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The role of gut microbiota in the development of type 1, obesity and type 2 diabetes mellitus

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Abstract

Diabetes is a group of metabolic disorders characterized by persistent hyperglycemia and has become a major public health concern. Autoimmune type 1 diabetes (T1D) and insulin resistant type 2 diabetes (T2D) are the two main types. A combination of genetic and environmental factors contributes to the development of these diseases. Gut microbiota have emerged recently as an essential player in the development of T1D, obesity and T2D. Altered gut microbiota have been strongly linked to disease in both rodent models and humans. Both classic 16S rRNA sequencing and shot-gun metagenomic pyrosequencing analysis have been successfully applied to explore the gut microbiota composition and functionality. This review focuses on the association between gut microbiota and diabetes and discusses the potential mechanisms by which gut microbiota regulate disease development in type 1 diabetes, obesity and type 2 diabetes.

Keywords

Gut microbiota; Type 1 diabetes; Type 2 diabetes; Obesity

Introduction

Diabetes mellitus (DM) is a heterogeneous group of metabolic diseases characterized by hyperglycemia, which may result in long-term complications leading to damage to many of the body's systems, especially kidneys, nerves, eyes, and blood vessels. The prevalence of diabetes has increased substantially in the past few decades. About 347 million people worldwide had diabetes in 2013 and it is estimated that death due to diabetes will double between 2005 and 2030. The morbidity and early mortality associated with diabetes have become a leading global economic and healthcare burden (http://www.who.int/diabetes/en/). There are two major types of diabetes. Type 1 diabetes (T1D) occurs when auto-reactive T cells attack islet β-cells resulting in insufficient insulin production. Type 2 diabetes (T2D) is a complex metabolic disorder in which islet beta cell failure occurs together with insulin

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resistance where the body becomes resistant to the insulin it produces. Insulin resistance is often associated with obesity. Beyond the widely-accepted concept that genetic factors play an important role in diabetes susceptibility, growing evidence has demonstrated that environmental factors (such as commensal bacteria, viruses, chemicals, diet) can also modify diabetes development. Of these factors, gut microbiota have been shown to play an important role in influencing development of both T1D and T2D. This has been supported by the results from both human studies and animal research, especially the evidence from discordant incidence of diabetes in monozygotic twins, who are genetically identical. The gut microbiota have co-evolved with humans in a symbiotic relationship and the total bacterial genome contains more than 3.3 million non-redundant genes (150 times more than human genes). With recent development of high-throughput sequencing and metagenomic analytical technologies, and the availability of germ-free facilities and gnotobiotic mice, the information from the gut microbiome has been widely explored and the alteration of gut microbiota composition has been linked to disease development in both patients and animal models. Understanding the interaction between diabetes development and gut microbiota will help us to dissect the underlying mechanisms. However, whether the gut microbiota initiate diabetes development or act as a disease enhancer remains controversial and this issue has not been completely elucidated.

1. Type 1 diabetes and gut microbiota

Type 1 diabetes is an autoimmune disease characterized by auto-reactive T cell-mediated destruction of insulin-producing pancreatic β-cells in the islets of Langerhans, accounting for about 10% of all cases with diabetes. The discordant diabetes incidence in monozygotic twins (less than 50% developed T1D) strongly suggests that non-genetically determined factors regulate the development of T1D. In this regard, gut microbiota have recently been studied and found to play a role in disease development. Growing evidence has shown that gut microbiota can shape mucosal immunity in many ways, such as contributing to development of gut associated lymphoid tissues, compartmentalizing pathogens, promoting and/or enhancing adaptive immune responses to pathogens [1]. However, inflammatory bowel disease (IBD) and autoimmune diseases may result from imbalanced or altered gut microbiota [2, 3]. Recent evidence from animal models of T1D – the Non-Obese Diabetic (NOD) mouse and the BioBreeding (BB) rat, as well as human studies indicate that gut microbiota are strongly associated with diabetes development [4, 5].

1.1 Altered gut microbiota affect type 1 diabetes development

In an effort to determine whether gut microbiota could affect T1D development, we compared the diabetes incidence of innate immune-deficient NOD mice (MyD88$^{-/-}$NOD, deficient in MyD88, an adaptor for multiple innate immune receptors that recognize microbial stimuli) either in specific-pathogen-free (SPF) or in germ-free (GF) conditions. It was very interesting that SPF-reared MyD88$^{-/-}$NOD mice were diabetes free, but GF-reared mice developed robust diabetes [6]. The composition of gut microbiota was different between MyD88-deficient and MyD88-sufficient NOD mice, indicating that the host MyD88-deficiency modified the gut microbiota [6]. This strongly suggests that commensal bacteria are an important factor in diabetes development. A recent study by Alkanani and

colleagues also showed that induction of diabetes in the RIP-B7.1 C57BL/6 mouse model was critically dependent on TLR3 and MyD88 pathways and an altered intestinal microbiome was responsible for the diabetes modulation [7]. Experimental data from BB rats performed by Roesch and colleagues also showed the altered gut microbiota were linked to the onset of diabetes in BB-DP (diabetes-prone) compared to BB-DR (diabetes-resistant) rats [8]. Moreover, Hara et al showed that antibiotic treatment could prevent Kilham rat virus-induced insulitis and T1D in the LEW1.WR1 rat model [9], suggesting that microbiota might also be involved in virus-induced diabetes. Transfer of gut microbiota from diabetes-protected MyD88-deficient NOD mice at an early age could stably alter the composition of the gut microbiota of recipient NOD mice, reduce insulitis and significantly delay the onset of diabetes [10]. Taken together, these data indicate altered gut microbiota are strongly associated with T1D and modulation of the gut microbiota by transfer of so-called "protective gut flora" could delay and/or prevent diabetes development. However, how gut microbiota are altered and what mechanisms are involved in the immune regulation by the commensal bacteria in diabetes development need to be further investigated.

1.2 Diet and gut microbiota

Both human and animal studies have indicated that diet influences development of T1D, which includes factors such as cow's milk, dietary wheat and gluten diet [11–13]. Gluten-free (GLF) diets have been shown to have an anti-diabetogenic effect in mouse models [14–16]. However, the mechanism by which these diets affect diabetes development is not fully understood. Marietta and colleagues compared the fecal microbiomes of NOD mice fed with either gluten-containing chows or GLF chows and found decreased *Bifidobacterium*, *Tannerella* and *Barnesiella* species but increased *Akkermansia* species in the gut microbiota of mice fed with GLF chows, compared with those fed with gluten-containing chows [17]. Adding back gluten in the diet reversed the anti-diabetogenic effect with decreasing *Akkermansia* species but increasing *Bifidobacterium*, *Tannerella* and *Barnesiella* species [17]. This indicated that the presence of gluten was directly responsible for the pro-diabetogenic effects of the diets, through changing the gut microbiota. A recent study showed that the anti-diabetogenic effect of GLF diet also affected diabetes development in the offspring. Hansen and colleagues fed pregnant NOD mice with GLF diets until the pups were weaned, when the diet was switched to standard chow. Insulitis and the incidence of diabetes in the offspring were dramatically decreased compared to the offspring of the NOD mice reared on standard diet [18]. This study suggested that the GLF environment in the early life of the NOD mice plays a critical role in diabetes development later in life. Gut microbiota analysis by 16S rRNA gene sequencing revealed a pronounced difference between both mothers and their offspring, characterized by increased *Akkermansia*, *Proteobacteria*, and *TM7* in the GLF diet group. Furthermore, GLF-fed offspring had increased i) pancreatic T regulatory cells ($Foxp3^+$Tregs), ii) M2 macrophage gene markers and tight-junction-related genes in the gut, iii) intestinal Tregs, while intestinal gene expression of pro-inflammatory cytokines was reduced [18, 19]. A recent study also found that a GLF diet could lower NKG2D and ligand (DX5) expression in the intestines of BALB/c and NOD mice [20]. Another recently-reported study by Larsen and colleagues also found that dietary gluten increased murine NK-cell activity against the pancreatic beta-cell line MIN6 cells *in vitro* and induced the expression of NKG2D and CD71 on NKp46+ cells

in all lymphoid organs in BALB/c and NOD mice *in vivo*, compared to a GLF diet [21]. It is known that NK cells are required for diabetes development [22, 23]. Taken together, a GLF diet protected mice from insulitis and T1D development through regulation of the immune response, including an increase in Tregs and reduced production of pro-inflammatory cytokines, mediated by alteration of intestinal microbiota.

Human studies have shown that T1D is associated with celiac disease (CD) - both diseases share similar genetic susceptibilities and the prevalence ranged from 0.49% to 16.4% in various reports [24]. A GLF diet is very important for CD patients. The effect of GLF diet on the development of human T1D has also been investigated and the results so far remain controversial. A recent report by Abid et al (2011), following the recommendation of The International Society for Pediatric and Adolescent Diabetes, showed that introduction of a GLF diet displayed short-term benefits by reducing gastrointestinal symptoms and hypoglycemia in British children aged from 6 to 11 year-old who had T1D, while the insulin requirement significantly increased [25]. Several other studies also found short-term benefits of GLF diet in T1D patients, including increased insulin sensitivity, reduction of HbA1c and prolonged remission, while other reports showed no effect [26–29]. However, the effect of GLF diet on the gut microbiota has not been explored in these studies. It would be interesting to investigate the influence of GLF diet on the gut microbiota and its relationship with T1D development in humans.

1.3 Effect of pH on gut microbiota

Early human studies showed that the pH of the gastric juice influenced growth of the bacteria in the stomach and intestine, and therefore, pH affected both the quality and quantity of the gastrointestinal microbiota [30]. A recent study demonstrated that female NOD mice maintained on acidic drinking water developed insulitis and hyperglycemia rapidly, compared to those on neutral drinking water [31]. The 16S rRNA-targeted pyrosequencing revealed a significant change in the composition and diversity of gut microbiota when the pH of drinking water was altered [31]. However, another report by Wolf et al (2014) showed opposite results, where NOD mice on neutral drinking water exhibited increased development of diabetes compared to those on acidic drinking water [32]. These mice had a decrease in *Firmicutes* and an increase in *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* in their intestinal microbiota, confirmed by 16S rRNA pyrosequencing [32]. The alteration of gut microbiota composition further shaped adaptive immunity, in which NOD mice drinking neutral water had a decrease in the level of Foxp3 expression in $CD4^+Foxp3^+$ cells, as well as decreased $CD4^+IL\text{-}17^+$ cells, and a lower ratio of IL-17/IFN-γ $CD4^+$ T cells [32]. The conflicting results from the two studies are likely to be related to different experimental settings, since other environmental factors including the origin of the NOD mice, diet and the cage bedding also affect disease development. Nevertheless, both studies were in agreement that the pH of drinking water strongly influenced the composition of gut microbiota and modulated diabetes development.

1.4 Gender bias on gut microbiota

Many autoimmune diseases demonstrate a gender bias, with women being more susceptible to autoimmunity than men [33, 34]. Sex hormones are considered to play a critical role in

gender-biased autoimmunity and androgens are widely accepted to be protective [34]. T1D has a strong gender bias in the NOD mouse model, with female to male ratio ranging from 1.3 to 4.4 depending on the colonies [35]. However, this gender bias is sensitive to environmental influences. Markle and colleagues investigated if gut microbiota played a role in the gender bias in NOD mice [36]. As expected, female NOD mice had a higher diabetes incidence, with earlier onset, in SPF conditions compared to male NOD mice. Surprisingly, these differences were abolished if the mice were housed in GF conditions, indicating that sex differences in T1D susceptibility are dependent on the presence of commensal bacteria. Transfer of gut microbiota from adult males to immature females altered the recipient's microbiota, attenuated the autoimmune phenotypes, elevated testosterone and led to T1D protection [36]. The authors showed that these changes were dependent on androgen receptor activity [36]. These data suggested that gut microbiota could affect sex hormone levels and regulate the fate of autoimmune disease in genetically susceptible individuals. Yurkovetskiy and colleagues further dissected the mechanisms involved in the gender bias in NOD mice in a recent study [35]. Colonization of GF NOD mice with defined microbiota revealed that some lineages were over-represented in males, which affected diabetes protection [35]. Their data supported the contention that the gender bias in T1D in NOD mice is directly associated with some defined gut bacteria. Although protection from T1D development in male NOD mice did not correlate with blood androgen concentration in their study, hormone-supported expansion of selected microbial lineages may work as a positive-feedback mechanism contributing to the sexual dimorphism of autoimmune diseases [35]. In addition to the gut microbiome, IFN-γ pathways have also been identified to be involved in protection of males from T1D by microbiota [35]. Taken together, the cross talk between hormones and the gut microbial community can promote immune-protective pathways in T1D development.

1.5 Altered gut microbiota in human T1D

The animal studies strongly suggest that gut microbiota play a pivotal role in T1D development. However, the gender bias in NOD mice does not translate to humans, as T1D is one of the few autoimmune diseases where the more males develop diabetes than females, after puberty [37]. Nevertheless, the gut microbiome also plays an important role in T1D development in humans and altered gut microbiota have been demonstrated to be associated with β cell autoimmunity in children who are at risk of developing T1D, similar to the results found in animal studies [38, 39]. Brown and colleagues compared the composition of gut microbiota between 4 young Finnish children with high genetic risk for T1D and 4 age-matched healthy controls, using high throughput 16S rRNA pyrosequencing. The gut microbial communities in the high-risk children was distinct from those of healthy controls, characterized as less diverse and less dynamic, i.e., stable. Using second generation metagenomic analysis, the Illumina shotgun approach, the Diabetes Prevention and Prediction (DIPP) study also found that the gut microbiome of four pairs of age and sex-matched children with new-onset T1D was very different from the healthy controls at both taxonomic and functional levels [40]. The results of the DIPP study suggested that a consortium of lactate- and butyrate-producing bacteria induced mucin synthesis to maintain gut integrity in healthy controls, while non-butyrate-producing lactate-utilizing bacteria prevented mucin synthesis, thus causing β cell autoimmunity and T1D [40]. De Goffau and

colleagues (2013) further expanded the subject group size and studied the composition of the gut microbiome in Finnish children who had at least two diabetes-associated autoantibodies (n=18, aged 4–14 years) compared with age- and sex-matched children who were autoantibody-negative (n=18). The study subjects also had similar early feeding history and HLA risk genotype [41]. The authors observed a low abundance of lactate- and butyrate-producing bacterial species, a dearth of the two most dominant *Bifidobacterium* species, *Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum*, and an increased abundance of the *Bacteroides* genus in children with islet autoantibodies, indicating that the altered gut microbiota were associated with β cell autoimmunity [41]. A similar alteration in gut microbiota was further confirmed in both young Spanish (7-year-old) and Mexican children (7–18 years old) with newly diagnosed T1D [39, 42]. These human studies confirmed that altered gut microbiota, in either composition or/and function, are strongly related to β cell autoimmunity and T1D, although the diversity and richness of the gut microbiota of the human subjects can be affected by geographical and ethnic diversities. Nevertheless, whether the alteration is involved in disease causation or is a consequence of selection by the host remains to be investigated.

The colonization by intestinal bacteria in both humans and mice begins immediately after birth, and soon the gut microbiome is established as a dynamic ecosystem, which is stabilized during the first 2–3 years in humans. However, the microbial composition increases with age, in both diversity and richness, and reaches the highest complexity in the adult human [43]. To determine the effect of microbiota on T1D development in early childhood, de Goffau and colleagues compared gut microbiota composition from children aged 1–5 years with new-onset type 1 diabetes (n=28) and age-matched healthy controls (n=27) from eight European countries using phylogenetic microarray analysis with a Human Intestinal Tract Chip (HITChip) [44]. The authors found that in pairs younger than 2.9 years of age, the combined abundance of the class *Bacilli* (notably *streptococci*) and the phylum *Bacteroidetes* was higher in diabetic children, whereas the combined abundance of members of *Clostridium* clusters IV and XIVa was higher in the healthy controls [44]. Control subjects older than 2.9 years of age were characterized by a higher fraction of butyrate-producing species within *Clostridium* clusters IV and XIVa than was seen in the corresponding diabetic children or in children from younger age groups. These butyrate-producing species of *Clostridium* clusters IV and XIVa could specifically colonize mucin in the gut and the bacteria have been shown to have beneficial effect against inflammation in inflammatory bowel diseases [45, 46]. These results confirmed the phylogenetic differences in gut microbiota composition between diabetic children and healthy controls and the importance of early development of healthy microbiota in the first few years of life. In a different study reported by Endesfelder et al (2014), the authors compared the microbiome of fecal samples, which were taken from the age of 6 months up to 3 years, between 22 children who developed anti-islet cell autoantibodies and 22 matched control children who remained islet antibody-negative in follow-up. There were no differences between these two groups in bacterial diversity, microbial composition, or single genus abundances, although the microbiome changed markedly during the first year of life, and was further affected by breast-feeding, food introduction, and birth delivery method [47]. Instead, substantial alterations in microbial interaction network were observed between the ages of 0.5 and 2

years in the children who developed anti-islet cell autoantibodies [47]. These findings strongly underscore a role for gut microbiota in the pathogenesis of anti-islet cell autoimmunity and T1D in early life. We can speculate that the altered microbial interaction in early life would result in aberrant microbial development in later life, affecting diversity and richness of specific bacterial species, which has been confirmed by the previous human studies.

1.6 Perspectives on mechanisms of gut microbiota regulating T1D

Both animal and human studies have demonstrated a link between gut microbiota and β cell autoimmunity. Maintaining gut integrity and a healthy gut microbiome from early ages will be critical to prevent diabetes development. However, how the gut microbiota interact with host immunity and change β cell autoimmunity is not clear. Gut microbiota are essential to the normal development of the immune system in the gut and the bacteria have the capacity to shape the adaptive immunity outside the gut. Although changes in gut microbiota have been linked with altered immune responses in some human and animal T1D studies, the underlying mechanisms by which the gut microbiota modulate immunity are not yet fully elucidated. It is known that segmented filamentous bacteria (SFB) are associated with the differentiation of Th17 cells and SFB can protect female NOD mice from diabetes development accompanied by a substantial population of Th17 cells in the small intestine lamina propria [48]. However, a recent study suggested that the effect of SFB on diabetes development in NOD mice was associated with other gut bacteria, as single colonization by SFB in GF NOD mice did not protect the mice from diabetes development [35]. However, when GF mice were first colonized with a group of commensals, SFB induced protection in these mice [35]. Our unpublished data suggest that IL-17 is, in fact, a diabetes-promoting cytokine, as IL-17 deficient NOD mice are protected from diabetes development, although the protection is not 100% (YY Li and L Wen, manuscript in preparation). Regardless, studies with SFB indicate that specific bacterial species can potentially regulate adaptive immunity and modify the health status of the hosts. A recent report by Yang and colleagues explored the mechanisms by which SFB induce Th17 cells differentiation in a mouse model. The investigators found that antigen-specific $CD4^+$ T cells in the gut differentiated into either Th1 or Th17 cells, depending on the antigens delivered from luminal bacteria [49]. SFB-derived antigens stimulated the intestinal antigen-specific Th17cells to differentiate and circulate beyond the small intestine [49]. This study provides direct evidence that cognate antigens derived from gut bacteria dictate the differentiation of antigen-specific T cells. These results also suggest that commensal bacteria-derived microbial peptides can activate antigen-specific T cells and thus contribute to inflammatory and autoimmune diseases.

Another example of a defined bacterial strain that can profoundly regulate the immune system is *Bacteroides fragilis* [50–52]. *Bacteroides fragilis* can induce IL-10 producing T regulatory cells (Tr1) and protect against experimental colitis, autoimmune encephalitis (EAE) and arthritis [51, 53, 54]. Here, polysaccharide A (PSA) in *Bacteroides fragilis* interacts with the host immune system and promotes IL-10 production by Tr1 cells [50, 53, 55]. The immune regulatory and disease-protective effects of *Bacteroides fragilis* are abolished if the PSA gene is deleted from *Bacteroides fragilis* [51, 53, 54]. We have tested the immunoregulatory role of *Bacteroides fragilis* in T1D development in NOD mice and

found, surprisingly, that *Bacteroides fragilis* has no effect on T1D development (G Efthimiou and L Wen, unpublished data). Similar results were also observed by other investigators (Chervonsky, personal communication). Thus, it is a fundamental requirement to determine whether commensal bacteria can induce antigen-specific pathogenic T cells involved in the initiation of T1D development. However, it should also be noted that, despite the important role that the gut microbiota play in health and disease, it would be unlikely that there will be a single "magic bullet" targeting all conditions. It is also unlikely that there is a single mechanism or one pathway behind the phenotypes and that the gut microbiome should be considered to be an important environmental modulator of the susceptibility to diabetes but not the only factor.

2. Obesity, type 2 diabetes and gut microbiota

Obesity is a major public health concern and has been rapidly spreading in both industrialized and the developing countries in the past few decades. Obesity increases the likelihood of chronic metabolic disorders, particularly insulin resistance, T2D, cardiovascular disease, fatty liver disease, hypercholesterolemia and a number of cancers [56, 57]. All of these have been linked to significantly reduced life expectancy and high mortality. Excessive food intake and lack of physical activity are major causative factors for obesity, although a few cases are primarily determined by genetic factors [58, 59]. Although not the only factor, obesity has been a major driving force in the rapidly expanding T2D epidemic worldwide in recent decades.

Excessive fat accumulation and low-grade systemic and chronic inflammation are the main characteristic phenotypes of obesity. There is growing evidence that gut microbiota also play an important role in the development of obesity. Musso and colleagues highlighted interactions between gut microbiota and host metabolism, predisposing to obesity and diabetes [60]. Gut microbiota can provide host energy by hydrolyzing the undigested plant polysaccharides (cellulose, xylan and pectin) and partially digested starch which the host consumes daily. Meanwhile, numerous plant- and host-derived glycoconjugates including cellulose, chondroitin sulfate, hyaluronic acid, mucin, and heparin will support intestinal bacterial ecosystems, which supply the host with pyruvate via glycolysis and short-chain fatty acids (SCFA, including acetate, propionate, and butyrate). These metabolites can modulate biologically active fatty acid composition, bile acid-activated metabolic pathways and dietary choline bioavailability. Fermentation end products, such as SFCAs, can engage the epigenetic regulation of inflammatory reactions via FFARs (free fatty acid receptors) and other SFCA receptors, while modulating the gut microbiota communities by selecting and promoting the "pathogenic" strain- *Faecalibacterium prausnitzii* and inhibiting beneficial strains- *Clostridium cluster IV* and *Clostridium cluster XIVa* in T2D patients versus healthy control subjects [61]. Furthermore, the gut microbiota and SFCA excretion can be influenced by the type and quantity of dietary fat and carbohydrate in the population at risk of developing metabolic syndrome [62]. "Balanced" gut microbiota are critical to maintain the energy equilibrium relationship between energy generation and expenditure, thus influencing host fat storage.

2.1 Different gut microbiota determined by diet and genetic factors in obesity

A wide array of studies using 16S rRNA pyrosequencing has demonstrated that the composition of gut microbiota of obese animals and human subjects differs from their healthy leaner counterparts, not only at phylum and class levels but also at species level [63–66]. The difference in the abundance lies in two major bacteria phyla, *Bacteroidetes* and *Firmicutes*, where higher *Firmicutes* and lower *Bacteroidetes* were found in obese subjects, while the opposite was found in lean and healthy controls [67, 68]. Studies performed by Armougom and colleagues (2009) confirmed that a reduction of *Bacteroidetes* was accompanied with an increase in *Lactobacillus* species that belong to the *Firmicutes* phylum in obese patients compared to normal-weight healthy control subjects [69]. The ratio of *Firmicutes* to *Bacteroidetes* (*F/B*) was also positively related to obesity, observed in a human weight-loss study [70]. However, there are also studies that do not support the association of an altered ratio of *F/B* in obesity with no difference found in *F/B* in obese and lean subjects in both humans and mice [68, 71–73]. It is not clear what factors contribute to the conflicting results; however, it is clear that this is a complex disease and much more research in large cohorts is needed.

Both genetic variations and diets can affect gut microbiota of mice and humans as discussed earlier for studies in T1D. Obesity is a highly heritable disease driven by complex interactions between genetic and environmental factors. To define the factors determining the obesity-related gut microbiota, Zhang et al (2010) used Apoa-1 knockout mice (impaired glucose tolerance and increased body fat) compared with wild-type counterparts to assess the effect of host genetics and diet on shaping the gut microbiota. The authors found that dietary change was associated with 57% of the total structural variation in gut microbiota, whereas genetic mutation accounted for no more than 12%, indicating that diet has a dominant role in shaping gut microbiota [73]. However, in a genome-wide association study, with more than 100 inbred mice, in response to a high-fat/high-sucrose diet, Parks et al (2013) found that genetic factors controlled obesity and gut microbiota composition. The authors identified 11 significant loci associated with obesity traits, several of which overlap with the loci identified in human studies [74]. There were significant relationships between gut microbiota and metabolic traits, and host genetic factors influenced the plasticity of gut microbiota in response to diet [74]. However, the investigators did not exclude the possibility that other factors could also partly contribute to the observed differences, since the gut microbiota varied significantly even in well-controlled cohorts [74]. Clearly, further comprehensive studies are needed to systematically examine the relationship between genetic background, diet, and gut microbiota composition. Defining an obesity-related microbiome profile or revealing the specific regulatory bacterial species would be more relevant than a general comparison of the gut microbiota composition.

2.2 Toll-like receptors in obesity

Innate immunity is the first line of defense of the body in response to exogenous insults such as bacterial, viral and fungal infections and innate immunity acts through highly conserved pattern-recognition receptors, such as Toll-like receptors (TLR), to coordinate the innate inflammatory response to both endogenous and exogenous stimuli. After engaging with their ligands, the downstream inflammatory responses of most TLRs are mediated through the

MyD88-dependent pathway, except TLR3, which is TRIF dependent. A low level of chronic inflammation is one of the major characteristics of obesity [75]. However; it remains controversial whether inflammation contributes to obesity-related insulin resistance. For the past two decades, inflammation in obesity has been considered to trigger insulin resistance. Nerverthless, recent studies by Tang el al (2010) using transgenic or knockout strategies to induce NF-kB transcriptional activities in mouse models demonstrated that high levels of inflammatory cytokines (TNF-α and IL-6) in the serum and adipose tissue were associated with increased energy expenditure and improved insulin sensitivity [76]. Lee et al (2011) also demonstrated that inflammation was not required for initiating HFD-induced insulin resistance but long term insulin resistance was dependent on macrophage-induced inflammation [77]. This suggests other factors including energy surplus, dietary fatty acids and environmental factors may also play a critical role in regulating obesity and insulin resistance, in addition to an imbalance between pro- and anti-inflammatory influences [78, 79]. Studies using TLR knockout mice have revealed that TLRs can regulate the gut microbiota and affect the metabolic syndrome. TLR5 is highly expressed in the intestine and can specifically recognize bacterial flagellin. Vijay-Kumar et al (2010) showed that TLR5-deficient C57BL/6 mice developed a metabolic syndrome phenotype, when reared in SPF conditions, including hyperlipidemia, hypertension, insulin resistance, and increased adiposity. To test the role of gut microbiota in this phenotype, the authors transferred gut bacteria from SPF-reared TLR5-deficient mice to GF wild-type (WT) mice. Impressively, following the transfer of gut bacteria, many features of the metabolic syndrome were seen in the GF WT recipients [80]. TLR2 is another TLR family member that recognizes cell-wall components such as peptidoglycan, lipoteichoic acid and lipoprotein from gram-positive bacteria, and lipoarabinomannan from mycobacteria. Previous studies showed that TLR2-deficient mice were resistant to HFD-induced obesity possibly due to decreased lipid uptake or oxidation in different tissues [81, 82]. However, Caricilli et al (2011) found that TLR2-deficient mice, housed in conventional pathogen-free conditions, exhibited a phenotype reminiscent of the metabolic syndrome, including increase in LPS absorption, subclinical inflammation, insulin resistance, glucose intolerance, and finally obesity. 16S rRNA sequencing analysis revealed different gut microbiota, with a 3-fold increase of *Firmicutes* but modest decrease of *Bacteroidetes* in TLR2-deficient mice, compared to wild-type controls. A reduction of regulatory T cells in visceral fat was also observed in TLR2-deficient mice [83]. These results demonstrated that TLRs play a critical role in governing the composition of gut microbiota, which may be linked with obesity, although contradictory findings were observed in different research groups. Interestingly, Ubeda et al (2012) argued that the impact of TLR deficiency on the composition of gut microbiota is minimal under homeostatic conditions and after recovery from antibiotic treatment, although the microbiota of MyD88- and TLR-deficient C57BL/6 mouse colonies differed significantly, with each mouse strain exhibiting a distinct and distinguishable bacterial profile [84]. However, this claim may not exclude the effect of TLRs on gut microbiota in mice of different genetic backgrounds or following specific treatment, such as HFD. Further studies will be needed to elucidate the effect of the TLR-MyD88 signaling pathway on the gut microbiota.

2.3 Antibiotics and obesity

Antibiotics have been used for 60 years to treat various infections. Recent studies have shown that antibiotics can promote weight gain in agricultural animals and have also been linked to obesity in humans who had been given antibiotics during early infancy [85–87]. However, the observation that GF mice were resistant to HFD-induced obesity argues for the pathogenic role of gut microbiota in the development of obesity. Vancomycin is an antibiotic used for the treatment of a number of bacterial infections, mostly against gram-positive bacteria. Million et al (2013) investigated the body weight and gut microbiota changes in patients with bacterial endocarditis who were treated with either vancomycin or amoxicillin. Vancomycin was associated with a 10% BMI increase and acquired obesity, accompanied by increased proportions of *Firmicutes*, *Bacteroidetes* and *Lactobacillus* and decreased *M. smithii* [88]. Our mouse study supported the observation that early life exposure to vancomycin significantly promoted body weight gain in C57BL/J6 mice on a normal diet (Yuksel, Guo and Wen, unpublished data). However, Murphy et al (2013) found that C57BL/J6 mice on a HFD, treated with vancomycin, gained less weight, had lower fasting blood glucose, plasma TNFα and triglyceride levels compared with controls without vancomycin treatment but on the same HFD [89]. 16S rRNA sequencing showed that vancomycin treatment led to significant reductions in the proportions of *Firmicutes* and *Bacteroidetes* and a dramatic increase in *Proteobacteria*, with no change in *Actinobacteria* [89]. Interestingly, the authors found that administration of a bacteriocin-producing probiotic strain, *L. salivarius* UVV 118 Bac^{+},could not improve metabolic profiles in HFD-induced obese mice [89]. The bacteriocin-producing probiotic strain had no significant impact on the proportions of *Firmicutes* but resulted in a relative increase in *Bacteroidetes* and *Proteobacteria* and a decrease in *Actinobacteria* compared with the mice treated with a non-bacteriocin-producing probiotic control strain *L. salivarius* UVV 118 Bac^{-} [89]. These findings demonstrated that antibiotic treatment could alter the gut microbiota composition and thus modulate the progression of obesity.

2.4 Gut microbiota causing obesity

The gut microbiota coevolve with the host and altered gut microbiota have been strongly associated with host obesity. Modification of gut microbiota may be a potential therapeutic strategy to prevent or reverse obesity, as studies have shown that transferring healthy microbiota to a second host can reverse and prevent metabolic syndrome [90]. Aberrations in gut microbiota are strongly associated with obesity. However, whether the alteration of the gut microbiota profile during obesity is a characteristic of the phenotype or a causative contributor remains unclear. Discordance for obesity in monozygotic or dizygotic twins provides a good opportunity to examine interrelations between obesity and associated gut microbiota without genetic interference. Ridaura and colleagues (2013) selected twin pairs discordant for obesity and transferred their uncultured fecal microbiota from four pairs or cultured collections from one obese (Ob) and one lean (Ln) co-twin into GF mice that were fed with a mouse chow with defined fat content. The uncultured and culturable bacterial component of Ob fecal samples induced significantly greater increases in body mass and adiposity than those of Ln fecal samples. The metabolic analysis found that the increased body mass in Ob-transferred mice was correlated with i) decreased production of SCFAs, ii)

increased metabolism of branched-chain amino acids, iii) reduced microbial transformation of bile acid species and iv) up-regulation of host farnesoid X receptor signaling compared to Ln-transferred mice [91]. Cohousing Ln and Ob transferred mice altered the metabolic profile of the microbiota from Ob-transferred mice to that of the Ln-transferred cage-mates and thus protected them from increased adiposity and body mass [91]. Transformation correlated with appearance of members of *Bacteroidales* from Ln into Ob microbiota. The authors also showed that the change of gut microbiota and phenotypic rescue were diet-dependent [91]. These results revealed that transmissible and modifiable interactions between diet and microbiota can influence host biology and metabolism. A study in rats also demonstrated a difference in the gut microbial composition between obese-prone (OP) and obese-resistant (OR) rats fed with the same HFD; this showed that OP rats possess a higher *Firmicutes* to *Bacteroidetes* ratio and there were significant genera differences [92]. GF mice that received gut microbiota from OP but not OR rats reproduced the characteristics of the OP phenotype, which included reduced intestinal and hypothalamic satiation signaling, hyperphagia, increased weight gain and adiposity, and enhanced lipogenesis and adipogenesis [92]. Specific species from *Oscillibacter* and *Clostridium* clusters XIVa and IV were detected in OP donors and GF recipient animals but were completely absent from OR animals [92]. These data suggest that susceptibility to obesity is characterized by an unfavorable microbiome predisposing the host to peripheral and central inflammation, and promoting weight gain and adipogenesis during obesogenic feeding.

The most effective medical intervention for weight loss, thus far, has been found to be Roux-en-Y gastric bypass (RYGB) surgery, which also reduces the risk of T2D and cardiovascular disease for obese patients. This surgery leads to changes in acid exposure in the gastric remnant and proximal small bowel, and restricts the ingested amount and types of food, limiting nutrient absorption. Furthermore, it may result in intestinal dysmotility, all of which would contribute to altering the gut microbiota and result in rapid weight loss, reduced adipogenesis, and improved glucose metabolism. In a human study, Zhang et al (2009) found that the gut microbiota communities in the individuals after RYGB surgery were different from either normal-weight or obese individuals [93]. Specifically, *Firmicutes* dominated in normal-weight and obese individuals but were significantly decreased in individuals who were post-gastric-bypass, who had a proportional increase of *Gammaproteobacteria*. Liou et al (2013) confirmed the alteration of gut microbiota post-RYGB surgery in mouse models, which showed a rapid and sustained increase in the relative abundance of *Gammaproteobacteria* (*Escherichia*) and *Verrucomicrobia* (*Akkermansia*) compared to those after sham surgery or sham surgery coupled to caloric restriction. Transfer of the gut microbiota from RYGB-treated mice to GF mice resulted in weight loss and decreased fat mass in the recipient mice, compared to the recipients given microbiota from sham surgery donors, potentially due to the altered microbial production of SCFA [90]. These findings suggest that changes in the gut microbiota contribute to the reduced weight gain and adiposity of the host after RYGB surgery. In conclusion, these transfer experiments strongly support the suggestion that the gut microbiota can act as a causative determinant in the induction and regulation of obesity.

2.5 Gut microbiota and T2D

T2D is a metabolic disorder characterized by hyperglycemia in the context of insulin-resistance, accounting for about 90% of all the patients worldwide with diabetes. T2D is strongly linked to genetic predisposition but also closely associated with obesity and insufficient physical activity. Nevertheless, the degree of obesity varies greatly in people with T2D, from almost 90% in North America to less than 40% in South East Asia [94]. More interestingly, a subset of obese people called metabolically healthy obese persons is insulin sensitive and euglycemic [95, 96]. These individuals have challenged traditional concepts to define obesity and leanness related to T2D. Several factors need to be considered, including body mass index, waist circumference, ethnicity and age, to provide the optimal therapeutic approach to treat T2D in clinical practice [94]. However, this not only supports the role of genetic factors but also suggests that there are other mechanisms involved in the regulation of T2D, including environmental factors. Recently, growing evidence has shown that gut microbiota play a critical role in the regulation of development of T2D. Characterization of the gut microbiota between patients who have T2D and healthy control subjects would help understanding of the pathogenic mechanisms and add insights into modulation of the microbial community for preventative purposes.

Larsen et al (2010) compared the gut microbiota from 18 male adults with T2D, who had a broad range of age and body-mass indices (BMI), to 18 healthy controls with similar range of age and BMI. The authors found the compositional changes in the gut microbiota were associated with T2D at both phylum and class levels [97]. A recent study investigated three groups of human subjects – those with newly- diagnosed T2D, prediabetes (Pre-DM) and normal glucose tolerance (NGT), showed that the gut microbiota were different in the three groups [98]. Using 16S rRNA based high-throughput sequencing, the authors found that there was a T2D-related dysbiosis, where there were a total of 28 taxonomic units associated with T2D, whereas the NGT group expressed a higher abundance of butyrate-producing *A. Muciniphila* ATCCBAA-835 and *Faecalibacterium prausnitzii* L2–6 than the pre-DM group. Both the pre-DM and T2D groups had a lower abundance of *Verrucomicrobiae* compared to the NGT group [98]. These results clearly indicate that not only do the gut microbiota of patients with T2D differ from healthy control subjects but the changes in gut microbiota are also associated with the progression of glucose intolerance.

To further explore the taxonomic and functional changes of gut microbiota related to T2D, a metagenome-wide association study (MGWAS) was carried out in a large cohort of 345 Chinese patients with T2D and healthy control subjects. The authors found that patients with T2D had a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens, while other microbial functions conferring sulphate reduction and oxidative stress resistance were also increased [99]. Using a similar strategy to study the gut microbiota of 145 70-year-old European women with normal, impaired or diabetic glucose tolerance profiles, Karlsson et al (2013) developed a mathematical model based on metagenomic profiles that identified T2D with high accuracy, which could be applied to identify women with metabolic abnormalities similar to those found in patients with T2D [100]. The metagenomic markers identified using these mathematical models are different between

Chinese and European women; however, the subjects in the study by Qin and colleagues were from different age groups and included both genders[99]. These results indicate that the predictive tools for metagenomic identification may need to consider both age and geographic location of the subjects to be studied.

In summary, the gut microbiota have been considered a super-organ, which has coevolved with the host. Gut microbiota not only provide energy for the host, but also can shape mucosal and systemic immunity through their metabolites or bacterial peptides. Both animal models and human studies have demonstrated a strong association between gut microbiota and host in health and disease. Growing evidence suggests that altered gut microbiota composition could play a causative role in the development of T1D, obesity and T2D; however, more controlled studies including those investigating geographic variations, age, environmental and genetic factors, and the studies of cellular and molecular mechanisms are much needed for verification. Dissecting the gut microbiota through a more global approach rather than using classical methods will help us to understand the underlying pathogenic mechanisms, especially focusing on identification of specific regulatory species and functional analysis. These will provide essential information to build tools for disease prediction and design specific strategies to modulate the gut microbiota for therapeutic purposes.

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References

1. Kamada N, et al. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol. 2013; 13(5):321–335. [PubMed: 23618829]
2. Geuking MB, et al. The interplay between the gut microbiota and the immune system. Gut Microbes. 2014; 5(3)
3. Vieira SM, Pagovich OE, Kriegel MA. Diet, microbiota and autoimmune diseases. Lupus. 2014; 23(6):518–526. [PubMed: 24763536]
4. Nielsen DS, et al. Beyond genetics. Influence of dietary factors and gut microbiota on type 1 diabetes. FEBS Lett. 2014
5. Vaarala O. Human intestinal microbiota and type 1 diabetes. Curr Diab Rep. 2013; 13(5):601–607. [PubMed: 23934614]
6. Wen L, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008; 455(7216):1109–1113. [PubMed: 18806780]
7. Alkanani AK, et al. Induction of diabetes in the RIP-B7.1 mouse model is critically dependent on TLR3 and MyD88 pathways and is associated with alterations in the intestinal microbiome. Diabetes. 2014; 63(2):619–631. [PubMed: 24353176]
8. Roesch LF, et al. Culture-independent identification of gut bacteria correlated with the onset of diabetes in a rat model. ISME J. 2009; 3(5):536–548. [PubMed: 19225551]
9. Hara N, et al. Prevention of virus-induced type 1 diabetes with antibiotic therapy. J Immunol. 2012; 189(8):3805–3814. [PubMed: 22988033]
10. Peng J, et al. Long term effect of gut microbiota transfer on diabetes development. J Autoimmun. 2014

11. Dahlquist G, Kallen B. Maternal-child blood group incompatibility and other perinatal events increase the risk for early-onset type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1992; 35(7):671–675. [PubMed: 1644246]
12. Knip M, Simell O. Environmental triggers of type 1 diabetes. Cold Spring Harb Perspect Med. 2012; 2(7):a007690. [PubMed: 22762021]
13. Rosenbauer J, Herzig P, Giani G. Early infant feeding and risk of type 1 diabetes mellitus-a nationwide population-based case-control study in pre-school children. Diabetes Metab Res Rev. 2008; 24(3):211–222. [PubMed: 17968982]
14. Funda DP, et al. Gluten-free diet prevents diabetes in NOD mice. Diabetes Metab Res Rev. 1999; 15(5):323–327. [PubMed: 10585617]
15. Lefebvre DE, et al. Dietary proteins as environmental modifiers of type 1 diabetes mellitus. Annu Rev Nutr. 2006; 26:175–202. [PubMed: 16848704]
16. Schmid S, et al. Delayed exposure to wheat and barley proteins reduces diabetes incidence in non-obese diabetic mice. Clin Immunol. 2004; 111(1):108–118. [PubMed: 15093559]
17. Marietta EV, et al. Low incidence of spontaneous type 1 diabetes in non-obese diabetic mice raised on gluten-free diets is associated with changes in the intestinal microbiome. PLoS One. 2013; 8(11):e78687. [PubMed: 24236037]
18. Hansen CH, et al. A Maternal Gluten-Free Diet Reduces Inflammation and Diabetes Incidence in the Offspring of NOD Mice. Diabetes. 2014; 63(8):2821–2832. [PubMed: 24696449]
19. Ejsing-Duun M, et al. Dietary gluten reduces the number of intestinal regulatory T cells in mice. Scand J Immunol. 2008; 67(6):553–559. [PubMed: 18476878]
20. Adlercreutz EH, et al. A gluten-free diet lowers NKG2D and ligand expression in BALB/c and non-obese diabetic (NOD) mice. Clin Exp Immunol. 2014; 177(2):391–403. [PubMed: 24673402]
21. Larsen J, et al. Dietary gluten increases natural killer cell cytotoxicity and cytokine secretion. Eur J Immunol. 2014
22. Gur C, et al. The activating receptor NKp46 is essential for the development of type 1 diabetes. Nat Immunol. 2010; 11(2):121–128. [PubMed: 20023661]
23. Poirot L, Benoist C, Mathis D. Natural killer cells distinguish innocuous and destructive forms of pancreatic islet autoimmunity. Proc Natl Acad Sci U S A. 2004; 101(21):8102–8107. [PubMed: 15141080]
24. Scaramuzza AE, et al. Type 1 diabetes and celiac disease: The effects of gluten free diet on metabolic control. World J Diabetes. 2013; 4(4):130–134. [PubMed: 23961323]
25. Abid N, et al. Clinical and metabolic effects of gluten free diet in children with type 1 diabetes and coeliac disease. Pediatr Diabetes. 2011; 12(4 Pt 1):322–325. [PubMed: 21615651]
26. Antvorskov JC, et al. Dietary gluten and the development of type 1 diabetes. Diabetologia. 2014; 57(9):1770–1780. [PubMed: 24871322]
27. Hummel S, et al. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care. 2011; 34(6): 1301–1305. [PubMed: 21515839]
28. Kaukinen K, et al. No effect of gluten-free diet on the metabolic control of type 1 diabetes in patients with diabetes and celiac disease. Retrospective and controlled prospective survey. Diabetes Care. 1999; 22(10):1747–1748. [PubMed: 10526749]
29. Leeds JS, et al. High prevalence of microvascular complications in adults with type 1 diabetes and newly diagnosed celiac disease. Diabetes Care. 2011; 34(10):2158–2163. [PubMed: 21911773]
30. Gray JD, Shiner M. Influence of gastric pH on gastric and jejunal flora. Gut. 1967; 8(6):574–581. [PubMed: 4865576]
31. Sofi MH, et al. pH of drinking water influences the composition of gut microbiome and type 1 diabetes incidence. Diabetes. 2014; 63(2):632–644. [PubMed: 24194504]
32. Wolf KJ, et al. Consumption of acidic water alters the gut microbiome and decreases the risk of diabetes in NOD mice. J Histochem Cytochem. 2014; 62(4):237–250. [PubMed: 24453191]
33. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014; 35(3):347–369. [PubMed: 24793874]

34. Zandman-Goddard G, Peeva E, Shoenfeld Y. Gender and autoimmunity. Autoimmun Rev. 2007; 6(6):366–372. [PubMed: 17537382]
35. Yurkovetskiy L, et al. Gender bias in autoimmunity is influenced by microbiota. Immunity. 2013; 39(2):400–412. [PubMed: 23973225]
36. Markle JG, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013; 339(6123):1084–1088. [PubMed: 23328391]
37. Gale EA, Gillespie KM. Diabetes and gender. Diabetologia. 2001; 44(1):3–15. [PubMed: 11206408]
38. Giongo A, et al. Toward defining the autoimmune microbiome for type 1 diabetes. ISME J. 2011; 5(1):82–91. [PubMed: 20613793]
39. Murri M, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med. 2013; 11:46. [PubMed: 23433344]
40. Brown CT, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One. 2011; 6(10):e25792. [PubMed: 22043294]
41. de Goffau MC, et al. Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. Diabetes. 2013; 62(4):1238–1244. [PubMed: 23274889]
42. Mejia-Leon ME, et al. Fecal microbiota imbalance in Mexican children with type 1 diabetes. Sci Rep. 2014; 4:3814. [PubMed: 24448554]
43. Ringel-Kulka T, et al. Intestinal microbiota in healthy U.S. young children and adults--a high throughput microarray analysis. PLoS One. 2013; 8(5):e64315. [PubMed: 23717595]
44. de Goffau MC, et al. Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. Diabetologia. 2014; 57(8):1569–1577. [PubMed: 24930037]
45. Van den Abbeele P, et al. Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J. 2013; 7(5):949–961. [PubMed: 23235287]
46. Van Immerseel F, et al. Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. J Med Microbiol. 2010; 59(Pt 2):141–143. [PubMed: 19942690]
47. Endesfelder D, et al. Compromised gut microbiota networks in children with anti-islet cell autoimmunity. Diabetes. 2014; 63(6):2006–2014. [PubMed: 24608442]
48. Kriegel MA, et al. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci U S A. 2011; 108(28):11548–11553. [PubMed: 21709219]
49. Yang Y, et al. Focused specificity of intestinal TH17 cells towards commensal bacterial antigens. Nature. 2014; 510(7503):152–156. [PubMed: 24739972]
50. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453(7195):620–625. [PubMed: 18509436]
51. Ochoa-Reparaz J, et al. A polysaccharide from the human commensal Bacteroides fragilis protects against CNS demyelinating disease. Mucosal Immunol. 2010; 3(5):487–495. [PubMed: 20531465]
52. Surana NK, Kasper DL. The yin yang of bacterial polysaccharides: lessons learned from B. fragilis PSA. Immunol Rev. 2012; 245(1):13–26. [PubMed: 22168411]
53. Ochoa-Reparaz J, et al. Central nervous system demyelinating disease protection by the human commensal Bacteroides fragilis depends on polysaccharide A expression. J Immunol. 2010; 185(7):4101–4108. [PubMed: 20817872]
54. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010; 107(27):12204–12209. [PubMed: 20566854]
55. Mazmanian SK, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005; 122(1):107–118. [PubMed: 16009137]
56. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. J Clin Endocrinol Metab. 2004; 89(6):2595–2600. [PubMed: 15181029]
57. Haslam DW, James WP. Obesity. Lancet. 2005; 366(9492):1197–1209. [PubMed: 16198769]

58. Bleich S, et al. Why is the developed world obese? Annu Rev Public Health. 2008; 29:273–295. [PubMed: 18173389]
59. Lau DC, et al. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. CMAJ. 2007; 176(8):S1–S13. [PubMed: 17420481]
60. Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med. 2011; 62:361–380. [PubMed: 21226616]
61. Remely M, et al. Effects of short chain fatty acid producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity. Gene. 2014; 537(1):85–92. [PubMed: 24325907]
62. Fava F, et al. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. Int J Obes (Lond). 2013; 37(2):216–223. [PubMed: 22410962]
63. Karlsson F, et al. Assessing the human gut microbiota in metabolic diseases. Diabetes. 2013; 62(10):3341–3349. [PubMed: 24065795]
64. Kotzampassi K, Giamarellos-Bourboulis EJ, Stavrou G. Obesity as a consequence of gut bacteria and diet interactions. ISRN Obes. 2014; 2014:651895. [PubMed: 24977101]
65. Moreno-Indias I, et al. Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. Front Microbiol. 2014; 5:190. [PubMed: 24808896]
66. Turnbaugh PJ, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228): 480–484. [PubMed: 19043404]
67. Ley RE. Obesity and the human microbiome. Curr Opin Gastroenterol. 2010; 26(1):5–11. [PubMed: 19901833]
68. Zhao L. The gut microbiota and obesity: from correlation to causality. Nat Rev Microbiol. 2013; 11(9):639–647. [PubMed: 23912213]
69. Armougom F, et al. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. PLoS One. 2009; 4(9):e7125. [PubMed: 19774074]
70. Ley RE, et al. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444(7122):1022–1023. [PubMed: 17183309]
71. Duncan SH, et al. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond). 2008; 32(11):1720–1724. [PubMed: 18779823]
72. Schwiertz A, et al. Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring). 2010; 18(1):190–195. [PubMed: 19498350]
73. Zhang C, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J. 2010; 4(2):232–241. [PubMed: 19865183]
74. Parks BW, et al. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. Cell Metab. 2013; 17(1):141–152. [PubMed: 23312289]
75. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006; 116(7): 1793–1801. [PubMed: 16823477]
76. Tang T, et al. Uncoupling of Inflammation and Insulin Resistance by NF-kappa B in Transgenic Mice through Elevated Energy Expenditure. Journal of Biological Chemistry. 2010; 285(7):4637–4644. [PubMed: 20018865]
77. Lee YS, et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. Diabetes. 2011; 60(10):2474–2483. [PubMed: 21911747]
78. Lee JY, Zhao L, Hwang DH. Modulation of pattern recognition receptor-mediated inflammation and risk of chronic diseases by dietary fatty acids. Nutr Rev. 2010; 68(1):38–61. [PubMed: 20041999]
79. Ye J. Mechanisms of insulin resistance in obesity. Front Med. 2013; 7(1):14–24. [PubMed: 23471659]
80. Vijay-Kumar M, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010; 328(5975):228–231. [PubMed: 20203013]
81. Davis JE, et al. Absence of Tlr2 protects against high-fat diet-induced inflammation and results in greater insulin-stimulated glucose transport in cultured adipocytes. J Nutr Biochem. 2011; 22(2): 136–141. [PubMed: 20434320]

82. Ehses JA, et al. Toll-like receptor 2-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet. Diabetologia. 2010; 53(8):1795–1806. [PubMed: 20407745]

83. Caricilli AM, et al. Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. PLoS Biol. 2011; 9(12):e1001212. [PubMed: 22162948]

84. Ubeda C, et al. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. J Exp Med. 2012; 209(8):1445–1456. [PubMed: 22826298]

85. Azad MB, et al. Infant antibiotic exposure and the development of childhood overweight and central adiposity. Int J Obes (Lond). 2014

86. Million M, Raoult D. The role of the manipulation of the gut microbiota in obesity. Curr Infect Dis Rep. 2013; 15(1):25–30. [PubMed: 23129415]

87. Murphy R, et al. Antibiotic treatment during infancy and increased body mass index in boys: an international cross-sectional study. Int J Obes (Lond). 2013

88. Million M, et al. Lactobacillus reuteri and Escherichia coli in the human gut microbiota may predict weight gain associated with vancomycin treatment. Nutr Diabetes. 2013; 3:e87. [PubMed: 24018615]

89. Murphy EF, et al. Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. Gut. 2013; 62(2):220–226. [PubMed: 22345653]

90. Liou AP, et al. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med. 2013; 5(178):178ra41.

91. Ridaura VK, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013; 341(6150):1241214. [PubMed: 24009397]

92. Duca FA, et al. Replication of obesity and associated signaling pathways through transfer of microbiota from obese-prone rats. Diabetes. 2014; 63(5):1624–1636. [PubMed: 24430437]

93. Zhang H, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci U S A. 2009; 106(7):2365–2370. [PubMed: 19164560]

94. P B. The lean patient with type 2 diabetes: characteristics and therapy challenge. Int J Clin Pract Suppl. 2007; (153):3–9. [PubMed: 17594388]

95. Camhi SM, Katzmarzyk PT. Differences in body composition between metabolically healthy obese and metabolically abnormal obese adults. Int J Obes (Lond). 2014; 38(8):1142–1145. [PubMed: 24216712]

96. Karelis AD. Metabolically healthy but obese individuals. Lancet. 2008; 372(9646):1281–1283. [PubMed: 18929889]

97. Larsen N, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One. 2010; 5(2):e9085. [PubMed: 20140211]

98. Zhang X, et al. Human gut microbiota changes reveal the progression of glucose intolerance. PLoS One. 2013; 8(8):e71108. [PubMed: 24013136]

99. Qin J, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012; 490(7418):55–60. [PubMed: 23023125]

100. Karlsson FH, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013; 498(7452):99–103. [PubMed: 23719380]