REVIEW

Can We Prevent Obesity-Related Metabolic Diseases by Dietary Modulation of the Gut Microbiota?[1]

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ABSTRACT

Obesity increases the risk of type 2 diabetes, cardiovascular diseases, and certain cancers, which are among the leading causes of death worldwide. Obesity and obesity-related metabolic diseases are characterized by specific alterations in the human gut microbiota. Experimental studies with gut microbiota transplantations in mice and in humans indicate that a specific gut microbiota composition can be the cause and not just the consequence of the obese state and metabolic disease, which suggests a potential for gut microbiota modulation in prevention and treatment of obesity-related metabolic diseases. In addition, dietary intervention studies have suggested that modulation of the gut microbiota can improve metabolic risk markers in humans, but a causal role of the gut microbiota in such studies has not yet been established. Here, we review and discuss the role of the gut microbiota in obesity-related metabolic diseases and the potential of dietary modulation of the gut microbiota in metabolic disease prevention and treatment. *Adv Nutr* 2016;7:90–101.

Keywords: gut microbiota, diet, prebiotics, probiotics, obesity-related diseases

Introduction

The prevalence of obesity has increased epidemically during the past 4 decades, and worldwide more than half a billion adults are now obese (BMI $\geq$ 30 kg/m^2) (1). It has been reported that the obesity epidemic is leveling off in certain European countries and in the United States (2, 3), but even when the slower growth is taken into consideration, it has been estimated that ~42% of the adult US population will be obese in 2030 (4). Obesity represents a major health risk because it can lead to impaired quality of life (5) and increased risk of a wide range of diseases including type 2 diabetes (T2D)[2] (6), cardiovascular diseases (7), nonalcoholic fatty liver disease (NAFLD) (8), and certain types of cancer (9). Metabolic syndrome is a clustering of metabolic risk factors related to abdominal obesity. It can be defined by the presence of abdominal obesity and any 2 of the following factors: increased fasting plasma glucose, increased TGs, reduced HDL cholesterol, and hypertension (10). Metabolic syndrome often precedes the onset of T2D and cardiovascular diseases (10, 11). Worldwide, it has been estimated that approximately one-fourth of the adult population has metabolic syndrome (10) and that the joined burden of obesity-related diseases causes 2.8 million deaths annually (1). Thus, effective strategies to reduce morbidity and mortality caused by obesity are important.

Obesity is a consequence of a prolonged imbalance between energy intake and expenditure caused by a complex interplay between genetic susceptibility and nutritional, physiological, social, and environmental factors (12). Because of the multifactorial character of the disease, strategies toward prevention and treatment can be multifaceted. It has been hypothesized that the gut microbiota is implicated in the pathogenesis of obesity and related diseases (13–17). Human intervention studies have shown improvements in metabolic risk markers after dietary interventions that induced specific alterations in the gut microbiota composition (18–20) and after duodenal infusion of fecal microbiota from healthy donors to individuals with metabolic syndrome (21), suggesting that the gut

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[2] Abbreviations used: GF, germ-free; HP; high protein; LGI, low glycemic index; NAFLD, nonalcoholic fatty liver disease; T2D, type 2 diabetes; TJ, tight junction; 16S rRNA, 16S ribosomal RNA.

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microbiota could constitute a target for metabolic disease prevention (22).

The aim of this review is to provide an overview of the role of the gut microbiota in obesity-related metabolic diseases and to discuss the potential of dietary modulation of the gut microbiota in metabolic disease prevention and treatment.

The Gut Microbiota

The human gut hosts trillions of microbes from all domains of life: eukaryota, bacteria, and archaea, with a dominance of bacterial cells (23). Most of the gut microbes reside in the colon, the last part of the digestive system where bacterial cells are present in concentrations of 10^9–10^{12} CFU/mL (24), comprising >1000 different species (25). For comparison, bacterial cells in the stomach and duodenum and in the jejunum and ileum are present in concentrations of 10^1–10^3 CFU/mL and 10^4–10^8 CFU/mL, respectively (24). Approximately 90% of the bacterial species in the adult gut belongs to just 2 phyla: Firmicutes and Bacteroidetes (26, 27). The dominant Firmicutes phylum is a diverse group that comprises gram-positive bacteria from >200 different genera including *Catenibacterium*, *Clostridium*, *Eubacterium*, *Dorea*, *Faecalibacterium*, *Lactobacillus*, *Roseburia*, *Ruminococcus*, and *Veillonella* (28). The second most prevalent phylum, the Bacteroidetes, comprises gram-negative bacteria from ~20 genera, including *Bacteroides*, *Odoribacter*, *Prevotella*, and *Tannerella* (28). Other common but less abundant phyla of the gut microbiota include Actinobacteria (*Bifidobacterium*, *Collinsella*), Proteobacteria (*Bilophila*, *Desulfovibrio*, *Escherichia*), and Verrucomicrobia (*Akkermansia*) (26).

The gut microbiota gives the human host a number of vital functions, including the ability to extract energy from otherwise indigestible dietary compounds, synthesize essential vitamins, and regulate the immune system (29). Because bacteria have short generation times, rapid mutation rates, and an ability to exchange genes, they also enable the human host to make rapid adaptations to new environmental exposures such as unknown toxins or new food sources (30). The gut microbiota develops immediately after birth, and host genotype, mode of delivery, and early nutrition have influence on which bacteria become the first inhabitants (29). The early microbiota composition in vaginally delivered infants resembles the vaginal microbiota of their mothers with a dominance of *Lactobacillus* and *Prevotella* species, whereas infants delivered by Cesarean section establish an early microbiota that resembles that of their mothers' skin including *Staphylococcus* species (31). In addition, the microbiota in infants can be distinguished by whether they are breast- or formula fed (32). During the first 3 y of life, the microbial diversity increases to reach a level similar to adulthood (33, 34). The composition of an individual's gut microbiota is generally considered stable (27, 35), but the relative abundance of bacterial species and the microbial diversity do vary with the physiological state of the individual during adulthood. This is demonstrated by the altered gut microbiota in pregnancy (36), inflammatory bowel diseases (25), obesity (13), T2D (14, 37), atherosclerosis (15), and NAFLD (16).

Analyses of >1200 gut metagenomes from European, Chinese, and American adults have shown that the collective genome of the gut microbiota (the gut microbiome) consists of almost 9.9 million nonredundant microbial genes and the size of the gut microbiome in an individual of around 763,000 microbial genes (38). Despite the large interindividual variation in the gut microbiome, it has been suggested that individuals across continents independent of age and phenotype can be assigned to one of just 3 different metagenomic profiles (enterotypes) dominated by either *Bacteroides*, *Prevotella*, or *Ruminococcus* (39). In addition, it has been estimated that ~40% of the gut microbial gene pool is shared among individuals (25), and there seems to exist a core microbiome, which is a set of microbial genes shared among the vast majority of healthy individuals that enables conservation of several important functional pathways including pathways involved in energy metabolism (13, 27). Bacterial genes identified as rare and present in only a smaller subset of individuals (<1%) have been suggested to also have vital functions for health because they are enriched in functions such as DNA replication, recombination and repair, and cell wall/membrane biogenesis, as compared with the common genes that appear to be mainly enriched in functions such as energy production, carbohydrate and amino acid transport, and metabolism (38). This is interesting because this more variable microbiome appears to be vulnerable to environmental factors, such as diet and drugs, to a higher degree than the core microbiome (40). Hence, bacteria with beneficial gene functions within the variable microbiome might represent a promising target for dietary interventions aimed at improving host metabolic health.

The Gut Microbiota and Barrier Function

The colonic lumen is surrounded by epithelial cells (colonocytes) and an underlying layer of connective tissue (lamina propria), which together form the mucosa (41). Goblet cells within the mucosa produce mucins, mainly mucin 2, which constitute the first physical barrier that protects the internal sites from luminal antigens (42). However, the main barrier is composed of the colonocytes and the seal between them provided by the tight junctions (TJs), which are transmembrane protein complexes (43). This selective gut barrier helps to maintain homeostasis, where passage of nutrients, ions, and water across the epithelium into the mesenteric blood stream is permitted and translocation of dietary antigens and microbes is prevented (43). Transport of nutrients from the lumen can occur by paracellular diffusion through pore-forming TJ complexes or by transcellular transport mediated by transporters and channels located at the apical and basolateral cell surfaces (43). Impaired barrier function can be caused by epithelial damage or by dysregulation of TJ proteins (42). Epithelial damage will have more severe consequences with regard to translocation of potential antigens than TJ dysregulation, but abnormalities in expression of TJ proteins might trigger immune activation and later

development of inflammatory diseases in susceptible individuals (42, 43). Disease processes that involve an inflammatory component can compromise the gut barrier function and contribute to persistent overstimulation of the immune system, where the interaction between luminal antigens and host-immune cells increases expression of permeability-enhancing factors (43). However, it is unclear whether a compromised barrier function can lead to inflammatory diseases in humans or whether the gut barrier function is affected only after systemic inflammation is established. Yet, there is evidence suggesting that increased translocation of luminal toxins can precede the onset of T2D (44) and inflammatory bowel diseases (45), pointing to impaired barrier function as a causative factor in disease pathology.

The microbiota enhances the gut barrier function and protects against translocation of bacterial toxins by competition with potential pathogenic bacteria for nutrients and adhesion sites and by production of antimicrobial compounds (46). The SCFAs such as acetate, propionate, and butyrate produced by the microbiota are the main energy sources for the colonocytes (47). Butyrate is of particular interest for gut barrier function because it is preferred over other SCFAs as nutrient for the colonocytes, and because it appears to enhance gut barrier function by regulation of TJ proteins and mucins (48). It is likely that the mucosa-associated microbiota contribute to the protection of host cells and the gut barrier function to a higher degree than the luminal microbiota. The mucosa-associated microbiota reside in the columnar epithelium and form a biofilm together with the outer mucus layer (49); because of this direct interaction with host cells, including cells in gut-associated lymphoid tissues, the mucosa-associated microbiota could be more vital for host immunological functions than the luminal microbiota (49, 50), which mainly affect host cells indirectly by exchange of metabolites (51). Because of sampling difficulties, less is known about the composition of the mucosa-associated microbiota than the luminal microbiota, but mucin-degrading bacteria in the human gut include *Akkermansia muciniphila*, *Bifidobacterium longum*, *Faecalibacterium prausnitzii*, *Ruminococcus torques*, and several *Bacteroides* species (52).

Modulation of the Gut Microbiota

Pre- and probiotics can modulate the gut microbiota in specific ways. A prebiotic can be defined as a nonviable food component that confers health benefit on the host associated with modulation of the microbiota (53). For a food component to be classified as a prebiotic, it must be neither hydrolyzed by the host enzymes nor absorbed in the upper part of the gastrointestinal tract and it must be a selective substrate for one or a limited number of beneficial bacteria in the colon (54). Common prebiotics include inulin, fructo-oligosaccharides, and galacto-oligosaccharides; emerging prebiotics include resistant starch, xylo-oligosaccharides, and arabinoxylan-oligosaccharides (53). Effects of prebiotics are generally attributed to *1*) stimulation of beneficial bacteria and SCFA production and, consequently, improved barrier function and resistance to inflammatory stimuli; *2*) increased mineral absorption; and *3*) modulation of lipid metabolism, possibly by suppression of lipogenic enzymes and thus decreased synthesis of lipoproteins and triglycerides (55). Moreover, prebiotics have been suggested to improve glucose homeostasis by stimulation of glucagon-like peptide 1 secretion (56–58). Dietary fibers induce several health effects that are similar to the function of prebiotics, such as stimulation of SCFA production and regulation of glucose and lipid metabolism (59), and it can be difficult to assess whether beneficial metabolic functions can be attributed to a dietary fiber per se or to a prebiotic effect of the fiber mediated through the microbiota. A common principle when evaluating the potential prebiotic effect of a food component is the ability to stimulate growth of *Bifidobacteria* and *Lactobacillus* that are considered beneficial bacteria (60). However, as our knowledge about the gut microbiota develops, our view on the criteria for which specific bacteria a prebiotic must stimulate expands.

Probiotics are living microorganisms that, when ingested, provide health benefits, either directly through interactions with host cells or indirectly through effects on other bacterial species (61). Common probiotics include a large number of *Bifidobacteria* and *Lactobacillus* species (62). Mechanisms that underlie beneficial effects of probiotics vary between bacterial strains (62) but are in general attributed to *1*) exclusion of pathogenic microorganisms by production of bactericides and competition for nutrients and adhesion sites; *2*) modulation of inflammatory responses through interaction with immune cells in the gut; and *3*) modulation of gene expression affecting host metabolism and gut barrier function (46). In addition, probiotics have been suggested to regulate lipid metabolism because of their ability to produce bile salt hydrolase enzymes that can de-conjugate bile acids (63). De-conjugated bile acids are less efficient at promoting lipid absorption than conjugated bile acids, and they are excreted through feces to a higher degree than their conjugated counterparts, which leads to increased hepatic uptake of serum cholesterol in order to synthesize new bile acids (63).

Synbiotics refers to combinations of pre- and probiotics (64). Synbiotics have the potential to induce more substantial effects on the gut microbiota and host health than isolated intake of pre- or probiotics, because they provide the probiotic bacteria in combination with a prebiotic component that stimulates probiotic bacteria survival and growth in the gastro-intestinal tract.

The overall composition of the diet also has a major impact on the gut environment shown by the imprint of long-term dietary intake on the microbiota (34, 65, 66), the changes in the microbiota after shifts in macronutrient composition (67), intake of animal- compared with plant-based diets (68), and after energy restriction (20, 69). Carbohydrates typically constitute the main part of the human diet, and consequently most gut bacteria are saccharolytic (70). Bacterial fermentation of carbohydrates, which have

reached the colon undigested, yields SCFAs. A high degree of carbohydrate fermentation lowers the pH value in the colon within the normal pH range of 5.5–7.0 (71), creating a mildly acidic environment that inhibits overgrowth of pH-sensitive pathogenic bacteria and favors the growth of certain beneficial bacteria such as the butyrate-producers *Roseburia intestinalis*, *Eubacterium rectale*, and *F. prausnitzii* (47, 67, 72). A shift from a normal diet in which carbohydrates provide 40–70% of the energy (73) to a diet with increased relative intake of energy from fat and protein most likely will change the gut microbiota composition, the pH of the colon, and the metabolites available for the host cells.

Studies in mice have shown that high-fat feeding can cause metabolic endotoxemia, a subclinical inflammatory state, with activation of NF-κB–induced pathways that may contribute to metabolic disease (74). The underlying mechanism appears to be increased translocation of gram-negative bacteria and bacteria-derived components, such as LPS, into the bloodstream, either directly or incorporated into chylomicrons (74–76). Thus, metabolic endotoxemia can be a consequence of increased postprandial chylomicron diffusion after a high-fat meal and/or a consequence of impaired gut barrier function. This mechanism also appears to apply to humans, in whom circulating LPS and LPS-binding protein have been associated with obesity, metabolic syndrome, and T2D (77–81). In addition, it has been suggested that germ-free (GF) mice are protected from proinflammatory consequences of a high-fat diet (82) and that intake of prebiotic fibers in combination with a high-fat diet can counteract diet-induced metabolic disturbances (83, 84), supporting a key role for the intestinal microbiota in the metabolic response to the diet.

Randomized controlled dietary interventions with high protein (HP) content and low glycemic index (LGI) have been shown to enhance weight loss and weight maintenance in overweight and obese adults (85, 86). However, HP diets have been hypothesized to be harmful for colonic health because they lead to increased fermentation of undigested protein in the colon, which produces potential harmful metabolites, such as ammonia, phenols, hydrogen sulfide, and amines (71, 87, 88). Interestingly, it has been shown in healthy volunteers that a HP diet with a high amount of red meat can cause carcinogenic lesions in DNA in rectal epithelial cells but that these lesions can be prevented by supplementing the HP diet with a fiber that yields butyrate upon fermentation (89). If fermentable dietary fibers can neutralize harmful effects on the colon after HP diets, it supports the use of diets with HP and LGI as a strategy to control body weight, because LGI diets typically are rich in fibers that are highly fermentable by the colonic microbiota (90). It may also partly explain why an LGI diet has been shown to improve low-grade inflammation in overweight and obese adults (91).

Altogether, these findings suggest that nondigestible dietary components that target the gut microbiota can prevent harmful consequences of diets high in animal protein and fat.

The Gut Microbiota and Host Metabolism

A link between obesity and the gut microbiota was initially suggested based on studies in GF mice (92). These mice were found to be leaner than conventionally raised mice, but to massively expand their fat mass and increase insulin resistance after colonization with cecal microbiota from the conventionally raised mice, despite a significant reduction in energy intake. Next, it was shown that colonization of GF mice with cecal microbiota from obese mice, when compared with microbiota from lean mice, resulted in a greater increase in body fat (93), suggesting that the gut microbiota affects phenotypic characteristics of the host. Studies in both mice and humans found that obesity was accompanied by an altered gut microbiota composition with differences in the abundance of the dominant bacterial phyla, which distinguished the obese and metabolically susceptible microbiota from the lean and healthy (13, 94). More recently, metagenomic studies have confirmed that it is possible to distinguish metabolically unhealthy from healthy individuals based on the characteristics of their gut microbiota with a high specificity and sensitivity (14, 37). However, the differences are not found at the overall phylum or enterotype level, but at the species level and by characterization of microbial gene functions. Several plausible hypotheses explaining a relation between the gut microbiota and metabolic health have been proposed. Obesity and related metabolic disturbances after colonization of the GF mice can be explained partly by increased absorption of SCFAs (92). The increased absorption of nutrients leads to increased energy storage in adipose and nonadipose tissue and, consequently, stimulation of hepatic lipogenesis. The microbiota has also been suggested to suppress the expression of AMP-activated protein kinase, which leads to decreased fatty acid oxidation in muscles (95), and to suppress the expression of angiopoietin-related protein 4, which leads to increased TG storage in the liver and adipose tissue (92, 96, 97). In addition, the increased expression of inflammatory signals from adipocytes in obese individuals is suggested to impair the gut barrier function and lead to translocation of proinflammatory molecules, such as gram-negative bacteria and LPS (98).

There are still uncertainties about the mechanisms that link the gut microbiota with metabolic diseases. It is unclear whether a given microbiota composition causes obesity and related metabolic diseases in humans or whether alterations in the microbial environment only are symptoms of metabolic disease. However, the studies in GF mice (92, 93), in which gut microbiota transplantation led to physiological changes such as increased fat storage and impaired metabolism in the receiving host, and the study in men with metabolic syndrome in which improvement in metabolic markers was induced by colonization with gut microbiota from healthy donors (21), indicate that

changes in the gut microbiota can be the cause and not just the consequence of metabolic disease.

Methods to Characterize the Gut Microbiota

Most of our knowledge of the human gut microbiota originates from analyses of stool samples because these are easily accessible. However, comparisons of microbiota from mucosal biopsies and stool samples have shown that there are compositional differences between the mucosa-associated and the luminal (fecal) microbiota (99). Ideally, studies of the gut microbiota should include mucosal biopsies and luminal content from different sites in the gut, and ensure collection of samples under anaerobic conditions, in order to avoid changes in the microbiota composition due to oxygen exposure. However, these types of samples are difficult to obtain from humans. Yet, stool samples do have the strengths of being host specific and representative of the interindividual variance (26, 100), which to some degree justifies the use of stool samples for comparison, although they do not provide an accurate picture of the entire gut microbiota.

Studies of the human gut microbiome require a number of considerations, from the collection and processing of samples and analyses of the microbiome composition to the interpretation of the data. It is crucial for the comparison of data from different studies that the applied methods are comparable, and the International Human Microbiome Standards project has developed standard operating procedures for the human microbiome field (101).

For the analyses of the gut microbiota composition, several techniques can be applied. Traditionally, culturing techniques in which microorganisms are isolated and characterized with the use of growth media have been used. However, because a majority of the bacteria in the colon are anaerobic and cannot be cultured under aerobic conditions, only an estimated 30% of the gut bacteria has been characterized by this method (102). Instead, culture-independent DNA-based methods have provided the opportunity to study the gut microbiota more extensively. By application of the DNA-based methods, changes in the diversity and characteristics of the gut bacteria can be identified to the species level during different physiologic states and after environmental exposures, such as dietary changes. These methods can be categorized roughly as targeted studies of gene fragments or studies of the whole microbiome (103).

Targeted gene fragment methods include probe hybridization techniques such as fluorescence in situ hybridization and DNA microarrays, in which phylogenetic identification is based on hybridization of specific oligonucleotide probes (102). Targeted fragment studies are also frequently based on analysis of 16S ribosomal RNA (16S rRNA), which is a part of the small subunit of the 70S ribosome (102). 16S rRNA is the preferred molecule for identification of bacteria because it is universally distributed and contains both conserved regions that are identical for all bacteria and 9 variable regions with highly specific sites that are unique for individual bacteria. These variable regions enable species-level identification (104). A common and cost-effective method for analysis of 16S rRNA is real-time qPCR (102).

Recently, metagenomic analysis has partly replaced the one gene approach, because it provides a broader view of the microbiome diversity (103). Metagenomics refer to the collective study of all genomes within a sample (105) and can be performed by shotgun sequencing, in which representatives of all gene fragments present are sequenced (103). A limitation to metagenomics is that we only get information on the encoded functional capacity of the microbiome and not on whether or to what extent the predicted genes actually are expressed (106). Future studies will probably integrate the genomic data with analyses of RNAs, proteins, and metabolites present in a given ecosystem.

In terms of probiotics, the culture-independent methods provide quicker and more accurate ways to enumerate viable strains than culture-based methods. However, it is common practice that microorganisms must be capable of replicating in order to be defined as viable, and culture-based methods are necessary to obtain information about replication (107). It can be argued, however, that culturing will underestimate the number of viable microbes, because the enumeration will exclude microbes that are metabolically active but not capable of forming colonies under the given experimental conditions. This would favor the use of culture-independent methods that identifies viable microbes, irrespective of a culturable or nonculturable state.

Association Between the Gut Microbiota and Metabolic Health in Humans

Comparisons between the gut microbiota of healthy and diseased individuals offer an opportunity to characterize the normal microbiota and to identify specific disease-associated alterations (**Tables 1** and **2**). Interestingly, it has been shown that microbial gene markers correlate better with T2D than common anthropometric risk markers (37) and common variation in the human genome (14). In general, bacterial gene functions enriched in individuals with obesity-related metabolic diseases provide increased capacity for energy metabolism, membrane transport, potential proinflammatory functions such as mucus degradation and production of toxins, and an increased potential to manage oxidative stress (13, 14, 17, 37). The microbiome in metabolically healthy individuals appears to be enriched in bacterial gene functions involved in cell motility, metabolism of co-factors and vitamins, and production of butyrate (14, 15, 37). A consistent finding is a decreased abundance of butyrate-producing bacteria, in particular *F. prausnitzii,* in individuals with obesity-related metabolic disturbances (17) and T2D (14, 37). However, the abundance of *F. prausnitzii* species appears to be negatively correlated with intake of dietary fat, and the link between *F. prausnitzii* and markers for insulin resistance has been shown to disappear after adjustment taking into consideration dietary fat intake (115). Studies that have used qPCR to characterize the gut microbiota have identified increased abundance of *Bifidobacteria* species in healthy individuals compared with individuals with obesity (108, 109) and T2D

TABLE 1 Association between the gut microbiota and obesity[1]

Study (reference)	Description	Results
Le Chatelier et al. 2013 (17)	Danish obese (n = 169) and nonobese (n = 123) adults; case-control; metagenomics	15,894 bacterial genes differed between groups. Bimodal distribution of bacterial genes: 23% of individuals with low microbiome richness (<480,000 genes), 77% with high microbiome richness (>480,000 genes); 9 species and 51 intestinal metabolic pathways differed between individuals with low and high microbiome richness.
Turnbaugh et al. 2009 (13)	North American obese and normal weight monozygotic (n = 31) and dizygotic (n = 23) adult twin pairs, and their mothers (n = 46); cross-sectional design; pyrosequencing	383 microbial genes differed between obesity and normal weight. Reduced microbial diversity in obesity.
Kalliomäki et al. 2008 (108)	Finnish overweight (n = 25) and normal weight (n = 24) children; longitudinal cohort study (prospective); FISH and qPCR	Bifidobacterium species increased and *Staphylococcus aureus* decreased in infancy in children with normal weight compared to in children who were overweight at age 7.
Simões et al. 2013 (109)	Finnish obese or nonobese adult monozygotic twin pairs (n = 20); cross-sectional; qPCR and DGGE	No difference in bacterial counts between normal weight, overweight, or obesity.
Million et al. 2012 (110)	French obese (n = 68) and normal weight (n = 47) adults; case-control; qPCR and culture	*L. paracasei, L. plantarum*, *B. animalis*, and *M. smithii* associated with normal weight; *L. reuteri* associated with obesity.
Štšepetova et al. 2011 (111)	Estonian obese and nonobese adults (n = 61); cross-sectional; qPCR	Lactobacillus species positively correlated with BMI. *L. paracasei* negatively correlated with FBG. *L. fermentum* marginally negatively correlated with FBG.
Schwiertz et al. 2010 (112)	German obese and nonobese adults (n = 98); cross-sectional; qPCR	Firmicutes (mainly *C. leptum*) reduced and Bacteriodetes (mainly *Bacteroides*) increased in overweight and obesity. *Bifidobacterium* and *Methanobrevibacter* negatively correlated with BMI.
Armougom et al. 2009 (113)	French adolescents and adults with obesity (n = 20) or anorexia nervosa (n = 9) and healthy controls (n = 20); case-control; qPCR	Bacteroidetes and Lactobacillus species increased in obesity compared to normal- and underweight.

[1] All studies are based on analyses of stool samples and are listed in order of priority according to the quality of the methods used. Description is given as population (n); design; technique. DGGE, denaturing gradient gel electrophoresis; FBG, fasting blood glucose; FISH, fluorescence in situ hybridization.

(116). Yet this association appears to be confounded by dietary intake as well, because *Bifidobacteria* have been shown to correlate positively with an intake of carbohydrates including dietary fibers, and adjustment for carbohydrate intake appears to abolish the association with markers for insulin resistance (115). Specific bacterial species that are consistently associated with metabolic markers in different studies and after adjustment for variation in host physiology and long-term dietary intake also exist; these include *Bilophila wadsworthia* and *Clostridium bolteae* that are associated with insulin resistance (14, 115).

It has been suggested that an additional characteristic of the gut microbiota in individuals with an unhealthy metabolic profile is a higher abundance of Lactobacillus species, as shown in individuals with obesity (110, 111), T2D (37), and NAFLD (16). Because bacterial species within the genus of *Lactobacillus* are among the most common probiotics and frequently added to dairy products, functional foods, and dietary supplements (117), reports of associations between Lactobacillus species and metabolic disease (16, 37) require consideration. However, beneficial associations between specific Lactobacillus species and metabolic traits have also been reported (111, 112, 118) (Table 2), and clinical trials that have explored the effect of interventions with Lactobacillus species on metabolic markers have shown either improvement in metabolic markers (119–121) or no effect (122–124). Thus, there are no intervention studies, to our knowledge, to substantiate that an intake of Lactobacillus species can lead to metabolic disturbances, which suggests that the reported associations between Lactobacillus species and metabolic diseases do not reflect a causal relation.

There is no clear consensus about the size and composition of a healthy gut microbiome, but it has been suggested that individuals with low microbiome richness, defined by <480,000 bacterial genes, are characterized by higher fat mass, insulin resistance, dyslipidemia, and low-grade systemic inflammation than individuals with higher microbiome richness (13, 17, 20) (Table 1). An association between microbiome richness and metabolic risk might be due to a stronger defense against pathogenic and proinflammatory microbes in a gut environment with a rich abundance of different bacterial genes (29). Not all studies have confirmed an association between measures for microbiome diversity and obesity (113), and it is possible that it is not the obese state per se but rather the presence of obesity-related metabolic diseases that are linked to reduced gut microbiome diversity. Yet, patients with T2D do not appear to have decreased microbiome diversity compared with healthy controls (Table 2) (14, 114, 116). It is possible that

TABLE 2 Association between the gut microbiota and T2D[1]

Study (reference)	Description	Results
Karlsson et al. 2013 (37)	Swedish elderly women with T2D (n = 53), impaired glucose tolerance (n = 49), or normal glucose tolerance (n = 43); metagenomics	MGSs most significantly depleted in T2D included *Desulfurispirillum indicum*, *Bacteroides intestinalis, Clostridium thermocellum*, *C. botulinum*, *C. beijerinckii, F. prausnitzii,* Roseburia, and Eubacterium species. MGSs most significantly enriched in T2D included *Lactobacillus gasseri, L. salivarius, L. antri, L. oris, L. crispatus, L. reuteri*, *Clostridium clostridioforme*, and *Streptococcus mutans*. Lactobacillus species positively correlated with FBG and HbA1c. Clostridium species negatively correlated with FBG, HbA1c, insulin, C-peptide, and TGs and positively with adiponectin and HDL cholesterol. *B. intestinalis* negatively correlated with insulin and waist circumference.
Qin et al. 2012 (14)	Chinese adults with T2D (n = 171) and healthy controls (n = 174); metagenomics	Bacterial gene markers enriched in controls largely assigned to butyrate-producing species within Faecalibacterium, Roseburia, and Eubacterium, but also to *Haemophilus parainfluenzae*. Bacterial gene markers enriched in T2D belonged to mucin degrading (*Akkermansia muciniphila*) and potential pathogenic species such as *Bacteroides caccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*, *Eggerthella lenta*, and *Escherichia coli*. No differences in microbial diversity between patients and controls.
Larsen et al. 2010 (114)	Danish adults with T2D (n = 18) and healthy controls (n = 18); pyrosequencing	Firmicutes including *Clostrida* reduced in T2D. *Betaproteobacteria* enriched in T2D and positively correlated with FBG. Roseburia species marginally negatively correlated with FBG. Lactobacillus species marginally positively correlated with FBG. No difference in bacterial diversity between groups.

[1] All studies have a case-control design, have used gene sequencing to characterize the microbiota, and are based on analyses of stool samples. Description is given as population (n); technique. FBG, fasting blood glucose; HbA1c, glycated hemoglobin; MGS, metagenomic species; T2D, type 2 diabetes.

differences in the gut microbiome between patients diagnosed with T2D and individuals with undiagnosed metabolic disease are due to the use of anti-diabetic medication by the patients, because studies in mice have shown that the antidiabetic drug metformin can induce compositional changes in the microbiota (125, 126). Another possible explanation is that the suggested link between gut microbiome diversity and metabolic markers is confounded by variations in dietary intake and that patients with T2D have modified their lifestyle as a consequence of the diagnosis. Gut microbiome richness has been shown to be positively correlated with an intake of protein from dietary sources other than meat (115), and individuals with low microbiome richness (bacterial gene count <480,000) have been shown to consume less fruit, vegetables, and fish than individuals with high microbiome richness (20). A confounding role of diet could also explain the large difference observed in the prevalence of individuals with low microbiome richness in obese cohorts, ranging from 8% (124) to 40% (20), despite comparable body fat mass, glucose, and lipid metabolism. Likewise, it might provide an explanation for the discrepancy between the 10–30% of obese individuals who can be classified as being metabolically healthy based on normal insulin sensitivity, visceral fat mass, and adipose tissue function (127), and the 60–92% that could be classified as metabolically healthy if based on gut microbiome richness (20, 124).

Thus, observational studies show that the composition of the gut microbiota in individuals with obesity-related metabolic diseases differs from that in metabolically healthy individuals, indicating that specific gut microbes are implicated in the pathology of metabolic diseases and that modulation of the gut microbiota could be a strategy in disease prevention. However, the suggested use of microbiome richness for stratification of healthy and unhealthy obese individuals would probably only capture a fraction of those who are at increased risk of developing metabolic disease, possibly partly due to confounding by dietary intake between healthy and unhealthy individuals.

Dietary Interventions Modulating the Gut Microbiota

Only a limited number of clinical trials have explored the effect of prebiotics on microbiota composition and metabolic markers simultaneously (**Table 3**). Interventions with different types of dietary fibers including prebiotics have been shown to modulate the gut microbiota and improve insulin sensitivity, low-grade chronic inflammation, and lipid metabolism (18, 19, 124, 128). Such findings could suggest that the prebiotic-induced changes in the microbiota lead to the improvement in host metabolism. However, clear conclusions cannot be drawn based on observations of parallel changes in the gut microbiota and metabolic markers as reported in the studies by Vulevic et al. (19) and Lecerf et al.

TABLE 3 Effect of prebiotic interventions on gut microbiota and metabolic risk markers[1]

Study (reference)	Description	Intervention	Results
Brahe et al. 2015 (124)	Danish obese women (n = 58); RCT, blinded; metagenomics	*Lactobacillus paracasei* F19 (9.4×10^{10} CFU/d), flaxseed mucilage (10 g/d), or placebo (maltodextrin); 6 wk	Flaxseed mucilage group: increased *Bilophila wadsworthia*, *Parabacteroides merdae*, and *Parabacteroides johnsonii*. Improved insulin sensitivity (OGTT) compared with placebo. Gut microbiota changes could not explain improved insulin sensitivity. *L. paracasei* F19 group: minor alterations in gut microbiota, not significant compared with placebo. No effect on metabolic markers.
Vulevic et al. 2013 (19)	British overweight adults (n = 45) with metabolic disturbances; RCT, crossover, double-blind; FISH	GOS (5.5 g/d) or placebo (maltodextrin); 12 wk, 4 wk washout	GOS modulated the gut microbiota. Bifidobacterium species increased. *Clostridium histolyticum, Bacteroides*, and *Desulfovibrio* species decreased. Biochemical changes: serum insulin, TC, TGs, CRP, and fecal calprotectin significantly reduced compared with placebo.
Dewulf et al. 2012 (18)	Belgish obese women (n = 30); RCT, double-blind; DNA microarray and qPCR	Inulin-type fructans (16 g/d) or placebo (maltodextrin); 12 wk	Inulin-type fructans modulated the gut microbiota. At genus level: increase in *F. prausnitzii*, Bifidobacterium, and Lactobacillus species. Decrease in *Bacteroides intestinalis*, *B. vulgatus*, and *Propionibacterium*. Biochemical changes: improved glycemia (OGTT), tendency toward reduced fat mass, and serum LPS compared with placebo. *Bacteroides intestinalis*, *B. vulgatus*, and *Propionibacterium* positively correlated with fat mass and glycemia. *F. prausnitzii* and *Bifidobacterium* negatively correlated with LPS.
Lecerf et al. 2012 (128)	French normal weight adults (n = 59); RCT, double-blind; qPCR	XOS (5 g/d) or XOS (1 g/d) + inulin (3 g/d) or placebo (maltodextrin); 4 wk	XOS and inulin modulated the gut microbiota: *Bifidobacterium* increased in both groups compared to placebo. Butyrate and propionate production increased in both group compared to placebo. Acetate production decreased in the XOS group compared to placebo. Biochemical markers: plasma LPS decreased in the XOS + inulin group compared to placebo.

[1] All microbiota analyses are based on stool samples. Description is given as population (n); design; technique. CRP, C-reactive protein; FISH, fluorescence in situ hybridization; GOS, galacto-oligosaccharides; OGTT, oral glucose tolerance test; RCT, randomized clinical trial; TC, total cholesterol; XOS; xylo-oligosaccharides.

(128), or even based on findings of correlations between the changes in metabolic markers and the gut microbiota as reported by Dewulf et al. (18) (Table 3) because these approaches do not exclude that the observed improvements in metabolic markers are mediated by the physicochemical properties of the dietary fibers (90) and not by the specific changes in the microbiota. At present, to our knowledge, no health benefits of pre- and probiotics on metabolic risk markers are found to be substantiated by scientific evidence when assessed by an independent authority such as The European Food Safety Authority. An improved approach to performing clinical trials in humans that addresses the link between dietary modulation of the gut microbiota and host metabolic health could be performing follow-up studies in which the specific bacterial strains associated with beneficial changes in metabolic markers during the dietary intervention are provided. Another less advanced strategy to address the cause-and-effect relation could be to combine the diet-induced alterations in the microbiota and the metabolic markers in the statistical processing of the data. We applied this approach in a clinical trial where we found specific alterations in the gut microbiota in parallel with improved insulin sensitivity after 6 wk intake of flaxseed mucilage. We found that the observed changes in the microbiota, when included as explanatory variables in mixed models, could not explain the improved insulin sensitivity (124) (Table 3).

Thus, there are indications that diet-induced alterations in the gut microbiota can improve host metabolic health, but so far it has not been shown that the microbiota modulation mediates the improvements in metabolic risk markers.

Conclusion

Obesity and obesity-related metabolic diseases are characterized by specific alterations in the human gut microbiota. Experimental studies with gut microbiota transplantations in mice and humans indicate that a specific gut microbiota composition can be the cause, and not just the consequence, of metabolic disease, suggesting a potential for gut microbiota modulation in prevention and treatment of obesity-related metabolic diseases. In addition, dietary intervention studies have suggested that modulation of

the gut microbiota can improve metabolic risk markers in humans, but a causal role of the gut microbiota in such experiments has not been established. There is a need for clinical trials that explore the role of diet-induced modulation of the gut microbiota on metabolic risk markers, rather than trials that explore effects on metabolic and metagenomic markers separately. Such trials will help us to clarify whether diet-induced modulation of the gut microbiota can improve host metabolic health.

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