Review

Impact of gut microbiota on diabetes mellitus

G. Blandino, R. Inturri, F. Lazzara, M. Di Rosa, L. Malaguarnera*

Department of Biomedical and Biotechnological Sciences, School of Medicine, University of Catania, Catania, Italy

Received 7 January 2016; received in revised form 4 April 2016; accepted 7 April 2016

Abstract

Various functions of the gut are regulated by sophisticated interactions among its functional elements, including the gut microbiota. These microorganisms play a crucial role in gastrointestinal mucosa permeability. They control the fermentation and absorption of dietary polysaccharides to produce short-chain fatty acids, which may explain their importance in the regulation of fat accumulation and the subsequent development of obesity-related diseases, suggesting that they are a crucial mediator of obesity and its consequences. In addition, gut bacteria play a crucial role in the host immune system, modulation of inflammatory processes, extraction of energy from the host diet and alterations of human gene expression. Dietary modulation of the human colonic microbiota has been shown to confer a number of health benefits to the host. Simple therapeutic strategies targeted at attenuating the progression of chronic low-grade inflammation and insulin resistance are urgently required to prevent or slow the development of diabetes in susceptible individuals. The main objective of this review is to address the pathogenic association between gut microbiota and diabetes, and to explore any novel related therapeutic targets. New insights into the role of the gut microbiota in diabetes could lead to the development of integrated strategies using probiotics to prevent and treat these metabolic disorders.

Keywords: Diabetes mellitus; Diabetic complications; Gut microbiota; Inflammation

1. Introduction

“Father of Medicine” Hippocrates’ famous statement that “all disease begins in the gut” recognized the essential role played by the gut and diet in many of the vital homeostatic functions of the human body. Indeed, the role of the human gut microbiota is crucial, as it is populated by a number of different microbial groups [1]. Every one of us has our own exclusive microbiota and gut microbiome (the microorganisms’ genes). The number of genes of the microbiota outnumbers human genes by a hundredfold [2]. Studies examining the influence of nutrients (such as dietary fibres and fats) and dietary habits (whether omnivores, vegetarians or vegans) in different populations have allowed stratification of the human population based on three principal bacteria and their microbiome’s genetic abundance [3]. Gut microbiota from different individuals have been classified into enterotypes, depending on their function, metabolism of dietary components, and ability to tolerate and metabolise drugs [4]. A healthy gut microbiome is characterized by the presence of microbes that enhance metabolism, and are resilient to infection and inflammation and resistant to autoimmune and cancer [5].

Increasing evidence indicates that gut microbiota are strongly associated with diabetes development [6,7]. In addition, the autoimmune mechanisms involved in the pathogenesis of type 1 diabetes (T1D) might also implicate peptidergic enteric neurons, which regulate immune-cell function and influence pro- and anti-inflammatory cytokine production, resulting in neurodegeneration [8]. Lymphokines produced in the pathogenic cascade involved in the development of autoimmune islet-cell damage could also lead to myenteric neuropathy [9]. Notably, the gut microbiota affect the intestinal mucosa via interactions with epithelial cells and the enteric nervous system, leading to changes in gut motility, sensory functions and pain perception (microbiota–brain–gut axis) [10]. The present review aims to provide some mechanistic insights by highlighting the role of the gut microbiota in diabetes to prompt the development of innovative therapeutic targets for the prevention, treatment and slowing of diabetes and other metabolic-associated disorders.

http://dx.doi.org/10.1016/j.diabet.2016.04.004
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2. Gut microbiota

Several studies from the Human Microbiome Project and the European Commission’s Metagenomics of the Human Intestinal Tract (MetaHIT) consortium have contributed to our better knowledge and understanding of the healthy composition and functional properties of the gut microbiota [1]. These studies indicate that host health is associated with the diversity and stability of the gut microbiome [11]. Gut microbiota constitute a dynamic entity that is modified by diet, lifestyle, antibiotics and genetic background [12]. Microbiota gut colonization probably starts at birth, as no large variations appear during healthy life. Interestingly, the type of microorganisms that colonize the gut of newborns depends on the delivery procedure. Infants born vaginally display a microbiota composed of Lactobacillus, Prevotella and Streptococcus spp coming from the maternal vaginal tract. In contrast, newborns delivered by caesarean section display predominantly Staphylococcus, Corynebacterium and Propionibacterium spp [13]. During early childhood, Actinobacteria, and particularly of the genus Bifidobacterium, dominate the gut microbiota of breastfed infants. Also at this time, the microbiota acquires a variety of new strains influenced by changes in diet, such as the introduction of solid foods, and by disease, such that gradually over time, it begins to resemble the adult composition [14]. Moreover, physical exercise is able to modulate gut microbiota, and increasing physical activity can increase the abundance of beneficial microbial species [15].

To date, around 100 large groups of bacteria, known as “phyla”, have been identified, each with a different repertoire of biochemical capabilities. In the adult gut microbiota, the majority of the microbial populations belong to the phyla Actinobacteria and Proteobacteria, and approximately 90% to the Bacteroidetes and Firmicutes phyla [16]. These phyla are differentially distributed throughout the gut and determine different microbial ecosystems [17]. In the Firmicutes phylum, the Clostridium cocoides group is the dominant population in the gut microbiota, with a large number of cultured and uncultured spp. A recent study showed that changes in the diversity of the C. cocoides group population in the gut microbiota correlates with age, and it was hypothesized that these changes can affect the health of the host [18]. Gut microbiota fulfill structural and histological functions, and play important metabolic roles for health maintenance, including amino-acid synthesis and the absorption of dietary fats and fat-soluble vitamins, and affect the protective actions that prevent pathogenic colonization and the composition of converted bile acids (Fig. 1) [19]. Bile acids bind to cellular receptors, which are internalised, and which activate distinct pathways involved in glucose homeostasis and lipid energy metabolism [20–22]. Furthermore, the gut microbiota help the host to eliminate calories from indigestible complex carbohydrates and plant polysaccharides via enzymes that are not encoded within the human genome [23]. Non-digestible carbohydrates are fermented by colonic microbes, leading to the production of short-chain fatty acids (SCFAs) such as butyrate, which has trophic effects on intestinal epithelium [24].

On the basis of the clustering patterns seen in the world’s population with variations in the levels of dominant microbiota genera, three enterotypes have been proposed: Bacteroides; Prevotella; and Ruminococcus [25]. Differences among these enterotypes are dependent on different combinations of microbial trophic chains. Bacteroides (enterotype 1) develops energy principally from fermentation of carbohydrates, as this genus has a very broad saccharolytic potential. Prevotella (enterotype 2) degrades mucin glycoproteins of the gut mucosal layer, while Ruminococcus (enterotype 3) binds mucins, and transports and degrades the constituent sugars. In addition, Bacteroides and Prevotella are enriched by the biosynthesis of different vitamins [25]. These enterotypes have been associated with long-term dietary patterns. Bacteroides spp have been correlated with diets dominated by high levels of animal protein and saturated fats, as found in the typical Western diet, whereas Prevotella is predominant in people with higher consumption of carbohydrates and simple sugars, as observed in agrarian and vegetarian societies [4].

In addition, microorganisms in the microbiota can regulate intestinal architecture by altering gut permeability. Intestinal epithelium is not only responsible for the assimilation of ingested food and nutrients, but also for the prevalence of crosstalk with the external surface of the body as well as between gut microbes. Epithelial cells constitute a physical barrier, impeding the translocation of the luminal contents of the inner tissues. The two main types of interconnecting junctions are the adherens junctions (AJs) and tight junctions (TJs). AJs are predominantly formed by cadherins linked to the actin cytoskeleton through a family of catenins, while TJs are the result of interactions of occludin, claudins and junctional adhesion molecule (JAM)-A, linked to the actin cytoskeleton via zonula occludens proteins (ZO-1, ZO-2) and α-catenin [26]. Myosin phosphorylation and contraction of the actin–myosin complex regulate the permeability of the epithelial barrier [27]. Damage to intestinal permeability allows the passage of endoluminal molecules into deeper layers which, in turn, weakens the intercellular connections and triggers activation of the inflammatory response [28]. Thus, enterohaemorrhagic Escherichia coli (EHEC) and enteropathogenic E. coli (EPEC) have the ability to adhere to intestinal epithelial cells (IECs) and disrupt the integrity of the barrier through TJ alterations [29]. Subsequent activation of the inflammatory response leads to increased concentrations of proinflammatory mediators, such as interferon (IFN)-γ and tumour necrosis factor (TNF)-α, which can both modulate the expression of several TJ proteins, such as ZO-1, JAM-A, occludin, claudin-1 and claudin-4 [30].

Thus, the intestinal epithelium and intestinal innate immune system are symbiotic, and cooperate in interactions between gut microbiota and the host. This synergy arises through a mechanism that can destroy pathogens while equally tolerating the presence of commensals, using strategies that generate ecological niches for beneficial gut microbiota [31]. Recognition of pathogen-associated molecular patterns (PAMPs) by epithelial cells via pathogen recognition receptors (PRRs) is important for this equilibrium. To date, the system linking gut microbial and host signals, and the onset or progression of metabolic alterations...
associated with high-fat feeding, have not been fully elucidated. Recently, however, a study in a murine model demonstrated that inducible IEC-specific deletion of the myeloid differentiation primary response gene 88 (MyD88) can partially protect against diet-induced fat storage, inflammation and diabetes via mechanisms directly involving the gut microbiota [32]. MyD88 is a PRR at the interface of the interaction between microorganisms and host, and one of the principal adaptor molecules for the majority of toll-like receptors (TLRs) [33,34]. As suggested by Everard et al. [32], MyD88 in IECs serves as a sensor, changing host metabolism according to diet, and influencing the composition of gut microbiota, energy metabolism, and the development of obesity and associated disorders. Nevertheless, there are no human studies showing that the host IEC MyD88 controls gut microbiota composition and that this is associated with metabolic disorders.

Thus, further investigations of human IECs need to be performed to confirm that host intestinal epithelial MyD88 controls gut microbiota composition and is associated with metabolic disorders. Moreover, it has been proposed that IECs produce interleukin (IL)-18, which contributes to the preservation of the intestinal barrier mainly by inducing epithelial cell proliferation, thereby enhancing the regeneration of damaged epithelium [35,36]. Interestingly, it was also found that high-fat feeding decreases IL-18 expression in the intestine, whereas IEC MyD88 deletion normalizes this parameter. This result indicates that intestinal MyD88 contributes to the regulation of IL-18 expression during high-fat feeding and thus helps to improve gut barrier function [32].

3. Obesity and gut microbiota

Overweight and obesity are metabolic disorders that have spread all over the world. The occurrence of type 2 diabetes (T2D) is mainly attributable to overweight and obesity. The interplay between behaviour and genetic and environmental factors are the principal contributors to obesity and T2D incidence. Among ambient determinants, human overall gut bacteria appear to be one of the crucial mediators of obesity and diabetes pathogenesis [37,38]. The microbiota possess protective functions in metabolic regulation, and play an active part in glucose and lipid metabolism [15]. A number of extrinsic and intrinsic factors can initiate perturbations of the gut microbiota. The consequence of these perturbations is a shift from normobiosis to dysbiosis, as documented by a deficiency of microbiota compositional and functional diversity (Fig. 3).

Obesity is linked to dysbiosis, a state characterized by alterations in microbiota composition, changes in bacterial metabolic activity and/or shifts in the local distribution of bacterial communities. Changes in gut microbiota composition could promote intestinal monosaccharide absorption and energy withdrawal from indigestible food components (principally carbohydrates) via SCFA production and de novo hepatic lipogenesis (Fig. 3) [39]. Furthermore, this dysbiosis could intensify fatty acid
loading in adipocytes, limiting the fasting-induced adipose factor in the gut which, in turn, increases lipoprotein lipase enzyme activity [40]. The main supposed mechanisms connecting a balanced gut microbiota composition to protection against diet-induced obesity in germ-free mice could be the inhibition of cellular energy-dependent protein kinase activation [41], followed by an association with SCFA signalling molecules, G-protein-coupled receptor activation and energy storage (Fig. 3) [42].

At present, intestinal dysbiosis is crucial for understanding the pathophysiology of obesity and diabetes. One of the mechanisms proposed to explain the crosstalk between the gut microbiota and regulation of fat storage and development of obesity-related diseases is metabolic endotoxaemia [43]. In particular, abnormal gut microbiota composition may trigger a state of chronic low-grade inflammation, rendering the host susceptible to systemic exposure to lipopolysaccharide (LPS) [44]. This large glycolypid molecule, derived from the outer membrane of Gram-negative bacteria, is a potent inducer of the innate immune-system response linked to adiposity, insulin resistance (IR) and de novo synthesis of triglycerides. By binding to TLR4 and its co-receptors, LPS triggers a cascade of responses, ultimately resulting in the release of proinflammatory molecules that interfere with modulation of glucose and insulin metabolism (Fig. 2). Studies of gut microbiota in both humans and animal models have helped to clarify its involvement in the pathogenesis of obesity. In obese humans, it was found that the relative proportion of Bacteroidetes was decreased in comparison to lean people [45]. Moreover, a shift was demonstrated towards a higher relative abundance of Bacteroidetes and a decreased number of Firmicutes in mice fed high-fat diets (HFDs), but which lost weight on low-calorie diets [43].

Other findings have demonstrated that treatment with Bifidobacterium strains restored alterations in the microbiota, reducing the excess numbers of Firmicutes and LPS-producing Proteobacteria, as well as the production of B cells, macrophages and cytokines (IL-6, MCP-1, TNF-α, IL-17), thereby improving systemic inflammation [44]. These effects were accompanied by improvements in metabolic dysfunction, including lowered levels of cholesterol, triglycerides, glucose and insulin, reduced body weight gain, and re-establishment of oral glucose tolerance and insulin sensitivity [32]. Moreover, human oligofructose supplementation, which increases bifidobacteria content, also reduced inflammatory status, and plasma and adipose tissue proinflammatory cytokines [46]. Obese adults with less bacterial diversity gained more weight over a 9-year follow-up period [47]. Therefore, low bacterial richness appears to be associated with more marked overall adiposity.

In obese/diabetic individuals, weight loss, rapid diabetic remission and metabolic improvement can be accomplished by a type of bariatric surgery – namely, Roux-en-Y gastric bypass (RYGB). In the gut microbiota of faecal samples from 30 obese individuals before and after RYGB, it was found that the Bacteroides/Prevotella groups were reduced in these subjects before the surgery, but increased 3 months after it. In addition, after 3 months, E. coli spp were increased, and were inversely correlated with fat mass and leptin levels, independently of changes in food intake. In contrast, the decrease after 3 months in lactic
acid bacteria (LAB), including the Lactobacillus, Leuconostoc and Pediococcus groups and Bifidobacterium, correlated with improvements in oral glucose tolerance and insulin sensitivity. This finding indicates that weight loss and changes in overall inflammatory status can modify gut microbiota ecology [48].

The gut microbiota profile may, in the near future, allow the prediction of which obese individuals are most likely to lose weight in response to energy-restricted diets, which would be a helpful strategy for obesity treatment [49]. In a group of obese or overweight subjects who underwent a 6-week programme of energy restriction, followed by another 6-week period of weight stabilization, the subjects who lost the least amount of weight – and, thus, more rapidly regained it – were those who had higher Lactobacillus, Leuconostoc and Pediococcus numbers in their faecal samples at baseline [49]. Given the cost of microbiota profiling (data sequencing and analysis), diet restriction seems a realistic first option. Colonization of germ-free mice with “obese microbiota” resulted in a significant increase in total body fat and IR compared with colonization with “lean microbiota” [8].

These results confirm that gut microbiota composition is an additional contributory factor to the pathophysiology of obesity and IR [50]. Remarkably, it has been shown that germ-free mice transplanted with the caecal microbiome of obese mice developed the phenotype of the donor [51]. Similarly, after colonizing germ-free mice with caecum-derived microbiota from conventionalised mice, the total amount of body fat increased and insulin sensitivity decreased, even with no changes in their diet [40]. Taken together, these findings suggest that the gut microbiota are active players in the development of obesity [43,52].

4. Gut microbiota in diabetes

In diabetic humans, there is a lack of uniformity in gut microbiota profiles. A human metagenome-wide association study showed significant correlations with specific gut microbes, bacterial genes and metabolic pathways in T2D patients [38]. These patients displayed higher levels of Lactobacillus spp compared with non-diabetics [53]. Lactobacillus spp correlated positively with fasting glucose and glycated haemoglobin (HbA1c) levels, while Clostridium spp correlated negatively with fasting glucose, HbA1c, insulin, C-peptide and plasma triglycerides, and positively with adiponectin and high-density lipoprotein (HDL) cholesterol [54]. In addition, based on the onset of metagenomic clusters (MGCs) in genetically obese and diet-induced leptin-resistant mice, 26 differentially abundant clusters were found when comparing diabetic mice with those with normal glucose tolerance [55].

Studies in different population have also shown that diabetic gut microbiota have lower concentrations of Roseburia intestinalis and Faecalibacterium prausnitzii (both butyrate-producing bacteria), and higher levels of Lactobacillus gasseri, Streptococcus mutans and Clostridiales members. Also, metformin administration caused an increase of Akkermansia muciniphila, a mucin-degrading Gram-negative bacterium, in the mucous layer [56]. Several experimental studies have shown that A. muciniphila concentrations correlate inversely with the presence of obesity and diabetes [57,58], and induce improvements in metabolic function (weight loss), glucose tolerance and systemic inflammation [38–59]. Furthermore, administration of a prebiotic such as oligofructose dramatically increased A. muciniphila levels, with beneficial effects on metabolic control. A recent study demonstrated that giving vancomycin to non-obese diabetic (NOD) mice increased their concentrations of A. muciniphila and enhanced glucose homeostasis [60]. Other studies reported that the proportions of Firmicutes and Clostridium spp were significantly reduced in diabetics compared with controls [60]. Likewise, the ratios of Bacteroidetes to Firmicutes and Bacteroides/Prevotella groups to C. cocoides/Eubacterium rectale groups correlated positively with plasma glucose levels. Similarly, the Betaproteobacteria class was highly enriched in diabetics vs non-diabetics and was positively correlated with plasma glucose (Fig. 3) [61]. It has also been suggested that there might be an inflammation-triggering effect of the intestinal microbiota in the development of autoimmune diabetes [62]. A pathological cascade that perturbs the intestinal immune system is a critical element in the development of autoimmune T1D. The link between the gut microbiota and the development of autoimmune diabetes can be explained by the shared lymphocyte-homing receptors in the gut and inflamed pancreas [63].

5. Gut microbiota, immunity and diabetes

The interaction between intestinal microbiota and the innate intestinal immune system has been recognized as an epigenetic factor that can modify the predisposition to T1D. It is possible that pancreatic damage originates from an initial cross-reaction of the immune system directed against a dietary antigen [64]. The association with microbiota can arise because gut epithelial cells express microbe-associated molecular pattern (MAMP) receptors, principally TLRs, which lead to a proinflammatory response that activates the nuclear factor (NF)-κB pathway (Fig. 4B). The activated TLRs and microbiota patterns result in the production of cytokines, chemokines and antibacterial products. It is known that high-fat feeding augments plasma LPS-containing microbiota at a concentration sufficient to increase body weight, fasting glycaemia and inflammation (Fig. 4B) [65]. Bacterial LPS inhibits IL-1 receptor-associated kinase (IRAK) M, a modulator of IRAK1 necessary for NF-κB activation. Also, the ubiquitination and degradation of IκB is inhibited by reactive oxygen species (ROS) induced by the microbiota, and peroxisome proliferator-activated receptor (PPAR)-γ, a product of TLR4 activation by LPS, shunts NF-κB from the cell core (Fig. 4B) [66]. Subsequently and initiated by bacterial recognition, the differentiation of effector T-helper (Th) 1, Th2 and Th17 cells, the development of regulatory T (Treg) cells and the production of secretory immunoglobulin A (sIgA) occurs.

Many bacterial communities can induce the production of inflammatory T cells. In such cases, segmented filamentous bacteria colonize the gut by coming into direct contact with the epithelium, facilitated by dendritic cells (DCs; Fig. 4B). This elicits a specific effector host response, characterized by
Fig. 3. Gut microbiota and how they differ in intestinal homoeostasis vs dysbiosis. SCFA: short-chain fatty acid.

a cascade of proinflammatory signals that culminates in the production of Th17 and Th1 cells, mediated by IL-1, IL-6 and IL-12, which can lead to autoimmunity [66]. Commensal microbiota in the colon, such as Clostridium clusters IV and XIVa, through their SCFA production stimulate the expression of FOXP3 in CD4+ T cells and induce the differentiation of Treg cells (http://www.mdpi.com/2072-6643/7/11/5461/htm–B27-nutrients-07-05461) [67]. Alterations in the gut microbiota profile, such as dysbiosis or inadequate introduction of foods during the first months of life, can increase susceptibility to, and generate the development of, autoimmune diseases locally in the gut or at a systemic level. In American and Finnish children, the fat intake from bovine milk products as well as proteins from fresh milk led to an increase in the risk of advanced β-cell autoimmunity and subsequent progression to T1D [68,69]. In fact, the presence of high titres of anti-β-casein at the time of diagnosis of T1D and latent autoimmune diabetes of adults (LADA) suggests that the antibody response to this protein may be relevant to autoimmune diabetes (Fig. 4A) [70].

Moreover, it has been proposed that high-gluten diets could be among the primary drivers of gut dysbiosis associated with T1D development (Fig. 4A) [71]. Interestingly, coeliac disease (CD) is more common in patients with T1D, and is associated with poorer glycaemic control and lipid profiles [72]. This is related to the timing and amounts of dietary gluten fed to infants. The progressive introduction of gluten-containing foods (in terms of quantity) to the diet at between 3 and 7 months after birth can lower the risk of T1D-associated autoimmunity [73]. Gluten has numerous effects on intestinal homoeostasis. Several CD studies have reported that gluten increases gut permeability by affecting TJs [74,75]. As a result, long gliadin peptides can enter in between epithelial cells into the lamina propria. From there, DCs can then migrate to other sites, such as the pancreatic lymph nodes, to activate autoreactive T cells (Fig. 4A) [73]. Pre-T1D children with multiple autoantibodies and those newly diagnosed with T1D present with a T-cell response against gliadin at a lower frequency and intensity than do healthy controls and patients with longer T1D durations [73]. This result supports the idea of an aberrant immune response related to the development of T1D.

As already mentioned, the intestinal microbiota of obese patients have been associated with higher levels of proinflammatory cytokines (Fig. 4B) [76]. Obese patients usually develop chronic adipose tissue inflammation because the consumption of an HFD in conjunction with the obese phenotype is associated with changes in gut microbiota, a decrease in IFA, and an increase in LPS and ileal inflammation. Enterocytes internalise LPS from the apical surface and convey it to the Golgi complex [77], which also contains chylomicrons, lipoproteins responsible for the transport of dietary long-chain fat through the blood and mesenteric lymph. It has been observed that chylomicrons promote intestinal LPS absorption. Thus, an excess of chylomicron formation during high-fat feeding facilitates endotoxin translocation via a reduction in intestinal alkaline phosphatase (IAP) activity, inducing the intestinal inflammation found in obesity and insulin-resistant states.

Obesity and its metabolic complications are associated with macrophage infiltration, responsible for almost all the adipose tissue TNF-α and IL-6 expression involved in inflammatory pathways. Moreover, it has been demonstrated that an HFD increases plasma levels of LPS, leading to low-grade endotoxaemia, and that IR is induced by LPS in differentiated adipocytes.
Fig. 4. Differences in the immunopathogenesis of type 1 (A) and type 2 (B) diabetes. LADA: latent autoimmune diabetes of adults; MCP1: monocyte chemoattractant protein-1.
The activation of macrophages is dependent on LPS/CD14 [78], a combination that serves as a ligand for TLR4 (Fig. 4B) [79]. TLR4 inactivation reduces food intake and inflammatory responses, but with no significant modification of body weight [79]. Thus, the LPS/CD14/TLR4 system appears to set the threshold at which an HFD can induce IR and the onset of diabetes and obesity. CD14 knockout mice lacking functional LPS receptors are hypersensitive to insulin, while increased endotoxaemia is associated with increased CD14 expression and increased IL-6 levels after a mixed meal containing lipids in healthy humans [80]. An HFD can affect IEC integrity, leading to impaired barrier function and increased intestinal permeability to bacterial fragments. The endotoxins can then trigger the proinflammatory cascade and activate TLR4 signalling [81]. In mice, chronic exposure to low-dose LPS resulted in liver steatosis, increased IR, dyslipidaemia, adipose tissue macrophage infiltration and obesity similar to what is observed with an HFD [31]. Interestingly, these effects were absent when TLR4-deficient mice were fed an HFD or when ob/ob mice fed an HFD were treated with antibiotics [33].

Besides LPS, other bacterial components can interact with inflammatory pathways [82]. Activation of innate immune responses by gut microbiota-derived molecular patterns is mostly due to bacterial cell-wall components such as flagellin [83] and polysaccharide A (PSA) [84], as well as commensal genomic DNA [85]. It has been reported that Bacteroides fragilis-derived PSA, a TLR2 ligand, can induce IL-10-producing CD4T cells and reciprocally suppress Th17 responses [86]. TLR5 is a flagellin-specific pattern-recognition receptor expressed in the gut mucosa that contributes to the defence against infection. Interestingly, TLR5+/− mice develop a metabolic phenotype comprising modifications of gut microbiota composition, hyperlipidaemia, hypertension, IR and obesity. As with other studies, colonizing wild-type germ-free mice with TLR5+/− mouse caecum microbiota transfers its metabolic phenotype to the recipient [80]. Further mechanistic studies indicate that DNA from gut flora plays a major role in intestinal homeostasis through TLR9 engagement [87]. Therefore, malfunction of the innate intestinal immune system may play an important role in the development of the metabolic syndrome through modification of the gut bacterial profile. Similarly, TLR9-deficient mice display an elevated frequency of FOXP3+ Treg cells at intestinal effector sites, with suppressed constitutive IL-17− and IFN-γ-producing effector T cells [88]. A NOD mouse model of T1D also revealed that a deficiency of the master adaptor protein MyD88 led to resistance to the development of T1D through alteration of gut bacteria [89].

All these observations suggest that different innate immune-activating components of gut bacterial origin have a different role in the regulation of gut immune homeostasis, and provide the first evidence of a ‘missing link’ between gut microbiota and innate immunity in T1D development. Many findings demonstrate that the presence/absence of specific microbes can modulate and programme life-long changes in immunity [90]. Nevertheless, as T1D is an autoimmune disease, it would be of interest to determine whether dysbiosis precedes or follows the development of diabetes. Also, future studies need to evaluate more deeply how dysbiosis influences metabolic disease progression.

6. Microbiota and mechanisms of gut motility in diabetes

The evidence indicates that gastrointestinal (GI) symptoms, especially those related to motility disorders of the upper GI tract, are more frequently seen in diabetic patients [33]. Diabetes is frequently associated with a variety of GI motility abnormalities in which nitricergic enteric neuropathy may be the primary dysfunction, independently of vagal dysfunction [91]. A multitude of different, uncommon symptoms seen in diabetic patients could be associated with the complex functions of the lower GI tract. In general, diabetics with advanced disease [3] suffer from watery and painless nocturnal diarrhoea alternating with periods of constipation and intermittent normal bowel function. Changes in the brain–gut system and different parts of the cortical network in diabetic patients could further modulate and contribute to the development, maintenance and subjective characteristics of GI symptoms in patients with diabetes [92–97]. In addition, peptidergic enteric neurons, as targets of inflammation, can modulate immune-cell function and therefore stimulate proinflammatory cytokine production, resulting in neurodegeneration [94]. Thus, the pathogenic cascade, which triggers the development of autoimmune diabetes through secreted lymphokines, could also result in altered neuro-immune interactions and provoke myenteric neuropathy.

Of the potential environmental triggers implicated in the development of diabetes-related myenteric neuropathy, the intestinal microbiome is considered a primary candidate. Accordingly, diabetes affects gut motility, thus influencing the microbiota in the intestine and colon. Evidence indicates that the gut microbiota communicate with the brain through the vagus nerve, transmitting information from the luminal environment to the central nervous system (CNS). Microbiota can interact via the gut–brain axis through modulation of afferent sensory nerves, enhancing their excitability by inhibiting the opening of calcium-dependent potassium channels, thereby modulating gut motility and pain perception, compromising the intestinal barrier and TJ, and so interfering with all gut functions [25]. Furthermore, microbiota can influence enteric nervous system (ENS) activity by producing molecules such as GABA, serotonin, melatonin, histamine and acetylcholine that act as local neurotransmitters [97], and by generating biologically active forms of catecholamines in the gut lumen [98]. Also, lactobacilli use nitrate and nitrite to generate nitric oxide [99] and to produce hydrogen sulphide, which modulates gut motility by interacting with the vanilloid receptor on capsaicin-sensitive nerve fibres [100]. Vagal and non-vagal muscarinic pathways influence the intestinal phase of insulin secretion, but regulation of gastric inhibitory peptide (GIP) secretion appears to be independent of vagal and muscarinic neural control mechanisms [101].

Microbiota may interact with gut motility through several mechanisms. First, by compromising the intestinal barrier and TJ, it can interfere with all gut functions. Decreased motility of the GI tract alters the gut microbial flora, which changes...
neurotransmissions by gastric afferents through the brain–gut axis [102]. In addition, the intestine is a huge and important endocrine organ of the body. Enteroendocrine cells in the intestine are mostly limited to deeper portions of the mucosa. In diabetes, the most important enteroendocrine intestinal cells are the K and L cells that produce GIP and glucagon-like peptide-1 (GLP-1) incretin hormones, respectively. K cells are localized to the duodenum and jejunum, whereas L cells are located preferably in the ileum, but also throughout the entire intestinal tract. The effect of incretin is impaired in T2D patients with autonomic neuropathy, compared with those without such neuropathy [103]. The vagus nerve mediates the gastric inhibitory effects of GLP-1

Table 1
Clinical and experimental studies on diabetes mellitus performed with probiotic strains.

<table>
<thead>
<tr>
<th>Study design/subjects</th>
<th>Probiotic strains</th>
<th>Quantity</th>
<th>Principal results</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>Clinical studies</strong></td>
<td></td>
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<tr>
<td>Randomized study</td>
<td>L. rhamnosus GG</td>
<td>10^{10} CFU/day</td>
<td>↓ Insulin</td>
<td>Laitinen et al. [114]</td>
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<td>Pregnant women</td>
<td>B. lactis Bb-12</td>
<td>10^{10} CFU/day</td>
<td>↓ Insulin sensitivity</td>
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<td></td>
<td></td>
<td>× 18 months</td>
<td>↓ Blood glucose</td>
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<td>Prospective</td>
<td>L. rhamnosus GG</td>
<td>10^{10} CFU/day</td>
<td>↓ Risk of gestational diabetes</td>
<td>Luoto et al. [115]</td>
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<tr>
<td>randomized study,</td>
<td>B. lactis Bb-12</td>
<td>10^{9} CFU/day</td>
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<tr>
<td>Mother–baby pairs</td>
<td></td>
<td>× 33 months</td>
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<td>Double-blind,</td>
<td>L. acidophilus La 5</td>
<td>7 × 10^{9} CFU/day</td>
<td>↑ Total antioxidant capacity</td>
<td>Ejtahed et al. [116]</td>
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<td>placebo-controlled,</td>
<td>B. lactis Bb-12</td>
<td>6 × 10^{9} CFU/day</td>
<td>↓ Fasting blood glucose, HbA1c</td>
<td></td>
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<tr>
<td>randomized study, T2D</td>
<td></td>
<td>× 6 weeks</td>
<td>↑ Erythrocyte SOD and GPx</td>
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<td>patients</td>
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<td><strong>Experimental studies</strong></td>
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<tr>
<td>Female NOD mice</td>
<td>L. acidophilus MB443</td>
<td>9 mg/week of VSL#3</td>
<td>↑ IL-10</td>
<td>Calcinaro et al. [117]</td>
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<td></td>
<td>L. delbrueckii subsp.</td>
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<td>↓ Insulin and decreased rate</td>
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<td>bulgaricus MB453</td>
<td></td>
<td>of β-cell destruction</td>
<td></td>
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<td></td>
<td>L. casei MB 451</td>
<td></td>
<td>↓ Incidence of auto-immune</td>
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<td></td>
<td>L. plantarum MB452</td>
<td></td>
<td>diabetes</td>
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<td></td>
<td>B. longum Y10</td>
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<td>B. infantis Y1</td>
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<td></td>
<td>B. breve Y8</td>
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<td></td>
<td>S. salivarius subsp.</td>
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<td></td>
<td>thermophilus MB 455</td>
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<tr>
<td>NOD mice</td>
<td>L. acidophilus MB443</td>
<td>1.5 × 10^9 CFU/day of</td>
<td>↑ Sensitivity of insulin</td>
<td>Ma et al. [118]</td>
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<tr>
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<td>L. delbrueckii subsp.</td>
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<td>VSL#3</td>
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<td></td>
<td>bulgaricus MB453</td>
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<td>↓ IkB activity</td>
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<td>L. casei MB 451</td>
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<td>↓ Hepatic NKT cell depletion</td>
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<td></td>
<td>L. plantarum MB452</td>
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<td>↓ NF-κB binding activity</td>
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<td>B. longum Y10</td>
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<td>thermophilus MB 455</td>
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<tr>
<td>C57BL/6J mice</td>
<td>L. plantarum DSM15313</td>
<td>25 × 10^8 CFU/day</td>
<td>↓ Blood glucose</td>
<td>Andersson et al. [119]</td>
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<td></td>
<td></td>
<td>× 20 weeks</td>
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<tr>
<td>Sprague-Dawley</td>
<td>L. reuteri GMNL-263</td>
<td>10^8 CFU/day</td>
<td>↓ Blood glucose and HbA1c</td>
<td>Lu et al. [120]</td>
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<tr>
<td>diabetic rats</td>
<td></td>
<td>× 4 weeks</td>
<td>↓ JAK2 and STAT1 phosphorylation</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>↓ PAI-1</td>
<td></td>
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<tr>
<td>Caco-2 cells BB rats</td>
<td>L. johnsonii N6.2</td>
<td>10^{10}–10^{11} CFU/L (cells)</td>
<td>↑ Paneth cell</td>
<td>Kingma et al. [121]</td>
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<tr>
<td>HT-29 cells</td>
<td>L. rhamnosus GG</td>
<td>10^7–10^9 CFU/mL</td>
<td>↓ LPS-induced IkBo degradation</td>
<td>Lee et al. [122]</td>
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<td>↓ NF-κB nuclear translocation</td>
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<tr>
<td>Rats</td>
<td>L. plantarum TN627</td>
<td>0.9 × 10^9 CFU/day</td>
<td>↓ Blood glucose</td>
<td>Bejar et al. [123]</td>
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<td></td>
<td>× 4 weeks</td>
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</table>

T2D: type 2 diabetes; SOD: superoxide dismutase; GPx: glutathione peroxidase; NOD: non-obese diabetic; NKT: natural killer T; NF-κB: nuclear factor-kappaB; JAK2: Janus kinase 2; STAT1: signal transducer and activator of transcription 1; PAI-1: plasminogen activator inhibitor-1; LPS: lipopolysaccharide.

a Type of cell and/or animal model used in experimental studies.
and, therefore, its effect on gastric motility may be altered in the presence of vagal neuropathy. GLP-1 displays neuroprotective effects in both the CNS and peripheral nervous system [104] and, thus, may have potential beneficial effects on impaired enteric nerves by improving their function in diabetes.

7. Probiotics and their therapeutic potential in diabetes

In general, the above-mentioned findings, which confirm a strong link between the GI system and diabetes, have focused considerable attention on the use of biotherapy to regulate intestinal microbiota function. Probiotics are live microorganisms that, when administered in sufficient quantities, provide health benefits to the host [105, 106]. However, the idea of a ‘universal strain’ that offers all of the beneficial improvements simultaneously is unlikely. In fact, the positive effects obtained with probiotics are probably strain-specific; thus, different strains of the same species may exert distinct effects [106]. The probiotic components associated with positive effects include a variety of cell constituents, such as peptidoglycan, teichoic acids, polysaccharides, fimbrial/pili constituents and bacteriocins [107]. Within the gut, probiotics are in competition for nutrients, metabolites and antimicrobial proteins, thereby modulating gut microbiota population diversity in various ways [9–14].

Probiotic administration can stimulate the immune response, improve lactose tolerance, avoid diarrhea, restore obesity-linked gut dysbiosis and exert anti-inflammatory effects [108–110]. In particular, the beneficial effects of probiotic strains include the prevention and treatment of obesity and inflammation, as well as of associated metabolic disorders such as diabetes. These positive effects arise through a direct influence on the mucosal barrier and, in particular, on the surrounding cells, which can hamper chronic inflammation [109]. In the context of obesity and metabolic disorders, probiotic supplementation can help to reduce hyperphagia, so improving control of weight gain, fat mass loss and glucose tolerance. Moreover, these positive effects may be obtained with no modulation of caloric intakes [111]. The majority of the probiotic strains showing positive effects on glucose metabolism in humans belong to the Lactobacillus genus (Firmicutes phylum) and, to a lesser extent, the Bifidobacterium genus (Actinobacteria phylum) [33, 60–111]. To demonstrate the benefits of probiotics for improving metabolic disorders, researchers now have access to a variety of assays for plasma and liver cholesterol, free fatty acids, alanine and aspartate transaminases (haptotaxicity markers), and gene and protein expression (involved in inflammatory and metabolic pathways). Two recent reviews [112, 113] demonstrated the beneficial effects of probiotics (mostly Lactobacillus spp) in the prevention and management of T1D and T2D. A summary of all clinical and experimental studies of diabetes involving popular probiotic strains is presented in Table 1 [114–123].

Propionibacterium freudenreichii, a promising well-known, non-LAB species, produces 1,4-dihydroxy-2-naphthoic acid (DHN), which can reduce inflammation in IL-10-deficient mice by suppressing proinflammatory cytokines [124]. Moreover, P. freudenreichii ssp shermanii JS, in combination with Lactobacillus rhamnosus GG, has anti-inflammatory effects on HFD-induced inflammation in ApoE−3-Leiden mice by decreasing intestinal mast-cell numbers [125]. More important, it would be of interest to investigate other “beneficial” microorganisms that are decreased in the gut microbiota of diabetic patients. Of the ones that might potentially be used in the treatment of T2D, A. muciniphila is of particular interest [56–61]. The administration of A. muciniphila MucT (ATCC BAA-835) to a diet-induced mouse model of T2D has been proved to exert beneficial effects on glucose metabolism by alleviating glucose intolerance in HFD-induced diabetic mice [126], suggesting that A. muciniphila could prevent the deleterious increase of gluconeogenesis in diabetic mice. In addition to A. muciniphila, other bacteria might be beneficial in the treatment of diabetes. For instance, F. prausnitzii plays an important role in preserving the gut barrier and controlling inflammation and diabetes progression [58, 127]. A traditional Chinese berberine-containing herbal formula given to T2D patients [128] changed the gut microbiota by increasing F. prausnitzii, which was negatively correlated with fasting blood glucose, HbA1c and postprandial blood glucose levels, and positively correlated with homeostasis model assessment of beta-cell function (HOMA-B). As F. prausnitzii is the least abundant spp found in T2D patients [62, 129], it may be of particular interest.

Although clinical and experimental studies have revealed the important potential of these probiotic strains in the management of diabetes, further investigations are still required to elucidate the molecular mechanisms involved in order to develop more effective strategies against diabetes and its complications.

Disclosure of interest

The authors declare that they have no competing interest.

References


