

## Gut Microbiota and Its Possible Relationship With Obesity

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Obesity results from alterations in the body's regulation of energy intake, expenditure, and storage. Recent evidence, primarily from investigations in animal models, suggests that the gut microbiota affects nutrient acquisition and energy regulation. Its composition has also been shown to differ in lean vs obese animals and humans. In this article, we review the published evidence supporting the potential role of the gut microbiota in the development of obesity and explore the role that modifying the gut microbiota may play in its future treatment. Evidence suggests that the metabolic activities of the gut microbiota facilitate the extraction of calories from ingested dietary substances and help to store these calories in host adipose tissue for later use. Furthermore, the gut bacterial flora of obese mice and humans include fewer Bacteroidetes and correspondingly more Firmicutes than that of their lean counterparts, suggesting that differences in caloric extraction of ingested food substances may be due to the composition of the gut microbiota. Bacterial lipopolysaccharide derived from the intestinal microbiota may act as a triggering factor linking inflammation to high-fat diet-induced metabolic syndrome. Interactions among microorganisms in the gut appear to have an important role in host energy homeostasis, with hydrogen-oxidizing methanogens enhancing the metabolism of fermentative bacteria. Existing evidence warrants further investigation of the microbial ecology of the human gut and points to modification of the gut microbiota as one means to treat people who are overweight or obese.

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Fiaf = fasting-induced adipocyte factor; LPS = lipopolysaccharide; rRNA = ribosomal RNA

Obesity is a growing epidemic in many developed countries, including the United States, and is arousing increasing concern in developing countries, which have historically dealt with the burden of undernutrition.<sup>1</sup> The prevalence of obesity in adults has increased by more than 75% since 1980; currently, more than half of the US population is overweight, with nearly 1 in 3 adults being clinically obese.<sup>2</sup> Children are also increasingly overweight, suggesting that this epidemic will continue to worsen. Obesity is a major health problem because of its serious health consequences, including type 2 diabetes mellitus, cardiovascular diseases, pulmonary hypertension, obstructive

sleep apnea, gastroesophageal reflux disease, musculoskeletal disorders, a variety of cancers, and a number of psychosocial concerns.<sup>1,2</sup> Obesity has also been shown repeatedly to be associated with an increased risk of mortality.<sup>2</sup> The social and economic costs of obesity and its associated comorbidities are enormous and threaten to overwhelm an already overburdened health care system.<sup>2</sup>

Obesity results from alterations in energy balance, ie, how the body regulates energy intake, expenditure, and storage. Because starvation poses a greater danger to an organism than overabundance, our biological systems are geared to better protect against weight loss than weight gain (ie, a *thrifty genotype*). Considerable effort has been made to improve the availability and stability of the food supply, resulting in an abundance of inexpensive, palatable, and energy-dense foods. Consequently, organisms adapted for a situation of insufficiency are now confronted with the easy availability of such foods.

The physiologic processes that regulate weight and metabolism, including peripheral hunger and satiety signals, the central integration of this information, and the integrated gastrointestinal response to food intake, have received intense investigation, particularly during the past decade.<sup>3-6</sup> A person's weight and body composition are likely determined by interaction between his/her genetic makeup and social, cultural, behavioral, and environmental factors. Although energy intake has increased and physical activity has declined during the past few decades, these changes are difficult to quantify.<sup>7</sup> An increased intake of energy-dense foods, especially when combined with reduced physical activity, surely contributes to the high prevalence of obesity<sup>8</sup>; however, the existence of complex systems that regulate energy balance requires that this paradigm be considered in a larger context.<sup>9,10</sup>

Recent evidence suggests that the trillions of bacteria that normally reside within the human gastrointestinal tract, collectively referred to as the gut microbiota, affect nutrient acquisition and energy regulation; it further suggests that obese and lean people have different gut microbiota. These findings raise the possibility that the gut microbiota has an important role in regulating weight and may be partly responsible for the development of obesity in some people. This article examines the evidence supporting these claims and explores whether modifying the gut microbiota could one day be a treatment option for obesity.

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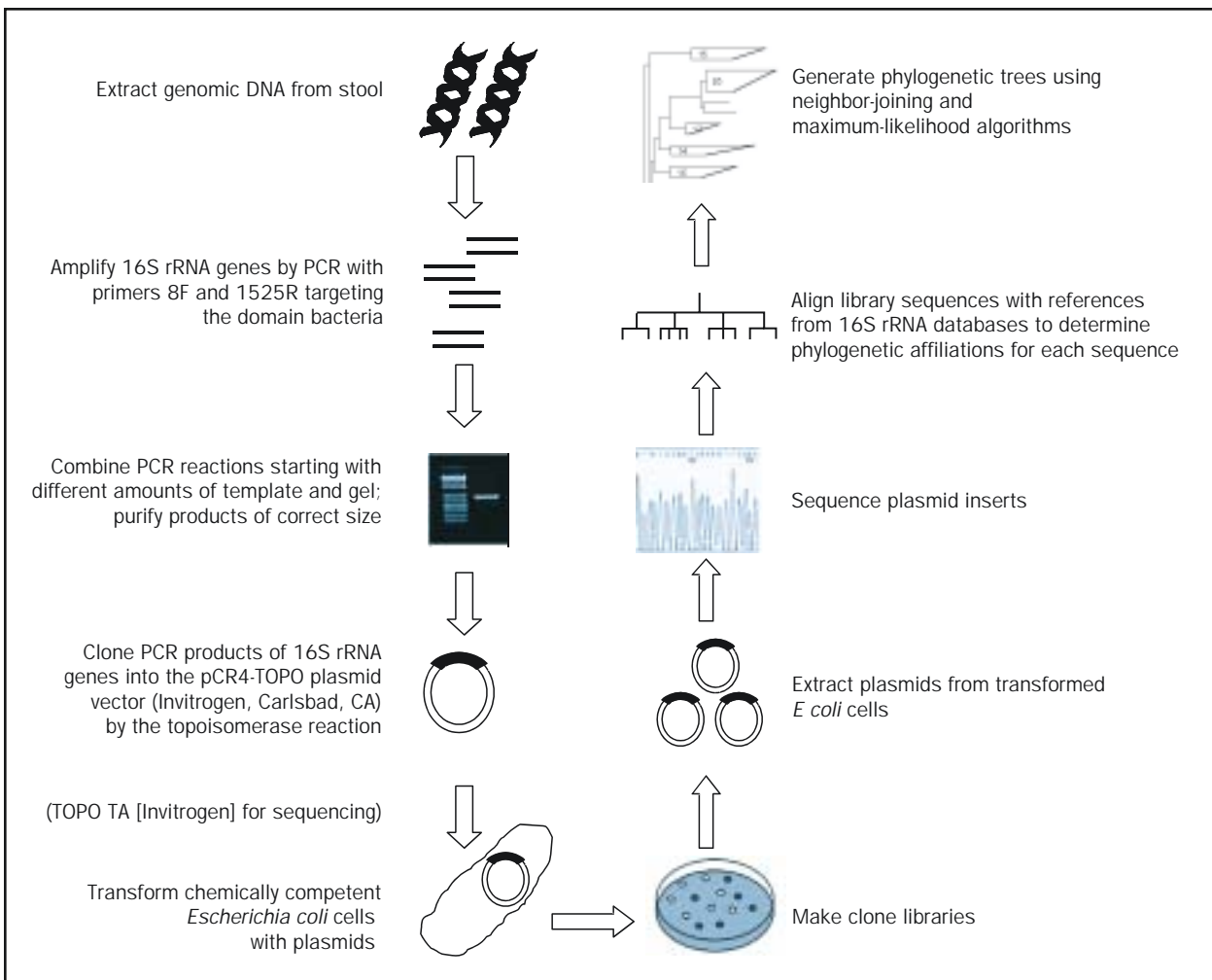


FIGURE 1. Steps for building a clone library to fingerprint a complex microbial community. PCR = polymerase chain reaction; rRNA = ribosomal RNA.

## INDIGENOUS GUT MICROBIOTA

### IDENTIFICATION

Until recently, our understanding of the human intestinal microbiota has been limited by reliance on conventional microbiological techniques (ie, selective culturing) and by our inability to culture many organisms in the gastrointestinal tract. With the development of methods for identifying gut microflora that do not require culturing (ie, molecular fingerprinting and ecological statistical approaches), a much more thorough and reliable assessment of the gut microbiota is now possible.<sup>11-13</sup> Specifically, the sequencing of 16S ribosomal RNA (rRNA) genes from amplified bacterial nucleic acid extracted from fecal material or mucosal samples has greatly facilitated the identification and classification of bacteria.<sup>14</sup> The study of entire microbial communities using metagenomic approaches based on

these molecular methods has revealed a much greater diversity in the bacterial and archaeal domains than was previously thought to exist and has helped determine the community structure of several other previously unknown ecosystems.<sup>11,14-17</sup> For the purposes of this article, *metagenomics* refers to the study of all genes existing within the human genome and within the gut microbial genomes. Metagenomic approaches have tremendous potential to improve our understanding of the ways in which commensal and pathogenic microorganisms adapt themselves in humans. Figure 1 summarizes the steps involved in building a clone library, the most commonly used technique for molecular fingerprinting.

Using these techniques, investigators have estimated that the gastrointestinal tract in an adult human contains approximately  $10^{12}$  microorganisms per milliliter of luminal content and harbors approximately 500 to 1000 distinct

TABLE. Major Bacteria and Archaea Phyla and Genera Found in the Human Gut Microbiota<sup>a</sup>

Phyla	Representative genera
Bacteria	
Firmicutes	<i>Ruminococcus</i> <i>Clostridium</i> <i>Peptostreptococcus</i> <i>Lactobacillus</i> <i>Enterococcus</i>
Bacteroidetes	<i>Bacteroides</i>
Proteobacteria	<i>Desulfovibrio</i> <i>Escherichia</i> <i>Helicobacter</i>
Verrucomicrobia <sup>b</sup>	
Actinobacteria	<i>Bifidobacterium</i>
Cyanobacteria <sup>b</sup>	
Synergistes <sup>b</sup>	
Archaea	
Euryarchaeota	<i>Methanobrevibacter</i>

<sup>a</sup> Prokaryotic phyla were identified by using an alignment of the 18,348-sequence dataset from reference 18.

<sup>b</sup> Not related to any known genera.

bacterial species.<sup>11,13,18</sup> A very recent report suggests that this number is in fact much higher, with at least 1800 genera and between 15,000 and 36,000 species of bacteria.<sup>19</sup> Life forms are divided into 3 domains: Eukaryota, the members of which contain a defined nuclear membrane separating the genome from cellular materials, and Bacteria and Archaea, which are prokaryotes lacking a DNA-containing nucleus. Prokaryotes are classified based on phylogeny (ie, 16S rRNA sequence similarities and differences). Although Archaea and Eukaryota domains are also represented in the gut, Bacteria clearly predominate.

Sequencing the 16S rRNA gene from clone libraries has shown that uncultivated species and novel microorganisms constitute a substantial fraction of the gut microbiota (Table). Using a cloning technique, Eckburg et al<sup>13</sup> recently conducted a comprehensive examination of the human colonic microbiota, finding that Bacteroidetes and Firmicutes account for more than 90% of all phylotypes of Bacteria and that *Methanobrevibacter smithii*, a hydrogen-consuming methanogen, dominates the Archaea domain.

As more microbial sequences become available, primers for real-time polymerase chain reaction will make it possible to quantify specific groups or species.<sup>20</sup> The growing database also allows design of molecular probes for quantitative real-time polymerase chain reaction, fluorescent in situ hybridization (FISH), and DNA microarray chips that identify specific bacterial species.

#### DEVELOPMENT

Despite our limited understanding of the composition of the indigenous gut microbiota, evidence suggests that it is established within the first year of life<sup>12,21</sup> and that the

transformation to adult-type microbiota is likely triggered by multiple host and external factors,<sup>22,23</sup> including the effects of the microbiota itself, developmental changes in the gut environment, and transition to an adult diet. The gut microbiota of the infant has long been thought to resemble that of the mother because most bacterial species are acquired during the birthing process.<sup>22</sup> However, this paradigm has been brought into question by recent evidence obtained using molecular techniques showing that children's stool samples do not resemble those of their parents more than those of other adults.<sup>12</sup> The gut microbiota remains remarkably constant after transformation to adult-type microbiota; however, transient changes can occur, and, as recently demonstrated by Ley et al<sup>24</sup> using culture-independent molecular methods, dietary factors can lead to long-term changes. This general stability is made possible by the recognition and tolerance of the infant-acquired microbiota by the gut immune system,<sup>25</sup> which, by being exposed to and sampling microbial antigens, identifies them as normal. In contrast, the gut microbiota of one person can differ markedly from that of another; greater diversity is also seen in luminal (ie, stool) vs mucosal (ie, epithelial) compositions.<sup>13</sup> Comparative studies of adults with varying degrees of relatedness have shown that host genotype is more important than diet, age, and lifestyle in determining the composition of the gut microbiota.<sup>26,27</sup>

The specific concentration and type of bacteria in the gastrointestinal tract are influenced by microhabitat variations throughout the gut, such as those in pH, oxygen, and nutrient availability. Figure 2 illustrates the key physiologic features of the human gut and the microbiological characteristics associated with them. Traditional culture-dependent microbiological studies have shown that the lower portion of the gastrointestinal tract has a higher bacterial count than the upper portion and that it is populated primarily by anaerobic bacteria, whereas the upper portion is populated largely by aerobic bacteria.<sup>21,28</sup> Indeed, the terminal ileum is said to represent a transition zone between the aerobic microflora found in the proximal gut and the anaerobic organisms found in the colon.<sup>21</sup> Once across the ileocecal valve, bacterial counts increase from 10<sup>7</sup> to 10<sup>9</sup>/mL in the terminal ileum to approximately 10<sup>10</sup> to 10<sup>12</sup>/mL in the colon.<sup>21</sup> Recent molecular analyses indicate that the same bacteria phyla are present in the different anatomic regions of the gut and that only the relative abundance of the subgroups of the prevalent phyla varies.<sup>19</sup>

#### METABOLIC FUNCTIONS

Studies using germ-free (ie, gnotobiotic) mice have shown that the gut microbiota is critical for maintaining normal gastrointestinal and immune function and normal digestion of nutrients.<sup>21,29-31</sup> Although incompletely understood, the

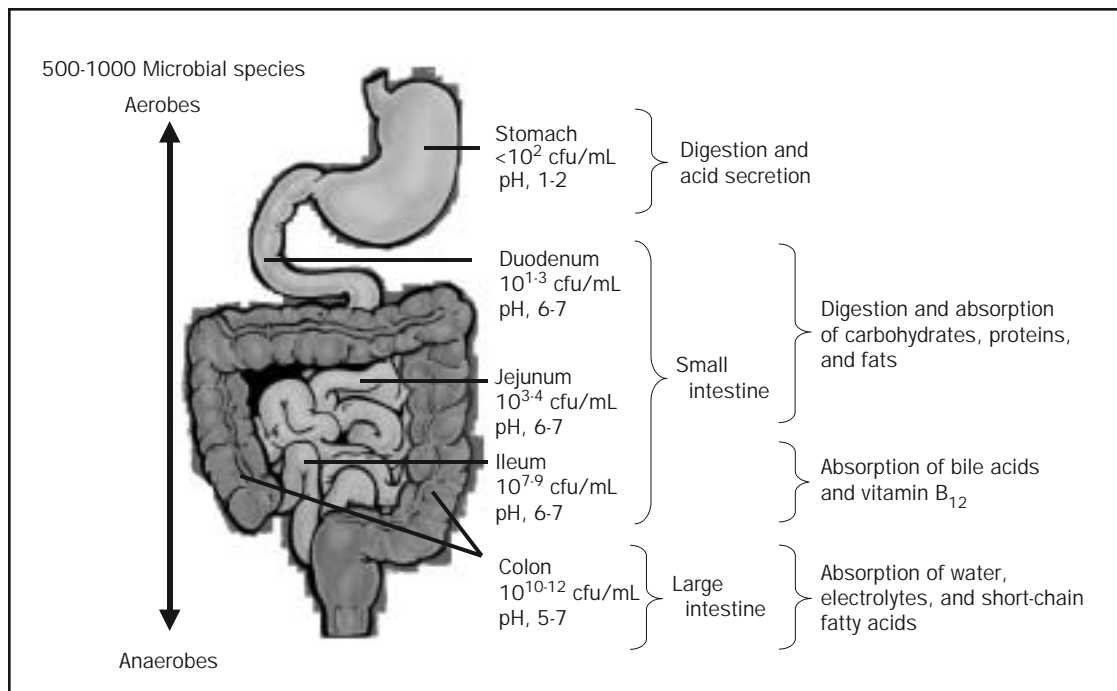


FIGURE 2. Key physiologic and microbiological features of the gut. Relative concentrations of bacteria and the pH at various locations within the adult gut are also noted. cfu = colony-forming unit.

gut microbiota is implicated in a variety of host functions involving intestinal development and function, including epithelial turnover, immune modulation, gastrointestinal motility, and drug metabolism.<sup>25,28,32-35</sup> The gut microbiota also has important metabolic functions, breaking down dietary toxins and carcinogens, synthesizing micronutrients, fermenting indigestible food substances, assisting in the absorption of certain electrolytes and trace minerals, and affecting the growth and differentiation of enterocytes and colonocytes through the production of short-chain fatty acids.<sup>31,36,37</sup> Finally, the normal gut microbiota helps prevent luminal colonization by pathogenic bacteria, such as *Escherichia coli* and *Clostridia*, *Salmonella*, and *Shigella* species.<sup>38,39</sup> Findings from a recent study by Gill et al<sup>11</sup> emphasize the important symbiotic contributions (in diversity and function) to the human metabolism made by the collection of microbial genomes known as the *microbiome*. After analyzing the fecal microbial community in healthy human participants, these investigators searched the DNA libraries for gene sequences that encode for enzymes known to participate in metabolism. They compared the translated enzyme sequences of the microbes to the ones in the human host and identified enzymes that affect host metabolism by maximizing the energy value of ingested food, promoting host homeostasis, and decontaminating the intestine.

## MICROBIAL CONTRIBUTIONS TO OBESITY

The metabolic activities of the gut microbiota facilitate the extraction of calories from ingested dietary substances, help to store these calories in host adipose tissue for later use, and provide energy and nutrients for microbial growth and proliferation. Individual differences in energy recovery may provide a physiologic explanation for the observation that some obese patients do not seem to overeat. Indeed, it has been suggested that a person's gut microbiota has a specific metabolic efficiency and that certain characteristics of the microbiota composition might predispose to obesity.<sup>34</sup>

### DIETARY ENERGY EXTRACTION

In an elegant series of experiments, Backhed et al<sup>40</sup> found that young conventionally reared mice have a 40% higher body fat content and 47% higher gonadal fat content than germ-free mice even though they consumed less food than their germ-free counterparts. The distal gut microbiota from the normal mice was then transplanted into the gnotobiotic mice (a process known as conventionalization), resulting in a 60% increase in body fat within 2 weeks without any increase in food consumption or obvious differences in energy expenditure. This result supports the hypothesis that the composition of the gut microbiota af-

fects the amount of energy extracted from the diet. The increase in body fat was accompanied by insulin resistance, adipocyte hypertrophy, and increased levels of circulating leptin and glucose.

To elucidate potential underlying mechanisms, these investigators showed that the microbiota promoted absorption of monosaccharides from the gut and induced hepatic lipogenesis in the host, responses mediated by 2 signaling proteins, carbohydrate response element-binding protein (ChREBP) and liver sterol response element-binding protein type-1 (SREBP-1). Finally, using genetically modified (fasting-induced adipocyte factor [Fiaf]–knockout) mice, they showed that gut microbes suppress intestinal Fiaf, also known as angiopoietin-like protein 4. Fasting-induced adipocyte factor inhibits lipoprotein lipase activity, thereby catalyzing the release of fatty acids from lipoprotein-associated triacylglycerols, which are then taken up by muscle and adipose tissue. In the study, Fiaf suppression resulted in increased lipoprotein lipase activity in adipocytes and promoted storage of calories as fat, leading Backhed et al to postulate that energy regulation by the gut microbiota occurs through a number of interrelated microbial mechanisms. These mechanisms include fermentation of indigestible dietary polysaccharides to absorbable forms, intestinal absorption of monosaccharides and short-chain fatty acids with their subsequent conversion to fat within the liver, and regulation of host genes that promote deposition of fat in lipocytes.

In a separate study designed to further explore the mechanism(s) underlying the resistance to obesity in germ-free mice, Backhed et al<sup>41</sup> studied germ-free mice consuming a Western-style, high-fat, sugar-rich diet. They determined that germ-free animals were protected from diet-induced obesity by 2 complementary but independent mechanisms that result in increased fatty acid metabolism: (1) elevated levels of Fiaf trigger the production of peroxisome proliferator-activated receptor  $\gamma$  coactivator, which is known to increase expression of genes encoding regulators of mitochondrial fatty acid oxidation; and (2) the activity of adenosine monophosphate–activated protein kinase, an enzyme that monitors cellular energy status, is increased. These findings suggest that the gut microbiota can affect both sides of the energy balance equation, influencing energy harvest from dietary substances (Fiaf) and affecting genes that regulate how energy is expended and stored.<sup>41</sup>

In a proof-of-principle study, Turnbaugh et al<sup>42</sup> sought to understand how the gene content in the gut microbiota contributes to obesity. First, they characterized the distal gut microbiomes of genetically obese leptin-deficient (*ob/ob*) mice and their lean (*ob/+* and *+/+*) littermates. Mice were used to avoid confounding variables such as diet, environment, and genotype that make such studies in humans difficult to interpret. In a series of experiments incor-

porating comparative metagenomics, these investigators showed that the microbiota in the *ob/ob* mice contained genes encoding enzymes that break down otherwise indigestible dietary polysaccharides. They also found more end products of fermentation (eg, acetate and butyrate) and fewer calories in the feces of the obese mice, leading them to speculate that the gut microbiota in these mice facilitate the extraction of additional calories from ingested food.

To further show that the composition of the gut microbiota is important in determining weight, the investigators transferred the gut microbiota of either *ob/ob* mice or lean mice to lean gnotobiotic mice. After 2 weeks, the recipients of the microbiota from the *ob/ob* mice extracted more calories from food and also showed a significantly greater fat gain than did mice that received the microbiota from lean mice (mean percent of fat gain  $\pm$  SD, 47% $\pm$ 8.3% vs 27% $\pm$ 3.6%; representing a difference of 4 kcal or 2% of total consumed calories based on the assumption that there are 9.3 kcal in a gram of fat).<sup>42</sup> These results suggest that differences in caloric extraction of ingested food substances may be determined by the composition of the gut microbiota, further supporting a microbial component in the pathogenesis of obesity. They also raise a number of questions. Do these small changes in energy extraction contribute to clinically meaningful differences in weight? How do conditions in the host (ie, genetic mutation in leptin in the *ob/ob* mouse) result in differences in the composition of the gut microbiota? Do these differences persist over time? Future studies are needed to clarify these issues.

#### CHRONIC SYSTEMIC INFLAMMATION

On the basis of the recent demonstration that obesity and insulin resistance are associated with low-grade chronic systemic inflammation,<sup>43</sup> Cani et al<sup>44</sup> postulated another mechanism linking the intestinal microbiota to the development of obesity. They hypothesized that bacterial lipopolysaccharide (LPS) derived from gram-negative bacteria residing in the gut microbiota acts as a triggering factor linking inflammation to high-fat diet–induced metabolic syndrome. In a series of experiments in mice fed a high-fat diet, they showed that (1) a high-fat diet increases endotoxemia and affects which bacterial populations are predominant in the intestinal microbiota (ie, it reduced both gram-negative [*Bacteroides*-related bacteria] and gram-positive bacteria [*Eubacterium rectale*—*Clostridium coccoides* group and bifidobacteria], favoring an increase in the gram-negative to gram-positive ratio), and that (2) chronic metabolic endotoxemia induces obesity, insulin resistance, and diabetes. Using CD14 mutant mice fed a high-fat diet, they showed that metabolic endotoxemia triggers the expression of inflammatory cytokines (eg, tumor necrosis factor  $\alpha$ , interleukin 1, interleukin 6, and plas-

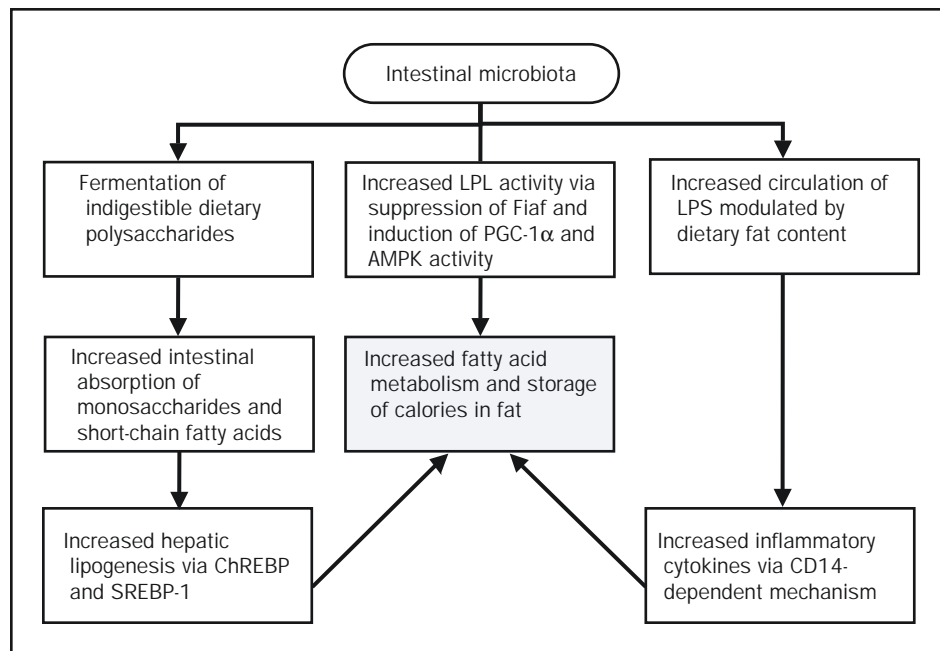


FIGURE 3. Mechanisms by which the intestinal microbiota may contribute to obesity. AMPK = adenosine monophosphate-activated protein kinase; ChREBP = carbohydrate response element-binding protein; Fiaf = fasting-induced adipocyte factor; LPL = lipoprotein lipase; LPS = lipopolysaccharide; PGC-1 $\alpha$  = peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ ; SREBP-1 = sterol response element-binding protein type 1.

minogen activator inhibitor 1) via a CD14-dependent mechanism. A key molecule binding LPS at the surface of innate immune cells, CD14 triggers the secretion of proinflammatory cytokines.<sup>45</sup> It has been suggested that the LPS/CD14 system sets the tone of insulin sensitivity and regulates the onset of obesity and diabetes.<sup>44</sup> Human studies have provided support for these findings. Treatment of humans with polymyxin B, an antibiotic that specifically targets gram-negative organisms, was shown to reduce LPS expression and hepatic steatosis.<sup>46</sup> A more recent study reported that patients with type 2 diabetes had higher LPS levels than did a well-matched group of control participants without diabetes.<sup>47</sup> Figure 3 summarizes the possible mechanisms by which the gut microbial community can contribute to obesity.

#### GUT MICROBIOTA COMPOSITION IN OBESE VS LEAN MICE

To assess the relative abundance of various types of gut bacteria in obese and lean mice, Ley et al<sup>18</sup> analyzed bacterial 16S rRNA gene sequences from the cecal microbiota of genetically obese (*ob/ob*) mice, their lean *ob/+* and *+/+* siblings, and their *ob/+* mothers, all fed the same polysaccharide-rich diet. They found that the *ob/ob* mice had 50% fewer Bacteroidetes and correspondingly more Firmicutes than their lean littermates, a finding unrelated to differences in food consumption. These changes were seen

throughout the division and were not due to an increase or decrease in the numbers of a few Bacteroidetes or Firmicutes members. The mechanisms responsible for this difference require further study. Ley et al also showed a strong association between kinship and distal gut microbial diversity, but the differences seen in obese mice occurred independently of kinship and sex. These results suggest that differences exist in the gut microbiota of obese vs lean mice, raising the possibility that the manipulation of gut microbiota could be a useful strategy for regulating energy balance in obese people.

#### GUT MICROBIOTA COMPOSITION IN OBESE VS LEAN HUMANS

To show the relevance of the animal experiments to humans, Ley et al<sup>24</sup> serially monitored the fecal gut microbiota in 12 obese participants in a weight-loss program for a year, randomly assigning them to either a fat-restricted or carbohydrate-restricted low-calorie diet. As in the mice experiments, members of the Bacteroidetes and Firmicutes divisions dominated the microbiota, and bacterial flora showed remarkable intraindividual stability over time. Before diet therapy, obese participants had fewer Bacteroidetes and more Firmicutes than lean control participants. After weight loss, the relative proportion of Bacteroidetes increased, while Firmicutes decreased, a

finding that correlated with the percentage of lost weight and not with changes in dietary caloric content. Bacteroidetes constituted approximately 3% of the gut bacteria before diet therapy and approximately 15% after successful weight loss. It is unknown why obese people have more Firmicutes. The host gut may have uncharacterized properties that select this bacterial phylum, which contains more than 250 genera and has diverse metabolic capabilities. For example, many of the *Bacillus* species are facultative aerobes, whereas the *Clostridium* species are obligate anaerobes. The vast diversity within Firmicutes may contribute to more efficient energy extraction from a variety of complex organic matter. Clearly, additional work is needed to better clarify the cause-and-effect relationship between obesity and the gut microbiota.

#### ENERGY HOMEOSTASIS

Although Bacteroidetes and Firmicutes are the dominant microbial organisms in the gut, methanogenic Archaea are also present. Archaeal methanogenesis improves the efficiency of polysaccharide fermentation by preventing the buildup of hydrogen and other reaction end products. In contrast, the formation of methane creates a large electron and energy sink; that energy is then unavailable for uptake by an animal. Cattle growers try to suppress methanogenesis in the cow's rumen for this reason. Unlike the rumen, which harbors acetate-utilizing methanogens such as *Methanosarcina* species,<sup>48</sup> the human gastrointestinal tract is dominated by hydrogen- and formate-oxidizing *Methanobrevibacter* species,<sup>13</sup> suggesting that acetate and butyrate produced by fermentative bacteria in the colon are not consumed by methanogens. By removing hydrogen and formate, *Methanobrevibacter* species may help the bacterial community produce more acetate and butyrate, which are important carbon sources for colon epithelium cells. As a result, this type of Bacteria-Archaea syntrophism in humans may lead to increased energy extraction from indigestible polysaccharide diets.

To better understand the contributions of specific microbes, Samuel and Gordon<sup>49</sup> colonized the gut of germ-free mice with *M smithii*, *Bacteroides thetaiotaomicron*, or both. *Bacteroides thetaiotaomicron* is a common colonic bacteria that is highly efficient in glycan metabolism, allowing otherwise indigestible sugars to be metabolized and harvested as additional energy.<sup>50</sup> *Methanobrevibacter smithii* is the most prominent Archaea in humans, constituting 10% of all anaerobes in the colons of healthy adults<sup>31</sup>; however, the role of Archaea in human health remains uncertain. Samuel and Gordon<sup>49</sup> found that cocolonization with *M smithii* and *B thetaiotaomicron* increased the efficiency of energy extraction from dietary polysaccharides and the amount of host adiposity more than did colo-

nization with either organism alone. Furthermore, Samuel et al<sup>51</sup> found that *M smithii* influenced the metabolism of *B thetaiotaomicron*, prompting it to consume mainly fructose-containing polysaccharides that break down into several substances, including formate, an important energy source of *M smithii*. These findings not only suggest a contribution of Archaea to digestive health but also show that interactions among microorganisms in the gut have a role in host energy homeostasis. These findings also raise the intriguing possibility of *M smithii* as a therapeutic target for reducing energy harvest in obese humans.

#### MODIFYING THE ECOSYSTEM AS A THERAPEUTIC STRATEGY

The best nonsurgical strategy for reversing obesity in the population may be to promote small but long-term changes in diet and physical activity that take advantage of our biological systems for regulating energy balance and preventing positive energy balance.<sup>3</sup> Although the role of the gut microbiota in energy regulation remains undefined, the existence of systems that regulate energy balance suggests that microorganisms might have a substantial cumulative effect over time. Although clearly no substitute for proper diet and exercise, manipulation of the gut microbiota may represent a novel approach for treating obesity, one that has few adverse effects. Use of antibiotics, prebiotics, and probiotics may result in nonspecific modulation of the gut microbiota.

#### ANTIBIOTICS

Recently, Brugman et al<sup>52</sup> showed that antibiotic treatment decreased the incidence and delayed the onset of diabetes in a diabetes-prone rat model. Using FISH, they showed that the gut bacterial composition of rats that developed diabetes differed from that of those that did not. Specifically, rats that did not develop diabetes displayed a lower number of *Bacteroides* species. The investigators speculated that the antibiotic-induced alteration in the gut microbiota led to a reduction in the antigenic load and subsequent inflammation that usually leads to pancreatic  $\beta$ -cell destruction. Although the study by Brugman et al did not directly address obesity, it demonstrates the potential of modulating the intestinal microbiota as a therapeutic strategy.

#### PREBIOTICS

Prebiotic agents are nondigestible oligosaccharides that act as "fertilizers" of the colonic microbiota, enhancing the growth of beneficial commensal organisms (eg, *Bifidobacterium* and *Lactobacillus* species).<sup>53</sup> Fructo-oligosaccharides are prebiotic agents that are fermented by a number of colonic bacteria to modulate the growth of beneficial

colonic bacteria. Inulin and oligofructose, naturally occurring fructo-oligosaccharides that are not digested in the upper gastrointestinal tract, have several functional and nutritional properties, including the ability to stimulate the growth of beneficial commensal organisms.<sup>53,54</sup> In 2 recent studies in which rats were fed a standard<sup>55</sup> or high-fat diet,<sup>56</sup> the addition of oligofructose to the diet reduced energy intake and consumption and protected against weight gain and fat-mass development, effects shown to be mediated by the modulation of endogenous gut peptides involved in appetite and weight regulation.<sup>57</sup> This result seems to contradict previously discussed findings suggesting that nondigestible polysaccharides may be responsible, at least in part, for increased weight in genetically obese mice because of increased energy extraction.<sup>42</sup> This discrepancy could result from a number of factors, including specific modulation of the gut microbiota (as yet poorly understood) and other physiologic effects of fibers, such as slowed gastric emptying and increased satiety.<sup>58</sup> Additional support for the role of prebiotics in reducing weight gain was provided by a study in which the addition of inulin and lupin-kernel fiber, also nondigestible starches, as fat replacers in a sausage patty was shown to reduce fat and energy intake in healthy humans.<sup>59</sup> In a single-blind, crossover pilot study of 10 healthy people of normal weight, a 2-week treatment with oligofructose was shown to increase satiety after breakfast and dinner and to markedly reduce hunger and prospective food consumption after dinner, leading to a total energy intake per day that was 5% lower than that of the placebo group.<sup>60</sup>

In a recent study of the link between prebiotics and endotoxemia, Cani et al<sup>61</sup> found that oligofructose increased the gut bifidobacterial content of high-fat diet-fed mice and that endotoxemia significantly and negatively correlated with *Bifidobacterium* species but with no other bacterial group. They also showed that, in high-fat oligofructose-treated-mice, a significant and positive correlation existed between *Bifidobacterium* species and improved glucose tolerance, glucose-induced insulin secretion, and normalized inflammatory tone. Although indirect, these lines of evidence present a rationale to warrant further investigation of the use of prebiotic supplementation to modify the gut microbiota in the management of food intake in people who are overweight and obese.

#### PROBIOTICS

Probiotics are nonpathogenic live microorganisms that, when ingested, confer health benefits to the host.<sup>62</sup> Probiotics have generated considerable interest in recent years because studies investigating their use in a variety of clinical conditions, particularly diarrheal disorders, have yielded encouraging results.<sup>63</sup> A potential role for pro-

biotics in the treatment of obesity has been suggested by 2 recent reports. Lee et al<sup>64</sup> investigated the antiobesity effect of *Lactobacillus rhamnosus* PL60, a bacterium of human origin that produces conjugated linoleic acid, in diet-induced obese mice. Conjugated linoleic acid has been suggested to have a number of potential health effects in animal studies, including the ability to reduce body fat.<sup>65</sup> After 8 weeks of oral feeding with *L rhamnosus* PL60, mice lost weight without reducing energy intake. Further studies by these investigators suggested that the antiobesity effects were possibly related to apoptosis and messenger RNA expression in white adipose tissue. However, it should be noted that *L rhamnosus* PL60 did not reduce cell size in epididymal adipose tissue, and thus the decrease in the weight of white adipose tissues was due to reduction in cell number rather than in cell size. Because the number of adipose cells is constant in adult humans and only the cell size changes with obesity, the role of *L rhamnosus* PL60 in humans is unclear. Further supporting the inefficacy of this particular probiotic approach are the results of a recent randomized controlled study in which 122 obese humans were treated with 3.4 g of conjugated linoleic acid or placebo for 1 year.<sup>66</sup> Sonnenburg et al<sup>67</sup> colonized germ-free mice with *B thetaiotaomicron* and *Bifidobacterium longum*, a commonly used probiotic. They found that, when *B thetaiotaomicron* encountered *B longum*, it expanded the range of polysaccharides targeted for degradation and did so independently of host genotype; however, it did not do so for all bifidobacteria (eg, *Bifidobacterium animalis*) with which it was cocultured. Similar metabolic effects were achieved with a probiotic from another division of Bacteria (*Lactobacillus casei*). In another demonstration that probiotics can exert metabolic effects on the host, Martin et al<sup>68</sup> administered probiotic beverages to germ-free mice that had been conventionalized with human baby flora. Using high-density data-generating spectroscopic techniques in combination with multivariate mathematical modeling, they showed that probiotic exposure resulted in distinct changes in the microbiome with associated metabolic alterations in a variety of tissues affecting energy, lipid, and amino acid metabolism. The importance of these findings to energy homeostasis and overall health in humans remains to be determined; however, they suggest that probiotics can alter the dynamics of the entire gut microbiota and show that these molecular approaches can be used to study the metabolic effects of probiotics on the host and the host's microbiome.

#### FUTURE DIRECTIONS

Studies are needed to clarify a number of issues related to the relationship between the gut microbiota and obesity.

First, it remains to be determined whether small changes in caloric extraction, seen in several past studies, can result in clinically meaningful differences in weight in humans. In principle, small but persistent changes in energy homeostasis, in this case from increased energy extraction, should lead to changes in body composition and weight.<sup>7</sup> Second, the possible relationship between the gut microbiota—including previously cultured and uncultivated strains, as well as rare and abundant microbes—and the regulation of weight must be proved or disproved. In particular, it is essential to demonstrate unequivocally whether differences in gut microbiota in obese vs lean people are the cause or the result of obesity. In this regard, hormonal or other signals that potentially direct changes in the make-up of the gut microbiota need to be elucidated. Of immediate benefit will be metagenomic studies of the gut microbiota in mice with diet-induced obesity and microbiota transplant studies similar to those previously described in genetically induced obese mice. Third, the mechanisms responsible for the relative proportions of Bacteroidetes, Firmicutes, and Archaea in mice and humans must be explored. In particular, the environmental and genetic factors that determine the distinctive characteristics of each person's microbiota must be identified. Fourth, differences in surface-adherent mucosal microbial colonies (vs those found within the bowel lumen) must be defined for people who are obese and for those who have successfully lost weight. Similarly, the factors that affect the local microbial ecology, particularly of the adherent microbes, must be investigated and identified. Finally, a means to deliberately modify the gut microbiota must be proposed and then examined in well-controlled and closely monitored studies. Clinical trials assessing the efficacy of prebiotics and probiotics should evaluate participants' intestinal microbiota before and after therapy. Furthermore, given recent reports questioning the safety of probiotics,<sup>69,70</sup> further research is needed to establish the safety of this approach.

## CONCLUSION

The worldwide obesity epidemic has intensified efforts to identify host and environmental factors that affect energy balance. One emerging finding is that the host and its microbiota have mutually beneficial and cooperative interactions. The evidence reviewed in this article, much of it obtained recently using powerful tools such as sequencing of 16S rRNA gene clone libraries, metagenomics, DNA microarrays, microbiota transplant, and gnotobiotic knock-out mice, suggests that the gut microbiota has a role in the regulation of energy balance and weight. It further suggests a role for a gut-derived factor, such as LPS, in the pathogenesis of obesity-related type 2 diabetes. Although these

findings are promising, studies are needed both to better understand the causal relationship between gut microbiota of varied composition and the propensity to be obese or lean and to assess whether modulating the gut microbiota could help to reduce obesity.

## ADDENDUM

After the manuscript had been accepted for publication, an article was published with relevance to our review. Kalliomäki et al<sup>71</sup> prospectively followed children from birth to age 7 years. Fecal samples collected at ages 6 and 12 months were analyzed using a variety of molecular techniques. Higher numbers of bifidobacteria and lower numbers of *Staphylococcus aureus* were found in children who were normal weight at age 7 years than in those who were overweight-obese, suggesting that differences in the composition of the gut microbiota precede overweight-obesity.

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