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Review

The Metabolic Role and Therapeutic Potential of the Microbiome

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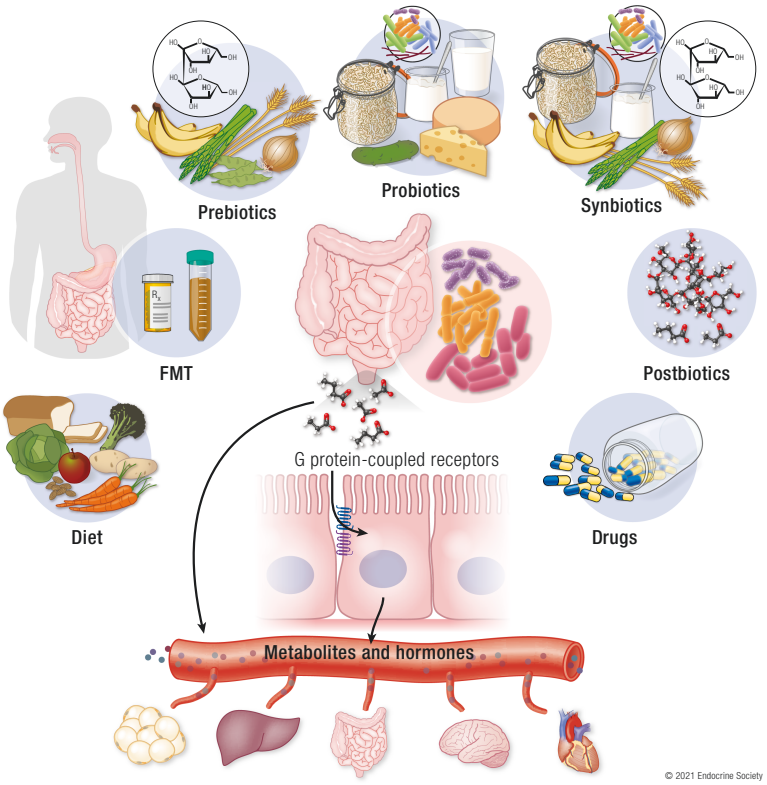
Abstract

We are host to an assembly of microorganisms that vary in structure and function along the length of the gut and from the lumen to the mucosa. This ecosystem is collectively known as the gut microbiota and significant efforts have been spent during the past 2 decades to catalog and functionally describe the normal gut microbiota and how it varies during a wide spectrum of disease states. The gut microbiota is altered in several cardiometabolic diseases and recent work has established microbial signatures that may advance disease. However, most research has focused on identifying associations between the gut microbiota and human diseases states and to investigate causality and potential mechanisms using cells and animals. Since the gut microbiota functions on the intersection between diet and host metabolism, and can contribute to inflammation, several microbially produced metabolites and molecules may modulate cardiometabolic diseases. Here we discuss how the gut bacterial composition is altered in, and can contribute to, cardiometabolic disease, as well as how the gut bacteria can be targeted to treat and prevent metabolic diseases.

Graphical Abstract



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Key Words: gut microbiome, obesity, type 2 diabetes, cardiovascular disease, metabolites, therapeutics, SCFA, intestine, liver, brain, adipose tissue.

Abbreviations: BCAA, branched-chain amino acid; BMI, body mass index; CNS, central nervous system; CONV-R, conventionally raised; CVD, cardiovascular disease; FMT, fecal microbiota transfer; FXR, farnesoid X receptor; GF, germ-free; GLP-1, glucagon-like peptide 1; GLP-1R, glucagon-like peptide 1 receptor; GPR, G protein–coupled receptor; HFD, high-fat diet; LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; PYY, peptide YY; SCFA, short-chain fatty acid; SGLT1, sodium glucose cotransporter-1; TMA, trimethylamine; TLR, Toll-like receptor; TPH1, tryptophan hydroxylase 1; TMAO, trimethylamine N-oxide.

ESSENTIAL POINTS

- We have more bacterial cells in the gut than human cells in the body
- The gut microbiota should be considered an endocrine organ affecting host physiology and metabolism
- The gut microbiota structure and function are altered in cardiometabolic diseases
- The gut microbiota produces vast amounts of bioactive molecules
- The gut microbiota is explored for novel therapeutics

During the past 2 decades, the gut microbiota has received dramatically increased attention. The gut microbiota is now considered an important modulator of host physiology that can contribute to disease in humans. It can be viewed as an endocrine organ that metabolizes nutrients in the diet and produces numerous metabolites. These metabolites can be absorbed and act on receptors in many organs including the intestine, liver, brown and white adipose tissue, and central nervous system (CNS). Many key features of metabolic diseases, including obesity, type 2 diabetes, cardiovascular disease, and liver steatosis, have been shown to be modulated by microbial products and metabolites, and evidence supports a causal role of the gut microbiota in development of these diseases. This review discusses how the microbiota is established at birth, develops, and functions as a complex microbial ecosystem, and discusses alterations in the gut bacterial composition observed in different metabolic diseases. It further focuses on causality studies, the metabolic effects of microbial products, and microbially produced metabolites in different organs, as well as the therapeutic potential of targeting the gut microbiota.

The Gut Microbiota

Vaginally born infants are colonized by microorganisms from the mother's vagina and gut at birth (1), whereas infants born through C-section are colonized by microorganisms from the skin and environment (2, 3). The colonization pattern is similar among children, but the kinetics of colonization varies. It follows discrete trajectories: some microbes can be considered early colonizers, while others are late colonizers (4). The early colonizers including *Bifidobacterium*, which is the most abundant genus at 4 months of age, thrive on human milk oligosaccharides. These early colonizers provide a more reduced environment and thus pave the way for more anaerobic microorganisms that start to expand when the infant transitions from breastfeeding to solid food, leading to a significant increased alpha diversity during the first 5 years of life (4). Even so, the gut microbiota composition in a 5-year-old child has not yet reached an adult's microbial complexity and late colonizers, including hydrogenotrophic archaea and bacteria, such as *Methanobrevibacter*, *Desulfovibrio*, and *Bilophila* (4), further contribute to the increased alpha diversity and complexity in adults (Table 1).

Studies in healthy individuals and in the general population show that environmental factors and host genetics shape the gut microbial composition in humans (Fig. 1). Many studies have shown the importance of the diet for the microbial composition. For example, cessation of breastfeeding is required for the maturation of the infant microbiota toward an adult-like composition enriched in *Bacteroides*, *Bilophila*, *Roseburia*, *Clostridium*, and *Anaerostipes* (3). In adults, the diet is associated with the gut microbiome composition (5-8), and switching between animal- and plant-based diets causes rapid and reproducible changes in the gut microbiota (9). These changes depend on differences in the microbes' ability to metabolize and utilize different dietary components for their growth. However, even if short-term extreme changes in the diet cause significant changes in microbial composition, mild changes in the diet only results in minor changes in the gut microbiome (10, 11). Other factors, including physical activity, immune system, infections, as well as antibiotics and other medications, also affect the gut microbial composition (7, 8, 12-17)). In addition to these environmental factors, host genetics have been shown to play a role (18, 19). The gut microbiota composition was found to be more similar between twins than unrelated individuals, and monozygotic twin pairs had more similar microbiotas than dizygotic twin pairs (18). However, the contribution of host genetics in shaping the gut microbiota is likely small, while environmental factors are more dominant determinants (20), yet explaining relatively little variation in the microbiome (8).

The gut microbiota is not a collection of independent microorganisms, but rather a complex microbial ecosystem in which the microorganisms communicate, cross-feed, recombine, and coevolve (21). These largely unexplored, complex polymicrobe–host interactions are closely related to the diet. Depending on the dietary composition as well as the presence of different microbes, an array of microbially produced metabolites is formed. This suggest that even if 2 individuals have similar dietary intake, the produced metabolites can differ significantly depending on the individuals' microbial composition (22). Individuals with comparable gut microbiota but divergent diets could also have different microbially produced metabolites (23). These metabolites are important for microbial interaction and cross-feeding, but could also affect the host's physiology by binding to receptors in the host. Much is still unknown regarding these metabolites: how they are produced, their cognate receptors, and the functions in the host. However, large-scale metabolome screening approaches have recently been set up to identify novel interactions between microbially produced metabolites and receptors (24), increasing our understanding of these signaling pathways.

Table 1. Definitions/explanations of microbiota-related terms used in the text.

Term	Definition/Explanation
Alpha diversity	Diversity of species in a single ecosystem or site.
Commensal gut microbes	Microbes living in a symbiotic relationship with the host. This relationship is often mutualistic, ie, both organisms benefit from the relationship.
Enterotype	A classification of living organisms based on the bacteriological composition of their gut microbiota.
Endotoxemia	Presence of endotoxin, ie, bacterial components such as lipopolysaccharide in the blood.
Metagenome	Metagenomics is the study of genetic material recovered directly from environmental samples.
Microbiome	The microbiome is the sum of all the microbes and their genomic elements in a particular environment such as the body or a part of the body.
Prebiotic	A substrate that is selectively utilized by host microorganisms conferring a health benefit.
Probiotic	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.
Postbiotic	A preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.
Shannon diversity	A measure of alpha diversity.

Colonization of commensal microbes and the interaction between these and the host are essential for the host's healthy development. The beneficial effects of the gut microbiota include maturation of immune tissues and fine tuning of immune responses (25). Several of the microbially produced metabolites and microbial components act locally but also in distant parts of the body to modulate immune function and other physiological processes (25, 26). In addition to their effects on the immune system itself, commensal microbes also have an important role in preventing pathogens to colonize the host by limiting space and nutrients available for pathogens. Host–microbe symbiosis is also necessary for metabolic health. Microbes produce vitamins, which is crucial since humans lack biosynthetic capacity for most vitamins (27). Even though vitamins are present in food, vitamin deficiency is still occurring due to undernutrition or poor eating habits. Thus, microbially produced vitamins could be metabolically beneficial for the host. In addition to these effects, the gut microbiota affects metabolic functions in a vast number of organs including the gastrointestinal tract, liver, white and brown adipose tissue, skeletal muscle, and brain. These effects and the therapeutic potential of targeting the microbiota will be covered in more details in this review.

Alterations in Gut Microbiota Composition and Metabolic Disorders

Alterations in gut microbial composition have been extensively studied in relation to various diseases including metabolic, immunological, and neurological diseases. Obesity is an increasing health problem worldwide, with several serious comorbidities including type 2 diabetes, cardiovascular disease (CVD), and liver steatosis. Several studies have investigated human fecal microbial composition in relation to obesity, but few consistent findings have been observed. A meta-analysis showed that most of the studies were underpowered (28). Only 1 of the 10 studies included had the power to detect a 5% difference between the groups, indicating the need of sufficiently powered studies. By combining the studies, a 2.07% difference in the Shannon diversity indices of nonobese and obese individuals was observed. The reduced alpha diversity in the obese subjects could potentially be an effect of a Western-style dietary pattern since such decreased diversity has been observed in rodents fed a Western diet (29). However, in addition to the reduced alpha diversity observed in obese individuals, an enrichment of Christensenellaceae has been detected in lean individuals (18). Since this link was initially detected, several studies from countries around the world have observed a similar inverse association between Christensenellaceae and body mass index (BMI) (30). BMI has also been shown to correlate with the prevalence of the Bacteroides2 enterotype, an intestinal microbiota configuration high in *Bacteroides* and low in *Faecalibacterium* and microbial cell density (31).

Type 2 diabetes and CVD are other metabolic diseases, tightly linked to obesity, in which the gut microbiota's involvement in the pathogenesis has been investigated. Similar to obesity, these diseases are also associated with low gene richness in the microbiome (32, 33). Le Chatelier et al showed that individuals with low richness are characterized by adiposity, insulin resistance, and dyslipidemia, and that the gene count correlated with metabolic parameters such as serum insulin levels and homeostasis model assessment of insulin resistance (32). Another study found that the overall microbiome composition was predictive for a large panel of cardiometabolic blood markers, including fasting and postprandial glycemic, lipemic, and inflammatory indices (34). This study also found that some microbes, such as *Prevotella copri* and *Blastocystis* spp., were indicators of favorable postprandial glucose metabolism. *P. copri* has also been shown to play a role in the interindividual response to barley kernel supplementation of bread. Individuals that responded with improved glucose metabolism when fed bread supplemented with barley kernels, rich in beta-glucans had higher abundance and expanded their population of *P. copri* compared with those that did not improve their glucose metabolism (35). Supplementation of *P. copri* to mice colonized with a microbiota from a nonresponder improved glucose metabolism compared with control mice when fed a chow diet rich in fibers but not when fed a Western-style diet. In contrast to these positive effects of *P. copri*, Pedersen et al. found that *P. copri* correlated with production of branched-chain amino acids (BCAAs) that has been linked to type 2 diabetes and that colonization with *P. copri* in high-fat diet (HFD)-fed mice induced insulin resistance, aggravated glucose intolerance and augmented circulating levels of BCAAs (36). This discrepancy, is currently unclear, but may be the result of strain specific effects and different interactions with the diet.

Observations from numerous studies show a consistent depletion of butyrate-producing bacteria in individuals with

type 2 diabetes including depletion of *Faecalibacterium*, *Clostridium*, *Alistipes*, *Pseudoflavonifractor*, *Oscillibacter*, and *Roseburia* (37-42). Such depletion of butyrate producers is present in both prediabetic and diabetic subjects naïve for diabetes treatment (39). Hypertension has also been linked to microbiota dysbiosis with a reduction in acetate- and butyrate-producing bacteria (33). Depletion of butyrate producers is not limited to metabolic diseases, but have also been observed in other diseases such as inflammatory bowel disease (43). Several beneficial health effects can be assigned to butyrate, which are discussed in detail below, but additional attributes associated with butyrate-producing bacteria may also contribute to improved host physiology. Taken together, these results show that metabolic diseases are associated with an altered gut microbial composition.

Causal Role of the Microbiota in Metabolic Disease

The altered gut microbial composition in individuals with metabolic disease calls for studying causality. Germ-free (GF) mice have been extensively used to determine the role of the gut microbes on host physiology and disease development as well as the underlying mechanisms involved. When fed a chow diet, rich in complex carbohydrates, GF mice have a reduced body weight and adiposity compared with conventionally raised (CONV-R) mice (44). Similarly, colonization of GF mice leads to weight gain and increased adiposity (44). At least part of these weight differences is due to increased energy harvest from the diet (45). The gut microbiota metabolizes otherwise indigestible complex carbohydrates leading to the formation of short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. These SCFAs can be absorbed by the host and used as energy. In the Western world, this additional energy harvest is estimated to account for 10% of the energy absorbed, but may be higher in societies with more plant-based foods (46). GF mice are not only leaner when fed a chow diet, but are also protected against diet-induced obesity when fed a diet rich in fat and low in complex carbohydrates. However, there are discrepancies between studies (47-51). While GF mice fed a Western diet, high in sucrose and in fat, are protected again obesity, GF mice fed a high-fat, normal sucrose diet are not protected (51). Furthermore, C57BL/6 mice appear to be more protected than Swiss Webster mice (49). These results suggest that the protection against diet-induced obesity is dependent on the diet's macronutrient composition and the mouse strain.

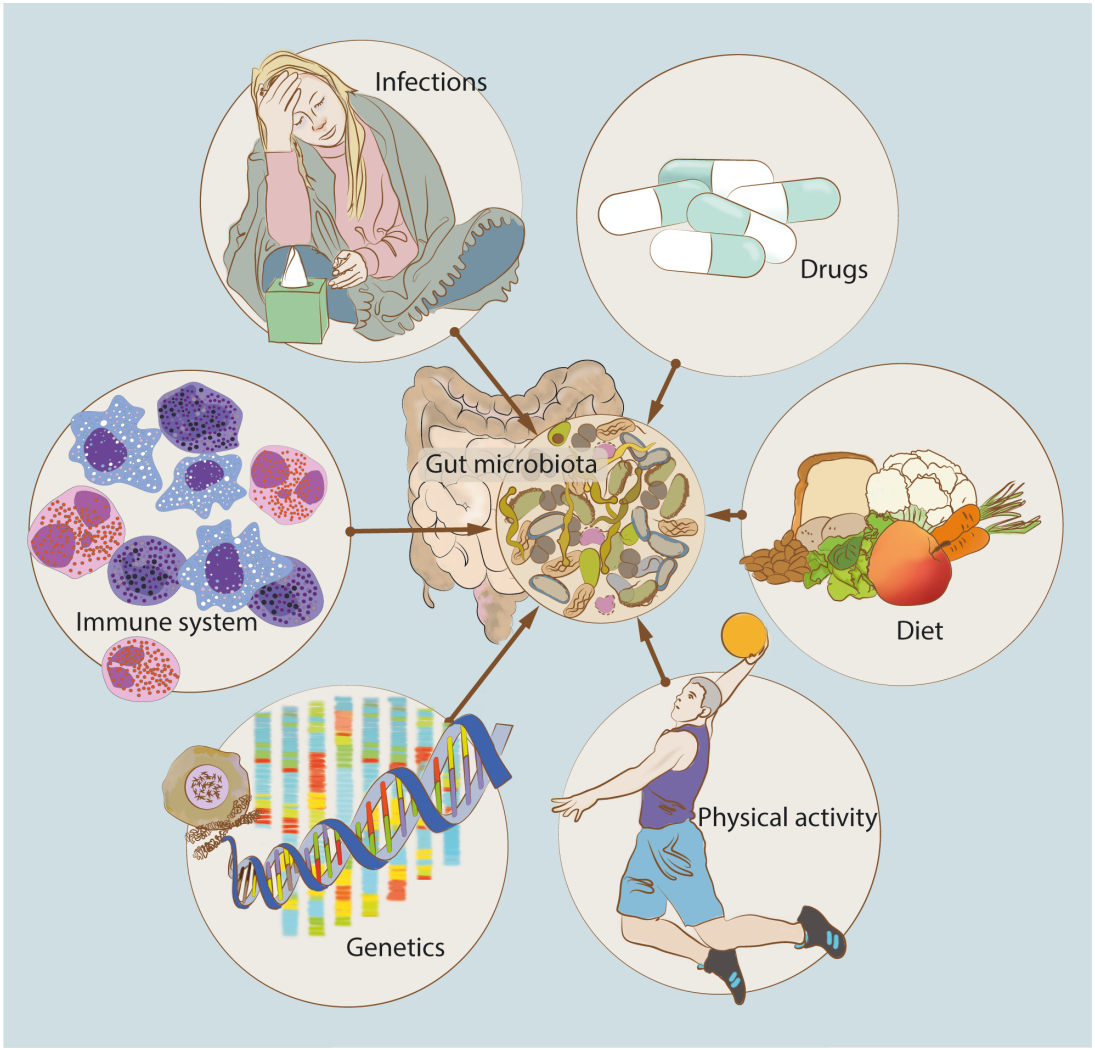

Figure 1. Factors influencing the gut microbial composition. Several factors, including host genetics, host immune system, diet, drugs, physical activity, and infections, influence the gut microbial composition.

However, additional factors may affect the interpretation of results in these studies including differences in baseline body weight between GF and CONV-R mice, energy absorption in their respective chow-fed controls, as well as differences in gut microbiota composition and development of diet-induced obesity between different mouse strains (52-54). Experimental outcome may depend on which mouse vendor is used since the gut microbiota composition has been shown to differ between different vendors, leading to vendor-specific alterations in microbial abundance and metabolic phenotype when the mice are fed high- or low-fat diets (54-56). This emphasizes the need for better controlled studies with weight-matched mice at baseline, knowledge about the microbial composition, as well as inclusion of proper chow-fed controls to be able to evaluate and compare studies. It is important to recognize that most studies are performed using laboratory mice that are raised in very controlled environments where they are fed defined diets. In contrast, wild mice have a dramatically different microbiome resulting in a thicker and more dense mucus layer (56, 57). Thus, studying microbiota from wild-caught animals may enhance the understanding of how the microbiota interacts with the host and the resulting effects on the host fitness (56, 58).

Even though the GF mouse model has been extensively used, the model has also been questioned due to its limited relevance in humans (59). Several developmental differences exist between GF mice and CONV-R mice including effects on the immune system, intestinal tract, CNS, and enteric nervous system. For example, colonization leads to morphological and functional changes along the intestinal tract including morphological changes in villi length and crypt depth, blood vessel density, mucus layer properties, stem cell proliferation, innervation of the colonic epithelium, and maturation of mucosa-associated lymphoid tissues (60-63). In the CNS, the gut microbiota has been suggested to affect the blood–brain barrier, neurogenesis, microglia maturation, and myelination (64). These differences should be considered when using the GF mouse model since they may modulate the response to certain intervention, making it difficult to extrapolate results from GF mice to the human situation. In addition to GF mice, the role of the microbes can be determined by depleting the gut microbiota in adult CONV-R mice using broad-spectrum antibiotic treatment. This model also has limitations. Antibiotics can for example exert intrinsic effects that are not related to the depletion of the microbiota. Furthermore, antibiotic treatment does not completely deplete the microbiota and there is a risk of selected growth of resistant microbes (65). To overcome the weaknesses with these 2 models, it has been suggested that at least some key experiments should be performed in both models to rule in or out effects of developmental differences as well as off-target effects of antibiotics (65). In addition to the previously mentioned studies, focusing on the GF mouse model, several studies have investigated the role of the gut microbiota in weight gain and adiposity using antibiotic-treated mice. Antibiotic treatment has been shown to reduce HFD-induced weight gain and adiposity, potentially by decreasing metabolic endotoxemia, inflammation, and intestinal permeability (66, 67).

Focusing on glucose homeostasis, both GF and antibiotic-treated mice have been shown to have improved glucose tolerance compared with CONV-R controls (68-70), and colonization of GF mice leads to impaired glucose tolerance (71), suggesting that acute modulation of the gut microbiota can affect glucose homeostasis. Furthermore, long-term antibiotic treatment can lead to reduced adiposity (67), which in turn improves glucose tolerance. To separate the effects of reduced body weight and fat mass from direct effects of the gut microbiota on glucose homeostasis, pair-feeding together with weight- and adiposity-matching could be applied. Using a pair-feeding protocol leading to matched body weights, Carvalho et al observed improved glucose tolerance in HFD-fed mice treated with antibiotics, an effect that was associated with reduced lipopolysaccharide (LPS) levels and inflammation (70). Other mechanisms for the improved glucose tolerance have also been suggested. Zarrinpar et al show that antibiotic-induced microbiome depletion alters glucose homeostasis independent on body weight and suggest that this improvement is due to a shift in the colonocyte's energy utilization from SCFAs to glucose (68). In addition, Martin et al showed that antibiotic treatment improved glucose tolerance via a peripheral serotonin-dependent mechanism (72). These results suggest that the interaction between the gut microbiota and the host's glucose metabolism is complex and that several mechanisms are likely involved, emphasizing that further studies are required to disentangle these interaction and to verify that these mechanisms are observed in humans.

Transfer of human fecal microbiota to GF mice has been shown to be a useful tool to study the effect of gut microbial composition and the effect of specific modulations (73, 74). It is now commonly used to determine if a specific gut microbial composition, associated with disease in humans, can transfer the disease phenotype to the mice (59). Such colonization experiments have been performed to determine the causal relationship between Christensenellaceae and weight. Goodrich et al found that when GF mice were colonized with stool samples from obese and lean individuals, the weight gain in the colonized mice negatively correlated with the abundance of Christensenellaceae. Furthermore, addition of *Christensenella minuta* to donor stool led to reduced weight gain in the recipient mice (18). These results suggest a causal role of Christensenellaceae in weight development. Other fecal microbiota transfer (FMT) experiments also suggest a causal role of the gut microbiota in weight gain. FMT from lean and obese twins to GF mice showed that the donors' phenotype could be transferred, in other words mice receiving FMT from the obese donors developed obesity and obesity-associated metabolic phenotypes compared with mice receiving FMT from the lean donors (75). Evidence from studies in humans also suggest a causal role for the gut microbiome in metabolic phenotypes. When transferring fecal microbiota from a donor to a recipient, metabolic phenotypes has been shown to be transmitted (76-78). These results will be discussed in more detail below.

Several limitations have been identified when human microbiota is transferred into mice (Fig. 2) (59) that have to be considered when interpreting the results. First, as already indicated, the GF mice have developmental differences compared with CONV-R mice and may therefore respond differently to the transferred gut microbiota than a previously colonized mouse would (71). Second, many of the taxa residing in the human gut are not transferred to the mouse gut, leading to significant differences between the inoculum and the resulting mouse microbiota (79). Third, the interactions between the microbes and the host and ecological factors are likely different in the surrogate recipient mice compared with the native human host that coevolved with the microbes over

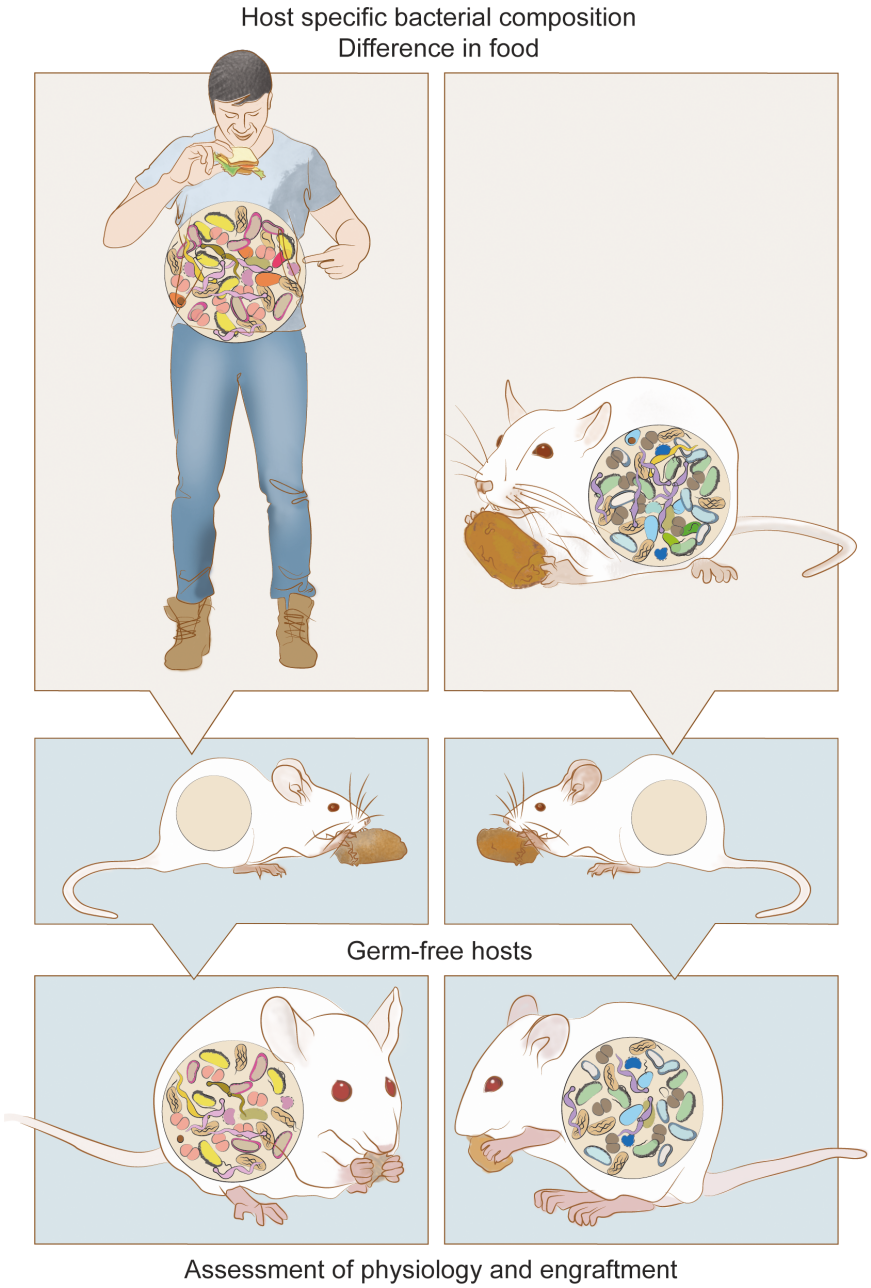

Figure 2. Fecal microbiota transplantation (FMT) experiments. FMT experiments are often performed to study the causal role of the gut microbiota in host physiology and pathogenesis. Fecal gut microbiota from human or mouse donors are used to colonize GF mice. However, this experimental model has serval potential limitations. First, the gut microbiota composition differs between humans and mice, and many of the taxa residing in the human gut are not transferred to the recipient mice, potentially altering the effects of the gut microbiota. Second, the diet differs between humans and mice, leading to a different array of metabolites that are formed and that exert effects in the host's body. It may also shift the microbiota in the recipient mice compared with the human donors. Third, the GF mice have developmental differences compared with the colonized donors, which may lead to a different response to the transferred gut microbiota.

a long time period. Despite these limitations, a systematic literature review showed that such transfer experiments have an exceptionally high "success" rate, demonstrating that 95% of all reported transfer studies showed that the gut microbiota could transfer at least 1 disease phenotype, leading to criticisms about the study design and request of more robust study protocols (59). The authors suggested improved study protocols including increased number of donors, no pseudoreplication or pooling of samples, confirmation of microbiome engraftment, and transfer of the dysbiotic pattern as well as mechanistic insight. Such improvements will decrease the number of false-positive associations observed and results in more reproducible studies. Another problem that could contribute to the high "success" rate is a bias for publishing studies with positive data compared with studies with negative data. Thus, it is important that also studies with negative data are published to provide an unbiased understanding for the role of the gut microbiota.

The Gut Microbiota Exerts Local and Peripheral Metabolic Effects

The gut microbiota exerts local metabolic effects within the gastrointestinal tract, where it affects the ability to digest, extract energy, absorb nutrients, and excrete byproducts from the food ingested. The functions modulated by the microbiota include intestinal permeability, intestinal motility, and secretion of gastrointestinal hormones. However, the gut microbiota does not only have local effects in the intestine (Fig. 3). The microbes produce numerous small molecules that can affect the host's physiology, both in the intestine and in other organs (22, 80). The concentration of these metabolites can reach millimolar concentrations, and may vary substantially among individuals, suggesting large interindividual variations in metabolite–receptor signaling. Since the gut is directly linked with the liver via the portal vein, most microbially produced metabolites that are absorbed from the gastrointestinal tract enter the circulation via the portal vein, allowing the liver to act as a second protection against harmful substances absorbed in the intestine (81). However, some metabolites escape degradation in the liver and other metabolites can be converted into biologically active metabolites by hepatic enzymes (82-84). Accordingly, gut microbiota can exert a metabolic effect in other part of the body through microbially produced metabolites and metabolite-induced hormonal release from the gut.

Microbially Produced Metabolites

Short-chain Fatty Acids

As previously mentioned, microbes ferment otherwise indigestible complex carbohydrates to form SCFAs, including acetate, propionate, and butyrate. These microbially produced metabolites have been extensively studied during the last decade, and several beneficial effects have been assigned to the SCFAs, especially to butyrate (85). Butyrate can promote epithelial barrier function via effects on tight junctions and by production of antimicrobial peptides (86). It also exerts an anti-inflammatory effect by histone deacetylases inhibition and activation of G protein–coupled receptors present in intestinal epithelial and immune cells (86). For example, it has been shown to suppress LPS-induced NF-κB activation via G protein–coupled receptor (GPR)109A in vitro, whereas in monocytes it induces prostaglandin E2 release and

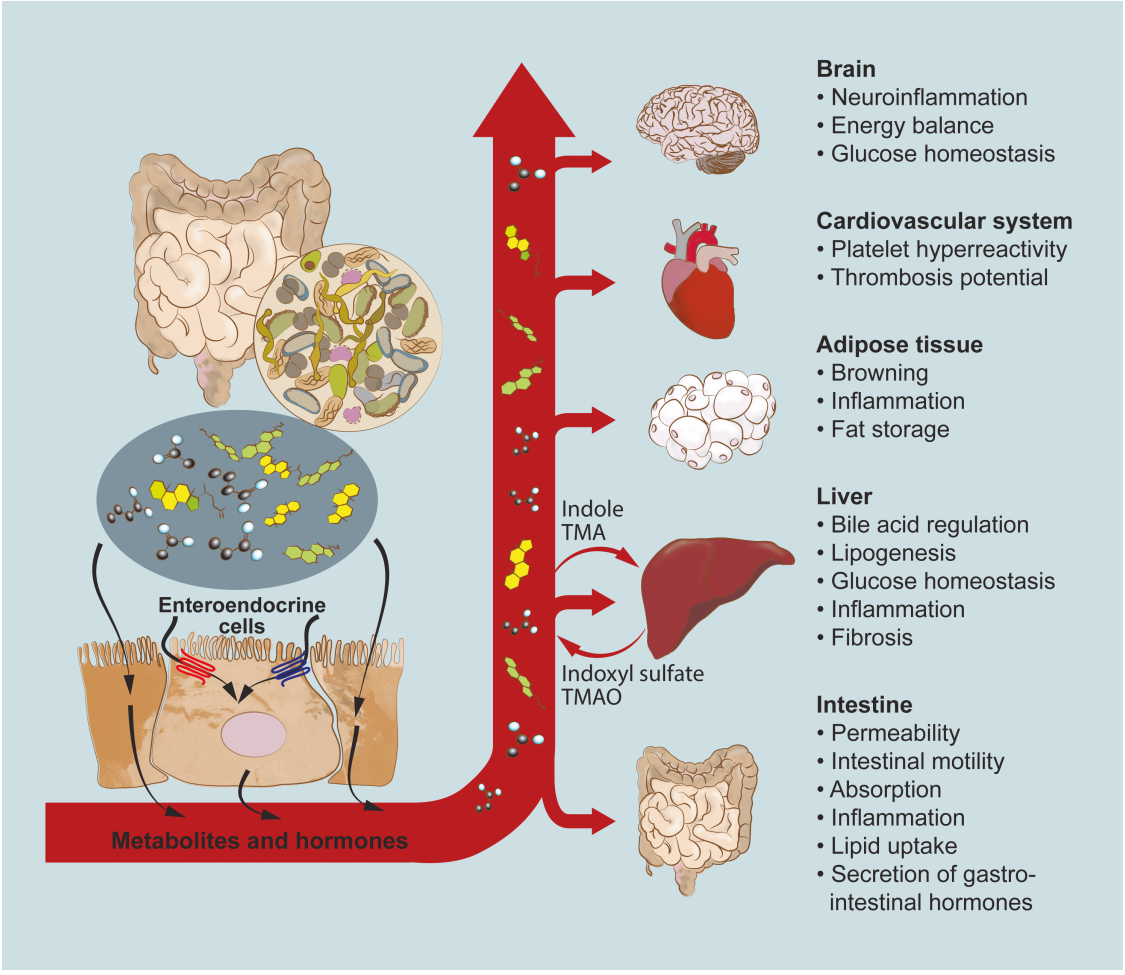

Figure 3. Microbially produced metabolites can exert local and peripheral effects in the host. The gut microbiota produces numerous metabolites that can act directly in the intestine or that can be absorbed in the host and effect other organs. These metabolites can modulate secretion of gastrointestinal hormones, which in turn can have peripheral effects. The liver is directly exposed to the microbially produced metabolites via the portal vein and metabolizes some of the metabolites leading to the production of a different set of metabolites. The absorbed metabolites and the metabolite-induced gastrointestinal hormones affect different organs in the body causing alteration in the host's metabolism.

expression of the anti-inflammatory cytokine interleukin-10. SCFAs (mainly butyrate) inhibit histone deacetylase activity, promoting histone acetylation, and thereby affecting gene regulation of cell proliferation, differentiation, and inflammatory response. In humans, circulating SCFAs have been found to negatively correlate with whole-body lipolysis, triacylglycerols, and free fatty acids levels (87). While circulating butyrate levels also correlated negatively with fasting plasma glucose levels, propionate correlated positively with insulin sensitivity (87). SCFAs are ligands to GPR43 (also known as free fatty acid receptor 2) and GPR41 (free fatty acid receptor 3), which are expressed in the intestine, adipose tissue, skeletal muscle, liver, and pancreas (85, 87, 88). Expression of the receptors in these tissues allows SCFAs to exert metabolic effects. When mice are fed HFD supplemented with SCFAs, they exhibit an increased energy expenditure and reduced weight gain compared with mice given a comparable HFD without supplementation (89, 90). SCFA supplementation also improves glucose tolerance, an effect that is likely due to reduced body weight gain and adiposity in the mice given the supplementation (89, 90). Li et al found that acute oral, but not intravenous, butyrate administration suppressed the activity of the hypothalamic orexigenic neuropeptide Y (NPY) neurons, decreased neuronal activity within the brainstem and decreased food intake (91). These effects were only observed when butyrate was administrated via intragastric gavage and not intravenously, and subdiaphragmatic vagotomy completely abolished butyrate-induced satiety, indicating that the effect of butyrate on satiety is likely mediated via vagal inputs to NPY neurons. Chronic butyrate supplementation prevented diet-induced obesity, hyperinsulinemia, hypertriglyceridemia, and hepatic steatosis, effects that were largely due to reduced food intake (91). Butyrate also modestly promoted fat oxidation and increased brown adipose tissue thermogenic capacity (91).

SCFAs are the main energy source for colonocytes, lining the intestinal wall. Thus, in the absence of microbes, the colonocytes become energy deprived (92) and switch to glucose fermentation to obtain ATP. In GF and antibiotic-treated mice, the insufficient energy availability in the colon leads to an adaptive response involving the intestinal hormone glucagon-like peptide 1 (GLP-1). Energy deprivation leads to increased secretion of GLP-1, which in turn slows intestinal transit allowing more time for energy extraction (93). When GF mice are colonized, GLP-1 levels decrease resulting in faster transit time, thereby potentially preventing bacterial overgrowth (93). It is possible that similar mechanisms are preserved in humans as increased GLP-1 levels and delayed gastrointestinal transit time have been reported in patients with anorexia nervosa (94, 95).

Besides the signaling ability, SCFAs can also be absorbed, utilized as energy by the host, and exert a lipogenic effect. Thus, in addition to the beneficial effects of SCFAs, they can also contribute to metabolic disease by increasing lipogenesis. Such detrimental effects have been shown to cause metabolic disease in Toll-like receptor (TLR) 5-deficient mice (96). These mice have gut microbial overgrowth, and increased levels of cecal butyrate and propionate, which was associated with increased hepatic de novo lipogenesis and metabolic impairment. Thus, in certain conditions such as innate immune deficiency, leading to bacterial overgrowth, excess SCFA levels may increase susceptibility to metabolic diseases. Further experimentation is required for clarifying how different SCFAs contribute to host metabolism in different contexts.

Secondary Bile Acids

Primary bile acids are produced from cholesterol in the liver and excreted into the duodenum upon food ingestion. The primary bile acids produced in humans and rodents differ: while chenodeoxycholic acid and cholic acid are the primary bile acids in humans, rodents produce cholic acid and muricholic acids (97). Furthermore, bile acids are conjugated to glycine and taurine in humans, but are almost extensively conjugated to taurine in rodents. These differences change the properties of the bile acids and need to be considered when extrapolating results from rodent studies to humans. In an attempt to overcome these differences and be able to study the role of bile acids in human diseases, mice lacking the enzyme Cyp2c70 have now been generated resulting in mouse models with a humanized bile acid pool (98-100). In the intestine, microbes convert the primary bile acids to secondary bile acids by deconjugation of taurine and glycine and through dehydrogenation, dihydroxylation, and epimerization, leading to alterations in the bile acids' properties (97). Bile acids act as detergent and facilitate fat absorption in the intestine. They are also important signaling molecules that bind to receptors, particularly farnesoid X receptor (FXR) and G protein–coupled bile acid receptor-1 (also known as TGR5) in the liver, intestine, muscle and brown adipose tissue to regulate host physiology (101). Signaling via FXR regulates expression of genes that is involved in the synthesis, uptake, secretion, and intestinal absorption of bile acids (101). Tissue-specific knockout mice with deletion of FXR specifically in the intestine or in the liver have shown that intestinal FXR activation predominately suppresses the hepatic expression of *Cyp7a1*, the rate-limiting enzyme in bile acid synthesis, via the induction of fibroblast growth factor 15 (FGF15) (102). Hepatic FXR activation, via the induction of SHP, is less important in suppressing *Cyp7a1* expression. In contrast to CYP7A1, both intestinal FXR/FGF15 and hepatic FXR/small heterodimer partner (SHP) pathways suppress expression of *Cyp8b1*, another enzyme involved in bile acid synthesis (102). When signaling via TGR5, bile acids could affect energy expenditure in brown adipose tissue and muscle (101). The gut microbiota modulates bile acid signaling by converting primary bile acids to secondary bile acids. For example, the gut microbiota reduces the natural occurring FXR antagonist tauro-beta-muricholic acid by deconjugating it and thus promoting FXR signaling in mice (103). Thus, differences in the microbial composition in humans could potentially affect the bile acid pool and thereby bile acids signaling as well as absorption of nutrients. However, further studies are needed to understand these interactions.

Amino Acid-derived Metabolites

The gut microbiota has also been shown to be involved in the production of metabolites that exert detrimental health effects. Imidazole propionate is 1 example of a microbially produced amino acid–derived metabolite, elevated during metabolic disease. Histidine is metabolized to urocanate which is further metabolized by bacteria expressing the enzyme urocanate reductase to imidazole propionate (82). Imidazole propionate is highly produced by a diabetes-associated microbiota and is elevated in the serum from subjects with type 2 diabetes compared with controls (82, 104). Furthermore, higher serum levels of imidazole propionate are also associated with reduced gut microbial richness (104). We have shown that imidazole propionate impairs

insulin signaling at the level of insulin receptor substrate through activation of the p38γ/p62/mTORC1 pathway in mice (82). Moreover, we found increased activation of p62 and mTORC1 in liver from subjects with type 2 diabetes and the imidazole propionate levels correlated with p62 and S6K1 phosphorylation in human liver. These results suggest a clinical importance of imidazole propionate affecting hepatic insulin signaling in type 2 diabetes.

Another group of metabolites that can be produced by the microbiota is the BCAAs. The 3 BCAAs, leucine, isoleucine, and valine, are essential amino acids, in other words humans lack the enzymes to produce these amino acids. Instead, these amino acids can be obtained from the food or be produced by the gut microbiota (105). Several studies have linked BCAAs to insulin resistance. For example, a BCAA-related metabolic signature was shown to correlate with insulin sensitivity in obese individuals (106), and leucine, isoleucine, and valine were all strongly associated with development of type 2 diabetes in a prospective study (107). In HFD-fed mice, BCAAs contribute to development of obesity-associated insulin resistance via a mTOR-dependent mechanism (106). Furthermore, another study showed that the gut microbiota in individuals with insulin resistance are characterized by an increased potential to produce BCAAs, which was linked to increased serum levels of BCAAs in these individuals (36, 108).

BCAAs can also be metabolized to branched-chain fatty acids. It was recently shown that the dietary protein composition of the diet can alter the gut microbial composition and subsequent production of branched-chain fatty acids (109). Mice fed a Western diet with a mixed protein source had aggravated diet-induced obesity and insulin resistance compared with mice fed a Western diet with casein as the only protein source. Part of this phenotype was transferrable through FMT from donor mice to GF recipient mice fed a Western diet. These results indicate the importance of considering the protein source and its effects on the gut microbial composition and their metabolites.

Other examples of microbially produced metabolites that can have detrimental effects include indoxyl sulfate and phenylacetylglutamine. Indoxyl sulfate is produced from tryptophan by gut microbial tryptophanase activity leading to indole formation that is further converted to indoxyl sulfate in the liver (83). Indoxyl sulfate is a uremic toxin, that has been associated to CVD in patients with chronic kidney disease (110). Microbially produced phenylacetylglutamine has also been associated with CVD and incident major adverse cardiovascular events (myocardial infarction, stroke, or death) (111).

Additional Metabolites

Another microbially produced metabolite with detrimental health effects is trimethylamine N-oxide (TMAO). Plasma levels of TMAO have independently been associated with incident CVD development and adverse event risks in several large-scale clinical studies (112-115). In animal experiments, plasma TMAO levels were positively associated with *Allobaculum*, and negatively associated with *Candidatus arthromitus* and Lachnospiraceae (112). Nutrients abundant in a Western style diet (phosphatidylcholine, choline, and carnitine) are used by the gut microbes to produce trimethylamine (TMA) and the reactions are catalyzed by several distinct microbial choline TMA lyases, including the proteins encoded by the *cutC/D* (choline utilization C/D) genes (84). After absorption in the intestine, TA is oxidized in the liver to form TMAO. Several studies have shown that TMAO can contribute to platelet hyperreactivity and enhanced thrombosis potential, thereby potentially affecting development of CVD (84, 112). Altogether, these studies show that metabolic diseases are associated with an altered microbially produced metabolite profile.

Gut Microbiota and Intestinal Function

Gut Permeability and LPS-induced Low-grade Inflammation

One of the main functions of the intestinal epithelium is to maintain a proper barrier function which allows nutrients, water, and ions to be absorbed but prevents pathogens and harmful substances to enter. The barrier consists of a mucus layer and a monolayer of epithelial cells connected by tight junctions. In several diseases, including type 2 diabetes and obesity, alterations in gut permeability are observed, leading to endotoxemia and low-grade inflammation (116). Intestinal permeability also correlates to visceral adiposity and liver fat in humans (117). Feeding mice HFD alters the gut microbiota composition, which in turn can promote inflammatory immune changes within the intestine and increase gut permeability (116). When mice are fed HFD, expression of tight junction proteins including Zonula occludens-1 and occludin decrease, which in turn is associated with increased levels of circulating lipopolysaccharide (66). Mimicking the endotoxemia observed during HFD feeding by subcutaneous LPS infusion resulted in weight gain, inflammation, and elevated fasting glucose levels, suggesting that the elevated LPS levels can directly contribute to obesity and insulin resistance (118). Furthermore, antibiotic treatment in HFD-fed mice restored expression of tight junction proteins, and the gut permeability in these mice remained similar to that in chow-fed mice. Thus, the gut microbiota may affect intestinal permeability and thereby the low-grade inflammation observed in metabolic diseases such as obesity. However, the endotoxemia observed after high-fat feeding could also be a result of an increased LPS absorption and release into the circulation via a chylomicron-dependent pathway, which can explain how the relatively large amphipathic LPS molecule can be transferred from the gut lumen (119). LPS binds the cell surface receptor TLR4, which initiates an inflammatory response (120). The receptor is widely expressed, such as in the liver, adipose tissue, and brain (121), and increased circulating LPS levels can cause low-grade inflammation in these tissues.

Microbial Regulation of Gastrointestinal Hormones

GLP-1 is mainly produced in L-cells, located in the small and large intestine, and in the brainstem (122). Microbial regulation of intestinal GLP-1 secretion is complex. While SCFA deficiency in GF and antibiotic-treated mice leads to elevated circulating GLP-1 levels (93), microbially produced SCFAs also increase GLP-1 plasma levels in rodents via a GPR43-dependent mechanism (123, 124). In a therapeutic perspective, the SCFA-induced GLP-1 levels may be more important. In humans, fasting circulating levels of acetate, propionate, and butyrate concentrations have been shown to positively associate with fasting GLP-1 concentrations (87). In addition to its effects on intestinal transit, GLP-1 is an incretin hormone that improves glucose-stimulated insulin secretion in the pancreas. However, the elevated levels of GLP-1 in GF

mice compared with CONV-R mice does not improve the incretin response (93). GLP-1 also exerts an anorectic effect by acting in the brain (125), which will be discussed in more detail below.

Furthermore, L-cells produce and secrete the gastrointestinal hormones peptide YY (PYY) and GLP-2. Similar to GLP-1, the gut microbiota can increase PYY and GLP-2 levels in the circulation via production of SCFAs and activation of specific GPRs, such as GPR43 and GPR41 (126-128). PYY is an anorexic hormone that acts in the brain to reduced appetite (129). It has also been shown to slow down intestinal transit (128, 130). Brooks et al showed that the fermentable carbohydrate inulin acts via GPR43 to increase PYY cell density and circulating PYY, reduce food intake, and prevent diet-induced obesity (127). Another study has shown that GPR41-deficient mice have reduced PYY levels compared with littermate controls, an effect that is dependent on the gut microbiota. However, even though these mice had reduced peptide YY, GPR41-deficient mice colonized with a fermentative community were leaner and weighed less than their littermate controls, an effect that was also dependent on the gut microbiota. The increased intestinal transit rate in the mutant mice was thought to decrease energy extraction and absorption in the mutant mice (128). GLP-2 has been shown to be important in maintaining intestinal barrier function (126). Selective gut microbiota modulation, associated with increased GLP-2 production, can have beneficial effects on gut barrier function (131). In addition to SCFAs, gut microbes could also modulate the secretion of GLP-1 and 2 as well as PYY via the conversion of primary bile acids to secondary bile acids and its signaling via TGR5 (101, 132). Thomas et al showed that TGR5 signaling increases intestinal GLP-1 secretion, which in turn improved liver and pancreas function and enhanced glucose tolerance in obese mice (132).

Approximately 90% of the body's serotonin is synthesized in the enterochromaffin cells lining the gut wall. GF mice have reduced levels of serotonin in serum as well as in colon, and colonization of GF mice restores the levels (133, 134). Microbially produced SCFAs as well as secondary bile acids may regulate gut-derived serotonin production by increasing the expression and activity of tryptophan hydroxylase 1 (TPH1), the rate-limiting enzyme for mucosal serotonin synthesis (72, 134). Indigenous spore-forming bacteria from mouse and human microbiota promote serotonin biosynthesis in the enterochromaffin cells, which in turn can decrease gastrointestinal motility as well as increase platelet activation and aggregation (133). In addition to these functions, the gut microbiota can also affect the glucose homeostasis by regulating peripheral serotonin. Intraperitoneal injection of serotonin increases plasma glucose and insulin (135). Furthermore, antibiotic treatment and inhibition of TPH1 improve glucose tolerance compared with vehicle-treated mice. However, combined treatment of TPH1 inhibition and antibiotics did not result in additive effects (72). Altogether, these studies indicate that the gut microbiota can modulate gastrointestinal hormonal production and secretion and thereby the host's metabolism.

Gut Microbiota and Metabolic Effects Beyond the Intestine

Bacterial Encroachment and Translocation

It is possible that bacteria from the gut translocate into the host and thereby contribute to disease. Several studies have detected bacterial components in the blood, adipose tissue and liver (136-140). Bacterial DNA and live bacteria have been detected in adipose tissue depots including omental, mesenteric and subcutaneous fat depots. Proteobacteria and Firmicutes were the most abundant phyla in adipose tissue, and the highest bacterial diversity was observed in the mesenteric depot (138). Proteobacteria was also the dominant phylum in blood (136, 139). Furthermore, bacterial composition in blood, liver, and adipose tissue was associated with metabolic disease (136-140), and 18 genera were differentially abundant in adipose tissue or blood between subjects with or without type 2 diabetes (138). A higher amount of bacterial DNA in the blood and the liver has also been associated with liver disease and obesity (139, 140). Taken together, several studies indicate that bacterial translocation occurs and that the amount and composition of the translocated bacteria are associated with metabolic disease. However, the contribution of the bacterial translocation to metabolic disease is unclear, and further studies are needed to examine this relationship.

Gut Microbiota and Liver Function

There is bidirectional communication between the gut microbiota and the liver which occurs via the portal vein, biliary tract, and systemic circulation. As mentioned above, the gut is closely linked to the liver via the portal vein and most absorbed metabolites enter the circulation via this vein. The liver is thus exposed to an array of microbially produced metabolites and microbial products, which can affect hepatic metabolism. Examples of such metabolites and microbial products are imidazole propionate, SCFAs, and LPS, which can have detrimental metabolic effects in the liver. We have for example shown that imidazole propionate impairs insulin signaling in the liver, at the level of insulin receptor substrate, causing metabolic disturbances (82). Furthermore, increased energy absorption in form of SCFAs can during certain conditions increase de novo lipogenesis and liver fat content, which in turn can increase serum lipids levels (96). Microbial and viral product, including LPS, can also enter the circulation and act on TLRs in the liver. The role of the hepatic TLR4 signaling has been determined using mice with liver-specific deletion of TLR4. When these mice are fed a HFD, they exhibit improved glucose tolerance, enhanced insulin sensitivity, and ameliorated hepatic steatosis compared with control mice suggesting a role of hepatic LPS–TLR4 signaling in development of metabolic diseases (141). As described above, primary and secondary bile acids are also examples of molecules involved in the liver–gut microbiota communication, and signaling via FXR in liver and intestine affects the host's metabolism. Other metabolites that may act on the liver to regulate metabolism and metabolic diseases including nonalcoholic fatty liver disease (NAFLD) are TMA, which is converted to TMAO, 3-(4-hydroxyphenyl) lactate, and ethanol (142). When studying patients with NAFLD, specific molecular networks have been identified linking the gut microbiome to disease (143). As with obesity and type 2 diabetes, Hoyles et al showed that patients with NAFLD were characterized by low microbial gene richness (143). This study further suggested that the microbial metabolite phenylacetic acid significantly increases hepatic BCAA utilization and hepatic lipid accumulation and is associated with liver steatosis.

Gut Microbiota and Adipose Tissue Function

In addition to the increased energy harvest, which by itself can cause differences in weight and adiposity, the gut

microbes also alter the host's adipose tissue metabolism by numerous mechanisms that ultimately can affect fat storage and adipose tissue function. Chow-fed GF mice are lean compared with CONV-R mice. Reduced lipid deposition in the adipose tissue and increased browning can contribute to this phenotype. The gut microbiota suppresses the circulating lipoprotein lipase inhibitor angiopoietin-like protein 4 and can thereby regulate lipid uptake in adipose tissue (44). Furthermore, by comparing GF, CONV-R, and antibiotic-treated mice, Suárez-Zamorano et al showed that microbiota depletion promotes browning of inguinal subcutaneous adipose tissue as well as perigonadal visceral adipose tissue, effects that were associated with improved glucose tolerance and insulin sensitivity (144). However, in contrast to this study, Li et al found that microbiota depletion did not promote browning of white adipose tissue at room temperature, but observed that the adaptive thermogenic capacity of brown and white adipose tissue was impaired under cold challenge when the microbiota was depleted (145). Cold exposure in rodents have also been shown to increase the activity in brown adipose tissue and lead to altered gut microbiota composition (146). Colonization with cold-adapted microbiota transferred at least part of the metabolic phenotype (146). In contrast to these rodent studies, cold-exposed brown adipose tissue activity in humans was not related to the characteristics of the fecal microbiota and was not transmissible through fecal transplantation to mice (147). Together with the known differences in brown adipose tissue function between rodents and humans (148), these results question the use of rodents for studying brown adipose tissue activity, and indicate that extrapolating results from these models to humans may not be appropriate. Thus, further studies are needed to determine how the gut microbiota affects the thermogenic capacity of brown and white adipose tissue in humans.

When rodents are fed a HFD they develop adipose tissue inflammation which in turn can impair the adipose tissue function and lead to insulin resistance and elevated blood glucose (149). The inflammatory response in adipose tissue depends on the dietary lipids, and how they affect the gut microbiota composition. Mice fed a lard diet, rich in saturated lipids, have increased TLR activation in the systemic circulation, increased inflammation in adipose tissue, and impaired insulin sensitivity compared with mice fed a fish oil–based diet, rich in polyunsaturated lipids (150). When GF recipient mice were transplanted with gut microbiota from lard-fed or fish oil–fed mice and subsequently fed a lard-based HFD, the mice receiving the gut microbiota from fish oil–fed mice had reduced weight gain and reduced adipose tissue inflammation compared with the mice receiving gut microbiota from lard-fed mice, indicating that the gut microbiota contributes to the phenotypic differences between mice fed lard and mice fed fish oil (150).

Gut Microbiota and the Gut–Brain Axis

The gut–brain axis is the bidirectional communication between gut and brain. The routes of communication include neural, endocrine, and immune mechanisms, allowing microbes to directly signal to the brain. Specialized enteroendocrine cells that lines the lumen of the gut contact the vagus nerve. These cells synapse directly with the vagal nodose neurons allowing sensory stimuli from the gut lumen to transduce within milliseconds using glutamate as a neurotransmitter, connecting the intestinal lumen with the brainstem in 1 synapse (151). These cells could potentially sense microbially produced metabolites and microbial products and rapidly relay such signals to the brainstem. Furthermore, vagal afferents expressing GLP-1 receptor (GLP-1R) or serotonin receptors can also mediate microbial signals to the brain (152, 153). The bidirectionally communication also allows the brain to affect the structure and function of the gut microbiota through the autonomic nervous system, by modulating regional gut motility, intestinal transit and secretion, and gut permeability, and potentially through the luminal secretion of hormones that directly modulate microbial gene expression (154).

The gut microbiota regulates feeding behavior (155) and microbially regulated GLP-1 exerts an anorectic effect by acting in the brain (125), but it has been questioned if the gut-derived GLP-1 can act directly in the brain or if it acts via vagal afferents. The half-life of the active form of GLP-1 in the circulation is less than 2 minutes due to degradation by the enzyme dipeptidyl peptidase 4 (125). There are studies supporting both a direct effect of circulating GLP-1 in the brain and an effect mediated by vagal afferents. Ruttimann et al used subdiaphragmatic vagal deafferentations to determine if vagal afferents mediate the GLP-1 effects. Vagotomy attenuated the GLP-1-mediated effects on meal size when GLP-1 was administrated via the intraperitoneal route (156). GLP-1 acting on the GLP-1R-expressing vagal afferents has further been shown to relay anorexigenic signals to parabrachial nucleus neurons and thereby control meal termination as well as improve glucose tolerance (152). In contrast to intraperitoneal administrated GLP-1, vagotomy had no effect when GLP-1 was infused into the hepatic portal vein (156), suggesting that the afferent nerves are not required to mediate intravenous GLP-1's effects on food intake.

GLP-1 also exerts anti-inflammatory and neuroprotective effects (125). We recently showed that GF and antibiotic-treated mice are protected against Western diet–induced hypothalamic inflammation via GLP-1R-dependent mechanisms (67). Hypothalamic inflammation is believed to contribute to diet-induced leptin resistance and weight gain (157, 158). Our results suggest that GLP-1 acts on GLP-1R in astrocytes and reduces hypothalamic inflammation and thereby modulates inflammatory response in the brain.

Bile acids can also signal in the brain. A recent study showed that bile acids act on TGR5 in hypothalamus leading to activation of the sympathetic nervous system which in turn decreased food intake, increased energy expenditure, and reduced body weight and fat mass (159). Furthermore, taurochenodeoxycholic acid, a primary bile acid, is increased in the small intestine and plasma but also in the dorsal vagal complex in the brain after a short-term HFD (160). It acts as an FXR agonist and is involved in nutrient sensing via a taurochenodeoxycholic acid–FXR axis in the upper small intestine and in the ileum (161). It also activates FXR in the dorsal vagal complex, and thereby decreases host insulin action (160). Transplantation of a healthy gut microbiota to the small intestine of HFD-fed rats restores the levels of taurochenodeoxycholic acid and insulin's suppression of glucose production. Both insulin and leptin act centrally to regulate hepatic glucose production (162). Notable, activation of intestinal FXR leads to diminished

secretion of GLP-1 in the gut (163). Since GLP-1 can improve leptin sensitivity in HFD-fed mice (67) and leptin has been postulated to activate a hypothalamic glucose lactate long-chain fatty acids–coenzyme A axis and neuronal relay to the dorsal vagal complex to lower hepatic glucose production, it is possible that taurochenodeoxycholic acid integrates the 2 pathways in regulating hepatic glucose production.

Most studies, focusing on the effect of the gut microbiota on feeding behavior and the involved neuronal circuits, have used bulk depletion of the microbiota in animal models to provide proof-of-concept that an intact microbiota is required for a specific function (155). Further insights into this regulation are needed, including determining which specific microbes and metabolites are involved. Corresponding human studies that manipulate specific microbes and metabolites will be important to assess host–microbe interactions in humans. Such information will be necessary in the development of microbiota-targeted treatment strategies.

Therapeutic Potential of the Microbiome

The increasing amount of evidence showing a causal role for gut microbes and their microbial products and metabolites in metabolic diseases opens the possibility to target the gut microbiota in these diseases. There are several ways in which the gut microbial composition and function can be modulated, including diet intervention, pre-, pro-, and postbiotics, microbiota transfer, or by certain drugs. In the past years, it has however become clear that individuals respond differently to interventions targeting the gut microbiota. Since the gut microbiota is a complex microbial ecosystem with delicate interactions between microorganisms, certain interventions may have a personalized response depending on composition of the pre-existing microbial community. For example, probiotics, namely administration of a beneficial microbe, may not have the desirable effect unless the microbe is able to engraft in the pre-existing microbial community. Furthermore, individuals with type 2 diabetes that already have high alpha diversity containing a high number of butyrate producers may not benefit as much from a microbiota-targeted intervention as individuals with low alpha diversity and low number of butyrate producers. Accordingly, the gut microbiota may facilitate into stratification of patients and personalized medicine.

Diet Intervention

The diet is 1 of the most prominent environmental factors that affects the microbial composition. However, while short-term extreme changes in the diet are able to significantly alter microbial composition in a rapid and reproducible way (9), mild changes in diet only result in minor changes in the gut microbiome (10, 11). Furthermore, studies suggest that different individuals respond differently to dietary changes and that personalized interventions may be required. For example, in a study including 800 individuals, Zeevi et al found high variations in postmeal blood glucose levels among individuals that consumed identical meals (164). Applying an algorithm that integrated clinical and microbiome features, they could accurately predict individual blood glucose responses after real-life meals, assessed using continuous glucose meters, and were even able to apply the algorithm to predict responses in a validation cohort. They found that personalized dietary interventions based on this algorithm induced lower postprandial glucose responses, which were accompanied by gut microbiota alterations. Some consistent alterations in the gut microbiota were observed in the population after the individuals had been given a personalized "good" or "bad" diet. For example, while *Bifidobacterium adolescent* decreased after a good diet and increased after a bad diet, *Roseburia inulinivorans*, *Eubacterium eligens*, and *Bacteroides vulgatus* increased after a good diet and decreased after a bad diet. These results suggest that personalized diet interventions, based on clinical and microbiome features, could be used to improve postprandial glucose response and improve metabolic disease.

Fecal Microbiota Transplantation

Another way to change the gut microbiota is to transfer fecal microbiota from a healthy donor to an unhealthy recipient. Today, such treatment has only been approved for recurrent *Clostridium difficile* infection, in which it has been shown to be effective (165). FMT was previously performed by either duodenal infusion or colonoscopy, but can now also be administrated through capsules containing freeze dried stool (166, 167). FMT has also been tested to improve metabolic parameters. Vrieze et al showed that insulin sensitivity was improved in obese individuals with metabolic syndrome 6 weeks after infusion of microbiota from lean donors (76). In contrast, autologous microbiota infusion did not improve insulin sensitivity. Kootte et al showed similar improvement in insulin sensitivity after FMT from lean donors to obese recipients with metabolic syndrome (78). However, even though such treatment improved metabolic parameters short term (76, 78), the positive effects was transient. While fecal transfer from lean donors to recipients with metabolic syndrome altered the gut microbiota and improved insulin sensitivity 6 weeks after the FMT, the gut microbiota and the metabolic parameters did not differ from baseline values 18 weeks after the FMT (78). Furthermore, the study by Kootte et al showed the importance of the baseline microbiota in determining the outcome of a gut microbiota intervention. Obese individuals with reduced gut microbiota diversity at baseline responded better to FMT and improved their insulin sensitivity more than obese individuals who had higher diversity at baseline (78).

Today, several randomized clinical trials, studying the effects of FMT on obesity, are registered in the NIH clinical trials registry (https://clinicaltrials.gov) as ongoing or recently completed. Using capsules to administrate donor FMT or placebo, 1 completed study shown no effect of FMT on obesity (168). However, a clearer picture of the effect of FMT on metabolic diseases will hopefully provided in the near future when several of the ongoing studies are completed and are reported. It should be noted that published studies showed large intra-individual variation in donor engraftment (168). Ecological variables such as low recipient and high donor alpha diversity and relative species abundance, as well as clinical variables such as antibiotic pretreatment, bowel lavage, and multiple rounds of FMT have been suggested to be associated with increased donor microbiota engraftment. When comparing FMT outcomes in 142 patients with ulcerative colitis, recurrent *C. difficile* infection, metabolic syndrome, infection with extended-spectrum beta-lactamase–producing

bacteria, or Crohn's disease, Schmidt et al observed high interindividual variability in strain-level outcomes following FMT. Furthermore, clinical outcome was not significantly linked with donor strain colonization, recipient strain displacement, or recovery of specific microbial functions. While they did not observe strong evidence that any species were inherently more invasive or resilient than others, several "gatekeeper" species in the recipient, in particular of the genus *Bacteroides*, inhibited colonization by other species. Moreover, they could accurately predict which recipient strains were going to be displaced, but their model could not as accurately predict which donor strain takes over. Performing FMT is not without risk, and harmful microorganisms have been transferred via FMT to recipients (169, 170). Therefore, it may be safer to use a defined microbial consortium, which also has the potential to elicit an effective and durable clinical response due to optimized symbiosis between the microbes (171). However, further studies are needed to determine if colonization of such a defined consortium could be a treatment option in metabolic disease.

Pre-, Pro-, Post-, and Synbiotics and Microbial Metabolites

A prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (172). Among the commonly used prebiotics are fermentable fibers such as inulin, fructooligosaccharides, and galactooligosaccharides. These prebiotics have all been used in trials to determine the beneficial effects in metabolic disease (172). Supplementation with inulin or inulin-propionate ester in overweight or obese subjects improve insulin resistance (173). Furthermore, a meta-analysis showed that inulin-type fructans improved fasting blood glucose, glycosylated hemoglobin, fasting insulin, and the homeostasis model assessment of insulin resistance in prediabetic and type 2 diabetic patients (174). Even though many studies suggest that prebiotics have beneficial effects in metabolic disease, few prebiotic health claims have been authorized and in-depth characterization of the effects of the prebiotics in the host is needed (172).

A probiotics is defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (175). Even though *Bifidobacterium* (*adolescentis*, *animalis*, *bifidum*, *reuteri*, *breve*, *and longum*) and *Lactobacillus* (*acidophilus*, *casei*, *fermentum*, *gasseri*, *johnsonii*, *paracasei*, *plantarum*, *rhamnosus*, and *salivarius*) are commonly used probiotics, neither the FDA nor the EFSA have today approved any claims for probiotics. Several randomized placebo-controlled trials have been performed in type 2 diabetic subjects, using mostly species from the genera *Lactobacillus* and *Bifidobacterium*, and meta-analyses suggest that probiotic intervention can improve glucose homeostasis in patients with type 2 diabetes (176-178). The effects of *C. minuta* on body weight are now also being tested in a clinical trial including normal weight, overweight, and obese subjects (NCT04663139). However, there are challenges when administrating live bacteria to the distal part of the intestine and improved delivery and engraftment may enhance the therapeutic effects. First, the bacteria have to survive unfavorable conditions during storage and during transit through the gastrointestinal tract, including exposure to oxygen, low gastric pH, bile acids, and enzymes. Microencapsulation can be used to protect the live bacteria against these unfavorable conditions and increase viability (179). Second, the beneficial effects of the probiotics may require bacterial engraftment in the intestine, which sometimes requires the coadministration of other microorganisms or different substrates, in other words administration of synbiotics. A synbiotic is defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" (180). Such synbiotic treatment has recently been tested in subjects with type 2 diabetic (181). This study found that 12-week administration of synbiotics containing inulin, *Akkermansia muciniphila*, *Clostridium beijerinckii*, *Clostridium butyricum*, *Bifidobacterium infantis*, and *Anaerobutyricum hallii* improved glucose metabolism after a standard 3-hour meal tolerance test. The synbiotic treatment was associated with altered fecal gut microbial composition with specifically consistent and increased levels of fecal *A. muciniphila* and *B. infantis*.

The International Scientific Association of Probiotics and Prebiotics has recently defined postbiotics as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (182). They state that "effective postbiotics must contain inactivated microbial cells or cell components, with or without metabolites, that contribute to observed health benefits". Furthermore, purified microbial metabolites may also be administrated. Supplementation of butyrate, alone or in combination with inulin, has been given to humans with some beneficial effects on inflammatory status and cardiometabolic phenotypes (183, 184). Canfora et al showed that colonic infusions of SCFA mixtures increased fat oxidation, energy expenditure, and plasma PYY in overweight/obese men (185). In contrast, Bouter et al did not observe metabolic benefits following butyrate administration in individuals with metabolic syndrome (186). This suggests that butyrate may have local effects in the colon that can improve host metabolism.

Gut Microbiota–Drug Interactions

A growing number of drugs has been shown to alter the gut microbiota and thus suggests that specific drugs may be developed to reshape the microbiota in a beneficial way. There are complex, bidirectional interactions between gut microbes and commonly used nonantibiotic drugs. While the gut microbial composition can be altered by drugs, the microbes can also modulate an individual's response to a drug by enzymatically transforming the drug's structure and altering its bioavailability, bioactivity, or toxicity (187). A large-scale in vitro screening showed the extensive impact of drugs on gut bacteria growth (13). When more than 1000 marketed drugs were screened against 40 representative gut bacterial strains, 24% of the drugs with human targets were found to inhibit the growth of at least 1 strain in vitro. One of these drugs was the cholesterol-lowering simvastatin. Further evidence for a role of statins in modulating the gut microbial composition comes from the cross-sectional MetaCardis Body Mass Index Spectrum cohort. As indicated above, the prevalence of Bacteroides2 enterotype correlates with BMI in this study. However, the Bacteroides2 enterotype was not as common in obese participants treated with statins as in those without treatment, suggesting that statins can potentially modulate the gut microbial composition directly or indirectly (31). Metformin is another example of a drug that alters the gut microbiota and that such change in the microbiome contributes

to its therapeutic effects (38, 188). In a randomized, double-blinded study, 4 months of treatment with metformin had strong effects on the gut microbiome with a potential increased ability to produce SCFAs including butyrate. Furthermore, when fecal samples from metformin-treated donors were transferred to GF mice, glucose tolerance was improved in mice that received metformin-altered microbiota compared with mice receiving the baseline microbiota (188). Several different alterations in microbial composition and their metabolite profile may mediate the beneficial effect of metformin. Sun et al showed that 3 days of metformin treatment in individuals with type 2 diabetes led to decreased levels of *Bacteroides fragilis* and increased levels of the bile acid glycoursodeoxycholic acid (189). They further showed that glycoursodeoxycholic acid is an intestinal FXR antagonist that improves the metabolic phenotype in a mouse model of obesity, and that FXR signaling is required for the effect of metformin. Colonization with *B. fragilis* on the other hand led to a more severe glucose intolerance in obese mice. Other studies indicate that the gut microbiota can affect nutrient sensing in the gut (190, 191), and that metformin can affect upper small intestinal glucose sensing via a sodium glucose cotransporter-1 (SGLT1)–GLP-1R–dependent pathway (191). While short-term HFD feeding led to diminished glucose-SGLT1 sensing and reduced abundance of *Lactobacillus* in the upper small intestine in rats, metformin treatment increased the abundance of *Lactobacillus* and restored SGLT1-dependent glucose sensing in these rats. Furthermore, metformin-pretreated microbiota from the upper small intestine in rats transplanted to HFD-fed recipient rats restored SGLT1-dependent glucose sensing. Thus, metformin and statins may affect gut microbial composition and function, which thereby potentially contribute to the efficacy of the drugs.

As mentioned, there is a bidirectional interaction between commonly used drugs and the microbiota. The effects of drugs can be modified by gut microbes directly by enzymatic conversion, changing the activity or the toxicity of the drug and leading to altered efficiency and side-effects. There is already a long list of drugs that are modified by gut microbes via enzymatic reactions, namely reduction, hydrolysis, oxidation, or acylation, and interindividual variation in enzymatic activity has been observed (192). Since the number of microbes residing in the distal part of the intestine is much higher than in the proximal part, the rate of absorption of a specific drug likely affects its microbial modification. In some cases, it may also be beneficial to modulate the gut microbiota before a certain therapy. An example of such a situation comes from cancer therapy. When cancer patients, previously not responding to anti–PD-1 (programmed cell death 1) therapy, were treated with FMT followed by reinduction of anti–PD-1 therapy, some of the patients started to respond to the therapy (193). Taken together, these results suggest that interindividual variation in gut microbial composition could affect drug efficiency and toxicity.

Some microbially produced metabolites, such as TMAO, have detrimental health effects and elevated levels of such metabolites could contribute to metabolic diseases. The possibility to specifically target the microbial enzymes involved in TMA production have now been explored. A structural analog of choline, 3,3-dimethyl-1-butanol has been shown to inhibit microbial TMA lyases leading to reduced TMAO production and attenuated choline diet–enhanced atherosclerosis in a mouse model (194). Following up on this study, second-generation inhibitors have been identified with increased potency, no observed toxicity, and sustainable suppression of TMAO levels that reverse choline diet–enhanced platelet responsiveness and thrombus formation (195). By specifically screening for inhibitors that accumulate in the microbes and are poorly absorbed, systemic exposure in the host can be limited and side effects could be minimized. These studies show that the more we understand about microbially produced metabolites, their signaling in host physiology, and their role in pathogenesis, the more sophisticated drugs can be developed that specifically targets certain microbial enzymes and minimizing side effects.

Conclusion and Future Perspective

Metabolic diseases, such as obesity, type 2 diabetes and CVD, are heterogenic diseases, and the etiology and clinical features differ between patients (196, 197). While diet, such as Western-style or high intake of red meat, has long been considered a risk factor for development of cardiometabolic disease, the importance of the gut microbiota has only recently been more recognized. We view the gut microbiota as an endocrine organ that acts as a filter through which the ingested nutrients need to pass. The diet–gut microbiota interaction determines which metabolites are formed and absorbed. Many key features of metabolic diseases including insulin resistance, atherogenic serum lipid profile, platelet hyperreactivity, thrombosis potential, and low-grade inflammation have been shown to be modulated by microbial products and metabolites (66, 82, 112, 198), suggesting that the gut microbial composition can contribute to different etiologies of cardiometabolic disease. Different factors including genetic predisposition, diet, physical activity, smoking, stress, and gut microbial composition, may interact and lead to a unique disease profile in each individual.

Ahlqvist et al could divide type 2 diabetic patients into 5 replicable clusters, which had significantly different patient characteristics and risk of diabetic complications (199). Similarly, Wagner et al found that such pathophysiological heterogeneity could already be identified in prediabetic individuals, potentially allowing early identification of individuals with increased risk of developing diabetes and complications (200). Better classification of patients allows us to predict a patient's response to a certain treatment regimen. We expect that it will be difficult to pinpoint 1 treatment strategy that is more beneficial than others targeting the gut microbiota. One patient subgroup may respond better to a certain treatment, while another patient subgroup may respond better to another treatment. For example, a subgroup characterized by deficiency of butyrate-producing bacteria may benefit from adding the missing microbes as pro- and synbiotics and/or the metabolite. Another subgroup of patients may have elevated levels of detrimental metabolites such as TMAO and imidazole propionate, and would benefit more from inhibitors designed to target microbial enzymes involved in the production of these metabolites. Accordingly, focus should be on validating which mechanisms contribute to disease in humans and thereafter adapt treatments, potentially in an individualized fashion. It is possible that the heterogeneity of these diseases together with variation in patients' baseline microbial composition cause the large interindividual variation in response to gut microbiota–targeted intervention, which has been observed in numerous studies (35, 78, 164, 201). Studies characterizing patients in-depth before and after an intervention, combining metagenomics, metabolomics, host tissue

transcriptomics, and clinical data could assist in identification of patient signatures that allow us to predict response to a specific intervention (202).

Much of the work to date has focused on cross-sectional observations, such as observing differences in the gut microbial composition between patients and healthy individuals at a certain timepoint when the disease is already manifested. However, such differences may only reflect the disease and not provide a complete picture of the role of the gut microbiota in disease development. Accordingly, longitudinal prospective studies in which individuals are followed before disease onset would be valuable to address causality and if microbiota, or their products, can be used as diagnostic markers (203). Similarly, longitudinal studies can provide understanding for intra- and interindividual variations over time in health and in response to different stresses (203, 204). Thus, future longitudinal studies will be important to identify microbial and metabolite signatures that cause metabolic disease, and to develop personalized treatment regimens targeting the gut microbiota.

There are several challenges en route to novel potential therapies beyond classical probiotics. Large-scale FMTs will likely have safety concerns and accordingly much focus has turned to next-generation probiotics. These are scientifically based; for example, bacteria that are reduced under certain conditions or patient groups have been isolated. However, many of these are strictly anaerobic, which will provide challenges for both production and storage. Furthermore, the regulatory framework on what is required for safety assessment and also classification of drugs vs food needs to be further clarified.

When considering the therapeutic potential of the gut microbiome, several questions remain to be answered. Can we for example prevent or treat metabolic diseases in the long term? Short-term studies indicate that gut microbiota modulation can have a beneficial effect, but long-term studies are required to determine the long-term outcome. If so, which are the best strategies to obtain a long-lasting effect, especially considering compliance from the patients and the individuals at risk of developing disease? Furthermore, much remains unknown regarding the complex interaction between gut microbes, diet, and other risk factors. Future studies are needed to disentangle these interactions, paving the way for tailored/individualized treatment strategies. Other questions relate to safety and drug interactions. Are there any risks of adverse events associated with different gut microbiota–targeting therapies? Furthermore, since patients with metabolic disease often use several different drugs, how does gut microbiota modulation affect the bioavailability, bioactivity, and toxicity of these drugs? Can gut microbiota–targeted therapy be combined with commonly used drugs for a more beneficial result or should certain combinations of drugs and microbiota-targeted therapy be avoided?

In conclusion, increasing number of studies suggest that the gut microbiota can be targeted, and that modulation of the gut microbiota and its function could be beneficial. Based on the baseline microbiome and/or clinical biomarkers, we may be able to identify optimal individualized treatment regimens targeting the gut microbiota. However, much is still unknown regarding host–microbe interactions and future work is needed to further characterize these interactions and the effects that a microbiota-targeted intervention could have on the host.

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Disclosure Summary

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