The Microbiome and Obesity: Is Obesity Linked to Our Gut Flora?

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The human gut is a lush microbial ecosystem containing about 100 trillion microorganisms, whose collective genome, the microbiome, contains 100-fold more genes than the entire human genome. The symbiosis of our extended genome plays a role in host homeostasis and energy extraction from diet. In this article, we summarize some of the studies that have advanced the understanding of the microbiome and its effects on metabolism, obesity, and health. Metagenomic studies demonstrated that certain mixes of gut microbiota may protect or predispose the host to obesity. Furthermore, microbiota transplantation studies in germ-free murine models showed that the efficient energy extraction traits of obese-type gut flora are transmissible. The proposed methods by which the microbiome may contribute to obesity include increasing dietary energy harvest, promoting fat deposition, and triggering systemic inflammation. Future treatments for obesity may involve modulation of gut microbiota using probiotics or prebiotics.

Introduction

The human body harbors a large contingent of microorganisms, but nowhere is there more abundant and intricate host-microbial interaction than in the colon. There are about 100 trillion microorganisms in the human colon, most of which are bacterial species [1]. There are 10^{11-14} microbes per deciliter, which makes it the most biodense niche known [2•]. Genetic material from these bacteria represents 100-fold more genes than the entire human genome [3••]. We are just beginning to unravel the complex relationships between the human host and our colonic petri dishes. Recent literature has focused on the link between our metabolism and the gut flora. In this article, we summarize some of the studies that have advanced the understanding of the microbiome and its effects on metabolism, obesity, and health.

The prevalence of obesity among US adults has more than doubled since 1980 [4]. Currently, 65% of the adult population are overweight and 32% are obese [4,5]. Furthermore, the prevalence of obesity among children has more than tripled since 1980, suggesting that this problem continues to worsen [4]. Obesity is associated with medical conditions including type 2 diabetes, cardiovascular disease, obstructive sleep apnea, and multiple malignancies. A strong correlation exists between obesity and increased mortality risk [6]. The enormous costs of obesity and its comorbidities to our already overburdened health care system are alarming.

Multiple factors drive the obesity epidemic, including genetic and environmental contributions such as increased food availability, high-fat diet, widespread use of high-fructose corn syrup, and physical inactivity [7]. For each individual, weight is determined by poorly defined interactions among genetic predisposition and social, dietary, behavioral, and environmental factors. Epidemiologic evidence suggests that the constant increase in obesity cannot be fully accounted for by genetics, food availability, and behavioral changes alone [8]. There is increasing evidence that our gut microflora plays a critical role in energy balance and metabolism, implicating it as a major factor in the development of obesity.

We have only recently begun to appreciate the importance of the symbiotic relationship with our microbial inhabitants. These consist mostly of anaerobic bacteria, but also archaeal spp, yeasts, and parasites, collectively known as microbiota [1]. Although the upper gastrointestinal tract is sparsely populated because of the luminal medium and propulsive forces (10^{2}–10^{4}), the human colon has the highest density of any known natural bacterial ecosystem (10^{11}–10^{14}), harboring at least 1000 and potentially as many as 36,000 species [2•,9]. This impressive bacterial load is equal in mass to a single kidney and as metabolically active as the liver [10]. Indeed, 90% of the cells in our body are microbial, such that we may be viewed as passengers in our mobile colonic petri dishes.

The Microbiome

The collective genome of the microbiota (known as the microbiome) contains 100-fold more genes than the entire human genome [3••]. Our microbiota have evolved with us, and their genes provide traits that we did not need to evolve on our own. The symbiosis with our extended genome of millions of microbial genes plays a role in metabolism, immune function, and gene expression [11]. The complex composite of human and microbiome has been described as a biologic "super organism" [12].

The microbiome endows the host with diverse and powerful health benefits. The vast array of enzymatic reactions, often distinct but essential to those encoded by the human genome, plays a role in host homeostasis, metabolism, synthesis of micronutrients, detoxification, epithelial development, and immune function [13]. One of the key activities of microbiota is efficient extraction of calories from ingested food (energy harvest), particularly through fermentation of otherwise indigestible polysaccharides and provision of short-chain fatty acids. The net effect may increase the caloric extraction from our diet by more than 100 kcal/d [10]. The microbiota are also involved in the production of vitamin K, multiple B vitamins, H_2, CO_2, methane gas, lysine, and the conversion of urea to ammonia [14]. It also metabolizes ingested foreign compounds (xenobiotics) and modulates the enterohepatic circulation of compounds detoxified by the liver and excreted in the bile [11]. The microbiome stimulates the growth of enterocytes and aids in the development of the immune system [15]. Commensal organisms protect the host from pathogens such as *Clostridia* by producing bacteriocins, blocking adhesion of pathogens, modulating the immune response, and stimulating the production of secretory IgA [11].

How the microbiome not only affects the health of the host but also influences disease states is not understood. Small-intestinal bacterial overgrowth has long been studied as a potential etiology of malabsorption, dyspepsia, rosacea, and irritable bowel syndrome [16,17]. More recently, the microbiome was implicated in the pathophysiology of localized intestinal diseases such as inflammatory bowel disease, irritable bowel syndrome, infectious diarrhea, and colon cancer, as well as systemic conditions such as type 2 diabetes and obesity [18•,19•].

Metagenomics: Who's There?

Until recently, our ability to study human microbiota was limited by reliance on selective culturing techniques that failed to detect 70% of microorganisms [20]. Development of molecular techniques for genetic analysis of commensal bacteria has revolutionized the study of the microbiome [15]. Advances in metagenomics promises to more reliably identify and characterize the composition of the microbiome and expand our understanding of the complex interaction between the host and microbiota function [11]. The 16S ribosomal RNA (rRNA) gene is found in all microorganisms. After extraction from fecal or mucosal samples and amplification using polymerase chain reaction (PCR), it serves as an ideal reference for metagenomic analysis of microbiota [20]. It contains nucleotide base sequences that are highly conserved in all bacteria, allowing accurate alignment but also enough variable regions to allow specific classification by species or strain [21]. Metagenomic techniques coupled with rapidly advancing microarray technology have propelled medical microbiology into a new era, allowing us to study the microbiome in ways that were previously impossible [22]. The US National Institutes of Health has invested $115 million in the Human Microbiome Project, with the goal of sequencing genomes from the many representatives of the human gut flora [3••].

The first comprehensive molecular survey of microbiota was performed by Ekberg et al. [23] in 2005, obtaining 13,335 16S rRNA sequences from fecal and mucosal biopsy samples of three healthy adult humans. Despite finding significantly greater microbial diversity than previously appreciated, the great majority were anaerobic bacteria belonging to two (of > 70 identified) divisions: the *Bacteroidetes* (48%) and *Firmicutes* (51%). The *Bacteroidetes* spp, in particular *Bacteroides thetaiotaomicron*, hydrolyzes otherwise indigestible polysaccharides and accounts for 10% to 15% of caloric requirement in humans [24]. Human colonocytes derive 50% to 70% of their energy from butyrate, which is derived from complex carbohydrates metabolized by *Firmicutes* spp via fermentation [25]. Other bacterial phylotypes identified in the human gut include *Bifidobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Fusobacteria*, *Cyanobacteria*, and *Spirochaetes* spp. Among nonbacterial microorganisms, *Methanobrevibacter smithii*, a hydrogen-oxidizing methanogen, dominated the *Archaea* domain.

Despite the consistent predominance of *Bacteroidetes* and *Firmicutes* at the division level, the makeup of human microbiota at the species and strain levels is as individual as a fingerprint [11]. Molecular studies showed that humans may share as little as 1% of the identical species [23]. Although there is also spatial variation within the same colon, the differences between individuals is greater than differences between various sample sites within the same colon [26]. The composition of bacterial species may be subject to transient changes; however, serial stool collection studies showed that the unique composition of an individual's microbiota remains remarkably stable over time [27]. This stability is thought to be in part due to the selective recognition and tolerance of the commensal microorganisms by the host immune system [28]. It is unclear how such diversity between individuals and temporal stability of individual composition is maintained.

Developmental Microbiology

We have begun to understand how our microbial inhabitants "got there" through studies of the developing

intestinal microbiota in infants and children. Evidence suggests that the composition of gut microbiota is established during the first year of life [29•], and incidental environmental exposure plays a key role in determining the eventual composition that persists through adulthood [8]. In utero, the fetal intestinal tract is sterile. The initial exposure is determined by the type of delivery. Vaginal delivery exposes the infant to maternal bacteria, whereas cesarean section exposes the infant to a different set of isolates, including more *Clostridia* spp [15]. In addition, breastfeeding exposes infants to flora composed predominantly of *Bifidobacteria* (90%), whereas bottle feeding is associated with a more diverse bacterial population, including *Bacteroidetes* and *Clostridia* spp [30]. Introduction of solid food marks the transformation to adult-type flora [31].

After a dynamic first year, the established adult-type microbiota remains remarkably constant through life. The microbiota are susceptible to transient changes and are influenced by dietary changes, medications including antibiotics, infectious pathogens, and intestinal surgery [15]. Elderly individuals experience losses in the number and diversity of *Bifidobacteria* and *Lactobacillus* and corresponding increases in *Bacteroidetes* and *Clostridia* bacteria [27]. We have yet to understand how the modern global shifts in lifestyle, diet, and other environmental factors influence the internal evolution of human microbiota and whether this has an impact on health and susceptibility to conditions such as obesity [3••].

The Microbiome and Energy Harvest

Our ability to study the effects of gut microbiota was greatly enhanced by experiments involving germ-free (gnotobiotic) animals using metagenomic techniques. Mice provide a suitable model for experimentation, because their colon microbiota have a distribution similar to humans, with a predominance of *Firmicutes* (60%–80%) and *Bacteroidetes* (20%–40%) [26]. The experimental colonization of germ-free mice, either wild-type or genetically engineered, with simplified microbial communities provided a powerful means of studying the properties and effects of microbiota while controlling variables such as host genotype, microbial diversity, and environmental factors (eg, diet) [3••].

A landmark study by Backhed et al. [32] found that germ-free mice had 42% less total body fat than their normal counterparts, even though they consumed 29% more food. Furthermore, transplantation of cecal microbiota from normal mice into the germ-free mice (a process known as conventionalization) resulted in a 57% increase in total body fat and insulin resistance without any increase in food consumption or changes in energy expenditure. These findings suggest that the presence of gut microbiota significantly increases the energy harvest from diet and promotes fat deposition.

This was supported by another study from the same group [33••], which found that the absence of gut microbiota protected against weight gain in mice consuming a Western-style, high-calorie diet (41% fat, 41% carbohydrate). After 8 weeks, germ-free mice gained significantly less weight than their conventionalized counterparts (2.1 ± 0.5 g vs 5.3 ± 0.8 g; $P < 0.05$). Interestingly, when assessing activity by monitoring gastrocnemius muscle activity, mice with normal gut flora had diminished locomotor activity compared with germ-free mice.

There is increasing evidence that not only the presence but also the relative proportions of the microbial divisions correlate with obesity. This was demonstrated by Ley et al. [26], who compared genetically obese leptin-deficient (*ob/ob*) mice and their lean (*ob/+* and *+/+*) siblings and *ob/+* mothers. The obese mice had 50% fewer *Bacteroidetes* and correspondingly more *Firmicutes* than their lean littermates ($P < 0.01$). These changes in the relative proportions of *Bacteroidetes* and *Firmicutes* were division wide and were not due to blooms or extinctions of a few species. Furthermore, a single runted *ob/ob* animal that consumed less food and had body mass similar to lean siblings still had relative proportions of *Firmicutes* (71%) and *Bacteroidetes* (26%) similar to obese *ob/ob* siblings, suggesting that the microbial shifts cannot be explained simply by differences in food consumption or total body mass.

Using the same murine model, Turnbaugh et al. [34••] assessed the effects of obese-type microbiota by transplanting the gut microbiota from either obese *ob/ob* mice or lean (*ob/+* or *+/+*) mice into lean wild-type, germ-free mice. After 2 weeks, the recipients of the obese-type microbiota had significantly greater dietary caloric extraction and fat gain compared with recipients of lean-type microbiota (47% ± 8.3% vs 27% ± 3.6% fat gain), despite no differences in food consumption and energy expenditure. This well-designed study demonstrates that the relative abundance of microbiota predisposes to obesity, and the efficient energy extraction traits of obese-type microbiota are transmissible. This not only further supports the role of microbiota in obesity but also raises the possibility of gut microbiota manipulation as a strategy for regulating energy balance in obese individuals.

In a small human study, Ley et al. [35••] examined the microbial profiles of 12 obese individuals. They found that obese individuals had fewer *Bacteroidetes* and more *Firmicutes* compared with lean controls. They were then randomly assigned to either a fat-restricted (FAT-R) or carbohydrate-restricted (CARB-R) low-calorie diet. Their gut microbiota was monitored over the course of 1 year by sequencing 16S rRNA genes from serial stool samples. On either diet, the relative proportion of *Bacteroidetes* increased with a corresponding decrease in *Firmicutes*. This shift away from *Firmicutes* to *Bacteroidetes* correlated with percentage weight loss as opposed to changes in caloric intake over time. Alterations in gut microbiota were only seen after 6% loss of body weight on the FAT-R

diet and 2% of the CARB-R diet. Similar to animal studies, the shifts were division wide and not due to blooms or extinctions of specific bacterial species. This study supports the potential for dietary modulation to manipulate gut microbiota in humans, which may consequently impact host metabolism.

In a recent study, Kalliomaki et al. [36•] analyzed stool samples collected from children at 6 and 12 months of age. The children were then followed until 7 years of age to determine whether early gut microbiota composition predicted weight development later in childhood. The children who were overweight or obese at age 7 (n = 25) had fewer *Bifidobacteria* (P = 0.02) and more *Staphylococcus aureus* (P = 0.013) at 6 and 12 months of age than children who were normal weight (n = 24). This implies that differences in the composition of gut microbiota may precede the development of obesity.

The invariable co-dominance of the *Bacteroidetes* and *Firmicutes* implies that there is minimal competition for resources between these two divisions, either through cooperation or specialization. It is unclear what factors in the setting of obesity tip the scales in favor of the *Firmicutes* over *Bacteroidetes*. Perhaps the *Firmicutes* possess more diverse enzymatic capabilities that more efficiently extract energy when a variety of complex organic matter is available. The observation that changes in the proportions of microbiota were division wide suggests that the factors driving these broad shifts across all species must depend on highly conserved bacterial traits [35••]. This emphasizes that the metabolic and energy extraction functions are fundamental to microbiota, such that all are affected by alterations in nutritional state.

How Could the Microbiome Cause Obesity?

However, this is a two-way street: just as host conditions influence microbiota, the presence and properties of microbiota have profound effects on host health and susceptibility to obesity. Proposed mechanisms by which the microbiome may contribute to the development of obesity include 1) increasing dietary energy harvest, 2) promoting fat deposition, 3) triggering systemic inflammation, 4) perhaps modifying locomotor activity, 5) and having central effects on satiety. One of the highly conserved traits of dominant microbiota is the capacity to metabolize otherwise indigestible dietary polysaccharides. We have seen that germ-free mice, in the absence of microbiota, gained less body fat despite more food consumption, until they received normal microbiota and gained efficient energy harvest and dramatic increases in body fat [32]. When germ-free mice were transplanted with the microbiota of either their obese or lean littermates, the recipients of obese-type microbiota had more end products of fermentation (acetate and butyrate) and fewer calories remaining in their stool, indicating more efficient dietary caloric extraction by obese-type microbiota [34••].

The presence of other microorganisms may indirectly influence energy harvest, such as *M. smithii*, which is the most common archaeal spp, accounting for up to 10% of all anaerobes in the colon [13]. Fermentation of starches by microbiota such as *Firmicutes* provide acetate and butyrate, key energy sources for colon epithelial cells. However, digestion of starch produces hydrogen buildup, inhibiting further digestion. *M. smithii* is capable of converting hydrogen into methane (methanogenesis), thereby allowing further and more efficient production of butyrate [37]. Samuel and Gordin [38] found that colonization of germ-free mice with *M. smithii* and *B. thetaiotaomicron* increased the efficiency of energy extraction and body fat gain more than colonization with either microorganism alone. This illustrates just one of undoubtedly many complex metabolic interactions among microorganisms which influence host energy balance. This also highlights *M. smithii* as an intriguing therapeutic target for energy harvest reduction in obese individuals [13].

Microbiota may also regulate host genes that promote deposition of absorbed fat into adipocytes. Fasting-induced adipocyte factor (Fiaf) decreases fat storage by inhibiting lipoprotein lipase, while promoting release of fatty acids by inducing peroxisomal proliferator-activated receptor coactivator (Pgc-1a) [32]. Gut microbiota was shown to suppress intestinal Fiaf, resulting in increased storage of calories as fat. In contrast, lean germ-free mice had elevated levels of Fiaf with reduced body fat deposition, even when fed a high-fat, high-sugar diet [33••]. Therefore, the gut microbiota can influence both sides of the energy equation, modulating the efficiency of energy harvest (input) and energy storage or expenditure (output).

Growing evidence has linked low-grade chronic systemic inflammation with obesity and insulin resistance [39]. Bacterial lipopolysaccharide (LPS) derived from gut microbiota acts as a trigger for systemic inflammation through binding with CD14 receptors [13]. Cani et al. [19•] found that mice fed a 4-week, high-fat diet had plasma LPS levels two to three times higher than normal, and theorized that chronic metabolic endotoxemia results in obesity and insulin sensitivity. Antibiotics previously were shown to reduce LPS and hepatic steatosis [40]. Similar metabolic profiles were found in mutant mice lacking CD14, linking the microbiome with systemic inflammation through the LPS/CD14 pathway [19•].

Antibiotics, Probiotics, and Prebiotics

By gaining a better understanding of the microbiome as an integral part of our physiology, we may eventually unlock its potential as a diagnostic marker for health or predisposition to diseases. We are also only beginning to define the factors needed to implement an effective strategy for manipulating the human microbiome and optimizing its performance to promote host health [3••]. Therapeutic interventions including antibiotics, probiotics, and prebi-

otics may offer novel treatments for a variety of diseases including obesity, diabetes, and fatty liver disease [41•]. Probiotics are nonpathogenic, living microorganisms that confer health benefits when ingested. Commonly used species include *Bifidobacteria*, *Lactobacillus*, and *Saccharomyces*. Prebiotics are nondigestible dietary substances that stimulate the growth and activity of beneficial commensal microorganisms. Examples include fructo-oligosaccharides, lactulose, and lactosucrose. Symbiotics are combinations of probiotics and prebiotics.

Human intervention to promote weight gain in farm animals by modification of gut flora has been widely used for more than 50 years [42]. Antibiotics and probiotics, such as *Lactobacillus* and *Bifidobacterium*, were used in cattle as growth promoters. It was suggested that the indiscriminant use of antibiotics and probiotics in cattle may be inadvertently contributing to the obesity epidemic [8]. A major prebiotic was introduced into the US diet in the 1950s in the form of high-fructose corn syrup. Has this unintentional prebiotic exposure led to a drift toward *Firmicutes*-predominant obesity-type microbiomes? With new metagenomic approaches, perhaps a better understanding of the microbial effects of these therapeutic agents can help us avoid exacerbating the obesity epidemic and perhaps attain the desired effect of weight control.

Although probiotics have shown efficacy in pouchitis, there is limited literature regarding the effects of probiotics on obesity and metabolism in humans [43]. Lee et al. [44] investigated the antiobesity effect of *Lactobacillus rhamnosus* PL60 on diet-induced obese mice. *L. rhamnosus* is a human-derived bacterial species that produces conjugated linoleic acid, which was shown to reduce body fat in animal studies [45]. After 8 weeks, obese mice receiving *L. rhamnosus* had reductions in body weight and white adipose tissue despite no changes in energy intake. However, the antiobesity effect observed was due to apoptosis rather than reduction of adipocyte size; therefore, the clinical applicability of these findings is uncertain because human obesity is primarily driven by changes in adipocyte size [13]. Futhermore, a study of 101 human subjects randomly assigned to conjugated linoleic acid or placebo for 1 year found no differences in maintaining body fat or weight loss [46]. In a study by Martin et al. [47•], germ-free mice were conventionalized with human baby microbiota and fed daily *Lactobacillus paracasei*, *L. rhamnosus*, or placebo. Compared with placebo, probiotics altered hepatic lipid metabolism, decreased plasma lipoprotein levels, and stimulated glycolysis.

Our understanding of prebiotic effects on obesity is limited as well. A single-blinded, crossover study by Cani et al. [48] found that a 2-week treatment with oligofructose in 10 healthy nonobese humans increased satiety after breakfast and dinner and reduced food consumption after dinner, leading to a total daily energy intake that was 5% lower than placebo. In a more recent study by Cani et al. [49•], mice fed a high-fat diet were treated with oligofructose. Prebiotic exposure restored normal levels of *Bifidobacterium* spp and reduced the low-grade systemic inflammation thought to be associated with obesity and glucose intolerance. Although these early findings are promising, it is unlikely that any one treatment can alter the gut flora to "cure" obesity. However, there is clearly potential for probiotics, prebiotics, or symbiotics to influence risk of obesity through reduction of energy harvest, endotoxemia, fat deposition, and promotion of satiety and energy expenditure.

Future Directions

Many questions remain about the role of our microbial inhabitants on health and obesity, and current evidence is mostly based on in vitro and animal studies. The environmental, dietary, and host factors responsible for the composition of gut flora must be further explored. Are differences in gut microbiota between lean and obese humans the cause or result of obesity, or both? What microbial properties or host conditions cause shifts in the relative abundance of *Bacteroidetes* and *Firmicutes* during changes in weight? Must a shift occur in our gut microflora to a lean-type composition before we can lose weight? Do the small reductions in energy extraction caused by shifts in gut microbiota result in clinically significant weight changes over years or a lifetime? Understanding the complex transgenomic metabolic interactions between gut microbiota with the host and other microbial species perhaps poses the ultimate challenge in deciphering and learning how to optimize our microbiome.

Clearly, there are still more questions than answers. However, the potential implications of this exciting and rapidly advancing field are staggering. Future treatments for obesity may be possible through the modulation of gut microbiota using antibiotics, probiotics, prebiotics, and possibly even microbiota transplants. A better understanding of dietary effects on the microbiome may allow individualized nutritional recommendations and guide food production and distribution. The microbiome may change the future of health care, providing new diagnostic biomarkers of health and new pharmacologic agents derived from members of the human microbiota or their chemical products. The ability to accurately profile each individual's microbiome may open the possibility of personalized medical treatments and prevention strategies [50].

Conclusions

The human gut microbiome is a complex ecosystem that has evolved with us and interacts with all of our daily functions. In this article, we discussed the new science that suggests the microbiome is critical to our metabolism and weight. By increasing or decreasing our energy harvest from ingested food, our microbiota provide or limit calories. Despite changes in diet, certain mixes of microbiota may protect us from excessive weight gain. The microbiome may also exert effects on fat storage and systemic inflammatory

states. Aided by advances in metagenomics and metabolonomics, we are beginning to comprehend the wide-reaching effects of our microbiome and grasp the potential for optimizing its beneficial functions. Is there a "holy grail" of gut flora that allows one to eat large amounts of calories and stay lean? The answer is undoubtedly negative, but there is no question that wielding a greater understanding of our microbial inhabitants has much to offer us, including diagnostic biomarkers for host health and novel therapeutic strategies for obesity management.

Disclosure

No potential conflicts of interest relevant to this article were reported.

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