Influence of Factors Altering Gastric Microbiota on Bariatric Surgery Metabolic Outcomes

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ABSTRACT Little is known about the influence of gastric microbiota on host metabolism, even though the stomach plays an important role in the production of hormones involved in body weight regulation and glucose homeostasis. Proton pump inhibitors (PPIs) and *Helicobacter pylori* alter gut microbiota, but their impact on gastric microbiota in patients with obesity and the influence of these factors on the metabolic response to bariatric surgery is not fully understood. Forty-one subjects with morbid obesity who underwent sleeve gastrectomy were included in this study. The *H. pylori* group was established by the detection of *H. pylori* using a sequencing-based method ($n = 16$). Individuals in whom *H. pylori* was not detected were classified according to PPI treatment. Gastric biopsy specimens were obtained during surgery and were analyzed by a high-throughput-sequencing method. Patients were evaluated at baseline and 3, 6, and 12 months after surgery. β-Diversity measures were able to cluster patients according to their gastric mucosa-associated microbiota composition. *H. pylori* and PPI treatment are presented as two important factors for gastric mucosa-associated microbiota. *H. pylori* reduced diversity, while PPIs altered β-diversity. Both factors induced changes in the gastric mucosa-associated microbiota composition and its predicted functions. PPI users showed lower percentages of change in the body mass index (BMI) in the short term after surgery, while the *H. pylori* group showed higher glucose levels and lower percentages of reduction in body weight/BMI 1 year after surgery. PPIs and *H. pylori* colonization could modify the gastric mucosa-associated microbiota, altering its diversity, composition, and predicted functionality. These factors may have a role in the metabolic evolution of patients undergoing bariatric surgery.

IMPORTANCE The gut microbiota has been shown to have an impact on host metabolism. In the stomach, factors like proton pump inhibitor treatment and *Helicobacter pylori* haven't been suggested to alter gut microbiota; however, the influence of these factors on the metabolic response to bariatric surgery has not been fully studied. In this study, we highlight the impact of these factors on the gastric microbiota composition. Moreover, proton pump inhibitor treatment and the presence of *Helicobacter pylori* could have an influence on bariatric surgery outcomes, mainly on body weight loss and glucose homeostasis. Deciphering the relationship

between gastric hormones and gastric microbiota and their contributions to bariatric surgery outcomes paves the way to develop gut manipulation strategies to improve the metabolic success of bariatric surgery.

KEYWORDS microbiota, stomach, obesity, *Helicobacter pylori*, proton pump inhibitors, sleeve gastrectomy

In the last few years, there has been a boost in the number of studies evaluating the implications of gut microbiota in health and disease. The role of gut microbiota in host metabolism has been extensively probed, being shown to be involved in energy storage and body weight regulation (1). However, many of these studies have been made using fecal samples, which are representative of the large intestine microbiota. Although these studies have shed light on the composition and functionality of the gut microbiota, the number of studies evaluating the microbiota of the upper gastrointestinal tract is limited, especially for gastric microbiota.

Most of the studies evaluating gastric microbiota have been made under conditions related to the organ, such as gastric ulcer, dyspepsia, gastritis, gastroesophageal reflux disease, or *Helicobacter pylori* infection. However, the gastric microbiota in patients with other dysfunctions, and especially metabolic disorders like obesity, has hardly been studied (2). It should be taken into consideration that in the stomach, and especially in the gastric fundus, hormones involved in body weight regulation and glucose homeostasis are produced (3). However, few studies have been made using gastric biopsy specimens (4–6), and the microbiota of the gastric fundus is yet to be analyzed.

In animal models, a high-fat diet has been shown to induce dysbiosis in the gastric microbiota that could have a role in the development of metabolic diseases (7). But many other factors have been suggested to have an impact on gastric microbiota (2), two of which are *H. pylori* infection and proton pump inhibitor (PPI) treatments, which modify the gastric pH and may contribute to altering the gastric microbiota.

H. pylori has been shown to be involved in the development of diseases like gastritis, peptic ulcer disease, or gastric cancer (8). *H. pylori* infection has been suggested to alter gastric microbiota (9, 10), but in many cases, *H. pylori* infection is asymptomatic and affected individuals do not seek medical attention, so the information about the impact of *H. pylori* colonization on the gastric microbiota in these undiagnosed individuals is limited.

PPIs are widely used for the treatment of acid-related gastroduodenal diseases. PPIs inhibit gastric acid secretion, increasing the pH in the stomach, which can alter the gut microenvironment. Indeed, PPI use could modify the fecal microbiota composition (11, 12), but information about the effect of PPIs on gastric microbiota is limited.

A matter of debate is whether *H. pylori* colonization could be implicated in postoperative complications like perforations and ulcers after bariatric surgery (13, 14). However, there is no information about metabolic outcomes after bariatric surgery. Previously, we have shown that *H. pylori* eradication may have an impact on glucose and lipid homeostasis (15, 16), but it is not clear whether *H. pylori* colonization could alter the metabolic response to bariatric surgery (17). Moreover, there is no information about the influence of PPI use on bariatric surgery metabolic outcomes.

The aims of the study were to (i) investigate the composition of gastric mucosa-associated microbiota in patients with obesity, (ii) investigate the impacts of PPIs and *H. pylori* on the composition and function of gastric mucosa-associated microbiota in these patients, and (iii) investigate the influence of these factors on the metabolic response to bariatric surgery.

RESULTS

Diversity in gastric mucosa-associated microbiota. A principal-coordinate analysis plot of weighted UniFrac distances was used to visualize complex relationships within the microbial communities. This β-diversity analysis (weighted UniFrac distance) allowed two different clusters to be distinguished according to *H. pylori* presence (permutational multivariate analysis of variance [PERMANOVA], pseudo-*F*, 10.93; *P* = 0.001) (Fig. 1a), as well as

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b

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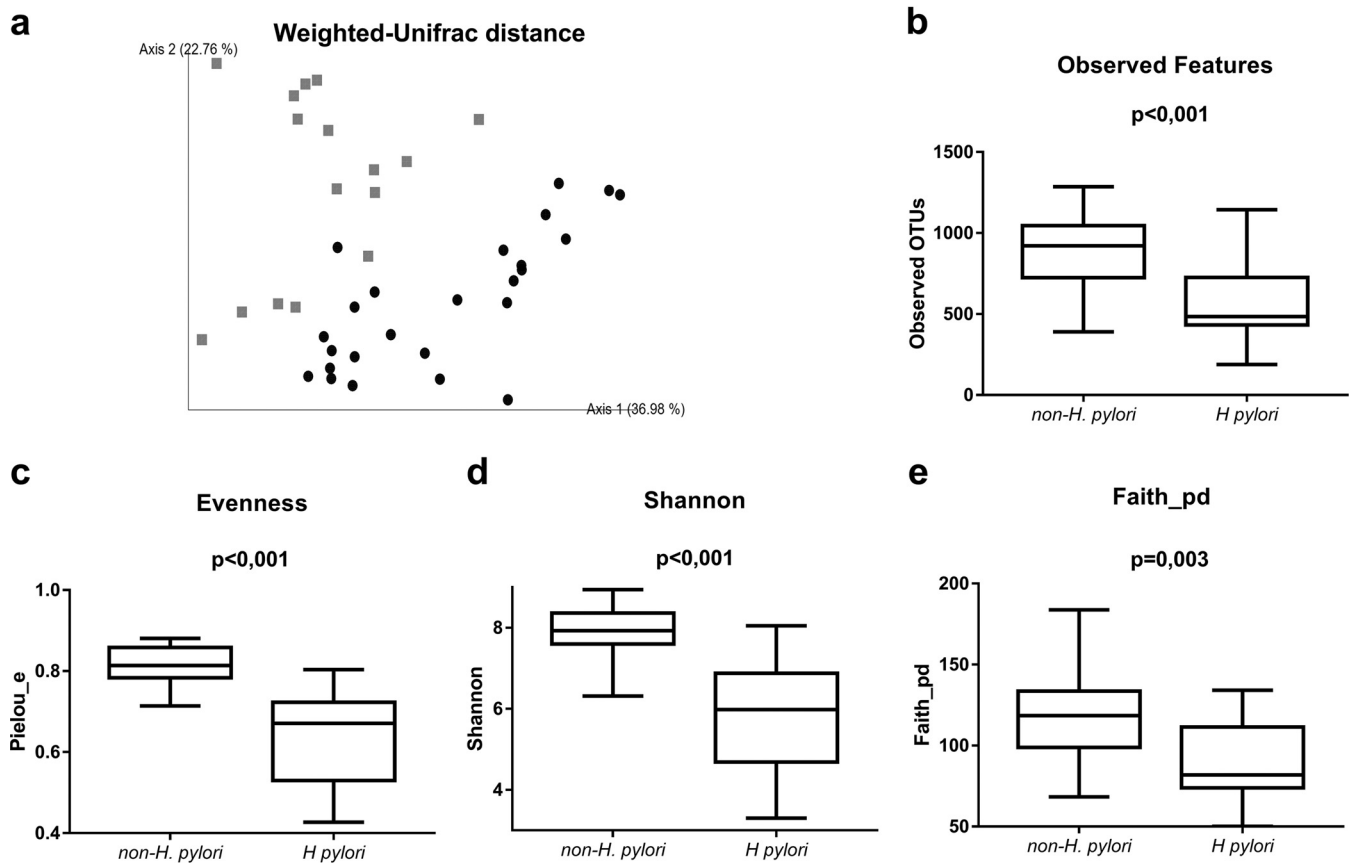
FIG 1 Diversity indexes of subjects classified according to the presence or absence of *H. pylori*, detected by a high-throughput sequencing method. (a) Weighted UniFrac distances. Gray squares, *H. pylori*; black circles, non-*H. pylori*. (b) Observed features. (c) Pielou evenness index. (d) Shannon index. (e) Faith phylogenetic diversity index.

the unweighted UniFrac distance (PERMANOVA, pseudo-*F*, 1.87, *P* = 0.041) (data not shown). A further α-diversity analysis of richness and evenness indexes also showed significant differences depending on *H. pylori* presence (Fig. 1b to e).

Deepening into the non-*H. pylori* group and following the same strategy, the unweighted UniFrac distance measure showed different clusters according to PPI use (PERMANOVA, pseudo-*F*, 1.79; *P* = 0.047) (Fig. 2a). However, no statistically significant differences according to α-diversity were found between these two clusters (Fig. 2b to e).

Anthropometric and biochemical characteristics. Based on the differences detected in microbial diversity analysis, subjects were classified into three groups: PPI users, non-PPI users, and the *H. pylori* group. These groups were used throughout the study. The main characteristics of the studied groups are represented in Table 1. At baseline, the mean age of the non-PPI users was significantly lower than the mean ages of the PPI users (*P* = 0.021) and the *H. pylori* group (*P* = 0.006). No differences were found in the rest of the studied variables at baseline.

Gastric core microbiota of patients with morbid obesity. The gastric mucosa-associated microbiota of all subjects included in the study are represented in Fig. S1 in the supplemental material. Focusing on the gastric mucosa-associated microbiota of those subjects without factors that alter gastric microbiota, that is, the non-PPI group, this group was mainly represented by six phyla: *Firmicutes* (39.73%), *Bacteroidetes* (22.55%), *Proteobacteria* (21.66%), *Actinobacteria* (7.45%), *Fusobacteria* (1.83%), and *Cyanobacteria* (1.78%). The predominant families were *Streptococcaceae* (18.92%), *Bacteroidaceae* (16.18%), *Prevotellaceae* (6.87%), *Carnobacteriaceae* (6.42%), *Enterobacteriaceae* (4.99%), *Micrococcaceae* (4.15%), *Neisseriaceae* (3.30%), and *Lachnospiraceae* (3.24%). At the genus level, *Streptococcus* was the genus most represented (23.47%), followed by *Bacteroides* (16.45%), *Prevotella* (6.44%),

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c   **d**   **e**
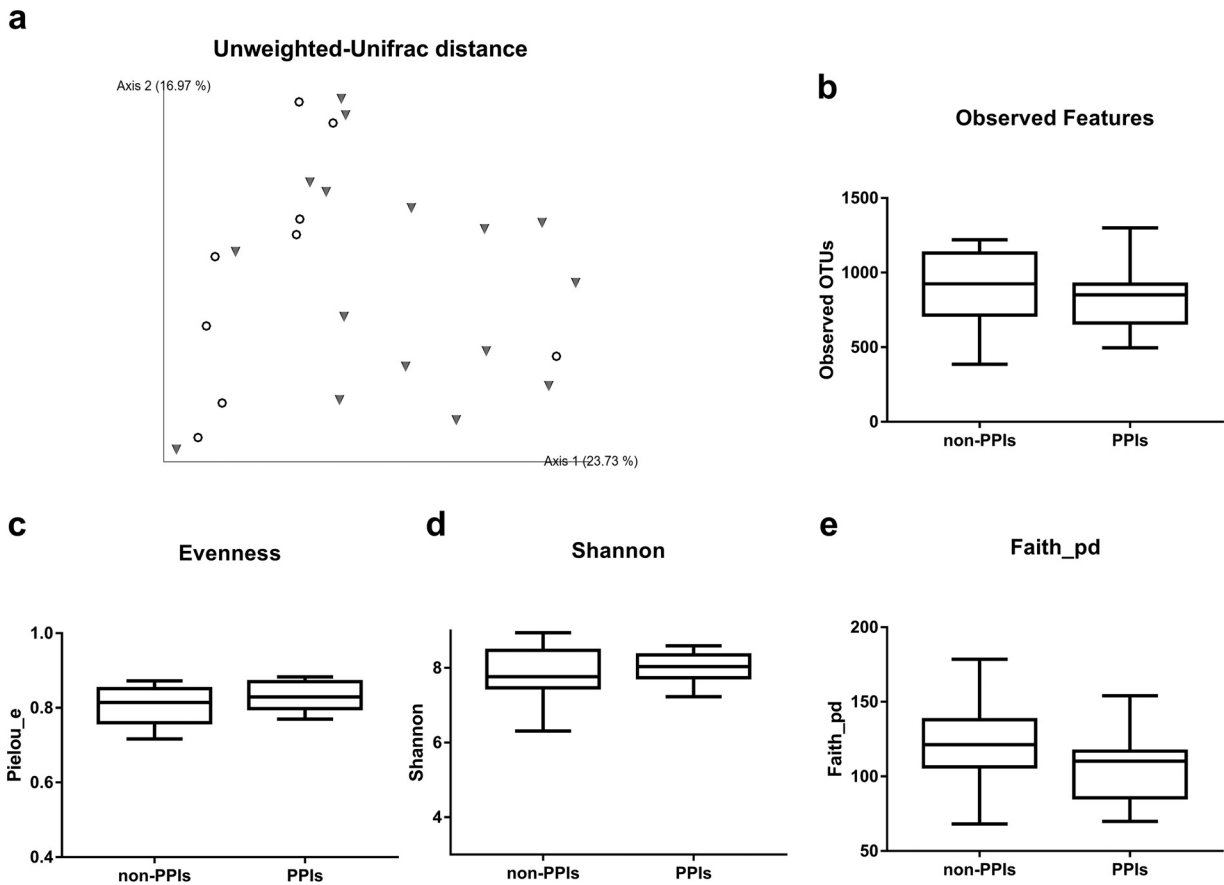
FIG 2 Diversity indexes of non-*H. pylori* subjects classified according to PPI use. (a) Unweighted UniFrac distance. Inverted triangles, non-PPI users; white circles, PPI users. (b) Observed features. (c) Pielou evenness index. (d) Shannon index. (e) Faith phylogenetic diversity index.

Alkalibacterium (5.96%), *Rothia* (4.97%), *Neisseria* (4.05%), *Shewanella* (3.89%), *Pseudomonas* (2.99%), and *Actinomyces* (2.93%). And finally, at the species level, *Bacteroides* species were the most predominant species (24.93%), followed by *Rothia mucilaginosa* (9.10%), *Alkalibacterium olivapovliticus* (7.24%), *Faecalibacterium prausnitzii* (3.80%), *Prevotella copri* (3.63%), and *Actinomyces odontolyticus* (3.36%) (Fig. 3).

Impacts of PPI use and *H. pylori* on the gastric mucosa-associated microbiota core. In 100% of the studied samples, the families *Bacteroidaceae*, *Streptococcaceae*, *Carnobacteriaceae*, and *Micrococcaceae* were detected, as well as members of the *Bacteroides* and *Streptococcus* genera and *Bacteroides* species (Fig. S2a).

At the family level, *Prevotellaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Shewanellaceae*, *Clostridiaceae*, *Lactobacillaceae*, *Coriobacteriaceae*, *Campylobacteraceae*, and *Enterobacteriaceae* were found in 100% of samples in both the PPI and the non-PPI group, while *Porphyromonadaceae*, *Fusobacteriaceae*, *Peptostreptococcaceae*, and *Flavobacteriaceae* were found in all non-PPI users and *Actinomycetaceae*, *Neisseriaceae*, *Bacillales incertae sedis*, *Erysipelotrichaceae*, and *Clostridiales* Family XI were found in all PPI users (Fig. S2a).

At the genus level, *Alkalibacterium* and *Shewanella* were identified in 100% of the PPI and non-PPI groups, while *Fusobacterium* and *Eubacterium* were detected in non-PPI users and *Actinomyces*, *Rothia*, *Granulicatella*, *Prevotella*, *Neisseria*, *Gemella*, *Veillonella*, *Atopobium*, *Clostridium*, and *Oribacterium* were identified in PPI users (Fig. S2b).

At the species level, only *Alkalibacterium olivapovliticus* was represented in 100% of the PPI and non-PPI groups, while *Rothia mucilaginosa*, *Granulicatella adiacens*, *Atopobium parvulum*, *Oribacterium sinus*, *Gemella haemolysans*, and *Streptococcus pseudopneumoniae* were identified in PPI users (Fig. S2c).

TABLE 1 Anthropometric and biochemical characteristics of the patients included in the study at different study time points

Characteristic[a]	Time point	Mean value ± SD for[b]:		
		Non-PPI users	PPI users	H. pylori
Sex (M/F)		3/13	1/8	5/11
Age (yr)		36.87 ± 7.76	48.56 ± 10.43*	47.27 ± 9.43†
Weight (kg)	Baseline	137.12 ± 19.23	128.32 ± 24.03	128.29 ± 24.48
	3 mo	110.29 ± 17.53	105.96 ± 20.33	103.85 ± 22.19
	6 mo	99.57 ± 15.13	87.63 ± 12.98	94.11 ± 22.90
	1 yr	85.46 ± 14.06	81.28 ± 8.9	92.02 ± 18.82
BMI (kg/m²)	Baseline	50.06 ± 4.77	49.69 ± 7.93	48.02 ± 9.87
	3 mo	39.42 ± 4.61	39.85 ± 6.26	39.27 ± 9.29
	6 mo	35.14 ± 4.30	34.07 ± 6.25	35.94 ± 9.94
	1 yr	30.29 ± 4.23	32.19 ± 4.32	34.99 ± 8.97
Glucose (mg/dl)	Baseline	90.88 ± 13.41	94.89 ± 19.21	104.19 ± 38.49
	3 mo	75.5 ± 8.28	85.33 ± 7.23	85-67 ± 13.74
	6 mo	77.33 ± 11.60	82.00 ± 7.61	87.6 ± 12.66
	1 yr	73.00 ± 8.74	80.00 ± 9.52	87.91 ± 15.01†
Insulin (μUI/ml)	Baseline	26.45 ± 13.97	21.67 ± 15.10	23.46 ± 17.15
	3 mo	9.23 ± 5.37	9.81 ± 5.61	11.84 ± 4.88
	6 mo	9.28 ± 5.03	9.36 ± 3.22	12.32 ± 11.46
	1 yr	6.69 ± 2.18	7.19 ± 4.00	8.86 ± 6.89
HOMA-IR	Baseline	5.95 ± 3.12	5.64 ± 5.15	6.47 ± 5.78
	3 mo	1.7 ± 0.94	2.12 ± 1.36	2.47 ± 0.99
	6 mo	1.86 ± 1.32	1.86 ± 0.55	2.66 ± 2.67
	1 yr	1.21 ± 0.46	1.48 ± 0.97	1.84 ± 1.63
HbA1c (%)	Baseline	5.56 ± 0.56	5.66 ± 0.45	6.11 ± 1.11
	3 mo	5.1 ± 0.47	5.40 ± 0.21	5.40 ± 0.31
	6 mo	5.07 ± 0.51	5.37 ± 0.19	5.45 ± 0.34
	1 yr	5.092 ± 0.46	5.33 ± 0.23	5.32 ± 0.28
Cholesterol (mg/dl)	Baseline	172.38 ± 27.43	171.00 ± 34.52	183.00 ± 35.19
	3 mo	171.83 ± 28.19	173.5 ± 31.88	183.25 ± 32.14
	6 mo	166.42 ± 35.18	194.5 ± 21.76	199.4 ± 33.10
	1 yr	177.41 ± 41.56	186.83 ± 44.49	184.36 ± 32.39
Triglycerides (mg/dl)	Baseline	112.19 ± 50.48	127.33 ± 53.63	166.87 ± 109.253
	3 mo	100.17 ± 29.11	110.83 ± 50.95	122.58 ± 64.55
	6 mo	83.17 ± 20.07	91.75 ± 37.74	104.5 ± 57.16
	1 yr	65.67 ± 15.04	104.83 ± 40.99	90.73 ± 53.35
HDL-chol (mg/dl)	Baseline	43.44 ± 10.45	51.89 ± 11.53	46.25 ± 10.54
	3 mo	38.67 ± 6.38	48.17 ± 7.05	46.42 ± 11.74
	6 mo	41.25 ± 3.67	59.5 ± 11.50	50.50 ± 15.99
	1 yr	49.33 ± 9.84	61.33 ± 18.25	53.54 ± 12.94
LDL-chol (mg/dl)	Baseline	106.5 ± 25.98	93.64 ± 28.98	104.46 ± 33.13
	3 mo	113.08 ± 28.65	103.16 ± 25.46	112.31 ± 27.11
	6 mo	108.53 ± 30.51	116.65 ± 27.61	128.00 ± 30.22
	1 yr	115.00 ± 36.66	104.60 ± 33.34	112.67 ± 28.02

[a]BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance index; HbA1c, glycated hemoglobin.
[b]*, Kruskal-Wallis test, $P < 0.05$ between non-PPI users and PPI users; †, $P < 0.05$ between non-PPI users and H. pylori group.

Impacts of PPI use and *H. pylori* on the gastric mucosa-associated microbiota composition. At the phylum level, linear discriminant analysis (LDA) effect size (LEfSe) analysis revealed that the non-PPI group was enriched in *Bacteroidetes* and TM7, while *Firmicutes* was predominant in the PPI group and the *H. pylori* group was enriched in *Proteobacteria* (LDA > 2; false discovery rate [FDR]-adjusted *P* value, <0.05) (Fig. 4a).

At the family level, the non-PPI group was enriched in *Bacteroidaceae, Prevotellaceae, Enterobacteriaceae, Shewanellaceae, Pseudomonadaceae, Ruminococcaceae, Desulfovibrionaceae, Comamonadaceae, Moraxellaceae, Rikenellaceae, Acidaminococcaceae, Sutterellaceae, Hyphomicrobiaceae, Flavobacteriaceae, Staphylococcaceae, Bacillaceae, Sphingomonadaceae,*
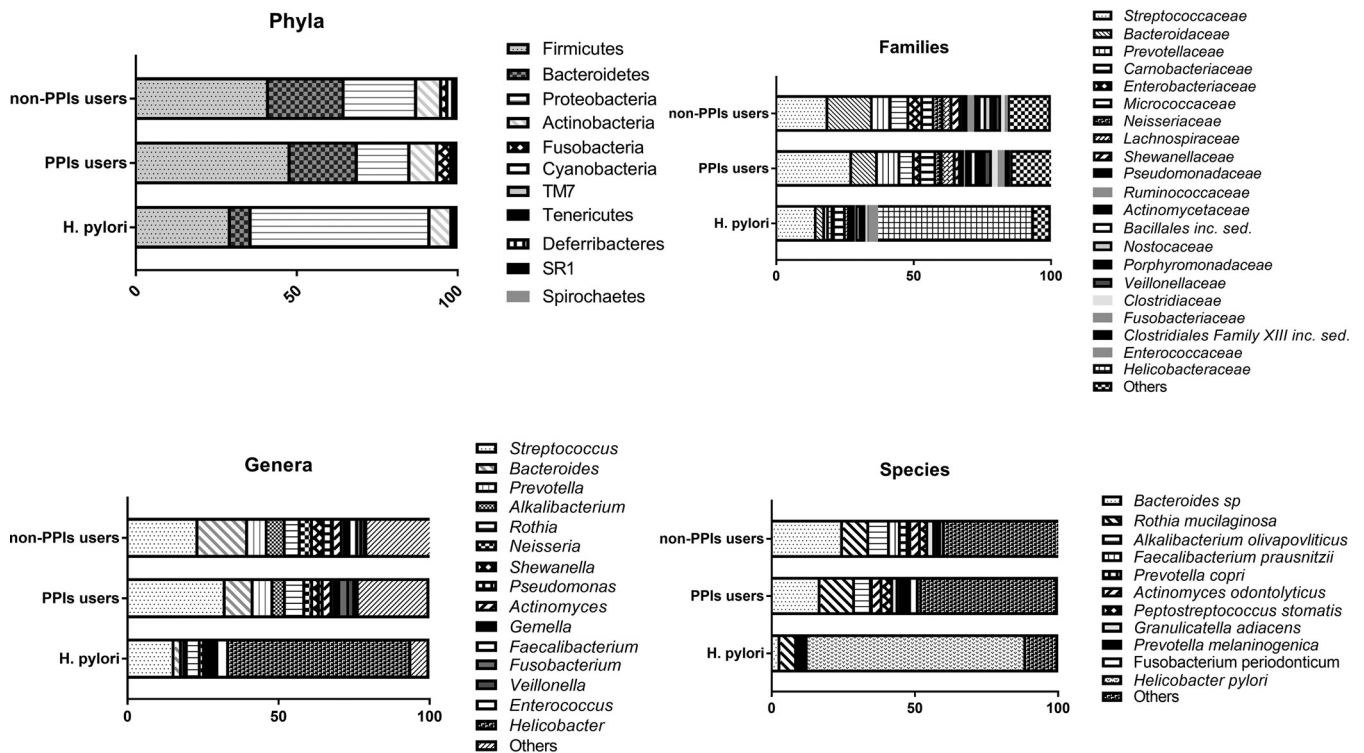
FIG 3 Most-highly represented taxa in gastric mucosa-associated microbiota of patients with morbid obesity, grouped into PPI users (non-*H. pylori*) and non-PPI users (non-*H. pylori*) and the *H. pylori* group.

Propionibacteriaceae, and *Caulobacteraceae*. The PPI group was enriched in *Carnobacteriaceae*, *Lachnospiraceae*, *Clostridiales* Family XIII *incertae sedis*, *Clostridiaceae*, *Peptostreptococcaceae*, *Clostridiales* Family XI *incertae sedis*, *Porphyromonadaceae*, *Fusobacteriaceae*, *Veillonellaceae*, *Coriobacteriaceae*, *Eubacteriaceae*, *Campylobacteraceae*, *Erysipelotrichaceae*, *Peptococcaceae*, and *Burkholderiaceae*, while the *H. pylori* group was characterized by an enrichment in *Helicobacteraceae* (LDA > 2; FDR-adjusted *P* value, <0.05) (Fig. 4b).

At the genus level, the non-PPI group was enriched in *Bacteroides*, *Prevotella*, *Shewanella*, *Pseudomonas*, *Faecalibacterium*, *Alistipes*, *Blautia*, *Staphylococcus*, *Propionibacterium*, *Serratia*, *Vibrio*, *Collinsella*, *Rhodanobacter*, *Parasutterella*, *Desulfovibrio*, *Slackia*, and *Chryseobacterium*. The PPI group was enriched in *Alkalibacterium*, *Peptostreptococcus*, *Fusobacterium*, *Eubacterium*, *Mogibacterium*, *Clostridium*, *Odoribacter*, *Veillonella*, *Lachnoanaerobaculum*, *Atopobium*, *Campylobacter*, *Butyrivibrio*, and *Yersinia*, while in the *H. pylori* group, enrichment in *Helicobacter* and *Sphingomonas* was found (LDA > 2; FDR-adjusted *P* value, <0.05) (Fig. 4c).

At the species level, the non-PPI group was enriched in *Prevotella copri* and *Bacteroides dorei*, while the PPI group was enriched in *Bacteroides* species, *Alkalibacterium olivapovliticus*, *Peptostreptococcus stomatis*, *Oribacterium sinus*, *Lachnoanaerobaculum orale*, and *Mogibacterium diversum*. As expected, *H. pylori* was the predominant species in the *H. pylori* group (LDA > 2; FDR-adjusted *P* value, <0.05) (Fig. 4d), with abundances that ranged from 5.49% to 56.13%.

Predicted metabolic pathways in gastric mucosa-associated microbiota. MetaCyc pathway analysis was performed to gain a better understanding of the role of gastric microbiota in each of the studied groups. This analysis estimated that the *H. pylori* group was enriched in pathways involved in the biosynthesis of tetrapyrroles, proteinogenic amino acids (L-lysine, L-isoleucine, L-threonine, and L-tryptophan), heme b, quinol and quinone, cell wall, phospholipids, nucleosides, nucleotides, and sugars and also in pathways involved in the generation of precursors of metabolites and energy (Table S1), while a decrease was shown in predicted pathways involved in the

a. Phyla

b. Families

c. Genera

d. Species
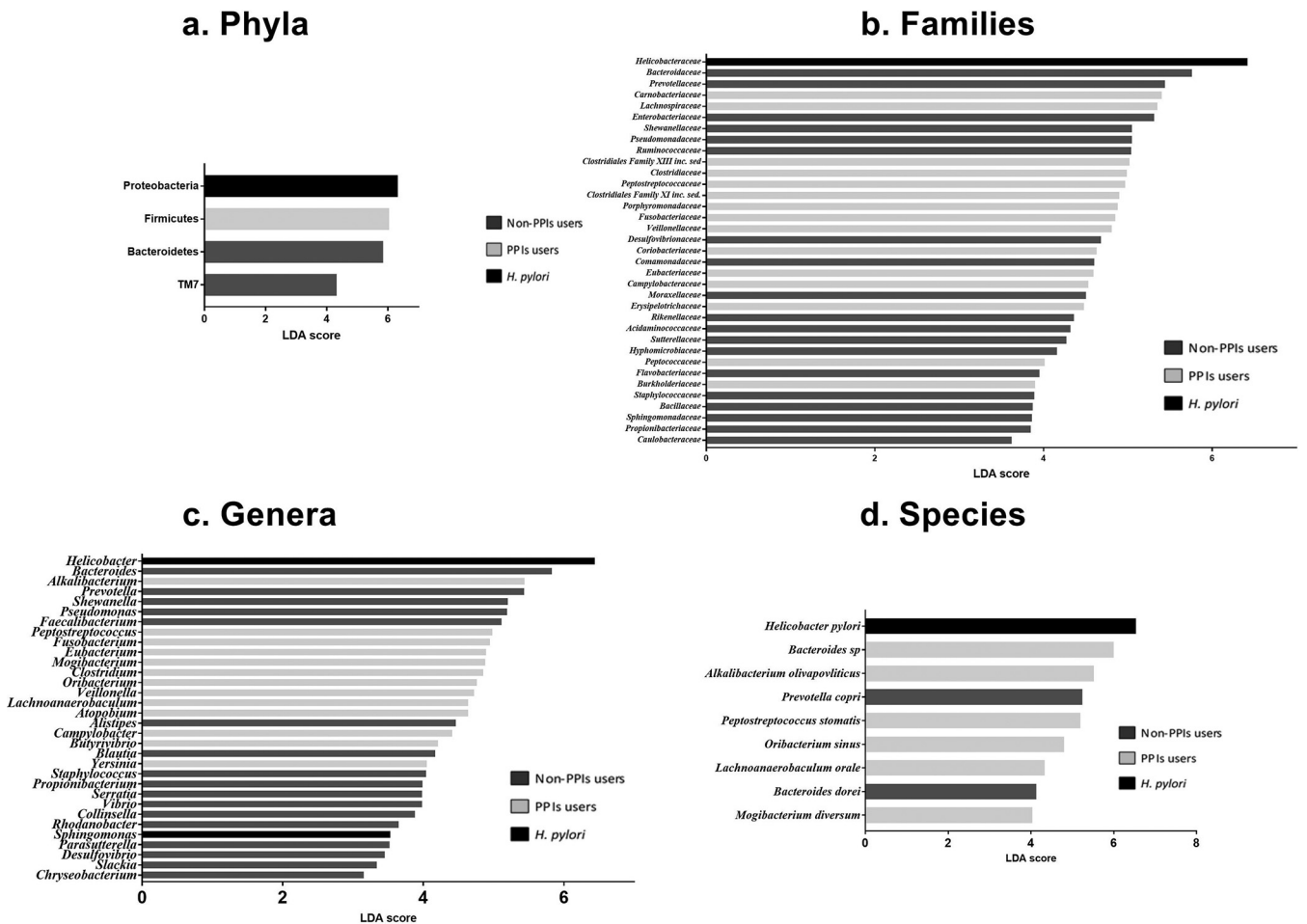
FIG 4 Linear discriminant analysis score of LEfSe analysis at different levels. Dark gray bars, non-PPI users; light gray bars, PPI users; black bars, *H. pylori* patients.

degradation of aromatic compounds, L-arginine, polysaccharides (glycan and glycogen), and sugar acids (D-galactarate, D-galacturonate, and D-glucarate) and also in pathways involved in the fermentation of pyruvate and fermentation to short-chain fatty acids and the biosynthesis of proteinogenic amino acids (L-arginine, L-histidine, and L-arginine), fatty acids (palmitate, stearate, oleate, and palmitoleate), thiamine, and sugar nucleotides (Table S1).

Comparing PPI users with non-PPI users, we found that there was an enrichment in sugar degradation in PPI users, while the non-PPI group was enriched in pathways involving aromatic compound degradation (benzoyl-CoA, protocatechuate, and vanillin) and nucleoside and nucleotide degradation, among others (Fig. S3).

Impacts of PPI use and *H. pylori* on the evolution of metabolic response to bariatric surgery. Glucose levels at the 1-year follow-up after bariatric surgery were significantly higher in the *H. pylori* group than in non-PPI users ($P = 0.042$). There were no statistically significant differences between groups in the rest of the variables at the different study time points (Table 1).

If variables are analyzed as percentages of change throughout the follow-up, the changes in weight and in body mass index (BMI) at 3 months were significantly higher in the non-PPI than in the PPI group (-21.81 ± 3.67 [mean $\pm$ standard deviation] versus -17.84 ± 2.42 [$P = 0.028$] and -21.81 ± 3.66 versus 17.89 ± 2.70 [$P = 0.042$], respectively). The percentages of changes of weight and BMI at the 1-year follow-up were significantly higher in non-PPI users than in the *H. pylori* group (-38.96 ± 7.48 versus -28.38 ± 5.63 [$P = 0.005$] and -38.95 ± 7.49 versus -28.39 ± 5.63 [$P = 0.005$]).
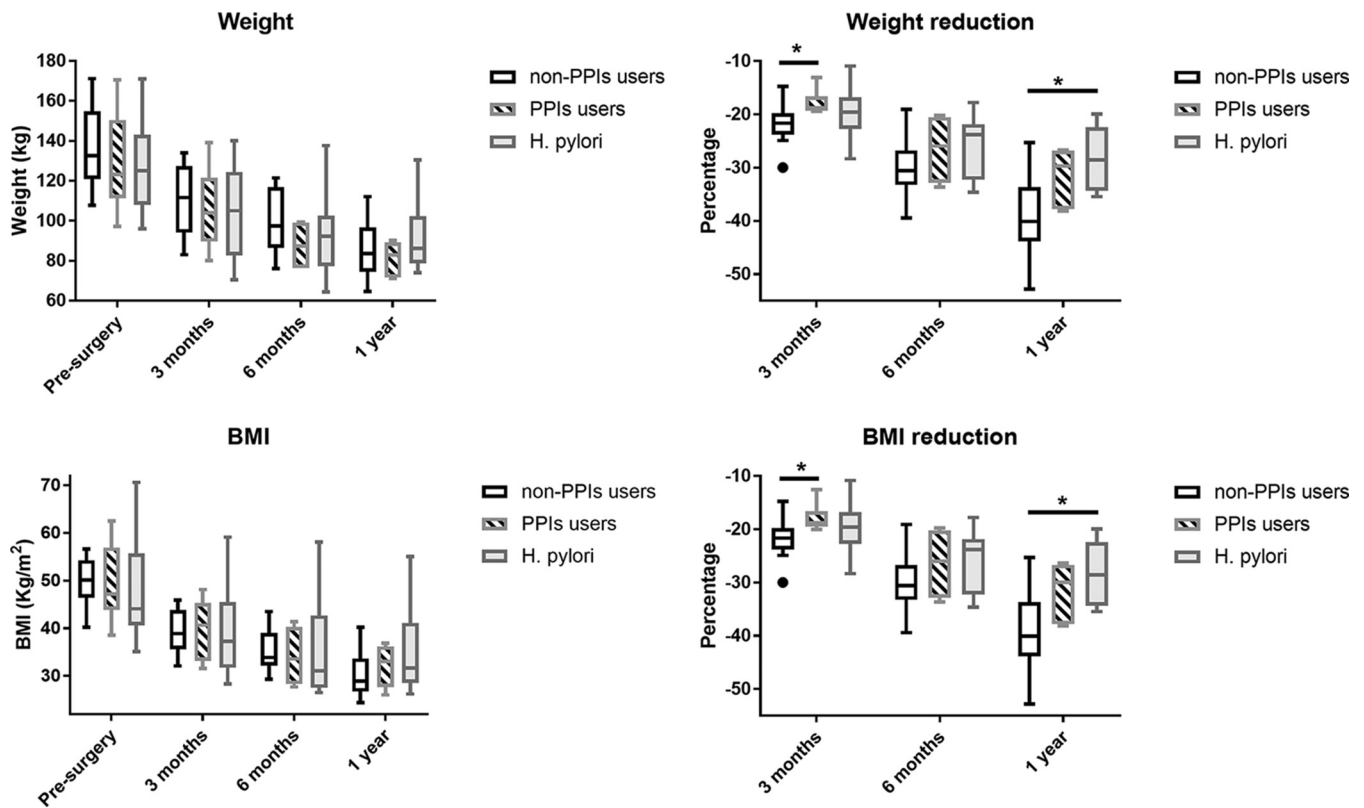
FIG 5 Weight/BMI trajectories and their percentages of reduction during follow-up. *, $P < 0.05$ (Kruskal-Wallis test adjusted by the Bonferroni correction for multiple tests).

The weight/BMI trajectories and their percentages of reduction during the follow-up are shown in Fig. 5. There were no statistically significant differences between groups in the rest of the variables at the different study time points (Table S2).

DISCUSSION

Gastric cancer, *H. pylori* infection, dyspepsia, and gastritis, among other diseases, have drawn the attention of gastric microbiota studies. Even though the gut microbiota has been shown to have an impact on host metabolism and the stomach has an important role in the production of hormones involved in body weight regulation and glucose homeostasis, knowledge about the relation between gastric microbiota and obesity is scarce. The present study describes the gastric mucosa-associated microbiota in patients with morbid obesity, and what is more important, we have shown the utility of the gastric mucosa-associated microbiota to find similar patterns among patients. Indeed, we showed the impact of commonly used medications, such as PPIs, and alterations, such as *H. pylori* presence, on the gastric microbiota composition and functions. On the one hand, although it produced alterations in the gastric microbiota composition, PPI treatment did not have a direct impact on its diversity; besides this, it produced a smaller BMI change in the short term after bariatric surgery. On the other hand, individuals not diagnosed with *H. pylori* showed lower microbiota diversity and also smaller reductions in body weight/BMI and higher glucose levels after bariatric surgery, something that deserves further investigation.

So far, there are few articles that have evaluated the gastric microbiota using biopsy specimens and high-throughput analysis for 16S rRNA sequencing approaches (18, 19), particularly in patients with morbid obesity, hindering the comparison of results between studies. In our study, as expected, the main phyla were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*, as has been previously described (18–20). Our study provides knowledge about the gastric mucosa of

patients with morbid obesity. The results showed that the predominant families were *Streptococcaceae* and *Bacteroidaceae*, as well as their respective genera *Streptococcus* and *Bacteroides*. *Streptococcus* is an acid-resistant bacterium (21), while *Bacteroides* is one of the most abundant bacteria in the gastrointestinal tract and a mutualistic bacterium that is key for the normal operation of the rest of the microbiota members (22). Thus, both bacteria seem to be key for the gastric microbiota function.

The presence of a halotolerant bacterium (*Alkalibacterium olivoapovliticus*) in the human gastric microbiota should be highlighted. Previous studies have reported the presence of halophilic and halotolerant microorganisms in human microbiota (23). Moreover, a link between halophilic microbiota and obesity has been suggested (24). Further studies are warranted to determine the role of halophilic and halotolerant bacteria in the human gastrointestinal tract and its possible link with diseases like obesity.

Previously, it has been suggested that *H. pylori* infection can alter the gastric microbiota composition (9, 10, 25). In this regard, our results showed that *H. pylori* colonization had an impact on the α- and β-diversity of gastric microbiota, although others, also using a sequencing approach, did not find discrepancies in diversity between patients positive and negative for *H. pylori* (19). However, in the latter study, the authors detected that individuals who were *H. pylori* negative when tested by conventional methods could hide the presence of *H. pylori* that was detected with the sensitive high-throughput sequencing method, which could hide the real impact of *H. pylori* colonization on gastric microbiota diversity and composition (19), supporting our approach.

LEfSe analysis showed an enrichment in the *Sphingomonas* genus in the *H. pylori* group. *Sphingomonas* has been identified as a player in the immune response, producing glycosphingolipids that correlate with natural killer T cells (26) Moreover, the lack of *Sphingomonas* after an *H. pylori* eradication treatment has been related to a persistent inflammatory state (27), indicating some kind of crucial role in the gastric microbiota but at a correct amount, although further studies are warranted to elucidate the role of *Sphingomonas*.

PPI use is increasing because of their multiple indications. Previous studies have been focused on the impact of PPIs on distal gut microbiota. A recent systematic review concluded that PPIs do not affect diversity but that they are associated with taxonomic alterations in the upper and distal gut microbiota (28). However, few studies have been done using gastric biopsy specimens. These studies have pointed out that PPI-treated patients were enriched in *Streptococcus* (5, 6). Although *Streptococcaceae* have been found as part of the core group of the gastric microbiota, we did not find this association between *Streptococcus* and PPI treatment. The direct consequence of the use of PPIs is the increase in the gastric pH, which allows higher colonization of the gastric environment by oral and upper gastrointestinal tract commensals, something reflected in our study, where PPI-treated patients' gastric microbiota showed enrichment in taxa frequently found in oral microbiota (29, 30). Our results are consistent with those of the prospective cohort study published by Amir et al. In that study, the authors showed that in gastric fluid, PPI treatment produced an enrichment in the oral microbiota members *Erysipelotrichaceae* and *Clostridiales*, while *Moraxellaceae*, *Comamonadaceae*, and *Flavobacteriaceae* decreased (31).

PPI-treated patients showed enrichment of *Peptostreptococcus stomatis*, a known member of the oral microbiome that has been associated with colorectal and gastric cancer (32, 33), as well as of *Lachnoanaerobaculum orale* and *Mogibacterium diversum*, both of which have been isolated in oral cavities, although there is no information about their possible role and functions in the gastrointestinal tract (34, 35). However, members of the oral microbiota have been related to a variety of systemic diseases when their abundances increase beyond the mouth (36).

Our results, based on patients with morbid obesity, showed enrichment in *Prevotella copri* and *Bacteroides dorei*. Although it has been shown to be one of the dominant species in the gastric microbiota (37), *P. copri* has shown contradictory associations with both

benefits to the host and disease in the human gut, being related to glucose metabolism improvement, mainly through the formation of short-chain fatty acids, but also to proinflammatory status promotion and the ability to potentiate the pathogenicity of other bacteria (38). So far as we know, *B. dorei* has not been previously detected in the gastric microbiota. In the intestinal microbiota, there are contradictory results regarding the inflammatory or anti-inflammatory role of *B. dorei* (39, 40). Further studies are necessary to elucidate the functions that the microbiota carries out in the stomach.

In the *H. pylori* group, predicted MetaCyc pathways showed an enrichment in pathways that are the basis of cellular activities involved in physiological processes like nucleoside and nucleotide biosynthesis, phospholipid biosynthesis, and the generation of precursor metabolites and energy. Also, heme biosynthesis and the intermediates of its biosynthesis as porphyrins were shown to be enriched. Heme represents a key iron source and an essential growth factor for bacteria that contributes to their virulence (41). Previous studies have shown that heme biosynthesis is increased in *H. pylori* infection, and *H. pylori* eradication has been shown to downregulate tetrapyrroles, which are involved in heme biosynthesis (42). In fact, although this has not been observed in our patients, *H. pylori* infection increases the risk for low iron status (43), as degradation of lysosomal ferritin may facilitate *H. pylori*'s pathogenesis and contribute to the bacterial persistence in the human stomach (44). Cytochromes are proteins containing heme groups, and both quinones and cytochromes are components of the *H. pylori* respiratory chain (45). In this manner, our results showed that the quinol and quinone biosynthesis predicted pathway was enriched in patients with *H. pylori*.

H. pylori eradication prior to bariatric surgery has been suggested to avoid postoperative complications, although the clinical evidence is scarce (13, 14). However, to the best of our knowledge, there is no study that has evaluated the impact of the presence of *H. pylori*, not detected before bariatric surgery, on the metabolic response to surgery. Our results showed that 1 year after bariatric surgery, glucose levels were significantly higher in the *H. pylori* group than in non-PPI users, although we did not find differences in the percentages of change in glucose levels during the follow-up, while the percentages of weight/BMI reduction were significantly lower. In a recent study, the impact of *H. pylori* eradication on the metabolic response to bariatric surgery has been evaluated, showing a greater reduction in BMI at 3 months after surgery in patients treated for *H. pylori* (17). Previous studies have suggested that *H. pylori* infection may affect gastric hormones involved in appetite regulation, such as ghrelin and obestatin (46), that are mainly produced in the gastric fundus, so the relation between *H. pylori* infection, gastric hormones, and bariatric surgery is an issue that deserves further investigation. Indeed, the role of gastric hormones in bariatric surgery outcomes has been brought to light by a recent study that suggests a reprogramming of endocrine cell differentiation after bariatric surgery (47). Elucidating the impact of gastric microbiota in this adaptative process is an issue that will be worth further research.

Several limitations to this research must be acknowledged. Patients were not tested for *H. pylori* infection by conventional methods before surgery, so we cannot claim infection, only presence. and biopsy specimens were obtained from only one location in the stomach. The study only included patients who underwent sleeve gastrectomy, but Roux-en-Y gastric surgery also induces stomach rearrangements, so further studies will be necessary to elucidate the role of gastric microbiota in the outcomes of different bariatric surgery procedures.

In conclusion, in patients with morbid obesity, PPI treatment, although it did not alter microbiota diversity, had an impact on the composition and function of gastric mucosa-associated microbiota. The presence of *H. pylori* produced a reduction in gastric mucosa-associated microbiota diversity, and it was associated with smaller weight/BMI reductions 1 year after surgery and could have had an impact on glucose metabolism. This study remarks the importance of gut microbiota in the metabolic machinery of the host and the necessity to study other gastrointestinal sections apart from the large intestine to fully understand the role of gastrointestinal microbiota in the homeostasis

of the host. Moreover, this study points out the use of the gastric mucosa-associated microbiota in particular, and gut microbiota in general, for the development of personalized medicine.

MATERIALS AND METHODS

Subjects. The study was undertaken in 41 subjects with morbid obesity (BMI > 40 kg/m^2) or with a BMI of >35 kg/m^2 with comorbidities (the BMIs ranged from 35.1 kg/m2 to 70.6 kg/m^2) who underwent sleeve gastrectomy from 2014 to 2018 at the Regional University Hospital and Virgen de la Victoria University Hospital, Málaga, Spain. Subjects were excluded if they had type 2 diabetes and were receiving insulin treatment or had cardiovascular disease or acute inflammatory or infectious disease. Any use of antibiotics or probiotic or prebiotic agents that could modify the microbiota in the previous 3 months were grounds for exclusion. PPI use was recorded. Patients followed a very-low-calorie diet (600 to 800 kcal) for 15 days before surgery (Optisource; Nestlé Health Care Nutrition), supplemented with proteins (1 g/kg of ideal body weight per day; ideal weight was defined by the weight corresponding to a body mass index [BMI] of 25).

All the participants included in the study gave their informed consent, and the study was reviewed and approved by the Ethics and Research Committee of the Regional University Hospital and Virgen de la Victoria University, Málaga, Spain. Samples from subjects were processed and frozen immediately after their reception in the Regional University Hospital Biobank (Andalusian Public Health System Biobank).

Anthropometric and laboratory measurements. Patients were followed up for 1 year after surgery. Anthropometric and laboratory measurements were recorded before surgery and 3, 6, and 12 months after surgery. Weight and height were measured according to standardized procedures, and BMI was calculated as weight (kg)/height^2 (m^2).

At all study time points, blood samples were collected after a 10- to 12-h fast. The serum was separated and immediately frozen at −80°C until analysis. Biochemical variables were measured in duplicate. Glucose, cholesterol, and triglycerides (Rando Laboratories, Antrium, UK) were determined by standard enzymatic methods. Insulin was determined by radioimmunoassay (DIASource ImmunoAssays SA, Nivelles, Belgium). The HOMA-IR (homeostasis model assessment of insulin resistance index) value was calculated with the following equation: HOMA-IR = fasting insulin (μIU/ml) × fasting glucose (mmol/liter)/22.5.

Analysis of gastric mucosa-associated microbiota. Gastric samples were obtained during the sleeve gastrectomy bariatric surgery. Samples were frozen in liquid nitrogen and maintained at −80°C until analysis. Gastric mucosa was scraped, and DNA was obtained using the PowerFecal DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, with an initial enzymatic treatment consisting of a lysis buffer containing 20 mM Tris-HCl, 2 mM EDTA, 1.2% Triton X-100, 10 mg/ml lysozyme, and 100 U/ml mutanolysin.

Amplicon library preparation was performed using the Ion 16S metagenomics kit and Ion Plus fragment library kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) as previously described (48).

Emulsion PCR was carried out using the Ion Chef System (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Sequencing of the amplicon libraries was carried out on an Ion 520 chip using the Ion Torrent S5 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions.

Sequence data analysis. Base calling and run demultiplexing were performed using Torrent Suite software version 5.4.0 (Life Technologies) with the default parameters (bead loading ≤30, key signal ≤30, and usable sequences ≤30). Data were analyzed using Quantitative Insights Into Microbial Ecology 2 (QIIME2) version 2019.1. Sequencing reads were denoised and clustered into amplicon sequence variants using DADA2 (49). Sequences were taxonomically classified against Greengenes 13_8 97% operational taxonomic unit (OTU) reference sequences. A randomly subsample at the lowest number of reads were used to evaluate alpha (Shannon's diversity, Pielou's evenness, Faith's phylogenetic diversity, and observed amplicon sequence variants [ASVs]) and beta diversity (Unweighted Unifrac and Weighted Unifrac distances). Samples with fewer than 1,500 sequences were removed prior to the analysis. Features with a count sum of less than 10 across all samples and those presented only in one sample were excluded from further analysis.

ASV tables were analyzed at different taxon levels using LEfSe to test differences in abundance between groups within the MicrobiomeAnalyst webtool with the default parameters of the developer (50). The plug-in feature-table in QIIME2 was used to calculate the core microbiomes, and the visualization was performed using the Venny web tool (51).

Identification of predicted metagenome functions was performed using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States plug-in (PICRUST2). MetaCyc pathways (52) were normalized within QIIME2 and analyzed using the STAMP (Statistical Analysis of Metagenomics Profiles) software with Welch's *t* test option (53).

Anthropometric and biochemical statistical analysis. Statistical software package SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) was used to study differences in anthropometric and biochemical variables between groups, using the Kruskal-Wallis test adjusted by the Bonferroni correction for multiple tests. Values were considered to be statistically significant when the P value was <0.05. The results are given as the mean values or percentages ± standard deviations.

Data availability. Raw 16S rRNA sequencing data for all samples have been deposited in the NCBI short read archive under accession number PRJNA706562.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 3.9 MB.

REFERENCES

1. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. 2004. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101:15718–15723. https://doi.org/10.1073/pnas.0407076101.

2. Rajilic-Stojanovic M, Figueiredo C, Smet A, Hansen R, Kupcinskas J, Rokkas T, Andersen L, Machado JC, Ianiro G, Gasbarrini A, Leja M, Gisbert JP, Hold GL. 2020. Systematic review: gastric microbiota in health and disease. Aliment Pharmacol Ther 51:582–602. https://doi.org/10.1111/apt.15650.

3. Moffett RC, Docherty NG, Le Roux CW. 2021. The altered enteroendocrine reportoire following Roux-en-Y-gastric bypass as an effector of weight loss and improved glycaemic control. Appetite 156:104807. https://doi.org/10.1016/j.appet.2020.104807.

4. Mailhe M, Ricaboni D, Vitton V, Gonzalez J-M, Bachar D, Dubourg G, Cadoret F, Robert C, Delerce J, Levasseur A, Fournier P-E, Angelakis E, Lagier J-C, Raoult D. 2018. Repertoire of the gut microbiota from stomach to colon using culturomics and next-generation sequencing. BMC Microbiol 18:157. https://doi.org/10.1186/s12866-018-1304-7.

5. Paroni Sterbini F, Palladini A, Masucci L, Cannistraci CV, Pastorino R, Ianiro G, Bugli F, Martini C, Ricciardi W, Gasbarrini A, Sanguinetti M, Cammarota G, Posteraro B. 2016. Effects of proton pump inhibitors on the gastric mucosa-associated microbiota in dyspeptic patients. Appl Environ Microbiol 82:6633–6644. https://doi.org/10.1128/AEM.01437-16.

6. Parsons BN, Ijaz UZ, D'Amore R, Burkitt MD, Eccles R, Lenzi L, Duckworth CA, Moore AR, Tiszlavicz L, Varro A, Hall N, Pritchard DM. 2017. Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. PLoS Pathog 13:e1006653. https://doi.org/10.1371/journal.ppat.1006653.

7. He C, Cheng D, Peng C, Li Y, Zhu Y, Lu N. 2018. High-fat diet induces dysbiosis of gastric microbiota prior to gut microbiota in association with metabolic disorders in mice. Front Microbiol 9:639. https://doi.org/10.3389/fmicb.2018.00639.

8. Kusters JG, Van Vliet AHM, Kuipers EJ. 2006. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev 19:449–490. https://doi.org/10.1128/CMR.00054-05.

9. Zhao Y, Gao X, Guo J, Yu D, Xiao Y, Wang H, Li Y. 2019. Helicobacter pylori infection alters gastric and tongue coating microbial communities. Helicobacter 24:e12567. https://doi.org/10.1111/hel.12567.

10. Das A, Pereira V, Saxena S, Ghosh TS, Anbumani D, Bag S, Das B, Nair GB, Abraham P, Mande SS. 2017. Gastric microbiome of Indian patients with Helicobacter pylori infection, and their interaction networks. Sci Rep 7:15438. https://doi.org/10.1038/s41598-017-15510-6.

11. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJM, Dijkstra G, Franke L, Xavier RJ, Jonkers D, Wijmenga C, Weersma RK, Zhernakova A. 2016. Proton pump inhibitors affect the gut microbiome. Gut 65:740–748. https://doi.org/10.1136/gutjnl-2015-310376.

12. Jackson MA, Goodrich JK, Maxan M-E, Freedberg DE, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE, Bell JT, Spector TD, Steves CJ. 2016. Proton pump inhibitors alter the composition of the gut microbiota. Gut 65:749–756. https://doi.org/10.1136/gutjnl-2015-310861.

13. Smelt HJM, Smulders JF, Gilissen LPL, Said M, Ugale S, Pouwels S. 2018. Influence of Helicobacter pylori infection on gastrointestinal symptoms and complications in bariatric surgery patients: a review and meta-analysis. Surg Obes Relat Dis 14:1645–1657. https://doi.org/10.1016/j.soard.2018.06.020.

14. Mocanu V, Dang JT, Switzer N, Skubleny D, Shi X, de Gara C, Birch DW, Karmali S. 2018. The effect of Helicobacter pylori on postoperative outcomes in patients undergoing bariatric surgery: a systematic review and meta-analysis. Obes Surg 28:567–573. https://doi.org/10.1007/s11695-017-3024-8.

15. Martín-Núñez GM, Cornejo-Pareja I, Coin-Aragüez L, Roca-Rodríguez MDM, Muñoz-Garach A, Clemente-Postigo M, Cardona F, Moreno-Indias I, Tinahones FJ. 2019. H. pylori eradication with antibiotic treatment causes changes in glucose homeostasis related to modifications in the gut microbiota. PLoS One 14:e0213548. https://doi.org/10.1371/journal.pone.0213548.

16. Martín-Núñez GM, Cornejo-Pareja I, Roca-Rodríguez MDM, Clemente-Postigo M, Cardona F, Fernández-García JC, Moreno-Indias I, Tinahones FJ. 2020. H. pylori eradication treatment causes alterations in the gut microbiota and blood lipid levels. Front Med (Lausanne) 7:417. https://doi.org/10.3389/fmed.2020.00417.

17. Goday A, Castañer O, Benaiges D, Pou AB, Ramón JM, Iglesias MDM, Barranco Priego L, Flores Le-Roux JA. 2018. Can Helicobacter pylori eradication treatment modify the metabolic response to bariatric surgery? Obes Surg 28:2386–2395. https://doi.org/10.1007/s11695-018-3170-7.

18. Stearns JC, Lynch MDJ, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, Croitoru K, Moreno-Hagelsieb G, Neufeld JD. 2011. Bacterial biogeography of the human digestive tract. Sci Rep 1:170. https://doi.org/10.1038/srep00170.

19. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perez-Perez G, Blaser MJ, Relman DA. 2006. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A 103:732–737. https://doi.org/10.1073/pnas.0506655103.

20. Bashir M, Prietl B, Tauschmann M, Mautner SI, Kump PK, Treiber G, Wurm P, Gorkiewicz G, Högenauer C, Pieber TR. 2016. Effects of high doses of vitamin D3 on mucosa-associated gut microbiome vary between regions of the human gastrointestinal tract. Eur J Nutr 55:1479–1489. https://doi.org/10.1007/s00394-015-0966-2.

21. Matsui R, Cvitkovitch D. 2010. Acid tolerance mechanisms utilized by Streptococcus mutans. Future Microbiol 5:403–417. https://doi.org/10.2217/fmb.09.129.

22. Comstock LE. 2009. Importance of glycans to the host-Bacteroides mutualism in the mammalian intestine. Cell Host Microbe 5:522–526. https://doi.org/10.1016/j.chom.2009.05.010.

23. Seck EH, Dufour J-C, Raoult D, Lagier J-C. 2018. Halophilic & halotolerant prokaryotes in humans. Future Microbiol 13:799–812. https://doi.org/10.2217/fmb-2017-0237.

24. Seck EH, Senghor B, Merhej V, Bachar D, Cadoret F, Robert C, Azhar EI, Yasir M, Bibi F, Jiman-Fatani AA, Konate DS, Musso D, Doumbo O, Sokhna C, Levasseur A, Lagier JC, Khelaifia S, Million M, Raoult D. 2019. Salt in

stools is associated with obesity, gut halophilic microbiota and Akkermansia muciniphila depletion in humans. Int J Obes (Lond) 43:862–871. https://doi.org/10.1038/s41366-018-0201-3.

25. Schulz C, Schütte K, Koch N, Vilchez-Vargas R, Wos-Oxley ML, Oxley APA, Vital M, Malfertheiner P, Pieper DH. 2018. The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection. Gut 67:216–225. https://doi.org/10.1136/gutjnl-2016-312904.

26. Wei B, Wingender G, Fujiwara D, Chen DY, McPherson M, Brewer S, Borneman J, Kronenberg M, Braun J. 2010. Commensal microbiota and CD8+ T cells shape the formation of invariant NKT cells. J Immunol 184: 1218–1226. https://doi.org/10.4049/jimmunol.0902620.

27. Sung JJY, Coker OO, Chu E, Szeto CH, Luk STY, Lau HCH, Yu J. 2020. Gastric microbes associated with gastric inflammation, atrophy and intestinal metaplasia 1 year after Helicobacter pylori eradication. Gut 69:1572–1580. https://doi.org/10.1136/gutjnl-2019-319826.

28. Macke L, Schulz C, Koletzko L, Malfertheiner P. 2020. Systematic review: the effects of proton pump inhibitors on the microbiome of the digestive tract—evidence from next-generation sequencing studies. Aliment Pharmacol Ther 51:505–526. https://doi.org/10.1111/apt.15604.

29. Wang L, Yin G, Guo Y, Zhao Y, Zhao M, Lai Y, Sui P, Shi T, Guo W, Huang Z. 2019. Variations in oral microbiota composition are associated with a risk of throat cancer. Front Cell Infect Microbiol 9:205. https://doi.org/10.3389/fcimb.2019.00205.

30. Wang B, Zhang Y, Zhao Q, Yan Y, Yang T, Xia Y, Chen H. 2020. Patients with reflux esophagitis possess a possible different oral microbiota compared with healthy controls. Front Pharmacol 11:1000. https://doi.org/10.3389/fphar.2020.01000.

31. Amir I, Konikoff FM, Oppenheim M, Gophna U, Half EE. 2014. Gastric microbiota is altered in oesophagitis and Barrett's oesophagus and further modified by proton pump inhibitors. Environ Microbiol 16: 2905–2914. https://doi.org/10.1111/1462-2920.12285.

32. Yu J, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, Tang L, Zhao H, Stenvang J, Li Y, Wang X, Xu X, Chen N, Wu WKK, Al-Aama J, Nielsen HJ, Kiilerich P, Jensen BAH, Yau TO, Lan Z, Jia H, Li J, Xiao L, Lam TYT, Ng SC, Cheng AS-L, Wong VW-S, Chan FKL, Xu X, Yang H, Madsen L, Datz C, Tilg H, Wang J, Brünner N, Kristiansen K, Arumugam M, Sung JJ-Y, Wang J. 2017. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. Gut 66:70–78. https://doi.org/10.1136/gutjnl-2015-309800.

33. Coker OO, Dai Z, Nie Y, Zhao G, Cao L, Nakatsu G, Wu WKK, Wong SH, Chen Z, Sung JJY, Yu J. 2018. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 67:1024–1032. https://doi.org/10.1136/gutjnl-2017-314281.

34. Nakazawa F, Poco SE, Sato M, Ikeda T, Kalfas S, Sundqvist G, Hoshino E. 2002. Taxonomic characterization of Mogibacterium diversum sp. nov. and Mogibacterium neglectum sp. nov., isolated from human oral cavities. Int J Syst Evol Microbiol 52:115–122. https://doi.org/10.1099/00207713-52-1-115.

35. Hedberg ME, Moore ERB, Svensson-Stadler L, Hörstedt P, Baranov V, Hernell O, Wai SN, Hammarström S, Hammarström M-L. 2012. Lachnoanaerobaculum gen. nov., a new genus in the Lachnospiraceae: characterization of Lachnoanaerobaculum umeaense gen. nov., sp. nov., isolated from the human small intestine, and Lachnoanaerobaculum orale sp. nov., isolated from saliva, and reclassification of Eubacterium saburreum (Prévot 1966) Holdeman and Moore 1970 as Lachnoanaerobaculum saburreum comb. nov. Int J Syst Evol Microbiol 62:2685–2690. https://doi.org/10.1099/ijs.0.033613-0.

36. Olsen I. 2015. From the Acta prize lecture 2014. The periodontal systemic connection seen from a microbiological standpoint. Acta Odontol Scand 73:563–568. https://doi.org/10.3109/00016357.2015.1007480.

37. Liu X, Shao L, Liu X, Ji F, Mei Y, Cheng Y, Liu F, Yan C, Li L, Ling Z. 2019. Alterations of gastric mucosal microbiota across different stomach

microhabitats in a cohort of 276 patients with gastric cancer. EBioMedicine 40:336–348. https://doi.org/10.1016/j.ebiom.2018.12.034.

38. Claus SP. 2019. The strange case of Prevotella copri: Dr. Jekyll or Mr. Hyde? Cell Host Microbe 26:577–578. https://doi.org/10.1016/j.chom.2019.10.020.

39. Thomson P, Medina DA, Ortúzar V, Gotteland M, Garrido D. 2018. Anti-inflammatory effect of microbial consortia during the utilization of dietary polysaccharides. Food Res Int 109:14–23. https://doi.org/10.1016/j.foodres.2018.04.008.

40. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z, Su L, Li X, Li X, Li J, Xiao L, Huber-Schönauer U, Niederseer D, Xu X, Al-Aama JY, Yang H, Wang J, Kristiansen K, Arumugam M, Tilg H, Datz C, Wang J. 2015. Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun 6:6528. https://doi.org/10.1038/ncomms7528.

41. Weinberg ED. 2009. Iron availability and infection. Biochim Biophys Acta 1790:600–605. https://doi.org/10.1016/j.bbagen.2008.07.002.

42. Yap TW-C, Leow AH-R, Azmi AN, Callahan DL, Perez-Perez GI, Loke M-F, Goh K-L, Vadivelu J. 2017. Global fecal and plasma metabolic dynamics related to Helicobacter pylori eradication. Front Microbiol 8:536. https://doi.org/10.3389/fmicb.2017.00536.

43. Hudak L, Jaraisy A, Haj S, Muhsen K. 2017. An updated systematic review and meta-analysis on the association between Helicobacter pylori infection and iron deficiency anemia. Helicobacter 22:e12330. https://doi.org/10.1111/hel.12330.

44. Flores SE, Aitchison A, Day AS, Keenan JI. 2017. Helicobacter pylori infection perturbs iron homeostasis in gastric epithelial cells. PLoS One 12: e0184026. https://doi.org/10.1371/journal.pone.0184026.

45. Marcelli SW, Chang HT, Chapman T, Chalk PA, Miles RJ, Poole RK. 1996. The respiratory chain of Helicobacter pylori: identification of cytochromes and the effects of oxygen on cytochrome and menaquinone levels. FEMS Microbiol Lett 138:59–64. https://doi.org/10.1111/j.1574-6968.1996.tb08135.x.

46. Ibrahim AS, Eldeeb MM, Salama OA, Rashad MM, Okasha KM. 2018. Possible association of ghrelin/obestatin balance with cardiometabolic risk in obese subjects with Helicobacter pylori. Endocr Regul 52:101–109. https://doi.org/10.2478/enr-2018-0012.

47. Ribeiro-Parenti L, Jarry A-C, Cavin J-B, Willemetz A, Le Beyec J, Sannier A, Benadda S, Pelletier A-L, Hourseau M, Léger T, Morlet B, Couvelard A, Anini Y, Msika S, Marmuse J-P, Ledoux S, Le Gall M, Bado A. 2021. Bariatric surgery induces a new gastric mucosa phenotype with increased functional glucagon-like peptide-1 expressing cells. Nat Commun 12:110. https://doi.org/10.1038/s41467-020-20301-1.

48. Gutiérrez-Repiso C, Moreno-Indias I, Martín-Núñez GM, Ho-Plágaro A, Rodríguez-Cañete A, Gonzalo M, García-Fuentes E, Tinahones FJ. 2020. Mucosa-associated microbiota in the jejunum of patients with morbid obesity: alterations in states of insulin resistance and metformin treatment. Surg Obes Relat Dis 16:1575–1585. https://doi.org/10.1016/j.soard.2020.04.008.

49. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869.

50. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. 2017. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45:W180–W188. https://doi.org/10.1093/nar/gkx295.

51. Oliveros JC. 2007. VENNY. An interactive tool for comparing lists with Venn diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html.

52. Caspi R, Billington R, Keseler IM, Kothari A, Krummenacker M, Midford PE, Ong WK, Paley S, Subhraveti P, Karp PD. 2020. The MetaCyc database of metabolic pathways and enzymes—a 2019 update. Nucleic Acids Res 48: D445–D453. https://doi.org/10.1093/nar/gkz862.

53. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30:3123–3124. https://doi.org/10.1093/bioinformatics/btu494.