Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Review Article

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

Jianchi Li[1,2], Huang X[3], Pan Y[2]* and Pan X[2]*

[1]Department of Orthopaedics and Traumatology, Shenzhen Tenth People's Hospital Affiliated to Jinan University, University of Chinese Academy of Sciences Shenzhen Hospital (Guangming), China

[2]Department of Orthopaedics and Traumatology, People's Hospital of Baoan District of Shenzhen, The Second Affiliated Hospital of Shenzhen University, China

[3]Shanghai Medical College, Fudan University, China

***Corresponding author:** **Yu Pan and Xiaohua Pan**
Department of Orthopaedics and Traumatology, People's Hospital of Shenzhen Baoan District, The 8th people's Hospital of Shenzhen, Affiliated Baoan Hospital of Shenzhen, Southern Medical University, The Second Affiliated Hospital of Shenzhen University, Baocheng Longjing 2nd Road No.118, Bao'an District, Shenzhen, Guangdong 518101, China.

Received: November 29, 2022; **Accepted:** December 29, 2022**; Published:** January 05, 2023

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

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.

Citation: Jianchi Li, Huang X, Pan Y, Pan X. Gut Microbiota Related Metabolites Changes - New Breakthrough Point for Treating Diabetes. J Bacteriol Mycol 2023; 10(1): 1200.

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, t 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-Chaina Mino 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 Alterationsin 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 themetabolomic 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-phospholipid 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 a-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.

References

1. Turner N, Zeng XY, Osborne B, Rogers S, Ye JM. Repurposing drugs to target the diabetes epidemic. Tlends Pharmacol Sci. 2016; 37: 379-389.
2. Chowdhury MK, Turner N, Bentley NL, Das A, Wu LE, et al. Niclosamide reduces giucagon sensitivity via hepatic PKA inhibition in obese mice: implications for glucose metabolism improvements in type 2 diabetes. Sci Rep. 2017; 7: 40159.
3. Wampach L, Heintz-Buschar A, Hogan A, Muller EEL, Narayanaswamy S, et al. Colonization and succession within the human gut microbiome by archaea, bacteria, and microeukaryotes during the first year of life. Front Microbiol. 2017; 8: 738.
4. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005; 307: 1915-1920.
5. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, et al. Diversity of the human intestinal microbial flora. Science. 2005; 308: 1635-1638.
6. Zoetendal EG, Ben-Amor K, Akkermans AD, Abee T, de Vos WM. DNA isolation protocols affect the detection limit of PCR approaches of bacteria in samples from the human gastrointestinal tract. Syst Appl Microbiol. 2001; 24: 405-410.
7. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, et al. Diversity of the human intestinal microbial flora. Science. 2005; 308: 1635-1638.
8. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO, et al. Development of the human infant intestinal microbiota. PLoS Biol. 2007; 5: e177.
9. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol. 2008; 6: 776-788.
10. Nicholson JK, Holmes E, Kinross JM, Darzi AW, Takats Z, et al. Metabolic phenotyping in clinical and surgical environments. Nature. 2012; 491: 384-392.
11. Tzoulaki I, Ebbels TM, Valdes A, Elliott P, Loannidis JP. Design and analysis of metabolomics studies in epidemiologic research: a primeron-omictechnologms. AmJEpidemiol. 2014;180:129-139.
12. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa e Melo F, Roelofs JT, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. Gut. 2016; 65: 575-583.
13. García-López R, Pérez-Brocal V, Diez-Domingo J, Moya A. Gut microbiota in children vaccinated with rotavirus vaccine. Pediatr Infect Dis J. 2012; 31: 1300-1302.
14. DeGruttola AK, Low D, Mizoguchi A, Mizoguchi E. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis. 2016; 22: 1137–1150.
15. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, et al. Gut dysbiosis is linked to hypertension. Hypertension. 2015; 65: 1331-1340.
16. Battson ML, Lee DM, Jarrell DK, Hou S, Ecton KE, et al. Suppression of gut dysbiosis reverses western diet-induced vascular dysfunction. Am J Physiol Endocrinol Metab. 2018; 314: E468-E477.
17. Arrick DM, Mayhan WG. Cerebrovascular Disease in Type 1 Diabetes: Role of Oxidative Stress[M]// Studies in Diabetes. Springer New York, 2014.
18. Wen L, Ley RE, Volchkov PV, Strangers PB, Avanesyan L, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008; 455: 1109-1113.
19. Malik A, Morya RK, Bhadada SK, Rana S. Type 1 diabetes mellitus: Complex interplay of oxidative stress, cytokines, gastrointestinal motility and small intestinal bacterial over growth. European Journal of Clinical Investigation. 2018; 48: e13021.
20. Brown CT, Davisrichardson AG, Giongo A, Gano KA, Crabb DB, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. Plos One. 2011; 6: e25792.
21. Murri M, Leiva I, Gomezzumaquero JM, Tinahones FJ, Cardona F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. Bmc Medicine. 2013; 11: 46-46.
22. Qin J, Li Y, Cai Z, Li S, Zhu J, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012; 490: 55-60.
23. Yun KL, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA. 2011; 108: 4615-4622.
24. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008; 57: 1470-1481.
25. Mcdermott AJ, Huffnagle GB. The microbiome and regulation of mucosal immunity. Immunology. 2014; 142: 24-31.
26. Gavin PG, Mullaney JA, Loo D, Cao KA, Gottlieb PA, et al. Intestinal Metaproteomics Reveals Host-Microbiota Interactions in Subjects at Risk for Type 1 Diabetes. Diabetes Care. 2018; 41: 2178-2186.
27. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biology. 2012; 13: R79.
28. McCarthy M. Nearly one in 10 US residents has diabetes, CDC reports. BMJ. 2014; 12: 348:g3962.
29. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, et al. Gut microbiota and metabolic syndrome. World J Gastroenterol. 2014; 20: 16079-16094.
30. Qin J, Li Y, Cai Z, Li S, Zhu J, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012; 490: 55-60.
31. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med. 2002; 347: 911-920.

32. Siegfried U, Shiho F, Ronald KC. Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. Molecular Metabolism. 2016; 5: 795-803.

33. Everard A, Cani PD. Diabetes, obesity and gut microbiota. Best Practice & Research Clinical Gastroenterology. 2013; 27: 73-83.

34. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016; 535: 376-381.

35. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, et al. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006; 116: 3015-3025.

36. Tzoulaki I, Ebbels TM, Valdes A, Elliott P, Loannidis JP. Design and analysis of metabolomics studies in epidemiologic research: a primer on-omic technologms. Am J Epidemiol. 2014; 180: 129-139.

37. Zhang X, Wang Y, Hao F, Zhou X, Han X, et al. Human Serum Metabonomic Analysis Reveals Progression Axes for Glucose Intolerance and Insulin Resistance Statuses. J Proteome Res. 2009; 8: 5188-5195.

38. Wang TJ, Larson MQ, Vasan RS, Cheng S, Rhee EP, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011; 17: 448-453.

39. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, et al. A branched-chain amino acid-related metabolic Signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009; 9: 311- 326.

40. Sun T, Rong Y, Hu X, Zhu Y, Huang H, et al. P1asma alkylresorcinol metabolite, a biomarker of whole-grain wheat and rye intake, and risk of type 2 diabetes and impaired glucose regulation in a Chinese population. Diabetes Care. 2018; 41: 440-445.

41. Koeth RA, Wang, Z, Levison, BS, Buffa JA, Org E, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013; 19: 576-585.

42. Oellgaard J, Winther SA, Hansen TS, Rossing P, von Scholten BJ. Trimethylamine N-oxide (TMAO) as a new potential therapeutic target for insulin resistance and cancer. Curr Pharm Des. 2017; 23: 3699–3712.

43. Patti GJ, Yanes O, Siuzdak G. Innovation: metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol. 2012; 13: 263-269.

44. Tanaka S, Tanaka S, Iimuro S, Yamashita H, Katayama S, et al. Cohort Profile: The Japan Diabetes Complications Study: a long-term follow-up of a randomised lifestyle intervention study of type 2 diabetes. Int J Epidemiol. 2014; 43: 1054-1062.

45. Zhao J, Zhu Y, Hyun N, Zeng D, Uppal K, et al. Novel metabolic markers for the risk of diabetes development in American Indians. Diabetes Care. 2015; 38: 220-227.

46. Shan Z , Sun T , Huang H , Chen S, Chen L, et al. Association between microbiota-dependent metabolite trimethylamine-N-oxide and type 2 diabetes. American Journal of Clinical Nutrition. 2017; 106: ajcn157107.

47. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, et al. Acetate mediates a microbiome-brain-B-cell axis to promote metabolic syndrome. Nature. 2016; 534: 213-217.

48. Chen T, Ni Y, Ma X, Bao Y, Liu J, et al. Branched-chain and aromatic amino acid profiles and diabetes risk in Chinese populations. Sci Rep. 2016; 6: 20594.

49. Chen T, Zheng X, Ma X, Bao Y, Ni Y, et al. Tryptophan predicts the risk for future type 2 diabetes. PLoS One. 2016; 11: e0162192.

50. Cobb J, Eckhart A, Motsinger-Reif A, Carr B, Groop L, et al. α-Hydroxybutyric acid is a selective metabolite biomarker of impaired glucose tolerance. Diabetes care. 2016; 39: 988-995.

51. Zhao L, Ni Y, Ma X, Zhao A, Bao Y, et al. A panel of free fatty acid ratios to predict the development of metabolic abnormalities in healthy obese individuals. Sci Rep. 2016; 6: 28418.

52. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Nature Reviews Gastroenterology & Hepatology. 2016; 13: 508-516.

53. Fangmann D, Theismann EM, Türk K, Schulte DM, Relling I, et al. Targeted Microbiome Intervention by Microencapsulated Delayed Release Niacin Beneficially Affects Insulin Sensitivity in Humans. Diabetes Care. 2018; 41: 398-405.

54. Vrieze A, Nood EV, Holleman F, Salojarvi J, Kootte RS, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012; 143: 913-916.

55. Yadav H, Jain S, Sinha PR. Oral administration of dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei delayed the progression of streptozotocin-induced diabetes in rats. J Dairy Res. 2008; 75: 189-195.

56. De la Cuesta-Zuluaga J, Mueller NT, Corralesagudelo V, Mejia EPV, Carmona JA, et al. Metformin Is Associated With Higher Relative Abundance of Mucin-Degrading Akkermansia muciniphila and Several Short-Chain Fatty Acid-Producing Microbiota in the Gut. Diabetes Care. 2017; 40: 54-62.

57. Plovier H, Everard A, Druart C, Depommier C, Hul MV, et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nature Medicine. 2017; 23: 107-113.

58. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nature Medicine. 2017; 23: 850 -858.

59. Forslund K, Hildebrand F, Nielsen T, Falony G, Chatelier E, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015; 528: 262-266.

60. Pasini E, Corsetti G, Assanelli D, Testa C, Romano C, et al. Effects of chronic exercise on gut microbiota and intestinal barrier in human with type 2 diabetes. Minerva Med. 2019; 110: 3-11.