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Programming of Host Metabolism by the Gut Microbiota

by Frederik Bäckhed

Key insights

The human gut environment is an important factor that contributes to metabolism. Studies with germ-free mice demonstrate protection against developing diet-induced obesity. Further evidence suggests that obese individuals have an altered microbiota.

Current knowledge

From a sterile gut at birth, a complex ecosystem evolves with the human host. As a result, there are 150-fold more bacterial genes than human genes in the adult body. Less is known about the impact gut microbiota have on human physiology. Improved glucose tolerance and insulin sensitivity, calorie restriction and resistance to developing obesity was demonstrated in germ-free mice; microbiota transplantation from obese and normal controls into germ-free mice showed increased body fat, suggesting that gut microbiota contributes to the pathogenesis of obesity.

Practical implications

These findings and further observations suggest possible microbial manipulation in the human gut during pregnancy or early childhood could offer a unique approach for obesity prevention and/or treatment.

Recommended reading

Bäckhed F, Manchester JK, Semenkovich CF, Gordon JL: Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007; 104: 979–984.

↑ Gut microbiota
↑ Energy extraction

Microbiota may contribute to disease progression by affecting:

- Enterohepatic gene expression
- Genes involved in immunity and metabolism

Obese phenotype and the gut. Gut microbiota is an environmental factor contributing to host metabolism. In the altered microbiota of obese individuals, several microbially modulated mechanisms reveal far-reaching effects, e.g. modulation of lipid metabolism in peripheral organs (see text for details).

Programming of Host Metabolism by the Gut Microbiota

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Key Messages

- The gut microbiota is an environmental factor that contributes to host metabolism.
- Germ-free mice are protected against developing diet-induced obesity.
- Obese individuals have altered microbiota.

Key Words

Gut microbiota • Host microbe interactions • Insulin resistance • Metabolism • Metagenome • Obesity

Abstract

The human gut harbors a vast ensemble of bacteria that has co-evolved with the human host and performs several important functions that affect our physiology and metabolism. The human gut is sterile at birth and is subsequently colonized with bacteria from the mother and the environment. The complexity of the gut microbiota is increased during childhood, and adult humans contain 150-fold more bacterial genes than human genes. Recent advances in next-generation sequencing technology and mechanistic testing in gnotobiotic mice have identified the gut microbiota as an environmental factor that contributes to obesity. Germ-free mice are protected against developing diet-induced obesity and the underlying mechanisms whereby the gut microbiota contributes to host metabolism are beginning to be clarified. The obese phenotype is associated with increased mi-

crobial fermentation and energy extraction; however, other microbially modulated mechanisms contribute to disease progression as well. The gut microbiota has profound effects on host gene expression in the enterohepatic system, including genes involved in immunity and metabolism. For example, the gut microbiota affects expression of secreted proteins in the gut, which modulate lipid metabolism in peripheral organs. In addition, the gut microbiota is also a source of proinflammatory molecules that augment adipose inflammation and macrophage recruitment by signaling through the innate immune system. TLRs (Toll-like receptors) are integral parts of the innate immune system and are expressed by both macrophages and epithelial cells. Activation of TLRs in macrophages dramatically impairs glucose homeostasis, whereas TLRs in the gut may alter the gut microbial composition that may have profound effects on host metabolism. Accordingly, reprogramming the gut microbiota, or its function, in early life may have beneficial effects on host metabolism later in life.

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Introduction

The human gut harbors a complex ecosystem that has co-evolved with the human host. During recent years, the interest in cataloging and understanding this gut microbiota has increased dramatically. Bacteria constitute the vast majority of the human gut microbiota, but archaea, viruses, and protozoans are also present. Recent data suggest that the human gut is inhabited by some 150–200

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prevalent and up to ~1,000 less common bacterial species [1, 2]. The potential impact of the gut microbiota on human health can best be illustrated by the fact that the gut bacteria outnumber our own somatic cells by an order of magnitude and that the gut microbiome, the collective genomes of the gut microbiota, encodes at least 150-fold more genes than in our genomes. Despite the dramatically increased knowledge of the gut microbiome and which bacteria are present in our gut, less is known about the physiologic impact the gut microbiota has on human physiology. The usage of gnotobiotic animal models, which were developed >75 years ago, has allowed mechanistic testing of how the gut microbiota is assembled, selected, and how it affects the mammalian host. In the following, I will review the composition of the gut microbiota, how an altered microbiota may contribute to human disease, and by which mechanisms the gut microbiota may affect host physiology and metabolism.

Composition of the Human Gut Microbiota

The human gut microbiota is dynamic and responsive to dietary changes (table 1), which may have profound effects on the gut microbial composition [3–5]. Nevertheless, several studies have revealed that an individual’s microbiota always is more similar over time than to other individuals. However, in childhood before the establishment of a stable and diverse microbiota this may be different. The fetus is sterile in utero and is rapidly colonized by environmental bacteria at birth and during vaginal delivery, and most of them are derived from the vaginal and fecal microbiota [6]. The initial microbiota is characterized by low diversity and mainly facultative anaerobic bacteria belonging to Proteobacteria and Actinobacteria. The gut microbiota then becomes more diverse, and bacteria belonging to Firmicutes and Bacteroidetes are dominant [1, 2, 7].

The development of the adult microbiota has been suggested to be the result of both positive and negative selection [8]. Some of these positive selection mechanisms could be host factors that allow specific bacteria to adhere, for example, glycolipids on the epithelium or specific glycans in the host mucus. Similarly, the diet is a major factor in shaping the gut microbiota. In contrast, the host immune system may alter the gut microbial community by killing of specific bacterial groups. For example, mice deficient in TLRs (Toll-like receptors) or NOD (nucleotide-binding oligomerization domain) receptors, which recognize conserved microbial signatures, or downstream signaling molecules have altered gut micro-

Table 1. Composition of the human gut

Stage	Characteristics
Fetus	Sterile
At birth	Colonized by environmental bacteria; maternal vaginal and fecal microbiota
Neonate	Initially low diversity, facultative anaerobic bacteria (Proteobacteria/Actinobacteria); progressing to more diversity and dominated by Firmicutes and Bacteroidetes
Adult	Diverse, with an individual’s microbiota more similar over time than to other individuals; selection mechanisms may be involved; positive selection: host factors, e.g. glycolipids on the epithelium, specific glycans in the host mucus; negative selection: diet and immune system
Adult with inflammatory bowel disease or type 2 diabetes	Reduced microbial diversity, particularly <i>Faecalibacterium prausnitzii</i>
Adult with obesity	Reduced bacterial diversity, particularly Bacteroidetes

bial composition [9–11]. Furthermore, epithelial-expressed effector molecules of the innate immune system also affect the gut microbial composition and may act protectively in patients with inflammatory bowel disease (IBD) [12–14]. Interestingly, several studies have now demonstrated that the gut microbiota of IBD patients has reduced microbial diversity, and animal models have provided direct evidence that the gut microbiota may be involved in the development of IBD [15].

Host Effects in Response to Microbial Colonization

Most studies identifying the early intestinal adaptations to microbial colonization have been obtained from animal models. Germ-free mice have elongated and thinner intestinal villi, which provides a larger surface area and may facilitate nutrient absorption from the gut. Furthermore, germ-free mice have increased expression of genes encoding transporters throughout the gut [Larsson and Bäckhed, unpubl. observation]. However, the elongated villus structure is more vulnerable to infections and accordingly the cellular composition is dramatically altered upon colonization with a normal gut microbiota. Upon

Fig. 1. The relationship between the human genome, the environment (diet shown here), and the gut microbiota affects our metabolism.

colonization, the villus is shortened and widened and the expression of molecules that are essential for increased barrier functions is upregulated [16]. The altered villus structure is associated with increased vascularization [17]. This process requires functional tissue factor and protease-activated receptor signaling [Reinhardt et al., unpubl. observation]. Furthermore, colonization of the gut also promotes recruitment of immune cells to the small intestine and formation of gut-associated lymphoid tissue [18]. Importantly, recent elegant studies have demonstrated that the innate and adaptive immune systems complement each other to maintain host microbiota mutualism [19].

Metabolic Functions of the Gut Microbiota

The gut microbiota is situated on the intersection between the diet and host genome and thus has important implications for food processing and making nutrients available to the host (fig. 1). Furthermore, the gut microbiota is an important ‘detoxifier’ of xenobiotic compounds that we ingest and may also affect drug metabolism [20]. Despite increased food intake, germ-free mice have reduced capacity to harvest energy from the diet [21]. The germ-free state is accordingly associated with reduced energy storage in the liver and skeletal muscle [22, 23]. AMPK (AMP-activated protein kinase) is an important energy sensor in all organisms from yeast to mammals and promotes energy-generating pathways, while shutting off an-

abolic pathways [24]. Accordingly, germ-free mice have increased AMPK activation in both liver and skeletal muscle [23]. These findings suggest that the germ-free state resembles calorie restriction, which is associated with longevity. Indeed, germ-free mice have increased lifespans and appear to be healthier in many aspects, including its resistance to develop obesity [23, 25–29]. However, the resistance to develop diet-induced obesity appears to be dependent on specific diet-microbiota interactions [28].

Microbiota in Obesity

The finding that the gut microbiota may be considered an environmental factor that modulates obesity has spurred studies to test if the gut microbiota is altered in obese individuals. Several studies comparing different cohorts of obese and lean individuals or obese individuals on a weight reduction program have been performed. However, the results of these studies have not reached the same conclusions, which may at least in part be explained by the small study cohorts in each study and the different methods used [reviewed in ref. 30]. However, a recent study including twins concordant for obesity and their mothers revealed that obesity is associated with reduced microbial diversity [2]. This finding suggests that there may be some common etiology between IBD and obesity as both diseases are associated with reduced microbial diversity and inflammation. Furthermore, a recent study demonstrated that *Faecalibacterium prausnitzii*, which is reduced in patients with IBD [31], also is reduced in patients with type 2 diabetes [32]. Taken together, these studies demonstrate that healthy individuals are associated with a diverse gut microbiota and elevated numbers of *F. prausnitzii*. However, the mechanisms whereby *F. prausnitzii* protects against IBD and diabetes remain to be clarified.

Several studies have reported reduced amounts of bacteria belonging to the phylum Bacteroidetes in obese individuals [2, 32, 33]. However, others did not observe these differences [34, 35]. Furet et al. [32] demonstrated that the levels of Bacteroidetes did not directly correlate with obesity, but rather with energy intake, which may provide some explanation for the discrepancy between the studies. However, most obese individuals have increased energy intake and their gut should thus contain reduced numbers of Bacteroidetes. The association between energy intake and Bacteroidetes was also demonstrated by Ley et al. [33]: the numbers of Bacteroidetes dramatically increased when obese patients were subjected to either a low-fat or low-carbohydrate diet.

Metagenome and Obesity

Whereas the gut microbiota is highly variable between individuals, which may also in part explain the above discrepancy between studies, the microbiome is relatively consistent between individuals [2]. Interestingly, in the obese microbiome, the numbers of genes favoring energy extraction and those involved in carbohydrate degradation are increased [2]. Animal studies with obese mice demonstrated a similar difference in the microbiome, which correlated with increased capacity to harvest nutrients from a carbohydrate-rich diet and the production of short-chain fatty acids (SCFAs) that can be used as substrates for gluconeogenesis and lipogenesis [36]. Murphy et al. [37] recently confirmed these findings, but also demonstrated that the results were dependent on animal age. Taken together, the gut microbiota is a highly dynamic 'organ' whose cellular composition is affected not only by diet, age and immune status, but also by host physiology such as obesity. Additional metagenomic studies are required to demonstrate how the microbiome is altered in obesity and type 2 diabetes.

Bariatric Surgery and Gut Microbiota

Several strategies have been introduced to decrease obesity including lifestyle changes, behavioral therapy and pharmacotherapy; however, these strategies have only been marginally beneficial [38]. In contrast, it was recently demonstrated that surgical approaches, gastric bypass, resulted in sustained weight loss, even for >15 years [39, 40]. In addition, these procedures rapidly improved glucose metabolism, and, in contrast to weight loss which can take between 3 months and 1 year to develop, the resolution of diabetes typically occurs within days to weeks after surgery [41]. The improvement in diabetes is caused by weight-independent and weight-dependent mechanisms, and although the exact mechanisms are unknown, the procedure is associated with several environmental changes, such as alterations in bile flow, reduction of gastric size, anatomic gut rearrangement and alterations in the flow of nutrients, vagal manipulation, and enteric gut hormone modulation [42], all of which may affect the gut microbial composition. Recent studies have demonstrated that gastric bypass surgery indeed dramatically alters the gut microbial composition

and that a subset, but not all, of these changes could be attributed to altered caloric intake [32, 43]. Accordingly, some of the beneficial effects of bariatric surgery may be attributed to the altered gut microbiota.

Direct Involvement of the Gut Microbiota in Causing Obesity

The above results suggest that the gut microbiota is altered in obesity but does not separate cause and consequence, i.e. does the gut microbiota contribute to the pathogenesis of obesity, or is the altered gut microbiota merely a consequence of obesity? As mentioned above, germ-free mice have reduced adiposity and do not develop diet-induced obesity. Direct evidence that the gut microbiota of obese individuals contributes to the pathogenesis of obesity was obtained by transplanting the gut microbiota from obese mice and lean controls into germ-free recipients. Mice receiving the gut microbiota from obese donors gained significantly more body fat compared with mice receiving the lean microbiota [11, 36, 44]. Accordingly, the obese phenotype can be transplanted between organisms through the microbiota.

Involvement of the Gut Microbiota as a Causative Agent of Other Metabolic Diseases

Obesity is associated with several comorbidities such as dyslipidemia, insulin resistance, type 2 diabetes, hypertension and cardiovascular disease. Significantly less is known about the role of the gut microbiota as a contributor to insulin resistance. However, we and others have demonstrated that germ-free mice have improved glucose tolerance and insulin sensitivity [22, 23, 29]. Furthermore, the increased insulin sensitivity of germ-free mice was associated with elevated levels of Akt phosphorylation in adipose tissue [29]. Only a few studies aimed to investigate if the gut microbiota is altered in patients with type 2 diabetes. Larsen et al. [45] used 454-pyrosequencing to determine the microbial composition in 18 Danes with type 2 diabetes over wide age and body mass index ranges. Diabetes was associated with increased levels of

Proteobacteria and reduced levels of Firmicutes, especially the class Clostridia. In agreement with this finding, Furet et al. [32] observed reduced levels of *F. prausnitzii* in obese patients with diabetes. Accordingly, an altered

Taken together, the gut microbiota is a highly dynamic 'organ' whose cellular composition is affected not only by diet, age and immune status, but also by host physiology such as obesity.

Fig. 2. The gut microbiota affects host metabolism and secretion of hormones from the gut, which can have profound effects on host metabolism in the periphery. Microbial suppression of Angptl4 expression in the gut increases lipoprotein lipase (LPL)-mediated triglyceride storage in adipose tissue as well as reduces fatty acid oxidation in skeletal muscle by a yet unidentified mechanism. Furthermore, lipopolysaccharide (LPS) derived from the gut may augment adipose inflammation and reduce insulin sensitivity.

microbiota in patients with type 2 diabetes may also contribute to disease progression.

Since obesity and insulin resistance may promote the development of atherosclerosis, we recently investigated whether the gut microbiota was altered in patients with atherosclerosis [46]. We did not observe any significant differences in the gut microbial composition, which may be explained by the relative shallow sequencing depth. Interestingly, we observed that the same bacterial species were identified both in atherosclerotic plaques, in the gut and especially in the oral cavity in the same patient. These findings suggest that microbiota formed in the oral cavity or gut may translocate to atherosclerotic plaques and thus promote inflammation, which may increase the risk for plaque rupture. Furthermore, specific bacteria correlated with serum cholesterol levels, suggesting that the gut microbiota may regulate cholesterol metabolism. By performing lipidomics on germ-free and conventionally raised mice, we demonstrated that the gut microbiota affects host lipid metabolism [47]. A putative mechanism for how the gut microbiota may contribute to host lipid and cholesterol metabolism could potentially be explained by microbial regulation of bile acid synthesis and metabolism. Conversion of cholesterol to bile acids occurs in the liver, but further microbial metabolism is required for fecal excretion. In addition, bile acids are im-

portant for emulsification of dietary lipids, and an altered gut microbiota could affect lipid metabolism, dyslipidemia and atherosclerosis in the host. However, this link remains to be demonstrated.

Mechanisms by Which the Gut Microbiota Contributes to Metabolic Disease

In contrast to other organs in the human body, the gut microbiota is a dynamic organ that rapidly alters its cellular composition as well as its gene transcriptional network in response to dietary shifts [3–5, 44, 48]. However, recent data suggest that it is not only the macronutrient composition that affects gut microbial ecology but also the caloric intake [32].

These findings suggest that microbiota formed in the oral cavity or gut may translocate to atherosclerotic plaques and thus promote inflammation, which may increase the risk for plaque rupture.

The effects between the gut microbiota and calorie density are reciprocal; caloric density does not only modulate the gut microbiota, the gut microbiota may also affect the amount of energy extracted by the host from the gut microbiota [36, 49]. Despite increased energy intake, germ-free mice are leaner compared with colonized counterparts [22]. This phenotype was rapidly ameliorated by colonizing the germ-free mice with an unfractionated cecal microbiota from a conventionally raised mouse, e.g. a microbiota transplant. Importantly, these effects on host adiposity appeared to be generalizable as germ-free mice of different strains and sex are leaner compared with colonized counterparts.

Evidence obtained from animal models over the past 5 years demonstrates that the gut microbiota promotes obesity and insulin signaling by several pathways (fig. 2). The gut microbiota can affect food intake that leads to obesity [11, 22]. In fact, orexogenic gene expression in the hypothalamus is regulated by the gut microbiota and furthermore demonstrates that the effect of the gut microbiota goes beyond the gut [Schéle et al., unpubl.]. The presence of the gut microbiota promotes increased glucose uptake from the small intestine by a yet unidentified mechanism [22]. However, colonization of germ-free mice with *Bacteroides thetaiotaomicron* increases Sglt1 expression in the small intestine, which provides a plausible mechanism. The increased levels of glucose, as well as SCFAs, can be used for de novo lipogenesis and the increased levels of glucose correlated with increased hepatic gene expression of lipogenic genes and elevated hepatic triglyceride levels [22, 47]. The increased lipogenesis was associated with increased very-low-density lipoprotein production, which transports triglycerides to the adipose tissue for storage. In addition to promoting lipogenesis, the gut microbiota suppresses intestinal expression of angiopoietin-like protein 4 (Angptl4; also known as fasting-induced adipose factor), a potent suppressor of LPL. LPL activity is required for hydrolysis of very-low-density lipoprotein and subsequent transport into adipose tissue. Accordingly, conventionally raised mice have increased LPL activity and increased adiposity [22]. Using mice deficient in Angptl4 we could directly demonstrate that the elevated levels of Angptl4 in germ-free mice, at least in part, contributed to the reduce adiposity.

Gut Microbiota and Inflammation

Metabolism and inflammation are tightly connected and recent findings have identified that particularly the innate immune system, which protects us from infections, may contribute to obesity and insulin resistance [50]. Apart from microbial effects on host metabolism, the gut microbiota also contributes to metabolic abnormalities by promoting low-grade metabolic inflammation [51]. Endotoxin is taken up from the gut together with chylomicrons or alternatively through increased gut permeability [reviewed in ref. 52]. Activation of Toll-like-receptor-4 in macrophages recruited to the adipose tissue promotes inflammation, which reduces insulin sensitivity [53]. As expected, germ-free mice have reduced

adipose inflammation (fig. 2) and improved insulin sensitivity compared with colonized counterparts [22, 23, 29, 54]. Interestingly, treatment of obese mice with antibiotics reduces plasma endotoxin levels, adipose inflammation, adiposity and liver triglycerides, and improves host glucose metabolism [55, 56]. Taken together, these findings suggest that the gut microbiota can directly contribute to host metabolism by affecting energy harvesting capacities from the diet and by modulating metabolic and/or inflammatory signaling pathways.

Regulation of Enteroendocrine Function

Fermentation of fibers in the distal gut produces SCFAs, which does not only salvage energy from the diet but also has important signaling functions in the gut through the G-coupled receptors GPR41 and GPR43. Enteroendocrine cells express the SCFA receptor GPR41 and their function may thus be regulated through the fermentation capacity of the gut microbiota. There are no apparent differences in the body composition of germ-free wild-type and *Gpr41*-deficient mice [57]. However, colonized *Gpr41*-deficient mice were leaner, which was associated with decreased expression of the hormone PYY, but the role of PYY in mediating these responses is has not been elucidated yet.

GPR43 was first identified as a modulator of inflammatory responses in the gut as a chemoattractant receptor on neutrophils [58, 59]. Bjursell et al. [60] recently found that *Gpr43*-deficient mice were resistant to diet-induced obesity. Protection against developing diet-induced obesity was, at least in part, explained by increased energy expenditure in *Gpr43*-deficient mice and the

Despite increased energy intake, germ-free mice are leaner compared with colonized counterparts.

reduced fat mass was accompanied by improved glucose tolerance. Despite no difference in adipocyte size, *Gpr43*^{-/-} mice contained fewer macrophages in the white adipose tissue, which may explain the improved glucose metabolism.

Metabolism of bile acids is another example of mammalian-microbial co-metabolism that may have physiological effects [61–63]. The gut microbiota is important for deconjugation, dehydrogenation, and dehydroxylation of bile acids, which results in secondary bile acids and increases the chemical diversity of these signaling molecules [64]. Accordingly, germ-free animals have very simplified bile acids characterized by conjugated cholic and muricholic acids, which are absorbed before they reach the distal gut [61, 63, 65; Islam et al., in preparation]. Lithocholic acid, which is produced from cholic acid by the microbiota in the colon, activates the G-coupled receptor TGR5 (also known as GPBAR1, M-BAR and BG37). Stimulation of TGR5 increases energy expenditure in brown adipose tissue by producing active tri-iodothyronine, which subsequently increases metabolic rate and energy expenditure [66]. Accordingly, stimulation of TGR5 prevents diet-induced obesity [67]. Recent data demonstrated that TGR5 is also expressed by L-cells in the colon, and activation of TGR5 in L-cells induces secretion of the incretin GLP-1, which promotes improved pancreas function and glucose metabolism in obese mice [67, 68]. Thus, it is plausible that differences in an individual's metagenome result in differences in the capacity to produce SCFAs and lithocholic acid and thereby have different levels of GLP-1 and glucose tolerance (fig. 2).

Does the Gut Microbiota Affect Metabolic Programming?

The incidence of type 1 diabetes among children and adolescents has, for unknown reasons, increased markedly in the Western countries during recent decades, suggesting significant environmental involvement [69]. In contrast to type 2 diabetes and insulin resistance, where the presence of a gut microbiota augments disease progression, the gut microbiota appears to be protective against type 1 diabetes [10]. Interestingly, metabolic abnormalities precede islet autoimmunity in children who later progress to type 1 diabetes independent of HLA-associated genetic risk [70], and these alterations resemble

those we have identified in germ-free mice [47]. Accordingly, rather than primarily affecting the immune system, the gut microbiota may alter the metabolic milieu, which may have profound effects on the immune system.

Since the altered metabolome was already observed in the cord blood, we hypothesize that the gut microbiota of the mother is transferred to the child who progresses to type 1 diabetes; thus, the child may be devoid of important constituents and/or has an impaired gut function that predisposes to type 1 diabetes. Taken together, these findings suggest that manipulation of the gut microbial composition during pregnancy or early childhood can provide a novel therapeutic approach to prevent or treat this disease.

Conclusions and Future Prospects

Development of next-generation sequencing techniques during the past decade has revolutionized the knowledge about the composition and function of the gut microbiota. However, to date, the metagenomes of relatively few individuals have been sequenced. Detailed studies on the metagenome early in life as well as regional metagenomes

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are required, e.g. mucus-associated versus luminal microbiota and microbiota in specific habitats of the small intestine, to determine whether or not it can be reprogrammed. Sequencing of the human host genome and the microbiome may reveal the differences in

the selection of the microbiota in individuals. Given that the cost for sequencing is rapidly declining, the major challenge will be data analysis rather than data generation. Transplantation of metagenomes from obese patients/patients with diabetes and healthy controls into genetically modified germ-free mice may provide tools to determine how the gut microbiota causes metabolic diseases.

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