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REVIEW ARTICLE

Understanding the role of the gut ecosystem in diabetes mellitus

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ABSTRACT

Diabetes mellitus is a type of metabolic disorder whereby patients are unable to regulate glycemia. It is currently a worldwide public health issue, and is a burden to society because of its disabling and common complications. Diabetes is multifactorial, and also induces the onset of other diseases. In the present report, we review the labyrinth encompassing the gut microbiota and gut microbiota-derived metabolites in type 1 diabetes and type 2 diabetes pathogenesis. There have been exceptional improvements in deoxyribonucleic acid sequencing and mass spectrometry technologies throughout these past years, and these have allowed the comprehensive collection of information on our unique gut ecosystem. We would like to advocate incorporating metagenome and metabolome information for a comprehensive perspective of the complex interrelationships between the gut environment, host metabolism and diabetes pathogenesis. We hope that with this improved understanding we would be able to provide exciting novel therapeutic approaches to engineer an ideal gut ecosystem for optimal health.

DIABETES

Diabetes mellitus, also generally referred to as diabetes, is a type of metabolic disorder whereby patients are unable to regulate glucose metabolism. Type 1 and type 2 diabetes are the most common, representing approximately 10% and 90% of cases, respectively[1]. The pathogenesis of type 1 diabetes, which is commonly prevalent in children and adolescents, is due to the inability of the endocrine system to produce insulin because of the immune-mediated destruction of β-islet cells[2]. Therefore, management of type 1 diabetes always involves the external administration of insulin. Although the causative mechanisms of type 1 diabetes are currently unknown, its probable causes are currently attributed to: genetic predisposition (with more than 40 genetic loci known to affect susceptibility), and several environmental factors including stress and viruses[3]. In contrast, the more common form of diabetes, type 2 diabetes, most often presents in adults. It usually presents as a combination of insulin resistance and insulin deficiency[4], as compared with an absolute deficiency of insulin in type 1 diabetes. Although the exact causes of type 2 diabetes have yet to be completely elucidated, various previous reports have associated type 2 diabetes with excessive visceral obesity[5], inactive lifestyle, lack of exercise and poor dietary habits[6], along with genetic factors. As compared with type 1 diabetes, type 2 diabetes has a larger selection of treatment options including peritoneal insulin administrations and non-insulin pharmaceuticals, as well as conscious modifications to lifestyle and dietary habits[1].

Diabetes is becoming a worldwide public health issue, prevailing at approximately 10% globally among adults[7]. It has been predicted by the International Diabetes Federation that by 2035, there will be 592 million cases with an additional 175 million undiagnosed diabetes cases[8]. A great deal of the strain from having diabetes stems from the complications that are commonly presented together with the disease that result in discomfort, and in serious cases, disability[9]. For example, young adults with type 1 diabetes are 10-fold more prone to the onset of cardiovascular diseases as compared with their healthy counterparts[10], and the most prevalent cause of fatality among type 2 diabetes patients is cardiovascular-related[11].

In the present review, we describe the relationships between the gut microbial environment in type 1 diabetes and type 2 diabetes patients, and hope that with increased understanding of these relationships, novel therapeutic interventions can be developed.

Gut Microbiota

The resident microorganisms in the gastrointestinal tract are collectively referred to as the gut microbiota. In mammalians,

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the gut microbiota mainly comprises of four main phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. These phyla are vital in the host metabolism and physiology regulation[12]. The number of both prokaryotic cells and host eukaryotic cells in the gut totals to approximately 100 trillion, which is threefold that of the total number of human body cells[13]. As such, we often consider our unique gut environment as a functional and measurable organ[14]. The composition of gut microbial communities vary along the gastrointestinal tract, and remodels within and between individuals as the dietary lifestyle and nutritional status of the individual varies[15]. It is only in these recent years that technological advances have allowed us to further comprehensively understand the holistic impact of the gut microbiota on the whole host metabolic system.

WHAT IS METABOLOMICS?

Technological breakthroughs, such as genomics, transcriptomics, proteomics, metabolomics and metagenomics, have allowed scientists to extensively evaluate the genome, transcriptome, proteome, metabolome and gut microbiome, respectively, with high-throughput techniques and analytical tools[16] concurrently. In recent years, we have been able to collect vast amounts of data pertaining to the gut microbiome and its metabolome to comprehensively assess the extent of the influence of the gut microbiota on human health, which can be attributed to the dramatic improvements in deoxyribonucleic acid sequencing and mass spectrometry technologies[17]. Nuclear magnetic resonance and mass spectrometry are commonly used to profile the feces, blood and urine metabolites produced by microbiota and host cells, thereby determining disease biomarkers in the process in wide-range metabolomic analytical methods[18]. The information gathered from the comprehensive assessment of the organ and systemic metabolism is important in maintaining the health and nutritional status of the host[19]. By evaluating the concentrations and presence of metabolites comprehensively, clinicians are able to better understand how clinical regimes impact the host metabolic profile[19].

METABOLOMICS PROFILING AND THE GUT MICROBIOTA

Nowadays, one of the common techniques undertaken to analyze the metabolome profile is to directly compare it with gut microbiota metabolism and to correlate these changes to the final metabolic outcomes in the host. As we have reported previously, the synergistic activities of the gut microbiome and the host is a reflection of overall human metabolism at the systemic level[20,21]. To state a few examples, in a study investigating the effects of probiotics or prebiotics, or their combination in gnotobiotic mice colonized with human infant microbes[22], the gut microbiota community was significantly altered by probiotic/prebiotic intervention, and this thereby induced various systemic changes in the metabolic profiles of different tissues. It was observed that there were elevated concentrations of *Bifidobacterium breve*, *Bifidobacterium longum* and *Bacteroides distasonis*; and a decline in ratios of *Escherichia coli* and *Clostridium perfringens*. Fat metabolism was also improved as concentrations of glucose and hepatic triglycerides in the plasma in groups that were administered prebiotics were also lowered[22]. Wikoff *et al.*[23] assessed the effects of gut microbiota on the host between germ-free and conventionally raised mice. There were many plasma metabolites that were detected only in conventionally raised mice, and not in germ-free mice. In addition, in the case of commonly observed metabolites between the mice raised under conventional or germ-free environments, one-tenth of them differed by more than 50%[23].

GUT ECOSYSTEM AND DIABETES

Apart from digestion, the gut microbiota is important in upkeeping the optimal state of host health, but it is also implicated in the pathogenesis of numerous metabolic diseases, such as obesity[24–26], diabetes[26–29], chronic kidney disease[30,31] and atherosclerosis[32–34]; and intestinal diseases[21], such as inflammatory bowel diseases[35] and colorectal cancer[36–38].

For the past 50 years, the increased use of vaccinations and antibiotics, such as penicillin, and increasingly improved hygiene standards have significantly lowered the prevalence of several infectious diseases. There are some strains of bacteria that are resistant to antibiotics and therefore, antibiotics consumption will skew gut microbiota composition[39]. The lack of diversity in the gut microbiome is implicated in an underdeveloped immune system, resulting in the host being susceptible to a range of diseases[40]. During this period, there were also drastic changes to the human diet, with an increased intake of carbohydrates and fats as a result of the common consumption of highly processed foods. Dietary fiber intake was also significantly lowered[41]. This is an example of a typical "Western diet," where individuals consume approximately only half of the recommended intake of 30 g of fiber daily[42]. As fibers cannot be digested by the human digestive fluid, they are fermented by the gut microbiota, thereby generating short-chain fatty acids (SCFAs) as metabolites[43]. SCFAs exert systemic anti-inflammatory effects by producing immunoglobulin A and immunosuppressive cytokines[43]. Loss of early-life exposure due to the increased use of antibiotics and a decrease in fiber intake results in dysbiosis[44,45], which is implicated in the increased incidences observed in inflammatory diseases, including diabetes[46]. SCFAs play vital roles in type 2 diabetes. There have been several studies reporting that the number bacteria involved in SCFA production were significantly lower in people with type 2 diabetes. SCFAs cohere to G-protein coupled receptors, resulting in the following biological effects. SCFAs promote secretion of glucagon-like peptide-1, an important incretin hormone, which is made by enteroendocrine L cells. Glucagon-like peptide-1 impedes secretion of glucagon, hampers gluconeogenesis in the liver, improves insulin sensitivity and augments central satiety, thereafter resulting in bodyweight loss[47]. Furthermore, SCFAs can directly hinder the low-grade inflammatory response caused by bacteria migration from the intestines into the mesenteric adipose tissue and the blood (Fig. 1)[48].

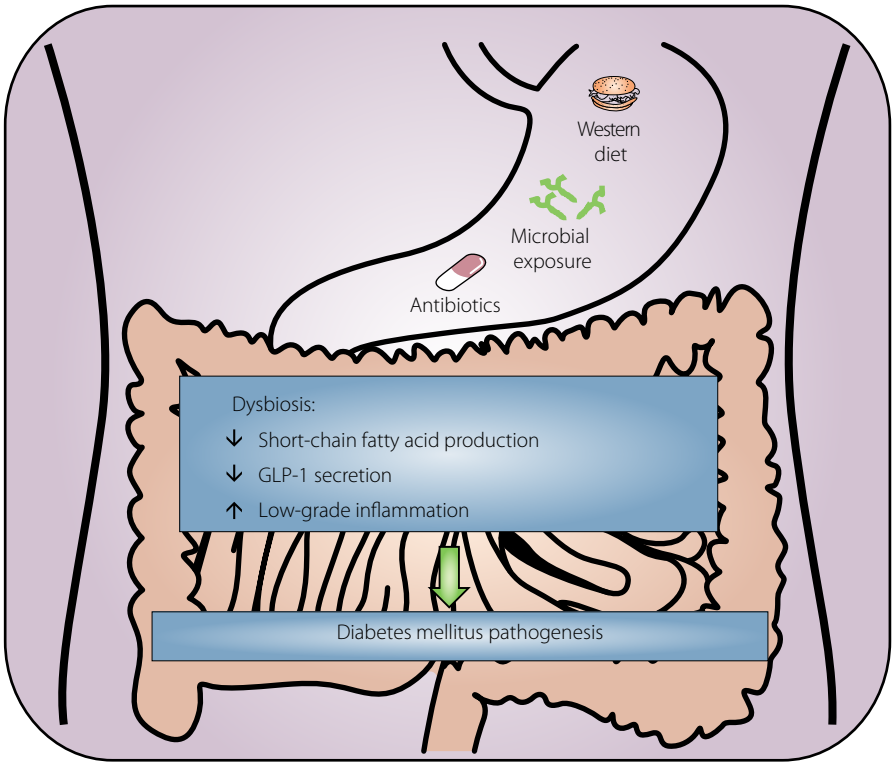

Figure 1 | Factors contributing to the pathogenesis of diabetes mellitus. Western diets, antibiotic consumption and microbial exposure have been reported to play a role in gut dysbiosis. This leads to a decrease in short-chain fatty acid production, lowered secretion of glucagon-like peptide 1 (GLP-1) and increased low-grade inflammation.

Just as the gastrointestinal tract supplies nutrients to cells and tissues, the metabolites originating from the gut microbiota provide this supply through the circulatory system. These numerous interactions amongst gut microbiota-derived metabolites, the gut microbiota and the host immune system is communicated through various signalling pathways. The host–microbe metabolic axes refers to the direct chemical communications between gut microbes, the host and immune pathways. These biological signals impact us system-wide, and directly influence organs. Within these axes, metabolic reactions are controlled by gut microbes producing choline, phenols, bile acids, and SCFAs by both the gut microbiome and host genome, which are vital to health[20]. These intricate interactions between the gut microbiome and its host play a hugely important role in maintaining good health, and might implicate the onset of diseases, such as diabetes. We will review these relationships in detail.

GUT MICROBIOTA AND TYPE 2 DIABETES

Obesity has been attributed to increasing the risk of multifactorial diseases, such as type 2 diabetes. Recently, it was reported that type 2 diabetes in humans was co-related to a lowered abundance of butyrate-producing microbes and an increased abundance of *Lactobacillus* sp[28,29,49]. Larsen *et al.*[49] noted that in human male type 2 diabetes patients, as compared with non-diabetic healthy subjects, there were significantly fewer Firmicutes, including Clostridia. There were positive correlations between plasma glucose levels and the ratios of Bacteroidetes to Firmicutes, and of the *Bacteroides–Prevotella* group to the *Clostridium coccoides–Eubacterium rectale* group. Furthermore, Betaproteobacteria was more abundant in type 2 diabetes patients than the controls. These observations hint that the Gram-negative Bacteroidetes and Proteobacteria might induce the pathogenesis of type 2 diabetes through an endotoxin-induced inflammatory response as the endotoxin, lipopolysaccharide, exists in high concentrations as a main outer cell membrane component[49]. Additionally, the gut microbiome might be a new biomarker for type 2 diabetes prediction, as gut metagenome-based computational models could predict the type 2 diabetes-associated phenotype in glucose-intolerant patients[29]. Vancomycin treatment in patients with metabolic syndrome reduced the abundance of Gram-positive bacteria that produce butyrate, and this was correlated with impaired insulin sensitivity. These results imply that lowered levels of butyrate-producing gut microbes in type 2 diabetes patients might lead to disease pathogenesis[50]. In another report, Vrieze *et al.*[51] transplanted fecal microbes from lean donors to insulin-resistant patients with metabolic syndrome. The results of that study showed that feces from lean subjects, improved insulin sensitivity and the abundances of butyrate-producing bacteria

were also increased[51]. Metagenome-wide-association-study evaluation of the gut microbial metagenome data of 345 type 2 diabetes patients and non-type 2 diabetes Chinese individuals by Qin *et al.*[28] showed that genes enriched in the type 2 diabetes group mainly comprised of opportunistic pathogens, such as *Bacteroides caccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*, *Eggerthella lenta* and *E. coli*, which have been documented to result in human infections. In contrast, almost all of the genes enriched in the non-diabetes control group were from various butyrate-producing bacteria, including *Clostridiales* sp. SS3/4, *E. rectale*, *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Roseburia inulinivorans*. Mucin-degrading microbial species, *A. muciniphila*, and sulfate-reducing species, *Desulfovibrio* sp., were also enriched in the type 2 diabetes group. Taking these results into account, type 2 diabetes patients only presented with elevation in abundances of several opportunistic pathogens and a reduction in numbers of beneficial butyrate-producing bacteria[28]. However, contrasting findings were reported in a European cohort[29]. In the European study, obese individuals with less severe metabolic syndrome had upregulated abundances of *A. muciniphila*, and this was associated with increased microbial diversity as compared with individuals who were metabolically compromised. These results show that associations between *A. muciniphila* and type 2 diabetes might be population-specific[29]. Forslund *et al.*[52] carried out a clinical study using 784 available human gut metagenomes to evaluate the effects of a popular antidiabetic drug, metformin. It was reported that the ameliorative effects of the drug could be attributed to the production of SCFAs, and the drug also induced microbiome shifts, as a relative increase in abundance of *Escherichia* species and depletion of butyrate-producing taxa were observed[52]. Diet-induced obese mice supplemented with *A. muciniphila* also presented with improved glycemic statuses (higher glucose tolerance and reduced inflammation)[53], and this change was due to lowered circulating lipopolysaccharide levels and intensified lipid oxidation[54].

RELATIONSHIPS BETWEEN BILE ACID METABOLISM, THE GUT MICROBIOTA AND TYPE 2 DIABETES

In the human liver, cholic acid and chenodeoxycholic acid are primary bile acids created from cholesterol. Gut microbiota transforms primary bile acids into secondary bile acids[55]. Deoxycholic acid, the most common and abundant secondary bile acid in humans, is converted from cholic acid by some species of *Clostridium* in the large intestine[56]. Bile acids are involved in glucose metabolism as signaling molecules and cellular receptor ligands. They activate both nuclear farnesoid X receptor (FXR) and the membrane-bound, G-protein-coupled receptor 1[57]. Through FXR, bile acids can suppress the *in vitro* expression of fructose-1, 6-biphosphatase-1, gluconeogenic phosphoenolpyruvate carboxykinase and glucose-6-phosphatase[58]. When the FXR gene was knocked down in *ob*/*ob* mice, diet-induced weight gain, hyperglycemia and glucose tolerance were observed. There was also higher glucose clearance and improved insulin sensitivity in adipose tissues[59]. FXR also plays a role in weight loss maintenance and improvement in glucose tolerance after vertical sleeve gastrectomy by increasing the systemic concentrations of bile acids and shifts in gut microbiota composition[60]. Activation of G-protein-coupled receptor 1 in enteroendocrine L cells induces the release of glucagon-like peptide-1, which is correlated to improvements in hepatic and pancreatic function. In addition, increased glucose tolerance in obese mice was also observed[47]. Bile acid sequestrants have been utilized to throw the enterohepatic circulation of bile acids into disarray by binding with bile constituents, thereby preventing gut reabsorption. This results in the reduction of low-density lipoprotein cholesterol. In type 2 diabetes patients, these molecules have also been shown to improve glycemic conditions through modulation of the gut microbiota and bile acid pool composition. These resulted in improvements in hepatic glucose metabolism and increased secretion of incretin hormones[56,61].

TYPE 1 DIABETES IN HUMANS

At present, little is known about the relationships between type 1 diabetes and the gut microbiota. A Diabetes Prediction and Prevention study reported that children with type 1 diabetes in Finland have lower relative abundances of Firmicutes and increased Bacteroidetes. In healthy individuals, within the Bacteroidetes phyla, the *Bacteroides ovatus* species represented >20% of the total increase as compared with the type 1 diabetes patients. Though that study was small, the findings reported the first-line evidence of specific changes in the gut bacteria in humans with type 1 diabetes[62]. In a clinical study carried out by Bosi *et al.*[63], where intestinal abnormalities were observed in 81 type 1 diabetes patients and 40 healthy subjects, it was shown that intestinal permeability was significantly increased in the type 1 diabetes patients as compared with healthy individuals, indicating that poor intestinal barrier function could contribute to type 1 diabetes pathogenesis. There are also larger cohort studies, such as The Environmental Determinants of Diabetes in the Young, which are currently in progress that further define alterations in the human gut microbiota composition, and the underlying mechanisms that lead to autoimmunity and onset of type 1 diabetes[64].

GUT MICROBIOTA-MEDIATED INTESTINAL PERMEABILITY

It has also been proposed that increased gut permeability might result in pancreatic β-cell damage due to the increased absorption of exogenous antigens[65]. Gut microbes are also reported to affect gut permeability, and thereby are important in type 1 diabetes pathogenesis[3]. Some microbial toxins have been reported to directly impair pancreatic β-cell function[66]. For example, the injection of *Streptomyces* toxin and bafilomycin A1 resulted in smaller sized islets and lowered pancreatic β-cell mass, and at the same time, impaired glucose

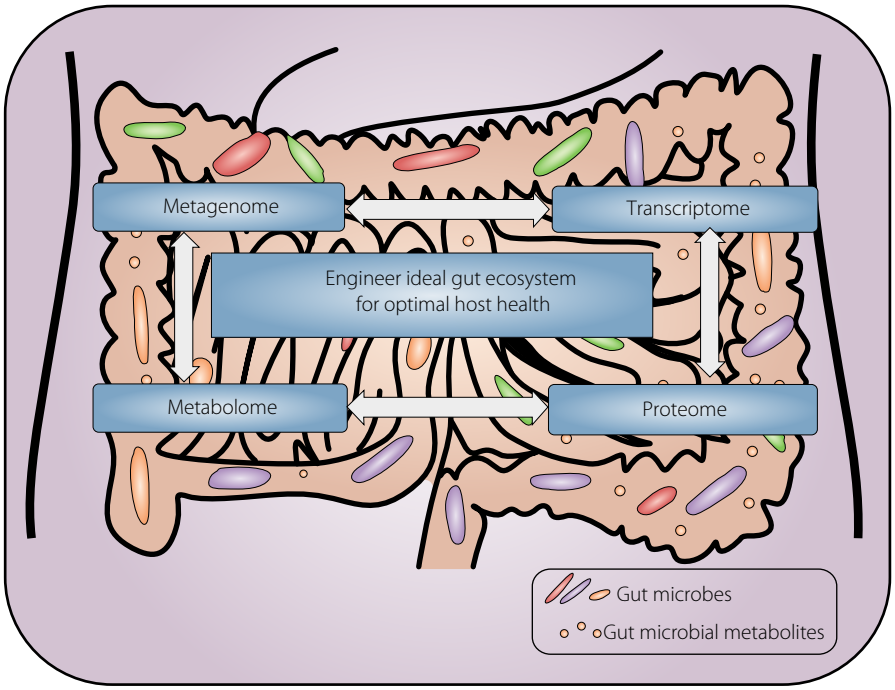

Figure 2 | The fusion of the information derived from microbiome, transcriptome, proteome and metabolome platforms. This information will allow us to understand the intricate interplay between gut microbiota and the host metabolism to suggest appropriate lifestyle and nutritional interventions by engineering an optimal gut environment towards the prevention and maintenance remission of diabetes.

tolerance[66]. There are several other microbial toxins, such as streptozotocin, that have been used in diabetes induction in mice[67]. Additionally, it was reported that wild-type non-obese diabetic (NOD) mice presented with type 1 diabetes, whereas NOD mice lacking MyD88 did not; yet germ-free MyD88-negative NOD mice developed type 1 diabetes, and this was attenuated when the mice were colonized with normal gut microbes[27].

TYPE 1 DIABETES IN ANIMAL MODELS

Diabetes-prone biobreeding rat

The role of gut microbiota in mechanisms of type 1 diabetes autoimmunity have been reported in studies involving animal models. Current data show that fluctuations in gut bacteria can be discerned before type 1 diabetes onset in both the diabetes-prone biobreeding (BBDP) and LEW1.WR1 rat. Brugman *et al.*[68] provided the first evidence showing the role of gut microbes in type 1 diabetes pathogenesis in the rat. It was observed that BBDP rats that progressed to type 1 diabetes had lower abundances of *Bacteroides* as compared with healthy rats. Antibiotic administration in combination with hydrolyzed casein dietary intervention attenuated type 1 diabetes through mechanisms correlated with gut microbiota modulation[68]. In addition, BBDP rats had lowered abundances of *Lactobacillus* and *Bifidobacterium* as compared with healthy diabetes-resistant biobreeding rats, implying that the gut microbial composition of the BBDP rat might be predisposed to type 1 diabetes[69]. The reasons behind the alterations in the gut microbiome of BBDP rats are currently unclear. However, as this murine model presents with severe lymphopenia, and as the immune system can significantly shape the gut microbial environment[68], the abnormal immune system present in this model could most probably be a result of the changes in the microbiome. However, the correlation between the altered intestinal microbiome and disease occurrence in the BBDP model is currently unknown[19]. The transfer of intestinal *Lactobacillus johnsonii* N6.2 from diabetes-resistant biobreeding rats to BBDP rats corresponded with a bacteria-specific delay in disease pathogenesis[44] through a mechanism that might involve upregulation of T helper 17 cells[70]. These findings are similar to the study where disease prevention and the upregulation of intestinal T helper 17 cells were observed after segmented filamentous bacteria were naturally transmitted to the NOD mouse[71]. These data propose that bacteria ameliorated disease states in both the aforementioned BBDP and the NOD murine models.

LEW1.WR1 rat

There have been experiments addressing the possibility that parvovirus Kilham rat virus infection results in alterations in the gut microbiota community as increases in the abundance of the Actinobacteria phylum and the *Bifidobacterium* genus were observed[72]. A temporal increase in the abundance of *Clostridium*, and fluctuations in bacterial community ratios shortly after infection suggests that gut microbiota might be involved

in destruction of islets in LEW1.WR1 rats[72]. These changes in gut microbiota composition after infection are most probably a result of the pro-inflammatory state activated by Kilham rat virus in Peyer's patches and other lymphoid organs[73]. It was also reported that treatment with the broad-spectrum antibiotic, Sulfatrim, downregulates Kilham rat virus-induced innate and adaptive immune responses, thereby preventing insulitis and islet destruction[72]. After infection, Sulfatrim treatment also lowered expression levels of interferon regulatory factor 7, C-X-C motif chemokine 10, interleukin-17A, and interleukin-6 in pancreatic lymph nodes and Peyer's patches[72]. These observations show that gut microbiota can shape innate and adaptive immunity beyond the gut. The data obtained from these murine models are consistent with earlier data derived from other diseases, and as such are clinically important as they they that gut microbiome engineering could potentially be used to intervene in disease onset.

Therapeutic Interventions in Diabetes using Prebiotic or Probiotic Dietary Supplementation

Amar *et al.*[48] proved that *Bifidobacterium animalis* subsp. *lactis* 420 could revert a low-grade inflammatory response[48]. In a randomized, placebo-controlled and parallel designed study by Asemi *et al.*[74], a multiprobiotic oral supplement was provided to test participants for 8 weeks. Multiprobiotics intake significantly reduced fasting plasma glucose, and improved oxidative status in type 2 diabetes patients as compared with the placebo group[74]. Concentrations of erythrocyte superoxide dismutase, glutathione peroxidase and total anti-oxidants were higher in the probiotic yoghurt group as compared with controls[74], providing evidence that probiotics exert anti-oxidative effects in type 2 diabetes patients. *Lactobacillus acidophilus* and *Lactobacillus casei* dietary intervention significantly attenuated streptozotocin-induced oxidative pancreatic damage by suppressing lipid peroxidation and formation of nitric oxide. Ejtahed *et al.*[75] also showed that probiotic dahi dietary intervention in the diets of a high-fructose-induced diabetic rat model, the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia and oxidative stress were significantly improved[75]. Nerstedt *et al.*[76] also documented that the administration of *L. acidophilus* NCFB1748 and *Lactobacillus paracasei F19* to germ-free mice enriched the colonization of the probiotic strains in the ileum as compared with the colon, and upregulated secretion of insulin-sensitizing hormones: adipsin and adiponectin were also observed[76].

CONCLUSIONS

The studies included in the present review emphasize that diabetes pathogenesis could be a result of specific pathogens, but metabolites produced by gut microbiota, such as bile acids, also play an important part. However so, the exact impact of gut microbes and their metabolites on the incidence and pathogenesis of diabetes have yet to be clearly elucidated. Taking these into consideration, we strongly stand by the methodology of integrating metagenomic and metabolomic information, as it is an important tool that aids further understanding of the gut microbiota–host metabolic flux ecosystem. As diabetes is multifactorial and can progress to other related metabolic diseases, such as cardiovascular problems, it is of utmost importance that the delicate interrelationships between gut microbiota and host metabolism are well understood in order to suggest appropriate lifestyle and nutritional interventions by engineering an optimal gut environment towards the prevention and maintenance remission of diabetes (Fig. 2). The fusion of the information derived from these omics platforms will allow us to understand the complex mammalian superorganism to a deeper extent. These measures would also contribute greatly towards promoting the optimal host health of society as a whole, and ensuring a higher quality of life for everyone.

DISCLOSURE

The authors declare no conflict of interest.

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