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DIABETES AND MICROBIOTA

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Abstract – This article reviews the main literature data published between April 2021 and March 2022 in the field of microbiota in diabetes. During the last year the microbial signature that separated type 1 diabetes patients from healthy subjects was determined as a potential early diagnostic sign. Moreover, the core gut microbial features, that could be associated with T2D risk, was identified by using Interpretable Machine learning framework with large-scale human cohort studies. In addition, the effect of prolonged use of antibiotics on perturbation of gut microbiota and gut permeability was noted as possible mechanism by which antibiotics potentially could lead to diabetes. Furthermore, importance of dietary fibers and short chain fatty acids (SCFAs) for prognosis of type 2 diabetes was evaluated. The results revealed that plant extracts could help to alleviate the gut microbiota composition alterations associated with T2D by increasing the abundance of beneficial and particularly SCFAs-producing bacteria.

Keywords: Gut microbiota, Diabetes, Dysbiosis, Short chain fatty acids (SCFAs).

INTRODUCTION

Modern times, together with new technologies, also increased the incidence in diseases tightly associated with modern lifestyles, with diabetes, among others, as potential 21st century epidemic disease[1,2]. According to data of International Diabetes Federation, number of diabetes mellitus patients has reached 537 million adults (20-79 years) and it is estimated to rise to 643 million by 2030 and 783 million by 2045[3]. Diabetes mellitus is metabolic disease, characterized by hyperglycemia, caused by inadequate insulin secretion, insulin action, or both[1]. Resulting chronic hyperglycemia is strongly connected with long-term damage, loss of function, and failure of numerous organ systems, including kidneys, nerves, heart and blood vessels. Type 1 diabetes (T1D) is referred to as an autoimmune disease, caused by a proinflammatory response against beta pancreatic cells[4]. Type 2 diabetes (T2D) is characterized by insulin resistance that contributes to higher demand of peripheral tissues for insulin, and eventually leading to functional failure of beta cells[5]. Apart from genetic factors, lifestyle and environmental factors that include diet and exposure to viruses, as well as dysbiosis of gut microbiota play a huge role in the development and onset of the disease[6,7].

GUT MICROBIOTA DYSBIOSIS AS POTENTIAL MARKER FOR EARLY DIAGNOSIS OF DIABETES

In the last year, it was shown that high *Firmicutes*/*Bacteroidetes* ratio, as well as instability of microbial composition, can be potentially used as an early sign of ongoing autoimmune disease development[8]. Moreover, distinct microbial signature that separated T1D subjects from healthy control group, was determined, further devising a model with possibility of

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making a difference between two aforementioned groups using only microbiome features. On functional level, several metabolic pathways that were significantly lower in adults with T1D were pinpointed, while several taxa and metabolic pathways were connected with host's glycemic control, suggesting additional mechanistic studies in order to identify the role of these bacteria for potential therapeutic strategies. The taxa most indicative of T1D, such as *Prevotella copri*, as well as *Ruminococcus gnavus* as a key taxon indicative of a healthy state, were discovered[9].

Novel approaches, using Interpretable Machine learning framework with large-scale human cohort studies, were designed with the aim of identifying core gut microbial features that could be associated with T2D risk[10].

A recent study also tried to link gut microbiota with plasma glucose levels and gestational diabetes mellitus (GDM), showing the relationship of second trimester of pregnancy altered gut microbiota composition before the GDM diagnosis and fasting serum level of metabolites, which may inform the diagnosis, prevention and treatment of GDM[11].

ANTIBIOTICS USE AS A CAUSE OF DYSBIOSIS AND DIABETES

Antibiotics affect gut microbiota and gut permeability, so it could be the possible mechanism by which antibiotics potentially could lead to diabetes, especially in already vulnerable populations[12,13]. One study found positive correlation between the use of narrow spectrum penicillins and prevalence of T1D[13]. More importantly, gut microbiota perturbation leads to depletion of short chain fatty acids (SCFAs), with a potential onset of increased insulin resistance and impaired glucose tolerance, which are both precursors to diabetes mellitus[14]. The use of plant extracts, like the one from *Berberis kansuensis* (BK extract), were found as helpful in alleviating symptoms of T2D in rat models, by regulating gut microbiota composition after antibiotic treatment[15]. The results showed that BK extract significantly reduced the body weight, and additionally reduced blood glucose, improved insulin resistance and inflammation, and increased insulin sensitivity. Also, it has been shown that BK extract improves the gut microbiota imbalance in T2D, by increasing the *Firmicutes*/*Bacteroidetes* ratio[15].

IMPORTANCE OF DIETARY FIBERS AND SHORT-CHAINED FATTY ACIDS

Humans, like many other animals, do not possess the enzymatic machinery necessary to metabolize some of the polysaccharides ingested through diet. However, a mutually beneficial interaction between animal hosts and specific microbial communities evolved over time that allowed hosts to tap into the nutritional value that those kinds of macromolecules hold. Those microbial communities reside mostly in the lower intestine where they have access to the undigested polysaccharides. It is through fermentation in the lower intestine that these microbes produce molecules that the host can take up and draw nutritional, metabolic and physiological value from. Most important end products are SCFAs, most notable of which are acetate, propionate and butyrate. These small organic molecules interact with the host in different ways. Butyrate is mostly metabolized locally by the epithelial cells of the colon for energy purposes, whereas acetate and propionate are transported to more distant places in the host, where they either act as substrates for gluconeogenesis and lipogenesis or act as important signaling molecules affecting numerous important processes in the body. Apart from having local metabolic effect within the gastrointestinal system, the gut microbiota can, thanks to various metabolites (e.g., SCFAs), exert changes in function of numerous endocrine organs, leading to various health disorders, including metabolic disorders and diabetes[16].

It has been examined how beneficial some foods and polysaccharides originating from those foods are in addressing metabolic disorders like diabetes. A recent study[17] investigated the potential hypoglycemic effect of Coix seed polysaccharides (CSP) on a mouse model of T2D with the aim of elucidating the underlying mechanisms of action of these polysaccharides. Treating the mice with CSP led to an increase in insulin and high-density

lipoprotein cholesterol levels, while at the same time decreasing the low-density lipoprotein cholesterol and triglyceride levels. The CSP treatment had further benefits, such as the repair of the intestinal barrier and by affected the gut microbiome composition in a way that led to increased abundance of SCFA-producing bacteria. Subsequent analysis of the colonic transcriptome showed that the hypoglycemic effect was associated with the activation of the IGF1/PI3K/AKT signaling pathway as well as with an increased abundance of SCFA-producing bacteria, further indicating their importance in regulating glucose and lipid metabolism. The increase in SCFA production upon treatment with CSP was additionally verified through GC-MS analysis of fecal SCFA levels. The authors also reported performing computational and molecular docking analysis, where they showed that SCFAs could directly bind to IGF1, PI3K and AKT[17].

Another study with similar aims was performed on *Dendrobium officinale* leaf polysaccharides (LDOP), investigating their potential use in treating sugar and lipid metabolism abnormalities, organ dysfunction and gut microbiota dysbiosis, in T2D mouse models. The potential of two fractions LDOP-A (147.45 kDa) and LDOP-B (9.91 kDa) to affect hyperglycemia, insulin resistance and other important parameters associated with diabetes was followed. Both LDOP-A and LDOP-B were shown to have positive effects on glucose homeostasis and insulin resistance. LDOP-A was shown to be more effective in modulating the energy metabolism, as indicated by its impact on body weight, food intake and lipogenesis in the mice treated with it. Pancreatic staining experiments showed that both LDOP-A and LDOP-B were capable of reducing the damage caused to the insulin-producing β-cells by a combination of a high-fat diet and administration of streptozotocin. LDOP-A supplementation led to increase in the SCFAs levels, especially that of butyrate. It is known that SCFAs like acetate, propionate and butyrate are capable of binding to FFA2 (GPR43) and FFA3 (GPR41), stimulating the release of GLP-1 in mixed colonic cultures *in vitro*[18]. Seeing as the expression of GPR41 was increased 3-fold and that of GPR43 1.5-fold upon supplementation with LDOP-A, the authors speculate that the SCFAs produced from LDOP-A and, to a lesser degree, LDOP-B may decrease inflammation inside the colon and help combat T2D. LDOP-A helped alleviate the gut microbiota composition alterations associated with T2D to some degree by lowering the *Firmicutes* to *Bacteroidetes* ratio and increasing the abundance of bacteria that are deemed beneficial, such as *Akkermansia*, *Bifidobacterium*, *Lactobacillus* and *Clostridiales*. LDOP-B did not lead to an increased abundance of SCFA-producing bacteria and the authors speculate that many of the differences between LDOP-A and LDOP-B in terms of their efficacy in treating aspects of diabetes stem precisely from this fact[19].

A number of similar studies were conducted, all of them with the aim of investigating the potential application of different polysaccharides in treating diabetes. Through metabolomics and 16S rRNA gene sequencing approaches, arabinoxylan was shown to increase the abundance of fiber-degrading bacteria and increase SCFA production, both of which were decreased in T2D rats, while at the same time decreasing the abundance of opportunistic pathogenic bacteria. In other words, it helped improve the altered gut microbiota composition in diabetes. Study also showed that arabinoxylan treatment led to the elevation of equol, indolepropionate and eicosadienoic acid levels[20]. This is significant because bacterially derived indolepropionate and other tryptophan metabolites are inversely associated with the risk of developing T2D, as shown in studies investigating the potential of dietary fibers to increase indolepropionate levels. In one such study[21], the impact of fibres on indolepropionate levels was explained by indolepropionate producing *Firmicutes* bacteria.

MODERN THERAPEUTIC APPROACHES TO TREATING DIABETES

Aside from prebiotics, such as dietary fibers, which can alter the gut microbiome composition in a favorable way, there are approaches based on probiotics as well as postbiotics. Numerous studies have shown the importance of the gut microbiome in metabolic homeostasis and, as shown above, a major way in which gut bacteria exert their positive influence on it is through the synthesis of SCFAs. It is this knowledge gained from fundamental research of the gut microbiome that has led to more innovative therapeutic approaches.

THERAPEUTICS BASED ON SHORT-CHAIN FATTY ACIDS

As described above, SCFAs play an important role in glucose and lipid metabolism, as well as in maintaining the integrity of the intestinal barrier, both of which are heavily implicated in the pathogenesis and progression of diabetes once damaged or impaired. However, because of their unfavorable pharmacokinetics, off-target effects, poor palatability and unpleasant odor, they have very limited clinical use. A recent study[22] looked into the possibility of employing enzyme metabolizable block copolymers as a way of delivering butyrate and propionate molecules in the form of esters. The polymers were designed to form self-assembling nanoparticles under physiological conditions and their therapeutic efficacy was tested in a mouse model of T2D through *ad libitum* drinking. Butyrate-nanoparticles (BNP) and propionate-nanoparticles (PNP) were tested and compared to conventional anti-diabetic drug exenatide. BNP showed an effect similar to that of exenatide in a glucose tolerance test performed on the mice treated with it, whereas the efficacy of free low molecular weight butyrate was insignificant in this regard, as well as that of PNP. In hematoxylin and eosin-stained pancreatic tissue, BNP treatment was shown to lead to an increase in the size of the Langerhans islets similar to the increase after treatment with exenatide[22].

Authors frequently cited the sustained release of SCFAs from the nanoparticles as the explanation for their higher potency in treating diabetes than that of free low molecular weight SCFAs, citing the relatively short half-life of SCFAs as well as potential toxicity of the high concentrations of SCFAs administered as free molecules as opposed to nanoparticles. It should be noted, however, that, when it comes to the comparison of nanoparticle-delivered SCFAs and free SCFAs, the mice tended to consume the free SCFAs much less than the ones bound to nanoparticles across all experiments, because of the unpleasant odor and taste. The authors cited higher consumption of the more palatable BNPs, prolonged residence time in the gastro-intestinal tract (48 h) owing to muco-adhesion and enzyme-mediated sustained release of butyric acid that led to negligible off-target effects thanks to the absence of abrupt rises in SCFA concentrations in the bloodstream as the mains reasons for the higher efficacy of BNPs than that of free SCFAs[22].

PROBIOTICS AND DIABETES

Probiotics, living microorganisms in the gut, have been shown to be able to induce various beneficial effects on the host. They have also been shown to be beneficial in numerous diseases, such as allergies, tumors, oral and intestinal disease, as well as many other. Recent studies have also indicated that probiotics supplementation could be of therapeutic benefit in chronic metabolic diseases, such as hyperglycemia, hyperlipidemia and hypertension[23].

One study[24] investigated the potential use of CFA-I (colonization factor antigen I)-expressing *Lactococcus lactis* as an orally administered probiotic with the aim to treat or prevent type I diabetes by induction or activation of T_{reg} cells specific for self-antigens. *Lactococcus lactis* expressing CFA-I (LL-CFA-I) was shown to be able to stimulate dendritic cells in a way that allows them to guide T cell differentiation in the direction of tolerogenic Treg cells, ultimately establishing a regulatory microenvironment in pancreatic tissue. Infection of bone-marrow derived dendritic cells with LL-CFA-I led to an increased production of IL-10, TGF-β and indoleamine-2,3-deoxygenase. Even though co-culturing these cells with naïve T cells did not promote Foxp3 expression, it did lead to the suppression of TNF-α and IFN-γ production and mice to which LL-CFA-I was administered orally did show an increase in IDO and TNF-β producting regulatory plasmacytoid dendritic cells in pancreatic lymph nodes[24]. Effect of probiotics in combination with plant extracts have also been investigated. A recent study[25] found that administration of *Lactobacillus casei* K11 alongside bitter gourd extract and mulberry leaf extract decreased both glucose levels as well as insulin resistance in diabetic mice and helped regulate lipid metabolism, oxidative stress and proinflammatory cytokine levels. Finally, the combined treatment led to an increase in free fatty acid receptor 2 expression, GLP-1 secretion and SCFA levels[25].

CONCLUSIONS

In this review, the importance of gut microbiota on diabetes onset and prognosis is evaluated from devastating effects of prolonged use of antibiotics to beneficial effects of plant extracts on gut microbiota, as a potential mechanism to affect diabetes (both type 1 and 2). In particular, the role of short chain fatty acids, as one of the most important microbial products influencing the host response was studied in respect on type 2 diabetes, as well as potential therapeutic agent.

Conflict of Interest

The authors declare no conflict of interest.

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