

The Human Gut Microbiome

A Review of the Effect of Obesity and Surgically Induced Weight Loss

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Recent advances in parallel genomic processing and computational mapping have been applied to the native human microbial environment to provide a new understanding of the role of the microbiome in health and disease. In particular, studies of the distal gut microbiome have proposed that changes in gut microbiota are related to obesity, the metabolic syndrome, and Western diet. We examined the changes in the distal gut microbiome composition as it relates to the lean and obese phenotypes, particularly after surgical weight loss. A PubMed search of publications from January 1, 2005, through December 31, 2012, used the search terms *weight*, *obesity*, *microbiome*, and *bariatric surgery*. We included studies that provided information on subjects' weight and/or body mass index and a formal assessment of the microbiome. Certain bacteria, specifically the archaeon *Methanobrevibacter smithii*, have enhanced ability to metabolize dietary substrate, thereby increasing host energy intake and weight gain. With weight loss, there is a decrease in the ratio of Firmicutes to Bacteroidetes phyla. One major finding from microbial sequencing analyses after Roux-en-Y gastric bypass is the comparative overabundance of Proteobacteria in the distal gut microbiome, which is distinct from the changes seen in weight loss without Roux-en-Y gastric bypass. This review provides the practicing surgeon with (1) an update on the state of a rapidly innovating branch of clinical bioinformatics, specifically, the microbiome; (2) a new understanding of the microbiome changes after Roux-en-Y gastric bypass and weight loss; and (3) a basis for understanding further clinical applications of studies of the distal gut microbiome, such as in Crohn disease, ulcerative colitis, and infectious colitis.

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The human gut microbiome (previously termed the *gut flora*) has been the subject of study for decades; however, the high diversity of organisms in the gut and the infeasibility of standard culture techniques in identifying those organisms historically have limited their study. Only within the past decade, with the advent of shotgun genomic sequencing and array-based microbial identification, has the whole breadth of the organismal diversity in the gut become apparent. An often-quoted estimate is that the human gut may

hold approximately 10^{14} cells (mostly prokaryotic), some 10-fold greater than the rest of the human body combined.¹ This organismal diversity carries with it an incredible genetic diversity, representing some 3 000 000 genes compared with the approximately 30 000 in the human genome, demonstrating a coevolutionary pathway. The microbiome has been shown to interact with the host in several ways in health and disease, including (1) modulating the inflammatory host response to the gut, (2) synthesizing small molecules and proteins that are taken up by the host, and (3) changing the amount of available energy in the diet. As such, several stud-

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ies have examined the role of the distal gut microbiome in obesity and the changes that occur after Roux-en-Y gastric bypass (RYGB) in humans and rodents. Although the differences in gut microbiota found in obesity and after RYGB have so far been mostly limited to simple comparisons in alternative states, our knowledge of the microbiome in health and disease is advancing rapidly, and the clinician needs to stay well informed on the current findings of the field and the techniques being used to study it.

METHODS

The techniques available for studying the composition of the microbiome are changing rapidly, and each technique has advantages and disadvantages.² Older techniques, such as fluorescent in situ hybridization and denaturing gradient gel electrophoresis, represented advances over simple cultures in that they could quantify relative amounts of known species. However, these techniques have largely fallen out of favor with the advent of and cost reductions in microarray-based and genomic techniques, which have the advantage of profiling a much larger percentage of the microbiome at once and with greater detail.

The most common currently used DNA-based methods rely on identifying microbial 16S ribosomal RNA (rRNA), which has conserved regions, making it easy to amplify, and highly variable regions, making it species specific. The first method of identifying microbiome species using 16S rRNA uses microarrays, wherein amplified-sample RNA (or just extracted DNA) is placed on a small chip preloaded with known species-specific 16S rRNA readings. This method allows rapid identification of known bacterial species in a sample; however, it has a significant limitation in that unknown species are not identified (because only known sequences can be preloaded on the microarray itself), and rare species may not be captured if their abundance is too limited. The output of such a study is thus a somewhat narrowed measure of the relative quantities of known species in a given sample. The other approach for 16S rRNA-based identification relies on sequencing of all amplified 16S rRNA in a given sample; this was originally accomplished with base-by-base Sanger sequencing but now is largely performed in one of many available, massively parallel sequencing methods (such as 454 pyrosequencing³). Sequencing of all 16S rRNA in a given sample has the advantage of identifying all species present, known and unknown, abundant and rare. The output of this method is a robust determination of all species and their relative abundances in a given sample, with greater precision than a microarray-based technique.

The final method is whole-genome shotgun sequencing. In this method, the entire DNA in a given sample is fragmented, sequenced, and then remapped (via overlapping sequence analysis) into the original genome.⁴ This information is then compared with preexisting databases to identify species and genes. This method has the advantage of identifying all species and all genes present (which may be a more useful measure of the functional impact of the microbiome). The method is computationally intense, relying on a considerable amount of bioinformatic mapping (to overlap sequences to recreate the genome, to match the recreated sequences to known species and genes, and then to recreate whole gene networks).^{5,6} However, these studies may provide the most clinically relevant data, because they are able to identify gene networks that may be over-represented in a particular microbiome (ie, vitamin synthesis or putrefaction), giving important clues to the function of changes in the physiology of the host.

RESULTS

THE DISTAL GUT MICROBIOME IN HEALTH AND OBESITY

The first 16S rRNA study of the human gut microbiome was performed in 1996, with only 1 subject and only 31% of identified rRNAs mapping to known species.⁷ Ten years later, Gill et al⁴ performed the first metagenomic sequencing of the human distal gut microbiome; in their report, only 2 individuals underwent sequencing. Since then, dozens of human sequences in healthy and diseased states have been published. Generally, these studies have shown that the gut microbiome is composed primarily of the phyla Bacteroidetes (largely *Bacteroides* or *Prevotella* species) or Firmicutes (largely *Clostridium* and *Lactobacillus* species). To better interpret findings, a healthy comparator group is useful. Arumugam et al⁸ sorted several dozen gut microbiomes (taken mostly from healthy Western European and Japanese volunteers) into several enterotypes based on principal component analysis. The 3 enterotypes were characterized most significantly by an over-representation of one each of the genera *Bacteroides* (phylum Bacteroidetes), *Prevotella* (phylum Bacteroidetes), or *Ruminococcus* (phylum Firmicutes). The 2 samples used by Gill et al⁴ were included in this enterotyping analysis and were the only 2 American subjects included. They would have constituted a fourth enterotype characterized by particularly low representation of the phyla Bacteroidetes with overexpressed Actinobacteria; however, the authors believed that these differences may have been due to technical issues in sample processing. More recently, the Human Microbiome Project Consortium⁶ published a landmark study of the microbiota at 18 different body sites in 242 healthy Western subjects, including the distal gut. The pace of research and the accumulation of data are thus accelerating rapidly. In the Human Microbiome Project Consortium study,⁶ in the gut, the phyla Bacteroidetes and Firmicutes dominated but to highly variable degrees; however, the composition of the metabolic pathways present (as evidenced by metagenomic sequencing) remained surprisingly stable, a finding that has been replicated several times.^{6,8,9}

With obesity as the preeminent digestive public health issue, several studies have examined the role of the distal gut microbiome in obesity. One of the early findings was that, in the gut of obese humans and mice, the ratio of Firmicutes to Bacteroidetes is elevated and falls with weight loss.¹⁰ Multiple studies have since examined this question and have confirmed the basic finding that increases in members of the phylum Firmicutes or decreases in the phylum Bacteroidetes (or in their subclasses) can be found in obese individuals and that the opposite is true when obese individuals lose weight (**Table**). A study of human fecal transplant into germ-free mice²⁰ showed that within hours of transition to a diet with high levels of sugar and fat, a shift toward increases in the phylum Firmicutes in the gut occurred, fulfilling one of Robert Koch's postulates that the cultured microorganism should cause disease when introduced into a healthy organism. Furthermore, this study

Table. The Microbiome and Weight

Source	Study Subjects	Comparison	No. of Subjects	Method	Taxonomic Findings	Functional Findings
Studies in Obesity and Medical Weight Loss						
Armougam et al, ¹¹ 2009	Humans	Anorexic, healthy, and obese	49	16S qPCR	Phylum Firmicutes overall had no changes between groups; <i>Lactobacillus</i> species (phylum Firmicutes) increased in the obese group; phylum Bacteroidetes decreased in obese group	NA
Arumugam et al, ⁸ 2011	Humans	No specific comparison; BMI in only 6 classified as obese	37	Metagenomics	Phyla not related to BMI	F-type ATPase levels increased with increasing BMI
Balamurugan et al, ¹² 2010	Adolescent humans	Obese vs nonobese	28	16S qPCR	<i>Bacteroides</i> , <i>Prevotella</i> , and <i>Porphyromonas</i> species (phylum Bacteroidetes), <i>Bifidobacterium</i> species (phylum Actinobacteria), and <i>Eubacterium rectale</i> (phylum Firmicutes) all unchanged; <i>Faecalibacterium prausnitzii</i> (phylum Firmicutes) increased in obesity	NA
Duncan et al, ¹³ 2008	Humans	Obese, nonobese, and obese using a weight-loss diet	47	FISH	Decreased phylum Firmicutes in obese subjects with weight-loss diet; no changes in phylum Bacteroidetes between nonobese and obese or in obese after weight loss	NA
Ley et al, ¹⁴ 2005	Mice	Ob/ob vs lean with a normal diet	19	16S Sequencing	Firmicutes to Bacteroidetes ratio increased in obesity	NA
Ley et al, ¹⁰ 2006	Obese humans	Before and after weight-loss diet (restricted fat or carbohydrate intake)	12	16S Sequencing	Firmicutes to Bacteroidetes ratio increased in baseline obesity and decreased with weight-loss diet; increases in phylum Bacteroidetes correlated with weight lost	NA
Nadal et al, ¹⁵ 2009	Obese adolescent humans	Before and after 10-wk calorie-restricted diet	39	FISH	Low-weight-loss group showed no significant differences; high-weight-loss group showed significant decreases in <i>Clostridium</i> (phylum Firmicutes) and increases in <i>Bacteroides</i> and <i>Prevotella</i> species (phylum Bacteroidetes)	Trend toward reduction in fecal energy content in high-weight-loss group
Santacruz et al, ¹⁶ 2009	Obese adolescent humans	Before and after diet and exercise for 10 wk	36	16S qPCR	Firmicutes to Bacteroidetes ratio decreased after weight loss and increased at baseline in group with less weight loss	NA
Schwartz et al, ¹⁷ 2010	Humans	Stratified by BMI (cutoffs 25 and 30)	98	16S qPCR	Firmicutes to Bacteroidetes ratio decreased in obesity; <i>Methanobrevibacter</i> species decreased in obesity	SCFA stool content increased in obesity
Turnbaugh et al, ¹⁸ 2006	Same animals as Ley et al ¹⁴ (PNAS)	Ob/ob vs lean with a normal diet	19	Metagenomics	Same findings as Ley et al ¹⁴	Phylum Bacteroidetes associated with carbohydrate metabolism genes and increased SCFA stool content in obesity
Turnbaugh et al, ²⁰ 2009	Human twins	Stratified by BMI (cutoffs 25 and 30)	154	16S 454 Pyrosequencing and metagenomics	Decreased phylum Bacteroidetes and increased phylum Actinobacteria in obese	Phylum Bacteroidetes associated with carbohydrate metabolism genes and greater diversity than Firmicutes-based genomes
Zhang et al, ¹⁹ 2010	Mice	Wild type vs ApoA-I negative; high-fat vs normal diet	20	16S Pyrosequencing and DGGE	No phyla-order differences observed; diet caused more difference than genotype; Erysipelotrichaceae (phylum Firmicutes) decreased with obesity	NA

(continued)

Table. The Microbiome and Weight (continued)

Source	Study Subjects	Comparison	No. of Subjects	Method	Taxonomic Findings	Functional Findings
Studies After RYGB						
Furet et al, ²³ 2010	Humans	Lean controls; obese at baseline and 3 and 6 mo after RYGB	43	16S qPCR	Decreased <i>Bacteroides</i> and <i>Prevotella</i> species in obesity (phylum Bacteroidetes), increasing after RYGB; increased <i>Lactobacillus</i> , <i>Leuconostoc</i> , and <i>Pediococcus</i> species (phylum Firmicutes) and <i>Bifidobacterium</i> (phylum Actinobacteria) in obesity, decreasing after RYGB	Decreased BMI and levels of leptin and inflammatory markers (eg, CRP, IL-6) after RYGB
Li et al, ²⁵ 2011	Rats	Sham surgery vs RYGB; preoperative vs 2, 4, 6, and 8 wk after RYGB	12	16S Pyrosequencing	<i>Bacteroides</i> species not measured; Lachnospiraceae and Ruminococcaceae (phylum Firmicutes) both decreased after RYGB	Increased fecal amines and cytotoxicity after RYGB
Li et al, ²⁴ 2011	Rats	Sham surgery vs RYGB; preoperative vs 2, 4, 6, and 8 wk after RYGB	30	16S Pyrosequencing	52-Fold increase in phylum Proteobacteria after RYGB; decrease in total phyla Firmicutes (4.5-fold) and Bacteroidetes (2-fold), and thus decreased Firmicutes to Bacteroidetes ratio after RYGB	Increased carbohydrate fermentation and protein putrefaction with concomitant rise in gut amines after RYGB
Woodard et al, ²¹ 2009	Humans after RYGB	RCT; probiotics (<i>Lactobacillus</i>) vs placebo	44	Hydrogen breath test	Patients given <i>Lactobacillus</i> probiotics (phylum Firmicutes) had increased weight loss compared to those receiving no supplements	Patients given <i>Lactobacillus</i> probiotics after RYGB had higher serum levels of vitamin B ₁₂ and less bacterial overgrowth via hydrogen breath test
Zhang et al, ²² 2009	Humans	Control vs obese vs after RYGB (8-15 mo); separate subjects constitute each group	9	16S Pyrosequencing	<i>Prevotellaceae</i> (phylum Bacteroidetes), <i>Coriobacteriaceae</i> (phylum Actinobacteria), <i>Erysipelotrichaceae</i> (phylum Firmicutes), and <i>Alcaligenaceae</i> (phylum Proteobacteria) increased in obese compared with controls <i>Enterobacteriaceae</i> (phylum Proteobacteria) and <i>Fusobacteriaceae</i> (phylum Fusobacteria) markedly increased in the RYGB group; <i>Clostridiales</i> (phylum Firmicutes) decreased in obese and RYGB compared with controls	NA

Abbreviations: ApoA-I, apolipoprotein A-I; ATPase, adenosine triphosphatase; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CRP, C-reactive protein; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescent in situ hybridization; IL-6, interleukin 6; NA, not available; ob/ob, obese phenotype leptin knockout mice; PNAS, prudent no added salt (diet); qPCR, quantitative polymerase chain reaction; RCT, randomized controlled trial; RYGB, Roux-en-Y gastric bypass; SCFA, short-chain fatty acids.

showed that fecal transplant from Westernized mice into germ-free mice was sufficient to induce obesity.²⁰ Only 2 studies have shown evidence contrary to the change in the Firmicutes to Bacteroidetes ratio in obesity: Schwartz et al¹⁷ and Zhang et al¹⁹ showed a decrease in the phylum Firmicutes in obesity. Overall, the causality of changes in weight vs changes in the gut microbiome has not been established; however, their correlation is now a relatively settled question.

A major area of inquiry besides taxonomic changes in the gut in obesity is functional changes in the gut. Omnivore digestion could not take place without the symbiotic gut microbiome. The gut microbiome plays an essential role in the breakdown of food, such as in fermentation of polysaccharides to short-chain fatty acids, and thus how much energy is available to the host.^{17,18} One study showed that mice colonized with the archaeon *Methanobrevibacter smithii* had enhanced ability to metabolize fructans to short-chain fatty acids, thereby increasing host energy intake and weight gain.^{26,27} Other studies have shown

that short-chain fatty acid levels are increased in the stool of obese humans and obese mice compared with control subjects. One study²⁸ of *Eubacteria* (phylum Firmicutes) and *Bacteroides* (phylum Bacteroidetes) species transplanted separately or jointly into the guts of gnotobiotic mice showed that colocalization of members of both phyla leads to a higher carbohydrate metabolism efficiency than in the presence of either alone, although butyrate production was significantly higher with *Bacteroides* alone than with *Eubacteria* species alone. Finally, although the total breakdown of the cluster of orthologous gene major categories (ie, the relative abundance of genes representing major functions, such as metabolism and cell growth) is largely stable across health and obesity, some studies have shown significant changes in the relative abundance of gene classes dealing with energy production and carbohydrate metabolism.^{8,9,18,29}

The goal of the studies in health and obesity is to find the correlations among the obese phenotype, taxonomic microbial changes, and functional gene groups that

change the way the gut metabolizes food. Once a desired change is identified, the tools at our disposal to try to enact it will include prebiotics (compounds given to try to encourage the growth of one gut species over another), probiotics (oral or rectal intake of biologic species), and antibiotics. Of significance for the general surgeon, probiotic supplements have already been shown to increase weight loss after RYGB.²¹

THE GUT MICROBIOME AFTER RYGB

Roux-en-Y gastric bypass in morbidly obese patients leads to significant weight loss and resolution of comorbid disease (such as type 2 diabetes mellitus, hypertension, and sleep apnea); it is the most durable treatment for obesity. Given the significant changes to gut anatomy and physiology in RYGB, several groups have tried to characterize the changes that occur in the distal gut microbiome. The causality of the changes in the microbiome is difficult to establish in RYGB because the diet, incoming pH and nutrient levels, and levels of bile and local hormones are all changing at once.

In the Table, the known studies to date examining RYGB and the distal gut microbiome are presented. Zhang et al²² first studied the effects of RYGB on the gut microbiome; unfortunately, the 3 subject groups studied were all composed of separate subjects without longitudinal preoperative and postoperative assessments, making interpretation somewhat difficult. Nevertheless, Zhang et al²² showed the standard decrease in *Clostridium* bacteria (phylum Firmicutes) after RYGB compared with controls that would be expected with weight loss. They also showed a particularly significant increase in Enterobacteriaceae bacteria (phylum Proteobacteria) in the RYGB group in a ratio not seen in non-RYGB gut microbiomes. The human study by Furet et al²³ included lean controls and obese patients at baseline and 3 and 6 months after RYGB. They showed the standard increased Firmicutes to Bacteroidetes ratio in the obese patients before RYGB and then a decrease in this ratio at 3 and 6 months postoperatively, in keeping with the patients' weight loss. This study also showed that leptin levels fell in inverse relation to rising levels of *Escherichia coli* and *Bacteroides* and *Prevotella* species. The final studies to have examined the gut microbiome in RYGB are by Li et al.^{24,25} They examined rats after sham surgery or RYGB. They, too, found the standard decrease in the Firmicutes to Bacteroidetes ratio; however, the rats were nonobese, and so weight loss was from a normal baseline. However, the authors found a striking 52-fold increase in phylum Proteobacteria after RYGB. The increase in phylum Proteobacteria matches the findings of Zhang et al,²² but the proportional increase is greater in the rat study. Nevertheless, both studies show a major shift in the gut microbiome to include phylum Proteobacteria. A recent study³⁰ of mice with obesity and increased insulin resistance induced by a high-fat diet found that, when given the antibiotics ampicillin, neomycin, and metronidazole by mouth, the mice had improved insulin sensitivity concomitant with a significant overrepresentation of phylum Proteobacteria. Thus, phylum Proteobacteria expression and insulin sensitivity may be linked.

Roux-en-Y gastric bypass takes most of the stomach out of the oral-intestinal flow; the removal of this high-acid environment makes post-RYGB patients a unique population for the use of probiotics because ingested organisms are less easily killed. Our group showed that, in post-RYGB patients, supplementation with lactobacillus probiotics (phylum Firmicutes) led to greater weight loss and less bacterial overgrowth (as measured by the hydrogen breath test) than in RYGB patients given placebo.²¹ The study demonstrates less hydrogen production with the probiotic arm. This finding is consistent with the observation that obese patients have an overrepresentation of the hydrogen-producing Prevotellaceae family.²² Interspecies hydrogen transfer between bacterial and archaeal species is speculated to be an important mechanism for increasing energy uptake by the human large intestine in obese persons. Furthermore, these findings show that we have the clinical ability to detect bacterial levels with a simple test and to effect physiologic changes by changing the gut microbiome. A greater understanding of the gut microbiome may thus uncover new targets within therapeutic reach.

COMMENT

The goal of studying the gut microbiome has been to elucidate its roles in the synthesis of metabolically active small molecules, the metabolism and breakdown of food products, and the gut inflammatory response. With the advent of massively parallel processing, the cost and time to accomplish a taxonomic analysis of the gut microbiome have fallen considerably. Common techniques, such as purpose-built gut microarrays, quantitative polymerase chain reaction analyses, and 16S pyrosequencing, can yield valuable taxonomic information about changes between a healthy and diseased state. Still, metagenomics, with its ability to identify not only the species present but also the gene networks present, holds promise for a true functional understanding of the gut microbiome. The reconstruction of the gut genome and the subsequent mapping to orthologous gene clusters is computationally intense, but the identification of different genetic functions in comparative studies may yield valuable therapeutic targets for prebiotic, probiotic, antibiotic, or pharmaceutical treatment. Furthermore, metagenomics can be combined with studies of other functional outcomes, such as metabolomics profiling via gas chromatography-mass spectroscopy or magnetic resonance imaging, which may yield valuable correlations among taxonomic changes, changes in functional gene networks in the gut, and changes in small molecules that drive functional processes (eg, short-chain fatty acids or vitamins).³¹

As the Human Microbiome Project Consortium continues to amass healthy samples, the baseline gut microbiome will be better understood. Firmicutes or Bacteroidetes bacteria clearly dominate the average healthy gut; however, the functional changes made by comparatively rare species (such as the archaeon *Methanobrevibacter*) can have important effects on host physiology. Although studies have shown various

taxonomic changes in the gut in health and disease, the overall amount of functional gene groups remains surprisingly constant between states. Nevertheless, multiple studies have uncovered small but significant gene network changes that may be responsible for the link between gut microbial taxonomy and physiologic change. Overall, a more nuanced understanding of the microbiome may be necessary to understand the causal relationship between changes in gut organisms and disease.

As the Western world continues to struggle with the obesity epidemic, more and more patients will undergo RYGB. As changes in the gut microbiome appear to be correlated with obesity, their importance in weight loss after RYGB may also be important. In addition to the standard decrease in the Firmicutes to Bacteroidetes ratio that accompanies weight loss, a major finding from microbial sequencing analyses after RYGB is the comparative overabundance of the phylum Proteobacteria in the distal gut microbiome. Because this finding is relatively recent, mechanistic interpretations of its contribution to post-RYGB physiology have not been published. Nevertheless, the simple fact that significant changes in the gut microbiome accompany surgical weight loss demands a further investigation of a possible causal connection.

One significant problem with most of the studies reviewed here has to do with sampling. Collecting a stool sample is easy and noninvasive and can be performed by a patient outside the office. However, the stool represents a significantly different environment than the wall of the gut, where microbiome-host interaction takes place.²⁰ Mucosal biopsies yield more accurate data about the interface between host and microbiome but are invasive and expensive. Furthermore, most nutrient uptake takes place in the small bowel, not the large bowel; unfortunately, there is no easy way to obtain uncontaminated samples of the small intestine (although some studies have examined the effluent from patients with ileostomies).

Overall, the advent of the new technologies of massively parallel genomic processing and computational genomic reconstruction and mapping have allowed for a significantly enhanced understanding of the physiology of the gut. Although beyond the scope of this review, several studies have begun to investigate the role of the microbiome in Crohn disease, ulcerative colitis, and irritable bowel syndrome; changes in the local inflammatory response may be significantly related to changes in the gut microbiome. Similarly, studies of the gut microbiome in other common clinical problems, such as *Clostridium difficile* colitis and graft-vs-host disease, may yield valuable clinical tools in restoring a healthy microbiome and curing disease.

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