

Gut microbiota characterisation in obese patients before and after bariatric surgery

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G. Campisciano^{1#}, S. Palmisano^{2,3#}, C. Cason², M. Giuricin³, M. Silvestri³, M. Guerra³, D. Macor^{2,4,5}, N. De Manzini^{2,3}, L.S. Crocè^{2,4} and M. Comar^{1,2*}

¹*Institute for Maternal and Child Health – IRCCS Burlo Garofolo, Via dell'Istria 65/1, 34137 Trieste, Italy;* ²*Department of Medical, Surgical and Health Sciences, General Surgery Clinic, University Hospital of Trieste, Strada di Fiume 447, 34100 Trieste, Italy;* ³*General Surgery Clinic, ASUITS, Strada di Fiume 447, 34149 Trieste, Italy;* ⁴*Clinica Patologie del Fegato, ASUITS, Strada di Fiume 447, 34149 Trieste, Italy;* ⁵*University of Verona, via S. Francesco 22, 37129 Verona, Italy;* [#]*these authors contributed equally;* manola.comar@burlo.trieste.it

Abstract

Intestinal microbiota analysis of obese patients after bariatric surgery showed that *Proteobacteria* decreased after laparoscopic sleeve gastrectomy (SG), while it increased after laparoscopic gastric bypass (LGB). Comparing to normal weight (NW) patients, obese patients that were selected for SG showed an almost equal amount of *Firmicutes* and *Bacteroidetes* and the ratio was not affected by the surgery. Obese patients before LGB showed a predominance of *Bacteroidetes*, whose amount regained a relative abundance similar to NW patients after surgery. Obese patients before LGB showed the predominance of *Bacteroides*, which decreased after surgery in favour of *Prevotella*, a bacterium associated with a healthy diet. The bacteria detected at the highest percentages belonged to biofilm forming species. In conclusion, in this study, we found that the characterization of the gut microbial communities and the modality of mucosal colonisation have a central role as markers for the clinical management of obesity and promote the maintenance of good health and the weight loss.

Keywords: obesity, microbiome, sleeve gastrectomy, laparoscopic gastric bypass

1. Introduction

Many recent studies have attempted to define links between gut resident bacterial communities and their function in obesity suggesting that microbiota plays an essential role since it creates a symbiotic dynamic relationship with the host (Sekirov *et al.*, 2010). It has been shown that certain characteristics of the gut microbiota composition confer metabolic efficiency and its dysbiosis is related to obesity. Most of the bacteria forming the adult intestinal microbiota belong to four phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. In obese subjects an intestinal microbiota dysregulation has been documented with changes mainly in the proportion of *Firmicutes* and *Bacteroidetes* in favour of an excess of *Firmicutes* and with a reduction in terms of diversity, although the dysregulation of many other bacteria has been associated to this metabolic disease (Peat *et al.*, 2015). Bacteria contribute to obesity not only from the metabolic point of view but also by promoting

processes, such as chronic inflammation (Arumugam *et al.*, 2011; Ley *et al.*, 2005; Segata *et al.*, 2012).

Using specific 16S rRNA probes, it was shown that the gut microbiota is composed of many bacteria, including *Enterococci*, *Bacteroides* and *Bifidobacteria*, occurring extensively in microcolonies. The presence of immunogenic bacterial species in microcolonies on epithelial surfaces may have implications for some forms of gut disease as greater numbers of bacteria would give rise to higher localized concentrations of bacterial antigens, toxins or other harmful secretory products (Macfarlane *et al.*, 2004). The gut microbiota is crucial in human metabolism, synthesizing unique and essential molecules, like vitamins, metabolizing ingested compounds, including dietary and pharmaceutical material, promoting the absorption of glucose, cooperating in the extraction of calories and their storage in host adipose tissue, providing energy and nutrients for microbial growth and proliferation, cooperating in the metabolism of bile

acids and is also an important factor for brain development. Furthermore, it stimulates the host immune system by contributing to its development and competitively inhibits the colonisation of the intestine by pathogenic bacteria (Thursby and Juge, 2017).

Bariatric surgery is currently the most effective treatment for obesity inducing a dramatic loss of fat mass and basically favouring metabolic improvement. The sensitivity towards lipolysis controlled by insulin and catecholamines is improved, adipokine secretion is altered and inflammatory markers decrease. A recent hypothesis for these effects includes changes in bile acid metabolism, gut microbiota and central regulation of metabolism (Peat *et al.*, 2015).

To better understand the relationship between gut resident microorganisms in bariatric patients, we will discuss data of microbiome composition from obese patients eligible for bariatric surgery, considered the first line of treatment in cases of morbid obesity not responding to dietary treatment and/or physical activity.

2. Material and methods

Patients selection

Twenty obese patients were selected after specific preoperative assessment. Obese subjects eligible for bariatric surgery were included in the study in accordance with the international guidelines. The inclusion criteria were: well-informed and motivated patients with acceptable operative risks, failure of non-surgical treatments, declared compliance to follow lifelong medical surveillance, aged 18 to 65 years, body mass index (BMI) of 40 kg/m² or between 35 and 40 kg/m² with obesity-related comorbidities. Exclusion criteria included the following: patients affected from documented liver cirrhosis, patients consuming an amount of alcohol >25 g/day, presence of other liver diseases like: patients infected with hepatitis B virus, hepatitis C virus, human immunodeficiency virus and presence of neoplasia. Surgical intervention was performed by surgeons of the General Surgery Clinic, Cattinara Hospital of Trieste. Ten patients underwent laparoscopic gastric bypass (LGB) and ten laparoscopic sleeve gastrectomy (SG). As control, twenty normal weight (NW) subjects with characteristics matching that of obese patients were recruited. Microbiota characterisation was performed by Ion Torrent next-generation high-throughput sequencing of the 16S rRNA gene on faecal samples before surgery and during the first follow-up visit (3 months later) at the Advanced Microbiological Laboratory, IRCCS-Burlo Garofolo, Trieste, Italy. All subjects provided informed consent before taking part in the study. The study was approved by the local ethical committee.

Surgical techniques

All patients were well informed about the surgical technique and the surgical risks and signed a full informed consent for the operation. Before starting with bariatric procedure, a liver surgical biopsy was performed. The LGB has both restrictive and malabsorptive effects. The first step (restrictive) of the procedure involves the creation of a stapled gastric pouch calibrated on a 34-French orogastric tube guide. The dissection begins between the first and second vascular arcades on the lesser curvature and a laparoscopic linear stapler is fired. The first suture line is horizontal, the second line runs parallel to the oesophagus towards the Angle of His. Once the gastric pouch is completely separated from the bypassed stomach, the omentum and the transverse mesocolon are lifted upwards and the ligament of Treitz is identified. The malabsorptive property is due to the total bypass of the duodenum and the proximal jejunum. The biliary limb is measured 100 cm distal to the ligament of Treitz and the alimentary limb is measured up to 120 cm. Both the gastrojejunostomy (between the gastric pouch and the alimentary limb) and the jejunojejunostomy (between the alimentary and biliary limbs) are created with linear staplers.

SG is a weight loss procedure that removes most of the stomach except a small banana-shaped pouch. An ultrasonic scalpel is used to make a window into the omental bursa proximal to the pylorus and the tissue that attaches the stomach to the omentum is then separated. The dissection proceeded to the Angle of His, the entire underside of the stomach from the greater curvature is completely freed from all the attachments up to the left crus of the diaphragm. A 34-French orogastric sizing bougie is then inserted by the anaesthesiologist and placed into the pyloric channel. An endoscopic linear cutting stapler is used to serially staple and transect the stomach staying close to the tube. The stomach is divided up to the level of the gastroesophageal junction. The greater curvature is completely freed and removed.

Sample processing, Ion Torrent sequencing and data analyses

DNA extraction from faeces was carried out using the NucliSENS® easyMAG® system (BioMérieux, Gorman, NC, USA). A real time EvaGreen® dye (Fisher Molecular Biology, Waltham, MA, USA) PCR was performed with the degenerated primer 27FYM and subsequently with Bt338F in conjunction with the U534R primer. The PCR targets the V1-V3 region. The size of the amplicons was checked on a 2% agarose gel. The amount of DNA after normalisation was quantified with a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The template preparation was performed using the Ion PGM Template OT2 200 kit on Ion OneTouch™ 2 System (Thermo Fisher Scientific,

Waltham, MA, USA) and then sequenced on the Ion PGM™ System machine, using the Ion PGM sequencing 200 KIT V2 (Thermo Fisher Scientific). Quantitative Insights Into Microbial Ecology (QIIME) 1.8.01 was used to process the sequence data. High quality (Q>25) sequences were demultiplexed and filtered by quality using `split_libraries_fastq.py` with default parameters, except for the length parameter (150 bp). Alpha diversity was assessed by the Chao1, observed Otus and phylogenetic diversity whole tree metrics. Differences in community composition between cohorts were investigated using the Kruskal-Wallis test. The sequences were aligned against the human intestinal microbiota 16S rRNA database (HITdb) (Ritari *et al.*, 2015).

3. Results

Microbiota composition

The three alpha diversity metrics (within-sample diversity) were not significantly affected based on the intestinal dysbiosis among patients before and after surgery and comparing to NW (Table 1). Nevertheless, in patients eligible for LGB, a slight decrease of the alpha diversity was observed while an increase of this value was detected after both surgeries.

Bacteroidetes, *Firmicutes* and *Proteobacteria* dominated the microbiome composition although the presence of *Actinobacteria* was detected (Figure 1). The relative

Table 1. Comparison of bacterial diversity between cohorts.^{1,2}

	NW	Before SG	After SG	Before LGB	After LGB
Chao1	623±228	698±169	722±176	609±51	812±129
Observed Otus	329±116	343±63	375±76	308±26	418±64
PD whole tree	17±0.61	18±3	18±2	17±0.6	19±2

¹ The results are expressed as the mean ± standard deviation at a rarefaction depth of 10,000 sequences per sample.

² LGB = laparoscopic gastric bypass; NW = normal weight; SG = laparoscopic sleeve gastrectomy; PD = phylogenetic diversity.

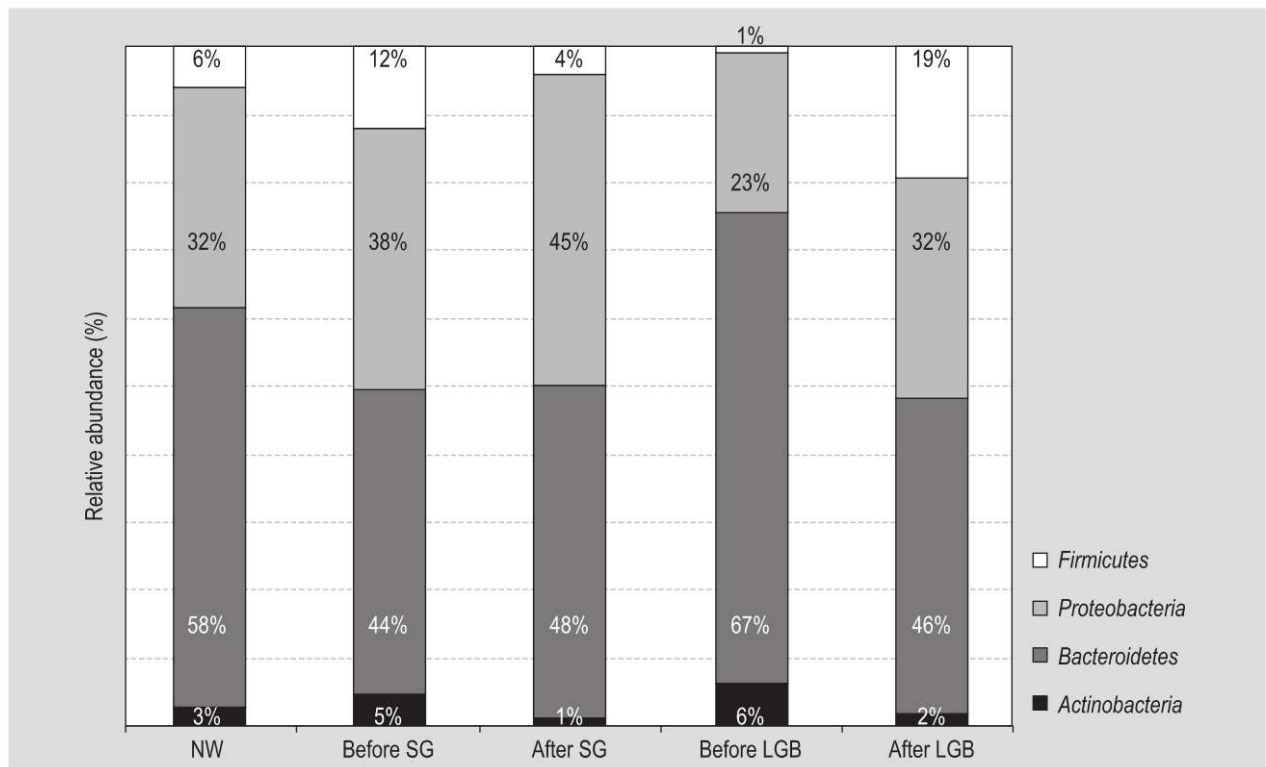


Figure 1. The intestinal bacterial communities from normal weight and obese patients. NW = normal weight; SG = laparoscopic sleeve gastrectomy; LGB = laparoscopic gastric bypass.

abundance of *Bacteroidetes* was similar between normal weight and obese patients while *Firmicutes* and *Proteobacteria* were significantly different (FDR P -value <0.05) (Figure 1). Considering the surgery techniques, the amount of *Proteobacteria* decreased after SG while it showed a significant increase after LGB (FDR $P < 0.05$). The *Firmicutes/Bacteroidetes* ratio, a surrogate marker of NW microbiome, was affected in this series by *Firmicutes* (Table 2). Comparing to NW patients, obese patients who were selected for SG showed an almost equal amount of both phyla and the ratio was not affected by the surgery. Conversely, the patients eligible for LGB showed a predominance of *Bacteroidetes*, whose amount regained a relative abundance, in 3 months after surgery, similar to NW patients. Moreover, based on recent evaluation documenting that the intestinal microbiota in adults clustered into two dietary enterotypes, distinguished by

greater abundance of genera *Prevotella* and *Bacteroides*, in this series the NW patients showed a doubled amount of *Bacteroides* comparing to *Prevotella* in line with the recent literature (Gorvitovskaia *et al.*, 2016; Figure 2). Obese patients before SG showed the same *Prevotella/Bacteroides* ratio before and after surgery, while patients after LGB showed an increase of *Prevotella*. With regard the presence of species involved in biofilm formation, in NW patients *Bacteroides uniformis* (9.23%), *Bacteroides vulgatus* (5.7%) and *Prevotella copri* (7.81%) were predominant. Obese patients before LGB, comparing to NW, showed a reduction of *B. vulgatus* (2.93%) and an increase of *B. uniformis* (15.28%) and *Bacteroides stercoris* (5.10%), this last specie found at very low percentages in NW patients. After LGB the percentages of *B. vulgatus* and *uniformis* regained values similar to NW (5.54 and 7.15%, respectively) and a high presence of *Hafnia alvei* (6.07%) was detected. Conversely,

Table 2. Firmicutes/bacteroidetes ratio.¹

	NW	Before SG	After SG	Before LGB	After LGB
<i>Firmicutes/Bacteroidetes</i>	0.50	0.80	0.90	0.34	0.70

¹ LGB = laparoscopic gastric bypass; NW = normal weight; SG = laparoscopic sleeve gastrectomy.

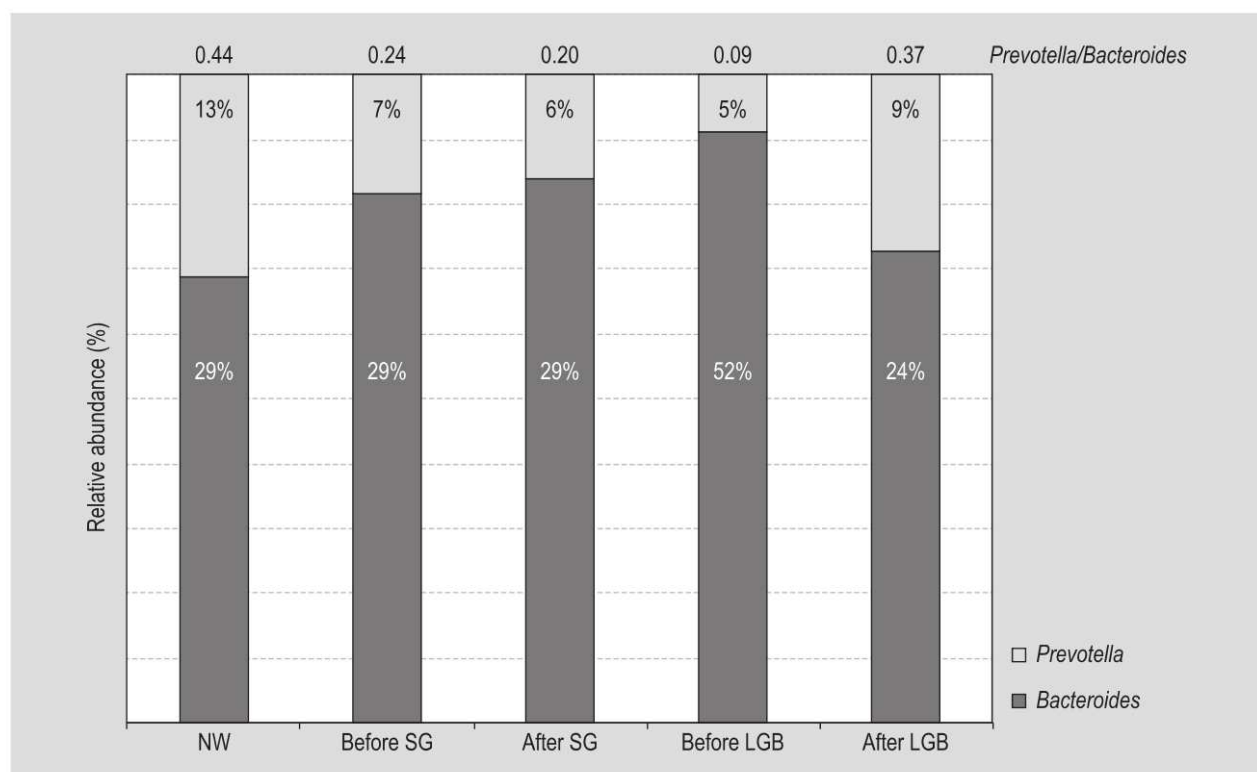


Figure 2. The *Prevotella/Bacteroides* ratio. The output of plot_taxa_summary.py of QIIME showing the relative abundance of the genera *Prevotella* and *Bacteroides*. On top of the bars, the ratio *Prevotella/Bacteroides* is shown. NW = normal weight; SG = laparoscopic sleeve gastrectomy; LGB = laparoscopic gastric bypass.

in patients before SG, comparing to NW and to obese patients eligible for LGB, a reduction of *B. uniformis* (3.2%) was observed. After SG the baseline bacterial composition, at species level, has not undergone deep changes, except for the increase of *B. uniformis* (6.91%) that, as observed in patients after LGB, tended to NW value.

4. Discussion and conclusions

Data from this study documented the influence of the bariatric techniques on restoring mucosal colonisation of bacterial species leading to important impact on the clinical management of obesity and on the maintenance of good health. Alpha diversity represents the diversity of a habitat or a community of microorganisms and describes its richness and evenness of individuals. Studies have documented the diversity of species characterising the composition of microorganisms that colonise the intestine (Backhed *et al.*, 2005; McCann, 2000) and it has been shown that obese people, compared to lean ones, have a dysbiosis in terms of quantity and variability of bacterial species (Okeke *et al.*, 2014). Confirming this statement, in the present study, we observed a generally improvement of the alpha-diversity parameter just at 3 months after bariatric surgery, showing as the alpha diversity could therefore be an interesting indirect marker to evaluate the efficacy of surgical treatment in term of restoring a functional microflora.

Numerous studies have shown that the composition of intestinal microbiota of an obese subject presents an increase in the proportion of bacteria belonging to the *Firmicutes* phylum and a reduction in those belonging to the *Bacteroidetes* (Kallus and Brandt, 2012; Ley *et al.*, 2005). A normal ratio ranges from 0.5 to 1 with predominance of *Bacteroidetes* and the alteration of this relationship reflects a state of dysbiosis. It has been reported that metabolic degradation of a source of energy is greater in the presence of a high amount of *Firmicutes* resulting in a strong increase of caloric absorption and therefore more weight gain (Kallus and Brandt, 2012). Moreover, the overgrowth of *Firmicutes* increases the number of lipid droplets resulting in gained absorption of fatty acids (Semova *et al.*, 2012). Thus a possible method to evaluate an intestinal efficient colonisation could be to assess the *Firmicutes/Bacteroidetes* ratio. The present study demonstrated that the relative abundance of *Firmicutes* and *Bacteroidetes* can exert intrinsic modification in its composition just at 3 months after surgery, although with different extent. Only in patients who underwent LGB the relative abundance of *Firmicutes* and *Bacteroidetes* tended to reach values similar to that of NW controls, while this was not observed in patients after SG. At the species level, the most abundant microflora was composed by microorganisms usually forming biofilms including *B. uniformis* and *B. vulgatus* (*Bacteroidetes*), *H. alvei* and

Roseburia intestinalis, belonging to *Proteobacteria* and *Firmicutes* phylum, respectively (Mirande *et al.*, 2010; Tan *et al.*, 2014). Of interest was the behaviour of *B. uniformis*, which was increased after SG and decreased after LGB, able to restore a normal colonisation of the gut mucosa. Recently, in obese mice, *B. uniformis* has been seen to ameliorate high fat diet-induced metabolic and immune dysfunction associated with intestinal dysbiosis and to be also able to reduce the production of some inflammatory cytokines (Gauffin Cano *et al.*, 2012).

Though, our study indicated that the *Firmicutes/Bacteroidetes* ratio may not be exhaustive for a predictive outcome of bariatric surgery. A more informative data derived by the analysis of the variation of the *Prevotella/Bacteroides* ratio, species belonging to *Bacteroidetes*, that seem to be strongly influenced by the diet regime (Fava *et al.*, 2013). Specifically, the increase of *Prevotella* is supported by a fibre-rich intake while *Bacteroides* are commonly associated with the consumption of animal fat and with a diet rich in proteins (Schnorr *et al.*, 2014). In our Italian cohort, an increase in *Prevotella/Bacteroides* was observed after LGB due to the bloom of *Prevotella*, which has been associated with a diet rich in fibres and polysaccharides (Gorvitovskaia *et al.*, 2016), while no relevant changes in *Prevotella/Bacteroides* was reported in patients after SG, suggesting a more efficacy effect of LGB intervention.

Proteobacteria, aerobe-anaerobe microorganisms, dominate together with *Bacteroides* and *Firmicutes* the microbiome composition of obese patients and represent an interesting group of bacteria triggering inflammatory mechanism. *Proteobacteria* are normally found at low proportion in the gastrointestinal microbiota and, basically, an increased amount is associated to a pro-inflammatory disorders (Mukhopadhyaya *et al.*, 2012). In this study, although the presence of *Proteobacteria* was detected after 3 months of surgical interventions as previously described in rat (Li *et al.*, 2011) and mice models (Liou *et al.*, 2013), we found that the amount of *Proteobacteria* decreased after SG and increased after LGB, event that may lead to post-surgical sequelae. Although the gastrointestinal tract (GIT) is physiologically an anaerobic environment, patients undergoing LGB are exposed to a transient aerobic condition that could promote *Proteobacteria* expansion (Shin *et al.*, 2015). Moreover, LGB procedure involves a GIT surgical reconfiguration, inducing several changes in the physical condition of the intestine, such as a modified pH that may affect the abundance of *Proteobacteria* (Duncan *et al.*, 2009), which are linked to inflammation (Mukhopadhyaya *et al.*, 2012). Indeed, *H. alvei* which belongs to the *Proteobacteria* phylum and is involved in a series of intestinal disorders increased after LGB (De Frutos *et al.*, 2017).

These results suggest that microorganisms of the dominant gut microbiota rapidly adapt in a starvation-like situation induced by bariatric surgery. Specifically, despite a similar loss of BMI, bariatric surgery techniques have different effects on restoring the microbial gut composition driving colonisation of mucosal surface just 3 months after surgery. Moreover, we suggest the characterisation of the gut microbial communities as marker for the clinical management of obesity, driving the maintenance of good health and weight loss.

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