The Intestinal Microbiota and Obesity

Samuel J. Kallus, MD and Lawrence J. Brandt, MD†*

Abstract: Obesity has been and continues to be an epidemic in the United States. Obesity has been addressed in multiple health initiatives, including Healthy People 2010, with no state meeting the proposed goal of a prevalence of obesity < 15% of the adult population. In contrast, obesity rates have continued to increase, with the self-reported prevalence of obesity among adults increasing by 1.1% from 2007 to the present. Indeed, since 2009, 33 states reported obesity prevalences of 25% or more with only 1 state reporting prevalence < 20%. There have been multiple approaches for the treatment of obesity, including fad diets, incentive-based exercise programs, and gastric bypass surgery; none of which have been optimal. In a murine model, it was shown that the majority of the intestinal microbiome consists of two bacterial phyla, the *Bacteroidetes* and the *Firmicutes*, and that the relative abundance of these two phyla differs among lean and obese mice; the obese mouse had a higher proportion of *Firmicutes* to *Bacteroidetes* (50% greater) than the lean mouse. The same results were appreciated in obese humans compared to lean subjects. The postulated explanation for this finding is that *Firmicutes* produce more complete metabolism of a given energy source than do *Bacteroidetes*, thus promoting more efficient absorption of calories and subsequent weight gain. Researchers were able to demonstrate that colonizing germ-free mice with the intestinal microbiome from obese mice led to an increased total body fat in the recipient mice despite a lack of change in diet. The converse, that, colonizing germ-free obese mice with the intestinal microbiome of thin mice causing a decreased total body fat in the recipient mice, has not yet been done. Other possible mechanisms by which the intestinal microbiome affects host obesity include induction of low-grade inflammation with lipopolysaccharide, regulation of host genes responsible for energy expenditure and storage, and hormonal communication between the intestinal microbiome and the host. The following review discusses the microbiome-obesity relationship and proposed mechanisms by which the intestinal microbiota is hypothesized to influence weight gain.

Key Words: intestinal microbiota, obesity, microbiome

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Over the past few decades, obesity has become more prevalent than malnutrition in the United States and has[1] reached pandemic status.[1] According to the most recent data published by the Centers for Disease Control and Prevention, 68% of United States adults older than 20 years of age are obese [having a body mass index (BMI) ≥ 30] or overweight (having a BMI ≥ 25).[2] BMI is calculated by dividing an individual's weight (in kilograms) by the square of the height (in meters) and is used to approximate one's level of body fat. Obesity is associated with many chronic conditions such as insulin resistance, inflammation, hepatic steatosis, cardiovascular disease, and type 2 diabetes mellitus.[3] Although obesity can be considered simply a reflection of a state in which energy intake exceeds energy expenditure, experience and data have shown that despite similar intake of calories and nutrients, and comparable levels of daily activity, some people are more susceptible to weight gain than others.[4] Indeed, obesity has proven to be a complicated state involving not only diet, but also lifestyle, genetics, environment, and, most recently, the microbiota within one's intestinal tract.

Our intestinal microorganisms, or microbiota, have been proven to affect energy harvesting and fat storage[5] and, therefore, to have the potential to influence the success or failure of weight loss and gain. In one study on the response of overweight and obese adolescents to a weight loss program of strict diet and regular exercise, the results were dependent on the subject's microbiota before the treatment,[6] suggesting that manipulation of the intestinal microbiota may be of benefit not only in weight loss but perhaps also in preventing weight regain after loss. In the study, there were two groups defined by how much weight loss occurred; the group with the more substantial weight loss was found to have greater amounts of *Bacteroides fragilis, Lactobacillus*, and *Bifidobacterium* relative to *Clostridium coccoides*, even though they were maintained on diet and exercise regimens that were identical to those of the group with less weight loss. The results indicated that interactions between the intestinal microbiota and body weight respond to lifestyle interventions to varying extents, and depend on an individual's initial microbiota structure. There are many ways in which intestinal microbiota can be altered; however, it should be noted that the current popularity of live-organism products such as probiotics, prebiotics, and synbiotics that are flooding the market are lacking substantial research to validate their health benefit claims.[7] It is important to conduct well-designed investigations of the determinants of the intestinal microbiota composition, and the role that (each) microorganism plays, alone or in combination with others, to foster weight loss or gain or maintenance of ideal or stable body weight.[8]

THE HOST AND INTESTINAL BACTERIA

The intestinal microbiota is a complex compilation of many thousands of bacteria that function as an organ system. There are many unknowns about the enteric bacteria in the human gastrointestinal (GI) tract because of the difficulties in culturing and growing out the many diverse species. In fact, although reference cultures remain valuable, cultural enumeration of GI luminal content has fallen out of favor as the gold standard for species

From the *Department of Medicine, Georgetown University Hospital, Washington, DC; and †Division of Gastroenterology, Montefiore Medical Center/AECOM, Medicine and Surgery, Bronx, NY.

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Reprints: Lawrence J. Brandt, MD, Emeritus Chief, Division of Gastroenterology, Montefiore Medical Center/AECOM, Medicine and Surgery, AECOM, 111 East 210th Street, Bronx, NY 10467 (e-mail: lbrandt@montefiore.org).

identification has now been replaced by genetic and molecular techniques of identification. Along the GI tract there is a lot of diversity with regard to type and amount of bacteria, although in general the number increases as one moves distally. There are approximately 300 to 500 unique bacterial species in the oral cavity, 10^3 bacteria per gram in the stomach, 10^8/g in the small intestine, and 10^{12}/g in the colon with more than 400 unique[9] species.[10,11] Generally, the bacteria in the proximal small intestine are Gram-positive facultative bacteria. In the distal small intestine and the colon, Gram-positive anaerobes often make up the majority of organisms with genera that include *Bifidobacterium, Clostridium, Eubacterium, Faecalibacterium,* and *Peptococcus*.[9,11] The major Gram-negative phylum is *Bacteroidetes*, which is also predominant in many individuals, with Gram-negative Proteobacteria, such as *Escherichia*, generally present in smaller numbers. Certainly, more information about which bacterial species and how many of each exist in the GI tract will continue to be defined as technology improves.

The evolutionary advantages of having commensal bacteria in the intestine include: helping to convert ingested complex nutrients to short chain fatty acids (SCFAs); transforming mucins and dietary fibers into simple sugars for host absorption; epithelial cell proliferation, which has been shown to be influenced by bacteria directing glycosylation of surface proteins on the intestinal epithelial cells[12] (highlighting the microbiota's ability to communicate with the host); nutrient and drug metabolism; and formation of a defense barrier considered to be essential in maturation and development of systemic and mucosal immune systems.[13–17] The microbiota produces essential vitamins such as vitamin K and folic acid, which contributes to the general nutritional status of the host. Intestinal bacteria also play a role in bile acid metabolism/recirculation by the activity of bile salt hydrolase. Bile salt hydrolase is highly conserved among the intestinal microbiota, most notably within the *Firmicutes, Bacteroidetes*, and *Actinobacteria* phyla, and the modified bile acid products protect against pathogen colonization through antimicrobial properties of the bile acids, which also up-regulate mucosal defenses, reduce cholesterol levels, and modulate lipid metabolism of the host.[18,19]

The intestinal flora also activates bioinactive compounds and inactivates bioactive compounds, with both beneficial and negative effects on the host. There are many ways in which compounds, such as, flavonoids and isoflavonoids, can be modified by the microbiota. Flavonoids are nonnutritive but are believed to be chemoprotective agents against cancer by their estrogenic effects, induction of cell-cycle arrest, antioxidant properties, and changes in cellular signaling.[20] Flavonoids and isoflavonoids are transformed by the microflora to metabolites, which possess biological properties that differ from the original compounds.[21,22] Thus, for example, daidzein, an isoflavone found in soy and beer, undergoes transformation to equol in the human GI tract, which is a nonsteroidal estrogen that may decrease the incidence of prostate cancer.[23] It is clear from these examples that the term intestinal microbiota does not simply describe the microorganisms that inhabit the human GI tract, but rather denotes a communicating and responsive diverse group of organisms that serves many purposes and actively influences the metabolism of the host.

RELATIONSHIP BETWEEN THE INTESTINAL MICROBIOTA AND WEIGHT

The intestinal microbiota is increasingly being recognized to play important roles in human adiposity and metabolism.[24] When the fecal contents of the ceca from obese or lean mice were introduced into the small intestine of the lean germ-free mice and equivalent food intakes provided, there was a greater increase in body fat in the mice that received microbes from obese donors than in those that received the intestinal contents of lean donors.[25] In a study by Hooper et al[26] germ-free mice were inoculated with a common commensal intestinal bacterium, *Bacteroides thetaiotaomicron*, and the transcriptional response of epithelial genes to the colonization was recorded using DNA microarrays. There was an increase in many components of the lipid absorption machinery in the recipient, including colipase, pancreatic lipase-related protein-2, apolipoprotein A-IV, and a fatty acid binding protein, as well as a decrease in fasting-induced adipose factor (Fiaf), a peroxisome proliferator-activated receptor (PPAR)-α target, which is repressed with fat feeding.[27] Hooper et al proposed epithelial cell transcriptional modulation of intestinal mediators as the means by which gut colonization regulates mucosal barrier function, nutrient absorption, metabolism, and many other functions.

Elli et al[28] have proposed that comparison of the intestinal microbiota of obese people and their lean relatives can reveal a core intestinal flora that is family specific, and which might predispose to obesity. Microbial colonization of the intestine occurs at birth and is complete, reaching the density and stability of an adult GI tract, at approximately 1 year after birth.[29] Altered intestinal bacteria in infancy may lead to the development of overweight and obesity later in life.[30] The microorganisms that colonize the intestine are derived from the surrounding environment, which is linked to the type of delivery and mode of feeding.[31] For example, the intestinal microbiota of infants delivered vaginally differs from that of infants delivered by cesarean section, both in the composition of the bacteria and the timing of colonization.[32] There have also been studies to show that *Bifidobacteria* dominates the intestinal microbiota of breast-fed infants by 4 to 6 weeks of age, and that formula-fed infants had a higher abundance of aerobic bacteria and fewer *Bifidobacteria*[33]; other studies have not shown such a difference.[34] The importance of the mode of delivery and feeding after birth requires further research regarding its short and long-term effects on the intestinal microbiota of infants.

In a study by Elli et al,[28] stool samples from 11 obese subjects and their blood-related lean family members were acquired and analyzed using denaturing gradient gel electrophoresis. It was concluded that there is a "familial fingerprint" of the intestinal bacteria that can be identified by a common core microbiota. The homology of the intestinal microbiota was much closer in blood-related individuals than in subjects of similar phenotype. Moreover, unrelated obese subjects had a lower degree of bacterial diversity in their fecal profiles than the stool of unrelated lean individuals. Interestingly, there were also fewer butyrate-producing bacteria in the obese subjects than in their blood-related lean family members. Turnbaugh et al[35,36] also looked at the role of genotype and early environmental exposure on the composition of the intestinal microbiome and found that the bacterial phyla in

the intestine were more similar in family members than unrelated individuals, regardless of body weight. The approach of comparing lean and obese family members may help identify bacterial groups that predispose certain members within a family to obesity and requires further investigation. The intestinal microbiota's contribution to an individual's predisposition to a certain body weight is intriguing and in the future is likely to change current thought on the role of bacteria and perhaps other microorganisms in obesity.

In mouse models of obesity, genetically obese mice (ob/ob) gained weight differently than high-fat fed obese mice. In looking at the bacterial genes present in the GI tracts of the genetically-induced and diet-induced obese mice, gene functions associated with lipid and carbohydrate metabolism (eg, phosphotransferase systems, glycoside hydrolases, polysaccharide esterases, and lipases) were enriched compared with those of lean control mice.[36,37] Although on the same diet, genetically obese mice had an increase in overall fat mass and a decrease in overall lean mass, whereas, the change in weight in high–fat-fed obese mice was attributable to increased fat mass alone. In both models, the proportions of intestinal *Firmicutes* increased progressively, but energy harvesting could not be correlated with the types and proportions of the major phyla.[6] Thus, there is a difference between environmental and genetic forms of obesity, at least in mouse models, and in the mechanisms by which obesity may occur.

Studies on weight loss after gastric bypass surgery shed further light on the relationship between the intestinal microbiota and weight control. After bypass surgery, there is decreased gastric acid secretion and decreased intestinal motility,[38] both of which can lead to bacterial overgrowth. Both the overgrowth and the altered proportions of bacterial species are hypothesized to affect calorie extraction. With administration of 6 months of probiotics (*Lactobacillus* species) in Roux-en-Y gastric bypass surgery patients, accelerated weight loss was observed compared with untreated patients after the same procedure. The investigators concluded that the intestinal microbiota contributes to weight loss and certain species, such as *Lactobacillus*, have positive effects on success rates of bypass surgery,[39] whereas, other bacteria may be detrimental to weight loss. These findings highlight the uncertain yet undeniable influence of bacteria in determining one's body weight.

POSSIBLE MECHANISMS WHEREBY THE INTESTINAL MICROBIOTA CAN CAUSE OBESITY

More Efficient Extraction of Calories From Ingested Food: Bacterial Species

It has been reported that there is a higher body fat content in conventionally reared mice than in germ-free mice even when the germ-free mice consume more food than the conventional ones[40]; however, a subsequent study that involved feeding a different high-fat diet[41] demonstrated the opposite results. In both studies the differences, which were evidently highly diet-dependent, appeared to be due to differences in energy expenditure rather than energy extraction from the diet.

Using 16S rRNA gene sequencing, it was shown that the bacterial communities in the ceca of genetically obese mice (ob/ob) had a different proportion of 2 dominant bacterial phyla (*Firmicutes* and *Bacteroidetes*) compared to the lean mice. The microbiota of the genetically obese mice had a greater representation of *Firmicutes* and proportionally lesser representation of *Bacteroidetes*,[42] as well as increased archaea (a unique domain that differs from eukaryotes and prokaryotes and consists of single-celled organisms).[25] Turnbaugh et al[25] found that both *Firmicutes*-dominant and *Bacteroidetes*-dominant microbiomes are enriched with genes encoding multiple enzymes that are involved in the early steps of indigestible polysaccharide metabolism, a finding suggested by the greater amount of weight gain in colonized mice compared with germ-free mice. To clarify, although *Bacteroidetes* do possess genes enriched for enzymes involved in lipid and carbohydrate metabolism, they have far fewer of these genes compared to *Firmicutes*, but more of them compared to noncolonized mice. The microbiome of obese mice had greater quantities of the genes encoding the glycoside hydrolases compared with wild-type controls, and enrichment of proteins involved in end product transport (ATP-binding cassette transporters) and metabolism (α-galactosidases and β-galactosidases) of the glycoside hydrolases. The cecum of the obese mice had elevated concentrations of butyrate and acetate, which are major fermentation end-products; this is consistent with the increased proportion of *Firmicutes*, which include butyrate producers.[43,44] Turnbaugh et al also showed that the genetically obese mice had significantly less energy in their feces relative to the lean mice, supporting the hypothesis of increased caloric extraction by the host, possibly from the enriched bacterial gene expression as discussed above.

There also have been human studies associating obesity with reduced representation of *Bacteroidetes*, a paucity of bacterial diversity, and enriched lipid-utilizing and carbohydrate-utilizing genes in the intestinal microbiome.[19] As seen in Table 1, numerous studies have been carried out investigating the relationship between the intestinal microbiota and body weight.[54] Some studies have found that there was no correlation between the proportions of *Bacteroidetes, Firmicutes*, and *Actinobacteria* and fecal energy content/extraction of calories,[6] whereas, others have found such a correlation.[54] Thus, there is much controversy regarding if and how changes in the microbial representation are related to weight. One possible explanation for these discrepancies is the wide variety in methodology of detecting bacterial phyla, (ie, polymerase chain reaction vs. 16S rRNA sequencing vs. fluorescent in-situ hybridization).

Another bacterial genus that has been implicated in obesity is *Bifidobacteria*. In rat studies, the number of *Bifidobacterium* species was markedly reduced in subjects with diet-induced obesity.[55] Some researchers report that an intestinal microbiota profile that has a higher number of *Bifidobacteria* may be protective against the development of obesity and weight gain, but the exact species of *Bifidobacteria* remain(s) unknown. It has been suggested that more detailed changes of the intestinal microbiota may be involved in the development of obesity, such as bacterial gene expression rather than simply, which bacterial phyla are present.[56] Turnbaugh et al identified a core gut microbiome preserved in unrelated individuals at the level of bacterial gene families rather than shared bacterial phylotypes. They found that unique combinations of species fulfilled similar functional roles for the host[3]; for example, two individuals could have enriched genes involved in carbohydrate absorption expressed by 2 different species of bacteria. Approximately 75% of the relevant

TABLE 1. Human Studies of Gut Microbial Ecology in Relation to Body Weight

Authors	Participants	Method (Sample Type)	Finding
Ley et al[42]	12 obese participants on 1 of 2 diets, carbohydrate or fat reduced, for 1 y; 2 lean controls	16S rRNA surveys by Sanger sequencing (feces)	Proportion of Bacteroidetes sequences increased over time, on average, and correlated with weight loss. No difference between diets
Turnbaugh et al[25,36,45,46]	154 participants, MZ and DZ twins and mothers, obese or lean	16S by Sanger and 454 pyrosequencing, metagenomics (feces)	Reduced levels of diversity, and reduced levels of Bacteroidetes in obese participants; metagenomes of obese participants enriched in energy-harvesting genes
Schwiertz et al[47]	30 lean, 35 overweight, and 33 obese participants	qPCR for Bacteroidetes, *Actinobacteria*, Archaea (feces)	More Bacteroidetes in overweight and obese vs. lean participants, and more Methanobrevibacter in lean participants
Collado et al[48]	Women before and during pregnancy, 18 overweight participants and 36 controls	FISH/flow cytometry and qPCR (feces)	Higher levels of Bacteroidetes and *Staphylococcus aureus* in overweight, positive correlation between Bacteroides levels and weight gain over pregnancy
Sotos et al[49]	8 obese and overweight adolescents during weight loss	FISH (feces)	Enterobacteriaceae and sulfate-reducing bacteria reduced in group with greatest weight loss. Reduced levels of *Roseburia* and *Eubacterium* in those with less weight loss
Duncan et al[43]	Participants on weight loss diets over 8 wk vs. weight maintenance	FISH counts (feces)	No difference in Bacteroidetes levels between groups; reduced levels of *Roseburia* and *Eubacterium*, and increased levels of *Clostridium* spp., correlate with reduced carbohydrate intake
Kalliomaki et al[50]	Obese and overweight children ($n = 25$) and normal weight children ($n = 24$); prospective study	qRT-PCR and FISH/flow cytometry (feces)	Children remaining lean at age 7 had higher levels of Bifidobacteria and lower levels of *S. aureus*, as infants
Santacruz et al[6]	36 adolescents on diet and physical activity, 10 wk	qPCR (feces)	*Bacteroides fragilis* abundance correlated with carbohydrate intake. Levels of *Bacteroides* and *Lactobacillus* increased with weight loss
Nadal et al[51]	39 adolescents on diet and physical activity, 10 wk	qPCR (feces)	*Clostridium histolyticum*, *Eubacterium rectale*, *and Clostridium coccoides* reduced with weight gain; increase in *Bacteroides* and *Prevotella* in high weight loss group
Sabate et al[52]	137 obese patients, 40 healthy controls	Glucosehydrogen breath test (for H2) and liver biopsy (breath, liver)	Bacterial overgrowth in small intestine more common in obese vs. lean participants
Zhang et al[53]	3 lean, 3 obese, and 3 postgastric bypass participants	Sanger and 454 sequencing of 16S rDNAs, qPCR (feces)	*Firmicutes* more abundant in lean participants, lowest after gastric bypass. Gamma-proteobacteria and Verrucomicrobia enriched after gastric bypass; higher Archaea in obese participants; overall communities of gastric bypass and obese participants more similar to each other than to lean participants

Reproduced with the permission of Ley[54] (Table 1).
DZ indicates dizygotic; FISH, fluorescent in situ hybridization; MZ, monozygotic; RT-PCR, real time-polymerase chain reaction.

genes that were predominant in obese individuals were derived from *Actinobacteria* and 25% were from *Firmicutes*. In contrast, lean individuals had similar genes mainly enriched from *Bacteroidetes*, with varying expression.[3,53] Phosphotransferase systems involved in microbial processing of carbohydrates also were found to be over expressed in the intestines of obese people, notably from *Prevotellaceae* and *Methanobacteriales*[57]; however, other bacterial phylotypes also had the ability to over express these same gene strains.

The intestinal microbiota is essential in breaking down resistant starch and dietary fiber into absorbable products such as SCFAs, including, propionate, acetate, and butyrate, which have different roles in energy-salvage.[58] Propionate is taken up by the liver and used as a precursor for liponeogenesis, gluconeogenesis, and protein synthesis[59]; acetate is used as a substrate for cholesterol synthesis in peripheral tissues[58]; and butyrate is the main energy supply for colonic epithelial cells.[60] Schwiertz et al[47] analyzed and compared the fecal SCFA concentrations of 98 human volunteers, of whom one third were of normal BMI, one third were overweight, and one third were obese. Obese volunteers were found to have increased serum glucose levels and SCFAs compared with their lean counterparts; however, no conclusive relationship between bacterial phylotypes and weight could be identified in this study. Both *Bacteroidetes* and *Firmicutes* produce SCFAs from nondigested dietary compounds that reach the colon, and the results of the study suggest that the amount of SCFAs produced is a more important predictor of obesity than the ratio of the 2 bacteria. Murphy et al observed that there were increased levels of SCFAs in the cecum of genetically obese mice and a reduced energy content of the feces compared with lean controls.[6] Yet another study found that the total SCFA content of the cecum was significantly greater in obese individuals compared with lean individuals, although these results should be interpreted carefully because the increased fecal concentration of SCFAs could have resulted from

increased microbial production, decreased absorption from the mucosa, or a change in the rate of transit time, all of which were not controlled for in the study.[47]

Nonbacterial species also play a role in the energy extraction from ingested food. In a murine model, Turnbaugh et al. showed that certain methanogenic archaea, for example, *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii*, increased the efficiency of intestinal bacterial fermentation by binding hydrogen, an end product of fermentation, thereby shifting the system toward further metabolism. In mice, co-colonization with *M. smithii* and *Bacteroidetes* increased efficiency and specificity of bacterial polysaccharide fermentation, which lead to more adiposity than when colonization was with either of these organisms alone.[60] These findings suggest that there are organisms other than bacteria in the GI tract that are involved in calorie extraction/absorption; this area of interest requires further investigation.

Other variables that must be considered when trying to understand the association between intestinal microbiota and obesity, in addition to the composition of the diet, are length of fasting periods, regularity and intensity of exercise, and age. It has been shown that mice fasting for 24 hours have a greater proportion of *Bacteroidetes* in their ceca than nonfasted mice eating at regular intervals,[61] despite equivalent percentages of body fat in both groups. In the same experiment, fasted mice were shown to use ketone bodies from lipolysis for energy rather than glucose from glycolysis, which was the main energy source in nonfasted mice; thus explaining the decreased adiposity in the fasted mice. High-endurance exercise in murine models (30 min of swimming) caused different amounts of muscle mass acquisition between germ-free and colonized mice, again showing the complicated association between the intestinal microbiota and weight.[62] As an individual ages, there is a significant decrease in the number of Bifidobacterial species (viewed as protective against weight gain) and increase in species detrimental to one's health, such as *Enterobacteria* and *Clostridia*.[62,63] It also has been shown that the ratio of *Firmicutes* to *Bacteroidetes* in the human intestinal microbiota increases with age, and therefore, must be taken into account when considering the role of weight change in the elderly.[64] Obesity appears to be a complex biological variable that must be looked at as reflecting much more than just the phyla of bacteria present in the GI tract of the host. A multitude of interactions among varied living microorganisms, bacteria, archea, and fungi must be also be considered.

Inflammation and Obesity

Chronic low-grade inflammation has been recognized to accompany both obesity and the metabolic syndrome.[65,66] It has been shown that the adipose tissue of genetic and diet-induced obese mice over expresses proinflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin-1, and interleukin-6,[67] which results in insulin resistance and is typical of the chronic low-grade systemic inflammation that may accompany obesity.[68] The mechanism by which TNF-α causes insulin resistance is by phosphorylation of a serine on insulin receptor substrate-1,[69] thereby rendering it inactive. The subsequent insulin resistance results in hyperinsulinemia, excessive adipose tissue, and hepatic lipid storage.

Mice with deleted lipopolysaccharide (LPS) receptors and CD14 mutants (CD14 is a coreceptor for toll-like receptor-4, which recognizes LPS) show hypersensitivity to insulin and delayed occurrence of obesity, diabetes, and insulin resistance when exposed to a high-fat diet. Therefore, one can conclude that high-fat diet-induced metabolic derangement occurs through the LPS/CD14 system.[70]

Although there is a proven relationship between obesity and inflammation, here the association of the intestinal microbiota with this inflammatory reaction is further explored. The intestinal microbiota has been associated with obesity through LPS (part of the cell wall of Gram-negative bacteria), which is hypothesized to be a trigger of inflammation. When mice were fed diets high in fat for 4 weeks, there was an increase in the concentration of circulating plasma LPS, a condition known as metabolic endotoxemia. The mechanism by which enteric LPS is absorbed remains unclear, but may be related to an increased filtration of LPS. An alternate hypothesis is that chylomicrons, which are formed from dietary triglycerides and strongly bind LPS, move LPS from the intestinal cell into the circulation, thereby causing or contributing to a chronic diet-induced inflammatory response.[69] LPS is produced within the intestine by the death of Gram-negative bacteria,[71] absorbed through intestinal capillaries,[72] and then transported in lipoproteins through the mesenteric lymphatic system.[73] Production of chylomicrons also promotes TNF-α mRNA production. Vreugdenhil et al hypothesized that chylomicrons bind LPS with its associated LPS-binding protein, which prevents cell activation acting as a local defense mechanism.[74] Further work is required to define the exact relationship between chylomicrons, LPS, and inflammation.

To further elucidate the relationship between LPS and obesity, LPS was injected subcutaneously, causing insulin resistance and weight gain in mice in the absence of a change in energy intake.[70] Similar to the study involving the CD14 mutants, insulin resistance and diet-induced obesity are not seen with subcutaneous LPS in mice that lack the toll-like receptor 4, which recognizes LPS.[75] The exact mechanism by which LPS induces inflammation is unknown, and continues to be investigated.

High-fat diets have been shown to increase circulating plasma concentrations of LPS and therefore lead to inflammation.[73] High-fat diets also have been shown to alter the composition of the intestinal microbiota, notably decreasing the number of the Gram-positive *Bifidobacteria* bacteria. Elevated levels of *Bifidobacteria* are associated with decreased intestinal leakiness, which has been suggested to allow less LPS to enter the host's serum.[74] Therefore, it is hypothesized that high-fat diets increase LPS absorption by modulating the intestinal bacteria that cause intestinal leakiness and inflammation. Such floral modulation is associated with a significant increase in fat mass, liver hepatic triglyceride accumulation, body weight gain, insulin resistance, and diabetes.[36] In order to evaluate the hypothesis that the intestinal microbiota was responsible for the aforementioned effects, Cani et al[76] treated mice with nonabsorbable antibiotics, and noted reduced plasma LPS levels, decreased intestinal permeability, and lowering of the inflammation in visceral adipose tissue. There was also decreased body weight gain and glucose tolerance in these antibiotic-treated mice. Therefore, one can conclude that an intestine in which microbial colonization is greatly reduced, exhibits decreased LPS absorption and intestinal leakiness, and disallows inflammation and weight gain. The relationship among diet, inflammation, and bacteria needs to be further explored, although the above mentioned cause and effect hypothesis remains intriguing.

Regulation of Host Genes is Responsible for How Energy is Expended and Stored

Although early on, it was believed that the intestinal microbiota increased energy extraction solely by more complete reclamation of energy from undigested dietary compounds, the relationship proved to be more complex. Another link between the microbiota and obesity is seen in the regulation of fatty acid metabolism. One mechanism whereby host lipid metabolism is regulated by the intestinal microbiota involves Fiaf, also known as angiopoietin-like protein 4, which regulates fatty acid oxidation in adipose and muscle tissue.[77,78] Fiaf is produced in white and brown fat, the liver, and intestine,[26] and inhibits lipoprotein lipase (LPL), which is involved in regulating fatty acid release from lipoproteins in fat, muscle, and the heart. When adipocyte LPL activity is increased, there is increased fatty acid uptake into cells, and accumulation of triglycerides in adipocytes.[79] Fasting reduces LPL activity, whereas feeding increases LPL activity through posttranscriptional regulation.

In murine models, the intestinal microbiota increased LPL activity by 122% in adipocytes and by 99% in the heart.[80] Intestinal microbes, most notably obligate anaerobes (eg, *Bacteroides*), suppressed the production of Fiaf, thereby increasing fatty acid release from lipoprotein-associated triacylglycerols, and increasing fatty acid uptake into adipose tissue and muscle.[22] When germ-free mice had bacteria introduced into their GI tract, Fiaf expression in the small intestine was suppressed, whereas Fiaf expression in white fat and the liver remained unchanged. The investigators of this study hypothesized that the microbiota stimulated hepatic triglyceride production through transcription factors that suppressed intestinal epithelial genes responsible for a circulating LPL inhibitor.[81] To test this hypothesis, germ-free Fiaf knockout mice were produced and found to have 67% higher fat pad LPL activity than their germ-free littermates with a functional Fiaf gene, proving that Fiaf inhibits LPL in vivo. Mice with functional variants of the Fiaf gene were found to have comparatively low levels of triglycerides in serum.[82] The role of Fiaf in the regulation of lipid metabolism in humans and its relationship to the intestinal microbes are subjects that require more detailed investigation but its role in animal fat metabolism is beginning to be far better appreciated.

There are two identified independent mechanisms why germ-free mice are not susceptible to diet-induced obesity, both of which involve increased fatty acid metabolism. The first mechanism is an increased production of the PPAR-γ coactivator, which is triggered by increased levels of Fiaf. The PPAR-γ coactivator is known to up-regulate the expression of mitochondrial fatty acid oxidation as discussed in the paragraph before. Germ-free mice have high levels of Fiaf and thus increased levels of PPAR-γ.

The second mechanism involves increased activity of adenosine monophosphate-activated protein kinase (AMPK), which is an enzyme involved in cellular energy status.[25] When AMPK activity is reduced (seen in mice with intestinal microbiota, ie, not germ-free), downstream targets have decreased phosphorylation, which leads to decreased mitochondrial fatty acid oxidation; this decrease is attributed to unphosphorylated acetyl-CoA carboxylase, which allows the enzyme to stay activated, thus leading to increased malonyl-CoA levels, and thereby inhibiting the entry of long-chain fatty acyl-CoA into the mitochondria for oxidation.[83] The presence of an intestinal microbiome suppresses the AMPK-dependent fatty acid oxidation in the liver and skeletal muscle, resulting in diet-induced obesity and diabetes.[84]

Hormonal Communication Between the Intestine and Host

Another way in which energy intake and expenditure are regulated is through endocrine signaling from the intestine to the brain. With nutrient intake, enteroendocrine cells in the intestine secrete hormones such as glucagon-like peptide 1 and 2 (GLP-1 and GLP-2). GLP-1 has been shown to slow gastric emptying, stimulate insulin release from the pancreas, and promote weight loss and satiety, whereas, GLP-2 reduces intestinal permeability and enhances intestinal glucose transport.[81,85]

As mentioned in the section about calorie extraction, the obesity-associated intestinal microbiome produces more SCFAs from carbohydrate fermentation than does that of nonobese controls.[25,86] Yet another proposed mechanism by which the intestinal microbiota communicates with the host is by G-protein coupled 41 and 43 (GP-41 and GP-43) receptors, which bind SCFAs.[87] GP-41 is broadly expressed in adipocytes, distal small intestine, and colon. When GP-41 is bound by SCFAs, it stimulates leptin expression and Peptide YY production. These 2 products have multiple effects including satiety and increased energy metabolism.[88] To determine the function of the GP-41 receptor, a study was conducted comparing wild-type, germ-free, and GP-41-deficient mice colonized with or lacking a microbial community possessing fermentative properties (*Bacteriodes thetaiotamicron* and *M. Smithii*).[86] GP-41 mRNA is highly expressed in enteroendocrine cells of small intestinal, colonic, and adipocyte epithelium, which relay information about nutrients and metabolic activity in the intestine to the host by peptide hormones that are unique to their location along the GI tract, and are secreted into portal and systemic circulation.[89] The study revealed that the level of microbial colonization in the intestine of all GP-41 knockout mice was the same as that of the wild-type and germ-free mice, suggesting that any difference in the 2 models was because of the GP-41 receptor and not the intestinal microbiome. The results of the study showed that the GP-41 knockout mice colonized with *M. Smithii* and *B. thetaiotamicron* gained significantly less weight than the colonized controls (wild type and germ free).

It was also found that colonized mice had increased levels of the hormone PYY, a hormone derived from the enteroendocrine cell that normally decreases intestinal transit rate and inhibits intestinal motility, increases recovery of SCFA from the diet, and increases fat accumulation in fat pads of mice.[86] When GP-41 signaling was absent (GP-41$^{-/-}$ mice), there were reduced levels of PYY in the plasma and increased intestinal motility, which led to decreased energy harvest from the diet.[90] The results indicated that GP-41-deficient mice have decreased intestinal absorption of SCFAs and reduced delivery to the liver, which decreases hepatic lipogenesis. The exact relationship between the intestinal microbiome and hormones is uncertain and requires further study, but it represents another mechanism by which the intestinal microbiota can communicate with the host, specifically in regard to weight.

CONCLUSION/FUTURE WORK

Although there are many causative and contributing factors to obesity, the intestinal microbiome is beginning to be recognized as one of major importance. The role of the

microbiome is certainly more complex than just ratios of bacteria, that is, *Firmicutes* to *Bacteroidetes*, as initially hypothesized.[45] Inflammation, gene, and hormonal regulation have been shown to be involved in the microbiome's effects on weight; however, exact mechanisms are only beginning to be defined.

It should be noted that Turnbaugh et al[46] reported in mice studies that adiposity is transmissible by fecal microbiotic transplant, which could have significant therapeutic implications in treating obesity once more information is known about the microbiome. Currently, when an individual fails to lose weight with diet, exercise, and psychiatric counseling, the only other option is surgery, such as restrictive or malabsorptive bariatric procedures. As the mechanisms of the microbiota's role in weight regulation are elucidated, one can envision transplanting intestinal contents from a thin individual into an obese individual, thereby altering the ratio of bacterial species, changing the way in which energy is expended and stored, and affecting hormonal regulation in the recipient. Such a novel approach to weight management is exciting and likely to be investigated, thereby contributing to our understanding of bacteria's role in obesity.

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