

While considered as a useful clinical tool to improve the clinical outcomes of patients with severe obesity, bariatric surgery is also a remarkable model to understand the fundamental mechanisms involved in drastic metabolic and inflammatory amelioration.

Amongst the myriad of potential mechanisms, changes in gut microbiota composition and related functional modification have been put forward with the availability of new sequencing tools. While GM changes can be observed and are associated with metabolic improvements in still relatively unpowered human studies, they are not always consistent and vary across population. Given these variations, further research efforts are needed to deepen the understanding of GM changes on improved metabolism post-BS, which may provide evidence for the need to act therapeutically on the GM to improve patient outcomes in the long term.

Authors contributions

JD contributed to the research, discussion of content, writing of this manuscript, J.A.W contributed to the research, discussion of content, writing and editing of this manuscript, and K.C. contributed to the discussion of content, writing and reviewing/editing the manuscript before submission All authors reviewed the manuscript.

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Disclosure of Potential Conflicts of Interest

None of the authors has anything to disclose relevant to this article.

Figure legend

Figure 1. Summary of the main changes in GM composition across literature and their link with modifications in clinical outcomes. ↑: Increase; BMI: Body mass index; CRP: C-reactive protein; HbA1C: Glycated hemoglobin; HOMA-IR: Homeostasis model assessment of insulin resistance; MCP-1: Monocyte chemoattractant protein 1; TNF-α: Tumor necrosis factor alpha.

Tables legends

Table 1. Gut microbiota changes described after bariatric surgery in both human and animal studies. @ represents studies where results regarding fecal GM transplants have been shown. The most commonly described GM changes are presented in bold, while conflicting results across studies are shown underlined. ↑: Increase; ↓: Decrease; AGB: Adjustable gastric banding; BIB: Biliointestinal bypass; BMI: Body mass index; BS: Bariatric surgery; DGGE: Denaturing gradient gel electrophoresis; DJB: Duodenal jejunal bypass; GLP-1; Glucagon-like peptide 1; GM: Gut microbiota; HFD: High fat diet; IGT: Impaired Glucose Tolerance; IHMS: International Human Microbiome Standards; MO: Morbidly obese, qPCR: Quantitative polymerase chain reaction; rRNA: Ribosomal ribonucleic acid; RYGB: Roux-en-Y gastric bypass; SG: Sleeve Gastrectomy; T2D: Type-2 diabetes; VBG: Vertical Banded Gastroplasty; VLCD: Very low calorie diet.

Table 2. Impact of BS-induced GM modulation on host metabolism, GM richness and clinical features. @ represents studies where results regarding fecal GM transplants have been shown. ↑: Increase; ↓: Decrease; =: no change; AGB: Adjustable Gastric Banding; BAs: Bile acids; BCAA: Branched Chain Amino-Acids; BMI: Body Mass Index; BS: Bariatric Surgery; CRP: C-reactive protein; DPP-4: Dipeptidyl peptidase-4; GM: Gut microbiota; GUDCA: Glyco-ursodeoxycholic acid; HbA1C: Glycated hemoglobin; HOMA-IR: Homeostasis model assessment of insulin resistance; LPS: Lipopolysaccharide; MCP-1: Monocyte chemoattractant protein 1; RYGB: Roux-en-Y Gastric Bypass; SCFA: Short Chain Fatty Acids; SG: Sleeve Gastrectomy; T2D: Type-2 Diabetes; TCDCA: Taurochenodeoxycholic acid; TNF-α: Tumor necrosis factor alpha;

Table 1.

Reference	Country	Design of the study	Number of patients with GM analyses	Surgery type (n of patients)	Samples	DNA extraction	Sequencing technique	Time-points sequenced	Changes in GM after BS	Impact of BS on fecal richness	Comments
Human studies											
Zhang *et al.*, 2009 [51]	USA	BS VS Obese VS lean individuals	6 MO patients and 3 lean individuals	RYGB (n=3)	Feces	QIAamp DNA Stool Kit (Qiagen)	Sanger & 16S rRNA pyrosequencing	8 to 15 months post-BS	↑ ***Gammaproteobacteria***, ***Verrucomicrobia***, *Fusobacteria* ↓ *Clostridia*	-	-
Furet *et al.*, 2010 [49]	France	BS VS lean individuals	30 MO (7 with T2D) patients and 13 lean individuals	RYGB (n=30)	Feces	Godon[84]	16S rRNA qPCR	Before, 3 months and 6 months post-BS	↑ *Bacteroides/Prevotella* ratio, ***Faecalibacterium prausnitzii***, ***Escherichia*** ↓ *Bifidobacterium*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*	-	-
Patil *et al.*, 2012 [85]	India	BS VS Obese VS lean individuals	5 thin, 5 lean, 5 obese and 5 obese-operated individuals	SG (n=3) and AGB (n=2)	Feces	QIAamp DNA Stool Mini Kit (Qiagen)	Sanger	-	↓ *Bacteroides* and Archaea	No changes	-
Kong *et al.*, 2013 [52]	France	BS	30 MO patients	RYGB (n=30)	Feces	Godon[84]	16S rRNA (V3-V4) pyrosequencing	Before, 3 months and 6 months post-BS	↑ *Bacteroides*, ***Escherichia***, *Alistipes* ↓ *Lactobacillus*, *Dorea*, *Blautia* and *Bifidobacterium*	↑ Number of genera and Chao1 index	-
Graessler *et al.*, 2013 [50]	Germany	BS	6 MO patients (n=5 T2D)	RYGB (n=6)	Feces	Nycodenz density gradient centrifugation, bacterial lysis and DNA digestion[86]	Shotgun metagenomic sequencing (Illumina)	Before and 3 months post-BS	↑ ***Proteobacteria***, ***Bacteroidetes/Firmicutes* ratio**, ***Verrucomicrobia*** ↓ ***Firmicutes***, *Cyanobacteria*	-	One patient received 6 days of penicillin 3 weeks prior the postoperative stool sample was collected
Ward *et al.*, 2014 [68]	USA	BS	8 MO patients	RYGB (n=8)	Feces	UltraClean Fecal DNA Kit (MO BIO, Inc.)	16S rRNA(V4) pyrosequencing	Before and 6 months post-BS	↑ ***Bacteroidetes***, ***Bacteroidetes/Firmicutes* ratio**, ***Proteobacteria*** (PPI users), ***Verrucomicrobia*** ↓ ***Firmicutes***, <u>*Proteobacteria*</u> (PPI non-users)	-	-
Damms-Machado *et al.*, 2015 [70]	Germany	BS VS VLCD	6 MO patients	SG (n=3)	Feces	PSP Spin Stool DNA Plus Kit with lyses enhancer (Stratec Molecular, Berlin, Germany)	Shotgun metagenomic sequencing (SOLiD)	Before, 3 months and 6 months post-BS	↑ ***Bacteroidetes***, ***Faecalibacterium pausnitzii*** ↓ Several ***Firmicutes*** (*Eubacterium*, *Faecalibacterium*, *Dorea*, and *Coprococcus*), *Bacteroides vulgatus*, <u>*Bacteroidetes/Firmicutes ratio*</u>	-	High inter-individual variability regarding the Bacteroidetes/Firmicutes ratio at baseline, despite relatively similar BMI
Tremaroli *et al.*, 2015 42 @	Italy	RYGB vs VBG vs MO patients	21 MO patients	RYGB (n=7) and VBG (n=7)	Feces	QIAamp DNA Stool Mini Kit columns	Shotgun metagenomic sequencing (Illumina)	About 10 years post-BS	↑ ***Proteobacteria*** (***Escherichia***, ***Klebsiella*** and *Pseudomonas*) ↓ ***Firmicutes***, *Eubacterium rectale* (VBG), Roseburia intestinalis (VBG)	-	The microbiota profiles were similar between RYGB and VBG patients, and differences in GM composition and genetic content are mostly due to the intervention and not BMI
Federico *et al.*, 2016 [72]	Italy	BS	11 MO patients	BIP (n=11)	Feces	Maxwell® 16 DNA Purification Kit (Promega)	qPCR-DGGE	Before and 6 months post-BS	↑ *Lactobacillus crispatus*, *Megasphaera elsdenii*, *Streptococcus spp.* ↓ *Butyrivibrio fibrisolvens*, *Roseburia hominis/faecis*, *Dorea longicatena*, *Blautia spp.*, *Ruminococcus spp.* and *Ruminococcus obeum*	-	The similarity was higher between subjects before the surgery than within the same subject before and after BS
Palleja *et al.*, 2016 [44]	Denmark	BS	13 MO patients (n=7 T2D and n=1 IGT)	RYGB (n=13)	Feces	IHMS 07V2	Shotgun metagenomic sequencing (Illumina)	Before, 3 months and 1-year post-BS	↑ ***Proteobacteria*** (including ***Escherichia coli*** and ***Klebsiella*** *pneumoniae*), *Streptococcus salivarius*, ***Akkermancia muciniphila*** ↓ <u>*Faecalibacterium prausnitzii*</u>, *Anaerotruncus colihominis*, *Megasphaera micronuciformis*	↑ Gene richness and Shannon diversity index during the first three months and stable afterwards	Surgery, baseline T2D status, metformin usage, GLP-1 levels (at each time point), and BMI (at each time point) explained most of the variation in terms of species composition
Patrone *et al.*, 2016 [73]	Italy	BS	11 MO patients (n=6 T2D)	BIB (n=11)	Feces	Maxwell® 16 DNA Purification Kit (Promega)	Shotgun metagenomic sequencing (Illumina)	Before and 6 months post-BS	↑ *Selenomonadales*, *Megasphaera*, *Lactobacillus*, ***Enterobactriaceae***, ***Gammaproteobacteria***, ***Proteobacteria*** ↓ *Lachnospiraceae*, *Ruminococcus*, <u>*Faecalibacterium*, *Blautia*</u>	↓ Chao1, Shannon and Simpson indexes	Decreased fecal pH after BS
Murphy *et al.*, 2017 [43]	New Zealand	BS	14 MO patients	RYGB (n=7) & SG (n=7)	Feces	Qiagen QIamp DNA stool mini kit	Shotgun metagenomic sequencing (Illumina)	Before and 1-year post-BSs	↑ RYGB: <u>*Firmicutes*</u>, *Actinobacteria*; SG: ***Bacteroidetes***	↑ Number of species (RYGB)	-

									↓ RYGB: *Bacteroidetes*		
Liu *et al.*, 2017 [48]	China	BS	23 MO patients	SG (n=23)	Feces	Nycodenz density gradient centrifugation, bacterial lysis and DNA digestion[86]	Shotgun metagenomic sequencing (Illumina)	Before, 1 month and 3 months post-BS	↑ *Bacteroidetes thetaiotaomicron*, ***Akkermansia muciniphila***, *Clostridiales bacterium* ↓ *Coprococcus comes* and *Dorea longicatena*	↑ Gene count, alpha-diversity	The GM composition of BS-operated obese patients shifted towards those of lean individuals
Aron-Wisnewsky *et al.*, 2018 [8]	France	BS	34 MO patients	RYGB (n=14+10) and AGB (n=10)	Feces	Godon[84]	Shotgun metagenomic sequencing (SOLiD)	1, 3, 12 months and up to 5 years post-BS	↑ *GU:99 Roseburia*, *GU:225 Butyricimonas virosa*, *GU:359 Butyricimonas*	↑ Gene richness 3 months after BS The increase similar proportion for both AGB and RYGB, and remained stable up to 5 years post-op.	The impact of RYGB was higher on the GM than that of AGB
Paganelli *et al.*, 2018 [71]	Netherlands	BS	45 MO patients	RYGB (n=23) and VSG (n=22)	Feces	Godon[84]	16S rRNA(V3-V4) shotgun sequencing (Illumina)	Before, 3 months and 6 months post-BS	↑ *Streptococcaceae*, ***Enterobacteriaceae*** ↓ *Bifidobacteriaceae*	No changes	-
Animal studies											
Li *et al.*, 2011 [57]	United Kingdom	BS vs Sham	12 Wistar rats under chow-diet	RYGB (n=6), Sham (n=6)	Feces	QIAamp DNA Stool Mini Kit (Qiagen)	16S rRNA (V1-V3) pyrosequencing	Before and 2, 4, 6- and 8-weeks post-BS	↑ Gammaproteobacteria ↓ Firmicutes, Bacteroidetes	-	The rats received an antibiotic treatment before the surgeries (amoxicillin/flucloxacillin)
Osto *et al.*, 2013 [87]	Belgium	BS vs Sham	16 Wistar rats under chow-diet	RYGB (n=8), Sham (n=8)	Samples collected across the length of the intestine	QIAamp DNA Stool Mini Kit (Qiagen)	qPCR	-	↑ *Bifidobacterium spp.* (across the intestine except the biliopancreatic limb), *Lactobacillus spp.* (caecum after RYGB), *Bacteroides/Prevotella* ratio (across the intestine except the biliopancreatic limb and caecum)	Increase total bacterial content in the alimentary limb after RYGB	-
Liou *et al.*, 2013 [40] @	USA	BS vs Sham vs calories-matched animals	13 C57Bl6 mice under HFD	RYGB (n=4), Sham (n=5)	Feces and samples collected across the length of the intestine	PowerSoil bacterial DNA extraction kit (MO-BIO)	16S rRNA(V4) shotgun sequencing (Illumina)	Fecal GM was analyses every two weeks during 12 weeks, and the GM of each intestinal segments was analyzed at 12 weeks (sacrifice)	↑ ***Bacteroidales***, ***Verrucomicrobiales***, ***Enterobacteriales***, Archaea ↓ *Clostridiales*, *Erysipelotrichales*, *Lactobacillales*	-	Changes of the GM composition were very rapid (1 week) and persistent The impact of RYGB was similar in both chow-fed and HFD-fed animals, suggesting a more pronounced effect of the surgery Increased gastric pH and decreased fecal pH
Arora *et al.*, 2017 [41] @	Sweden	BS	15 fa/fa rats under chow-diet	RYGB (n=5), DJB (n=5) and Sham (n=5)	Samples collected across the length of the intestine	QIAamp DNA Stool Mini Kit	16S rRNA (V1-V2) pyrosequencing & 16S rRNA(V4) shotgun sequencing (Illumina)	35 days after the surgeries (sacrifice)	↑ *Lactococcus spp.* (across the intestine after RYGB), *Bacteroides vulgatus* (ileum and colon after RYGB), ***Escherichia coli*** (across the intestine after RYGB) ↓ *Lactobacillus animalis* (across the intestine after RYGB), *Lactobacillus reuteri* (across the intestine after RYGB)	-	The GM composition is affected by RYGB but not by DJB The transfer of ileal GM from RYGB-operated rats induced an alteration of the glucose tolerance in the recipient mice, whereas the transfer of their cecal content slightly improved it
Duboc *et al.*, 2018 [88]	France	BS vs Sham	20 Male Wistar rats under HFD	RYGB (n=6), SG (n=5) and Sham (n=9)	Caecum	-	16S rRNA(V3-V4) shotgun sequencing (Illumina)	40 days post-BS (sacrifice)	↑ *Clostridium* (RYGB), *Ruminococcus*, ***Enterobacteriacae***	-	-

Table 2.

Reference	Metabolic changes	Link GM - clinical information
Human studies		
Furet *et al.*, 2010 [49]	-	*Faecalibacterium prausnitzii*, *Escherichia coli*, and the *Bacteroides/Prevotella* ratio were associated with inflammatory parameters, and correlated with changes of body weight, BMI, fat mass, leptin concentrations and food consumption aster the surgery
Patil *et al.*, 2012 [85]	↓ SCFA	-
Kong *et al.*, 2013 [52]	-	BS ↑ the number of bacterial genera associated with white adipose-tissue genes Most of the 14 genera modulated by BS were deeply correlated to clinical variables (HOMA-IR, fasting glucose, fat-mass etc.), although half of the associations were dependent on food intake
Graessler *et al.*, 2013 [50]	-	Several bacteria were correlated to both BMI and CRP post-BS, including *Lactobacillus acidophilus*, *Faecalibacterium prausnitzii*, *Coprococcus comes* *Faecalibacterium prausnitzii* correlates with plasma glucose levels, and *Thermomicrobium* and *Veillonella parvula* with HbA1C
Damms- Machado *et al.*, 2015 [70]	↑ Conjugated BAs (including GUDCA, TCDCA) ↓ Caloric extraction from nutrients, butyrate fermentation pathways, some secondary BAs = SCFA (no changes, confirmed in 10 other operated subjects)	-
Tremaroli *et al.*, 2015 [42] @	↑ Circulating post-prandial BAs ↓ SCFA	GM transplantation post-BS demonstrated a role of the GM in the reduction of adiposity observed after BS
Palleja *et al.*, 2016 [44]	↑ Oxygen tolerance, transport of macronutrients and micronutrients	-
Patrone *et al.*, 2016 [73]	↑ Relative levels of valerate and hexanoate ↓ Butyrate production (but levels were similar), relative levels of acetate and propionate	Significant positive associations were observed between *Clostridium* levels and insulin concentration, *Faecalibacterium* levels and triglycerides, *Gemmiger* (*Proteobacteria*) and serum glucose, total cholesterol and *Clostridium*, and a negative relationship between blood glucose concentration and the abundance of *Lactobacillus*. Among those, only the relations with *Gemminer*, *Lactobacillus* and *Faecalibacterium* remains significant after adjustment for calories intake.
Murphy *et al.*, 2017 [43]	↑ Import of carbohydrates (RYGB) and amino acid metabolism (RYGB & SG)	*Roseburia intestinalis* is associated with T2D remission both after SG and RYGB After BS, *Paraprevotella* and *Acidaminococcaceae* correlate with fiber intake and MCP-1, *Prorionibacteriaceae* and *Blautia* with TNF-a, *Bacteroidales* correlates inversely with HbA1C, *Slackia*, *Weissela*, *Anaerostipes*, *Coprococcus* and *Coprobacillus* with BMI
Liu *et al.*, 2017 [48]	↓ Carbohydrate fermentation, citrate cycle, glycosaminoglycan degradation, LPS synthesis pathway, BCAA synthesis	*Bacteroidetes thetaiotaomicron* is associated negatively with BMI and Glutamate levels, itself associated with the improvements of hyperglycemia, insulin-resistance and inflammatory markers
Aron-Wisnewsky *et al.*, 2018 [8]	↑ Glycine, acetyl glycine, methylmalonate ↓ Amino acid, BCAA, phenylalanine and tryptophan pathway metabolites	Positive correlations with BMI & fat mass: *Bacteroides finegoldii*, *Coprobacillus spp.*, *Anaerostipes hadrus* Negative correlations with BMI & fat mass: *Fusobacterium nucleatum*, *Dialister spp.*, and *Hungatella hathewayi* (correlating positively with HbA1C)
Paganelli *et al.*, 2018 [71]	-	Decreased HbA1c was associated with *Coriobacteriaceae* and *Clostridiales*
Animal studies		
Osto *et al.*, 2013 [87]	Increased DPP-4 activity in the alimentary limb and the serum	-
Liou *et al.*, 2013 [40] @	-	The GM of RYGB-operated animals was able to (i) decrease host adiposity and (ii) decrease fasting insulin levels and HOMA-IR upon gut microbiota transplantation
Arora *et al.*, 2017 [41] @	-	The transfer of ileal GM from RYGB-operated rats induced an alteration of the glucose tolerance and higher fat gain in the recipient mice, whereas the transfer of cecal GM induced a slight increase in glucose tolerance
Duboc *et al.*, 2018 [88]	↓ BAs deconjugation in the ileum of SG-operated animals	-

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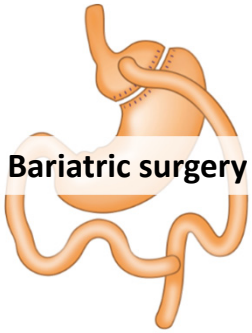

Bariatric surgery

Bacterial species increased after BS
Proteobacteria (*Escherichia*, *Klebsiella*), Verrucomicrobia (*Akkermansia*), Bacteroidetes (*Alistipes*)

↑ Gene richness and bacterial diversity

Bacterial species decreased after BS
Firmicutes (*Blaudia*, *Dorea*, *Ruminococcus*)

Clinical associations

Corpulence parameters
Body weight, BMI, fat mass

Metabolic parameters
Fasting glucose, HbA1c, HOMA-IR, fasting insulin concentration

Inflammatory parameters
CRP, MCP-1, TNF-α