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Chapter

Gut Microbiota and Bariatric Surgery

Natalia Bastón-Paz, Manuel Ponce-Alonso, José Avendaño, María Garriga and Rosa del Campo

Abstract

The gut microbiota comprise all the living organisms in our intestine. Microbiota has key roles in metabolic homeostasis, digestion and nutrient metabolism protection against pathogens or modulation of the immune system. Advances in techniques such as metagenomics or metabolomics have expanded our knowledge of the intestinal ecosystem. Beyond genetic, behavioral, or environmental factors, alterations of gut microbiota parameters such as composition, diversity, or metabolites including short-chain fatty acids, have shown to be associated with cardiovascular comorbidities. In this chapter, we described the role of the gut microbiota in obesity and type 2 diabetes pathophysiology, and the changes it undergoes during bariatric surgery, as well as explored the possibilities of modifying the microbiome to obtain potential clinical benefits.

Keywords: gut microbiota, obesity, type 2 diabetes, bariatric surgery, diet, probiotics, fecal microbiota transplant

1. Introduction

The human organism is a complex biological system composed of cells belonging to three domains: Eukarya, Bacteria, and Archaea, in addition to viruses [1, 2]. The microbiome has been considered as the last human organ [1] and can be defined as the whole genomic and metabolomic content of the microbial community that coexists and interacts with our cells [3]. The gut microbiota, the most complex and abundant microbiome [4], is the focus of this chapter because of its direct relationship with obesity and bariatric surgery.

The gut microbiota has traditionally been studied by culturing, with the aim of identifying and characterizing single isolated microorganisms related to acute or chronic infections [5]. Although culturing techniques are improving with various strategies [6, 7], their resolution is insufficient because most bacteria are uncultivable. Today, microbiota studies are focusing on the overall ecosystem, not only individual microorganisms; and to address the real effect of microbiota colonization on human health over prolonged periods.

Knowledge of the human microbiome has exploded in the last two decades due to the development of genomic strategies based on marker genes such as 16S ribosomal
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**Figure 1.**
Schematic representation of the usual workflow in the study of the bacterial composition in a sample by NGS of 16S rDNA amplicons.
DNA (rDNA), and massive sequencing techniques, also known as next-generation sequencing (NGS) (Figure 1). Multi-omics technologies, including metagenome, metatranscriptome, metaproteome, and metabolome approaches provide valuable information on microbial functions [8]. The high potential of combining various “omics” techniques to analyze host-microorganism interactions allows us to dissect the molecular mechanisms by which microbiomes influence human health. A summary of the characteristics of each technique is shown in Table 1. Bioinformatics analysis of these big data allows us to characterize the ecological biodiversity of a given microbial community and draw conclusions [9]. Nonetheless, this field of research is beyond the scope of this chapter.

### 2. The importance of sampling

Microbiota composition is typically studied in biological samples, such as stool, mucosal biopsy, intestinal aspirate, luminal brushing, etc.; for population studies, however, sampling should be noninvasive and performed mainly in healthy conditions. In practice, although fecal samples are the most used as a representation of the intestinal tract, significant differences have been demonstrated among the mucosal microbiota of each intestinal anatomic region [10, 11]. Its main limitations are that feces also contain DNA from microorganisms ingested with food, which are not part of our microbiota; that we are not able to differentiate between living and dead microorganisms; and given that diet causes fluctuations in its composition, longitudinal sampling is required to decipher the real core of the native microbiota.

After deposition, strict anaerobic bacteria begin to lose viability after contact with oxygen, an irrelevant factor if DNA techniques are used. However, numerous studies have demonstrated the influence on the results of collection, transport, storage, and processing of the samples [12]. All samples belonging to the same study should be collected, preserved, and processed simultaneously and identically, to minimize any source of variability [13]. As a rule, recommendations on feces collection consist of using a sterile container with a screw cap, which should be transported without delay to the processing center and frozen as soon as possible at −80°C, although it is also acceptable to perform a first freeze at higher temperatures (as low as −20°C). Freezing prevents changes in microbial communities until nucleic acid extraction can be performed, which is crucial for RNA analysis because it is more easily degraded.
than DNA in freeze–thaw cycles. Therefore, optimizing sampling methods should not be ignored. Future sampling procedures should include reducing invasiveness, performing non-cross-contamination sampling, and minimizing disturbance to normal intestinal physiology [12].

3. Gut microbiota composition

The parameters that help us to characterize the microbiome have been classically used in ecology and can be separated into those related to alpha diversity, which is a measure of microbiome diversity that allows us to define the total number of species and their relative contribution applicable to a single sample (Shannon or Chao1 indexes, i.e.); and beta diversity, which is a measure of similarity or dissimilarity between two communities, allowing us to compare ecosystems of different subjects or times (Bray-Curtis or UniFrac metrics, i.e.) [14, 15].

Currently, there is no consensus on the “normal” composition of the microbiota or universal cutoff points for classifying a microbiome as healthy or pathological according to the presence/absence or abundance of certain taxa in the overall ecosystem. It is generally considered that the greater the number of species present, and the more balanced the distribution of species, the healthier and more resilient the ecosystem [16].

The most dominant bacterial phyla of the human gut are Bacillota (formerly Firmicutes), Bacteroidota (formerly Bacteroidetes), Actinomycetota, and Pseudomonadota (formerly Proteobacteria), with *Bacteroides, Clostridium, Peptococcus, Bifidobacterium, Eubacterium, Ruminococcus, Faecalibacterium*, and *Peptostreptococcus* as the most abundant genera. Remarkably, *Bacteroides* family represents approximately 30% of the total bacteria, suggesting an important role in the global metabolism [4].

Beyond composition, the real impact of microbiota on human health is conditioned by their metabolism. This balanced host–microbe interaction can be defined as eubiosis, again habitually linked to high taxa diversity, high microbial gene richness, and stable microbiome functionality [8]. An imbalance in this functionality is defined as dysbiosis, a term typically used when the composition is different from that of healthy individuals; in our view, however, it is more a functional than compositional concept. This disturbance of gut microbiota can also modulate intestinal permeability as well as immune responses, favoring a proinflammatory state [5, 17, 18].

3.1 Factors affecting gut microbial composition

Several factors can influence microbiota composition. These factors include the mode of infant delivery and breastfeeding, diet, intake of antibiotics and other drugs, stress, disease, smoking, drinking, aging, and race, among others [18]. The main influencing factors are described below:

a. Mode of infant delivery and breastfeeding. The mode of delivery significantly affects gut colonization in newborns. Passage through the birth canal affords the neonate a microbiota like that of the mother’s vagina, whereas for infants born via Cesarean-section, the microbiota resembles the mother’s skin and environmental microorganisms [19, 20]. Breastfeeding also provides beneficial genera, such as *Bifidobacterium* and *Lactobacillus*, with a lower colonization rate by *Escherichia coli, Clostridium*, and *Bacteroides* [21, 22].
b. **Antibiotic exposure.** Various studies have shown both the short- and long-term impact of antibiotics on the gut microbiota [23, 24]. Among the short-term effects of antibiotic use reported are diarrhea and recurrent *Clostridioides difficile* infection [25]. The long-term effects include allergic conditions and obesity due to altered metabolic activity [26]. Lastly, systematic use of antibiotics can reduce bacterial species diversity, as well as selecting for antibiotic-resistant strains even after exposure has been eliminated.

c. **Diet.** Diet is the major factor conditioning gut microbiota composition. The Western diet has been associated with less bacterial alpha diversity compared with the Mediterranean diet and other diets with lower animal protein and high vegetable and fiber intake. Unlike the Western diet, which has been associated with a higher risk of obesity, diabetes, cancer, and cardiovascular disease due to high intakes of animal proteins, saturated fats, and simple sugars, the Mediterranean diet and plant-based diets prevent cardiovascular disease, reducing mortality risk and limiting weight gain. It has been reported that the consumption of a plant protein-based diet increases *Bifidobacterium* and *Lactobacillus* genera, as well as decreasing *Bacteroides* and *Clostridium* species [27, 28]. The intake of nondigestible carbohydrates, such as fiber and resistant starch, appears to have the highest impact of all nutritional components on gut microbiota composition, diversity, and metabolic profile [29].

d. **Lifestyle.** Lifestyle factors include physical activity, smoking, and the surrounding environment, to name a few. The individual's level of physical activity and their amount of exposure to pollutants are considered critical factors affecting microbiome composition [18]. Regarding physical activity, an active individual's microbiome possesses a greater abundance of beneficial bacteria, such as *Faecalibacterium*, *Roseburia*, and *Akkermansia* [30]. As for environmental exposure, certain pollutants have been associated with fewer taxa and higher numbers of *Bacteroides* and *Clostridium* species [31]. Lastly, when comparing the gut microbiome of smokers with that of non-smokers and former smokers, that of smokers was found to be enriched with *Bacteroides* and reduced in *Bacillota* and *Pseudomonadota* [32].

4. **Functions of gut microbiota**

A mature, healthy gut microbiota has significant functions in the human body [4]: protection against pathogens by colonizing mucosal surfaces and production of various antimicrobial substances; development and modulation of the immune system; digestion and nutrient metabolism; control of cellular proliferation and differentiation; modification of insulin resistance and its secretion; and facilitation of dynamic communication between the gut and multiple organs [33, 34].

In Ref. to its role in enhancing the immune system, immunological immaturity is observed in germ-free and laboratory mice compared with wild mice, and humans residing on farms exhibit greater functional microbial diversity and a lower susceptibility to chronic inflammatory diseases [35]. The gut microbiota has also been described as an important immunoregulator of bone's remodeling processes [36, 37].

One of the most relevant roles of gut microbiota is the metabolism of dietary elements into bioactive food components. Indigestible carbohydrates are metabolized into short-chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids,
which are mainly produced due to fermentation by Bacillota, Bacteroidota, and other anaerobic bacteria. These compounds supply significant energy for intestinal epithelial cells, strengthen the mucosal barrier, contribute to intestinal homeostasis, and reduce inflammation [38]. Moreover, the gut microbiota participates in the biosynthesis of certain essential amino acids and vitamins and is involved in the synthesis of bile acids, cholesterol, and conjugated fatty acids [17]. On the other hand, other microbial derived-metabolites, such as trimethylamine N-oxide (TMAO), have been associated with cardiovascular disease [39].

Therefore, understanding the metabolic pathways of derived microbial compounds is crucial for establishing a link to the metabolism of the healthy host or to the pathogenesis of metabolic diseases.

5. Gut microbiota in obesity and type 2 diabetes mellitus

5.1 Obesity

Obesity is a complex and multifactorial disease with significant morbidity and mortality, and it is a major public health problem, particularly in the developed world [40]. Among the risk factors contributing to obesity (genetic, behavioral, socioeconomic, and environmental), the gut microbiota has been recognized as a major contributor [3]. More than 10 years ago, a differential gut bacterial composition linked to increased Bacillota and a reduction in Bacteroidota was demonstrated in genetically obese (ob/ob) mice compared with lean (ob/+ ) and wild-type (+/+ ) mice that had been fed with the same polysaccharide-enriched diet [41]. Moreover, after transplanting the obese and lean microbiomes to germ-free recipients, the phenotypes of the mouse donors were reproduced.

Numerous studies have been designed to identify significant differences in the bacterial gut microbiota composition between lean and obese individuals [42] and to describe the impact of the bariatric surgery approach to obesity [43]. Regarding bacteria, the Bacillota/Bacteroidota ratio has been discarded because it was proven ineffective as a differential marker of the microbiota in patients with obesity, given that an expansion of Bacillota leads to a proportional reduction of the other phyla [44]. The bariatric surgery approach will be discussed in a later section.

According to the following meta-analysis, which reviewed the composition of the gut microbiota in obese and non-obese individuals [45], no significant differences were found in alpha diversity. On the other hand, at the genus level, lower relative proportions of Bifidobacterium and Eggerthella (Actinomycetota) were observed in the obese group compared with the non-obese group. The genera of Acidaminococcus, Anaerococcus, Catenibacterium, Dialister, Dorea, Eubacterium, Megaplasma, Roseburia, Streptococcus (all belonging to the Bacillota phylum), Fusobacterium (Fusobacteriota), Prevotella (Bacteroidota), Escherichia-Shigella, and Sutterella (Pseudomonadota) were significantly higher in the obese individuals. On the other hand, Verrucomicrobiota (Akkermansia muciniphila), Faecalibacterium, Methanobrevibacter smithii, and Lactobacillus species have a lower presence in obesity [46].

5.2 Type 2 diabetes mellitus

The etiology of type 2 diabetes mellitus (T2DM) involves a combination of genetic variants and environmental factors shared with obesity, in which most individuals are
either overweight or obese. Insulin resistance is followed by a compensatory higher biosynthesis and insulin secretion.

Although there are some inconsistencies among studies, it appears that in the early stages of T2DM, before patients have been treated with anti-hyperglycemic drugs, the gut microbiota could have loss of butyrate-producing taxa, a marked reduction of \textit{Akkermansia}, and an increase in proinflammatory bacterial genera such as \textit{Bacteroidota} \cite{47, 48}.

The effects of metformin on the gut microbiota have been studied in patients with T2DM, demonstrating a higher relative abundance of \textit{Akkermansia}, \textit{Butyrivibrio}, \textit{Bifidobacterium}, and \textit{Megasphaera} compared with individuals without T2DM \cite{49}. On the other hand, those with non-metformin treated T2DM had a higher relative abundance of Clostridiaceae and a lower abundance of \textit{Enterococcus casseliflavus} compared with individuals without T2DM. The authors found significant associations between metformin intake and gut microbiota composition.

Additional studies \cite{50, 51} have also observed shifts in gut microbiota in patients treated with metformin by increasing the abundance of \textit{Akkermansia} and SCFA-producing bacteria, which activate intestinal gluconeogenesis, resulting in lower glycemic levels. \textit{Akkermansia} participates in maintaining the cohesion of the mucin layer by reducing translocation of proinflammatory lipopolysaccharides and controlling fat deposition, adipose tissue metabolism, and glucose homeostasis. SCFAs, especially butyrate and propionate, trigger intestinal gluconeogenesis, benefitting glucose and energy homeostasis and reducing hepatic glucose production, appetite, and body weight.

Nevertheless, further large-scale studies are necessary to evaluate the interactions between the changes in gut microbiota and the effects of metformin to establish a potential target intervention from a microbiological perspective.

6. Impact of bariatric surgery on gut microbiota

As has been described throughout the present book, bariatric surgery (BS) is indicated as treatment for reducing body mass index (BMI) in severe obesity (BMI $\geq 40$ Kg/m$^2$ or $\geq 35$ Kg/m$^2$) with at least one obesity-related disease \cite{52}. This surgery improves glycemic control because of weight loss and calorie restriction, along with increased insulin sensitivity and secretion \cite{53}. Several changes in gut microbiota composition depending on the type of surgery have been observed.

The procedures vary, although the most common are sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB). While SG is a restrictive approach based on stomach reduction, RYGB combines restrictive and malabsorptive approaches by reducing the stomach and anatomically reorganizing the biliary and digestive tracts \cite{54}. These procedures alter the anatomy of the digestive and biliary tract, hormonal status, and the amount and choice of nutrients ingested, which could modify the composition of the microbiota and the quantity of several microbial metabolites \cite{54, 55}. However, whether the evolution of the microbiota is the cause or the consequence of weight loss and improvement of obesity-related diseases (or whether the changes are more related to the specificities of the surgical procedure) remains to be determined.

One of the most relevant lines of research proposes to predict weight loss after BS by examining the basal composition of the gut microbiota. Previous studies have indicated that BS modifies the gut microbiota profiles \cite{56, 57}. Changes in alpha diversity do not appear to be clear, but beta diversity analyses consistently show more profound changes for the RYGB approach with an expansion of the phylum...
Pseudomonadota. Redistribution of the bile acid circuit, whose antibacterial activity limits the expansion of gamma-Proteobacteria in the small intestine, appears to be the main cause of the increase in the members of this phylum found in samples from patients undergoing RYGB [58].
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In 2022, Salazar et al. [58] observed that BS caused a decrease in the genera *Roseburia*, *Faecalibacterium*, *Ruminococcus*, and *Bifidobacterium* and an increase in *Escherichia/Shigella* and *Akkermansia*. As observed in other studies [59], differences between samples at baseline and at the end of follow-up were much more profound in the RYGB group: the phyla *Pseudomonadota*, *Bacteroidota*, *Verrucomicrobiota*, and *Fusobacteriota* experienced a significant increase in number at 3 months after RYGB surgery, inversely to *Bacillota* and *Actinomycetota*. However, the changes were considerably less marked in the SG group, with a slight enrichment of certain *Bacillota*, such as *Streptococcus*, *Parvimonas*, *Hungatella*, *Lactobacillus*, and *Desulfovibrio*, along with a decrease in *Bacteroidota* and *Negativicutes*. Lastly, and despite these differences in bacterial composition, the authors emphasized that weight loss was uniform in both groups, independent of the initial gut microbiota composition (Figure 2).

6.1 Type 2 diabetes mellitus remission

Regarding T2DM remission after BS, discordant results have been published according to a meta-analysis review [60]. This discordance could be explained by the design of the studies, the sample size and statistical power to assess differences, as well as the duration of follow-up. In addition, the authors note that different remission criteria were used in the literature reviewed, which could have possibly led to discrepancies in the interpretation of the available evidence. However, other researchers have explored the possibility that remission of diabetes after RYGB and SG surgery may be associated with interindividual differences in microbiota composition.

Although post-surgical changes in gut microbiota richness and composition were observed, these were independent of T2DM remission status, and no specific postoperative gender signature was identified that discriminated patients who reached this metabolic outcome [59]. However, a distinct genus signature pre-RYGB was observed in patients with total T2DM remission (Figure 3).

Murphy et al. [61] found that body weight reduction, dietary changes, and T2DM remission were similar 1 year after both RYGB and SG. RYGB surgery resulted in an

Figure 3. Gut bacteria genera at the preoperative period in obese patients classified according to T2D remission after RYGB. These figures represent comparison at the preoperative period of gut bacteria genus profile between patients classified, after RYGB, according to presence (blue boxes; \( n = 8 \)) and absence of T2D remission (red boxes; \( n = 6 \)). There was a higher relative abundance of (A) *Asaccharobacter* \(( p = 0.028)\) and (B) *Atopobium* \(( p = 0.047)\) and a lower relative abundance of (C) *Gemella* \(( p = 0.038)\), (D) *Coprococcus* \(( p = 0.02)\), and (E) *Desulfovibrio* \(( p = 0.030)\) in the patients with T2D remission than in patients without, (from: Al-Assal K et al. [59]).
increased Bacillota and Actinomycetota phyla, but a decreased Bacteroidota phyla. On the other hand, the SG procedure resulted in an increased Bacteroidota phyla. An increase in *Roseburia* species was observed among those who achieved diabetes remission in both types of surgery, although greater changes in gut microbiota metabolism occurred after RYGB than after SG. Contrary to the findings of Al-Assal et al. [59], those with persistent diabetes postoperatively had more *Desulfovibrio* species before surgery.

Similar results were addressed in Davies et al. [62], a higher abundance of *Eubacteriaceae* and *Alistipes putredinis* was observed before surgery in those individuals with T2DM remission post-intervention. After BS, *Lachnospiraceae* and *Roseburia* species were more abundant in those who had achieved T2DM remission.

The differential bacterial abundance was analyzed in 8 patients who underwent RYGB with complete resolution of diabetes as reported by Salazar et al. in 2022 [58], showing a significant increase of Verrucomicrobiota phyla (*Akkermansia*) and *Fusobacteriota* (*Fusobacterium*) after surgery, whereas the relative abundance of the phyla Bacillota (*Faecalibacterium*, *Erysipelotrichia*, *Gemmiger*, and *Lactobacillus*) and Actinomycetota (*Bifidobacterium*) decreased.

7. Potential interventions for modulating gut microbiota

There is immense potential for microbiome-modulating therapies based on microbial replacement, which are emerging as a treatment option for several diseases. These new intervention approaches have been made possible by our growing understanding of host-microbiome interactions [63]. Various interventions are possible depending on the level of invasion, ranging from dietary changes to microbial replacement through fecal transplantation (Figure 4).

7.1 Diet

As mentioned previously, nutritional recommendations for a Mediterranean diet or plant-based diet, which are rich in polyunsaturated and monounsaturated fat, have

![Interventions to Modulate the Gut Microbiota](image)

*Figure 4.* Different level interventions for gut microbiota modulation.
been associated with higher bacterial diversity, higher levels of total SCFA [64, 65], and significant reductions in plasma cholesterol [66]. Increased physical activity has also demonstrated beneficial changes to the gut microbiome [67]. To establish a clear intervention effect on the gut microbiome through modulation of dietary fat, both in quantity and quality, more clinical trials are needed to establish nutritional recommendations [68].

7.2 The “biotics”: Prebiotics, probiotics, symbiotics, and postbiotics

Complex mixtures of bacterial strains (probiotics) and various fiber dosages (prebiotics), which are “symbiotic”, have been used in multiple studies regarding obesity treatment. In vitro studies have shown that symbiotics are more efficient at modulating gut microbiota than prebiotics or probiotics alone [69].

According to the consensus statement of the International Scientific Association of Probiotics and Prebiotics (ISAPP), postbiotics are defined as “preparations of inanimate microorganisms and/or their components that confer a health benefit on the host” [70]. SCFAs are currently the most common type of postbiotics used. These compounds increase brown adipose tissue and promote browning of white adipose tissue, as well as regulate appetite by interfering with the gut-brain axis [71]. Overall, probiotics, prebiotics, symbiotics, and postbiotics appear to exhibit beneficial effects on gut microbiota modulation. Nevertheless, further large-scale trials are required to evaluate their beneficial properties, safety profile, dosage, and the durability of their beneficial effects in the prevention and treatment of obesity [72].

7.3 Fecal microbiota transplantation (FMT)

FMT is a modulation strategy that transfers a complete microbial ecosystem from a healthy donor to a patient with the aim of ecologically restoring an aberrant microbiota [73]. The donor’s microbiota can be administered through colonoscopy or orally by capsules. This technique has been widely investigated for the treatment of recurrent Clostridioides difficile infection, with outstanding therapeutic success rates; however, there is no current indication for obesity [74].

Although several studies have been performed in patients with various inflammatory disorders, such as irritable bowel syndrome and obesity-associated metabolic disorders, therapeutic success rates were not as high, or no effect was observed [75–77].

Further studies are required to understand the mechanisms through which changes in gut microbial ecology and engraftment of microbiota affect metabolic outcomes for patients with obesity. In addition, further research is needed to better define the optimal fecal microbial preparation as well as dosing and method of delivery [78].

8. Final conclusions

• Even with valuable insights into the impact of the microbiome on human health and disease, our understanding is limited due to the highly individualized profile of the microbiome and its complex multi-directional interactions with the human host.
• The development of sampling methods is critical for future gut microbiota research, given that the correct sampling has a crucial effect on the accuracy of “omics” techniques.

• Future larger studies using high-throughput sequencing and metagenomic and metabolomic techniques will provide a better understanding of the composition of the microbiota and its functional evolution after BS.

• Although further investigation is required, combining various modulation strategies, such as diet, biotics, and in certain cases, FMT, might be the best approach to “normalize” the gut microbiota as prophylaxis therapy when patients are going under BS.

• The gut microbiota influences several aspects of human health, from innate immunity to energy and metabolism. Modulation of the gut microbiome could therefore potentially reduce obesity and should be based on dietary interventions and lifestyle changes.

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Conflict of interest

The authors declare no conflict of interest.
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