The role of the gut microbiota in metabolic health

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ABSTRACT **The global prevalence of obesity and related comorbidities has increased considerably over the past decades. In addition to an increase in food consumption and a reduction in physical activity, growing evidence implicates the microorganisms in our gastrointestinal tract, referred to as the gut microbiota, in obesity and related metabolic disturbances. The composition of the gut microbiota can fluctuate markedly within an individual and between individuals. Changes in gut microbial composition may be unfavorable and predispose an individual to disease. Studies in mice that are germ free, mice that are cohoused, and mice that are treated with antibiotics have provided some evidence that changes in gut microbiota may causally contribute to metabolic disorders. Several mechanisms have been proposed and explored that may mediate the effects of the gut microbiota on metabolic disorders. In this review, we carefully analyze the literature on the connection between the gut microbiota and metabolic health, with a focus on studies demonstrating a causal relation and clarifying potential underlying mechanisms. Despite a growing appreciation for a role of the gut microbiota in metabolic health, more experimental evidence is needed to substantiate a cause-and-effect relationship. If a clear causal relationship between the gut microbiota and metabolic health can be established, dietary interventions can be targeted toward improving gut microbial composition in the prevention and perhaps even the treatment of metabolic diseases.—Janssen, A. W. F., Kersten, S. The role of the gut microbiota in metabolic health. *FASEB J.* 29, 3111–3123 (2015). www.fasebj.org**

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THE GLOBAL PREVALENCE OF OBESITY has increased substantially over the past decades (1). As a consequence, there has been a rise in obesity-related comorbidities, such as diabetes mellitus, dyslipidemia, and hypertension, each of which serves as an independent risk factor for cardiovascular diseases (2–4).

The increase in obesity rates is believed to be the result of increased consumption of calorie-dense foods in combination with reduced levels of physical activity. Interestingly, growing evidence implicates the microorganisms residing within the gastrointestinal tract in obesity and its associated metabolic disturbances (5, 6). Initial studies indicated that diet-induced obesity (DIO) in mice is associated with changes in gut microbial composition (7–9). In addition, obese humans were shown to have an altered composition of the gut microbiota, as compared to that of lean individuals (10, 11). These early findings have fed the notion that the gut microbiota plays a role in obesity. Besides obesity, changes in the gut microbial community also have been found in patients with symptomatic atherosclerosis (12), in individuals with type 2 diabetes (13), and in patients with nonalcoholic fatty liver disease (NAFLD) (14, 15), suggesting that the gut microbiota has a role in these diseases as well.

However, these cross-sectional and correlative studies did not discriminate between the possibilities that the observed changes in the gut microbiota causally contribute to obesity and associated diseases or occur as a consequence of the disturbed metabolism or immune system. Therefore, in this review, we examine the role of the gut microbiota in mediating the effects of diet on metabolic health and explore the potential underlying mechanisms. In the first part, we describe the normal function of the gut microbiota in humans, and in the second part, we discuss the effect of diet on the gut microbiota and the potential relationship with metabolic diseases.

HUMAN GUT MICROBIOTA

All higher organisms live in an intimate relationship with microorganisms. Microorganisms that live in a particular environment such as the intestine are known collectively as a microbiota. A microbiota is composed of bacteria, viruses, fungi, archea, and protozoa. They reside at every surface that is in contact with the external environment, but in particular at mucosal surfaces of the gastrointestinal tract (16–18). The advent of new molecular techniques, such as 16s rRNA sequencing and dedicated DNA Chips, has made it possible to identify and study the composition of the human gut microbiome (19, 20). It is estimated that the number of bacteria in the human intestine easily reaches 10^{14} and is predominantly composed of Bacteroidetes and Firmicutes (90%),

Abbreviations: Angptl4, angiopoietin-like protein 4; Cb1, cannabinoid receptor 1; DIO, diet-induced obesity; FXR, farnesoid X receptor; NAFLD, nonalcoholic fatty liver disease; PPARγ, peroxisome proliferator-activator receptor γ; PYY, peptide YY; SAA, serum amyloid A; SCFA, short-chain fatty acid; SREBP, sterol regulatory element-binding protein; TGR5, G protein-coupled bile acid receptor; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide; WAT, white adipose tissue

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complemented with Actinobacteria, Proteobacteria, and Verrucomicrobia (5, 21, 22).

The gut microbiota lives in a mutualistic relationship with its host that is beneficial to both organisms, and it is especially important in the development of the immune system. The complete absence of bacteria in the GI tract leads to large defects in the development of gut-associated lymph tissues, low levels of secretory IgA antibodies in the intestine, and less and smaller mesenteric lymph nodes. Furthermore, the gut microbiota has an important role in protecting the host against invasion of intestinal pathogens and in maintaining tissue homeostasis (18, 23, 24). Without the gut microbiota, dietary fibers such as inulin, pectin, xylans, and mannans would leave the body unaffected. Fermentation of dietary fiber by bacteria yields energy, which is important in the growth and maintenance of the microbial community. In addition, fermentation leads to the formation of metabolic end products that are beneficial to the host (25, 26). The principal end products of carbohydrate fermentation are the short-chain fatty acids (SCFAs) acetate, propionate, and butyrate, as well as gases such as CO_2, H_2, and CH_4 (26, 27). SCFAs are very effectively absorbed in the colon, giving rise to minimal loss in the feces. The host utilizes SCFAs for a variety of different purposes. Whereas butyrate is an important energy source for colonic epithelial cells, acetate and propionate can be utilized by the liver for lipogenesis and gluconeogenesis, respectively (5, 25, 28).

CHANGES IN GUT MICROBIAL COMPOSITION AND METABOLIC HEALTH

The composition of the gut microbiota is not constant but differs between individuals and can fluctuate markedly within an individual. Interindividual variation in bacterial diversity is caused by differences in host genomes, but also by environmental factors, such as antibiotic use, lifestyle, hygiene, and diet. An altered gut microbial composition, defined as dysbiosis, may be unfavorable and may predispose an individual to disease (18, 29, 30).

How diet affects gut microbiota

Wong *et al.* (25) proposed that the gut microbiota evolved in conjunction with the consumption of diets containing large amounts of nondigestible fibers and complex carbohydrates, which presumably was the diet of our prehistoric ancestors. They reasoned that modern diets that are low in dietary fibers malnourish the gut microbiota and thereby negatively influence the health of the host. De Filippo *et al.* (31) examined to what extent consumption of a Western diet differentially affects human gut microbial composition as compared with the diets of our ancestors, which was characterized by large amounts of starch, fiber, and plant polysaccharides, and low amounts of fat and animal protein. In this study, the fecal microbiotas of 14 healthy children living in Burkina Faso, Africa, were compared with the fecal microbiotas of 15 healthy children from Florence, Italy. Compared to the feces of children from Italy, the feces of the African children contained higher amounts of Bacteroidetes and lower amounts of Firmicutes (31). However, not all studies that assessed microbial composition found similar associations. Wu *et al.* (32) showed that Bacteroidetes and Actinobacteria are positively associated with fat-rich diets and negatively associated with fiber-rich diets, whereas the opposite applied to Firmicutes and Proteobacteria. David *et al.* (33) found that an animal-based diet has a more pronounced impact on gut microbial clusters than does a plant-based diet. In addition, it has been found that Firmicutes associate negatively and Bacteroidetes positively with animal-based diets (33). In contrast, no effect of diet on any phyla was observed in a 10-wk controlled-feeding study in which obese individuals successively consumed a control diet, a diet rich in resistant starch or nonstarch polysaccharides, and a reduced carbohydrate weight-loss diet. The diets' effects were observed only at lower taxonomic levels (34). One of the possible reasons that changes in gut microbial composition differed in the various studies is the duration of the diet intervention. Gut microbial composition has been shown to be stable, even up to 10 d after the switch to a new diet (32). Besides the duration of the dietary intervention, the composition of an individual's gut microbiota before the intervention may also influence the effect of diet on gut microbial composition (34, 35). This notion is supported by a recent mouse study that showed that the gut microbial composition is not only determined by the current diet but also partly depends on dietary history (35). Additionally, a study in human subjects concluded that a change in dietary fiber can produce marked changes in the gut microbiota, but these depend on the initial composition of an individual's gut microbiota (34). In general, it can be concluded that diet markedly affects gut microbial composition at the phylum level and especially at the lower taxonomic levels (32, 34, 36, 37), although not necessarily in a manner that is consistent between individuals.

In addition to diet, host genetic makeup, and nondietary environmental factors, such as antibiotic use, lifestyle, and hygiene, may also affect microbial composition (18). It has been found that the within-twin similarity in fecal microbial composition is comparable between adult monozygotic twin pairs and dizygotic twin pairs, indicating that environmental factors play a dominant role in determining gut microbial composition (38). Some of these environmental exposures may exert their effects early in life, such as in the route of childbirth, feeding method (breast or bottle feeding), and weaning. For instance, the gut microbiota of newborns delivered *via* cesarian section more closely resembles the microbiota of the maternal skin, whereas in those delivered vaginally, the gut microbiota is derived primarily from the maternal birth canal and rectum, giving rise to an abundance of *Lactobacillus*, *Prevotella*, and *Atopobium* (39). Taken together, diet markedly influences intestinal microbial composition, but to date there is no evidence that particular dietary components have a specific and consistent effect on gut microbial composition, likely because of the major confounding effects of genetic makeup and various environmental factors.

To overcome the inherent limitations of cross-sectional studies and directly investigate the functional impact of the gut microbiota, investigators have performed studies in germ-free mice. These mice are bred and born without exposure to any microorganisms and can be colonized with specific microbial communities to create gnotobiotic mice.

These gnotobiotic mice are a powerful tool for exploring the interaction between the gut microbiota and the host, because host genotype, diet, microbial composition, and other environmental factors can be strictly controlled (40). A proof-of-principle study showed that the gut microbial composition of germ-free C57BL/6 mice colonized with adult human fresh or frozen fecal microbiota largely resembled the microbial composition of the donor. Switching to a Western diet with high levels of fat and sugar resulted in a significant decrease in Bacteroidetes and an increase in Erysipelotrichi, a class of bacteria from the phylum Firmicutes (41). A similar shift in the microbial community was observed in another study in which germ-free mice were cultured with human microbiota, followed by consuming a Western diet (42). Thus, studies in germ-free mice are instrumental in dissecting the role of the gut microbiota in mediating the effects of diet on metabolic health without interference by potential confounders.

Association between gut microbiota and metabolic health

As described above, diet markedly affects gut microbial composition. Diet also influences obesity, as seen in wild-type mice fed a high-fat diet rich in saturated fat, which is a frequently used model of DIO. An interesting finding is that, concurrent with enhanced weight gain, DIO mice have an altered gut microbial community, as compared with mice fed a low-fat diet, suggesting that obesity is linked to the gut microbiota (7, 43). Alterations in microbial composition were also found in genetically obese mice, which exhibited increased levels of Firmicutes and reduced levels of Bacteroidetes in the cecum in comparison with their lean wild-type and heterozygous littermates (8). An increase in the ratio of Firmicutes to Bacteroidetes was also observed in obese rats (44) and pigs (45), when compared with animals in the lean state. The above studies suggest that obesity influences gut microbial composition or *vice versa*. In contrast, Hildebrandt *et al.* (46) reported that intestinal microbial composition is primarily influenced by diet and not by the obese state. Using Resistin-like molecule β-knockout mice, which are resistant to DIO, they showed that switching from a chow to a high-fat diet changes gut microbial composition independent of the obese state. Furthermore, *ob/ob* mice, which are genetically altered obese mice characterized by a mutation in the leptin gene, showed no difference over time in the number of fecal Firmicutes, despite progressive obesity (47). It could thus be argued that high-fat feeding *per se*, independent of obesity, was the primary determinant of gut microbial composition in DIO studies.

Causal link between gut microbiota and metabolic health

The major limitation of the studies mentioned above is that they merely correlated microbial composition with obesity. Whether a relationship between the microbiota and obesity is in fact causal can be ascertained by performing studies of gnotobiotic animals, animals treated with antibiotics, or animals that are cohoused, allowing modulation of the gut microbial composition. The use of such approaches makes it possible to determine whether changes in gut microbial composition directly affect metabolic health or *vice versa*. Studies in germ-free mice have supported a causal role for the gut microbiota in obesity. Whereas conventionally raised mice develop DIO, germ-free mice are protected against the development of obesity (48–50). Furthermore, it has been observed that transplantation of the gut microbial community from conventionally raised obese mice to germ-free or antibiotic-treated mice causes more pronounced weight gain than colonization with microbiota from lean conventionally raised donors, indicating that the composition of gut microbiota can affect obesity (9, 51).

To examine whether the human gut microbiota may affect weight gain, germ-free mice were colonized with fecal microbiota from adult humans and placed on a Western diet, resulting in increased adiposity when compared to mice colonized with human microbiota followed by a low-fat, plant polysaccharide-rich diet. Strikingly, the feces from mice colonized with human microbiota and fed a Western diet caused higher levels of total body fat when introduced into germ-free recipient mice by gavage, as compared with feces from mice colonized with human microbiota and fed a low-fat, plant polysaccharide-rich diet (41).

As an extension to the above findings, Ridaura *et al.* (52) transplanted into germ-free mice the fecal microbiota of adult female twin pairs that were discordant in obesity. An increase in adiposity and total body mass was found in the recipients of the gut microbiota transplanted from the obese twin, as compared to the mice that received the lean twin's gut microbiota. Of particular note, the obese state was transferable, as observed, by cohousing mice that received transplants of either the obese or lean twin's microbiota. Because mice are coprophagic, cohousing causes transfer of the gut microbiota *via* the ingestion of other animals' feces.

Further suggesting a causal link between gut microbiota and obesity, treatment of mice with the antibiotic vancomycin, which caused a decrease in the relative amounts of Bacteroidetes and Firmicutes combined with an increase in the abundance of Proteobacteria, reduced weight gain in mice fed a high-fat diet (53). In a recent study, it was shown that antibiotic treatment early in life may set metabolic consequences in motion. Giving mice whose mothers were treated with penicillin before the birth of their pups a low dose of penicillin during the weaning period increased DIO and visceral fat accumulation, and affected hepatic gene expression and metabolic hormone levels such as peptide YY (PYY). A causal role for the microbiota was confirmed by transplanting the gut microbiota from antibiotic-treated mice to germ-free mice. Remarkably, cessation of the antibiotic treatment induced a marked recovery of microbial composition but did not change the metabolic phenotype (54).

Taken together, the evidence from studies in mice suggests a causal link between the gut microbiota and obesity. Complementing the animal studies mentioned above, several human studies support such a correlation. Specifically, humans with obesity have been shown to have an increased ratio of Firmicutes and Bacteroidetes in comparison with that of lean individuals (10). In addition, the abundance of Bacteroidetes increases with weight loss, either by fat- or carbohydrate-restricted low-calorie diets (10)

or after a gastric bypass (55). Because people who undergo gastric bypass also have to make major adjustments in their diet, the latter study did not provide any information on whether the changes in microbial composition are dictated by the changes in diet or by the reduced adiposity.

Although other studies have found changes in gut microbial composition in obese individuals, an increase in the Firmicutes:Bacteroidetes ratio in obesity and the increase in the presence of Bacteroidetes during weight loss have not been observed consistently (11, 38, 56–58). Confounding factors such as diet, fasting (59), hygiene, lifestyle (18), and the use of antibiotics (60) affect gut microbial composition and may explain the discrepancies between findings in these studies. These confounding factors, as well as species-specific factors, may also explain why vancomycin treatment has been associated with weight gain in adult subjects (61), whereas vancomycin reduces weight gain in mice (53). In conclusion, some human studies have suggested that intestinal microbial composition is altered in obesity, although more studies are necessary to determine the direction and causality of the relationship.

In addition to its association with obesity itself, the gut microbiota may also be connected with perturbations that are coupled to obesity, including systemic inflammation, insulin resistance, and NAFLD (14, 62, 63). Cohousing experiments revealed that the gut microbiota may have an important role in NAFLD. Numerous studies have demonstrated that activation of the inflammasome by endogenous danger signals, such as reactive oxygen species, are involved in the progression of NAFLD. Activation of the inflammasome causes cleavage and thereby activation of the proinflammatory cytokine IL-18 (64–66). In an intriguing finding, Henao-Mejia *et al.* (63) reported that mice deficient in IL-18 developed hepatic steatosis and inflammation. To investigate whether this phenotype is caused by an altered gut microbial composition, IL-18 knockout mice where cohoused with wild-type mice. Cohousing of these mice unexpectedly exacerbated hepatic steatosis and inflammation in the wild-type mice, indicating that the gut microbiota may promote development of NAFLD. A causal role of the gut microbiota in NAFLD is supported by the finding that antibiotic treatment reduced hepatic triglyceride accumulation in mice fed a high-fat diet (67) or a diet rich in fructose (68), but also reduced hepatic T cell infiltration in mice with concanavalin A-induced hepatitis (69).

A causal role for the gut microbiota in systemic inflammation and insulin resistance has been found in studies with gnotobiotic and antibiotic-treated animals. Whereas ileal TNFα expression does not differ between germ-free mice fed a low- or high-fat diet, TNFα expression is elevated in conventionally raised mice fed a high-fat diet, as compared with its expression in those fed a low-fat diet. Since TNFα is a biomarker of proinflammatory changes in the intestine, these data suggest that the gut microbiota, in combination with a high-fat diet, promotes intestinal inflammation. High TNFα levels have been shown to correlate significantly with the progression of obesity and the development of insulin resistance (50). A causal role of the gut microbiota in insulin resistance is supported by the finding that vancomycin treatment decreases plasma TNFα levels and improves fasting blood glucose levels in mice fed a high-fat diet (53). Compared to conventionalized mice, germ-free mice have been found to have improved insulin sensitivity, which is accompanied by lower plasma TNFα and serum amyloid A (SAA) levels (70). High SAA levels lower insulin sensitivity in adipocytes (71) and may therefore explain the insulin resistance observed in conventionalization studies (70, 72, 73). In addition, fasting plasma free fatty acid levels have been found to be higher in conventionally raised mice (70), also potentially contributing to insulin resistance (74). Membrez *et al.* (75) reported that, in 2 different mouse models of insulin resistance, treatment with norfloxacin and ampicillin lowered fasting and post–glucose tolerance test glucose and insulin plasma levels. Since norfloxacin and ampicillin suppress Enterobacteriaceae and bacteria from the genuses *Bacteroides*, *Lactobacillus*, and *Bifidobacterium*, it can be hypothesized that these bacteria have detrimental effects on glucose tolerance.

A recent high-profile study indicated that the gut microbiota may have antidiabetic effects in humans. Infusion of gut microbiota from lean subjects into individuals with metabolic syndrome increased fecal microbial diversity, including in butyrate-producing bacteria. Along with these changes in their microbial community, recipients of "lean" microbiota also showed increased insulin sensitivity (73). Although these findings need confirmation, they provide the first evidence of a causal link between gut microbiota and insulin resistance in humans.

HOW THE MICROBIOTA MAY AFFECT METABOLIC HEALTH

The studies presented above hint at the notion that instead of merely being a consequence of metabolic disorders, changes in intestinal microbial composition may in fact causally contribute to these disorders, although more evidence is needed. The uncertainty regarding the causal link between the gut microbiota and metabolic health has not prevented exploration of the potential underlying mechanisms that may connect the two, which include increased energy harvest, endotoxemia, altered SCFA signaling, and choline and bile acid metabolism.

Energy harvest and Angptl4

The gut microbiota provides the host with energy by extracting calories from otherwise indigestible carbohydrates *via* bacterial fermentation. In the intestine, complex carbohydrates are degraded by the gut microbiota into monosaccharides, which are subsequently fermented. Fermentation products include the gases H_2, CO_2, and CH_4 and the SCFAs, which can be absorbed by the host and used as an energy source (28). The notion that the gut microbiota provides energy to the host is supported by the finding that germ-free mice, which are not naturally colonized with microorganisms, gain less weight when fed a high-fat diet than do conventionally raised mice (48–50).

In support of a role of fermentation in excess fat storage during obesity, *ob/ob* mice exhibit increased fermentation in the cecum, as revealed by an increase in the SCFAs acetate and propionate (76). Metagenomic analysis has revealed that the *ob/ob* gut microbiome is enriched in

genes involved in extracting energy from food, which may suggest that the gut microbiota from obese mice extracts energy from the diet more efficiently. This notion was supported by two findings. First, *ob/ob* mice had significantly less energy in their feces than did their wild-type littermates. Second, transplantation of the gut microbiota from *ob/ob* mice to germ-free mice caused a significant increase in total body fat, as compared to transplantation from lean wild-type mice (76). Therefore, *ob/ob* mice may not only become obese because of increased food intake but also because of the increased energy harvest by altered gut microbiota.

The main products of bacterial degradation and fermentation are monosaccharides and SCFAs. As a result of increased energy harvest, it has been shown that conventionally raised mice have increased monosaccharide uptake from their gut compared to their germ-free counterparts. It has been postulated that the increase in monosaccharide uptake promotes hepatic triglyceride synthesis, the accumulation of triglycerides in adipocytes, and subsequently, an increase in body fat. In one study, conventionally raised mice had higher liver triglyceride levels and increased expression of the lipogenic transcription factors sterol regulatory element-binding protein (SREBP)-1 and carbohydrate-responsive element-binding protein (ChREBP), as well as their targets acetyl-CoA carboxylase and fatty acid synthase (72). These results are in accordance with those of another study that showed that mice conventionally raised on a high-fat diet gained more body weight than did germ-free mice, because of the more efficient conversion of ingested food to body weight (70). In addition, it has been suggested that germ-free mice and mice treated with antibiotics are better able to oxidize fatty acids in the liver, muscle, and adipose tissue because of increased AMPK activity and the consequent reduction in acetyl-CoA carboxylase activity, which may protect them from DIO (48, 77).

It is noteworthy that germ-free mice lacking the angiopoietin-like protein 4 (*Angptl4*) gene are not protected against body weight gain (48, 72). Intestinal Angptl4 expression has been shown to be significantly lower in conventionally raised mice than in germ-free mice (48, 72). Angptl4 is an inhibitor of the enzyme lipoprotein lipase, which is responsible for clearing triglycerides from the blood into the tissues. Accordingly, suppression of Angptl4 by the gut microbiota may enhance extraction of triglycerides from the blood and promote their storage in adipose tissue (72, 78). A recent study showed that Angptl4 may regulate intestinal lipid uptake by inhibiting intestinal lipase activity. Microbial suppression of Angptl4 may therefore promote weight gain *via* the loss of its inhibitory effect on intestinal lipase activity, leading to increased intestinal triglyceride hydrolysis and lipid uptake (79).

An overview of how microbiota might affect obesity *via* energy harvest and Angptl4 is displayed in **Fig. 1**.

Gut permeability

It has also been postulated that the microbiota affects metabolic health by modifying gut permeability. Membrez *et al.* (75) observed that the improved glucose tolerance in mice treated with norfloxacin and ampicillin was not due to differences in body weight but may be related to reduced inflammation and protection against endotoxemia, based on decreased plasma levels of LPS and jejunal TNFα expression in antibiotic-treated mice.

It has been suggested that endotoxemia can be triggered by diet. Fat-enriched diets alter gut microbial composition, as described earlier, but have also been shown to increase plasma LPS levels in mice (80) and humans (81). LPS is a major constituent of the gram-negative bacterial cell wall. Small amounts of endotoxin in the bloodstream may elicit a low-grade systemic immune response similar to that observed during obesity (82). A role for the gut microbiota in this process has been suggested because mice fed a high-fat diet have increased levels of cecal LPS-containing gram-negative bacteria (80) and also exhibit increased intestinal permeability with reduced expression of the tight junctions zonula occludens (ZO)-1 and occludin (62, 83). Besides reducing the expression of intestinal tight junctions, there is also evidence that the gut microbiota may regulate gut permeability *via* the endocannabinoid system. In a study by Muccioli *et al.* (84), modulation of gut microbial composition, either by colonizing germ-free mice or treating mice with antibiotics, reduced colonic and adipose tissue

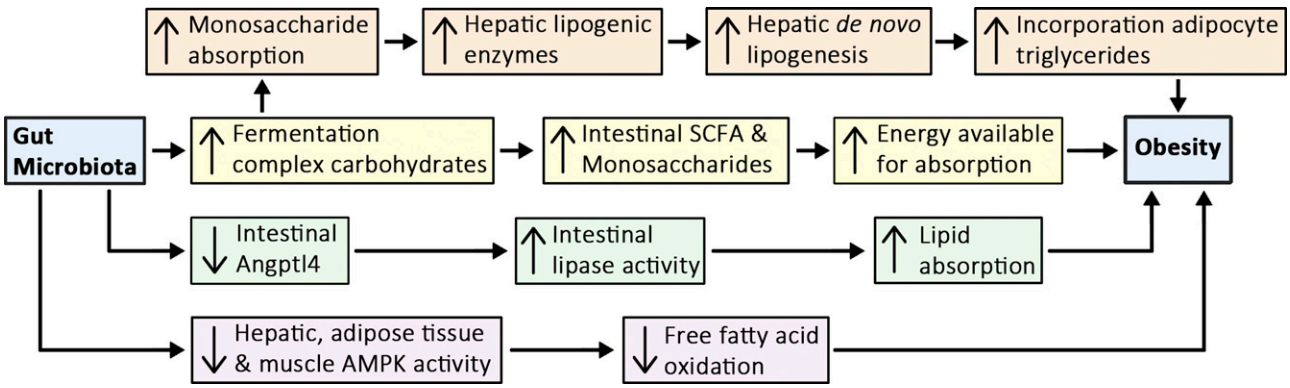

Figure 1. How microbiota may affect obesity *via* energy harvest and Angptl4. Gut microbiota may increase energy harvest from the diet by fermenting complex carbohydrates, yielding energy in the form of monosaccharides and SCFAs. Increased monosaccharide absorption may lead to increased hepatic lipogenesis and may favor weight gain. The gut microbiota may also contribute to obesity by reducing free fatty oxidation and increasing intestinal lipid absorption.

cannabinoid receptor (Cb)-1 expression, whereas feeding the mice a high-fat diet increased Cb1 expression. Blocking this receptor in mice through the infusion of a Cb1 receptor antagonist resulted in an improved intestinal integrity, reduced plasma LPS levels, and reduced adiposity and body weight gain, compared to the effects observed in saline-infused mice.

Lam *et al.* (62) found that the decrease in relative abundance of *Lactobacillus* and increase in *Oscillobacter* during high-fat feeding significantly correlated with decreased transepithelial resistance. In turn, Cani *et al.* (83) showed that changing gut microbial composition with antibiotic treatment in mice fed a high-fat diet significantly reduced gut permeability and increased tight junction expression, in association with reduced levels of circulating LPS and cecal LPS. Furthermore, it has been shown that reduced plasma LPS levels after antibiotic treatment improves metabolic health in mice fed a high-fat diet. Serum IL-6 and TNFα levels are lowered, indicating reduced systemic inflammation. Antibiotic treatment also results in less infiltration of macrophages in adipose tissue, less body weight gain, and improved insulin and glucose tolerance, all conditions related to systemic inflammation (77). Similar findings have been made in antibiotic-treated *ob/ob* mice. The metabolic and inflammatory effects of antibiotic treatment are mostly mimicked by deletion of the LPS receptor CD14 (83). In addition, LPS seems to have detrimental effects on the progression of NAFLD (85). Antibiotic treatment in mice with hepatic steatosis reduces hepatic lipid accumulation, which is associated with lower portal vein endotoxin levels (68).

Based on these and other findings, the gut microbiota may thus be involved in modifying intestinal permeability and trigger low-grade (metabolic) endotoxemia and related disturbances (49, 75). However, it should be mentioned that investigators in several studies have not been able to replicate the elevated plasma LPS levels that occur during high-fat feeding, despite causing obesity (86–88). It has also been suggested that a high-fat diet increases the sensitivity of mice to LPS without affecting its plasma level (88).

In addition to LPS, other bacterial cell wall components may be involved in mediating the effects of gut microbiota on metabolic health. Monocolonization of germ-free mice with *Escherichia coli* or a mutant *E. coli* variant has shown that only the wild-type *E. coli* strain raises plasma LPS levels. However, both *E. coli* strains impair insulin and glucose tolerance and increase adiposity (49). A possible role for the bacterial cell wall component peptidoglycans may be hypothesized, based on the finding that activation of the peptidoglycan receptor nucleotide-binding oligomerization domain-containing protein (NOD)-1 induces hepatic and peripheral insulin resistance in mice, whereas loss of NOD1 and NOD2 in mice fed a high-fat diet causes higher insulin sensitivity and lower adipose tissue inflammation (89).

The possible effects of the gut microbiota on gut permeability, glucose metabolism, and inflammation are depicted in **Fig. 2**.

Microbial regulation of metabolic processes

SCFAs

SCFAs are the main end products of bacterial fermentation in the intestine. The number of microbiota in the gut, the microbial composition, the gut transit time, and the available substrates for microbial fermentation are all factors that influence the production of SCFAs (25, 90), which serve not only as substrates for energy production, lipogenesis, and gluconeogenesis, but also can regulate biologic processes by serving as signaling molecules.

An important set of molecular targets for SCFAs are GPCR41 and -43. These proteins are expressed in numerous tissues, including adipose tissue and enteroendocrine cells, leading to activation of distinct downstream effects (91, 92). GPCR41 and -43 are activated by a similar set of ligands but differ somewhat in their specificity. Whereas GPCR41 is activated more potently by propionate as compared with acetate, GPCR43 displays a preference for acetate over propionate (93).

Samuel *et al.* (94) showed that the gut microbiota promotes adiposity and body weight *via* GPCR41-mediated signaling. They demonstrated that conventionally raised wild-type mice and germ-free wild-type mice cocolonized with *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii*—two prominent bacteria in the distal human gut—had significantly more adiposity and gained more body weight than their conventionally raised GPCR41-knockout littermates. The increased adiposity and body weight gain may be related to increased serum levels of PYY, leading to

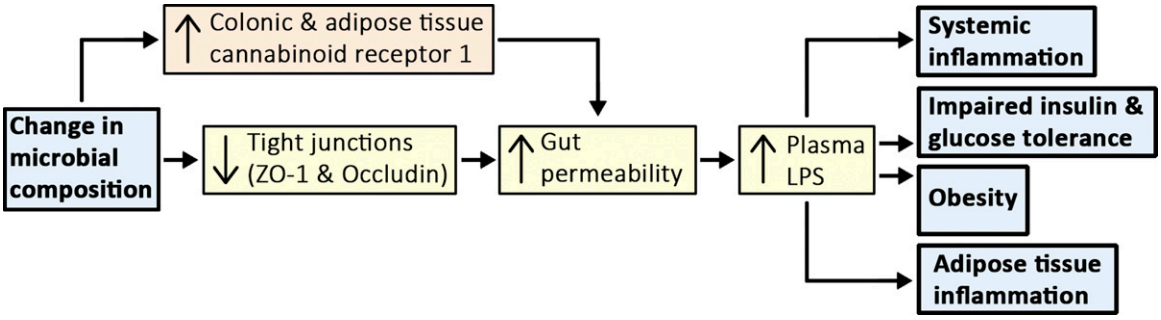

Figure 2. Possible effects of gut microbiota on gut permeability, glucose metabolism, and inflammation. High-fat diet-induced changes in microbial composition may increase gut permeability *via* Cb1, the expression of tight junctions, or both. The increase in gut permeability induces endotoxemia and thereby possibly systemic inflammation, obesity, impaired insulin and glucose tolerance, and adipose tissue inflammation.

a reduced gut transit rate. As a consequence, conventionally raised wild-type mice may extract calories from their diet more efficiently and absorb more SCFAs, resulting in enhanced hepatic *de novo* lipogenesis, as indicated by increased hepatic triglyceride levels. These features were blunted in germ-free wild-type and GPCR41-knockout mice, as well as in germ-free GPCR41-knockout mice that were cocolonized. In contrast, Lin *et al.* (95) found that body weight gain was similar between wild-type and GPCR41-knockout mice, suggesting a lack of effect of GPCR41 on adiposity. However, food intake was higher in GPCR41-knockout mice than in wild-type mice, suggesting less efficient energy harvest and thus a decrease in the food efficiency ratio in the GPCR41-knockout mice. Adding to the controversy, oral administration of butyrate and propionate was found to inhibit DIO independent of GPCR41. With respect to GPCR43, Kimura *et al.* (96) showed that acetate-mediated signaling *via* GPCR43 may prevent obesity. GPCR43-knockout mice fed a high-fat diet and treated with antibiotics and acetate had increased body and white adipose tissue (WAT) weights, which were reduced in wild-type mice treated with the same regimen. Consistent with an antiobesity effect of GPCR43, mice with adipose tissue-specific overexpression of GPCR43 had significantly lower body and adipose tissue weight than did their wild-type littermates. These mice also exhibited impaired insulin signaling in WAT, but not in muscle and liver, and had increased energy expenditure, which seemed to be attributable to a muscle-specific increase in the expression of glycolysis- and β-oxidation genes and reduced expression of gluconeogenesis genes. Taken together, SCFAs, mainly acetate, may activate adipose tissue GPCR43 to reduce uptake of fatty acids and glucose by adipocytes and improve systemic insulin sensitivity, leading to consumption of lipids and glucose by other tissues. It has been speculated that through this mechanism, adipose tissue GPCR43 activation may reduce adiposity and improve systemic insulin sensitivity. Whether circulating SCFA concentrations are sufficiently high to cause substantial activation of GPCR43 in adipose tissue requires further study.

In addition, GPCR43 activation in the intestine may have an antidiabetic effect. It has been shown that GPCR43 activation by SCFAs promotes the release of glucagon-like peptide-1 by intestinal enteroendocrine L cells, thereby stimulating the release of insulin and leading to improved glucose tolerance (97).

The above described actions of SCFAs *via* GPCR43 may provide a molecular explanation for the beneficial effects of dietary fiber on metabolic health (98). Diets rich in fiber increase the number of SCFA-producing bacteria and fecal SCFAs, as observed in a comparison of fecal microbiota from healthy European and African individuals (31). However, SCFA-mediated GPCR41 signaling seems to provoke opposite effects. Therefore, additional studies are needed to sort out to what extent GPCR43 and -41 mediate the metabolic effects of SCFA in the intestine and other organs. In a recent study, it was shown that, in addition to GPCRs, SCFAs may elicit their effects *via* the peroxisome proliferator-activator receptor γ (PPARγ). Specifically, it was observed that feeding mice a diet rich in inulin, which leads to enhanced SCFA production, induces PPAR target genes and pathways in the colon. *In vitro* studies have indicated that butyrate and propionate directly activate PPARγ (99). In another study, SCFA supplementation in mice fed a high-fat diet protected against body weight gain, which was accompanied by improved insulin sensitivity and decreased hepatic triglycerides. The reduction in hepatic steatosis by SCFAs was not observed in liver-specific PPARγ-knockout mice, whereas the reduction in weight gain and the improved insulin sensitivity after SCFA supplementation were abolished in adipose tissue-specific PPARγ-knockout mice, indicating that the SCFA-induced effects are mediated *via* a tissue-specific PPARγ-dependent mechanism (100).

An overview of the possible GPCR and PPARγ-mediated effects of SCFAs on obesity and diabetes is displayed in **Fig. 3**.

Choline metabolism

As described in this review, changes in gut microbial composition may be linked to the development of obesity and diabetes. Emerging evidence also links the gut microbiota to cardiovascular diseases (101–103).

Wang *et al.* (101) were the first to report a relationship between microbial choline metabolism and development of atherosclerosis. Choline is an essential nutrient that is present in foods such as eggs and red meat. It is a component of cell membranes and is also involved in lipid metabolism (28). Feeding mice with labeled phosphatidylcholine resulted in increased levels of plasma trimethylamine (TMA) and subsequently trimethylamine *N*-oxide (TMAO), both metabolites of choline. TMA is formed in the intestine, absorbed, and rapidly metabolized in the liver into TMAO by hepatic flavin-containing monooxygenases. It is of interest that plasma TMAO levels were not increased in phosphatidylcholine-fed germ-free mice or mice treated with antibiotics, suggesting that the gut microbiota is involved in metabolic processing of dietary choline (101).

Feeding atherosclerosis-prone ApoE-knockout mice a diet supplemented with 1% choline resulted in increased plasma TMAO levels and a large increase in the number of foam cells, probably due to the increased expression of the cholesterol influx receptors CD36 and SRA. In addition, an increase in atherosclerotic lesion size was observed after addition of choline to the diet. When the gut microbiota was suppressed by antibiotics, foam cell formation and atherosclerotic development were inhibited (101).

Similar results have been seen in humans. When humans are challenged with phosphatidylcholine, plasma TMAO levels increase. Plasma TMAO levels are almost completely blunted after antibiotic treatment and reappear when the use of antibiotics is discontinued. In addition, high plasma TMAO levels in humans have been found to be associated with a high risk for major adverse cardiovascular events, even when the hazard ratios are adjusted for the traditional risk factors (104).

Expanding on the above findings, Koeth *et al.*(102) found that supplementation with choline, L-carnitine—a nutrient in red meat that also contains a TMO—or TMAO reduces reverse cholesterol transport, which describes the movement of cholesterol from peripheral tissues back to the liver *via* the plasma. The reduced reverse cholesterol transport was abolished when the gut microbiota was suppressed with antibiotics. Taken together, diets rich in choline or L-carnitine raise plasma TMAO levels, possibly *via* their microbial conversion, which may thereby lead to

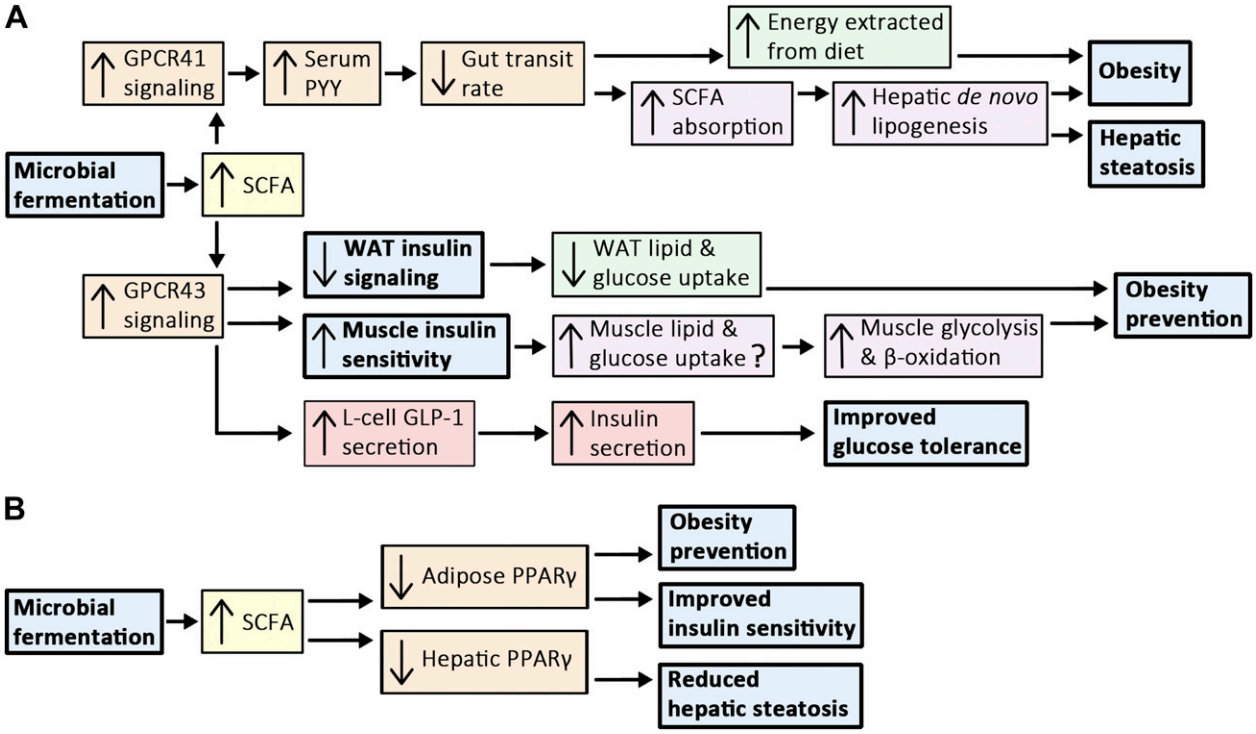

Figure 3. Possible GPCR- and PPARγ-mediated effects of SCFAs on obesity and diabetes. *A*) Microbial fermentation induces the production of SCFAs and subsequent signaling *via* GPCR. SCFA-mediated GCPR41 signaling may reduce the gut transit rate, allowing more time to extract energy from the diet and thereby promoting energy uptake and obesity. In contrast, SCFA-mediated GPCR43 signaling in WAT may prevent obesity by reducing insulin-mediated lipid and glucose uptake in WAT and improving muscle and liver insulin sensitivity, thereby promoting fatty acid and glucose consumption. GPCR43 signaling may also have antidiabetic effects by increasing muscle insulin sensitivity and insulin secretion. *B*) SCFAs may also act in a PPARγ-dependent fashion, as observed by SCFA supplementation in mice fed a high-fat diet. Whereas SCFA-mediated reduction in adipose PPARγ may prevent obesity and improve insulin sensitivity, the SCFA-attenuated hepatic PPARγ may reduce hepatic steatosis.

enhanced development of atherosclerosis. An overview of how the gut microbiota may affect atherosclerotic development *via* the conversion of choline or L-carnitine is displayed in **Fig. 4**.

Bile acid metabolism

Bile acids are formed in the liver from cholesterol. The primary bile acids cholic acid and chenodeoxycholic acid are secreted into the small intestine to assist with the emulsification and absorption of dietary lipids and fat-soluble vitamins. The diversity of the primary bile acids is increased through bacterial activity in the gut to form the secondary bile acids deoxycholic acid and lithocholic acid (105). Consequently, the absence of microbiota causes an increased abundance of primary bile acids and a decreased abundance of secondary bile acids, as seen in germ-free rats (106) and in humans after oral intake of vancomycin for 7 d (107). About 95% of the primary and secondary bile acids are reabsorbed in the intestine and returned to the liver *via* a process referred to as the enterohepatic cycle (105).

Bile acids also act as signaling molecules. An important molecular target of bile acids is the farnesoid X receptor (FXR). As the gut microbiota profoundly affects bile acid metabolism, it can be hypothesized that the microbiota may also have a role in FXR signaling. In fact, it has been found that the presence of microbiota downregulates genes

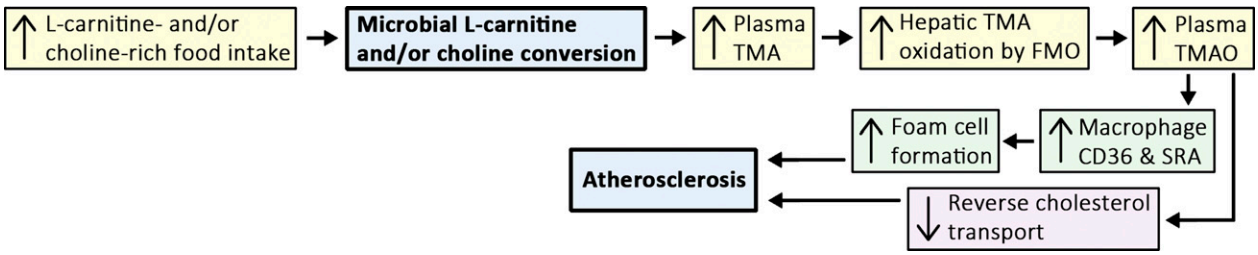

Figure 4. Proposed mechanism by which the gut microbiota may affect atherosclerosis *via* the conversion of choline and L-carnitine. The microbiota converts choline and L-carnitine to TMA, which is subsequently metabolized to TMAO. TMAO may increase foam cell formation and reduce reverse cholesterol transport, thereby increasing the risk of development of atherosclerosis.

involved in bile acid synthesis, such as the rate-limiting enzyme Cyp7a1, while up-regulating genes encoding bile acid efflux transporters (106, 108). The effects on bile acid synthesis seems to depend on microbial FXR signaling, because the reduced liver Cyp7a1 expression, as seen in conventionally raised mice compared to germ-free mice, was abolished in FXR-knockout mice (108). Besides affecting bile acid metabolism, the gut microbiota has also been shown to alter the expression of genes involved in glucose and lipid metabolism related to the FXR pathway. Indeed, hepatic expression of phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and SREPB-1 is upregulated in germ-free and antibiotic-treated rats as compared to conventionally raised rats (106). In contrast, antibiotic treatment of mice that had been fed a high-fat diet showed down-regulation of hepatic SREBP-1c expression, resulting in decreased hepatic *de novo* lipogenesis. The absence of gut microbiota leads to a significant increase in intestinal primary tauro-β-muricholic acid, which cannot be converted to secondary bile acids. Tauro-β-muricholic acid in turn inhibits intestinal FXR signaling, leading to inhibition of ceramide synthesis and reduced hepatic SREBP-1 expression and *de novo* lipogenesis (67). In addition to FXR, bile acids also signal *via* the membrane-bound G protein-coupled bile acid receptor TGR5 to modulate glucose metabolism (28, 106). A recent study found a positive correlation between the abundance of fecal secondary bile acids and peripheral insulin sensitivity after modulation of the gut microbial composition by vancomycin treatment (107). However, a potential role for the FXR or TGR5 pathway was not investigated.

Several studies have shown that dietary fat content influences the gut microbiota, which may be mediated by changes in bile acids (109–112). High-fat diets elevate bile secretion to enhance lipid emulsification and absorption and thereby also alter bile acid levels in the intestine. It has been shown that adding cholic acid to the diet modifies gut microbial composition in favor of the Firmicutes and leads to increased fecal deoxycholic acid concentrations. Consistent with the bactericidal properties of deoxycholic acid, addition of cholic acid to the diet reduced total microbial counts by 51% compared to those of the control group (110). Accordingly, it can be suggested that the changes in gut microbial composition triggered by a high-fat diet is the consequence of increased bile acid secretion. Next to its bactericidal property, deoxycholic acid can also induce DNA damage. Deoxycholic acid has been found to be significantly increased during high-fat feeding, promoting hepatocellular carcinoma in mice exposed to a chemical carcinogen. When the gut microbiota was suppressed with antibiotics, serum deoxycholic acid levels were lowered, and a significant reduction in the development of hepatocellular carcinomas was observed, indicating that changes in microbial composition and its metabolites can have detrimental effects on the host (113).

FUTURE PERSPECTIVES

In this review, we carefully analyzed the literature causally linking the gut microbiota to metabolic health with a special interest in potential underlying mechanisms. Conventionalization studies, studies in germ-free mice, and studies using antibiotics have provided evidence that changes in gut microbiota may be causally involved in metabolic disease, rather than merely being a consequence of it. Several different mechanisms have been proposed and explored that may mediate the possible effects of the gut microbiota on metabolic disorders, including increased energy harvest from the diet, increased gut permeability, changes in SCFA-mediated signaling, and altered bile acid metabolism.

Although several studies have provided evidence that the gut microbiota is causal in the development of obesity, reports are conflicting. Indeed, whereas Ceasar *et al.* (49) and Bäckhed *et al.* (48) found that germ-free mice are protected against DIO, Fleissner *et al.* (114) were unable to reproduce their findings. That the reduced diet-induced weight gain in germ-free mice as compared to conventionally raised mice was not observed in rats is intriguing (115). Reports on the metabolic effect of the microbial fermentation products SCFAs in obesity development are also contradictory. SCFAs were suggested to promote obesity *via* GPCR41 signaling (94), whereas they were postulated to be protective against obesity through GPCR43 signaling (96) (Fig. 2). These apparent contradictions may be explained by differences in how the gut microbiota is modulated. In some studies the composition of gut microbiota was modulated *via* the use of antibiotics, whereas other studies have used germ-free mice. Second, diet, age, and the genetic makeup of the host are all confounding factors that vary among studies. Furthermore, differences in the composition of the gut microbiota at the start render it difficult to compare studies and to identify the specific microbes responsible for the observed effects. A problem with the use of antibiotics is that certain bacteria are resistant to them, and other microbes such as yeast may take over, making it difficult to pinpoint the organisms responsible for the observed effects. Another concern is that nearly all studies characterize the microbiota composition in the feces, which may differ substantially from the microbial composition in the more proximal intestine (22).

A key question is whether studies of germ-free and conventionally raised animals appropriately reflect the role of microbiota in metabolic health in humans. The similarity in gut microbial composition between mice and humans is quite low. Ley *et al.* (8) found that although most of the phyla are present in both humans and mice, 85% of the bacteria genera found in mice are not found in humans. In addition, although studies with germ-free animals provide valuable information, these animals are quite ill, perhaps as a result of defects in immune system development, morphologic intestinal defects, or poor provision of vitamins to the host (23, 24). As a result, studies of germ-free mice may not properly reflect the human physiologic situation. Colonization of these mice with microbiota promotes obesity and reduces metabolic health, but it is questionable whether the observed findings are very informative about the role of microbiota in human metabolic health. An appropriate alternative approach to germ-free mice would be to use mice colonized with human intestinal communities and a proper functioning immune system and intestinal physiology. In this model, the effect of human microbial composition on metabolic health can be studied *via* modulation of the gut microbiota with

antibiotics. Furthermore, more emphasis should be placed on assessing changes in microbial gene expression, as opposed to measuring only the genomic composition.

Another approach to exploring the effects of the gut microbial composition on metabolic health is by performing intervention studies with pre- and probiotics. The term prebiotic describes "a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (116). In principle, all dietary fibers that are fermented are assumed to have prebiotic properties. A large body of evidence suggests that various types of dietary fibers beneficially affect metabolic health. For instance, inulin-type fructans have been shown to counteract high-fat-diet–induced obesity and insulin resistance (117). Studies using various prebiotics are an effective way to investigate the direct influence of changes in gut microbial activity on metabolic parameters.

Whereas prebiotics stimulate the growth and activities of gut microbiota, probiotics are orally delivered live bacteria that are assumed to affect health positively. Two reports have suggested that probiotics reduce body weight gain and have antidiabetic effects (118, 119). At the same time, two other studies have not shown any benefits of probiotics for metabolic health (53, 120). A meta-analysis by Million *et al.* (120) demonstrated that the same *Lactobacillus* strain may promote weight gain in undernourished individuals, whereas it may reduce weight gain in obese individuals (120). Accordingly, the effects of probiotics are not only strain dependent but likely also depend on the characteristics of the host. So far, the European Food Safety Authority has denied any health claims for probiotics. In contrast, numerous claims on prebiotics, including arabinoxylane, β-glucan, and guar gum, have been approved. Overall, more evidence supporting a positive influence of pre- and probiotics on metabolic health is needed, including data from randomized human intervention trials. Also, additional insight should be gained into their mechanism of action.

In conclusion, there is growing understanding of how changes in intestinal microbial composition may affect the metabolic status of the host. More human trials are needed to substantiate the role of the gut microbiota in metabolic health in humans. Also, targeted studies should be conducted to better identify which bacterial strains positively influence metabolic health. Once the causality between the gut microbiota and metabolic health of the host can be further demonstrated, dietary interventions with, for example, prebiotics, probiotics, or a combination of both can be used to modify an individual's gut microbial composition and activity toward the prevention and possibly even treatment of metabolic diseases. FJ

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