Intestinal microbes regulate metabolic function and energy balance; an altered microbial ecology is believed to contribute to the development of several metabolic diseases. Relative species abundance and metabolic characteristics of the intestinal microbiota change substantially in those who are obese or have other metabolic disorders and in response to ingested nutrients or therapeutic agents. The mechanisms through which the intestinal microbiota and its metabolites affect host homeostasis are just beginning to be understood. We review the relationships between the intestinal microbiota and host metabolism, including energy intake, use, and expenditure, in relation to glucose and lipid metabolism. These associations, along with interactions among the intestinal microbiota, mucus layer, bile acids, and mucosal immune responses, reveal potential mechanisms by which the microbiota affect metabolism. We discuss how controlled studies involving direct perturbations of microbial communities in human and animal models are required to identify effective therapeutic targets in the microbiota.

Keywords: Obesity; Energy Expenditure; Bile Acids; Gut Microbiota.
Obesity and insulin resistance are closely related to the presence of (visceral) adipose tissue inflammation. For example, adipose tissue produces numerous inflammatory cytokines, and there is evidence that visceral adipose tissue—and, to a lesser extent, subcutaneous adipose tissue—can promote insulin resistance. The presence of subcutaneous adipose tissue macrophages and crown-like structures (or accumulated CD68-positive macrophages) correlates with expression of genes that control inflammation, indicating a role for the innate immune system in adipose tissue inflammation. Changes in intestinal microbiota cause long-term changes in inflammation in obese subjects; bacterial endotoxins such as LPS activate pattern recognition receptors such as Toll-like receptors, leading to an innate immune response and insulin resistance. The intestinal microbiota also produces many molecules that promote inflammation, such as peptidoglycans and flagellins, which activate inflammatory pathways, resulting in obesity and insulin resistance.

Short-chain fatty acids (SCFAs) are produced by intestinal bacteria and may play important roles in dampening the adverse effects of intestinal pathogens on host metabolism. Diet-derived fibers are metabolized and fermented to SCFAs such as acetate, propionate, and butyrate by intestinal bacteria. Germ-free mice produce almost no SCFAs and therefore have altered responses to inflammatory stimuli. SCFAs may serve as an energy source for the intestinal epithelium and liver, given their transport predominantly via the portal vein after intestinal absorption. SCFAs might also modulate the immune response by reducing intestinal permeability. This hypothesis is supported by the observation that translocation of LPS from the gut to the portal vein produces obesity-associated low-grade inflammation and subsequent insulin resistance in mice, which can partly be reversed by administration of the propionate-producing bacteria Akkermansia muciniphila.

Besides obesity, the intestinal microbiota might also be involved in atherogenesis. Specific dietary nutrients characterized by trimethylamine groups (e.g., choline, phosphatidylcholine, and carnitine) are metabolized into the atherogenic compound trimethylamine-N-oxide (TMAO) by bacteria. Studies using germ-free mice or mice given broad-spectrum antibiotics showed that the intestinal microbiota is required for formation of trimethylamine (TMA) and TMAO. Further, bacterial colonization of germ-free mice increases their plasma levels of TMAO, indicating that the intestinal microbiota is required for generation of TMA from sources of dietary choline or carnitine (such as eggs, milk, and red meat). For example, carnitine is an abundant nutrient in red meat, and the intestinal microbiota mediates production of TMAO from dietary L-carnitine.

**Alteration of the Gut Microbiota in Obesity and Diabetes**

The intestinal microbiota is altered in humans and animal models of obesity. The intestinal (cecum-derived) microbiota of ob/ob mice has a 50% reduction in levels of Bacteroidetes and an increased proportion of Firmicutes compared with wild-type mice. The composition of the fecal microbiota of obese human subjects is similarly affected but changes with weight loss. Studies in germ-free mice provide insights into the effects of the intestinal microbiota on host metabolism. Germ-free mice fed high-fat, high-sugar diets did not have the same metabolic disturbances as the littermates that were not germ free. Transfer of intestinal microbiota from obese mice resulted in significantly greater adiposity in recipients than transfer of microbiota from lean donors.

One way in which intestinal microbes might affect host metabolism is by extracting calories from otherwise indigestible carbohydrates; these carbohydrates are fermented by intestinal microbes to produce SCFAs. SCFAs may act as an energy substrate as they are absorbed by the intestinal epithelium and metabolized in the liver. Mouse models of obesity and human obese subjects have increased intestinal (cecal) levels of SCFA and decreased energy content in their feces.

Studies have associated changes in proportions of Bacteroidetes and Firmicutes with obesity and metabolic syndrome. Metagenome-wide association studies by Qin et al (performed in China) and Karlsson et al (performed in Europe) reported metagenomic differences between a cohort of patients with type 2 diabetes mellitus and a group of healthy subjects. Clusters of genomic sequences were used as signatures for specific groups of bacteria, and each study found independently that the microbiota of subjects with type 2 diabetes mellitus had a lower proportion of butyrate-producing Clostridiales (Roseburia and Faecalibacterium prausnitzii), and greater proportions of Clostridiales that do not produce butyrate, as well as pathogens such as Clostridium clostridioforme. Other associations differed between the 2 studies. Karlsson et al detected an increased proportion of Lactobacillus gasseri and Streptococcus mutans (commensal bacteria in the mouth and upper intestinal tract) in their cohort with type 2 diabetes mellitus. Qin et al observed a greater proportion of Escherichia coli (which produce LPS to cause endotoxemia) in patients with type 2 diabetes mellitus. These studies raise interest in the association between type 2 diabetes mellitus and reduced production of butyrate because diets supplemented with butyrate were previously shown to prevent and reverse insulin resistance in mice that became obese on high-calorie diets and increase energy expenditure. Combined results from human and animal studies of obesity suggest that reduced butyrate production by the microbiota contributes to the development of insulin resistance.

**Microbiota and Bile Acids**

Bile acids are secreted as glycochenzyme, taurine, or sulfate conjugates. Compounds excreted in bile reach the intestinal tract, where they can be deconjugated by gut microbiota, aided by populations of microorganisms with their own hydrolytic enzymes such as β-glucuronidase and sulfatases. Most liver-secreted bile acids (95%) are reabsorbed in the ileum to be taken up by the liver in the enterohepatic...
cycle. Only a small part of the bile acid pool escapes the enterohpatic cycle and travels toward the large intestine to be excreted in the feces.

This excretion is accompanied by microbial deconjugation of glycine- and taurine-conjugated bile acid; germ-free mice were shown to have altered metabolism of bile acids. Follow-up studies showed that germ-free mice had increased levels of conjugated bile acids throughout the intestine, with no deconjugation, and strongly decreased fecal excretion. More recently, studies with antibiotics provided further support for the importance of intestinal microbiota in bile acid metabolism. Mice given vancomycin for 3 days increased biliary bile acid output 3-fold, whereas fecal output decreased 70%. We recently showed that vancomycin therapy (which eradicates gram-positive bacteria) of patients with insulin resistance also significantly altered the fecal bile acid pool, reducing the proportion of secondary bile acids, compared with primary bile acids, which is associated with reduced insulin sensitivity. Gram-positive bacteria in the intestine therefore affect the bile acid pool, which alters regulation of lipid and glucose metabolism via the FXR and TGR5 signaling pathways.

### Intestinal Microbiota and Host Physiology and Diseases

Recent advances in sequencing technology and large-scale information processing have increased our understanding of the diversity of the intestinal microbiota and its relationship to host physiology and diseases. Comprehensive characterizations of the microbiota have revealed rapid and profound effects of environmental and physiological changes on the composition and metabolic activities of the microbiota. Conversely, the microbial ecology has been correlated with specific diseases such as obesity and insulin resistance, and microbiota transfer studies in animals and humans have shown that alterations in the gut microbiota contribute to the pathogenesis of these disorders.

Studies of development of the microbiota in children have provided important information about interactions between host tissues and the microbiota and how disruptions in the microbiota can contribute to disease development. Newborns have essentially no intestinal microbiota. However, low levels of bacterial translocation through the placental circulation likely provide a primitive bacterial community before birth. Nevertheless, the newborn intestine begins colonization by maternal and environmental bacteria during birth. The structure of this primary microbiota varies with mode of birth (cesarean section vs vaginal delivery), feeding (breastfeeding vs formula), and level of sanitation in the environment. After the first year of life, each infant develops a distinct microbial community; by approximately 3 years, microbial profiles resemble those of adults in patterns and diversity. The progressive maturation of the intestinal microbiota is likely to be important for the establishment of commensal and pathogen recognition by the immune system and determine later host-microbe interactions and susceptibility to infection, autoimmunity, and inflammatory diseases.

The composition of the gut microbiota remains relatively stable from late childhood to old age, when changes occur again. These late changes often correspond with age-related changes in diet and digestive physiology, but it is not clear how these changes affect the gut microbiota. The structure of the gut microbiota varies spatially along the intestinal tract and cross-sectionally. The bacteria that reside in close proximity to the mucosal surface are believed to interact more closely with the immune system, whereas bacteria that reside in the lumen may be more closely associated with food and products of digestion, influencing nutrient assimilation, signaling, and metabolic function.

Cani et al used the term “metabolic endotoxemia” to describe the inflammatory state often associated with the metabolic syndrome. This systemic inflammation is believed to occur as bacteria translocates through the gastrointestinal tract, increasing circulating levels of LPS. Obese subjects with insulin resistance have increased circulating levels of LPS compared with matched controls; endotoxemia is independently associated with energy intake even in lean men. Toll-like receptor 4--null mice are protected from diet-induced obesity and associated insulin resistance.

Antibiotics, which have been used against pathogenic bacteria for nearly a century, also alter the commensal bacterial community, affecting metabolic and immune regulation. Short-term intake of antibiotics, via medication or dietary exposure to antibiotic-treated animals, causes shifts in the microbiota that have detrimental effects on metabolism. Recent studies have associated cumulative antibiotic exposure with rates of obesity and metabolic disorders. Administration of subtherapeutic doses of antibiotics to young mice increases their adiposity and promotes metabolic dysfunction. Similar observations have been reported from epidemiological studies highlighting the importance of commensal intestinal bacteria for metabolic homeostasis.

### Luminal Factors That Regulate Host Metabolism

Commensal gut bacteria regulate immune and metabolic functions via several mechanisms. Bacteria release fatty acids, peroxidases, proteases, and bacteriocins that prevent pathogens from expanding in the intestinal community; as previously mentioned, bacterial disaccharidases ferment unabsorbed dietary carbohydrates into SCFAs. Although increased generation of SCFAs could increase energy uptake from ingested plant carbohydrates, recent studies have shown that higher levels of SCFAs are generated by the microbiota in the small intestine of lean subjects. The signaling properties of the altered SCFAs might therefore be partly responsible for the metabolic and immunologic effects of the intestinal microbiota. The microbiota also regulates hepatic production of triglycerides by suppressing lipoprotein lipase inhibitors (angiotensin-like 4, or fasting-induced adipose factor), resulting in continued expression of lipoprotein lipase and continued production of liver triglycerides. The microbiota has also been shown to regulate insulin sensitivity and the development of nonalcoholic
fatty liver disease through its influence on the production of inflammatory cytokines such as tumor necrosis factor α. It is unclear whether these changes are mediated by gut luminal signaling events or the accumulation of bacterial products in the portal circulation.

Diet is an important determinant of the composition of the gastrointestinal microbiota. Indigestible dietary carbohydrates are the main substrate available to intestinal bacteria, specifically resistant starches, dietary fibers, unabsorbed sugars, and alcohols. These are broken down via bacterial metabolism to complex monosaccharides and oligosaccharides and then to SCFAs. Other metabolites such as carbon dioxide and hydrogen are also produced, including branched-chain fatty acids, ammonia, choline, and mercaptan. These are important nutrients not only for the host but also for the commensal bacteria in the gut lumen. Levels of choline in the diet have been associated with low levels of γ-proteobacteria and high levels of Erysipelotrichi as well as hepatic steatosis in rodents and humans. Altered levels of choline may promote the development of nonalcoholic fatty liver disease through the effects of toxic methylamines and have been correlated with cardiovascular disease. SCFAs affect mediators of inflammation (such as tumor necrosis factor α and β interleukin-6, interleukin-1β) and prevent activation of the transcription factor nuclear factor κB. Moreover, butyrate has been shown in animal and cell culture models to stimulate leptin production by adipocytes and GLP1 secretion by the L cells, whereas propionate is used for gluconeogenesis and lipogenesis in the liver. Butyrate may also have direct effects on gene expression by acting as a histone deacetylase inhibitor and has been shown to affect DNA methylation, proliferation, and differentiation in mammalian colonic epithelial cells. The prebiotic inulin caused a specific increase in Bifidobacterium species in mice with diet-induced obesity and a corresponding reduction in adiposity, most likely mediated via increased intestinal SCFAs. Identical findings were reported in obese mice on weight and glucose or lipid metabolism after dietary enrichment with pure butyrate. Likewise, dietary fats have distinct effects on the gut microbiota; high-fat diets increase numbers of Firmicutes and Proteobacteria and subsequent inflammatory tone, potentially within 24 hours after the dietary composition is altered. The ability of the gut microbiota to affect host metabolism is therefore mediated by interactions between 4 key components: host nutrient intake, bile acids, luminal mucus layer, and the gut microbiota (and the SCFAs they produce). These interactions affect expression of hormones and signaling molecules, neuron activities, and immune function (Figure 1). The gastrointestinal mucus gel is divided into a secreted, loosely associated layer and a layer firmly attached to the mucosa. Mucus and degradation products of epithelial cells are powerful substrates for bacterial metabolism. Goblet cells produce a protective mucus layer that forms a barrier and provides lubricant for the gastrointestinal tract. Glycans, secreted by cells in the mucus layer, are a rich source of carbohydrates, comprising 50% to 80% of oligosaccharides by weight. Those with a deficiency in the production of fucose, a hexose sugar, are unable to glycosylate the terminal end of mucus glycans in the gut. Changes observed in the gut microbiota of (antibiotic-induced) fucose-deficient animals support a model in which the mucus layer interacts with and affects the quality of commensal mucosal bacteria. These data corroborate studies of vancomycin showing significant alterations in the commensal bacteria, with significant increases in bifidobacteria in mice and increased thickness of the luminal mucus layer. As previously described, proportions of Akkermansia, a mucin-degrading bacterium from the Verrucomicrobia, have been inversely correlated with body weight. Interestingly, diet-induced obese mice have decreased proportions of Akkermansia, whereas mice that have undergone gastric bypass surgery have increased proportions compared with before surgery. A prebiotic-enriched diet that contains oligofructose, a major constituent of the luminal mucin layer that activates intestinal goblet cells, increases concentrations of Akkermansia.

The SCFAs can signal through several G protein–coupled receptors, including GPR41 and GPR43. (encoded by FFAR2) are expressed by enteroendocrine cells and activated by SCFAs and other microbial metabolites. GPR41-knockout mice with conventional microbiota have reduced adiposity compared with wild-type mice with conventional microbiota, whereas germ-free wild-type and GPR41-knockout mice have similar levels of adiposity. These observations indicate a role for SCFA receptors in fat deposition. 4-Cresol, a metabolite by-product of tyrosine metabolism, is believed to be produced by Clostridium, Bifidobacterium, and Bacteroides fragilis. Levels of 4-cresol metabolites in human urine correlate with diverse pathophysiology, including obesity and inflammatory bowel disease, which are conditions that also have been associated with differences in the composition of the gut bacterial community.

**Therapeutic Potential of the Microbiota**

The large amount of evidence that a healthy microbiota is required for human health and protects against disease has led to much interest in its therapeutic potential. Fecal microbial transplantation (FMT) has been shown to be an effective therapy for recurrent or refractory Clostridium difficile–induced colitis; its success in early trials has led to more widespread use. The ability of the microbiota to transfer metabolic phenotypes among mice indicates the potential of FMT and related approaches for treating metabolic disease in patients. FMT from healthy, lean subjects to obese subjects has been reported to improve peripheral insulin sensitivity. These results provide an important proof of concept for microbiota-directed treatment of metabolic disorders with a distinct effect of the donor’s intestinal microbiota signature (so-called superdonor). Microbial communities change with body weight in rodents and humans. Proportions of A...
Figure 1. Mechanisms of bile acid and SCFA metabolism in the (A) physiological and (B) pathophysiological state. Altered SCFA production by intestinal microbiota leads to perturbations in bile acid, lipid, and glucose metabolism as well as increased intestinal permeability, resulting in aggravated metabolic endotoxemia and subsequent low-grade inflammation.
muciniphila, a mucin-dependent butyrate-producing strain that resides in the mucus layer, are inversely correlated with body weight and metabolic profile. Oral gavage of A muciniphila led to a corresponding increase in gut colonization and reversed high-fat diet–induced metabolic changes such as gain in fat mass, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance; these effects were not observed after transfer of heat-killed organisms. Akkermansia is only one of several bacterial genera in which the proportions are associated with metabolic function. Its ability to affect the metabolic phenotype, however, provides evidence that it might be possible to treat patients with defined intestinal bacterial populations (single or multiple organisms) rather than incompletely defined, patient-derived products.

Increasing our understanding of the mechanisms by which the microbiota influence energy balance and metabolic function could lead to interventions (eg, foods or medications that target specific host factors or bacteria) that achieve the physiological effects of a healthy microbiota. The observation that bacterial expression of the cutC gene increased the risk of cardiovascular disease provides a case in point. The product of the cutC gene increases the conversion of choline to TMA, a potential precursor of cardiovascular pathology. Manipulating the microbiota to decrease the prevalence of cutC-expressing organisms is one therapeutic approach. Therapies aimed at decreasing cutC expression or those that counteract the yield of TMA or the effects of TMA itself could offer significant new alternatives for the treatment of these metabolic disorders.

To date, the most effective sustainable treatment for weight reduction is Roux-en-Y gastric bypass (RYGB) surgery. Patients who undergo RYGB surgery lose ~30% of their excess body weight and have significant improvements in glucose homeostasis within the first year after surgery. Although the mechanisms that produce these effects are not clear, the intestinal bacteria greatly change after the procedure, characterized by increased proportions of Gammaproteobacteria (Escherichia) and Verrucomicrobia (Akkermansia). To test the hypothesis that some of the effects of RYGB surgery are mediated by interactions between host cells and the microbiota, researchers created a mouse model of RYGB. When cecal contents were transferred from mice that underwent gastric bypass surgeries into germ-free mice, the recipients showed significant weight loss and decreased fat mass compared with the recipients of cecal contents from mice that underwent a sham procedure.

On the other hand, the ability of the microbiota to transmit weight gain was recently shown using feces from weight-discordant pairs of human twins. Furthermore, transfer of fecal material from obese and lean littermates to germ-free mice produced a 15% to 17% greater increase in adiposity in recipients of microbiota from obese mice than lean mice. In a second set of experiments, recipients of microbiota from lean mice were cohoused with recipients of microbiota from obese mice and nonconventionalized germ-free mice. The microbiota from lean recipients appeared to spread to all groups of mice affecting body weight.

There are few data on the metabolic effects of FMT in humans. Recent studies have described alterations in the microbiota that induce changes in glucose and bile acid metabolism. In the first study, intestinal microbiota was transferred from lean human donors to recipients with metabolic syndrome via a postpyloric enteral feeding tube. A separate group of subjects underwent autologous...
transplant of intestinal microbiota (reinfusion of their own microbiota [controls]). Insulin sensitivity was measured in recipients of the allogeneic stool preparation at baseline and after 6 weeks using the hyperinsulineemic euglycemic clamp technique. Six weeks after allogeneic FMT, insulin sensitivity significantly increased, with higher median rates of glucose disappearance. Gut microbial diversity also increased after allogeneic FMT but was unchanged in the control group. Fecal SCFAs were also significantly altered after allogeneic FMT compared with controls. These experiments suggest that increased bacterial diversity is associated with reduced insulin resistance. However, this was a small study and FMT from only specific donors had beneficial effects (eg, super-donor), so further studies are needed and currently ongoing in our department.

In conclusion, the intestinal microbiota is an important factor in the prevention and treatment of metabolic dysregulation. Studies that have examined the effects of transferring microbiota between conventional and germ-free rodents, humans and rodents, and humans and humans have shown its role in the development of obesity and subsequent insulin resistance. Whether and to what extent specific intestinal bacterial strains are relevant diagnostic and therapeutic targets remains to be proven.

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