Impact of the Gut Microbiota on the Development of Obesity: Current Concepts

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Energy balance is an equilibrium between the amount of energy extracted from the diet and the amount expended. Selective pressures throughout evolution have programmed animals to protect energy stores through the accumulation of adipose tissue; as diets have changed and energy-dense foods have become readily available, obesity rather than malnutrition has become the primary concern in developed nations. Nevertheless, factors other than the types of food and their availability appear to be important. Recent evidence suggests that the gut microbiota play a role in energy harvest, storage, and expenditure. The preponderance of the evidence demonstrates that germ-free mice are protected against obesity and that the transfer of gut microbes from conventionally raised animals results in dramatic increases in body fat content and insulin resistance. Moreover, the composition of the gut microbiota has been shown to differ in lean and obese humans and animals and to change rapidly in response to dietary factors. The gut microbiota may also influence the development of conditions characterized by low-level inflammation, such as obesity and type 2 diabetes, through systemic exposure to bacterial lipopolysaccharide derived from the intestinal microbiota. Together, these data suggest that modification of the gut microbiota may be a relevant therapeutic avenue for obesity and other metabolic disorders.

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INTRODUCTION

The intestinal microbiota have coevolved with the human host to perform a number of functions that affect the host's physiology and metabolism. Indeed, the host and its microbiota have mutually beneficial and cooperative interactions. In particular, the metabolic activity of the human gut microbes has been suggested to function as an auxiliary, virtual organ (1,2). In the past decades, interest in the metabolic role of the commensal gut microbes in humans focused on their potential to ferment indigestible nutrients, produce micronutrients, and reduce harmful toxins; however, it has become increasingly appreciated that host/microbe interactions help balance host vital functions and participate in health maintenance.

Over the past 25 years, the prevalence of obesity in the United States has increased by >75%. At present, nearly two-thirds of the US population is overweight and 1 in 3 adults are clinically obese (3). Obesity has serious health consequences, including increased risk for type 2 diabetes, cardiovascular disease, pulmonary hypertension, sleep apnea, and a number of cancers, and is strongly linked to an increased risk for mortality (3,4). In part, this epidemic is likely to be related to physiologic biases "programmed" into humans by selective pressures during the evolutionary process; hence, although the availability and stability of the food supply has improved over the past several centuries, humans remain physiologically predisposed to protect energy stores through the accumulation of adipose tissue. Complex systems clearly have developed over time to regulate energy balance, and recent research has implicated the gut microbiota as a critical determinant of nutrient uptake, energy regulation, and ultimately, weight and metabolic disorders.

This review will examine the role of the gut microbiota in energy harvest and fat storage, explore differences in the microbiota in obese and lean individuals, and evaluate potential mechanisms for modulating the gut microbiota to influence metabolic parameters in humans.

Gut microbiota and obesity

Gut microbes affect energy harvest and storage. Studies conducted in germ-free mice support a role for the gut microbiota in the harvest and storage of energy derived from ingested nutrients. Backhed *et al.* (5) demonstrated that the introduction of gut microbiota from conventionally raised mice into germ-free mice (i.e., "conventionalization") resulted in a 60% increase in body fat and

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insulin resistance within 2 weeks despite reduced chow consumption (by 29%) and increased activity (by 27%) compared with germ-free mice. It was subsequently shown that the transfer of the microbiota harvested from the intestine of conventionally raised, genetically obese (*ob/ob*) mice into germ-free mice resulted in a transfer of the obese phenotype (6). The obese mice were also found to harvest more fecal energy than their lean counterparts. A number of mechanisms have been implicated in the link between intestinal microbiota, increased fatty acid metabolism, and storage of calories as fat; these include: (i) the intestinal absorption of monosaccharides and short-chain fatty acids (SCFAs) following the fermentation of indigestible polysaccharides, together with increased hepatic lipogenesis via carbohydrate and sterol response-element binding proteins; (ii) the suppression of fasting-induced adipocyte factor (Fiaf), a circulating inhibitor of lipoprotein lipase, which catalyzes the release of fatty acids from lipoprotein-associated triacylglycerols that are then taken up by muscle and adipose tissues; (iii) the suppression of adenosine monophosphate-activated protein kinase, a cellular "fuel gauge" that is activated in response to metabolic stresses such as exercise, hypoxia, and glucose deprivation; and (iv) the interaction between products of polysaccharide fermentation (i.e., SCFAs) and G-protein-coupled receptor 41 (Gpr41) expressed by enteroendocrine cells in the gut epithelium that reduces the expression of PYY, an enteroendocrine cell–derived hormone that inhibits gut motility, thereby increasing intestinal transit rate and, possibly, reducing nutrient contact time (7).

Gut microbiota may be different in obesity. Together, these data suggest that gut microbes can influence both the harvest of energy from components of the diet and how energy is stored and expended. It is, therefore, reasonable to suggest that a subset of the intestinal microbiota may contribute to obesity. Indeed, deep sequencing of the distal gut microbiota of lean, wild-type mice and *ob/ob* mice (8) revealed consistent differences in the two major bacterial phyla, *Bacteroidetes* and *Firmicutes*. Specifically, the *Firmicutes* were higher whereas the *Bacteroidetes* were correspondingly reduced despite a similar diet and activity level. Metagenomic studies in the *ob/ob* microbiome demonstrated an enrichment in genes coding for enzymes involved in the initial steps of breaking down indigestible dietary polysaccharides and in genes coding for enzymes that import, metabolize, and generate the end products of these metabolic pathways (6). Turnbaugh *et al.* (9) showed similar findings in mice with diet-induced obesity. Interestingly, although the changes in the *Firmicutes* and *Bacteroidetes* were division-wide in the *ob/ob* mice, in the diet-induced obese mice, the difference was related mainly to a bloom in a single class of bacteria called *Mollicutes*.

Similar to the information presented in mice, evidence also suggests that differences in the gut microbiome exist between obese and lean humans. In a small study of individuals undergoing weight loss using either a low-carbohydrate or low-fat diet and followed for a year, at baseline, the subjects' ratio of *Firmicutes* to *Bacteroidetes* was substantially higher than that of the normal-weight controls (10). Importantly, in those subjects who had successful and sustained weight loss, the ratio returned to normal. Despite these intriguing findings, controversy persists regarding the contribution of the microbiota to the development of obesity in humans and the importance of the *Firmicutes/Bacteroidetes* ratio, in particular, due to conflicting findings from studies that have failed to confirm differences in the abundance of *Bacteroidetes* and *Firmicutes* between lean and obese humans (11–14).

In humans, it has been suggested that the relative composition of the gut microbiota during early life predicts the subsequent development of overweight and obesity. In a comparison of fecal samples from 25 overweight or obese children and 24 normal-weight children (15), bifidobacterial numbers in fecal samples during infancy were significantly higher in children remaining at a normal weight at age 7 years, whereas significantly greater numbers of *Staphylococcus aureus* in infancy were detected in children who subsequently became overweight. Notably, a second study found that the microbiota composition in pregnant overweight and normal-weight women is different, with *Bacteroides* and *Staphylococcus* relatively overrepresented in women who are overweight during pregnancy (16). Because the mother influences the original inoculum and subsequent development of the infant microbiota, it is possible that this is one underlying mechanism by which a propensity for obesity is conferred from the parent to the infant. Indeed, infant fecal composition has been found to be related to weight and weight gain of their mothers during pregnancy (17).

The microbial community within the gut is diverse, consisting of not only a broad range of bacterial species, but also *Archaea* and various microbial eukaryotes. Thus, syntrophic (i.e., "cross-feeding"), symbiotic, and competitive interactions among bacterial and archaeal species may also play key roles in promoting obesity. In this regard, methane-producing *Archaea* have been found to be present in greater abundance in obese mice and humans compared with lean individuals (6,11). Recently, a study in which germ-free mice were colonized with *Bacteroides thetaiotaomicron* (an adaptive bacterial forager of dietary polysaccharides), alone or together with *Methanobrevibacter smithii* or the sulfate-reducing bacterium, *Desulfovibrio piger*, found that cocolonization with *M. smithii* but not *D. piger* induced *B. thetaiotaomicron* to ferment dietary fructans to acetate, resulting in a significant increase in host adiposity compared with monocolonized or *B. thetaiotaomicron/D. piger* cocolonized mice (18). Further support for a direct interaction between gut microbes in humans was provided by a study conducted in normal-weight and morbidly obese subjects, and in subjects after gastric bypass. Phylogenetic analysis found that *Firmicutes* were dominant in normal-weight and obese individuals but significantly decreased in individuals after gastric bypass, who had a proportional increase in *Gammaproteobacteria* (11). Similarly, the numbers of hydrogen-producing *Prevotellaceae* were enriched in obese individuals as were *Archaea*, represented primarily by members of the order *Methanobacteriales* (hydrogen-oxidizing methanogens), which were present at a higher level in obese individuals compared with lean subjects or those after gastric bypass. The investigators hypothesized that hydrogen transfer between bacterial and archaeal species may increase energy uptake by the large intestine in obese persons via methanogens removing

fermentation intermediates, such as H_2 or formate, thus relieving thermodynamic limitations and allowing greater production of SCFAs that are then available to be absorbed across the intestinal epithelium. In contrast, Schwiertz *et al.* (14) found no difference in the abundance of *Archaea* in overweight or obese humans, which brings into question the utility of *Archaea* as a potential biomarker of obesity. Clearly, further investigation in the area is needed.

Influence of diet on the gut microbiome. Diet has recently been shown to strongly and rapidly influence the composition of the gut microbiota, raising the question of whether the diet independent of the obese phenotype is responsible for the changes in gut microbe composition (19,20). Using a resistin-like molecule-β (RELMβ) knockout mouse model that gains less weight than a wild-type mouse, it was shown that a high-fat diet is associated with a decrease in *Bacteriodetes* and an increase in both *Firmicutes* and *Proteobacteria* as previously reported; however, the change in microbiota occurred regardless of whether the mice were obese or not, suggesting that diet was the driving force behind this microbial change in abundance (19). Similarly, Fleissner *et al.* (20) compared conventionally raised and germ-free mice fed either a low-fat, high-fat, or Western diet and found that the germ-free mice fed the high-fat diet gained more weight than the conventional mice, suggesting that diet is more important than gut microbes. Interestingly, they also reported an increase in Fiaf levels in the obese mice, contradicting the mechanistic findings described previously (5). Using an *ob/ob* mouse model fed low-fat and high-fat diets and compared with wild-type mice, Murphy *et al.* (21) found an increased *Firmicutes/Bacteroidetes* ratio as previously described, but determined that the compositional changes were primarily the result of the high-fat diet rather than genetic obesity. Finally, results obtained from a mouse model with a humanized gut microbiome indicate that the gut microbes undergo a rapid shift in only a single day after switching from a low-fat, plant polysaccharide–rich diet to a high-fat, high-sugar, "Western" diet (22). This dietary change also induced adiposity, resulted in altered microbiome gene expression, and was shown to be transmissible via microbiota transplantation. Clearly, there exists a complex relationship between diet, age, host physiology, and genotype affecting microbiota composition.

Given the difficulty of studying controlled diet interventions in humans, much less is known. Nevertheless, a very recent report showed that short-term alterations in nutrient load induced rapid changes in the gut microbiota in lean, but not obese, humans (13). Specifically, in lean individuals, increases in nutrient load resulted in an increased relative abundance of *Firmicutes* (and a corresponding decrease in *Bacteroidetes*) and increased energy harvest. Interestingly, this relationship was not observed in obese subjects, suggesting that the microbiota of obese and lean individuals respond differently to changes in the caloric content of diet.

Metabolic endotoxemia, inflammation, and the immune system in obesity and the metabolic syndrome

Obesity is associated with a number of other metabolic disorders characterized by chronic, systemic, low-grade inflammation. Although endotoxin (lipopolysaccharide (LPS)), derived from the cell wall of Gram-negative bacteria, circulates at low concentrations in the blood of healthy individuals, the presence of genetic and diet-induced obesity and other metabolic disorders has been associated with a substantial increase in LPS concentrations, a condition termed "metabolic endotoxemia" (23,24).

Consumption of a high-fat meal in both animals and humans results in significant increases in endotoxin concentrations and changes in the gut microbiota composition (25,26). Increases in systemic endotoxin levels may result from increased intestinal permeability caused by the compositional changes in the microbiota (24). Endotoxemia may then contribute to the low-grade inflammation, insulin resistance, adipocyte hyperplasia, and decreased β-cell function that characterizes the metabolic syndrome. Antibiotic treatment of both high-fat-fed mice and *ob/ob* mice reduces metabolic endotoxemia and cecal LPS content (27). This CD14-driven effect is paralleled by decreased intestinal permeability and reductions in glucose intolerance, by body weight gain and fat mass development, and by markers of inflammation, oxidative stress, and infiltration of macrophages into visceral adipose tissue (27). These effects appear to be mediated, at least in part, by interactions between LPS and adipose tissue metabolism through endocannabinoid-driven adipogenesis (28).

Toll-like receptors (TLRs) are highly expressed transmembrane proteins (i.e., pattern recognition receptors) in the innate immune system that recognize structurally conserved molecules derived from microbes. Beyond the potential of high-fat diets to induce metabolic endotoxemia, the innate immune system may play a role in both regulating the gut microbiota and, by extension, influencing the development of metabolic disorders (**Table 1**). TLR4 (which recognizes LPS) knockout mice have been shown to be resistant to LPS and diet-induced weight gain (27). Mice genetically deficient for TLR5, which recognizes bacterial flagellin, exhibit hyperphagia and features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance, increased adiposity, and significant changes in the composition of the gut microbiota (29). Transfer of the gut microbiota from TLR5-deficient mice to wild-type, germ-free mice conferred features of the metabolic syndrome. In contrast, abrogation of the expression of TLR2, which recognizes a number of microbial products including peptidoglycan, lipoteichoic acid, and lipoprotein, protects mice from diet-induced adiposity, insulin resistance, hypercholesterolemia, and hepatic steatosis and is also associated with attenuation of adipocyte hypertrophy (30). Notably, members of the phyla *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* were more abundant in mutant mice than in wild-type mice (31), consistent with previous studies suggesting that the relative proportion of *Bacteroidetes* is reduced in obese mice (8). Similarly, loss-of-function mutations in TLR4 prevents diet-induced obesity and insulin resistance (32).

Therapeutic relevance

In addition to diet and antibiotics, a number of clinically relevant medical and surgical strategies exist for the manipulation of the gut microbiota. In contrast to antibiotics, probiotics are living, nonpathogenic microbes that, when ingested in sufficient amount,

Table 1. Impact of Toll-like receptors (TLRs) on metabolic disease

Author	TLR	Ligands	Results in TLR mutants
Vijay-Kumar *et al*. (29)	TLR5	Recognition of bacterial flagellin	Increased hyperlipidemia, hypertension, insulin resistance, increased adiposity; significant changes in the composition of the gut microbiota
Himes and Smith (30)	TLR2	Peptidoglycan, lipoteichoic acid, and lipoprotein from Gram-positive bacteria, among others	Protection against diet-induced adiposity, insulin resistance, hepatic steatosis; adipocyte hypertrophy attenuated; increase in *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*
Tsukumo *et al*. (32); Cani *et al*. (37)	TLR4	Lipopolysaccharide from Gram-negative bacteria	Protection against diet-induced obesity and insulin resistance

confer a therapeutic effect. Prebiotics such as fructooligosaccharides and inulin are nonabsorbed, selectively fermented short-chain carbohydrates that facilitate targeted changes in commensal gastrointestinal microbes. Synbiotics include both a probiotic and a prebiotic, the combination of which is intended to improve probiotic colonization. Finally, certain surgical weight loss procedures such as the Roux-en-Y gastric bypass (RYGB) have been shown to alter the gut microbiota. The clinical relevance of antibiotic and probiotic use is supported by their use as growth promoters in the farm industry.

Antibiotics. As previously noted, antibiotic treatment reduces metabolic endotoxemia and the cecal content of LPS in both high-fat-fed mice and *ob/ob* mice, resulting in reduced glucose intolerance, body weight gain, and markers of inflammation and oxidative stress (27). Antibiotic treatment, combined with a protective hydrolyzed casein diet, has been shown to decrease the incidence and delay the onset of diabetes in a rat model (33). However, in an age of rapidly increasing, multidrug-resistant pathogens, prescribing antibiotics to counteract human obesity should be carefully considered. Moreover, the widespread use of prophylactic antibiotics in the livestock industry as a means of growth production suggests that therapies designed to reduce obesity may have unintended consequences (34).

Prebiotics. The effect of prebiotics on metabolic parameters has been examined in animal and human studies. In rats, addition of oligofructose to standard or high-fat diets paradoxically reduced energy intake and attenuated weight gain and fat-mass development (35,36). This effect may be mediated by an increase in gut bifidobacterial content, which has been shown to reduce endotoxemia, improve glucose tolerance and glucose-induced insulin secretion, and normalize inflammatory markers (37).

Probiotics. Given the potential role of the intestinal microbiota in metabolic disorders, it is reasonable to hypothesize that restoration or supplementation of certain microbial populations may have a beneficial effect. In fact, administration of probiotic beverages to germ-free mice conventionalized with human infant microbiota results in a broad range of changes in the microbiome and alterations in energy homeostasis and lipid and amino-acid metabolism (38). Oral supplementation of newborn mice with *Bifidobacteria* may attenuate the steady rise in endotoxin levels seen during early life (39) and has been shown to prevent autoimmune diabetes in a nonobese diabetic mouse model (40). Lee *et al.* (41) evaluated the effect of *Lactobacillus rhamnosus* PL60, a human-originated bacterium that produces conjugated linoleic acid, on diet-induced obesity in mice. After 8 weeks of feeding, *L. rhamnosus* was associated with a significant reduction in body weight without reducing energy intake and a significant reduction of white adipose tissue, potentially as a result of increased apoptosis.

The long-term effect of perinatal probiotics on the development of obesity was assessed in 159 pregnant women who were randomized to either *L. rhamnosus* GG or placebo initiated 4 weeks before expected delivery and continued for 6 months postnatally (42). Children were followed for up to 10 years after birth. A two-phase pattern of excessive weight gain was observed, with initial weight gain occurring from the fetal period to 24 to 48 months of age and a second period starting after 24 to 48 months of age. Probiotic intervention moderated weight gain occurring during the first phase, particularly among children who later became overweight, but did not affect weight gain during the second period.

Roux-en-Y gastric bypass. Although the mechanisms of action of surgical weight loss procedures have been simplified into restrictive and malabsorptive, the alterations in bowel anatomy and physiology that occur following certain operations such as the RYGB have been demonstrated to cause changes to the gut microbiota that may have relevance for energy harvest and storage postoperatively (11,43). Zhang *et al.* (11) detected significantly higher numbers of H_2-utilizing methanogenic *Archaea* in obese individuals than in normal-weight or RYGB individuals. *Firmicutes* were dominant in normal-weight and obese individuals but significantly decreased in RYGB individuals, along with a proportional increase of *Gammaproteobacteria*. Furet *et al.* (43) further demonstrated that RYGB results in rapid adaptation of the microbiota. In this study, *Faecalibacterium prausnitzii* was found to be directly linked to the reduction in the low-grade inflammatory state in obesity and diabetes, independently of caloric intake. The potential role of the gut microbiota on weight loss after RYGB was also recently suggested by the demonstration of enhanced weight loss following administration of a probiotic postoperatively (44).

Conclusion

The accumulating evidence strongly suggests that the gut microbiota play an important role in the regulation of energy balance and weight in animals and humans and may influence the development and progression of obesity and other metabolic disorders, including type 2 diabetes. Although not a substitute for diet and exercise, manipulation of the gut microbiome represents a novel approach to treating obesity. However, a number of research

avenues must be pursued in order to leverage manipulation of the gut microbiome for therapeutic purposes. Importantly, the differences between the gut microbiota in lean and obese individuals remain incompletely understood, as does the impact of diet on the composition of the gut microbiome. Indeed, the cause-and-effect relationship of the findings described remains to be established as does the clinical relevance to humans. Furthermore, because the "ideal" composition of the gut microbiota, if one exists, remains poorly understood, modification must be pursued with caution.

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