Current progress in metabolomics of gestational diabetes mellitus

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Conflict-of-interest statement: The authors declare no conflicts of interest for this manuscript.

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Abstract

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders of pregnancy and can cause short- and long-term adverse effects in both pregnant women and their offspring. However, the etiology and pathogenesis of GDM are still unclear. As a metabolic disease, GDM is well suited to metabolomics study, which can monitor the changes in small molecular metabolites induced by maternal stimuli or perturbations in real time. The application of metabolomics in GDM can be used to discover diagnostic biomarkers, evaluate the prognosis of the disease, guide the application of diet or drugs, evaluate the curative effect, and explore the mechanism. This review provides comprehensive documentation of metabolomics research methods and techniques as well as the current progress in GDM research. We anticipate that the review will contribute to identifying gaps in the current knowledge or metabolomics technology, provide evidence-based information, and inform future research directions in GDM.

Key Words: Gestational diabetes mellitus; Pregnancy; Metabolomics; Biomarker

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disorders of pregnancy. As a metabolic disease, GDM is well suited to metabolomics study, which can monitor the changes in small molecular metabolites induced by maternal stimuli or perturbation in real time. This review provides comprehensive documentation of metabolomics research methods and techniques as well as the current progress in GDM research. We anticipate that the review will contribute to identifying gaps in the current knowledge or metabolomics technology, provide evidence-based information, and inform future research directions in GDM.

INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders of pregnancy and is defined as any degree of carbohydrate intolerance that occurs or is first recognized during pregnancy[1]. The prevalence of GDM has been rising worldwide, including in China, in the past few decades[2] and its prevalence ranges from 9.3% to 25.5% worldwide[3]. GDM can cause short-term and long-term adverse effects in pregnant women, including an increased risk of macrosomia, preterm delivery, preeclampsia, cesarean section, neonatal hypoglycemia, hyperbilirubinemia, respiratory distress syndrome, type 2 diabetes (T2D), hypertension, metabolic disorders, and even cardiovascular disease[4-8]. In addition, GDM also has a far-reaching impact on the health of offspring. Increasing data have shown that maternal hyperglycemia is positively correlated with health problems in offspring, including an elevated incidence of obesity[9], T2D, metabolic syndrome, hypertension[10], dyslipidemia, insulin resistance (IR), cardiovascular disease[11], autism, *etc*[12]. Therefore, given the unique treatment time window provided by pregnancy, early identification of high-risk individuals and good glycemic control in patients with GDM not only provide benefits in the short term but also reduce the occurrence of complications, in the long run, thus benefiting the next generation.

Systems biology inaugurated a new era of biological and biomedical research, which is different from the traditional one-gene-at-a-time approach, which discovers detailed molecular functions of individual genes or proteins[13]. Systems biology includes genomics, transcriptomics, proteomics, and metabolomics to comprehensively research the changes in biological systems under physiological and pathological conditions[14]. Among them, the concept of metabolomics was first put forward by Nicholson *et al*[15] in 1999. Metabolomics is the qualitative or quantitative analysis of a large number of small molecular metabolites (MW < 1000 Da) that are intermediate or final products of all the metabolic pathways in a living organism[16]. Therefore, the biggest difference between metabolomics and other 'omics' disciplines is that it directly reflects the activities that are occurring or have already taken place in the body, while genomics, transcriptomics, and proteomics explain the possible activities and processes in the body.

Metabolomics has the following characteristics: (1) Small changes in gene and protein expression will be magnified in metabolites, which makes the detection much easier; (2) Nonfunctional changes in genes and proteins will not be reflected in metabolites, so they play a "noise filtering" role in the process of transmitting information from upstream to downstream; (3) The number of metabolite varieties is far less than that of genes and proteins, and the molecular structure is much simpler; and (4) The structure of metabolites is similar in various organisms, so the study technology used in metabolomics is in common[17]. In the past decade, metabolomics research has resulted in rapid progress in various fields of life science, such as detecting biomarkers, diagnosing and treating diseases, interpreting life phenomena, exploring disease mechanisms, developing drugs, and promoting human cognition of life phenomena[18].

In previous studies, many risk factors for GDM have been identified, including advanced maternal age, family history of diabetes, prepregnancy overweight and obesity, and genetic components[14]. However, the etiology and pathogenesis of GDM

are still unclear. As a metabolic disease, GDM is very suitable to be studied by metabolomics, which can monitor the changes in small molecular metabolites induced by maternal stimuli or perturbation. The application of metabolomics in GDM can discover diagnostic biomarkers, evaluate the prognosis of the disease, guide the application of diet or drugs, evaluate the curative effect, and explore the mechanism. This article reviews the metabolomics research methods and techniques as well as the current progress in metabolomics in GDM research. We anticipate that the review will contribute to identifying gaps in current knowledge or in metabolomics technology, provide evidence-based information, and inform future research directions in GDM.

METABOLOMICS RESEARCH METHODS AND TECHNIQUES

Metabolomics research methods

Metabolomics detects a complete set of small molecular metabolites in cells, body fluids, or tissues based on modern analytic techniques with high throughput, high sensitivity, and high accuracy and analyzes the association of metabolites with pathophysiological changes under the influence of internal or external factors, which reflect the "end result" of environmental exposures, disease invasion, drug treatment, and genetic variation at a certain time in the organism[19,20].

At present, the two most commonly used strategies for metabolomics analysis are untargeted metabolomics and targeted metabolomics[21]. Untargeted metabolomics, also known as global metabolomics profiling, means that all or most possible metabolites are unbiasedly qualitatively or quantitatively analyzed in given biological samples[22,23]. This approach will be applied when it is not known which metabolites are of importance in the research question or when the aim is to discover unknown physiological patterns since it can produce a large number of complex molecules, provide directions for identifying biological markers and metabolic pathways, and offer a better understanding of internal metabolic physiology by high-throughput methods[24,25]. In contrast, targeted metabolomics refers to dozens to hundreds of metabolites, usually a specific class of metabolites with similar physicochemical properties (such as carbohydrates, lipids, and amino acids) or a class of metabolites involved in the same biochemical pathways (such as gluconeogenesis, beta-oxidation, or the citric acid cycle), being analyzed[26,27]. It is usually used to identify new biomarkers or deeply investigate metabolite functions and pathways as well as the relationship between metabolites and diseases[28]. However, though widely applied, this approach is often biased, artificially amplifying the effects of selected metabolites on the performance of the biological system and neglecting the metabolites not in the selection[24,28,29].

Due to the comprehensive nature of metabolomics, careful consideration of the following aspects is required: Selection and storage of the sample specimens, technology for the extraction of the metabolites, quality control of the analyses, and statistical methods to determine the metabolites undergoing significant change[30]. The basic procedures of metabolomics research consist of the experimental design, sample collection and pretreatment, metabolite extraction and detection, original data acquisition and processing, bioinformatics and statistical analysis, and annotation or identification of metabolites[31]. The main analysis workflow of the metabolomics analysis is shown in Figure 1.

Metabolomics research techniques

Human metabolites include thousands of known and unknown small molecular metabolites whose properties of polarity, size, and concentration vary enormously, ranging from low-molecular-weight, hydrophilic, polar metabolites (*e.g.* amino acids) to higher-molecular-weight, hydrophobic, nonpolar metabolites (*e.g.* lipids)[32]. This diversity brings great technical challenges to the detection, identification, and quantification of metabolites[33]. At present, there is no analytical method that can simultaneously detect or quantify all metabolites in human biological samples. Scientists try to use a variety of analytical techniques to analyze various complicated metabolites, among which nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the two most widely used techniques in the field of metabolomics[24, 34].

NMR technology is a spectral technique that converts resonant frequencies into molecular chemistry and structure information by making use of the different resonance frequencies produced by different nuclear absorption radiation[35]. It does not need chromatographic processing, and the sample preparation is simple. It can
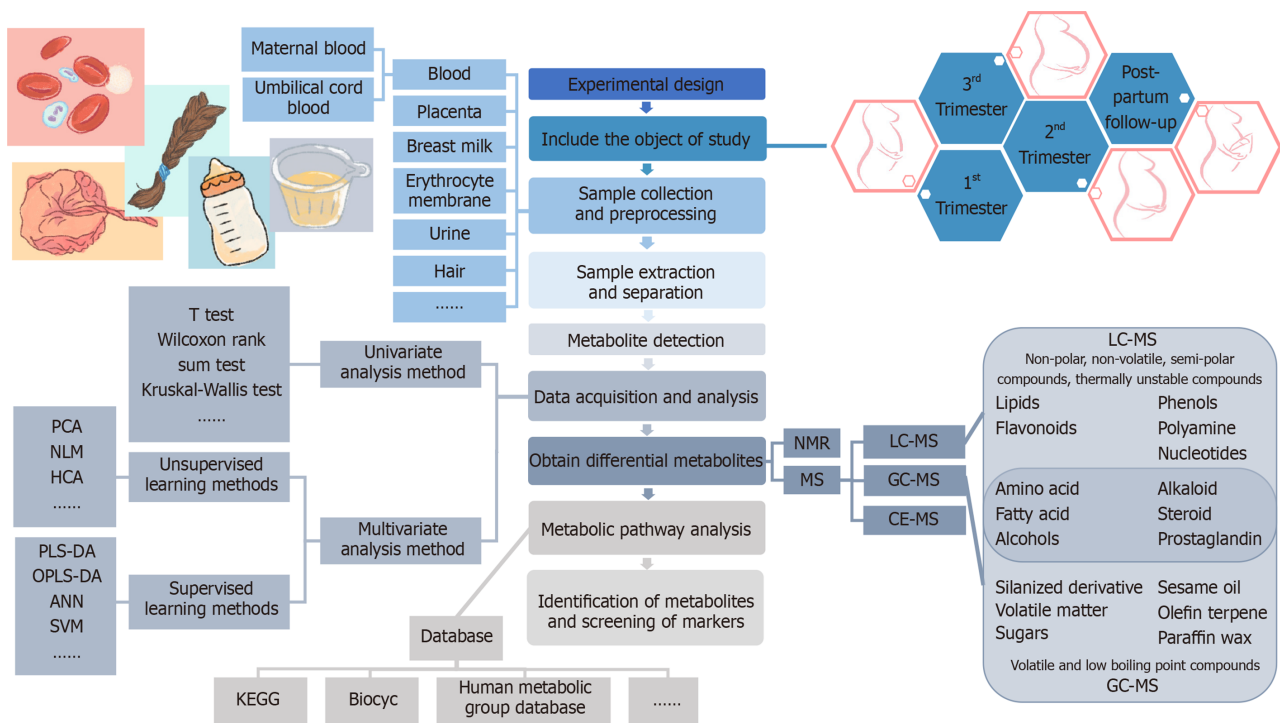
Figure 1 Main workflow of metabolomics analysis. PCA: Principal component analysis; NLM: Nonlinear mapping; HCA: Hierarchical cluster analysis; PLS-DA: Partial least squares discriminant analysis; OPLS-DA: Orthogonal partial least squares discriminant analysis; ANN: Artificial neural network; SVM: Support vector machine; NMR: Nuclear magnetic resonance spectroscopy; MS: Mass spectrometry; LC: Liquid chromatography; GC: Gas chromatography; CE: Capillary electrophoresis.

detect tissue samples *in situ*, providing accurate structural information on metabolites [36], so it is suitable for the qualitative and quantitative study of metabolites[24,30]. However, its deficiency lies in its low sensitivity and incomplete metabolite coverage in biomedical research. However, in recent years, with the progress of high magnetic field magnets, pulse sequences, and freezing probe technology[37], the sensitivity and resolution of NMR approaches have been significantly improved.

MS analysis is a method to separate and detect moving ions according to the ratio of mass to charge (m/z) by electric and magnetic fields[29]. Metabolomics based on MS analysis usually involves separation by chromatography combined with chromatography to reduce the matrix effect and ion inhibition effect. At present, MS hyphenated chromatography can be divided into three categories: Liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis-mass spectrometry (CE-MS). The choice of different chromatographic separation methods depends on the properties of the analyte (such as molecular weight and hydrophilicity/hydrophobicity): LC-MS is mainly used to identify nonpolar, nonvolatile, semipolar, and thermally unstable compounds[38]. LC-MS technology separates compounds in LC through the interaction between the mobile phase (solvent) and stationary phase and identifies the separated compounds in the mass spectrometer. LC-MS can cover a wider range of metabolites and is the mainstream method for metabolomic analysis at present. GC-MS is mainly used for volatile and low boiling point compounds. The derivation method can increase the volatility and thermal stability of nonvolatile compounds for GC-MS analysis. The separated analyte must be ionized before entering the MS instrument, and the ionization method is related to the type of MS detector[29,39].

Compared with NMR technology, the high sensitivity and high resolution of the MS method make it possible to detect thousands of metabolites in a wide dynamic range [40], which makes MS the first choice for researchers who need low abundance metabolite data or to separate large amounts of metabolites at the same time[41,42]. Both NMR and MS have obvious advantages and disadvantages (shown in Table 1), and they are highly complementary. The combination of these two technologies can improve the overall quality of the research. In recent years, researchers have begun to use NMR and MS to analyze the metabolic spectrum of samples, drug efficacy and toxicology, disease pathophysiology, and other aspects[30,43], and to explore the compatibility of NMR and MS analysis platforms[30].

Table 1 Comparison of characteristics of nuclear magnetic resonance spectroscopy technology and mass spectrometry technology

Comparison item	NMR	MS
Sensitivity	Low, less than 100 metabolites at a time	High, more than 1000 metabolites at a time
Selectivity	Poor	Good
Targeting analysis ability	Usually used for nontargeted analysis	Targeted and non-targeted analysis
Sample preparation	Simple	Complex
Sample measurement	Completed in a single measurement	Require different chromatographic techniques
Sample recovery	Nondestructive, recyclable, and long-term preservation	Unrecyclable, less sample consuming
Repeatability	High	Low
Tissue sample	Directly without pretreatment.	Need pretreatment such as tissue extraction

NMR: Nuclear magnetic resonance spectroscopy; MS: Mass spectrometry.

Metabolomics research in GDM

In recent years, metabolomics studies in GDM have shown the characteristics of universality and diversity. For example, there are a variety of biological samples, including blood plasma or serum from the maternal or fetal umbilical cord, erythrocyte membrane, urine, amniotic fluid, breast milk, placenta, and hair, but most samples used are plasma or serum. The time periods of study also have different variations from the first trimester, second trimester, third trimester, at delivery, to postpartum periods following delivery. At present, GDM metabolomics research is mainly focused on carbohydrates, amino acids, lipid metabolites [including fatty acids (FAs), phospholipids, sphingomyelin, *etc.*], purines, uric acid, bile acid, and other small molecular metabolites, as well as related metabolic pathways. The results of the papers published in recent years are shown in Table 2[44-58].

CARBOHYDRATE METABOLITES

It is well known that the characteristic clinical biochemical index of GDM is an increase in blood glucose content, which continues to be high with the development of pregnancy. A recent study tracked the metabolic changes in individuals from baseline (6-9 wk postpartum) to a 2-year follow-up (2 years postpartum) in 1035 women diagnosed with GDM and studied the trajectory of T2D progression with a longitudinal analysis[55]. The researchers found that hexose, which represents the sum of all 6-carbon monosaccharides including glucose and fructose, was the only significantly increased metabolite in all three analyses (at baseline, at follow-up, and longitudinal analysis)[55]. Shokry's study has also found that the sum of hexose (approximately 90%-95% glucose, 5% other hexoses) was significantly increased in GDM in both maternal plasma and, especially, umbilical cord plasma[56]. The association between hexose and future T2D risk was highly significant at baseline[55,59] and within 2 years of follow-up[60,61]. However, the increase in hexose (likely glucose) is not surprising, since diabetes and its severity are defined by the circulating blood glucose level. Although there is a continuous interaction between various nutrient metabolism pathways, in most cases, carbohydrate metabolism takes precedence over protein and fat in energy production. Hexose metabolism is the most regulated, which is also most closely related to the occurrence of T2D. Disorders in circulating hexoses may indicate problems with carbohydrate metabolism but may also be potential problems with amino acid or lipid metabolism.

In addition to perturbations in hexose metabolism, some disorders of glucose metabolism pathway molecules have also been detected in women with GDM. Mokkala *et al*[50] found that the level of citrate, which is an intermediate in the tricarboxylic acid cycle synthesized from acetyl-CoA and oxaloacetate, increased in women with GDM. Citric acid inhibits glycolysis and conversely stimulates gluconeogenesis and lipid synthesis, *i.e.*, energy storage. Thus citric acid is an important regulator of energy metabolism[62]. Therefore, in addition to its relationship with GDM, citric acid may be a determinant of adiposity in overweight and obese women, as observed in a study of nonpregnant participants with obesity[63]. Early studies,

Table 2 Selected gestational diabetes mellitus metabolomic studies in recent years

Study design	Study methods	GDM diagnostic guidelines	Sample collection time	Biological samples	Analytical platform(s)	Changes in direction (*vs* control)	Main differential metabolites	Ref.
Biological samples collected before GDM diagnosis								
Prospective study (overweight and obese), *n* = 82 GDM, *n* = 275 controls	Targeted NMR-based metabolomics approach	IADPSG	12.8–15.6 wk	Maternal serum (fasting)	NMR	Up	All-sized VLDL particles; medium-sized HDL particles; small-sized HDL particles; glucose; lactate; pyruvate; isoleucine; leucine; alanine; phenylalanine; GlycA	Mokkala *et al*[44], Finland, 2020
						Down	Very large HDL particles	
Nested case-control study, *n* = 243 GDM, *n* = 243 controls	Targeted Lysophosphatidylcholines and bile acids	IADPSG	9-11 wk	Maternal plasma (fasting)	LC-MS/MS	Up	LPC egg; LPC 15:0; LPC 17:0; LPC 18:0; LPC 18:1	Liu *et al*[45], China, 2020
						Down	Deoxycholic acid; glycoursodeoxycholic acid	
Case-control study, *n* = 65 GDM, *n* = 366 controls	Targeted amino acids	IADPSG	12-16 wk	Maternal serum	UHPLC-MS/MS	Up	Alanine; glutamate; isoleucine; phenylalanine; tyrosine	Jiang *et al* [46], China, 2020
						Down	None	
Case-control study, *n* = 121 p-GDM, *n* = 121 control	Targeted metabolomics	multi-step screening	16-19 wk	Maternal serum(randomly)	HILIC-MS/MS	Up	None	Sakurai *et al* [47], Japan, 2019
						Down	Glutamine; pyrophosphate; octulose-1,8-bisphosphate	
Case-controls study, *n* = 36 p-GDM, *n* = 31 controls				Maternal urine (randomly)	HILIC-MS/MS	Up	Shikimate-3-phosphate; 1,3-diphosphoglycerate; N-acetyl-L-alanine	
						Down	Ethanolamine; methionine	
Nested case-control study, *n* = 131 GDM, *n* = 138 controls	Nontargeted metabolomics	IADPSG	12 wk	Maternal serum (fasting)	UPLC-QTOFMS, UPLC-TQMS, GC-TOFMS	Up	C14:1(trans-9); C16:1 (cis-7); C17:1 (cis-10); C18:1 (cis-9); C19:1 (cis-10); C20:1 (cis-11); C12:0; C16:0; C17:0; C20:0; C16:2 (cis-9_12); C18:2 (9_11&10_12-cis&trans-conjugated-99%); C18:3 (cis-9_12_15); C22:3 (cis-13_16_19); C18:3 (cis-6_9_12); C18:2 (cis-9_12); C20:4 (cis-5_8_11_14); C20:3 (cis-8_11_14); C20:2 (cis-11_14); C22:5 (cis-7_10_13_16_19); THDCA; LCA; HDCA; isoDCA; 6_7_diketoLCA; leucine; isoleucine; valine; acetylaspartic acid; alanine; glutamic acid; 2-aminobutanoic acid; 2-oxo-4-methylvaleric acid; gamma-aminobutanoic acid; Aminomalonic acid; pyruvic acid; 1-monooleoylglycerol; 2-ethylhexanoic acid; mannose; threitol	Hou *et al* [48], China, 2018
						Down	CA; dehydro_LCA; cysteine; 2,3,4-trihydroxybutyric acid; maltose; threonic acid	

Biological samples collected after or simultaneously with GDM diagnosis								
Nested case-control study, *n* = 23 GDM, *n* = 77 controls	Targeted polar metabolites and lipids	IADPSG	24-28 wk	Maternal serum (2 h OGTT)	LC-MS	Up	Adenosine; taurolithocholic acid; glycoli thocholic acid; glycochenodeoxycholic acid	Geiaye *et al* [49], Peru, 2019
						Down	Methionine sulfoxide; C58:10 triacylglycerol; C58:9 triacylglycerol; C8 carnitine; C14:2 carnitine; C14:1 carnitine; C12 carnitine; C4-OH carnitine	
Case-control study (overweight), *n* = 100 GDM, *n* = 252 controls	Targeted NMR-based metabolomics approach	IADPSG	34.6-35.9 wk	Maternal serum (fasting)	NMR	Up	VLDL particles; glucose; citrate; isoleucine; leucine; phenylalanine; acetoacetate; GlycA	Mokkala *et al* [50], Finland, 2020
						Down	Small HDL particles	
Case-control study (overweight), *n* = 19 GDM with medication, *n* = 76 GDM with diet						Up	VLDL particles; lactate; isoleucine; leucine; GlycA	
						Down	Small HDL particles	
Case-control study, *n* = 45 GDM, *n* = 98 controls	Targeted lipids metabolomics approach	IADPSG	At admission, after admission, and up to 24 h after delivery	Maternal plasma (fasting)	LC-IMS-MS	Up	PE(P-20:0_18:1); PE(P-18:0_22:6); PE(P-20:0_22:6); PE(P-18:1_22:6); PC(P-18:0_22:6); PC(17:0_22:6)	Odenkerk *et al* [51], United States, 2020
						Down	PEBP1; TG(12:0_16:0_18:1); TG(14:0_16:0_18:1); TG(14:0_16:0_18:2); PDIA6; PDIA5;LYAG; EXT1; B4GA1	
Case-control study, *n* = 11 GDM only, *n* = 11 hyperlipidemia only, *n* = 12 GDM plus hyperlipidemia, *n* = 11 controls	Targeted lipids metabolomics approach	IADPSG	27–33 wk	Maternal serum (fasting)	GC-MS/MS	Up	3-Dehydrocarnitine; 6-hexanoyl-D-erythro-sphingosine; arachidoyl ethanolamide; CER; Che; DG; dMePE; eicosanoicacid; FA; hexadecanamide; LdMePE; LPA; LPC; LPEt; LPG; LPI; MG; N-hexadecyl-ethanolamine; OAHFA; octadecanoicacid; PAF; PC; PE; Pet; PG; phSM; PI; PIP,SM; So; TG	Liu *et al* [52], China, 2019
						Down	None	
Case-control study, *n* = 30 GDM, *n* = 30 controls	Nontargeted metabolomics	IADPSG	24-28 wk	Maternal serum	LC-MS	Up	TXB2; traumatic acid; PGC2; PGJ2; PGB2; PGA2; pravastatin; PGD2-d4; PGE2-d4; crotonoyl-CoA; methacrylyl-CoA; 2S-hydroxybutanoic acid; D(-)-beta-hydroxy butyric acid; 4-hydroxy-butyric acid; DPA; oleic acid; rumenic acid; linoleic acid; urocortisone; corticosterone; 11-deoxycortisol; tetrahydrocortisol; 2-hydroxyestrone; dehydroepiandrosterone sulfate; tetrahydrocorticosterone; LPA (0:0/16:0); LysoPC (20:4); psychosine	Li *et al* [53], China, 2019
						Down	PGG2; 6-keto PGE1; 11-dehydro-TXB2;	

							cholesterol; lathosterol; coenzyme Q10; lutein; zeaxanthin	
Case-control study, *n* = 32 GDM, *n* = 11 controls	Targeted lipidomic profile	WHO—2013 criteria	24-28 wk	Maternal erythrocyte membrane (fasting)	GC-MS	Up	Oleic acid; vaccenic acid; sapienic acid	Bukowiecka-Matusiak *et al*[54], Poland, 2018
						Down	Myristic, palmitic, and stearic acids	

Biological samples collected in longitudinal cohort study

Nested case–control studyBaseline (6–9 wk postpartum), *n* = 173 future T2D, *n* = 485 non-T2D	Targeted metabolomics	Carpenter and Coustan criteria	6–9 wk postpartum (baseline);	Maternal plasma (fasting)	FIA-MS/MSLC-MS/MS	Up (baseline)	Hexose; histidine; isoleucine; serine; tyrosine; leucine; methionine; glutamate; lysine; tryptophan; threonine; proline; acylcarnitines AC3; acylcarnitines AC10; acylcarnitines AC16; spermidine; diacyl-glycerophospholipids	Lai *et al*[55], United States, 2020
						Down (baseline)	Glutamine; kynurenine; sphingomyelins; lysophosphatidylcholines; acyl-alkyl-glycerophospholipids	
Follow-up (2~8 yr postbaseline), *n* = 98 future T2D, *n* = 239 non-T2D			Up to 2 yr postbaseline (follow-up).			Up (follow-up)	Hexose; glutamate; isoleucine; tyrosine; leucine; valine; alanine; PC; aa C32:1; acylcarnitines AC5	
						Down (follow-up)	Glycine; PC aa C38:1; PC aa C38:6; PC ae C36:2; PC ae C40:6; PC ae C34:3; PC ae C34:2; PC ae C36:3; PC ae C38:4; lysoPC a C17:0; lysoPC a C18:1; lysoPC a C20:4; lysoPC a C18:2; lysoPC a C16:0; lysoPC a C18:0; SM(OH)C22:2; SM(OH)C14:1; creatinine	
Case-control study, *n* = 90 GDM, *n* = 94 controls	Nontargeted metabolomics	IADPSG	1–3 d postpartum	Colostrum	GC-MS	Up	None	Wen *et al*[3], China, 2019
						Down	Heneicosane; G\glycine, N-(methoxyoxoacetyl)-methyl ester; 3-aminoisobutyric acid; glutamine; oxaloacetic acid; 4-aminobutyric acid	
			7-10 d postpartum	Transition milk	GC-MS	Up	Asparagine; malic acid	
						Down	D-Proline; glycine, N-(methoxyoxoacetyl)-, methyl ester; hydroxybenzoic acid; malonic acid; 9-heptadecenoic acid	
			4 wk postpartum	Mature milk	GC-MS	Up	Heneicosane; cysteine; lignoceric; malic acid	
						Down	Nonacosane; glycine; N-ethyl-N-(2-ethoxyethoxycarbonyl)-; 2-methoxyethyl ester; pyroglutamic acid; beta-alanine; 2-oxoadipic acid; 3-methyl- 2-oxovaleric acid; 4-aminobutyric acid; glutamine; oxalic acid; oxaloacetic acid; pimelic acid; 9-heptadecenoic acid; 10-pentadece- noic acid; 2-hydroxyglutaramic acid; nervonic acid	

Case-control study, n = 50 Healthy, overweight/obese, n = 45 GDM, n = 67 Healthy, normal weight	Targeted metabolomics	National Diabetes Data Group criteria	At delivery	Maternal plasma	LC–MS/MS	Up	Hexoses; Asn/Asp	Shokry *et al* [56], Spain, 2019
						Down	Phospholipids (PL); LPC16:0; PCaa38:3; PCaa 38:5; SM32.2	
Case-control study, n = 40 Healthy, overweight/obese, n = 27 GDM, n = 49 Healthy, normal weight				Cord plasma of offspring		Up	Hexoses; Asn/Asp; 3-methyl-2-oxobutanoic acid	
						Down	PCae38:0; Carn; short-chain AC; acetyl carnitine; NEFA26:1, malic; succinic acids	
Case-control study, n = 107 GDM, n = 107 controls	Nontargeted metabolomics	Criteria of the American Diabetes Association	First trimester (12.8 wk median)	Maternal serum	UHPLC-MS	Up	Proline; ornithine; glycerophosphocholine; glutamic acid; taurine; acetylcarnitine; uracil; hypoxanthine; aspartyl-isoleucine; pantothenic acid; lysoPC (14:0); LysoPC [16:1(9Z)]; linoleoyl carnitine; palmitoylcarnitine; LysoPC (16:0); histidine; succinic acid semialdehyde; malic acid; xanthosine	Zhao *et al* [57], China, 2019
						Down	Threoninyl-phenylalanine; asparaginyl-tryptophan; phenylalanyl-gamma-glutamate; valyl-isoleucine; aspartyl –phenylalanine; phenylalanyl-valine; DL-2-aminooctanoic acid; phenylalanyl-isoleucine; acetylglycine	
			Second trimester (26.1 wk median)			Up	Guanidoacetic acid; acetylcarnitine; propionylcarnitine; 2-octenoylcarnitine; LysoPC (14:0); LysoPC [16:1(9Z)]; palmitoylcarnitine; vaccenyl carnitine; LysoPC (16:0); LysoPE [0:0/18:1(11Z)]; LysoPC (17:0)	
						Down	Threoninyl-phenylalanine; phenylalanyl-gamma-glut-amate; phenylalanyl-isoleucine	
Case-control study, n = 15 GDM, n = 50 controls	Nontargeted metabolomics	IADPSG	6-8 wk	Maternal urine	UPLC-MS/MS	Up	Levoglucosan; polyethylene glycol; 6-hydroxy-5-methoxyindole glucuronide	Liu *et al*[58], China, 2019
						Down	1'-acetoxyeugenol acetate; 3,4-dimethyl-5-pentyl-2-furanundecanoic acid; 2-hydroxylauroylcarnitine; L-phenylalanyl-L-proline	

GDM: Gestational diabetes mellitus; p-GDM: Prior to diagnosis of gestational diabetes mellitus; T2D: Type 2 diabetes; IADPSG: International Association of Diabetes and Pregnancy Study Group; WHO: World Health Organization; OGTT: Oral glucose tolerance test; NMR: Nuclear magnetic resonance spectroscopy; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; UHPLC-MS/MS: Ultra-high-performance liquid chromatography-tandem mass spectrometry; HILIC-MS/MS: Hydrophilic interaction chromatography-tandem mass spectrometry; UPLC-QTOFMS: Ultra performance liquid chromatography-quadrupole-time-of-flight mass spectrometry; UPLC-TQMS: Ultraperformance liquid chromatography-triple triple-quadrupole mass spectrometry; GC-TOFMS: Gas chromatography-time-of-flight mass spectrometry; LC-MS: Liquid chromatography-mass spectrometry; LC-IMS-MS: Liquid chromatography-ion mobility spectrometry and mass spectrometry; GC-MS/MS: Gas chromatography-tandem mass spectrometry; GC-MS: Gas chromatography-mass spectrometry; FIA-MS/MS: Flow injection analysis-tandem mass spectrometry; UPLC-MS/MS: Ultra performance liquid chromatography-tandem mass spectrometry; VLDL: Very low density lipoprotein; HDL: High density

lipoproteins; GlycA: Glycoprotein acetylation; LPC: Lysophosphatidylcholine; CA: Cholic acid; LCA: Lithocholic acid; PE: Phosphatidylethanolamine; PC: Phosphatidylcholine; PEBP1: Phosphatidylethanolamine-binding protein 1; TG: Triacylglycerols; PDIA: Protein disulfide-isomerase A; LYAG: Lysosomal alpha-glucosidase; EXT: Exostoses; B4GA1: Beta-1,4-galactosyltransferase 1; CER: Ceramides; Che: Choleterylesters; DG : Diacylglycerols; dMePE: Dimethyl-phosphatidylethanolamine; FA: Fatty acids; LdMePE: Lysodimethylphosphatidylethanolamine; LPA: Lysophosphatidic acid; LPEt: Lysophosphatidylethanol; LPG: Lysophosphatidylglycerol; LPI: Lysophosphatidylinositol; MG: Monoglyceride; OAHFA: Octadecanoicacid; PAF: Platelet-activating factor; Pet: Phosphatidylethanol; PG: Phosphatidylglycerols; PI: Phosphatidylinositol; PIP: Phosphatidylinositol; SM: Sphingomyelins; So: Sphingoshine; TXB2: Thromboxane B2; PG: Prostaglandins; LysoPC: Lysophosphatidlycholine; AC: Acylcarnitine; PCaa: Diacyl-phosphatidylcholines; Asn: Asparagine; Asp: Aspartic acid; PCae: Acyl-alkyl-phosphatidylcholines; Carn: Carnitine; NEFA: Nonestesterified fatty acid; LysoPE: Lysophosphatidylethanolamine.

designed as case-control studies, showed that the circulating concentration of β-hydroxybutyric acid in GDM patients was higher than that in controls[64,65]. β-hydroxybutyric acid is a classic ketone body found alongside acetone and acetoacetate. Mokkala *et al*[50] also found elevated levels of the ketone body acetoacetate in patients with gestational diabetes. Previous studies have also detected increased concentrations of ketone bodies in pregnant women with high fasting blood glucose levels[66] and found that they are associated with GDM[67-69].

Similar to T2D, a failure of glucose utilization will lead to the formation of these ketone bodies. It is well known that in the latter half of normal pregnancy, maternal metabolism is transformed into catabolism, during which increased lipolysis provides FAs that can be utilized in ketogenesis[70]. The produced ketone bodies are used as maternal energy to replace glucose, which is mainly utilized by the fetus[71]. Elevated pyruvate and glucose anaerobic decomposition products (3.27 times higher than that in the normal group) were observed in the urine of GDM patients at 17 wk of pregnancy, in the research from Michelle A. Willims' group[72]. Such results on pyruvate are consistent with other reports[73,74]. These results indicate that abnormalities in glycolysis and tricarboxylic acid cycle metabolic pathways result in disturbed energy metabolism and dysregulation of glucose metabolism associated with GDM.

Amino acid metabolites

The association of increased serum amino acid levels with obesity and IR was observed more than 40 years ago[75]. Advances in metabolomic analysis have renewed interest in this field. By using metabolomic techniques, a large number of amino acid metabolites related to GDM have been detected, including amino acids (alanine, glutamine, and glycine), branched-chain amino acids (isoleucine, leucine, and valine), and aromatic amino acids (phenylalanine, tyrosine, and histidine). Among all these amino acids, the relationship between branched-chain amino acids (BCAAs) and IR has been deeply studied.

Abnormal amino acid metabolism can occur in the early stage of pregnancy. Mokkala *et al*[44] analyzed fasting serum samples *via* a targeted NMR approach in early pregnancy (median: 14.3 wk of gestation), and found that the concentrations of two branched-chain amino acids, namely, isoleucine and leucine, as well as phenylalanine and alanine, were already increased in women who developed GDM. Similar findings have been reported previously in which elevated BCAA levels including valine and phenylalanine, but not alanine, were detected in obese women with GDM[67]. In contrast, surveys of women with heterogeneous body mass indexes (BMIs) have reported that increased concentrations of valine[76] and alanine[76,77] in early pregnancy or no changes in BCAAs[77,78] were associated with the onset of GDM. Other studies have reported that glutamine[47], glutamic acid, and serine[79] in serum significantly differed between the prior to diagnosis of GDM (p-GDM) and control groups in the first trimester. Glutamine in serum showed an area under the receiver operating characteristic curve of 0.81 and may be a predictive metabolite for GDM[47]. Combined metabolomic analysis of plasma and urine revealed that serotonin, 5-HIAA, L-tryptophan, melatonin, and 6-hydroxymelatonin may be effective predictors of GDM[80]. However, the results of studies on the relationship between the concentrations of amino acids in early pregnancy (prior to diagnosis of GDM) and the risk of GDM are not consistent[67,77,81]. Based on findings from previous studies and considering that amino acids can induce IR[82], it can be proposed that amino acids may become effective indexes for the prediction and early diagnosis of GDM[76-78]. There is no consensus on amino acids for early screening of GDM[83]; however, many scholars still suggest that the measurement of amino acid concentrations in early pregnancy should be taken as part of routine testing in the future, so that clinicians can identify high-risk pregnant women with GDM as early as possible, to take effective intervention measures to prevent GDM[46,60,84,85].

Perturbed amino acid metabolism can not only predict the risk of GDM, but it also can predict the transition from GDM to T2D[86]. Allalou *et al*[59] enrolled 1010 GDM women without T2D at 6-9 wk postpartum (baseline) who were screened for T2D annually for 2 years. They found that 113 women progressed to T2D within 2 years and another 17 women developed T2D between 2 and 4 years. By a metabolomics study with baseline fasting plasma, it was found that 21 metabolites such as free FAs and amino acids (including isoleucine, leucine, tryptophan, tyrosine, alanine, and amino phenylalanine) were identified and could effectively predict the transition from GDM to T2D 2-4 years after delivery. Clinical trials have also demonstrated that the concentrations of BCAAs, such as leucine, isoleucine, and valine, are increased up to 7 years before the onset of T2D[84]. The latest study found that higher levels of BCAAs and 3-hydroxyisobutyric acid in plasma were associated with IR in the transition from GDM to T2D[86].

Disturbance of BCAAs and their metabolites is closely related to IR and decreased islet β-cell function in women with GDM[60,73,84,87-93]. It is the most common and most important abnormality of amino acid metabolism in GDM. The levels of leucine and isoleucine were associated with IR and decreased insulin sensitivity[82], while the tyrosine concentration was positively correlated with IR and insulin secretion[94]. The elevated levels of BCAAs in plasma increased the transport of FAs through endothelial cells to skeletal muscle, increased intracellular lipid accumulation, and weakened the insulin signaling pathway mediated by 3-hydroxyisobutyric acid[95]. BCAAs are involved in IR through several pathways, including fatty acid oxidation and the mTOR, JNK, and IRS1 pathways[96,97]. In addition, the increased BCAA concentrations may imply an increase in the absorption or production of intestinal microflora or a decrease in utilization/decomposition/degradation. In Mokkala *et al*'s study, higher concentrations of BCAAs (isoleucine and leucine) and phenylalanine were detected in women with GDM even after adjusting for pregestational BMI[44]. Other studies have reported an increase in leucine[98], isoleucine[46], and other amino acids such as alanine[99] and a decrease in L-valine[100] in patients with GDM compared with control patients. However, several studies have failed to detect differences in BCAA concentrations between patients with GDM and non-GDM in the first, second, or third trimester of pregnancy[77,101]. In addition, animal and human studies supplementing or restricting BCAA levels have reported conflicting effects[90,102-107]. Therefore, we still need to further explore and study amino acids, especially BCAAs, to track the individual changes in metabolism during disease progression, and explore the underlying molecular mechanisms.

Lipid metabolites

Lipids are involved in regulating a variety of life processes, including energy conversion, material transport, information recognition and transmission, cell development and differentiation, and apoptosis. However, abnormal lipid metabolism is closely related to some diseases, such as diabetes, arteriosclerosis, obesity, Alzheimer's disease, and tumorigenesis. In women with GDM, the physiological changes in lipids are magnified, which may indicate potential metabolic disorders during pregnancy[108]. At present, the understanding of the structures and functions of lipids still lags far behind that of genes and proteins, mainly due to the diversity and complexity of lipid molecular structures. In addition, the lag of analytical technology has hindered the systematic research on whole lipids and their complex metabolic network and functional regulation. The common lipids are fat, phospholipid, and sterol. The lipid differences closely related to GDM are mainly found in FAs, phospholipids, glycerolipids, glycerophospholipids, and sphingolipids.

FAs are the simplest lipid molecules and the main components of many complex lipids such as phospholipids, triacylglycerols, and sphingolipids. According to the saturation degree of the hydrocarbon chain, FAs can be divided into three categories: Saturated FAs (SFAs), monounsaturated FAs (MUFAs), and polyunsaturated FAs (PUFAs).

The SFA and MUFA families are the most abundant fatty acid species involved in metabolic disorders and are related to IR and related diseases[109,110] as well as diabetes-related pathways[111]. They are molecules of important biological and pathophysiological significance that are responsible for cell membrane fluidity, cell proliferation, lipid-mediated cytotoxicity, programmed cell death, unfolded protein response, the pathogenesis of obesity and cancer, and especially for metabolic diseases[112-114]. In the early studies of women with overweight and GDM, the free MUFAs in blood were greatly influenced by diet[115] and obesity[116]. Chen *et al*[117] studied circulating (serum) FAs and detected impaired fatty acid composition, not only in women with GDM but also in women with impaired glucose tolerance. There is a

strong correlation between the severity of maternal hyperglycemia and the concentration of serum FAs in the third trimester; however, plasma lipids are more dependent on dietary intake than tissues or cell membranes[117]. Bukowiecka-Matusiak *et al*[54] investigated the erythrocyte membrane fatty acid profiles of 32 pregnant women with GDM and 11 pregnant women with normal glucose tolerance by GC-MS and found that of the 14 measured FAs representing the characteristics of membrane lipids, three kinds of SFAs (myristic acid, palmitic acid, and stearic acid) of erythrocyte membranes in patients with GDM showed a downward trend. The relative content of MUFAs in the erythrocyte membrane of the GDM group was higher than that of the NGT group, especially the oleic acid and vaccenic acid contents, which were significantly increased[54]. There was no significant change in PUFAs in erythrocytes between the GDM and NGT groups. Based on the differences between the GDM and NGT lipidomic profiles, the authors postulated that stearic acid and cis-acetic acid can be regarded as dual biomarkers of specific SFA-MUFA transformation pathways. Stearic acid and cis-acetic acid are involved in erythrocyte membrane remodeling in women with GDM by the coupling of δ-9 desaturase and elongase enzymes[54]. In addition, the higher concentration of vaccenic acids in red blood cells was related to lower fasting glucose, better insulin sensitivity, and a reduced risk of T2D[118]. Because erythrocyte membrane composition reflects the dietary intake for the past 2-3 mo, the erythrocyte membrane fatty acid state can be more reflective. The results from a study on breast milk found increased levels of FAs in mature milk compared to those in colostrum and transition milk, while there was a considerable amount of decreased FAs (9-heptadecenoic acid, pimelic acid, 10-pentadecenoic acid, 2-hydroxyglutaramic acid, and nervonic acid) in breast milk from GDM women. This may be due to the fact that free FAs are the cornerstone of adipogenesis and neurons. Differences in free FA profiles may play a more profound role in breastfeeding-related developmental disorders or future health risks in offspring. Therefore, functional lipidomics based on membrane FAs and other tissues has become a convenient and relevant molecular tool to examine the nutritional and metabolic status of patients.

FAs can be categorized according to the length of the carbon chain into short-chain FAs (less than 6 carbon atoms), medium-chain FAs (6-12 carbon atoms), and long-chain FAs (more than 12 carbon atoms). It has been reported that long-chain acylcarnitines[119,120] and their precursors long-chain acyl-CoAs are related to IR. In the Zhao *et al*'s [57] study, long-chain acylcarnitines (such as palmitoyl carnitine and vaccenyl carnitine) increased in women with GDM both in the first and second trimesters. Another study observed 201 metabolites in maternal plasma at delivery and cord plasma obtained from 325 participants by LC-MS/MS[56]. The long-chain NEFAs and Krebs metabolites in cord blood from GDM offspring showed an overall decrease, and the most significant metabolites were NEFA26:1, malic acid, succinic acid, and 3-methyl-2-oxybutyric acid. In an analysis of fatty acyl groups in GDM[51], lipids containing 12:0, 14:0, 15:0, 18:3, 22:4, or 24:1 fatty acyls were downregulated, while 20:0 and 22:6 fatty acyls were upregulated, such as 22:6 for PE P- and PC and PE O- species. Furthermore, myristic acid or 14:0, which has previously been thought to be negatively associated with 22:6[121], was also downregulated in GDM but not in the same lipid species[51]. As previously reported, a higher level of palmitoleic acid is beneficial to insulin sensitivity in the case of metabolic diseases, and its administration in animal models can effectively reduce IR and hepatic lipid accumulation[122]. Therefore, different FAs may play different roles in maternal and infant health, and even the same FAs may have different effects due to different doses and exposure times.

The complex relationships among phospholipids and sphingomyelin metabolites and the risk of diabetes have not been widely studied and some studies have conflicting results. A longitudinal analysis revealed that six diacyl-glycerolphospholipids (the PC aa C group) were positively correlated with T2D at baseline (6–9 wk postpartum) and follow-up (from baseline to 2 years), while 11 acyl-alkyl-glycerolphospholipids (the PC ae C group) were negatively correlated with T2D risk[55]. In a case-cohort study, some diacyl-phosphatidylcholines were associated with a higher risk of T2D, while sphingomyelin and 1-acyl-alkyl-phosphatidylcholine (C18:2) were associated with a lower risk[89]. Moreover, in a subset of the hyperglycemia and adverse pregnancy outcome study, disturbed lipid metabolism of certain classes of phospholipids and lysophospholipids was detected in the diabetes risk group prior to hyperglycemia at 2 years of postpartum[89]. Wheeler's group found that several sphingomyelins decreased at baseline (6–9 wk postpartum) and follow-up (from baseline to 2 years)[55]. Interestingly, in contrast to the finding of a negative relationship between sphingomyelin metabolism and T2D risk by Wheeler's group[55, 123], another study[89] showed that sphingomyelins were increased in T2D patients

and positively associated with future T2D[124,125]. Thus, further research is needed to investigate the role of phospholipids and sphingomyelins in T2D risk among different populations.

In short, the chain length and the degree of desaturation of FA moieties in lipid molecules increase the complexity of assigning biological roles to various lipid classes. Moreover, lipids that are synthesized endogenously or obtained through the diet influence their accumulation and/or metabolism and subsequent biological roles. Therefore, it is not surprising that opposing views exist on the pathogenicity and mechanisms of specific lipids during the development of IR. However, new high-resolution metabolomic technology provides bright prospects to identify lipid subclasses and novel families of lipids.

Other metabolites

Purine is a key component of the cellular energy system. Hypoxanthine is a degradation product of adenosine triphosphate, which is a major bioenergy source, and is further converted to xanthine by xanthine oxidase (XO) and then converted to uric acid, the final oxide of purine metabolism[126]. The study found that hypoxanthine and xanthine in the GDM group were significantly higher than those in the control group during early pregnancy[57], which may be due to higher energy intake in women with GDM in early pregnancy. However, in the second trimester, the levels of hypoxanthine and xanthine in women with GDM were significantly lower than those in the first trimester, while the concentration of uric acid was significantly increased. This is because the activity of XO in the peripheral blood of pregnant women with GDM is higher than that of healthy women, resulting in excessive uric acid accumulation[127,128]. A high uric acid level is associated with IR[129] and is considered to be a risk factor for GDM[130] and T2D[131]. In addition, the levels of inflammatory markers such as glycoprotein acetylation (GlycA, a marker of low-grade inflammation)[44,67,68,132-134] and some sterol hormones such as 11-deoxycortisol, 17α-hydroxyprogesterone, progesterone[76], cortisol, androstenedione, dehydroepi-androsterone sulfate, and 11 deoxy-cortisol steroids[53]in pregnant women with GDM are different from those in normal pregnant women[135]. Disorders of these processes lead to metabolic and immune dysfunction and the development of pregnancy complications.

Li *et al*[136] previously reported that decreased concentrations of two secondary bile acids, deoxycholic acid (DCA) and glycoursodeoxycholic acid (GUDCA), in early pregnancy were associated with a significantly elevated risk of GDM. Recently, they investigated the associations between trimethylamine (TMA), trimethylamine nitrogen oxide (TMAO), and related metabolites (choline, betaine, L-carnitine) and the risk of GDM during early pregnancy[137]. The levels of TMAO and related metabolites decreased significantly in women with GDM, while the concentration of TMA was increased. The change in TMAO had a clear threshold effect, independent of DCA and GUDCA, to a great extent. It is commonly known that the conversion from betaine, choline, and L-carnitine to TMA depends on gut microflora, and the conversion of TMA to TMAO depends on the activity of flavin monooxygenase 3 (FMO3), especially FMO3 in the liver[138,139]. According to Rothman's multicausality theory[140], the significant additive interaction between abnormal gut microflora and decreased FMO activity in the liver may be one of the causes of GDM. In addition, Liu *et al*[52] also conducted microbiomics coupled with lipidomics analyses to characterize gut microbiota and lipometabolism in pregnant women with GDM, hyperlipidemia, or GDM plus hyperlipidemia. This study and other studies[48,141,142] further indicate that changes in the fecal microbiota and plasma lipidome can predict and characterize the development of GDM with lipid metabolic abnormalities.

Effects of diet or medicine on metabolomics of GDM

Huhtala *et al*[143] enrolled 217 GDM pregnant women treated randomly with metformin or insulin, and 126 GDM pregnant women who achieved sufficient glycemic control by diet and lifestyle modifications alone as a control. The levels of serum amino acids and lactic acid at diagnosis and 36 wk of gestation were measured by ^{1}H NMR. It was found that the majority of the amino acid concentrations increased with gestational age. Compared with the insulin-treated group, the metformin-treated group had a greater increase in alanine (16% *vs* 8%), isoleucine (11% *vs* 5%), and lactic acid (29% *vs* 14%). Another study[144] explored 71 pregnant women with GDM (including 28 patients who received dietary treatment only, 20 patients who received metformin, and 23 patients who received insulin) at three different gestational time points: 15-18^{+6} and 27-28^{+6} wk (before treatment) and 34-36^{+0} wk (after treatment). The results showed that prior to the initiation of treatment, metabolic differences were the

most obvious between the diet and insulin-treated groups, especially very-large-density lipoprotein (VLDL) and high-density lipoprotein (HDL) subclasses and components. Even after drug treatment, there were still differences in lipid composition and particle size among the three groups at 34-36 wk of gestation. In recent years, Mokkala *et al*[50] found no differences in lipids in VLDL, and very large and large HDL particles were detected in overweight or obese women who had been treated for GDM for approximately 10 wk. Variations among these results may be due to the inconsistency in population selection, sampling times, and the improvement in metabolic variables to a certain extent by treatment[67,145]. These results indicate that diet, metformin, or insulin therapy in pregnant women with GDM can affect amino acid or lipid metabolism-related IR. Thus, the pathophysiological process of blood glucose control can be revealed by metabolic profiles.

CLINICAL UTILITY OF METABOLOMICS IN GDM

Metabolomics research in GDM focuses on the abnormalities of small molecule metabolites such as carbohydrate, amino acids, lipid, sterol hormones, as well as bile acids and disordered metabolic pathways. In addition, signaling pathways and metabolic pathways analysis on results obtained from detected substances is an important part of metabolomics research, which helps to further explore the related metabolites, enzymes, and genes, so as to deeply understand their underlying biological phenomena.

The clinical utilities of metabolomics in GDM depend on the different time periods of the subjects. The main clinical utilities of research focused on the first trimester period are to identify early diagnostic or predictive molecules related to GDM disease [44-48]. The main clinical utilities of the studies concentrated on the second and third trimesters of pregnancy are to accurately evaluate the condition of GDM, identify differential metabolic markers and metabolic pathways, predict pregnancy outcomes and maternal and fetal prognoses, or evaluate the efficacy of drug/diet therapy[49-54, 144,145]. A postpartum follow-up study mainly predicts the long-term occurrences of T2D or other metabolic diseases in GDM pregnant women and their offspring as well as the metabolic impact of GDM on their offspring[3,55,146].

BOTTLENECKS OR LIMITATIONS OF CURRENT STUDIES

Although metabolomics is developing rapidly as a new discipline, it still has some limitations and faces some technical challenges.

First, as a new research method, metabolomics needs to be further improved with respect to analytical technology, data acquisition, and analysis. GC-MS and LC-MS are affected by the matrix effect and ion suppression/enhancement[147], which are potential threats to the accuracy of metabolomics experiments. In addition, the process of screening specific biomarkers by metabolomics technology is tedious and complicated, especially for metabolic pathway analysis. Thus, the coverage of metabolomics is still insufficient for global metabolomics experiments despite the expansion of the metabolomics database[148].

Second, in recent years, although large amounts of data have been obtained from metabolomics in GDM research, many data are contradictory. Dissimilarities among these findings may be due to differences in the GDM diagnostic criteria, ethnic origin, size of the study populations, specimens prepared for testing, and metabolite profiling platforms. It is important to highlight that future research needs to follow a strict experimental design to improve the repeatability and reliability of research results.

Finally, metabolomics research has strict requirements for sample quantity, quality, and sample processing technology. Metabolites are more affected by the environment and individual differences and are also very sensitive to the storage mode and time of biological sample collections[149,150], which greatly increases the difficulty in obtaining reliable samples. Thus, future studies, especially large-scale, multicenter, dynamic monitoring, and prospective cohort studies are required before metabolomics can be routinely applied in clinical practice.

CONSIDERATIONS FOR FUTURE STUDIES

Although the research task of metabolomics is arduous, the popularization of mass spectrometry has laid the foundation for clinical application. Future metabolomics research still has broad prospects, which are embodied in the following:

First, considering the complexity and individual differences in biological samples, as well as other relevant lifestyle factors, standardized sample collection and operational procedures should be developed to ensure the accuracy and repeatability of the experiment.

Second, cross-sectional designs limit the analysis of the relationship between metabolite disturbance and the risk of GDM, so it is necessary to increase the sample size, conduct longitudinal analysis, and establish reliable clinical cohort samples. Tracking the metabolic changes in the individual and describing the disease progression of each case can also provide a theoretical basis for early stratification and appropriate treatment.

Third, many studies have identified specific metabolites and their relative changes in the relative concentration without providing the absolute concentration of metabolites, as a result, metabolomics data from different laboratories could not be compared with each other. The absolute quantification of metabolites and the establishment of a general standard database closely related to GDM are important research directions in the future.

Finally, in the postgenomic era of modern high-throughput computational technologies, it is essential to integrate the increasing amount of data generated from metabolomics and its upstream genomics, including genomics, transcriptomics, and proteomics, by sophisticated computational biology tools. Thus, this valuable comprehensive analysis can provide further insight into the etiology and pathophysiology of GDM, which might help to clarify the mechanisms of the occurrence and development of GDM and provide precise medical treatments for GDM.

CONCLUSION

In brief, metabolomics related to GDM research is in a period of vigorous development. Given the rapid improvement in omics technologies such as metabolomics, together with updating metabolic databases, bioinformatics, and artificial intelligence, our understanding of metabolic regulation in GDM will rapidly advance and thus significantly benefit the prevention and treatment of GDM.

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