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REVIEW

Gut microbiome and metabolic diseases

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Abstract The prevalence of obesity and obesity-related disorders is increasing worldwide. In the last decade, the gut microbiota has emerged as an important factor in the development of obesity and metabolic syndrome, through its interactions with dietary, environmental, and host genetic factors. Various studies have shown that alteration of the gut microbiota, shifting it toward increased energy harvest, is associated with an obese phenotype. However, the molecular mechanisms by which the gut microbiota affects host metabolism are still obscure. In this review, we discuss the complexity of the gut microbiota and its relationship to obesity and obesity-related diseases. Furthermore, we discuss the anti-obesity potential of probiotics and prebiotics.

Keywords Gut microbiome · Metabolic syndrome · Obesity · Probiotics · Omics

Abbreviations

NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
MGWAS	Metagenome-wide association study
TLR4	Toll-like receptor 4
LPS	Lipopolysaccharide
NOD mouse	Non-obese diabetic mouse
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
FMOs	Flavin monooxygenases
CLA	Conjugated linoleic acid
GLP-1	Glucagon like peptide-1
SCFA	Short-chain fatty acid

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Introduction

A large variety of commensal (from Latin *com mensa*, meaning "share a table") microbes colonize the surfaces of our body, and our gut lumen is no exception [1]. In fact, the density of gut microbiota in the colon reaches as high as 10^{11}/g content, overwhelmingly exceeding the density of any other known bacterial niche of the globe. Our gastrointestinal tract contains more than 100 trillion commensal microbes classified into at least 1,000 different species [2]. Nevertheless, the diversity of gut microbiota is largely limited and biased; out of 28 phyla identified to date, gut microbiota are mainly composed of four phyla, namely, *Firmicutes, Bacteroidetes, Actinobacteria*, and *Proteobacteria*. This is thought to be attributable to the coevolution of the host (i.e., ourselves) and the commensal microbiota. The number of gut microbiota (more than 100 trillion cells) greatly exceeds that of the somatic cells constituting our body (~60 trillion cells). Moreover, gut microbiota in each individual contain ~600,000 genes [2], approximately 25 times more than the number of genes in our own genome. Thus, the gut microbiota is often likened to a measurable organ consisting of prokaryotic cells, which creates the unique gut ecosystem together with the host eukaryotic cells (Fig. 1). In light of these considerations, the Nobel laureate Joshua Lederberg proposed to deem the host and its commensal microbiota as a "superorganism" [3]. To understand the normal physiology and pathology of humans as the superorganism, therefore, it is vital to understand the gut

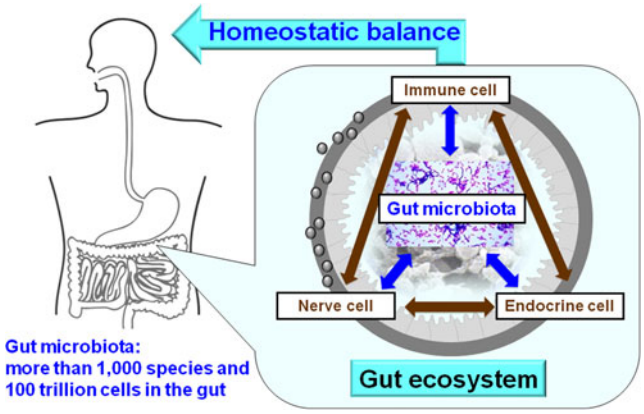

Fig. 1 Schematic overview of the gut ecosystem. Intestinal immune, nerve, and endocrine cells are tightly interlinked (*double-headed brown arrows*) and form a highly complex gut ecosystem together with the gut microbiota through host–microbial crosstalk (*double-headed blue arrows*), which contributes to maintain the balance of homeostasis in the host

ecosystem by a comprehensive analysis of the host, the gut microbiota, and their interactions.

There have recently been great strides in our understanding of the human gut microbiota, thanks largely to the emergence of next generation DNA sequencers [1, 2, 4–8]. In this type of analysis, DNAs isolated from human feces are directly subjected to shotgun sequencing followed by bioinformatic assembly of the sequence reads to generate metagenomic sequences, the collective genome sequences of all the gut microbes. An important advantage of this kind of "metagenome" analysis is that it enables us to identify genes in the genomes of both culturable and unculturable bacteria in the gut, the latter comprising the majority of the microbiota. The metagenomic sequences can also be used for quantitatively accurate estimation of the bacterial composition by mapping the reads to reference genomes of human microbes. These are available from a website (http://www.hmpdacc.org/), in which genomes of more than 2,000 bacterial strains have been deposited. These studies have provided us with novel and important insights into the gut microbial society. For example, the human gut microbiota from different individuals could be clustered into three predominant subtypes, termed "enterotypes", dominated by *Bacteroides*, *Prevotella*, and *Ruminococcus*, respectively. Although enterotypes appear independent of nationality, sex, age, or body mass index, they are strongly associated with long-term dietary habits, particularly high protein and animal fat (*Bacteroides* enterotype) or high carbohydrate consumption (*Prevotella* enterotype) [9]. A controlled feeding study has shown that enterotype identity of each individual remained stable during the 10-day study, suggesting that enterotypes could be strongly associated with long-term diet. Interestingly, it has been reported that the Japanese gut microbiota possess "special" genes not found in Caucasians, which encode enzymes specifically for degrading carbohydrates existing only in seaweeds ("nori" in Japanese) but not in terrestrial plants [10]. The authors of this study propose that these so-called "nori genes" are horizontally transferred from seaweed-associated marine bacteria to the gut microbiome of the Japanese, since they have a long history of eating seaweed. Taken together, these observations suggest that the gut microbiota has coevolved with the host and its feeding habitat.

The gut microbiota is thought to possess a variety of functional properties resulting in broad range impacts on human physiology and pathology. For example, they aid in host nutrition and energy harvest, by the production of vitamins, and fermentation of food components that are otherwise indigestible by the host [11–14]. They also contribute to intestinal epithelial homeostasis, development of the immune system, protection against pathogens, as well as drug metabolism [15–18]. In fact, beneficial commensal microbes, such as *Bifidobacterium* spp. and *Lactobacillus* spp., have long been consumed by people as "probiotics" [12, 19, 20]. Furthermore, certain materials, such as oligosaccharides, aid proliferation of the probiotic species and thereby are also beneficial for health. These are called "prebiotics" and are often consumed as functional foods [21]. On the other hand, imbalance of the gut microbiota, or dysbiosis, can predispose individuals to a variety of disease states ranging from gut-intrinsic disorders such as inflammatory bowel diseases [22–24], Crohn's disease and ulcerative colitis, and colonic cancer [25, 26] to systemic diseases such as allergic diseases [27, 28] and metabolic syndromes such as obesity [6, 29–32], diabetes [7, 33–35], arteriosclerotic diseases [36, 37], and nonalcoholic steatohepatitis (NASH) [38, 39]. Therefore, a comprehensive understanding of the gut microbiota and assessment of causal relationships between it and related disorders are necessary for generating therapeutic approaches to cure these diseases.

Gut microbiota and obesity

The first report of gut microbial difference between obese and lean phenotypes was in leptin-deficient (ob/ob) mice in 2005 [40]. This study showed that, proportionately, phylum Bacteroidetes was less and phylum Firmicutes was more abundant in obese ob/ob mice than in their lean littermates, when analyzed by 16S rRNA gene sequencing. As both groups of mice were fed with same diet, the results suggested that obesity could be due to the difference in gut microbial composition, although no causal association between these two phyla and the development of obesity was demonstrated. A subsequent study with obese human twins showed that a decrease in Bacteroidetes proportion with an increase in Firmicutes proportion was correlated with the enrichment of microbial genes encoding key enzymes related to

carbohydrate metabolism, which consequently might increase the ability to digest food and supply energy such as short-chain fatty acids (SCFAs) to the host [6, 29]. Interestingly, colonization of the gut microbiota from not only obese mice [41] but also from obese humans [29] into germ-free recipient mice reproduced the obese phenotype. It has also been reported that obese children already have different gut microbiota compared to lean children [42, 43]. This difference may imply that the gut microbial composition during early childhood is a key factor to becoming obese later in life, and that modulation of the gut microbiota in early life might be an effective strategy to prevent obesity.

It has also been reported that a high-fat diet affects the composition of the gut microbiota in mice. The *Clostridium coccoides* group and *Bifidobacterium* spp. were significantly reduced in obese mice, whereas *Lactobacilli/Enterococci* and *Bacteroides* were comparable [44]. Feeding of a high-fat diet for 14 weeks also induced similar changes, with a significant reduction in the *Eubacterium rectale*/*C. coccoides* group and in *Bifidobacterium* spp. [34]. Interestingly, oral administration of *Bacteroides uniformis* CECT 7771 ameliorated the high-fat diet-induced immune and metabolic disorders that correlated with gut microbial modifications in obese mice [45]. Furthermore, recent 16S rRNA gene pyrosequensing of mice with obesity-associated hepatocellular carcinoma also showed that the proportion of Bacteroidetes was remarkably decreased and gram-positive bacteria such as Clostridiales and Bacilli drastically increased in the mice fed a high-fat diet [46]. The severity of hepatocarcinoma is likely increased due to the presence of a larger proportion of Gram-positive bacteria because they produce a high level of deoxycholic acid, a gut microbial metabolite that can damage host DNA through cholic acid metabolism. Oral antimicrobial treatment against Gram-positive bacteria significantly decreased the severity of hepatocarcinoma in the high-fat diet-fed mice, suggesting that the high-fat diet-induced obesity followed by increment of clostridia and bacilli contribute to hepatocarcinoma development through deoxycholic acid production [46]. Besides these reports, Kim et al. [47] found that Ruminococcaceae and Rikenellaceae were enriched in mice fed a high-fat diet. Taken together, specific changes of the gut microbial composition induced by high-fat diet appear to contribute to the host obese phenotype.

A recent analysis of gut microbes-associated weight gain found that the number of *Akkermansia muciniphila* was dramatically decreased (100- to 1,000-fold) in both genetically and high-fat diet-induced obese mice [48]. This bacterial species has been reported as a novel mucin-degrading bacterium that colonizes in the mucus layer and constitutes 3–5 % of the microbial community [49]. Further study showed that the proportion of this bacterium is negatively correlated with body weight [43, 50–52], as well as type 1 [53] and type 2 [7] diabetes. Furthermore, when the proportion of *A. muciniphila* was normalized in obese mice either by *A. muciniphila* oral administration or by treatment with oligofructose as a prebiotic, there was an improvement in several metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance [48, 50]. The study also demonstrated that all these beneficial effects required viable *A. muciniphila* cells because heat-killed *A. muciniphila* treatment did not lead to any improvement in these metabolic disorders [48].

Obese gut microbiota seems to induce chronic low-grade inflammation in the host gut [44, 54, 55]. Chronic experimental metabolic endotoxemia-induced obesity, diabetes, and liver insulin resistance trigger the expression of several inflammatory factors [44]. de La Serre et al. have reported that obesity in rats induced by a high-fat diet resulted in changes in the composition of the gut microbiota and activation of toll-like receptor 4 (TLR4) signaling in the gut epithelia. The authors hypothesized that activation of the TLR4 pathway through gut microbial changes provoked gastrointestinal inflammation associated with the obese phenotype [55]. In a recent study, Fei and Zhao have demonstrated that mono-association of germ-free mice with an endotoxin-producing *Enterobacter cloacae* B29 strain isolated from an obese human subject induces obesity and glucose homeostasis disorders upon feeding with a high-fat, but not a normal diet [56]. These studies imply that lowering plasma endotoxin levels could be a potent strategy for the control of metabolic diseases. Besides, Vrieze et al. have reported that fecal transplantation of gut microbiota from lean healthy donors into human patients with metabolic syndrome through small intestinal infusions results in improved insulin sensitivity [57]. The improvement in insulin sensitivity in recipient patients correlated with an increase in the number of butyrate-producing bacteria, suggesting that microbial butyrate may help promote this improvement.

Gut microbiota and diabetes

In addition to the above reports on the relationship between the gut microbiota and obesity development, several studies have indicated that the composition of gut microbiota correlates with type 1 and 2 diabetes development. Larsen et al. [58] found that the gut microbiota of human male subjects with type 2 diabetes had significantly fewer Firmicutes, including Clostridia, than that of non-diabetic control subjects. The study also revealed that the plasma glucose concentration was positively correlated with both the ratios of Bacteroidetes to Firmicutes, and of the *Bacteroides–Prevotella* group to *C. coccoides–E. rectale* group. Furthermore, the diabetic subjects had more *Beta-proteobacteria* than non-diabetic control subjects. These findings suggest that the Bacteroidetes and Proteobacteria may promote type 2 diabetes through an endotoxin-induced inflammatory response because they are gram-negative bacteria

and have a high level of the endotoxin lipopolysaccharide (LPS) as a main component of their outer membranes. Membrez et al. have examined whether a depletion of gut microbiota influences glucose tolerance in ob/ob mice as a model of type 2 diabetes [59]. In this model, 2-week-antibiotic treatment significantly reduced the number of both aerobic and anaerobic microbes in the gut. As a result, antibiotic-treated ob/ob mice had lower liver triglycerides and plasma LPS concentrations, and higher liver glycogen and plasma adiponectin concentrations than non-treated ob/ob mice, indicating the efficacy of microbial depletion for improving glucose tolerance in ob/ob mice. These authors further speculate that the glucose tolerance improvement in ob/ob mice could be mediated by changes in metabolic and inflammatory pathways due to changes in the gut microbial community.

Recently Qin et al. [7] have developed a novel gut microbiota analytical platform, the metagenome-wide association study (MGWAS), to identify disease-associated metagenomic markers. The authors carried out MGWAS on gut microbial metagenome data of 345 Chinese individuals containing type 2 diabetes patients and non-type 2 diabetes control subjects. They found that almost all of the genes enriched in the control group were from various butyrate-producing bacteria, including *Clostridiales* sp. SS3/4, *E. rectale*, *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Roseburia inulinivorans*. By contrast, most of the genes enriched in type 2 diabetes group were from opportunistic pathogens, such as *Bacteroides caccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*, *Eggerthella lenta*, and *Escherichia coli*, which have previously been reported to cause or underlie human infections such as bacteremia and intra-abdominal bacterial infections. Interestingly, the well-known mucin degrading microbial species *A. muciniphila* and sulfate-reducing species *Desulfovibrio* sp. were also enriched in the type 2 diabetes group. From these results, these authors concluded that type 2 diabetes patients had only a moderate degree of gut microbial dysbiosis with increases in several opportunistic pathogens and a reduction in butyrate-producing bacteria, which may be a beneficial metabolite [7].

More recently, Karlsson et al. [60] also conducted shotgun metageomic sequencing of gut microbiota of 145 European women (all 64 years old) with normal, impaired or diabetic glucose control. These authors further developed a mathematical model to identify type 2 diabetes with high accuracy based on metagenomic profiles of gut microbiota. By applying this model to European women with impaired glucose tolerance, those who had diabetes-like metabolism could be identified. They also applied this mathematical model to a gut microbial metagenomic dataset of the Chinese cohort described above [7] and found that metagenomic markers to distinguish type 2 diabetics from others were different between the Chinese and European cohorts, suggesting that an age- and geographical location-matched dataset of the gut microbial metagenome will be required to develop an adequate mathematical model for discrimination of type 2 diabetes.

Several studies have also been conducted to elucidate the role of gut microbiota in type 1 diabetes development. Bosi et al. [61] compared the intestinal abnormalities in 81 type 1 diabetes subjects and 40 healthy control subjects. The intestinal permeability of type 1 diabetes patients was significantly increased as compared to that of healthy control subjects, suggesting that a weakened intestinal barrier function could be participating in the pathogenesis of type 1 diabetes. Vehik and Dabelea [62] also suggested that increased gut permeability may affect the absorption of exogenous antigen that may attack and damage pancreatic beta cells. It has also been reported that gut microbes can affect intestinal permeability and thus may play an important role in type 1 diabetes development [63, 64]. In addition to this gut microbe-mediated abnormal intestinal barrier theory, other groups have proposed the hypothesis that microbial toxin(s) can directly affect or damage the pancreatic beta-cell function. Myers et al. [65] reported that injecting *Streptomyces* toxin, bafilomycin A1, into mice resulted in smaller islets, reduced pancreatic beta-cell mass and impaired glucose tolerance. Other microbial toxins, such as streptozotocin, have been used to induce diabetes in experimental mouse models [66]. In addition, taking advantage of non-obese diabetic (NOD) mice lacking MyD88, a downstream TLR adaptor molecule, Wen et al. [33] found that wild-type NOD mice developed type 1 diabetes, whereas MyD88-deficient NOD mice did not; interestingly, germ-free MyD88-negative NOD mice developed diabetes, and this was suppressed by colonization with normal gut microbes. These findings indicate that gut microbes–host innate immune system interactions are critical factors for modifying type 1 diabetes development.

Gut microbiota and atherosclerosis

Atherosclerotic vascular disease is a complex pathologic phenotype that is caused by host genetic and environmental factors such as food and commensal microbes. Wang et al. reported that three phospholipid-associated molecules, choline, betaine, and trimethylamine N-oxide (TMAO) in the plasma seem to promote atherosclerosis and could be used as a biomarker for predicting the risk of cardiovascular diseases [37]. These three phospholipid-associated molecules were identified by a metabolome analysis of the plasma of 50 atherosclerotic disease patients using LC/MS compared to 50 age- and gender-matched control subjects. To understand the contribution of these three molecules to the risk of cardiovascular diseases, these authors next utilized a murine

model of atherosclerosis, the apoE-deficient mouse, and demonstrated that the plasma TMAO levels in apoE-deficient mice positively correlated with aortic lesion area. In addition, the expression level of the liver flavin monooxygenases, which convert trimethylamine (TMA) to TMAO, positively correlated with the plasma TMAO levels in mice and humans. Antibiotic treatment of apoE-deficient mice significantly reduced the plasma TMAO level and the size of the atheroma, suggesting that gut microbes significantly affect the development of atheroma in apoE-deficient mice. Finally, these authors showed that supplementing the diets of apoE-deficient mice with 1 % choline increased foam cell formation with an accompanying increase in the expression of CD36 and SRA1 scavenger receptors on macrophages and that this outcome was prevented by treatment with broad-spectrum antibiotics. Taken together, the authors found a novel pathway linking dietary lipid intake, the gut microbiota and atherosclerosis; dietary choline was converted to TMA by gut microbes and then the absorbed TMA was metabolized to TMAO, a pro-atherosclerotic metabolite, by hepatic flavin monooxygenases.

Recently, the same group also reported that dietary L-carnitine, which possesses a TMA structure similar to choline and is abundant in red meat, is also metabolized to TMA by gut microbes and further converted to TMAO in the liver, which accelerates atherosclerosis in mice [36]. 16S rRNA pyrosequencing of cecal microbiota in the mice fed with normal chow or an L-carnitine supplemented diet showed that the family Prevotellaceae and the genus *Prevotella* were enriched and positively correlated with the plasma TMA level in the mice fed the L-carnitine supplemented diet. Interestingly, plasma TMAO concentrations in omnivores were significantly higher than in the vegans/vegetarians. Correlation analysis of fecal microbiome composition and the plasma TMAO levels showed that the plasma TMAO concentration in the subjects with a *Prevotella* enterotype was significantly higher than in *Bacteroides* enterotype subjects. Oral L-carnitine challenge of human subjects who were vegans/vegetarians or omnivores indicated that the omnivorous subjects produced more TMAO than did vegans/vegetarians following ingestion of L-carnitine. These results suggest that dietary habits may modulate both the composition of the gut microbiota and their ability to metabolize TMA and TMAO from dietary L-carnitine.

Metagenomic analysis of the fecal microbiome also revealed that the genus *Collinsella* was enriched in symptomatic atherosclerosis patients, whereas the genus *Roseburia* and *Eubacterium* were enriched in healthy subjects [67]. Further metagenomic characterization of the functional capacity of the fecal microbiome revealed that genes encoding peptidoglycan synthesis were enriched and phytoene dehydrogenase was depleted in symptomatic atherosclerosis patients. In accordance with this, these patients had a reduced serum β-carotene levels. These findings imply that the inflammatory status of the symptomatic atherosclerosis patients may be associated with characteristic changes in the gut microbiome.

Gut microbiota and non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in many countries throughout the world. NAFLD is characterized by fat deposition (steatosis) in the liver that is unrelated to excessive alcohol consumption, and can be seen in states of insulin-resistance and the metabolic syndrome. NAFLD may progress from simple fatty liver to liver cirrhosis and hepatocellular carcinoma [68, 69]. NASH is the most severe form of NAFLD that affects 10–20 % of all NAFLD patients and is a major cause of cirrhosis of the liver [70]. The incidence of NASH is increasing; however, the underlying mechanisms remain obscure. It is assumed that various genetic, metabolic, inflammatory and environmental factors are contributing to its pathogenesis [39]. Many human and animal studies have investigated the relationships between the gut microbiota and NAFLD [30, 44, 71–77]. Bäckhed et al. have reported that conventionalized mice have a higher concentration of liver triglycerides than germ-free mice, although the amount of food intake was reduced in the conventionalized mice. These authors also showed that colonization with the gut microbiota was associated with a higher monosaccharide absorption from the gut lumen, which promotes de novo fatty acid synthesis and triglyceride production in the liver, as confirmed by increased activity of enzymes such as acetyl-CoA carboxylase and fatty acid synthase [76]. In addition, Cope et al. [77] have found that synthesis of microbial fermentation products including ethanol in the gut is a key factor to induce obesity in mice and may be related to the pathogenesis of fatty liver disease. As with obesity, Cani et al. have reported that microbial endotoxin-related chronic inflammation involves CD14-TLR4 signaling and that hepatic Kupffer cell activation in mice seems to contribute to the pathogenesis of NAFLD [30, 44, 71]. Gut microbiota also modulate bile acid metabolism. Swann et al. [72] have reported that the gut microbiota can indirectly promote hepatic steatosis and lipoperoxidation through farnesoid X receptor-mediated signaling, which affects bile acid secretion in mice. Collectively, these animal studies indicate that the gut microbiota can induce fatty liver through an increase in monosaccharide absorption [76], hepatotoxic ethanol production [38], microbial endotoxin-induced low-grade chronic inflammation [30, 44, 71], as well as modulation of bile acid metabolism [72].

Several human studies on the relationship between gut microbiota and NAFLD/NASH development have also been

conducted. Sabate et al. [73] have reported that gut microbial overgrowth in obese patients may be linked to hepatic steatosis. Wigg et al. have also reported that half of NASH patients have microbial overgrowth and increased serum tumor necrosis factor alpha levels, suggesting that NASH might be associated with gut microbial dysbiosis and systemic inflammation, although intestinal permeability is unchanged [78]. Miele et al. [74] have found that NAFLD development in human subjects is associated with increased intestinal permeability due to microbial overgrowth in the small intestine and disruption of intestinal mucosal tight junctions: Small intestinal microbial overgrowth in human NAFLD patients might contribute to hepatic fat deposition through the increased intestinal permeability caused by the disrupted intestinal tight junctions. Furthermore, as in NAFLD studies in animal models, Verdam et al. [75] have shown that chronic endotoxemia in human patients is correlated with the severity of NAFLD. In addition, systemic ethanol levels were significantly higher in NASH patients than the control group, indicating that ethanol-producing microbes might be related to the pathogenesis of NASH [79]. It has recently been demonstrated that dietary choline depletion might also play a role in human NAFLD development [80]. In this study, 15 female subjects were placed on well-controlled diets in which choline levels were adjusted. It was found that dietary choline deficiency modified the gut microbial composition and that the levels of bacterial class Gammaproteobacteria and Erysipelotrichi were positively correlated with changes in the liver fat content. Another group also reported that NASH patients had a lower percentage of the bacterial phylum Bacteroidetes compared to both healthy controls and simple steatosis patients, which seems to be similar to the gut microbial profile in obese human subjects [6, 29]. Collectively, these findings suggested that the difference in gut microbial profile among NAFLD, NASH, obese, and healthy controls might offer a marker for diagnosis, as well as a target for preventive/therapeutic medicine such as probiotic intervention, for these diseases.

Probiotic and prebiotic modulation on metabolic diseases

Probiotics, typically contained in dairy fermented products such as yogurt, are well known as healthy microbes that, when orally administered in adequate amounts, confer health benefits to the host. On the other hand, prebiotics are certain food materials such as oligosaccharides that promote proliferation of probiotics. Probiotics and prebiotics have been introduced in our life as health promoting supplements, and there have been many publications on the beneficial effects of probiotics and prebiotics such as improvement of gut environment [81], regulation of immune functions [82], and prevention of pathogenic microbial infection [20]. Furthermore, the anti-obesity potential of probiotics and prebiotics has also been described, as discussed below.

Conjugated linoleic acid (CLA) is a naturally occurring conjugated isomer of linoleic acid found in ruminant-derived meets and dairy products[83, 84] and has been shown to prevent colonic carcinogenesis, arteriosclerosis, as well as obesity in mice [85, 86]. The CLA-producing probiotic strain *Lactobacillus rhamnosus* PL60 has been reported to reduce body weight gain and mass of white adipose tissue with no effect on food intake in mice fed with high-fat diet. This effect was coupled with higher expression of uncoupling protein-2, while fatty acid synthase expression and serum leptin and glucose concentrations were reduced [87]. Another probiotic strain that produces CLA, *Lactobacillus plantarum* PL62, also resulted in decreased body weight gain and glucose concentration in obese mice induced by high-fat diet intake [88]. Furthermore, probiotics have also been reported to reduce adipocyte size in different adipose depots [89–91], which is regarded as a key parameter for assessing the anti-obesity potential of probiotics. The putative mechanisms seem to be the increase in fecal excretion of neutral sterols and bile acids, combined with a reduction in lymphatic absorption of triglycerides, phospholipids as well as cholesterol [89]. In the experiment with 3T3-L1 cells, a pre-adipose cell line, incubation with a *L. plantarum* KY1032 cell-free extract resulted in reduction of adipogenesis [92], and incubation with the insoluble fraction from the fermented milk product kefir resulted in reduced adipocyte differentiation [93]. Supplementation with *Lactobacillus paracasei* F19 resulted in decreased total body fat and a reduction in the amount of triglycerides in different lipoprotein fractions in mice fed a high-fat diet [94]. In addition, both germ-free (GF) and conventionalized mice orally administered with *L. paracasei* F19 showed an increased serum level of Angiopoietin-like 4, which is a lipoprotein lipase inhibitor regulating lipid deposition into adipocytes [94, 95]. Administration of *L. paracasei* F19 and *Lactobacillus acidophilus* NCFB1748 to GF mice resulted in enriched colonization of the probiotic strains in the ileum as compared to the colon, and upregulation of insulin-sensitizing hormones such as adipsin and adiponectin [96]. Supplementation of apoE-deficient mice with *Lactobacillus reuteri* ATCC4659 resulted in the reduction of body weight gain, adipose, and liver weights, as well as increased expression of carnitine palmitoyl transferase1A in the liver, suggesting that probiotic supplementation activated hepatic β-oxidation [97].

There are relatively few studies dealing with the changes in microbiota composition caused by probiotic supplementation toward anti-obesity functions. Supplementation of mice with *L. rhamnosus* GG and *Lactobacillus sakei* NR28 reduced the relative abundance of both Firmicutes and *Clostridium* cluster XIVa in the small intestine, and resulted in the reduction of body weight gain, fat mass and expression of lipogenesis-

related enzymes such as hepatic stearoyl-CoA desaturase-1, fatty acid synthase and acetyl-CoA carboxylase [98]. However, supplementation with *L. acidophilus* NCDC13 in diet-induced obese mice increased the number of total bifidobacteria in cecal contents and feces but did not reduce adiposity [99]. In another study, oral inoculation with *Lactobacillus ingluviei* increased the total abundance of fecal *Lactobacillus* spp. and Firmicutes in mice and resulted in increased body weight gain, liver weight, and metabolism [100]. In healthy overweight human subjects, oral administration of *Lactobacillus gasseri* SBT2055 was accompanied by a reduction of abdominal visceral and subcutaneous fat [101]. Supplementation of *L. rhamnosus* GG in infant formula for six months resulted in better growth with higher weight gain [102], and, in a follow-up study, pre- and postnatal administration of *L. rhamnosus* GG prevented excessive weight gain in the children [103]. Taken together, the physiological effects of probiotics on human subjects seem to be strain specific.

Hepatic steatosis is closely linked to metabolic syndrome. It is characterized by aberrant lipid storage in the liver and subsequent hepatic inflammation. Colonization of GF mice with microbiota from hyperglycemic mice has been shown to contribute to the development of NAFLD independent of obesity [104]. Oral administration of *Clostridium butyricum* MIYARI 588, a butyric acid-producing anaerobe, has also been shown to reduce NAFLD progression in rats with diet-induced steatosis [105]. Supplementation with VSL#3, the most highly concentrated probiotic supplement available with eight different naturally occurring strains of "good" bacteria, mediates a natural killer T cell-dependent improvement of diet-induced steatosis and hepatic insulin signaling, resulting in improved insulin sensitivity [106]. VSL#3 supplementation is also reported to reduce c-jun kinase activity and hepatic lipogenesis in leptin-deficient ob/ob mice [107]. In apoE-deficient mice, VSL#3 improves insulin resistance, prevents the development of histologic features of mesenteric adipose tissue inflammation and steatohepatitis, and reduces the extent of aortic plaques [108]. In another study, the effect of VSL#3 was tested on high-fat diet-induced oxidative and inflammatory damage in the liver of young rats, and again the probiotics blocked the increase in inflammatory markers compared to the control high-fat diet group [109]. Collectively, these studies suggest that, at least in the pre-clinical setting, various probiotics may improve fatty liver disease.

One of the most studied prebiotic supplements is inulin, naturally derived from plants, and related compounds such as fructooligosaccharides, which have different degrees of fructose polymerization. Inulin specifically enhances the growth of bifidobacteria, which is coupled with a reduction in body weight gain and improvement in glucose homeostasis [110–112] and obesity-related inflammation called metabolic endotoxemia [16, 34]. Pyrosequencing of gut microbes in ob/ob mice fed with prebiotic oligofructose revealed gut microbial changes in more than 100 taxa, of which 16 taxa displayed more than 10-fold change in abundance [50]. One of the identified species was *A. muciniphila*, which was negatively correlated with body weight as described above [52]. A major effect of inulin supplementation seems to be its influence on production of gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), ghrelin, and other related peptide hormones through microbial changes both in rats [110, 113, 114] and in humans [115–117]. These hormones modulate several physiological functions such as insulin secretion through incretin and gastrointestinal motility, implying that these functions may contribute to the anti-obesity potential of prebiotics. In fact, microbial production of SCFAs has been proposed to play a role in increasing secretion of gut hormones such as GLP-1 [118, 119].

Other studies have also shown that prebiotic fibers reduce the ratio of Firmicutes to Bacteroidetes in obese rats [113] and ameliorate NAFLD by decreasing hepatic de novo lipogenesis [120]. Supplementation of fungal chitin glucan increases the number of bacteria closely related to *Clostridium* cluster XIVa including *Roseburia* spp., which is accompanied by reduced weight gain and fat mass development [121]. It has been also reported that wheat-derived arabinoxylans restore the number of *Bacteroides*/*Prevotella* spp. and *Roseburia* spp. and markedly increase the number of *Bifidobacterium* spp., in particular *Bifidobacterium animalis* lactis, in cecal contents of mice fed with high-fat diet [122]. Supplementation of the diet

Fig. 2 Obesity and obesity-related disorders induced by gut microbiota. An imbalance of the gut microbiota may be involved in the development of metabolic disorders such as obesity, NASH, atherosclerosis, and diabetes. Lipopolysaccharide (*LPS*), a component of the outer membrane of Gram-negative bacteria of the microbiota, seems to be a crucial factor influencing the development of these metabolic disorders

with inulin increases *Bifidobacterium* spp. and *F. prausnitzii*, and decreases *Bacteroides intestinalis*, *Bacteroides vulgates*, and *Propionibacterium* in obese women [123]. In addition, consumption of galactooligosaccharides for 12 weeks increased several type of *Bifidobacterium* spp. and decreased the number of *Bacteroides* in healthy human subjects [124].

Conclusion

The increased prevalence of obesity and obesity-related disorders such as diabetes, atherosclerosis, and NAFLD is becoming a major problem for health care throughout the world. Dietary habit and lifestyle seem to be crucial factors influencing the development and progression of obesity. Recent studies have examined obesity with a new perspective and found that gut microbiota might be related to the development of these metabolic disorders (Fig. 2). Increase in serum LPS levels, defined as metabolic endotoxemia, occurring in individuals with obesity demonstrates that specific gut microbial components may trigger metabolic disorders. Animal experiments have clearly shown that the gut microbiota also influence host energy metabolism. These findings indicate that certain bacterial molecular targets involved in the control of obesity and obesity-related disorders might be identifiable. Many studies have reported the difference in the composition of gut microbiota between obese and lean individuals, both in animal models as well as in humans. However, we cannot conclude at this time that specific genera, classes, or species of gut microbes are always positively or negatively correlated with the obese phenotype. Therefore, more controlled human and animal studies are necessary to clarify these complex issues. Combination of metagenomic, transcriptomic, and metabolomic analyses could further elucidate the molecular basis of metabolic interactions between gut microbiota and host physiology. This integrative omics approach, combined with mechanistic studies with appropriate animal models, will help further understanding of the functions of distinct microbial groups or individual species of the gut microbiota and evaluating the effectiveness of prebiotic and probiotic approaches on the control of obesity and obesity-related diseases in humans.

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