

Review Article

Gut Microbiota Related Metabolites Changes - New Break through Point for Treating Diabetes

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Introduction

Diabetes, a metabolic disease characterized by hyperglycemia due to defects in insulin secretion or impaired insulin action, is represented as metabolic disorders of glucose and usually combined with abnormal metabolism of lipids and proteins. Long-standing hyperglycemia and metabolic disorders lead to the dysfunction of the whole body, especially the eyes, kidneys, cardiovascular and nervous system. Disability and mortality are extremely higher due to the chronic damage of blood vessels and nervous system, such as diabetic foot necrosis, and renal failure, etc. However, the prevalence of diabetes seems to be unstoppable which, according to the research of Turner and others, will double in the next 20 years [1]. At present, although there are drugs applied in the clinic that can control blood sugar and alleviate the symptoms of hyperglycemia, pathogenesis of

diabetes has not been clearly explained up to this date and there is still no treatment addressing root causes for diabetes. Therefore, scientists are striving to find an effective treatment that can cure this chronic diabetes disease [2]. Following genomics, proteomics, and transcriptomics, metabolomics is another rising branch of science to study the total biochemical changes quantitatively in living organism, which has been gradually used in diabetes researches, maybe a possible breakthrough point.

Gut microbiota, which harbor in the human intestinal tract with relatively stable population, plays an important and complex role in the human micro-ecological system. They take principal part in maintaining metabolic balance of almost all kinds of basic substances for the whole human body. In this article, we review the recent researches about mechanism and emerging treatments for diabetes mellitus from the perspective of gut microbiota-related metabolites in human body.

Abstract

Diabetes is a common disease characterized by high blood glucose level, combined with metabolic disorder of carbohydrates, lipids, proteins and other substances. The relationship between the human gut microbes and diabetes is still not clear. Human intestinal tracts harbor trillions of microbes that maintaining a symbiotic relationship with the host. Gut microbiota not only helps the host to digest certain food, but also produces a large number of biologically active signaling molecules involved in immune regulation, anti-tumor, anti-aging regulation, which are also related to the pathogenesis of diabetes. These molecules can be used as targets for prediction, diagnosis and treatment of diabetes. Metabolomics, as a rising technology to study the changes of metabolites in human body quantitatively, can be a helpful tool for researchers in this area. This article reviews recent progresses about mechanism and emerging interventions of diabetes mellitus from the perspective of metabolism of gut microbiota.

Keywords: Gut microbiota; Metabolites; Diabetes; Metabolomics; Metabolic diseases

Main Points:

1. Gut microbiota participates in the pathogenesis of diabetes.
2. Metabolomic alterations in diabetes relate to gut microbiota.
3. Targeting to the gut microbiota-related metabolomic alterations in diabetes can be potential breakthrough.

Introduction of Gut Microbiota and Metabolomics

Basic Knowledge of Gut Microbiota

Intestinal microorganisms can be one of the most important parts of the human micro-ecological system. They play roles in maintaining a symbiotic relationship between microorganisms, hosts and the human intestinal environment. Studies have shown that human gastrointestinal tract contains trillions of microbes, most of which is bacteria and archaea, fungi and micro eukaryotes [3]. The entire human gut microbe contributes 10 million genes, which greatly increases the coding ability of human as a super organism. There are nine kinds of bacteria identified in the human intestine, including Actinomycetes, Proteobacteria, Cyanobacteria, Fusobacterium, Micrococcus, Absidia, Bacteroides, Spirochetes, and VadinBE97 [4,5]. Ninety-eight percent of them are classified into four kinds, which are thick-walled bacteria (64%), Bacteroides (23%), Actinomycetes (3%) and Proteobacteria (8%). In recent years, the researches about gut microbes mainly based on metagenomics and 16S rRNA gene sequencing technology. These multiple analytical techniques give us various information about diversity and function of gut microbiota, which help to open a new field to study the intestinal bacteria [6,7].

The composition and diversity of gut microbes are affected by the various internal and external environment of the host, such as the physiological gastrointestinal structure of the host, self-genotype, age, gender, health status, immune system, diet style, living environment, region, social behavior, and so on [8,9]. Among the whole microbes in intestinal tract, the microflora of the colon is in the range of 10^{10} to 10^{12} CFU/mL, is the most intensive part in the intestinal microflora. Even in the same area of intestine, microbial flora is different in the different spatial distribution. Generally, gut microbiota has three biological layers in the intestinal lumen. The deep layer is a membrane flora mainly composing of Lactobacillus and Bifidobacteria. The major components of microbiota in middle layer are anaerobic bacteria, such as Faecal bacteria, Streptococcus pneumoniae, Weirong cocci, and Eubacteria. The microbes in the surface layer can move in the intestinal lumen, called luminal flora, mainly consisting of aerobic bacteria such as Enterococcus and Escherichia coli. One third of the dry weight of the feces is the microorganisms, and more than 98% of them are obligate anaerobic bacteria, which mainly are Bifidobacteria, Bacteroides, True bacilli and anaerobic Gram-positive cocci.

Brief Introduction of Metabolomics

Metabolomics is a novel technology to comprehensively analyze small compounds in living organisms based on modern systems biology. Metabolomics technology can help to summarize the whole metabolic characteristics of biological systems, and explore the metabolic mechanisms behind physiological pathology, and screen out biomarkers for earlier stage prediction, diagnostic classification, and drug efficacy [10]. The commonly used high-throughput metabolomics detection techniques include Nuclear Magnetic Resonance (NMR), Liquid Chromatography-tandem Mass Spectrometry (LC-MS), and Gas Chromatography-Mass Spectrometry (GC-MS) [11].

In recent years, metabolomics has applied to the study of gut microbiota and their metabolites. Clinically, among all kinds of metabolic disorders, diabetes is recently considered to be tightly related to gut microbiota, which attracts scientists to apply metabolomics technologies in their researches focusing on this area.

Gut Microbiota and the Metabolites in Diabetes

In recent years, more and more studies have shown that there is an interdependent and mutually constrained dynamic balance between such huge microbial pools in the intestine with the whole human body to ensure the routine physiological activities. For example, gut microbiota offers many functions to the host, such as functionalizing as the intestinal barrier, regulating host immunity, digesting food that not absorbed by the host, synthesizing certain vitamins and hormones, and preventing the colonization of pathogenic bacteria [12,13]. If there is any disorder, the imbalance of flora will cause many intestinal diseases, such as inflammatory bowel disease, diabetes, obesity, allergies, autism, colon cancer and cardiovascular disease [14-16].

The Participation of Gut Microbiota in the Pathogenesis of Diabetes

Many recent studies have shown that the development of diabetes is associated with abnormal changes in gut microbiota. Type 1 Diabetes (T1DM), formerly known as insulin-dependent diabetes mellitus, occurs mostly in children and adolescents with characterization of a lack of adequate insulin production and elevated blood glucose levels. The disease is usually caused by the destruction of oxidative stress and autoimmune of T cell-mediated pancreatic beta cells, resulting in partial or complete loss of insulin production [17,18]. The reason for the destruction of autoimmune of beta cells is not entirely clear. Another study has highlighted the role of the Gastrointestinal (GIT) microbiome in the process of T1DM [19]. Brown et al. reported that the disorder of intestinal micro-ecological is closely related to T1DM. Compared to the control group, the ratio of Actinomycetes, Bacteroides, and Proteus for T1DM group was increased, while the abundances of Bacterium, Fusobacterium, Magnaporthe oryzae were reduced [20]. In addition, the diversity of intestinal microbiota declines in T1DM patients suggesting that the intestinal ecosystem becomes simpler [21,22]. Studies of the GIT microbiome have shown that there may be multiple pathogenesis to cause the destruction of beta cell and T1DM disease simultaneously or independently. Firstly, Immune regulation associated with microbiome ecological dysregulation may lead to destruction of beta cells in T cell-mediated genetically susceptible individuals [23,24]. Secondly, the pathogenesis of T1DM is related with chronic low-grade inflammation caused by intestinal leakage, endotoxemia and immune disorders [25,26]. Thirdly, the imbalance of microbial ecology can induce metabolic-related oxidative stress and disrupt nutritional availability in metabolic syndrome [27].

Type 2 Diabetes (T2DM), adult-onset type diabetes, accounts for more than 90% of diabetic patients, which usually occurs after 35 to 40 years of age [28]. The patients usually have the common characters of obese, insulin resistance, decreased insulin sensitivity, and inappropriate insulin secretion related hyperglycemia. The etiology and pathogenesis of T2DM are related to many factors such as heredity, race, age, environment and lifestyle. Among them, the GIT microbiota has attracted more and more attention because that T2DM is closely related to intestinal microbial disorders. It is reported that T2DM patients have more pathogens in intestinal microbiota and less bacteria to produce butyric acid [29]. Metagenomic studies have revealed that there is the accumulation of sulfate reduction and antioxidant stress in T2DM patients and the reduction of certain butyrate-producing bacteria [30]. The short-chain fatty acids, especially butyrate produced at microbial fermentation,

can enhance integrity of intestinal wall and prevent metabolic endotoxemia, inflammation related diseases. In the Non-Obese Diabetic (NOD) sterile mouse model, diabetes develops spontaneously indicating that the disease of diabetes is closely related to microbes [31]. In addition, the gut microbiome can interact with the host genetically to influence the expression of proteins associated with human health and disease [32]. Studies have shown that the changes of intestinal microbial composition are associated with the activity of intestinal proteins and alkaline phosphatase, resulting in increasing intestinal permeability for insulin resistance [33]. The feature of serum metabolic component in an insulin resistant individual is the increased level of the Branched-Chain Amino Acid (BCAA) as the intestinal microbial metabolite, which is related with the gut microbiota [34]. S Shi H et al. have found that gut microbiota can also trigger inflammatory responses in patients with obesity and insulin resistance by secreting Lipopolysaccharide (LPS) [35].

Metabolomic Alterations in Diabetes

Diabetes is a kind of endocrine system disease with metabolic disorders of sugar, lipids, and proteins, especially the glucose metabolism dysfunction. Therefore, metabolomics is a very suitable approach to study the diabetes [36]. By comparing serum metabolomics of normal glucose tolerance patients with that of T2DM, scientists found that T2DM patients have certain metabolic abnormalities in sugar, lipid metabolism, amino acids [37]. At present, it is generally accepted that the level of serum BCAA as intestinal microbial metabolites is closely related to Insulin Resistance (IR), which could be a biomarker to predict DM [38]. The studies have found that the mechanism may be related to the low activity of mTOR and INK, and the rapamycin as the mTOR inhibitor could reverse the IR that caused by BCAA [39]. Some researchers have used LC/MS to quantify the concentration of alkylresorcin metabolite 3(3,5-Dihydroxyphenyl)-1-Propionic Acid (DHPPA) in plasma for type 2 diabetes and impaired glucose-regulated patients. It is possible that DHPPA is a protective factor for those patients when analyzing the quantitative data via the multivariate logistic regression method [40]. Some researchers have found that the acetate level in the stool of diabetes patients is also higher using metabolomics method, which could activate parasympathetic nerve and stimulate the islet B cells to secrete more insulin. And then it leads to the hyper-insulin glycemia, impaired glucose tolerance, and increased secretion of ghrelin in the stomach, resulting in an increased incidence of obesity and type 2 diabetes.

The gut microbiota could digest a variety of trimethylamine-containing compounds to produce Trimethylamine (TMA), which can be further oxidized by hepatic Flavin Monooxygenase (FMO) to Trimethylamine-N-Oxide (TMAO) [41]. Studies have shown that both type 2 diabetes and insulin resistant disease are associated with the level of TMAO [42]. These findings help to further understand the metabolomic alterations of diabetes and its possible relationship with gut microbiota [43].

Study of Gut Microbiota and Related Metabolites in Clinical Application for Diabetes

It is helpful to evaluate the risk of disease and diagnose in clinic by checking metabolic bio-markers. Studies have reported that both lipids and amino acids are the main bio-markers to assess the risk of diabetes [44]. Zhao et al have analyzed the small metabolites in the blood of type 2 diabetes patients based on LC-MS. They have found that certain molecules are significantly related to reduce the risk of diabetes, such as glycerol-phospho-

lipid choline, 7-Hydroxy-2,3,4,5,8-Trimethoxyisoflavan (HPMF), two tetrapeptides Metabolites (MEIR, LDYR) and an undefined small molecule (X-490). While 2-hydroxybiphenyl-matched metabolites (2HBP) and another unclear small molecular substances (X-1178) were significantly associated to increase the risk of diabetes [45]. Studies have shown that higher level of plasma TMAO is related with higher risk of newly diagnosed type 2 diabetes, and this association is not modified by the FMO3 rs2266782 polymorphism [46].

The acetate content in both blood and feces of high-fat diet rats gradually increased suggesting that acetate could be used as a potential biomarker for the diagnosis of diabetes and obesity [47]. Chen et al. have used ultra-high-performance liquid chromatography together with triple quadrupole mass spectrometry platform to quantitate serum amino acid levels in 429 diabetic patients at different stages of diabetes. They have found that the levels of five amino acids are increased in the early stages of diabetes including proline, leucine, isoleucine, tyrosine and styrene [48]. Chen et al also have found that patients with higher tryptophan level tend to present higher degree of insulin resistance and insulin secretion. In addition, serum tryptophan level is positively correlated with the risk of diabetes unveiling the potential of tryptophan as a new biomarker [49]. Impaired glucose tolerance is one of the factors for high risk of type 2 diabetes. Cobb et al. have studied all metabolites in the blood of impaired glucose tolerance using targeted metabolomics. They have found that α -hydroxybutyrate is a selective metabolite biomarker for impaired glucose tolerance patients, which could be used to predict the early stage of diabetes [50]. Zhao et al. have analyzed the level of fasting endogenous fatty acid metabolites in serum of healthy individuals, obesity, and the ones after 8 week of caloric restriction via the metabolomics method. They have found that obese patients have high risk to turn into diabetes when the level of oleic acid/stearic acid is high and the ratio of stearic acid/palmitic acid and arachidonic acid/digo-1-linolenic acid is low [51]. These findings also have demonstrated that the method to analyze metabolites via metabolomics could be a novel approach for the diagnosis of diabetes.

Studies have shown that the therapeutic way for diabetes based on gut microbiota could alleviate the symptoms of diabetes. Fecal Microbiota Transplantation (FMT) as a new therapeutic technology can improve peripheral insulin sensitivity for male patients with metabolic syndrome and increase the population of butyrate-producing bacteria in the fecal microbiota in a study for 18 people [52]. Recently, other studies have focused on niacin (Nicotinic Acid [NA] and Niacinamide [NAM]) and the gut microbiome in a group of more than 500 individuals with different metabolic phenotypes. The results have showed that regulation of NA level by microbial intervention may be an effective means to cure type 2 diabetes in the future [53]. In addition, recombinant *L. lactis* strains that genetically modified to produce GLP-1 also exhibited ability to stimulate insulin secretion and improve glucose tolerance in mice [54]. Besides that, lactobacillus yogurt can adjust diabetic status induced by streptozotocin and enhance antioxidant capacity [55]. In recent years, *Akkermansia muciniphila* has received more and more attention [56,57]. Studies in mice have shown that *Akkermansia muciniphila* can alleviate diabetes induced by high-fat diets [56]. Drugs to treat diabetes also have an effect on gut microbiota. Metformin as the most common drug to control hyperglycemia in T2DM is not clearly understood in the aspect of function mechanism. While some studies have reported that metformin can improve intestinal microbial diversity and regulate microbial activity [58,59].

Another study for 30 of T2DM patients in stable condition have also shown that slow exercise can be a way to treat T2DM by reducing the overgrowth of intestinal microbiota, intestinal leakage, and systemic inflammation [60].

With the development of science, technologies including deoxyribonucleic acid sequencing and mass spectrometry have made significant progress, which enable us to obtain more comprehensive information on intestinal ecosystems and metabolomics. We can further analyze the data and study the pathophysiological mechanisms of diabetes for prediction, diagnosis, and drug therapy. We believe that the novel exciting therapeutic method to control the status of diabetes can be realized by designing an ideal intestinal ecosystem.

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