REVIEW

Gut microbiota dysbiosis in diabetes mellitus

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INTRODUCTION

Diabetes has become a worldwide epidemic and presents one of the most challenging health problems of the 21st century because of its rising prevalence in both developed and developing nations ("International Diabetes Federation. IDF Diabetes Atlas 7th Edition. Brussels, Belgium," 2015; "World Health Organization. Global report on diabetes. ," 2016), its significant associations with excess cardiovascular disease and all-cause mortality, and inexorable health care costs (Emerging Risk Factors et al. 2010; Rao Kondapally Seshasai et al. 2011; Zimmet & Alberti 2016).

Both the World Health Organization (WHO) and the International Diabetes Federation (IDF) have provided estimates on the rising prevalence of diabetes worldwide since 1997. For example, in 1998, it was estimated that there were 135 million people with diabetes in 1995, and predicted 300 million people by 2025 (King et al. 1998). A later report suggested that there were 171 million people with diabetes in 2000, and predicted that 366 million people will have diabetes by 2030 (Wild et al, 2004). The most recent prediction by IDF is that in 2040 there will be 642 million people with diabetes worldwide ("International Diabetes Federation. IDF Diabetes Atlas 7th Edition. Brussels, Belgium," 2015).

The number of people with diabetes, particularly in developing countries, appears to be dramatically rising. For example, in Asia and the Pacific region, the rise of diabetes has been estimated to be more than 150% between 2000 and 2035 (Nanditha et al. 2016). Over 60% of the people with diabetes live in Asia, with about one-half in China and India combined (Nanditha et al, 2016). The Western Pacific, the world's most populous region, has more than 138.2 million people with diabetes, and the number is predicted to rise to 201.8 million by 2035 (Nanditha et al. 2016). The Philippines, for example, has over 3,721,900 cases of diabetes in 2017 ("International Diabetes Federation. IDF Diabetes Atlas 7th Edition. Brussels, Belgium," 2015). The scenario poses huge social and economic problems to most nations in the region and could impede national and, indeed, global development. More action is required to understand the drivers of the epidemic to provide a rationale for prevention strategies to address the rising global public health "tsunami." Unless drastic steps are taken through national prevention programs to curb the escalating trends in all of the countries, the social, economic, and health care challenges are likely to be insurmountable.

Diabetes mellitus represents a chronic heterogeneous disease that has been thought to result from the interaction of genetic and environmental factors (Akerblom et al. 1997; Bluestone et al. 2010; Kahn et al. 2014; Lyssenko et al. 2008). There are generally two major forms of diabetes. Type 1 diabetes (T1D), which occurs predominantly in young people (diagnosis at 30 years of age or younger), is caused by an immune-associated, organ-specific destruction of insulin-producing pancreatic beta cells, resulting in an absolute insulin deficiency and an absolute need for lifelong insulin replacement (Bluestone et al. 2010). Type 2 diabetes (T2D), the more common form, has been regarded as a progressive metabolic disorder that is caused primarily by insulin resistance in peripheral tissues (skeletal muscle and adipose tissue) followed by a relative lack of insulin secretion due to the late exhaustion of pancreatic beta cell

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function after the establishment of insulin resistance (Kahn et al. 2014). The pathogenesis of diabetes is complex and remains incompletely understood. Although genetic and environmental factors, such as diet and drugs, are well known to contribute to the disease, an increasing body of evidence has emerged suggesting that the microbial organisms that colonize the human intestinal tract may play key contributory roles in the development and management of both T1D and T2D.

Gut microbiota composition

In recent years, the rapid development of molecular sequencing technologies and advancement of powerful computational and bioinformatic tools have greatly enhanced our understanding of the composition of microbial organisms in the human intestinal tract in health and disease.

The intestinal tract (gut) of humans is inhabited by a highly diverse microbial community referred to as the gut microbiota. Gut microbiome refers to the total genomes of gut microbiota and is often used to describe the entity of microbial functions encoded by gut microbiotia. It has been estimated that the gut microbiota contains about 100 trillion (10^{14}) microbes, which comprises about ten-fold the number of human cells, and encodes 150-fold more genes than our own genome (Ley, Peterson et al. 2006; Qin et al. 2010). However, a recent estimate of the relative abundance of bacterial cells and human cells in the human body has been revised and showed that the total number of bacteria in a 70 kg "reference man" is 3.8×10^{13} and the total number of human cells is 3.0×10^{13} (Sender et al. 2016). The gut microbiota has co-evolved with humans such that it is mutually beneficial and essential in maintaining physiological homeostasis and metabolism in the host (Bäckhed et al. 2005).

Metagenomic studies using 16S rRNA gene sequencing technology indicate that most of the microbes residing in the human intestinal tract are dominated by members of two bacterial divisions, namely Bacteriodetes and Firmicutes, which comprise over 90% of the known phylogenetic categories (Bäckhed et al. 2005; Eckburg et al. 2005; Qin et al. 2010). Other bacterial phyla found in human gut include Proteobacteria, Actino-bacteria, Fusobacteria, and Verrucomicrobia phyla (Bäckhed et al. 2005; Eckburg et al. 2005; Qin et al. 2010). The diversity of microbes within the gut, defined as the number and abundance distribution of distinct types of microbial organisms, is immense and varies between and within individuals as a result of coevolution between microbial communities and their hosts (Eckburg et al. 2005; Ley, Peterson et al. 2006). Despite this inter-individual variation, the composition of gut microbiota appears to be relatively stable over time and resilient within an individual (Faith et al. 2013; Lozupone et al. 2012; Schloissnig et al. 2013). This has important clinical implications in determining differences in gut microbiota composition between normal and disease states and in evaluating host responses to dietary changes, drugs, and treatment of specific disease. An abundance of aerobic and facultative anaerobic species dwell in the upper gastrointestinal (GI) tract (i.e., stomach and small intestine), while anaerobic species reside in the lower GI tract (Gill et al. 2006).

It is well recognized that genetic and environmental factors influence the diversity and structure of the gut microbiota (Spor et al. 2011). Host genes that have been identified as important in determining microbial diversity in the gut have roles that impact host metabolism and innate immune function (Goodrich et al. 2014; Spor et al. 2011). Environmental factors include diet, individual hygiene, life style, mode of birth, and the use of medications and antibiotics; all of which collectively participate in colonization, adaption, and stabilization of the host gut microbiota (Jernberg et al. 2010; Lozupone et al. 2012; Muegge et al. 2011; Schloissnig et al. 2013; Sommer & Bäckhed 2013; Spor et al. 2011; Wu et al. 2011; Yatsunenko et al. 2012).

Gut microbiota function

The gut microbiota performs a variety of metabolic and physiological functions that are protective or beneficial to the health and well-being of the host, including among others, nutrient extraction/energy storage (Bäckhed et al. 2004), breakdown of polysaccharides, polyphenols and synthesis of vitamin, modification of bile acid metabolism (Joyce et al. 2014), maintenance of intestinal barrier permeability (Sharma et al. 2010), and immune regulation which are discussed extensively in separate reviews (Hooper et al. 2012; Rowland et al. 2018; Tremaroli & Bäckhed 2012).

In addition to the direct action on the gut mucosa and the enteric nervous system (ENS), the metabolic output of the gut microbiota reaches well beyond the local GI compartment. Thus, considering the ability to influence the function of distal organs and systems, in many respects, the gut microbiota has been considered as a "virtual endocrine organ" (Clarke et al. 2014; Evans et al. 2013).

Gut microbiota and glucose homeostasis

Several lines of evidence indicate that the gut microbiota participates in the initiation and regulation of glucose synthesis and homeostasis. Gut microbiota-derived short chain fatty acids (SCFAs), from fermentation of dietary non-digestible carbohydrates in the lower intestine that enter the systemic circulation and act as metabolic precursors for glucose and lipid metabolism in the host. For example, acetate is utilized for lipogenesis in the liver. Butyrate is the major fuel source for colonocytes. Propionate is also largely taken up by the liver, and is used as a substrate for hepatic gluconeogenesis. SCFAs also act as signaling molecules and ligands for at least two G protein-coupled free fatty acid receptors, FFAR2 (GPR43) and FFAR3 (GPR41), which are expressed in the distal small intestine, colon, and adipocytes (Brown AJ et al. 2003; Giongo et al. 2011; Kau et al. 2011; Khan & Jena 2015; Le Poul et al. 2003).

Besides serving as a source of energy, SCFAs have multiple actions in various tissues which can have positive or negative effects on glucose metabolism and energy homeostasis. For example, butyrate has been shown to improve insulin sensitivity and reduce adiposity in mice with diet-induced obesity (Lin et al. 2012). The SCFAs butyrate and propionate have also been shown suppress food intake and prevent high fat diet-induced weight gain in mice (Tolhurst et al. 2012). The inhibition of food intake and body weight was mediated in part through stimulation of the gut hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), by butyrate and propionate (Tolhurst et al. 2012). It was further shown that SCFAs stimulate the release of the gut-derived peptides, GLP-1 and peptide YY (PYY) through activation of G-protein-coupled receptor FFAR2 (GPR43) in the intestine to modulate nutrient absorption from the gut, intestinal motility, and appetite (Psichas et al. 2015; Tolhurst et al. 2012). Along similar lines, gut-microbiota derived SCFAs butyrate and propionate were shown to increase intestinal glucose production through activation of intestinal gluconeogenic (IGN) genes (De Vadder et al. 2014). The increased IGN gene expression induced by butyrate appears to be mediated by an increase in intracellular cyclic AMP, whereas IGN activation by propionate involves a gut-brain neural axis mechanism (De Vadder et al. 2014).

Altered gut microbiota in type 1 diabetes

A number of studies in animal models and humans have shown alterations in the composition and diversity of the gut microbial community in type 1 DM.

Animal studies

The gut microbiome has been implicated in chronic proinflammatory disorders, including T1D (Vaarala et al. 2008; Wen et al. 2008). Investigators have shown that intestinal bacteria are associated with T1D in the diabetes-prone BioBreeding rat (Brugman et al. 2006), the LEW1.WR1 rat model of virus-induced T1D (Hara et al. 2012), and the non-obese diabetic (NOD) mouse (Calcinaro et al. 2005; Matsuzaki et al. 1997). Investigators use diabetic rats and mice models to provide data showing gut microbiota causes inflammatory disorders.

Human studies

Only a few studies have investigated gut microbiota composition in humans with type 1 diabetes. Giongo et al analyzed the gut microbiota composition from stool samples obtained at 3 different time points in 8 Finnish children who developed autoimmune type 1 DM and found a decrease in bacterial diversity at the phylum level overtime in these autoimmune type 1 DM children relative to that of age-matched, genotype-matched, nonautoimmune controls (Giongo et al. 2011). There was a striking decline in Firmicutes and increase in Bacteroidetes in the gut microbiome overtime as children become autoimmune. By contrast, an increase in Firmicutes and a decrease in Bacteroidetes was noted in healthy children. A single species, Bacteroides ovatus, comprised nearly 24% of the total increase in the phylum Bacteroidetes in cases compared with controls. Conversely, another species in controls, represented by the human Firmicutes strain CO19, represented nearly 20% of the increase in Firmicutes compared with cases overtime.

In another study, de Goffau et al compared the gut microbiota of children aged 1-5 years with new-onset T1D with that of age-matched non-diabetic control children using the Human Intestinal Tract Chip (HITChip), a phylogenetic microarray that provides a deep global compositional analysis of the human intestinal microbiota (de Goffau et al. 2014). In this study, it was shown that for children younger than 2.9 years, 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. Controls older than 2.9 years showed 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 the younger age groups, while the diabetic children older than 2.9 years could be differentiated by having an increased microbial diversity.

In a study of 7-year-old Spanish diabetic children who had already had TID for 4 years, the Blautia coccoides/Eubacterium rectale group (Clostridium cluster XIVa) was found to be less represented in diabetic children (Murri et al. 2013).

Alkanani et al analyzed the gut microbiome from four subject cohorts with or without evidence of islet autoimmunity residing in the Denver metro area in U.S. and demonstrated that the abundances of four bacterial genera were altered in seropositive subjects compared with seronegative first-degree relatives (FDRs) (Alkanani et al. 2015). Furthermore, seropositive subjects and seronegative FDRs as well as new-onset patients have a reduction in the abundance of the Firmicutes genera Lactobacillus or Staphylococcus compared with unrelated healthy control subjects. The gut microbiota of healthy control subjects with no family history of autoimmunity had increased abundances of Lactobacillus spp. and Staphylococcus spp. versus new-onset patients and seropositive and seronegative FDRs. Further discriminant analysis showed that the microbiomes of seropositive subjects and seronegative FDR cohorts are similar to each other but distinct from those of new-onset patients and unrelated healthy control subjects and that the microbiota of new-onset patients is different than that of unrelated healthy subjects. Taken together, these findings suggest that alterations in the intestinal microbiome may be linked with diabetes susceptibility and Type 1DM onset.

The data suggest that gastrointestinal tract dysbiosis may be associated with disease progression. Murri et al found a pattern toward increased abundances of the Bacteroidetes genus Bacteroides and a reduction in the Bacteroidetes genus Prevotella and the phylum Firmicutes in subjects with multiple autoantibodies versus one autoantibody (Murri et al. 2013). These data are consistent with other studies showing that seropositive individuals with multiple autoantibodies have altered abundance of the Bacteroides genus.

These observations differ from data from previous reports that demonstrated increased microbial diversity in patients with diabetes or increased abundances of the Bacteroidaceae family and the Bacteroides genus in seropositive individuals compared with autoantibody-negative control subject. The reasons for these disparate results are not entirely clear as the gut microbiome is influenced by multiple environmental factors, such as diet, exposure to microbes, geography, climate, and cultural differences that exist between different countries and communities (Yatsunenko et al. 2012).

Brown et al found a higher proportion of Actinobacteria, Bacteroidetes, and Proteobacteria phyla in TID subjects, whereas, the control group had higher abundance of Firmicutes, Fusobacteria, Tenericutes, and Verrucomicrobia (Brown CT et al. 2011). A longitudinal study on young children suggested that bacterial diversity diminishes over time in genetically prone autoimmune children when compared with healthy control age-mates. Particularly, it was observed that Bacteroides ovatus contributed almost 24% of constituents in the phylum Bacteroidetes in the T1D subjects (Giongo et al. 2011). Results from other studies have also shown changes in the microbial ecology and a decline in bacterial diversity in T1D cases (Alkanani et al. 2015; de Goffau et al. 2014; Murri et al. 2013).

Altered gut microbiota in type 2 diabetes

Animal studies

Microbiota products such as lipopolysaccharide (LPS) can drive low-grade inflammation, which has been recognized as a potential cause for insulin resistance. Bäckhed et al reported that in contrast to mice with a gut microbiota, germ-free mice exhibited protection against obesity that develops after consuming a Western-style, high-fat, sugar-rich diet (Bäckhed et al. 2007). The lean phenotype has reduced energy harvest from ingested food and increased energy expenditure which is associated with increased skeletal muscle and liver levels of phosphorylated AMP-activated protein kinase (AMPK) (Bäckhed et al. 2007). AMPK plays a role in energy homeostasis and its activation protects germ-free mice from diet-induced diabetes.

Cani et al showed that normal endotoxemia increased or decreased during the fed or fasted state, respectively (Cani et al. 2007). A high-fat diet increases plasma LPS concentration two to three times, and increases the proportion of a LPS-containing microbiota in the gut. Similar to high-fat fed mice, induced metabolic endotoxemia resulted in an increase in fasted glycemia and insulinemia and an increase in whole-body, liver, and adipose tissue weight gain (Cani et al. 2007).

Mice with a discrete defect in innate immunity, such as absence of the flagellin receptor toll-like receptor 5 (TLR5), failed to manage their microbiota, resulting in altered composition, with microbiota encroachment or decrease in bacterial-epithelial distance (Carvalho et al. 2012; Vijay-Kumar et al. 2010). TLR5-deficient mice develop insulin resistance, which can be transferred to wild type germ-free mice via microbiota stool transplant. Thus gut barrier function includes not only intercellular junctions that directly impede passage of bacterial products but also host systems of mucus deployment and innate immunity that keep bacteria at a safe distance from the epithelium and help maintain stable microbiota composition.

Human studies

A study by Larsen et al included 36 male adults with18 subjects diagnosed with diabetes type 2. They had a mean age of 56 years, and a mean body mass index (BMI) of 30. The other 18 controls had a mean age of 59 years, and a mean BMI of 28. The total bacterial counts were similar in the diabetic and the control groups. By characterization of the intestinal microbiota by tag-encoded pyrosequencing, the authors demonstrated that the proportion of Firmicutes was significantly higher in the controls compared to the diabetic group. The phylum Bacteroidetes and Proteobacteria were somewhat but not significantly enriched in the diabetic group compared to controls (Larsen et al. 2010). The authors concluded that T2D is associated with compositional changes in the intestinal microbiota mostly apparent at the phylum and class levels. These results are in agreement with the recent evidence obtained for overweight persons by Schwiertz and colleagues (Schwiertz et al. 2010) though they contradict other previously mentioned studies. Given the aforementioned findings, a positive correlation between ratios of Bacteroidetes to Firmicutes and BMI could be expected. The reverse tendency was observed, which was regarded as indicative that overweight and T2D are associated with different groups of the intestinal microbiota.

Qin et al performed a metagenome-wide association study in 345 Chinese subjects with T2D subjects (Qin et al. 2012). It was designed as a case-control study with non-diabetic controls. Using a shotgun-sequencing approach, the authors detected a "moderate" degree of dysbiosis in T2D subjects with a decrease in the abundance of various butyrate producing bacteria, including Clostridiales sp. SS3/4, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis and Roseburia inulinivorans (all these bacteria belong to the phyla Firmicutes). Furthermore, they identified more opportunistic pathogens such as Bacteroides caccae, Clostridium hathewayi, Clostridium ramosum, Clostridium symbiosum, Eggerthella lenta and Escherichia coli. At the pathway level, the gut microbiota of T2D patients was functionally characterized by an enrichment in the membrane transport of sugars, branched-chain amino acid (BCAA) transport, methane metabolism, xenobiotics degradation and metabolism, and sulphate reduction. This does to some degree support the concept of an increased capacity for energy harvest proposed by Turnbaugh (Turnbaugh et al. 2006). By contrast, there was a decrease in the level of bacterial chemotaxis, flagellar assembly, butyrate biosynthesis and metabolism of cofactors and vitamins.

Karlsson et al investigated the faecal microbiota in 145 70-year old women—53 women had T2D; impaired glucose tolerance was present in 49 women, and 43 had normal glucose tolerance (NGT) (Karlsson et al. 2013). In contrast to previous reports on observations between lean and obese people, the faecal microbiota of non-diabetic, impaired glucose tolerance (IGT) and T2D women contained similar numbers of genes. In the diabetic group, the authors observed an increase in the abundance of four Lactobacillus species and decreases in the abundance of five Clostridium species. As for metabolic control, Lactobacillus species correlated positively with fasting glucose and glycated hemoglobin (HbA1c), whereas Clostridium species correlated negatively with fasting glucose, HbA1c and other metabolic markers. Both species were in no correlation with the BMI of the test subjects. In a metagenomic cluster model, they identified Roseburia and Faecali bacterium prausnitzii as highly discriminant for T2D. These bacteria are known as human gut colonizers and butyrate producers. Such bacteria have been reported to improve diabetic control and insulin sensitivity (Ley, Turnbaugh et al. 2006).

Mechanisms linking altered gut microbiota and diabetes

Altered gut microbiota or dysbiosis has been implicated in the pathogenesis of both T1D and T2D. The underlying mechanisms or pathways that link gut microbiota and the development of diabetes are incompletely understood. Several factors or mediators have been proposed, including increased energy harvest from the diet, altered increased intestinal production of SCFAs, increased intestinal permeability, gut microbiota-induced metabolic endotoxemia, systemic low-grade inflammation, modulation of signaling pathways and dysfunction of immune system, and alteration in the gut-brain axis. These items will be discussed further.

Increased energy harvest

Studies in mouse models and humans have demonstrated the inter-relationships between diet, gut microbiota composition, and energy equilibrium and suggested that the gut microbiota has the ability to alter energy balance in the host by modulating extraction of calories from the diet (Bäckhed et al. 2004). In their studies of normal and germ-free (GF) mice, Bäckhed et al demonstrated that transplantation of gut microbiota from normal mice into GF mice produced a marked and rapid increase in body fat (Bäckhed et al. 2004). The increase in body fat was observed even though the food intake in the recipient mice was reduced. This finding indicated that the gut microbiota by virtue of its capacity to harvest energy from the diet was responsible for increased fat storage in the host. Further direct biochemical evidence for a role of gut microbiota in energy harvesting is the observation that the presence of the microbiota promotes increased monosaccharide uptake from the gut lumen to the liver resulting in induction of de novo triglyceride production in the liver. This effect is mediated by transactivation of hepatic lipogenic enzymes and carbohydrate response element binding protein (ChREBP) and sterol response element binding protein 1 (SREBP-1), two transcription factors known to mediate hepatocyte lipogenic responses to insulin and glucose (Bäckhed et al. 2004). The microbiota was also shown to promote storage of triglycerides in adipocytes through suppression of fasting-induced adipocyte factor (Fiaf), a circulating lipoprotein lipase (LPL) inhibitor, resulting in increased LPL activity in adipocytes (Bäckhed et al. 2004).

The relationship between gut microbiota composition, dietary caloric load, and nutrient absorption has also been investigated in humans. In studies of obese individuals who were placed on two different low-calorie diets for 12 months, Ley et al showed an increase in the Firmicutes and a decrease in the Bacteroidetes phyla in the feces of obese subjects that was associated with adiposity (Ley, Turnaugh et al. 2006).

Altered short-chain fatty acid production

Another mechanism that links gut microbiota to diabetes is altered production of SCFAs by aberrant microbiota. Metabolites, such as the SCFAs, are produced by colonic microbiota through anaerobic fermentation of dietary fat and carbohydrate (Le Poul et al. 2003; Psichas et al. 2015; Rios-Covian et al. 2016). SCFAs, namely acetate, propionate, and butyrate enter the systemic circulation and act as metabolic

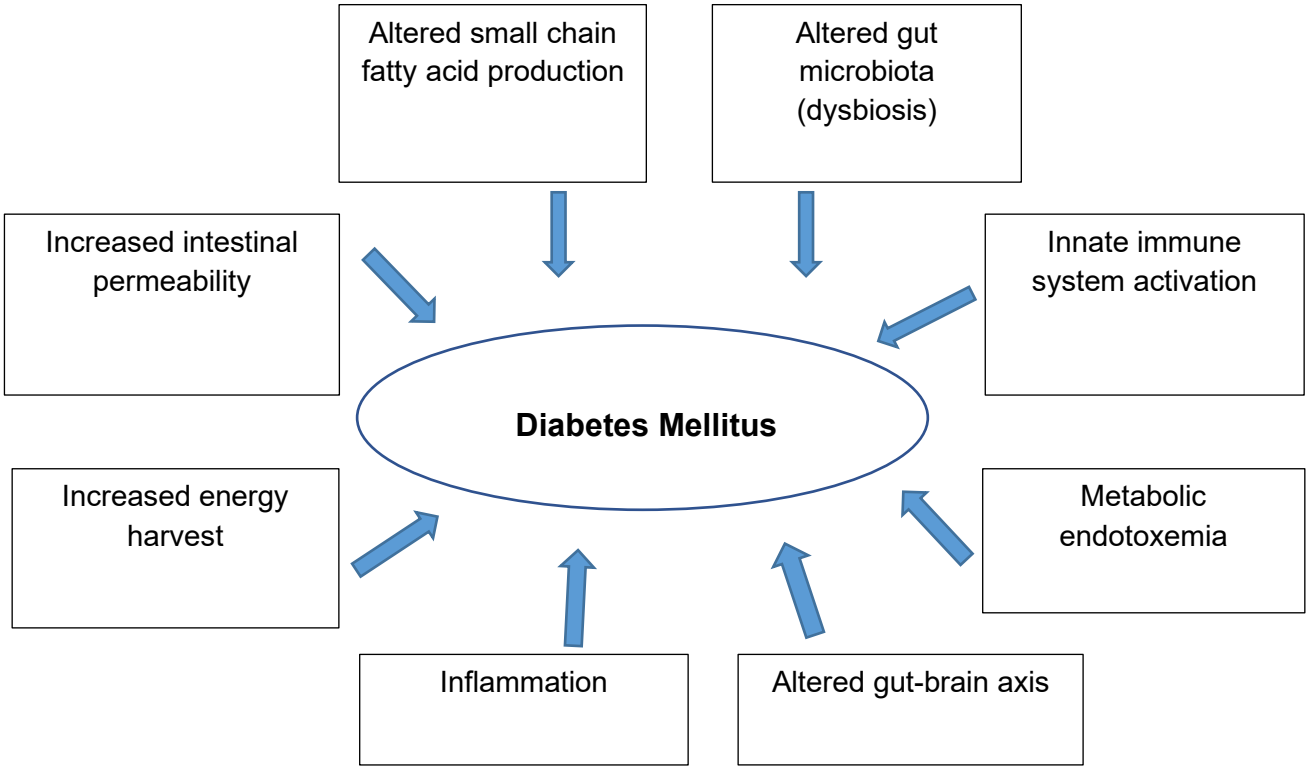

Figure 1: Mechanisms linking altered gut microbiota and diabetes

precursors for glucose and lipid metabolism in the host (Le Poul et al. 2003). For example, acetate is utilized for lipogenesis in

the liver. Propionate is also largely taken up by the liver, and is used as a substrate for hepatic gluconeogenesis. Butyrate is the major fuel source for colonocytes. SCFAs also act as signaling molecules and ligands for at least two G protein-coupled free fatty acid receptors, FFAR2 (GPR43) and FFAR3 (GPR41), which are expressed in the distal small intestine, colon, and adipocytes (Priyadarshini et al. 2018; Psichas et al. 2015; Rios-Covian et al. 2016; Tolhurst et al. 2012).

Changes in gut composition and function may adversely affect the development of diabetes through altered secretion of intestinal peptide hormones that impair insulin secretion/action and influence glucose metabolism induced by gut dysbiosis. For example, intestinal milieu regulates the secretion of hormones such as GLP-1, glucose-dependent insulinotropic polypeptide (GIP), ghrelin, gastrin, somatostatin, cholecystokinin (CCK), serotonin, peptide YY, GLP-2, all of which importantly influence metabolism in general and in particular glucose metabolism (Tolhurst et al. 2012).

Increased intestinal permeability

Increased intestinal permeability has been reported in patients with T1D and T2D (Bosi et al. 2006; Cox et al. 2017). Recent studies in animal models and humans suggest that altered gut microbiota in T1D and T2D is associated with increased permeability of the intestinal barrier or "leaky gut" which leads to translocation or passage of gut bacteria or their products into the systemic circulation (Amar et al. 2011; Meddings et al. 1999; Vaarala et al. 2008). Amar demonstrated that before the onset of diabetes, after only one week of a high-fat diet (HFD), live commensal intestinal bacteria are present in large numbers in the adipose tissue and the blood where they can induce inflammation (Amar et al. 2011). This translocation is prevented in mice lacking the microbial pattern recognition receptors nucleotide binding oligomerization domain containing 1 (Nod1) or CD14, but overtly increased in Myd88 knockout and ob/ob mice. This "metabolic bacteremia" is characterized by an increased co-localization with dendritic cells from the intestinal lamina propria and by an augmented intestinal mucosal adherence of non-pathogenic Escherichia coli translocation or passage of bacteria or their products into the systemic circulation (Amar et al. 2011).

In studies of mice with diabetes induced by a high fat diet, Cani et al have shown that high fat feeding resulted in altered gut microbiota composition and increased intestinal permeability by reducing the expression of genes coding for two tight junction proteins, zonula occludens (ZO-1) and occludin proteins, known to maintain normal intestinal barrier (Cani et al. 2008) . Moreover, oral antibiotic treatment of high-fat fed-diabetic mice restored normal intestinal epithelial integrity, indicating that the intestinal microbiota was involved in this process.

Inflammation

Inflammation has been cited as a cause for T1D and T2D. Cani and co-workers first reported and identified bacterial LPS, a major glycolipid constituent of the cell wall of gram-negative bacteria, as a triggering factor in insulin resistance in obesity and diabetes (Cani et al. 2007). These investigators demonstrated a marked increase in circulating levels of LPS, termed as "metabolic endotoxemia", in mice fed a high fat diet (HFD). In addition, HFD-fed mice had altered intestinal bacterial composition with an increase in the proportion of an LPS-containing microbiota in the cecum (Cani et al. 2008). Moreover, chronic subcutaneous infusion of exogenous LPS induced obesity in association with increased fasting plasma glucose and insulin levels and liver insulin resistance. Both HFD and LPS infusion were also associated with increased gene expression of inflammatory cytokines, (tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1), IL-6, and plasminogen activator inhibitor (PAI-1). These findings indicate that endotoxemia induced by endogenous LPS or by exogenous infusion of LPS may be causally involved in the induction of obesity and insulin resistance associated with high-fat feeding.

Recent studies in humans have also linked circulating endotoxemia with development of diabetes. For example, in a large Finnish population-based study, Pussinen et al have shown for the first time that fasting serum endotoxin or LPS activity (measured by kinetic Limulus Amebocyte Lysate test) was significantly associated with increased risk for clinically incident diabetes (Pussinen et al. 2011).

Chronic low-grade inflammation has been implicated in the pathogenesis of T1D (Bending et al. 2012; Clark et al. 2017), insulin resistance and T2D (Cefalu 2009; Hotamisligil 2006; Shoelson et al. 2006). In T1D, islet inflammation is an early event marked by infiltration of CD8+ and CD4 T cells around beta cells followed by eventual destruction and loss of function of beta cells (Clark et al. 2017). In T2D, the inflammatory process is characterized primarily by local macrophage infiltration in adipose tissue, the major site of insulin resistance in T2D. Macrophage activation in adipocytes leads to release of proinflammatory cytokines, such as TNF-α and IL-1β, and adipocyte chemokines, such as adiponectin, which activate c-jun N-terminal kinase (JNK) and IKKβ/NF-κB through classical receptor-mediated mechanisms resulting in insulin resistance (Cefalu 2009; Shoelson et al. 2006). In addition to the inflammatory reaction in adipose tissue, there is also evidence of islet inflammation with macrophage infiltration and its involvement in T2D (Eguchi & Manabe 2013; Ehses et al. 2007; Richardson et al. 2009).

In addition to the contribution of inflammation to insulin resistance, there is also evidence of islet inflammation and its causative involvement in β cell dysfunction in T2D. The 3T3-L1 studies have demonstrated that LPS activates TLRs to cause inflammation (Creely et al. 2007). LPS has also been shown to inhibit β-cell gene expression in a TLR4-dependent manner and via NF-κB signaling in pancreatic islets, suggesting a novel mechanism by which the gut microbiota might affect pancreatic β-cell function (Amyot et al. 2012). TLR4 is present in antigen-presenting cells, but also in non-immune cells such as adipocytes, myocytes, and pancreatic β-cells (Garay-Malpartida et al. 2011; Kiely 2009; Vives-Pi et al. 2003). This suggests that activation of TLR4 signaling can induce both insulin resistance and pancreatic β-cell dysfunction. Additionally, it has been shown that islet amyloid polypeptide (IAPP) found in beta-cells in T2D activates NOD-,LRR-and pyrin domain-containing protein (NLRP) inflammasome to produce proinflammatory cytokine IL-1β that causes beta cell destruction (Masters et al. 2010).

Innate immune system

TLRs are a family of pattern-recognition receptors that play a critical role in the host defense innate immune system by activating proinflammatory signaling pathways in response to microbial pathogens and maintaining tissue integrity (Medzhitov 2001). Creely et al. have shown increased TLR2 expression in adipose tissue from T2D patients with strong correlations of plasma endotoxin levels suggesting that gut microbiota related factors are involved in the development of T2D and obesity in humans (Creely et al. 2007). Dasu and colleagues have reported that significant increments in TLR2 and TLR4 along with their ligands and cofactors were found at both mRNA and protein levels in subjects with T2D (Dasu et al. 2010).

TLR4, the best-characterized TLR, binds to LPS of gram-negative bacterial cell walls resulting in inflammation which induces both insulin resistance and pancreatic β-cell dysfunction (Amyot et al. 2012; Garay-Malpartida et al. 2011; Vives-Pi et al. 2003). The concentration of FFA, well-known to play a key role in the pathogenesis of insulin resistance and non-insulin dependent diabetes mellitus, was directly associated with an increase in TLR4 gene and protein expression, which can be correlated with the severity of insulin resistance in obese and T2D subjects (Reyna et al. 2008).

Earlier research suggested that T2D might be associated with the dominance of gram-negative bacteria in the gut such as, Bacteroidetes and decrease in gram positive beneficial bacteria (Bäckhed et al. 2004; Raetz & Whitfield 2002). LPS is a major component of the gram negative bacteria envelope and a known inducer of inflammation in vivo. The proinflammatory component of LPS endotoxin (or lipid A) is composed of fatty acid and phosphate groups attached to a central glucosamine dimer. Specific serum binding protein TLR4 transports endotoxin to cells expressing its corresponding receptor through the glycosylphosphatidylinositol (GPI) linked protein necessary for LPS binding (Raetz & Whitfield 2002). Diet-induced obese mice exhibit a constant increase in the plasma LPS termed as "metabolic endotoxemia" which further improved by the reduction in Bacteroides-Prevotella spp. by antibiotics administration (Cani et al. 2008). The gut mucosal permeability is governed by the complex system of the tight junction proteins, including occludin and claudin. In mice, decrease in the population of bifidobacteria species in the gut loosens the tight junctions between the cells of the gut lining. The loose junctions or malfunction of the barrier increases the gut permeability and allows LPS from microbes to leak through the gut epithelium which causes a low-grade inflammation and can induce a number of metabolic disorders, including the insulin resistance leading to T2D (Cani et al. 2007).

The dysregulation of the immune response by gut dysbiosis leads to inflammation, oxidative stress, and insulin resistance. Chronic dysbiosis, leakage of microbiota and metabolic products traversing across the mucosal barrier may increase type 2 prevalence (Yoo et al. 2020). In animal studies, MyD88 protein (an adaptor for multiple innate immune receptors that recognize microbial stimuli) deficiency completely abrogates type 1 diabetes development in NOD mice that were kept in specific pathogen-free conditions. However, the protection was abolished when the mice were housed in germ-free conditions. (Wen et al. 2008) MyD88 deficiency also resulted in a reduction of antimicrobial peptides in the mouse intestine, thus altering gut microbiota composition and further shaping adaptive immunity (Tai N et al. 2016).

Alteration in the gut-brain axis

A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric nitric oxide-dependent and gut-brain axis. (Grasset et al. 2017, Grasset and Burcelin 2019). The ileum bacteria impairs the GLP-1-activated gut-brain axis for the control of insulin secretion and gastric emptying. Much remains to be studied in this area as the authors question 1) how the gut microbiota influences the neuronal detection of glucose and 2) how the diabetes mellitus-induced gut microbiota shit observed participates to the alterations of autonomic nervous system and the gut-brain axis activity (Grasset and Burcelin 2019).

Potential Benefits

Understanding the relationship between the gut microbiome and diabetes mellitus may open opportunities for drug management of diabetes by altering gut dysbiosis. Metformin, a drug commonly used to treat type 2 diabetes, may exert its beneficial effect, in part by altering the gut microbiota. Metformin increase Bacteroidetes and Verrucomicrobia and genera Akkermansia, Bacteroides and Escherichia, and decreases Intestinibacter (Rosario et al. 2018, Zhang & Hu 2020). Preliminary data suggest a role of pre- and probiotics on gut microbiota in the treatment of diabetes (Mishra et al. 2019, Amar et al. 2011, Calcinaro et al. 2005). The role of early nutrition appears to increase the risk of type 1 diabetes in those with genetic

predisposition (Verduci et al. 2020). Fecal microbiota transplantation (FMT) plays an emergent, promising technological innovation in restoring the gut microbiota composition in amelioration of obesity and diabetes (Zhang L et al. 2020, Zhang PP et al. 2020, Wang et al. 2019).

SUMMARY AND CONCLUSIONS

This review examines the available evidence from studies in animal models and humans with or without diabetes that have investigated the role of intestinal microbial composition on the development of diabetes. The underlying mechanisms or pathways that link altered gut microbiota composition (dysbiosis) to the development of both type 1 and type 2 DM have been discussed with special emphasis on gut microbiota-induced changes in intestinal short-chain fatty acids, metabolic endotoxemia, low-grade systemic inflammation, and innate immune system activation and their interrelationships to diabetes.

In summary, an increasing body of evidence from studies in animals and humans indicates that alterations in the composition and function of the gut microbiota can contribute to the development of both T1D and T2D. Several experimental studies in rodents (mice), have shown that there is altered composition and diversity of gut microbiota. Few studies in humans also suggest that diets rich in animal fat and protein can modify human gut microbiota composition. The mechanisms whereby altered gut microbiota composition and function (dysbiosis) may lead to the development of T1D and T2D are not completely clear. Several mechanisms have been discussed and include increased energy harvest from diet by gut microbiota, altered production of SCFAs, metabolic endotoxemia, inflammation, and dysfunction of the innate immune system. It is possible that there may be other factors or mediators yet to be discovered that link gut microbiota to diabetes. More research is needed to elucidate the molecular, cellular, and metabolic mechanisms underlying gut microbiota dysbiosis in the development of T1D and T2D. A better understanding of these interactions might prove useful in designing novel nutritional therapies in patients with diabetes.

CONFLICT OF INTEREST

This manuscript was written without a funding source. The authors declare no conflicts of interests.

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