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REVIEW

Application of Metabolomics in Various Types of Diabetes

Fangqin Wu, Pengfei Liang

Department of Burns and Plastic Surgery, Xiangya Hospital, Central South University, Changsha, Hunan, People's Republic of China

Correspondence: Pengfei Liang, Department of Burns and Plastic Surgery, Xiangya Hospital, Central South University, Changsha, Hunan, People's Republic of China, Tel +86-13875858144, Email liangpengfeijbm@163.com

Abstract: Metabolomics is the analysis of numerous small molecules known as metabolites. Over the past few years, with the continuous development in metabolomics, it has been widely used in the detection, diagnosis, and treatment of diabetes and has demonstrated great benefits. At the same time, studies on diabetes and its complications have discovered the metabolic markers that are characteristic of diabetes. However, the pathogenesis of diabetes has yet to be clarified, as well as no complete cure. The mechanism of diabetes has not been completely elucidated, and its eradication treatment is not available. Thus, prevention of the onset of the disease and its treatment have become very important. In this review, we focused on the recent progress in the use of metabolites in diabetes and their complications, as well as understanding the impact of diabetes metabolites.

Keywords: metabolomics, diabetes, diabetes complications, biomarkers

Introduction

Diabetes continues to wreak havoc in the world, resulting in close to 6.7 million deaths.[1] By 2045, the number of people with diabetes will reach approximately 780 million,[2] and the proportion of adults with diabetes in China will be approximately 11%.[3] Currently, the diagnostic criteria for diabetes primarily include fasting blood glucose, determination of glycosylated hemoglobin, and oral glucose tolerance testing.[4] The diabetic foot, a major complication, causes a high rate of death and disability.[5] Diabetes induces severe debilitating neuropathy, often associated with increased sensitivity to ulceration and infection, and can eventually lead to the amputation of the lower limbs.[6] As a result, early diagnosis and treatment of diabetes are especially important. Owing to the rise of metabolism, Technologies such as mass spectrometry and nuclear magnetic resonance are used in the diagnosis and treatment of diabetes, which will become a promising method.[7] Metabolomics mainly study the collection of small molecules (relative molecular weight of less than 1000). In organisms, metabolites are not only simple biomarkers, but also an important driver of metabolic processes.[8] Diabetes, a classical metabolic disease, is appropriate for metabolic research.[4] Next, an overview of metabolomics and its application in various types of diabetes will be presented.

Brief introduction to Metabolomics

Metabolomics is a discipline that emerged after genomics, transcriptomics, and proteomics. Metabolites are low-molecular-weight compounds such as amino acids, nucleotides, lipids, and sugars.[7] The main research target in metabolomics is small molecules in organisms and environments with complex surface features.[9] The "quantified" compounds have increased from nearly 17,000 to nearly 18,500, and the "unquantified" compounds have increased from about 2800 to about 3300.[10] Metabolomics has become the method of choice for biomarker discovery.[11] Metabolites play critical role as players in various physiopathological responses. First, the methylation of metabolites in DNA and RNA and covalent modification of protein post-translational modifications play a driving function. Second, metabolite-macromolecule noncovalent interactions are a second mode of regulation of cellular activity. Third, tumor metabolites can serve as prototypes of biological metabolic activity. Fourth, metabolites act as a major mediator of biological

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systems.[8] Fifth, the metabolic function of microorganisms has a special impact on the human ecosystem.[12] Metabolomics is highly sensitive, so it can be used to discover the normal and abnormal mechanisms of the body through subtle biological changes.[13] Therefore, according to the participation of metabolites in various reactions and the various metabolites produced, the development of a disease can be diagnosed, and the metabolic pathway can be interfered to achieve a certain therapeutic effect. Metabolites reflect the metabolism of the entire body's functions, and can better grasp the "big picture."

Metabolomics studies generally include sample preparation, metabolite determination, and data analysis. Sample preparation is relatively straightforward, usually carried out by noninvasive methods. Serum, plasma, and urine are the most commonly used samples for metabolic analysis and can be used to assess endogenous metabolites.[14] At present, the methods for the detection of metabolites mainly include NMR spectroscopy, liquid chromatography–mass spectrometry (LC-MS), and gas chromatography-MS (GC-MS). NMR spectroscopy does not require special sample preparation and is easier to operate than MS; MS is more sensitive than NMR.11 MS has the advantages in the detection and identification of potential metabolic markers in complex biological samples.[15] Metabolite pathways can also be more clearly understood by isotope tracing.8 Informatics and analysis techniques combined with orthogonal biological methods for analysis can be used to further expand the analysis of metabolites to understand the corresponding metabolite level.[13] Other analysis methods include fingerprint analysis and target analysis.[16,17] Some data analysis tools suitable for metabolomics have also been developed, such as a nonparametric method known as TIGER (Technical Variation Elimination with Integrated Learning Architecture), which is beneficial for future metabolomics data analysis.[18] Through metabolomic analysis and computational modeling, it was found that glutamine metabolism is related to breast cancer, and the data obtained are related to the prognosis of patients.[17] Various disease-related metabolic computing models have been developed, providing great help for disease research.

Application of Metabolomics

Diabetes is mainly divided into four categories, namely, type 1 diabetes (T1D), type 2 diabetes (T2D), special type of diabetes due to other causes, and gestational diabetes.[19]

Type 1 Diabetes (T1D)

T1D is a chronic autoimmune disease mainly caused by a decrease in the number of β-cells, leading to the decrease of insulin and production of hyperglycemia.[20] Pflueger M et al found that autoantibody-positive odd-chain triglycerides and phospholipids containing polyunsaturated fatty acids were higher in autoantibody-positive children than in autoantibody-negative children, and were independent of age at first autoantibodies. Children who developed autoantibodies before age 2 had methionine concentrations two times lower than those who developed autoantibodies in later childhood or were autoantibody-negative. And it is thought that the methionine pathway may be involved in the formation of antibodies in early infancy. Li, Z et al found that in the analysis process from insulin autoantibody seroconversion to diabetes analysis, with the rapid growth of children' height, the progression of T1D also increased.[21,22] Before the onset of T1D, the levels of lysophosphatidylcholine and methionine decreases, and the level of ceramides increases.[23] On the therapeutic side, Huff DR et al data suggest that myostatin inhibition may be a target for the effective treatment and management of the cardiometabolic and skeletal muscle dysfunctions that occur in T1DM.[24] Combination of the four omics will lead to a better understanding of the whole picture of T1D and search for more comprehensive potential biomarkers.[25] Metabolomics occupies an important position, and further work is needed to study the biological mechanism of T1D.

Type 2 Diabetes (T2D)

Diagnosis of Diabetes

T2D is characterized by insulin resistance (IR) of the body, and skeletal muscle mitochondrial dysfunction is one of the causes of IR.[26] Hyperglycemia induces progressive extensive metabolic changes in beta cells that significantly reduces mitochondrial metabolism and ATP synthesis. Many complications arise primarily from T2D, including renal failure, retinopathy, lower extremity amputations, and cardiovascular disease.[27,28] Metabolomics can determine the occurrence and development of the disease much earlier than when the corresponding symptoms of diabetes appear. Glycine and lysophosphatidylcholine were found to be the predictors of impaired glucose tolerance and T2D.[29] In recent years, some

metabolite disturbances have been discovered using metabolic techniques. Zhang et al used UPLC-oaTOF-MS technology to detect and analyze the serum of diabetic nephropathy (DN) in diabetic patients and healthy people. It was found that serum leucine, dihydrosphingosine and phytosphingosine were significantly altered, indicating that both amino acid metabolism and phospholipid metabolism were disturbed in diabetic patients.[30] In addition to altered amino acid metabolism, dyslipidemia is common in T2D, including elevated triglycerides, low level of high-density lipoprotein cholesterol, and presence of low-density lipoprotein cholesterol particles.[31] Among them, the cholesterol synthesis pathway occupies an important position in T2D.[32] Both systemic and local lipid metabolisms are altered.[6] Li et al used gas chromatography/time-of-flight mass spectrometry (GC′GC-TOFMS) combined with pattern recognition to analyze the plasma of diabetic patients and normal people in a control experiment. Through the above operations, several potential biomarkers were discovered and identified, including glucose, palmitic acid, 2-hydroxybutyric acid, and linoleic acid. An important pathophysiological factor in diabetes is free fatty acid, which also reflects the disorder of glucose metabolism or fatty acid metabolism,[33] and a large amount of free fatty acids can cause oxidative stress in the body, damaging the corresponding skeleton of cells and then leading to T2D.[33,34] Studies have also found that palmitic acid, linoleic acid, and 2-hydroxybutyric acid may play an important role in the diagnosis of diabetes.[33] Using metabolic technologies, we found that amino acid metabolism, fat metabolism, and other metabolic disorders can be diagnosed according to the corresponding metabolites, and new metabolic markers can be found. By comparing 220 patients and 216 healthy people, it was found that glutamate, oleic acid, and palmitic acid are positively correlated with diabetes, while asparagine was negatively correlated.[35] When the levels of phenylalanine and histidine are elevated, they become risk factors for T2D.[36] Although it has been found that biomarkers can be used as diagnostic criteria for diabetes, they are still limited by technology and lack of standardized compound databases for comparison and retrieval, requiring continuous exploration.

Diabetes Treatment

As a severe metabolic disease T2D, metabolomics plays an important role in drug and target therapy. The effects of metformin on different patients were evaluated using a pharmacometabolomics approach.[37] Gu et al compared 60 diabetic patients with traditional Chinese medicine (TCM) in a barbaric treatment group and the placebo group. Plasma was collected before and after the treatment, and analyzed using a comprehensive method of fingerprint analysis and target analysis. It was found that the concentrations of 13 fatty acids in the treatment group were significantly reduced, and 10 fatty acids were also statistically different from those in the placebo group. The results indicate that berberine may play a key role by downregulating the high levels of free fatty acids.[38] Studies on the mechanism of TCM in the treatment of diabetes provide an indispensable help. Studies have found that when the levels of glycine and glutamine in the blood are elevated, the risk of diabetes is correspondingly reduced.[27] Therefore, we can supplement the corresponding amino acids or reduce their content as a treatment method.

The above is mainly about the treatment of people with type 2 diabetes, and the researchers have also done some other studies in animals. Glutamine and glycine may also serve as a target for the treatment of diabetes. In addition to amino acid therapy, the corresponding enzymes can also be targeted for therapy. Dipeptidyl peptidase 4 inhibitors (DPP4i) have been used to treat T2DM and are safe and effective in most patients.[39] Miller et al knocked out mice of the hepatic glutaminase 2 (Gls2) gene and received a high-fat diet, but reduced fasting blood glucose levels compared with wild-type mice. In humans, this effect is more pronounced.[40] Altered glutamine metabolism was observed in diabetic patients, including decreased serum glutamine and alpha-ketoglutarate concentrations, but increased succinate concentrations. Control of macrophage polarization using glutamine metabolism may provide a potential target for diabetes-related pathologies.[41] Glutamine has become a hot topic in the current treatment of diabetes. Fasting blood glucose triglycerides and total cholesterol were downregulated in mice with T2DM treated with antiamylase 3. Urine was analyzed, and 29 metabolites were found, including amino acids. Finally, it is speculated that the tricarboxylic acid (TCA) cycle, amino acid metabolism, and lipid metabolism play a role in the therapeutic mechanism of RS3 on T2DM.[42] Methionine restriction reduces the visceral fat accumulation and maintains the insulin activity.[43] Diabetes is affected by many factors, among which diet therapy is also an important part of diabetes.

Diet is also especially important in diabetes treatment.Calabrese et al have shown that polyphenols are beneficial for diet to modulate the gut-brain microbiome axis, which can alter the blood glucose regulation and IR in prediabetic and diabetic patients.[44] For the design and control of the diet, it is beneficial to regulate the blood sugar level in diabetic patients and also shows potential to reverse dyslipidemia.[45]

Diabetic Complications

Diabetes is characterized by persistent hyperglycemia leading to macrovascular and microvascular complications, which is the most important cause of diabetes morbidity and mortality. However, there is no effective treatment to reverse and repair the damage to human organs.[46,47] This shows the importance of early prevention; otherwise, other organ functions may be affected. People with diabetic complications have a lower sense of smell.[48] Complications caused by oxidative stress may be reduced when the corresponding metabolite zinc is supplemented.[49] Meprin β can alter different metabolic pathways to affect diabetic complications.[50] Utilization of metabolomic high-throughput screening can provide important insights into the pathophysiological pathways of diabetes and help manage its impact.[51]

Diabetic foot, the most common complication, has altered microcirculation, muscle metabolism, and calcium metabolism in the foot skin.[52,53] Jalgaonkar MP et al found that SIRT1 and FOXO in diabetic complications can be potential therapeutic targets.[54] Flaxseed oil omega-3 fatty acids may indirectly cure DFU by improving metabolites.[55] The changes in the metabolic level before and after wound treatment are helpful to evaluate the treatment effect of diabetic complications. Hung et al treated 57 patients with diabetic foot. Among them, 38 patients were healed, and 19 patients had unhealed ulcers. Serum levels of leucine, isoleucine, arginine, and threonine were found to be significantly higher in the healed group compared to the nonhealed group.[56] A cause-to-cause cohort study of diabetic foot treatment and a cause-to-effect prospective study were also conducted. Jones et al conducted a prospective cohort study of 9 diabetic foot patients, and the treatment group showed a higher hydroxyproline concentration after supplementation with arginine, glutamine, and β-hydroxy-β-methylbutyric acid (HMB), and it was significantly higher than before and statistically significant.[57] Changes in these amino acids may become the evaluation criteria for the efficacy of diabetic foot treatment. In addition, microdialysis can provide the metabolite concentration differences, giving valuable information.[58] It makes the metabolic research of diabetic foot advance further.

DN diagnosis and treatment are also important goals, but not easy ones. Metabolomics studies search for powerful indicators by detecting small molecules in the kidney.[59] Gao Het al. Found through the study of diabetic mice and statistical analysis of patients with diabetic nephropathy, it was found that Citric acid may be a potential marker for the diagnosis of DN.[60] By comparing the blood and urine metabolites in DN in different periods, Li M et al found that the relative amount of TCA cycle intermediate metabolites in urine and serum can be used as a diagnostic indicator of renal injury.[15] The purpose of treatment can be achieved by interfering with the corresponding metabolic pathways. Activation of NO/sGC/PKG pathway by cinaciguat can improve DN and may also be a suitable treatment for T1D.[61] Lipid metabolism in podocytes plays a major role in DN. This demonstrated a novel role for JAML in regulating the podocyte lipid metabolism through SIRT1-mediated SREBP1 signaling. Therefore, JAML can be used as a therapeutic target for the treatment of DN.[62] Esmati P et al found that some amino acids and acylcarnitines were involved in the development of DN.[63] Supplementation with leucine was found to alleviate early DN as it reversed the disturbed TCA cycle.[64] Wang Z et al administered astragaloside IV to rats with DN; the results show that the metabolic disorder of functional metabolites such as amino acids was alleviated, further highlighting the application potential of metabolomics in DN.[65]

Diabetic patients may develop eye diseases, such as retinopathy, cataract, and iritis.[66] Diabetic retinopathy is the most important and typical complication of diabetic eye diseases. The prevalence of diabetic eye diseases is increasing, and it has become a major cause of blindness. Although early prevention of the disease is important, current screening methods include primary care only. This highlights the growing importance of metabolomics. However, the use of metabolites in this area of treatment is relatively small compared to diabetic foot and DN.[67] At present, studies on the metabolomics of diabetic eye diseases are still in the initial stage. Through the analysis of aqueous humor, plasma, and other body fluids and related major metabolic pathways, it is slowly entering the diagnosis and treatment phase.[68] Aiello LP et al showed that intensive glycemic control reduces the risk of surgery in diabetic patients with T1D.[69] We can try to screen other

metabolites and interfere or supplement through metabolomics to prevent and treat diabetic eye diseases and avoid serious impact on patients' lives.

Gestational Diabetes Mellitus (GDM)

GDM is common in pregnancy, and its prevalence is increasing in young women.[70] It has adverse effects on pregnant women and fetuses. When OGTT and a glucose challenge test were used to screen for diagnosis, the results were found to be insignificant.[71] There is no better method for early screening. GDM is a metabolic disease, so metabolomics has become a potential screening method. It can detect the changes in small molecules in the mother. GDM maternal plasma and the corresponding lipid extracts were searched for pre- and post-diagnostic metabolic biomarkers using NMR spectroscopy. The results showed comparable classification performance for both the plasma and extracts, and plasma enabled direct and faster analysis.[72] Hsa_circRNA_102682 can be used as a predictive marker for GDM, which may be involved in the regulation of fat metabolism.[73] When GDM blood sugar returns to normal, lipid metabolism still has metabolic disorders.[74] Metabolomics has strong operability and high performance. Miettinen HE et al used gas liquid chromatography to analyze the ratio of squalene and noncholesterol sterol to cholesterol serum and umbilical cord blood with GDM, and concluded that when GDM controls blood sugar, it does not affect neonatal cholesterol metabolism.[70,75] However, larger sample sizes are required for testing. According to the metabolite inference, it can also be determined to participate in certain metabolic pathways. Sun et al conducted a controlled experiment in 42 pregnant women with GDM and 39 normal pregnant women. Statistically significant differences were observed in the concentrations of 41 metabolites detected between groups, mainly in the lysine degradation pathway and aminoacyl tRNA biosynthesis pathway. It was also found that the levels of some amino acids and fat metabolism increased in GDM.[76] Tan et al successfully constructed a discriminant model for distinguishing the metabolic characteristics of GDM patients and PE pregnant women with a strong discriminative ability. The characteristic metabolites that can be screened can reflect various disorders of patients as early as possible, providing a certain reference and help for the discussion of the occurrence, development, and treatment of diseases.[77] Although the model is not very complete, it is a big step forward for GDM research. Changes in plasma metabolites involved in purine degradation, fatty acid oxidation, and IR during the early pregnancy are associated with the subsequent development of GDM.[78] In the first few years of diabetes onset, various metabolic disorders represented by AA dysregulation already existed and appeared in the early postpartum period of GDM. The properties of predicted metabolites take precedence over the results of some clinical parameters.[79] There are differences in the amount and nature of metabolism between the DM in pregnant and nonpregnant DM.[80] Feng Y et al found that the IR of GDM has a certain relationship with iron metabolism, and its changes may be related to the pathogenesis of GDM.[81] Lipids and triglycerides in GDM plasma have been altered for at least 10 weeks; however, the consequences and mechanisms of these differences are unknown.[82] Studies have shown that the pathogenesis may be different in the second trimester and the third trimester.[83] All of the above are studies of pregnant women with diabetes. Although the pathogenesis and mechanism of GDM are still unclear, further investigation is needed, but the information obtained now will provide certain directions for the future development of GDM. Miao M et al found that inulin improves blood glucose and fat metabolism by activating glucose transport through the translocation of GLUT4, which is mainly due to the decreased expression of RETN and the enhanced phosphorylation of IRS and Akt in GDM mice, allowing the repair of insulin signaling pathway.[84]

Conclusions

Studies on metabolomics is a problem that cannot be ignored under the historical conditions of increasing number of diabetics. In recent years, with the rapid development of metabolomics technology, metabolomics has also become an important research topic. Metabolites participate in the entire "metabolic chain" and they run through and have varying degrees of impact on other omics. When the detection technology of metabolomics is combined with computational biology and orthogonal experiments, the researchers could screen the metabolites of diabetes and speculated the metabolic pathways. Based on the existing foundation to treat diabetes and its complications, the diagnosis and treatment of diabetes have made great progress. Some representative potential metabolite disorder markers are currently screened (Table 1). However, there are still some shortcomings. For some diagnostic metabolic markers of diabetes, a large

Table 1 List of several metabolic markers of diabetes included in this review

List of Several Metabolic Markers of Diabetes Included in this Review	Disorder of Amino Acid Metabolism	Disorders of Fatty Acid Metabolism
Type 1 diabetes	Methionine↓	Lysophosphatidylcholine↓, Ceramide↑
Type 2 diabetes	Glycine↓, Asparagine↓, Glutamate↑, Phenylalanine↑, Histidine↑, Glutamine↓, Leucine↓.	Lysophosphatidylcholine ↓ Dihydrosphingosine↓, phytosphingosine↓, triglycerides↑, HDL cholesterol↓, LDL cholesterol particles↓, palmitic acid↑, linoleic acid↑, 2-hydroxybutyric acid.
Diabetic complications		
1) Diabetic foot	Leucine↓, Isoleucine↓, Arginine↓, Threonine↓ Glutamine↓	β-Hydroxy-β-methylbutyric acid↓.
2) Diabetic kidney	Citrate↑Leucine↓	Acylcarnitine↑.
3) Diabetic eye	/	/
Gestational diabetes mellitus	Lysine↓	Triglycerides↑

Notes: "↑" indicates positive correlation, "↓" indicates negative correlation.

number of cohort studies still needed to be validated to ensure their feasibility. In addition, we also need to consider the differences in some metabolites between the experimental animals and humans. When animal experiments are successful, human validation is essential, and a certain degree of safety must be guaranteed. Finally, because the influence of metabolism is multifactorial, it may be endogenous or exogenous. When some influencing factors change, the metabolism will change accordingly, and the metabolites produced will be different. This is also a major difficulty in metabolomics research. Nevertheless, we can identify the main influencing factors and metabolites to detect the pathophysiological process, etiological mechanism, early prevention, and evaluation of treatment effects. By intervening on its main influencing factors, the purpose of treating the disease can be achieved. Now, some success has been achieved by the researchers. However, the research on diabetes is still far from enough, and it still needs to be supplemented by subsequent researchers. The future prospect of metabolomics in diabetes research is not only that it can provide special information, but also that it is linked to biological systems and has important implications for disease research.

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Disclosure

The authors report no conflicts of interest in this work.

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