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BRIEF COMMUNICATION

The Effect of PPI Use on Human Gut Microbiota and Weight Loss in Patients Undergoing Laparoscopic Roux-en-Y Gastric Bypass

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Abstract Laparoscopic Roux-en-Y gastric bypass (LRYGB) achieves sustainable weight loss possibly by altering the gut microbiota. The effect of a proton pump inhibitor (PPI) on weight loss and the gut microbiota has not been explored. PPI use and the gut microbiota were assessed before and 6 months after LRYGB in eight patients. Bacterial profiles were generated by 16S ribosomal RNA (rRNA) gene sequencing. Prior to LRYGB, PPI users had a higher percent relative abundance (PRA) of Firmicutes compared to nonusers. PPI users at 6 months post-LRYGB had a higher PRA of Firmicutes [48.6 versus 35.6 %, p=nonsignificant (NS)] and a trend toward significantly lower percent excess weight loss (49.3 versus 61.4 %, p=0.067) compared to nonusers. PPI use post-LRYGB may impair weight loss by modifying gut microbiota.

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Abbreviations

PRA	Percent relative abundance
PPI	Proton pump inhibitor
RYGB	Roux-en-Y gastric bypass
LRYGB	Laparoscopic Roux-en-Y gastric bypass
PWL	Percent weight loss
PEWL	Percent excess weight loss
NS	Nonsignificant

Introduction

The bacteria that inhabit the gastrointestinal tract (gut microbiota) appear to play a role in obesity [1]. Obese humans and mice have a higher abundance of bacteria from the phylum Firmicutes and a lower abundance from the phylum Bacteroidetes compared to their lean counterparts [2, 3]. The nutrient load affects the gut microbiota in lean and obese adults differently. Specifically, when overfeeding a lean individual, a resulting 20 % increase in relative abundance of Firmicutes (with corresponding decrease of Bacteroidetes) was associated with an increase in energy harvest of 150 kcal [4]. Colonization of germ-free mice with the gut microbiota from obese mice increases total body fat compared to colonization with microbiota from lean mice, suggesting that differences in the gut microbiota between lean and obese individuals play a role in obesity and are not a mere consequence of obesity [3].

Roux-en-Y gastric bypass (RYGB) for severe obesity achieves 60 % excess weight loss long term [5]. Recent human studies suggest that the gut microbiome is altered after RYGB [6, 7], and Liou et al. showed that the transfer of gut microbiota from RYGB-treated mice to germ-free mice

resulted in weight loss and decreased fat mass compared to germ-free mice who received microbiota from sham-operated mice [8]. These findings support the hypothesis that changes to the gut microbiota help mediate weight loss after RYGB.

There is a considerable variability in weight loss following RYGB, and the reasons for this variability are unclear. Severely obese patients use proton pump inhibitors (PPIs) at high rates [9], and long-term PPI use has been associated with at least a 5 % weight gain in 36 % of patients using PPIs compared to only 4 % of controls gaining at least 5 % of body weight [10]. PPIs have been associated with small intestinal bacterial overgrowth [11] and a decrease in small intestinal pH [12]. A change in intestinal pH can alter the gut microbiota, which could have subsequent effects on weight loss post-RYGB. This study assessed the association between PPI use and the gut microbiota just prior to laparoscopic Roux-en-Y gastric bypass (LRYGB) and at 6 months post-LRYGB and the relationship of post-LRYGB PPI use with weight loss.

Methods

Ethics Statement

This protocol was approved by the Ohio State University Institutional Review Board and the Colorado Multiple Institutional Review Board, and all subjects gave written informed consent before participation.

Patient Population

Eight severely obese subjects (seven females and one male) who were eligible candidates for LRYGB at the Ohio State Wexner Medical Center (Columbus, Ohio) were recruited between December 2010 and December 2011. All subjects had a BMI≥40 kg/m^2. Adult subjects (18 to 70 years of age) were included if their weight was stable (±5 kg) for at least 1 month prior to surgery, and they were electively choosing to undergo LRYGB. Subjects were excluded if they were taking antibiotics, probiotics, steroids, or other immunosuppressants chronically or 30 days before submitting a fecal sample. Weight, height, and PPI use were recorded. PPI users were defined by the use of a PPI for 4 days or more per week for the preceding 4 weeks. Nonusers were defined as those who had not taken a single dose of a PPI or a histamine 2 blocker (H2B) in the previous 2 weeks (before the baseline stool collection) and had not been regular PPI (or H2B) users at any point in the preceding 3 months.

Fecal Samples

The subjects volunteered fecal samples before and 6 months after LRYGB. Fecal samples were self-collected, delivered to the study coordinator, reduced to 100-mg aliquots, and stored at −80 °C until future analysis. Preoperative stool samples were collected within 1 month before LRYGB and at the sixth month clinic visit. Fecal samples were collected from eight subjects prior to LRYGB, and in five of these eight subjects at 6 months post-LRYGB. The samples were analyzed at the University of Colorado Denver's Microbiome Research Consortium (MiRC).

High-Throughput DNA Sequencing and 16S Amplicon Library Construction

Bacterial profiles from the subjects were determined by broad-range amplification and sequence analysis of 16S ribosomal RNA (rRNA) genes following our previously described methods [13, 14]. In brief, DNA was extracted from the 100-mg fecal aliquots using the UltraClean Fecal DNA Kit (MO BIO, Inc.). Amplicons were generated using primers that target the V4 variable region of the 16S rRNA gene. PCR products were prepared using previously described techniques [15–17]. The bacterial DNA sequences were uploaded into a taxonomic software program called SINA.

Assembled sequences were aligned and classified with SINA (1.2.11) [18] using the 244,077 bacterial sequences in Silva 111 NR [19] as a reference configured to yield the Silva taxonomy. Operational taxonomic units (OTUs) were produced by clustering sequences with identical taxonomic assignments. The software package Explicet (v2.8.3; www.explicet.org) [20] was used for display, analysis, and figure generation of results.

The resulting sequence dataset consisted of 5,164,221 bacterial rRNA sequences with a median of 266,992 reads/sample (IQR 112,133–630,024). The median genus-level goods coverage score for libraries, a measure of completeness of sequencing, was 99.9 %, indicating that the depth of sequencing was sufficient to fully describe the biodiversity of the samples.

Statistical Analysis

The composition of the gut microbiota is expressed as a percent relative abundance (PRA) of the entire taxonomic library. An ecologic index [21] of diversity [Shannon's diversity (H_o)] was calculated with Explicet. This index was estimated through bootstrap resampling (1,000 replicates) and rarefaction of the OTU distributions obtained from each specimen. Mean percent relative abundances were compared between PPI users and PPI nonusers using an unpaired Student's *t* test. Weight loss was calculated at 6 months post-LRYGB. Weight loss outcomes were percent weight loss (PWL) and percent excess weight loss (PEWL). PWL was calculated with the following equation: PWL=((Preoperative weight) −(Weight at 6 months post-LRYGB))/(Preoperative weight)×100. PEWL was calculated with the following

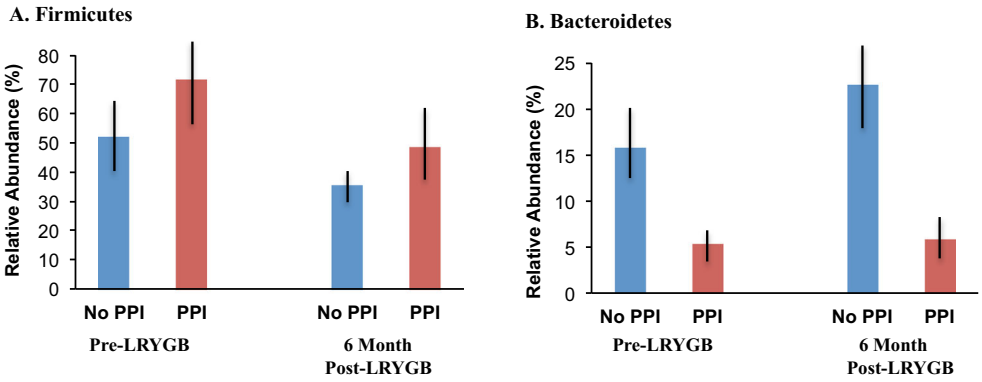

Fig. 1 Mean percent relative abundances of bacteria from the phyla Firmicutes (**a**) and Bacteroidetes (**b**) before and 6 months after laparoscopic Roux-en-Y gastric bypass (*LRYGB*) by proton pump inhibitor (*PPI*) use at each time point

equation: PEWL=((Preoperative weight)−(Weight at 6 months post-LRYGB))/((Preoperative weight)−(Ideal body weight))×100.

Results

A total of eight subjects (seven females; mean age 44.3 years) underwent LRYGB. The mean pre-LRYGB BMI (±SD) was 47.1±4.8 kg/m^2, with a mean weight (±SD) of 131.5±16.9 kg. Just prior to LRYGB, three patients were chronic PPI users, and five patients were nonusers. At 6 months post-LRYGB, five patients were chronic PPI users, and three patients were nonusers. The indication for PPI use in all cases was for symptoms related to GERD. A fecal sample was obtained from all patients pre-LRYGB and from five patients at 6 months post-LRYGB (three chronic PPI users and two nonusers). The resulting dataset of 5,161,446 bacterial rRNA sequences revealed 13 phyla. Four phyla were present in high abundance (≥5 %) and represented 95.9 % of the total sequences. The four high-abundance (>5 %) phyla were as follows: Firmicutes, Bacteroidetes, Proteobacteria, and Verrucomicrobia. Fifty-six different genera were detected. Six genera were present in high abundance (≥5 %) either before or at 6 months post-LRYGB and represented 48.6 % of the entire microbiota. The six high-abundance genera were the following: *Streptococcus*, *Clostridium*, *Blautia*, *Escherichia*, *Bacteroides*, and *Akkermansia*.

Phylum Firmicutes

Prior to LRYGB, the mean PRA of Firmicutes was higher (Fig. 1) in PPI users (71.6 %) compared to nonusers (52.1 %) [*p*=nonsignificant (NS)]. Although the PRA of Firmicutes was decreased overall at 6 months post-LRYGB, users of PPIs had a higher PRA (48.6 %) of Firmicutes compared to nonusers (35.6 %) (*p*=NS). The higher PRA of Firmicutes in PPI users prior to LRYGB was due to a higher PRA from the genera *Streptococcus*, *Clostridium*, and *Blautia*.

Phylum Bacteroidetes

Before LRYGB, the mean PRA of Bacteroidetes was lower (Fig. 1) in PPI users (5.4 %) compared to nonusers (15.8 %) (*p*=NS). At 6 months post-LRYGB, PPI users also had a lower PRA (5.9 %) of Bacteroidetes compared to nonusers (22.6 %) (*p*=NS). Specifically, the genus *Bacteroides* had a lower PRA in PPI users compared to nonusers both pre-LRYGB (3.5 versus 10.3 %, respectively) and at 6 months post-LRYGB (4.6 versus 18.4 %).

Phylum Proteobacteria

Prior to LRYGB, the PRA of Proteobacteria was lower in PPI users (6.8 %) compared to nonusers (19.5 %) (*p*=NS). At 6 months post-LRYGB, the PRA of Proteobacteria was similar in PPI users (13.8 %) compared to nonusers (12.4 %) (*p*=NS). Bacteria of the genus *Escherichia* were higher in PPI users compared to nonusers both pre-LRYGB (4.5 versus 0.4 %, respectively) and at 6 months post-LRYGB (10.8 versus 3.7 %).

Phylum Verrucomicrobia

Bacteria from the phylum Verrucomicrobia were entirely composed of bacteria from the genus *Akkermansia*. The

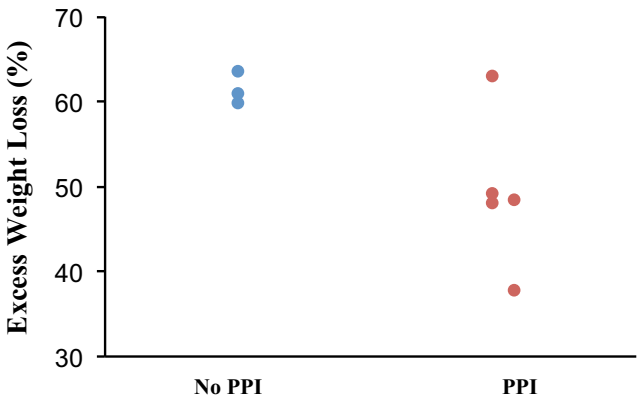

Fig. 2 Percent excess weight loss 6 months after laparoscopic Roux-en-Y gastric bypass by proton pump inhibitor (*PPI*) use at 6 months

PRA of *Akkermansia* was higher in PPI users (12.8 %) compared to nonusers (7.2 %) prior to LRYGB (p=NS). The PRA of *Akkermansia* was increased and similar between PPI users (28.0 %) and nonusers (26.3 %) at 6 months post-LRYGB (p=NS).

Diversity

The mean SDI was similar before and 6 months after LRYGB and did not differ by PPI use either before or at the 6-month post-LRYGB time point.

Weight Loss

At 6 months post-LRYGB, patients who were using PPIs had poorer weight loss. The mean PWL (±SD) for PPI users at 6 months was 27.4±4.6 % compared to 31.1±8.0 % for nonusers (p=NS). The mean PEWL (±SD) for PPI users at 6 months was 49.3±9.0 % for PPI users compared to 61.4±2.0 % in nonusers (Fig. 2) (p=0.067).

Discussion

This is the first study describing the association of PPIs and the gut microbiota before and after LRYGB. Our results support recent publications that the gut microbiota is altered following LRYGB [6, 7]. Bacteria from the phylum Firmicutes were decreased and bacteria from the phylum Verrucomicrobia (genus *Akkermansia*) were increased following LRYGB, a shift associated with leanness [22]. We also report three important and novel findings related to PPI use before and after LRYGB. First, PPI use before and after LRYGB was associated with an increase in Firmicutes and a decrease in Bacteroidetes (i.e., a more "obese" microbiota). Secondly, PPI use at 6 months was associated with a trend toward significantly poorer weight loss after LRYGB, consistent with the differences in the gut microbiota profiles. Finally, microbial diversity at 6 months post-LRYGB was preserved compared to diversity just prior to LRYGB, and PPI use at either time point did not clearly impact diversity.

Although this study was limited by the small sample size, the results for the association of PPI use with poorer weight loss post-LRYGB are consistent with our other data of 516 consecutive patients who underwent LRYGB at the University of Colorado Hospital [23]. These novel observations are important as they identify potential mechanisms (e.g., altered intraluminal pH) by which the gut microbiota is modified and influences body weight regulation. A potential confounder for PPI use and weight gain is gastric pouch size (unknown in this study). However, subjects did not report maladaptive eating in their postsurgical dietician visit. Further investigation into how PPIs impact the gut microbiota, nutrient absorption, and energy balance should be explored.

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Conflict of Interest No conflicts of interest exist for any of the authors.

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