Special Article

Fermentation potential of the gut microbiome: implications for energy homeostasis and weight management

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Energy homeostasis is regulated by twin factors, energy intake and energy expenditure. Obesity arises when these two factors are out of balance. Recently, the microflora residing in the human gut has been found to be one of the influential factors disturbing energy balance. Recent interest in this field has led to use of the term "gut microbiome" to describe the genomes of trillions of microbes residing in the gut. Metagenomic studies have shown that the human gut microbiome facilitates fermentation of indigestible carbohydrates to short-chain fatty acids that provide excess energy to the body, thus contributing to the obese phenotype. Alteration in the ratio of Bacteroidetes *and* Firmicutes *drives a change in fermentation patterns that could explain weight gain. Therefore, changes in the gut microbiome (induced by antibiotics or dietary supplements) may be helpful in curbing the obesity pandemic. This review provides information on the expansive role the gut microbiome is believed to play in obesity and other related metabolic disorders.*

INTRODUCTION

The adult human gut is home to trillions of bacteria living harmoniously and interacting remarkably in a mutualistic manner with the host.[1] The distal gut, in particular, has recently been recognized as an ecosystem in which each microbial inhabitant is involved in the redistribution of energy, either by facilitating energy extraction in the form of short-chain fatty acids (SCFAs) or chemical transformations. This largely unexplored area has attracted researchers around the globe and resulted in the initiation of metagenomic studies targeting the genomes of trillions of microbes residing in the gut, collectively called the "microbiome," and exploring their inheritance and association with the host.[2] It is a tedious task to unearth the genomes of such a diverse and densely populated ecological unit, in which total microbial members outnumber the human somatic and germ cells by a factor of ten. However, successful application of axenic mouse models, genomics, proteomics, and pyrosequencing tools, accompanied by rigorous statistical analysis, will likely pave the way towards better understanding of the host-microbiome relationships and their roles in ameliorating intestinal disorders, obesity, and the metabolic syndrome.

Obesity is rising at an alarming rate worldwide, but because of easy access to energy-rich foods in developed countries, people residing in them have been affected by the pandemic in greater numbers. In the United States, about 65% of the population is estimated to be overweight.[3] Obesity is basically the imbalance between energy intake and energy expenditure. It is governed by many factors including food intake, physical activity, emotional state, genetics, and type of diet.[4] The common failure of obese individuals to comply with weight-loss feeding regimens and the unavailability of effective drugs to treat obesity has accelerated research to find the causal factors and alternative approaches to combat this pandemic.[5] While earlier studies focused on genomics and endocrinology to unravel the underlying mechanisms of body-weight regulation, application of metagenomic methods

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has provided further insights into the diversity of the gut microflora and its participation in the regulation of energy homeostasis. This review aims to provide a comprehensive update on recent developments in the field.

OBESE VERSUS LEAN MICROBIOME: ROLE OF SHORT-CHAIN FATTY ACIDS

Dietary fiber containing fermentable carbohydrates have been part of the human diet since Paleolithic times.[6] The fermentable carbohydrates present in the human diet are subjected to bacterial fermentation, resulting in the production of acetate, propionate, and butyrate in the ratio of 60:25:15.[7] It is now widely accepted that there are marked differences in the microbiota and their fermentation profiles in obese and lean phenotypes. The prominent dominance of two bacterial divisions, *Firmicutes* (60–80%) and *Bacteroidetes* (20–40%), has been revealed by experimentation using the obese mouse model (ob/ob) lacking expression of the leptin gene. These studies revealed that the ratio of *Firmicutes* to *Bacteroidetes* helped determine obesity, with obese mice displaying 50% more *Firmicutes* than *Bacteroidetes*.[8,9] The ob/ob mice with a *Firmicutes*-enriched microbiome displayed increased expression of the enzymes involved in the breakdown of otherwise indigestible dietary carbohydrates and in the pathways for starch/sucrose, galactose, and butanoate metabolism. Higher concentrations of fermentation products, i.e., butyrate and acetate in the cecum, and lower fecal energy also confirmed the microbiome-derived energy salvage in obese (ob/ob) animals.[9]

Another bacterial class that emerged as an influential factor in host energy balance and obesity is the mollicutes.[10] Using conventionalization methods, which involve colonization of gnotobiotic mice with microbiota from conventionally raised mice, and a metagenomic approach, it was noticed that mollicutes class of the *Firmicutes* flourished better in the diet-induced obese mice fed a carbohydrate-rich Western diet. Shifting the mice to a carbohydrate-/fat-restricted diet resulted in a dramatic reduction in the population of mollicutes. Functional characterization of the mollicutes-enriched obese gut microbiome revealed the presence of a phosphotransferase system, with fructose and mannose metabolism pathways involved in the uptake and metabolism/fermentation of diverse sugar molecules.[10] However, the abundance of ABC transporters involved in the uptake of diverse sugar molecules was depleted.

Hildebrandt et al.[11] performed a study with RELM-β (a goblet cell-specific protein) knockout mice, which show resistance to diet-induced obesity upon switching to a high-fat diet (HFD). In both the wild-type mice (became obese with a HFD) and the RELM-β knockout mice (non-responsive to diet-induced obesity with an HFD), HFD resulted in the expansion of *Firmicutes* and *Proteobacteria* and reduction of *Bacteroidetes*, independent of an obese phenotype. Among the *Firmicutes*, the maximum bloom was in *Clostridiaceae*, while no significant increase was observed in the mollicutes population. The functional alignment of expanded genomes revealed upregulation of the genes encoding ABC transporters.

Obese fa/fa rats, having mutation in the leptin receptor gene, also displayed reductions in total bacterial and bifidobacterial counts, as compared to their lean counterparts. DGGE profiling revealed the presence of *Halomonas* and *Sphingomonas* strains in the cecal content of fa/fa rats. Researchers ascribed obesogenesis to both of these bacteria, with acetate production as the cause in the former and carbohydrate scavenging in the latter.[12]

Human beings harbor two dominant microbial populations i.e., *Bacteroidetes* and *Firmicutes*, despite marked interpersonal variations. Obese individuals, however, possess relatively more *Firmicutes*, a finding that is similar to those from murine studies. One study subjected obese individuals to dietary fat/carbohydrate restrictions and monitored the subjects' gut microbiomes for 1 year. While bacterial diversity within subjects remained constant over the course of the study, a division-wide decline in *Firmicutes* was observed.[13] In another quantitative real-time polymerase chain reaction-based study, higher numbers of *Lactobacillus* species belonging to the division *Firmicutes* and lower numbers of *Bacteroidetes* were observed in obese subjects.[14] Conversely, Schwiertz et al.[15] observed a shift in the ratio towards *Bacteroidetes* in obese subjects with a parallel increase in propionate concentration in overweight and obese volunteers. Another study found no change in *Bacteroidetes* proportions in obese and nonobese subjects. Weight-loss diets resulted in reductions in the numbers of butyrate-producing *Firmicutes* but no changes in *Bacteroidetes*.[16]

Sequencing and functional analysis of the healthy human microbiome has revealed that genomes of identified bacterial phylotypes are aligned with a number of functions like metabolism and fermentation of glycans, synthesis of essential amino acids and vitamins, methanogenesis, and detoxification of xenobiotics.[17] This supports the theory that energy derived from indigestible plant polysaccharides is followed by scavenging of hydrogen produced during fermentation by methane production. Kurokawa et al.[18] also revealed 14 families of glycosyl hydrolases and many enzymes involved in the metabolism of mono- and disaccharides in the healthy Japanese adult microbiome. The infant microbiome displayed 12 families of glycosyl hydrolases, which is similar to adults, and transporters with an over-representation

of the phosphotransferase system that may mediate the uptake of lactose from breast milk. They also found that horizontal gene transfer might have occurred between resident microbes in the human gut due to the presence of transposases and integrase/site-specific recombinases in their genome.

Gastric bypass surgery in obese people led to reductions in the levels of *Firmicutes* and increases in the levels of *Gammaproteobacteria*.[19] Interspecies hydrogen transfer between bacterial (belonging to the family *Prevotellaceae*) and archaeal members (*Methanobacteriales*) of the gut microbiota is proposed as the mechanism behind efficient energy uptake in obese individuals. Verberkmoes et al.[20] used a metaproteomics approach and found many bacterial proteins responsible for the active transport, uptake, and metabolism of a vast array of polysaccharides supplied in the human gut through diet. However, acetogenesis was proposed as an alternative route of hydrogen scavenging in humans, along with methanogenesis.

Thus, obesity is linked with blunted microbial diversity and lower levels of *Bacteroidetes*. The "core" microbiome contains genes important for life; these were identified from massive microbial gene sequences related to carbohydrate and amino acid metabolism, secretion systems, and membrane transports. Most of the *Bacteroidetes* were found to be associated with carbohydrate metabolism, while the *Firmicutes* were associated with transport systems. It was proposed that disturbance and deviation from this "core" microbiome leads to obesity.[21,22]

So far, it can be stated that gut microbiomes comprised largely of *Firmicutes* facilitate the uptake and fermentation of indigestible carbohydrates into SCFAs, which is the major mechanism responsible for the obese phenotype in both mice and humans. The extent to which SCFAs contribute to total energy content has been calculated in some studies. Under in vitro experimental conditions using swine feces, the absorbed SCFAs contributed about 17.6% of energy when pigs were fed high-fiber diets.[23] In humans, hind-gut fermentation could account for 10% of total energy requirements.[24]

However, a body of literature suggests an anti-obesity role played by fermentable fiber mediating its action through gut-related phenomenon linking SCFAs and regulation of gut hormone expression.[25–29] Emerging evidence also indicates that SCFAs play a role in controlling obesity. SCFAs act as ligands for two G-protein-coupled receptors, GPR41 and GPR43, identified recently in human colon. While GPR41 exhibits specificity in the order of propionate > butyrate > acetate, GPR43 is equally potent for all three SCFAs. Both GPR41 and GPR43 are co-localized with colonic enteroendocrine cells expressing peptide YY, which is known to inhibit food intake and gastrointestinal motility.[30,31] Acetate and propionate were shown to stimulate adipogenesis in an adipocyte cell line.[32] A reduction in lipolytic activity was also observed in the adipocytes treated with acetate and propionate.[33] Propionate has also been demonstrated to increase leptin release from adipose tissue in mice,[34] stimulate expression of leptin, and suppress the pro-inflammatory factor resistin in human adipose tissue depots.[35] All these observations suggest a potential role of SCFAs in the regulation of obesity and is further needed to be strengthened with experimentation.

MICROBIOME AND BODY WEIGHT MANAGEMENT

In an earlier report on the impact of microflora on body composition, Levenson[36] noted that axenic rat carcasses had lower body fat than conventional animals. Recently, Backhed et al.[37] used a process called conventionalization, by which germ-free (GF) mice are transplanted with microbiota of conventionally raised mice, and demonstrated a 57% increase in total body fat and a 61% increase in the weights of epididymal fat pads of the conventionalized mice within 14 days. One of the factors implicated in this phenomenon was reduced expression of fasting-induced adipocyte factor (Fiaf) in conventional animals, which is known to be a potent inhibitor of lipoprotein lipase.[37] An effort was then made to unravel its potential as a microbiome-influenced factor in GF Fiaf -/- mice. The knockout animals gained more weight and had lower expressions of peroxisome proliferator-activated receptor coactivator 1α (Pgc1α) followed by a parallel decrease in genes encoding the enzymes of fatty acid oxidation, i.e., carnitine: palmitoyl transferase-1 and medium-chain acyl coA dehydrogenase.[5] It could be concluded that expression of these genes is regulated by Pgc1-α, which in turn is controlled by the gut microbiome via Fiaf. The observations were also attributed to AMP kinase (AMPK), which had potentiated expression in the liver and muscle of GF mice accompanied by increased expression of fatty acid oxidation enzymes.[5] Thus, GF mice are able to endure diet-induced obesity due to elevated activity of AMPK and Fiaf, which target fatty acid oxidation and uptake, finally leading to the lean phenotype.

Another study, however, does not support the protection of GF mice from obesity. Gnotobiotic mice were shown to gain more body weight when administered a high-fat diet due to lower energy expenditure. Higher intestinal Fiaf expression was observed with both high-fat and Western-type diets but its circulating level was unchanged, indicating it has no role as a gut microbiota-regulated factor in fat storage.[38]

The conventionalization of gnotobiotic mice with microbiota from ob/ob mice also resulted in transmissibility of the adiposity trait via the microbiome. The

process produced obesity in the GF mice and they exhibited significantly more body fat than recipients of microbiome from control animals.[9]

One report contradicts the relationship between gut microflora and obesity. The treatment of ob/ob mice with the antibiotics norfloxacin and ampicillin resulted in improved oral glucose tolerance and reduced hepatic steatosis. The improvement in insulin sensitivity was correlated with reduced production of lipopolysaccharide, tumor necrosis factor-α , and elevated secretion of adiponectin in the mice treated with antibiotics. However, alterations in the gut microflora induced no changes in body weight or body fat mass, thus ruling out a contribution of gut microflora to weight management.[39]

In humans, weight loss is accompanied by alterations in the gut microflora. In a study of overweight adolescents, caloric restriction and physical activity for 10 weeks brought about a loss of body weight (>4 kg) accompanied by increased numbers of *Bacteroides fragilis* and lactobacilli. A marked reduction in *Clostridium coccoides* was also noticed. While post-intervention reductions in all the bifidobacterial species were recorded, only the decrease in *B. longum* was significant and the ratio of *Bifidobacteria* to *C. coccoides* increased.[40] In a similar study of adolescents losing weight (>4 kg), Nadal et al.,[41] using fluorescent *in situ* hybridization to enumerate bacterial groups, recorded reductions in *Clostridium histolyticum* as well as *Eubacterium rectale-Clostridium coccoides*, and elevations in the counts of the *Bacteroides-Prevotella* group of species. Total fecal energy was also significantly reduced. The authors suggested the observed increase in the propionate producer group, *Bacteroides-Prevotella*, could explain the weight loss because propionate is known to inhibit the incorporation of colonic acetate in lipid synthesis[42] and may favor a lean phenotype.

BACTEROIDES THETAIOTAMICRON: PROVIDING INSIGHT INTO HOST-MICROBIOME-OBESITY INTERACTIONS

Bacteroides thetaiotamicron, a highly obligate anaerobe and member of the human adult gut microflora, has attracted investigators because of its diverse foraging activity. Conventionalization of gnotobiotic mice with *B. thetaiotamicron* sheds light on the successful adaptation of this species and its response to environmental fluctuations, especially with regard to carbohydrate availability in the gut. Its genome is endowed with 163 paralogs of two outer-membrane proteins that bind and import starch, 226 predicted glycoside hydrolases, and 15 polysaccharide lyases. Interestingly, the shift from plant polysaccharides to host mucus glycans at the time of nutrient unavailability shows the flexibility this glycophile has in its dining habit.[43]

To gain a better understanding, the adaptability of this species was studied in the context of a shift from suckling to weaning gut niches in the monoassociated mouse. The results reflected the persistence of *B. thetaiotamicron* in the transition from mother's milk to an adult diet containing plant-derived polysaccharides. During the suckling period, *B. thetaiotamicron* thrived on host-derived mucus glycans and simple sugars like lactose, glucose, and galactose derived from milk. Changes in the gut environment, with an abundance of complex sugars in chow diet, brought about upregulation of genes expressing hydrolases, like arabinosidases, levanases, rhamnosidases, pectate, and lyases, and enhanced expression of transcriptome-encoding environmental sensors and capsular proteins involved in the import of sugars that coincided with the complex glycan availability.[44]

An association of *B. thetaiotamicron* with a representative archaeal member of the human gut microbiota, *Methanobrevibacter smithii*, was demonstrated in gnotobiotic mice fed a fructan-rich diet. *M. smithii* emerged as a "power broker" in the distal gut community, influencing the specificity of fermentation and fat storage. It utilized formate, rather than hydrogen, to produce methane, as evident from the enhanced expression of gene clusters encoding proteins metabolizing formate in mice harboring both *B. thetaiotamicron* and *M. smithii*. Biassociated mice also displayed a rise in acetate levels leading to upregulation of fatty acid synthase. Consequently, it also contributed to adiposity with an 80% increase over germ-free controls in the epididymal fat pad weights.[45]

As discussed, the fermentation products C2-C6 fatty acids bind to GPR 41, which is expressed in enteroendocrine cells. To better understand the link between SCFAs and host adiposity, GPR41-/- mice were reared with *B. thetaiotamicron-M. smithii.* Both colonized and conventionally raised GPR41-/- mice were leaner and had lower body weights. GPR-deficient mice cocolonized with *B. thetaiotamicron* and *M. smithii* also displayed lower leptin and PYY values. Though it did not affect chow consumption, caloric extraction from the diet was reduced in the cocolonized GPR41-/- mice. With PYY being the regulator of gut motility, its lower levels resulted in increased intestinal transit with reduced absorption of SCFAs; eventually, this led to reduced hepatic lipogenesis in GPR knockout mice.[46] Thus, the findings indicate GPR41 is a gut microbiota-regulated factor that plays an important role in energy balance.

To gain a better understanding of the interactions between two dominant phyla of the human gut, gnotobiotic mice were either singly or concomitantly colonized with *B. thetaiotamicron* and *Eubacterium rectale*, as representative members of *Bacteroidetes* and *Firmicutes*, respectively. *B. thetaiotamicron* responded by increasing the expression of genes degrading the host mucus glycans

that had no access to *E. rectale*. *E. rectale's* biassociation with *B. thetaiotamicron* resulted in the downregulation of 51 glycosyl hydrolases and upregulation of a number of peptide and amino acid transporters. Cocolonization also drove the channeling of acetate produced by *B. thetaiotamicron* towards butyrate production by *E. rectale*, which reduced the NADH levels facilitating glycolysis.[47]

Employing *B. thetaiotamicron* in monoassociation and biassociation with other representative bacterial phylotypes provides some sense of the complexity of the interactions and fluctuations in the dining preferences of gut bacteria members.

PROBIOTICS: IMPACT ON MICROBIOME AND OBESITY

Probiotics are the microbes that, when administered in adequate amounts, confer a beneficial effect upon host health. *Lactobacillus* and *Bifidobacteria* species are the most widely used probiotics.

There is much evidence to support the fat-lowering and anti-obesity potential of probiotics.[48–50] In another microarray-based study, the administration of either *L. paracasei* and *L. acidophilus* to axenic mice elicited genes regulating fat and sugar metabolism. Enhanced expression of the insulin-sensitizing hormones adipsin and adiponectin, and reduced expression of resistin-like β, which is known to induce insulin resistance, were observed. All the altered genes favor a reduction in body fat and insulin sensitization.[51] However, lactobacilli belong to the *Firmicutes* and increased amounts of *Firmicutes* have been correlated with the obese phenotype in both humans and mice. It is consequently suggested that caution is warranted when adding lactobacilli to the diet. A role of probiotics like lactobacilli in the weight gain observed in farm animals has been reported, and similar effects in humans may be anticipated.[52]

Various types of evidence also support the preventive role of *Bifidobacteria* in obesogenesis. Recently, a lower number of *Bifidobacteria* was correlated with obesity in an obese *fa/fa* rat model.[12] *Bifidobacteria* are also able to protect diet-induced obese mice against metabolic endotoxemia, which sets the tone for low-grade inflammation causing obesity. Increases in the amount of *Bifidobacteria* with oligofructose feeding and decreases in intestinal endotoxin levels may account for the reduced body weight gain and fat mass development observed in diet-induced obese mice.[53] Changes in gut flora associated with antibiotic intake also resulted in reductions of metabolic endotoxemia-induced inflammation and obesity.[54]

Luoto et al.[55] conducted a 10-year follow-up study and found that pre- and post-natal *Lactobacillus rhamnosus* GG intervention modified the growth patterns of children by inhibiting excessive weight gain during the first years of life. Kalliomaki et al.[56] reported that normal-weight children had higher numbers of *Bifidobacteria* and lower counts of *Staphylococcus aureus* at an early age than overweight children.

The interaction between probiotics and microbial residents of the gut was evaluated in a simplified germ-free mouse model colonized with *B. thetaiotamicron* and a well-known probiotic, *Bifidobacterium longum*. *B. longum* expanded the foraging ability of *B. thetaiotamicron*, especially with regard to the hydrolysis of mannose- and xylose-containing glycans. *L. casei* also displayed upregulation of genes encoding hexosaminidases and arabinosidases in *B. thetaiotamicron*. However, another *Bifidobacterium* species, *B. animalis*, dominantly upregulated genes associated with transcription and replication of *B. thetaiotamicron* without significantly affecting carbohydrate utilization capabilities.[57] Thus, disparity was revealed in the nature of expansion by species-specific alterations of different probiotics and the resulting impact on the host's intestinal environment.

TRANSMISSIBILITY OF MICROBIOME

The infant gut obtains its first inoculum from the mother during delivery. Since the microbiome is now correlated with obesity, one can speculate about the mother-to-child transfer of microbes responsible for the obese phenotype. Moreover, the mode of delivery and feeding also shape the composition of an infant's gut microbiota.[58] Overweight pregnant mothers are also known to transport excessive energy to the growing fetus, which can pose problems for the infant later in life.[59,60] Collado et al.[61] studied the composition of the microbiota in normal and overweight pregnant mothers. Normal-weight mothers had lower numbers of total *Bacteroides* and *S. aureus*. In the third trimester of pregnancy, *Bacteroides* were positively correlated with weight gain, while higher numbers of *Bifidobacteria* were related with lower weight gain. In another study, overweight pregnant mothers had lower numbers of *Bacteroides* and *Bifidobacteria* and enhanced numbers of *Staphylococcus*, *Enterobacteriaceae*, and *Escherichia coli*.[62]

Kinship behavior is also observed in the inheritance of microbial populations, with sister mothers and their offspring sharing similar microbial communities. The human gut microbiome is shared by family members with a high degree of similarity reported in monozygotic twins.[8] An interrelationship between the host genotype and microbiome has also been observed. In studies of twins, Zoetendal et al.[63] demonstrated that the host genotype affects microbial diversity. They also observed a positive relationship between similarity indices of the microbial populations and genetic relatedness of the host, with the highest correlations seen in monozygotic twins.

A DGGE banding pattern in marital partners living in the same environment showed lower similarity.

Although germ-free mice have been used in most of the experiments and their intestinal physiology is different from their conventional counterparts, metagenomics is, without a doubt, providing further insights into the host/microbiota relationships. Axenic mice have reduced vascularity, muscle wall thickness, and digestive enzyme activities.[64] They also display slower renewal of intestinal epithelial cells and higher locomoter activity.[2] All of these factors can account for the observed differences in energy balance and can determine the extent to which the microbiome can contribute to energy homeostasis and the metabolic syndrome. These discoveries have led to the new term "superorganism"[65] to describe a human being that encompasses microbiota genomes along with a 2.85 billion base pair human genome. These substantial findings strengthen the concept of personalized healthcare and lend considerable importance to the microbiome.

CONCLUSION

The gut microbiome is endowed with extensive fermentation capabilities and the ability to extract energy from plant-derived undigested polysaccharides in the diet. In addition to containing host digestive enzymes, the microbiome influences the patterns of carbohydrate digestion, in particular. An excellent example is *B. thetaiotamicron*, which possesses an array of hydrolases in its genome, which facilitate energy salvage and an appreciable tendency to adapt to the different substrates available in the gut. Microflora balance provides a key to all the anomalies, with the *Bacteroides* to *Firmicutes* ratio being the major player in determining the host's risk of obesity and other metabolic diseases. The obese phenotype has an excess of extracellular gut luminal *Firmicutes* that express a number of glycohydrolases, finally producing SCFAs. Other microbiome-regulated factors are also identified and known to regulate fatty acid uptake and its oxidation. The microbiome is inherited from mothers during birth and shows relatedness, with the similarity patterns being most prevalent in monozygotic twins. The amount of *Bifidobacteria* is correlated with reduced weight gain in both infants and adults. However, since the "energy extraction" hypothesis contradicts the well-known fat-lowering and weight-reducing potential of dietary fiber, it needs to be validated with more stringent protocols and experimentation.

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