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ORIGINAL CONTRIBUTIONS

Changes in Gut Microbiome after Bariatric Surgery Versus Medical Weight Loss in a Pilot Randomized Trial

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Abstract

Background Gut microbiota likely impact obesity and metabolic diseases. We evaluated the changes in gut microbiota after surgical versus medical weight loss in adults with diabetes and obesity.

Methods We performed 16S rRNA amplicon sequencing to identify the gut microbial composition at baseline and at 10% weight loss in adults with diabetes who were randomized to medical weight loss (MWL, $n = 4$), adjustable gastric banding (AGB, $n = 4$), or Roux-en-Y gastric bypass (RYGB, $n = 4$).

Results All participants were female, 75% reported black race with mean age of 51 years. At similar weight loss amount and glycemic improvement, the RYGB group had the most number of bacterial species (10 increased, 1 decreased) that significantly changed ($p < 0.05$) in relative abundance. Alpha-diversity at follow-up was significantly lower in AGB group compared to MWL and RYGB (observed species for AGB vs. MWL, $p = 0.0093$; AGB vs. RYGB, $p = 0.0093$). The relative abundance of *Faecalibacterium prausnitzii* increased in 3 participants after RYGB, 1 after AGB, and 1 after MWL.

Conclusions At similar weight loss and glycemic improvement, the greatest alteration in gut microbiota occurred after RYGB with an increase in the potentially beneficial bacterium, *F. prausnitzii*. Gut microbial diversity tended to decrease after AGB and increase after RYGB and MWL. Future studies are needed to determine the impact and durability of gut microbial changes over time and their role in long-term metabolic improvement after bariatric surgery in adults with type 2 diabetes.

Clinical Trial Registration NCTDK089557—ClinicalTrials.gov

Keywords Diabetes · Obesity · Gastric bypass · Gastric band · Gut microbiome · Randomized controlled trial

Introduction

With the twin global epidemic of obesity and diabetes, bariatric surgery, especially Roux-en-Y gastric bypass (RYGB), has emerged as a highly effective treatment for patients with obesity and type 2 diabetes (T2DM) [1–4]. Studies have shown that individuals with diabetes and obesity have decreased gut microbial diversity and gene richness as well as altered

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microbiota composition [5–9]. Whereas, weight loss after RYGB in adults with obesity was associated with increased gut microbial diversity and increase in potentially beneficial gut bacteria such as *Fecalibacterium prausnitzii* and *Akkermansia muciniphila* [10, 11].

Several experimental studies in mice indicate a causal link between gut microbiome and regulation of host weight. For example, colonizing germ-free mice with stools from obese and lean human twin pairs led to excess weight gain in recipients of microbiota from the obese donors but not the lean donors [12]. Similarly, transplantation of RYGB-associated gut microbiota into germ-free outbred Swiss Webster mice triggered loss of body weight and adiposity, thus suggesting a direct effect of RYGB-altered microbial communities on improved metabolic outcomes [11, 13]. Similar studies in humans are limited such as the transplant of gut microbiota of a lean donor into a subject with metabolic syndrome that led to improved insulin sensitivity after 6 weeks [14]. Together, these studies support the hypothesis that the gut microbiota contributes functionally to host metabolism and metabolic changes after bariatric surgery.

To date, however, there is few randomized trial evaluating the role of gut microbiota after weight loss in human [15]. Thus, our goals in this ancillary study to Improving Diabetes through Lifestyle and Surgery (IDeaLS) trial were to characterize and compare the changes in gut microbiota associated with medical and surgical weight loss at similar glycemic improvement and weight reduction in adults with diabetes.

Materials and Methods

Study Design We conducted a randomized, controlled pilot trial to determine the comparative effectiveness of a medical weight loss intervention versus 2 surgical procedures for weight loss in people with mild or moderate (BMI 30–40 kg/m^2) obesity with type 2 diabetes mellitus (T2DM), at a similar weight loss (~ 10%). A total of 15 participants were randomized in a 1:1:1 design to one of the following three groups, medical weight loss (MWL), RYGB, or laparoscopic adjustable gastric banding (AGB), and 12 participants completed the study. Randomization allocation was computer-generated using a password-protected, SAS-based randomization interface only accessed by designated study personnel without contact with participants. Study personnel generating the random allocation sequence and intervention assignment were separate from those enrolling participants. The MWL program was adapted from the Diabetes Prevention Program individualized counseling approach combined with the Look AHEAD study approach using meal replacements and frequent self-monitoring; goal calorie consumption per day was 1200–1500 kcal/d for adults weighing less than 220 lb; 1500–1800 kcal/d if more than 220 lb [16]. The final outcomes were assessed when 10% of initial body weight was lost or at 9 months in case the 10% weight loss was not achieved. This study was approved by the Institute Review Board of the Johns Hopkins University School of Medicine. All study participants provided written informed consent. This trial was registered in clinicaltrials.gov.

Sample Collection Study of the microbiome was added as an ancillary study to the trial prior to recruitment of the first patient. Stool samples were collected at home and was immediately dispersed in RNALater (QIAgen) and refrigerated overnight the day before the baseline clinic visit and at the time of final data collection. Upon receipt, samples were stored at − 80 °C for long term storage and analysis at a later time.

DNA Library Construction and Sequencing Extraction of genomic DNA from the stool samples were performed as previously described at the Institute of Genome Sciences at the University of Maryland [17]. Bacterial profiles were determined using multiplexed 16S rRNA amplicon sequencing (V3-V4 region) on the Illumina MiSeq platform (2x300bp) using the Caporaso laboratory protocol [18].

Bioinformatic Microbiome Analysis 16S rRNA amplicon sequences from 12 patients before and after 10% weight loss were analyzed for relative abundance, taxonomic classification, and diversity using the Quantitative Insight Into Microbial Ecology (QIIME) software suites [19]. The median number of reads per sample was 322,101 (range 161,740 to 453,556); each read was 301 base pair in length. Alpha-diversity rarefaction curves for each group were calculated from the profiles with QIIME using default options and the "observed_species," "phylogenic diversity (PD)_whole_tree," and "chao1" measures to estimate fecal microbial community richness and phylogenic diversity. Beta-diversity was calculated with the "weighted unifrac" measure [20]. The software MetaStats was then used to compare the relative abundance spectra before and after each treatment, and for the entire patient group (p value < 0.05, q-value < 0.05) [21].

Results

All participants were female, 75% reported black race with mean age of 51 and mean weight loss of 10%, 9.9%, and 6.3% for the RYGB, AGB, and MWL arms, respectively. The two surgical arms had a shorter median time (1.75 months for RYGB and 2.33 months for AGB) to data collection compared to the MWL arm (7.9 months). Two of four MWL participants did not achieve the goal 10% weight loss by 9-month final data collection time point, whereas all of the

surgical arm participants achieved the 10% weight loss goal well within the 9-month time cutoff (Table 1). Mean hemoglobin (Hb) A1c similarly decreased in all three arms (− 1.2%, − 1.0%, and − 1.2% median reduction for the RYGB, AGB, and MWL arms, respectively, $p = 0.99$).

Change in Relative Abundance of Gut Microbiota Before and After Weight Loss

We next compared the change in the relative abundance of individual bacterial taxa after surgical versus medical weight loss. At the phylum level, both surgical groups showed an increase in the relative abundance of *Proteobacteria*. The RYGB group additionally showed an increase in the relative abundance of *Actinobacteria* (Fig. 1). At the species level, the RYGB group had the most number of bacterial OTUs (11 OTUs, 10 increased, 1 decreased) that significantly changed ($p < 0.05$) in relative abundance after the intervention followed by AGB (4 OTUs, 2 increased, 2 decreased) or MWL (2 species, 2 increased) groups (Supplementary Table S1).

Lower Biodiversity in Gut Microbiome After AGB Compared to MWL or RYGB

The fecal microbial community richness and phylogenic diversity (alpha diversity) were similar at baseline across all three groups. However, post-intervention, alpha-diversity was significantly different across the three groups (observed species $p = 0.0249$, Chao1 $p = 0.0388$, PD $p = 0.0345$) and the subsequent pair-wise comparisons showed that it was lower in AGB group compared to MWL and RYGB (AGB vs. MWL, observed species $p = 0.0093$, Chao1 $p = 0.0155$, PD p = 0.0249; AGB vs. RYGB, observed species $p = 0.0093$, Chao1 $p = 0.0121$, PD $p = 0.0071$). In contrast, both the MWL and RYGB groups showed a modest trend for increased fecal microbial community richness and phylogenic diversity (Fig. 2). In addition to alpha diversity, we used weighted UniFrac analyses to compare the similarity of the gut microbial communities across the three groups. There was no distinct clustering before and after each intervention in PCoA analyses (not shown).

Change in Relative Abundance of Individual Bacterial Genera

The relative abundance of a putatively beneficial microbe, *Faecalibacterium* genus [22, 23], appeared to increase more in the RYGB group compared to AGB or MWL groups; specifically in 3 participants after RYGB, 1 after AGB and 1 after MWL. In contrast, the relative abundance of *Roseburia* was increased more in the MWL group compared to RYGB or AGB groups, specifically in 3 participants after MWL and none after AGB or RYGB. Interestingly, the one RYGB patient with a decrease in *Faecalibacterium* had the smallest improvement in hemoglobin A1C compared to the rest of the RYGB group with an increase in *Faecalibacterium* after surgery. Another putatively beneficial microbe, *Akkermansia* genus [24], increased similarly across the groups in relative

Table 1 Clinical characteristics of participants ($N = 12$)

	MWL ($n = 4$)	AGB ($n = 4$)	RYGB ($n = 4$)	All ($N = 12$)
Age, years	56 (32–62)	45 (41–53)	57 (43–60)	52.5 (32–62)
Female	4 (100)	4 (100)	4 (100)	12 (100)
Race				
Black	3 (75)	3 (75)	3 (75)	9 (75)
White	1 (25)	1 (25)	1 (25)	3 (25)
BMI, kg/m^2, baseline	38.5 (31.0–40.5)	35.8 (33.0–37.6)	35.1 (31.3–38.6)	37.0 (31.0–40.5)
Weight, kg, baseline	109.8 (87.1–112.3)	98.7 (88.1–99.9)	94.2 (80.0–111.0)	99.8 (80.0–112.3)
Percent change from baseline at study termination	− 6.3 (− 8.8, − 3.0)	− 9.9 (− 10.7, − 8.5)	− 10.0 (− 10.3, − 9.5)	
HbA1C, %, baseline	8.4 (6.3–9.9)	8.4 (7.2–10.0)	8.5 (6.3–9.8)	8.4 (6.3–10.0)
Change from baseline	− 1.2 (− 2.6, 0)	− 1.0 (− 2.2, − 0.4)	− 1.2 (− 1.5, − 0.3)	
Fasting glucose, mg/dl	147 (89–197)	202 (110–218)	150 (90–256)	163 (89–256)
Time to end of study, months	7.9 (4.4–9.6)	2.3 (2.1–4.3)	1.8 (0.9–5.6)	3.4 (0.9–9.6)
Duration of diabetes, years	4 (1–16)	9 (1–14)	6 (1–11)	6 (1–16)
Metformin use, baseline	2	4	4	10
Metformin use, follow-up	2	4	2	8

Data displayed are median (range) or *n* (% of column). *MWL* medical weight loss, *AGB* adjustable gastric band, *RYGB* Roux-en-Y gastric bypass, *BMI* body mass index, *HbA1C* hemoglobin A1C

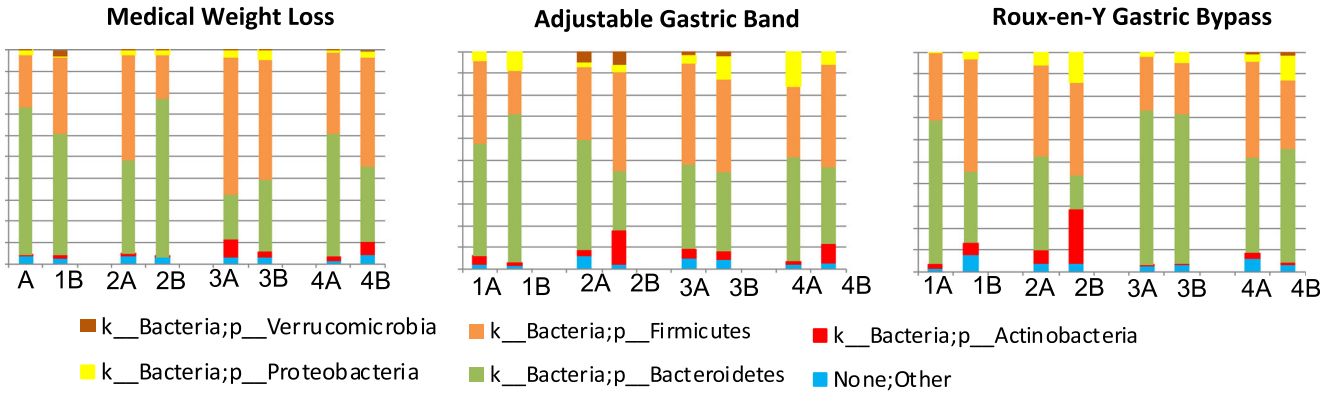

Fig. 1 Change in relative abundance of gut microbiota before and after weight loss. Y axis: percent ranging from 0 (bottom) to 100 (top) with each 10% increment noted by gray horizontal line, X axis: Each pair of bars per individual with A noting before and B after intervention

abundance, in 2 participants after RYGB, 3 after AGB, and 2 after MWL (Table 2).

Discussion

To our knowledge, this is the first randomized human study comparing the changes in gut microbiota after RYGB, AGB, or MWL and we report several key observations. First, we found that at similar weight loss and glycemic improvement, gut microbiota changes varied by weight loss intervention. Specifically, the relative abundance of *Proteobacteria* was increased after RYGB and AGB and *Actinobacteria* after RYGB (Fig. 1). A recent study also reported an increase in the relative abundance of *Actinobacteria* 1 year after RYGB [25]. However, whether this is an effect of surgical intervention and/or the specific procedure is unclear since hospitalization alone is known to rapidly change the microbiota as well as perioperative exposure to antibiotics in surgical groups. In contrast, the MWL group showed little change in relative abundance and types of gut microbiota after weight loss. Our finding supports the results of a recent systematic review of preclinical and clinical studies showing that *Proteobacteria and Verrucomicrobia* are increased after bariatric surgery [26]. Interestingly, *Proteobacteria* was significantly increased along with improved insulin sensitivity after the gut microbiota of obese mice were modulated with antibiotics, thus suggesting a beneficial role of *Proteobacteria* in glucose metabolism [27]. However, we did not see an increase in *Bacteroides* in our surgical group, which was also reported after bariatric surgery in the recent systematic review [26].

We also found that the gut microbial diversity and richness was lower after AGB compared to after RYGB or MWL. Reduced gut microbial diversity may be undesirable since it has been observed in individuals with metabolic diseases compared to healthy individuals, whereas it was increased after RYGB in several studies [10, 28, 29]. Both the MWL and RYGB groups in this study showed a trend for increased fecal microbial community richness and

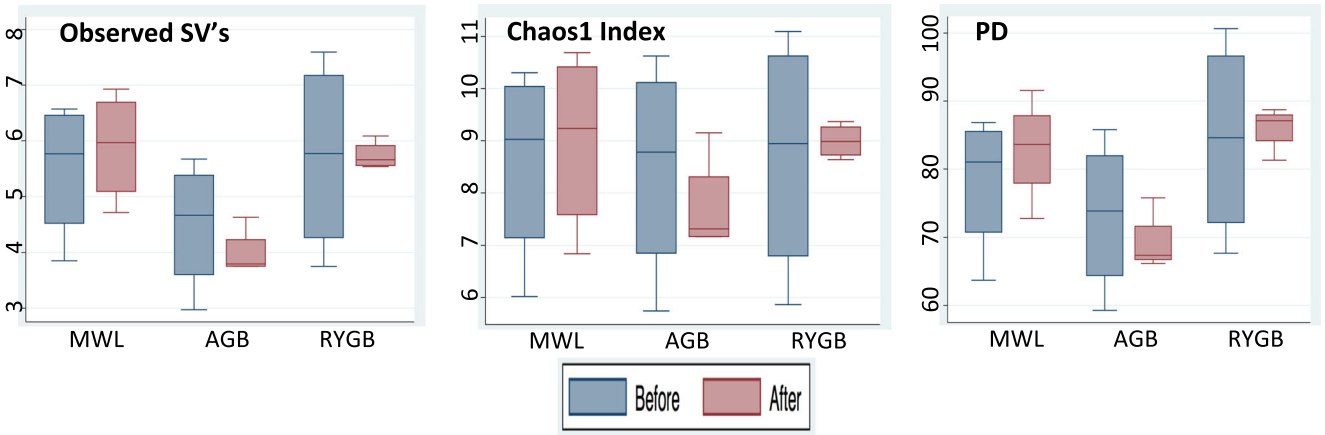

Fig. 2 Change in alpha diversity of gut microbiota after medical vs. surgical weight loss. Left panel, observed species; middle panel, Chao 1 index; right panel, faith's phylogenic diversity (PD). MWL: medical weight loss, AGB: adjustable gastric band, RYGB: Roux-en-Y gastric bypass, Y axis numbers for Observed SV's and Chaos 1 Index must be multipled by 1000

Table 2 Change in relative abundance of putatively beneficial bacteria after weight loss (%)

Microbe	Medical weight loss			Adjustable gastric band			Roux-en-Y gastric bypass		
	Before	After	Change	Before	After	Change	Before	After	Change
F. prausnitzii	1.18	2.57	+	0.48	0.36	–	0.05	0.11	+
	7.81	3.83	–	1.95	0.06	–	2.87	4.82	+
	7.18	3.09	–	2.75	0.66	–	0.70	0.93	+
	6.18	3.76	–	0.01	0.14	+	11.59	5.32	–
Akkermansia	0.08	3.04	+	0.01	0.03	+	0.00	0.10	+
	0.39	0.00	–	4.94	6.44	–	0.02	0.01	–
	0.01	0.1	+	1.69	1.99	+	0.04	0.04	=
	0.01	0.91	+	0.01	0.01	=	0.94	1.69	+
Roseburia	0.03	0.18	+	0.03	0.01	–	0.04	0.04	=
	0.62	0.16	–	0.28	0.01	–	0.07	0.01	–
	0.01	0.05	+	0.11	0.01	–	0.04	0.04	=
	0.01	0.16	+	0.28	0.01	–	0.07	0.01	–

phylogenic diversity. Indeed, a recent study comparing the change in gut microbiota after RYGB vs. AGB similarly showed an increase in gut microbial diversity after RYGB and not after AGB. This was thought to be due to an increase in facultative anaerobic microbes that are acid-sensitive and bile-resistant possibly related to reduced gastric acid exposure after RYGB [30].

We observed an increase in the relative abundance of the putatively beneficial microbe, *Faecalibacterium* genus, associated with those who underwent RYGB compared to AGB and MWL. Indeed, other studies reported that adults with metabolic disease such as type 2 diabetes have less *F. prausnitzii* colonization compared to healthy adults with increased relative abundance observed after weight loss [23, 31, 32]. The beneficial role of *F. prausnitzii* is thought to be mediated, in part, by its anti-inflammatory effect. *F. prausnitzii* was shown to be negatively correlated with inflammation in adults who underwent bariatric surgery and its anti-inflammatory effect may be mediated by its metabolites blocking nuclear factor-kB activation and subsequent secretion of pro-inflammatory mediators [22, 33]. In addition, *F. prausnitzii* may enhance insulin sensitivity by inducing GLP-1 secretion via its metabolites [34].

In contrast, an increase in the relative abundance of *Roseburia* genus was associated with those who underwent MWL compared to RYGB and AGB. This finding differs from a recent study showing an increase in *Roseburia* in adults with diabetes who achieved diabetes remission after bariatric surgery [25]. This difference may be partly explained by the fact that the finding by Murphy et al. is based on a longer follow-up (1 year post-surgery). Consistent with this idea, we observed an increase in the relative abundance of *Roseburia* in the gut microbiota of our MWL group who had a longer follow-up. The relative abundance of *Akkermansia* genus was increased in a similar number of individuals across all three groups. Across the total of 12 individuals in this study, 8 individuals showed an increase in the relative abundance of *Akkermansia* after weight loss. This finding confirms a recent study that reported a positive correlation between *Akkermansia muciniphila* and weight loss [30]. *Akkermansia muciniphila*, a mucin-degrading bacterium, may play a role in human gut barrier function and host metabolism by modulating the translocation of microbial molecules across the gut [35]. *Akkermansia* was shown to be less abundant in diseases such as obesity and diabetes while increasing its abundance was associated with improvement in diet-induced obesity and insulin resistance [24].

The strengths of this study include the randomized controlled study design, comparable reduction in weight and hemoglobin A1C across all three groups, and robust number of reads per microbiome sample thus supporting the high integrity of the microbiome samples collected. The limitations of this study include a small sample size as a pilot study, although comparable to other human studies to date focused on post-bariatric surgery changes in human gut microbiome and the longer time needed in the MWL group to achieve weight loss comparable to the surgery groups [11, 31]. However, the changes in gut microbiota after RYGB were previously reported to occur quickly and remain persistent, thus suggesting that our observation in RYGB group may have been similar at a later time point matching that of the MWL group [29]. We also acknowledge the important role of nutrition in shaping the gut microbiome and the differences in terms of the diet given across the three groups; namely, surgical patients underwent dietary changes typical after bariatric surgery including small frequent liquid diet immediately post-surgery and subsequent advancement to solids over the ensuing few months [15]. We also noted differences across the groups

with regard to diabetes medications use during the trial. Among the diabetes medications, metformin has been shown to modulate the gut microbiota and the use of metformin across all individuals in this study remained high [36]. It is also possible that our findings are confounded by the fact that the surgery groups routinely receive a dose of intravenous antibiotics perioperatively for infection prevention. However, specific data on the impact of limited doses of intravenous antibiotics on the gut microbiome have not yet been published. Furthermore, this is standard of care, so any influence of this would be expected for all surgical weight loss patients.

Conclusions

In summary, we found that at similar weight loss and glycemic improvement, the change in gut microbiota differed depending on the method of weight loss intervention. Specifically, the relative abundance of *Proteobacteria* was increased after RYGB and AGB and *Actinobacteria* after RYGB. The gut microbial diversity and richness were lower after AGB compared to after RYGB or MWL. We also observed that the putatively beneficial microbe, *Faecalibacterium* genus, increased in its relative abundance more often after RYGB compared to AGB and MWL. Whether the different speeds at which the three weight loss methods led to metabolic improvements can be partly explained by the changes in gut microbiome remains to be investigated. In addition, future studies are needed to determine the impact and durability of gut microbial changes over time and their role in long-term metabolic improvement after bariatric surgery in adults with type 2 diabetes.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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