Using metabolomics and proteomics to identify the potential urine biomarkers for prediction and diagnosis of gestational diabetes

*Jie Yu, Jing Ren, Yaolin Ren, Yifan Wu, Yuan Zeng, Qian Zhang,** and Xinhua Xiao**

Key Laboratory of Endocrinology, Ministry of Health, Department of Endocrinology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, 100730, China

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Summary

Gestational diabetes mellitus (GDM) is one of the most common metabolic complications during pregnancy, threatening both maternal and fetal health. Prediction and diagnosis of GDM is not unified. Finding effective biomarkers for GDM is particularly important for achieving early prediction, accurate diagnosis and timely intervention. Urine, due to its accessibility in large quantities, noninvasive collection and easy preparation, has become a good sample for biomarker identification. In recent years, a number of studies using metabolomics and proteomics approaches have identified differential expressed urine metabolites and proteins in GDM patients. In this review, we summarized these potential urine biomarkers for GDM prediction and diagnosis and elucidated their role in development of GDM.

Keywords: Gestational diabetes mellitus; Urine; Biomarker; Metabolomics; Proteomics

Introduction

Gestational diabetes mellitus (GDM) is a type of diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes before gestation.[1] With a steady increase in global morbidity observed over the last four decades, GDM has become one of the most common metabolic complications during pregnancy.[2] Nine to twenty-six percent of pregnant women are diagnosed with GDM based on different criteria.[3] Epidemiological studies have identified some risk factors for GDM, including ethnicity, increased maternal age, overweight/obesity, previous gestational diabetes history and a family history of type 2 diabetes mellitus (T2DM).[4] GDM can raise the risk of adverse pregnancy and neonatal consequences, including spontaneous abortion, congenital anomalies, preeclampsia, perinatal asphyxia, respiratory distress, fetal demise, macrosomia, cardiomyopathy and hyperbilirubinemia.[5] Although recognized and treated during pregnancy, GDM has a life-long impact on maternal and fetal health. Women with a history of GDM are more likely to develop T2DM, cardiovascular disease (CVD) and chronic kidney disease (CKD) later in life, and their offspring tend to have metabolic diseases, such as diabetes, hypertension and obesity.[6]

Abbreviation: 1 h-PG, One-hour postload glucose; 2DE, Two-dimensional electrophoresis; 5-HIAA, 5-Hydroxyindoleacetic acid; 5-HT, 5-Hydroxytryptamine; 8-OHdG, 8-Hydroxy-2-deoxyguanosine; AAA, Aromatic amino acid; AADC, Aromatic amino acid decarboxylase; AhR, Aryl hydrocarbon receptor; Akt, Protein kinase B; a-TL, A-tocopheronolactone; AUC, Area under the curve; BCAA, Branched-chain amino acid; CE, Capillary electrophoresis; Cer, Ceramide; CKD, Chronic kidney disease; COPD, Chronic obstructive pulmonary disease; CRH, Corticotropin-releasing hormone; CVD, Cardiovascular disease; DG, Diglyceride; FA, Fatty acid; FBG, Fasting blood glucose; FPG, Fasting plasma glucose; GC, Gas chromatography; GDM, Gestational diabetes mellitus; HAPO, Hyperglycemia and adverse pregnancy outcome; HILIC-MS/MS, Hydrophilic interaction chromatography tandem mass spectrometry; IDO1, Indoleamine 2,3-dioxygenase 1; IMP, Inosine monophosphate; IR, Insulin resistance; IRS-1, Insulin receptor substrate 1; ITI, Inter-alpha-inhibitor; ITIH4, Inter-alpha-trypsin inhibitor heavy chain H4; K/T, Kynurenine to tryptophan ratio; KYN, Kynurenine; LC, Liquid chromatography; L-FABP, Liver-type fatty acid-binding protein; LGA, Large for gestational age; MALDI-TOF, Matrix-assisted laser desorption ionization time-of-flight; MS, Mass spectrometry; mTORC1, Mammalian targets of rapamycin complex 1; MUFA, Monounsaturated fatty acid; NFPG, Neonatal fasting plasma glucose; NMR, Nuclear magnetic resonance; NW, Neonatal weight; OGTT, 75 g oral glucose tolerance test; PC, Phosphatidylcholine; PCae, Choline ether phospholipid; PCOS, Polycystic ovary syndrome; PPA2, Protein phosphatase 2A; PUFA, Polyunsaturated fatty acid; RCT, Randomized controlled trial; ROC, Receiver operating characteristic; ROS, Reactive oxygen species; SFA, Saturated fatty acid; SID-MS, Stable isotope dilution direct infusion electrospray ionization mass spectrometry; SM, Sphingomyelin; SRM/MRM, Selected/multiple reaction monitoring; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; TBC1D5a, TBC1 family member 5 isoform a; TCA, Tricarboxylic acid cycle; TDO, Tryptophan 2,3-dioxygenase; TPH, Tryptophan hydroxylase; Trp, Tryptophan; UPLC-MS, Ultra-performance liquid chromatography-tandem mass spectrometry; XO, Xanthine oxidase

*Corresponding author.
**Corresponding author.
E-mail addresses: xiaoxh2014@vip.163.com (X. Xiao), zhangqian6088@pumch.cn (Q. Zhang).

Currently, diagnostic criteria for GDM are unified between obstetric and diabetes organizations from different countries.[7–10] The one-step and two-step strategies, both implemented at 24–28 weeks of gestation, are widely used and accepted internationally.[1] Nevertheless, current criteria for diagnosing GDM on the basis of plasma glucose levels fail to identify all GDM patients and cannot distinguish pregnant women who may develop postpartum diabetes or CVD.[11] Overall, early interventions for GDM, such as physical activity, diet intervention and probiotic supplementation, have been proven effective in reducing GDM risk, as confirmed by several randomized controlled trials (RCTs).[12–14] Consequently, it is essential for early prediction, precise diagnosis and timely intervention of GDM, which not only protects mothers and offspring against disadvantageous influences but also decreases health care use and cost.

The mechanism of GDM is typically described as increased insulin resistance (IR) and pancreatic β-cell defects.[15] Appearance of GDM is a gradual process. Biomarkers act as a reliable tool to reflect the internal condition of an organism and are utilized to evaluate the risk of disease development and response to therapy.[16] Numerous studies about GDM biomarkers have been published, mainly focusing on maternal blood biomarkers.[17] Moreover, some insightful researchers have investigated urine biomarkers because urine measurement has several advantages in clinical applications. For example, urine is accessible in large quantities, can be collected noninvasively, provides an abundant ingredient profile to analyze, and has the capacity to reflect biochemical imbalances in the organism. Compared with other biofluids, handling of urine samples is also relatively simple.[18]

In the last decade, many studies have been conducted to discover potential urine biomarkers of GDM with the advancement in metabolomics and proteomics techniques. The aim of the studies focusing on the first and early second trimesters of pregnancy is to identify early predictive molecules, and the aim of the studies focusing on the second and third trimesters of pregnancy is to identify urinary biomarkers that are indicative of the diagnosis of GDM and maternal and fetal outcomes. Metabolites and proteins in urine have been confirmed to vary in GDM patients at different gestational weeks (Table 1). This review aimed to objectively and thoroughly summarize studies on the application of metabolomics and proteomics approaches in GDM, highlighting aspects of potential urine biomarkers for prediction and diagnosis. We also compared the sensitivity and specificity of different predictive and diagnostic models (Table 2), clarified the possible roles of urine metabolites and proteins in the pathogenesis of GDM, and proposed strategies for future metabolomic and proteomic studies of GDM.

Urine metabolomics

Approximately 4500 metabolites have been identified in urine in relation to approximately 600 human conditions.[33] The potential ability of urine metabolites to predict and diagnose diseases has been extensively researched, including but not limited to cancers,[34] kidney diseases[35] and metabolic diseases.[36] Metabolomics is the qualitative or quantitative analysis of a variety of small molecule metabolites that are intermediate or end products of all metabolic pathways in a living organism.[37] Metabolomics analysis strategies are generally categorized as untargeted and targeted. Untargeted methods are more appropriate for exploring candidate biomarkers or metabolic mechanisms in diseases when it is unclear which metabolites or metabolic pathways are crucial to the research question. In contrast, targeted methods are typically utilized for verification of biomarkers and investigation of specific biological pathways by analyzing metabolites with similar physicochemical properties or those participating in the same biochemical pathways.[37] Liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) coupled with mass spectrometry (MS) or proton nuclear magnetic resonance (NMR) are the most frequently employed platforms for metabolomics. MS has the advantages of high sensitivity, high resolution, low sample consumption, fast analysis speed and a wide range of data coverage; its disadvantages include complex sample preparation, requirement for different chromatographic techniques and poor repeatability of data.[38] NMR does not require chromatographic processing and sample preparation is simple. It can detect tissue samples in situ and provide accurate structural information about metabolites. However, its shortcomings are low sensitivity, incomplete metabolite coverage and higher detection cost. Since both NMR and MS have advantages and disadvantages and are highly complementary, the combination of these two techniques can improve the overall quality of the study.[37] The complex data produced by high-throughput metabolomics require advanced algorithms to process, including traditional approaches such as univariate analysis and logistic regression, as well as novel approaches such as machine learning.[39] Changes in metabolic composition directly reflect disease processes, environmental exposures, and toxicological and nutritional intakes at a certain time since metabolites act as substrates or products in metabolic pathways. After validation, these key metabolites can be applied for disease diagnosis, treatment evaluation, and even prediction of disease susceptibility.[40]

GDM is influenced by multiple-level factors, which involve various metabolic pathway disorders, such as amino acids, lipids and purines.[41] Due to its unparalleled capability to measure thousands of metabolites from intricate biological systems, metabolomics is

Authors	Countries	Techniques	Study design	Gestational weeks	Changes in urine
Miriam Leitner et al.[19]	Austria	SID-MS	cross-sectional study; GDM (n = 14) vs NGT (n = 18)	12~26 weeks	↑: L-tryptophan, serotonin, N-acetylserotonin, 5-HIAA; ↓: 5-methoxytryptamine, melatonin
Kai P. Law et al.[20]	China	UPLC-MS	longitudinal cohort study; GDM (n = 27) vs NGT (n = 34)	11~14 weeks, 23~27 weeks and 29~33 weeks	↑: indoleacetaldehyde, serotonin, 5-hydroxykynurenamine, xanthine, 1-methyladenosine, 1-methylhypoxanthine and N^4-acetylcytidine (in the 1st-3rd trimesters); indoleacetic acid, indole-3-acetamide, oxitriptan and hypoxanthine (in the 1st and 2nd trimesters); uric acid and 7-methylguanine (in the 1st and 3rd trimesters); xanthosine (in the 2nd trimester)
Danuta Dudzik et al.[21]	Poland	CE-TOF/MS	cross-sectional study; GDM (n = 20) vs NGT (n = 20)	22~28 weeks	↑: hystidine, glutamine, phenylalanine, tryptophan and cystine; ↓: carnitine
Yamilé López-Hernández et al.[22]	Mexico	UPLC-MS	cross-sectional study; GDM (n = 24) vs NGT (n = 11)	third trimester	↑: 5-carboxy-alpha-chromanol, aspartame, DG (24:0/14:1), L-tryptophan, L-urobilinogen, Cer (d18:0/23:0), SM (d18:0/22:0), 11-oxo-androsterone-glucuronide, cortolone-3-glucuronide, tetrahydroaldosterone-3-glucuronide, 5-androstene-3b,16b,17a-triol, 21-deoxycortisol, 11b,17a,21-triydroxypreg-nonolone and cucurbitacin c
Xing Wang et al.[23]	China	GC/MS	cross-sectional study; GDM (n = 59) vs NGT (n = 48)	N/A	↑: D-galactose, 3-methoxytyrosine and D-glucose; ↓: 2-O-methyl-L-ascorbic acid, citraconic acid, N-methylglutamic acid, cysteine-glycine, glutamine, glycine, galactitol, trehalose, isocitric acid, 2-hydroxyhexanoic acid, nicotinic acid and 3-dehydroshikimic acid
Chunfang Qiu et al.[24]	USA	LC-MS/MS	nested case-control study; GDM (n = 25) vs NGT (n = 25)	16~17 weeks	↑: ethylmalonate and pyruvate; ↓: adipate
Kenichi Sakurai et al.[25]	Japan	HILIC-MS/MS	nested case-control study; GDM (n = 36) vs NGT (n = 31)	first and early second trimesters	↑: 1,3-diphosphoglycerate, shikimate-3-phosphate and N-acetyl-L-alanine; ↓: ethanolamine and methionine
Chunfang Qiu et al.[26]	USA	ELISA	nested case-control study; GDM (n = 55) vs NGT (n = 43)	around 16 weeks	↑: 8-OHdG
Mehmet Oğuz Erbağcı et al.[27]	Japan	LC-MS/MS	longitudinal cohort study; GDM (n = 33) vs NGT (n = 84)	11~14 weeks	↑: 8-OHdG
Wenjin Fu et al.[28]	China	ELISA	cross-sectional study; GDM (n = 101) vs normal pregnant women (n = 20) vs nonpregnant women (n = 24)	N/A	↑: L-FABP (GDM > normal pregnant women > nonpregnant women)
Zhiying Hu et al.[29]	China	MALDI-TOF/MS	case-control study; GDM (n = 74) vs NGT (n = 31)	second and third trimesters	↑: ITIH4
Zhiying Hu et al.[30]	China	MALDI-TOF/MS	case-control study; GDM (n = 60) vs normal pregnant women (n = 31) vs nonpregnant women (n = 31)	second and third trimesters	↑: coagulation factor IX, TBC1D5a and immunoglobulin kappa constant (GDM > normal pregnant women and nonpregnant women)
Brian J. KOOS et al.[31]	USA	UPLC-MS/MS	nested case-control study; GDM (n = 46) vs NGT (n = 46)	around 12 weeks	↑: argininate, saccharopine, nicotinate ribonucleoside, 7,8-dihydroneopterin and phenol glucuronide
Joana Pinto et al.[32]	Portugal	NMR	case-control study; non-treated GDM (n = 18) vs NGT (n = 34)	second and third trimesters	↑: choline, creatine, galactose and threonine; ↓: hippurate, 3-HIVA, lysine and PAG

Abbreviation: GDM: gestational diabetes mellitus; SID-MS: stable isotope dilution direct infusion electrospray ionization mass spectrometry; NGT: normal glucose tolerance; 5-HIAA: 5-hydroxyindoleacetic acid; UPLC-MS: ultra-performance liquid chromatography-tandem mass spectrometry; CE-TOF: capillary electrophoresis time-of-flight; MS: mass spectrometry; DG: diglyceride; Cer: ceramide; SM: sphingomyelin; GC: gas chromatography; LC: liquid chromatography; HILIC-MS: hydrophilic interaction chromatography tandem mass spectrometry; 8-OHdG: 8-hydroxy-2-deoxyguanosine; L-FABP: liver-type fatty acid-binding protein; MALDI-TOF: matrix-assisted laser desorption ionization time-of-flight; ITIH4: Inter-alpha-trypsin inhibitor heavy chain H4; TBC1D5a: TBC1 family member 5 isoform a; NMR: nuclear magnetic resonance; 3-HIVA: 3-hydroxyisovalericacid; PAG: phenylacetylglutamine.

Table 1: **Summary of studies discovering altered urine components in GDM patients.**

becoming a widely utilized technique for biomarker discovery and in the field of GDM research.[42] Metabolites in urine have the potential to become biomarkers for GDM and provide further insight into the etiology and pathophysiology of GDM.

Amino acid metabolites

Growing evidence indicates a close association between amino acid metabolites and metabolic disorders, such as obesity, IR and T2DM.[43–45] With advancement of metabolomics, an increasing number of amino acid metabolites related to GDM have been detected, especially aromatic amino acids (AAAs) and branched-chain amino acids (BCAAs).

AAA

AAAs are amino acids possessing a phenyl ring structure that contains tyrosine, phenylalanine and tryptophan (Trp). Several studies have shown that there is a positive correlation between the circulating (serum or plasma) level of AAAs and development of GDM.[38] Tryptophan is an essential amino acid obtained from diet. There are three main pathways of tryptophan catabolism: (1) tryptophan can be metabolized to

Models	Study design	Gestational weeks	Sensitivity	Specificity	The AUC value
Urine serotonin metabolites (L-tryptophan, serotonin, 5-HIAA, melatonin and 6-hydroxymelatonin) and 11 plasma metabolites[19]	cross-sectional study; GDM (n = 14) vs NGT (n = 18)	12~26 weeks	N/A	N/A	0.990
Urine metabolites (trehalose and 3-dehydroshikimic acid), bacteria (Enterobacteriaceae and Enterococcaceae) and fecal metabolites (5-hydroxyindoleacetic acid and valine)[23]	cross-sectional study; GDM (n-59) vs NGT (n = 48)	N/A	N/A	N/A	0.957
Urine metabolites (dihydroorotate, argininate, phenylglucuronide, 7,8-dihydroneopterin, nicotinate ribonucleoside, saccharopine and lanthionine)[31]	nested case-control study; GDM (n = 46) vs NGT (n = 46)	around 12 weeks	97.8%	95.7%	0.993
Urine proteins (coagulation factor IX, TBC1D5a and immunoglobulin kappa constant), FPG and HbA1c[30]	case-control study; GDM (n = 60) vs normal pregnant women (n = 31) vs nonpregnant women (n = 31)	second and third trimesters	76.7%	64.5%	0.758

Abbreviation: GDM: gestational diabetes mellitus; AUC: area under the curve; 5-HIAA: 5-hydroxyindoleacetic acid; TBC1D5a: TBC1 family member 5 isoform a; FPG: fasting plasma glucose.

Table 2: **Urine biomarker models for prediction or diagnosis of GDM.**

serotonin via tryptophan hydroxylase (TPH) and aromatic amino acid decarboxylase (AADC); (2) tryptophan can enter the tryptophan-kynurenine (Trp-KYN) pathway, which is regulated by indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO); (3) tryptophan can be directly converted by the gut microbiota into several molecules, including indole and its derivatives, which are ligands of the aryl hydrocarbon receptor (AhR).[46,47] Trp metabolism can affect the function of islet β-cells, IR, intestinal barrier and angiogenesis in diabetes mellitus.[48] Notably, multiple studies have found that urine tryptophan catabolism is aberrant in GDM patients, which is worthy of specific discussion.[19–22]

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from tryptophan through a series of enzymatic processes. It regulates various functions, including but not limited to the well-known roles in emotion and feeding behaviors, as well as its influence on metabolic homeostasis.[49] A study by Miriam Leitner et al. employed untargeted GC-MS identification of plasma metabolites and targeted stable isotope dilution direct infusion electrospray ionization mass spectrometry (SID-MS) detection of urine metabolites of the tryptophan-melatonin-serotonin pathway.[19] Participants included fourteen women with GDM and eighteen women with normal glucose tolerance at 12~26 weeks of pregnancy, and plasma metabolites such as tryptophan significantly distinguished the GDM patients from healthy controls. The urine target analytes L-tryptophan, serotonin, N-acetylserotonin, and 5-hydroxyindoleacetic acid (5-HIAA) were increased and 5-methoxytryptamine and melatonin reduced in the GDM patients in comparison to the healthy pregnant women. After combining the data on urine serotonin metabolites (L-tryptophan, serotonin, 5-HIAA, melatonin and 6-hydroxymelatonin), the accuracy of the GDM predictive model was enhanced, with an area under the curve (AUC) value of 0.99.[19] In mouse models, evidence suggests that serotonin synthesis in pancreatic islets activated by lactogenic signaling during pregnancy stimulates pancreatic β-cell proliferation.[50] Serotonin within pancreatic β-cells induces insulin secretion through serotonylation, whereby serotonin is covalently coupled with the small GTPases Rab3a and Rab27a.[51] These findings support the hypothesis that elevated serotonin levels potentially function as a compensatory mechanism for sustaining standard glycemia in the event of a disturbed metabolic state, such as in GDM.[19] Accordingly, abnormalities in tryptophan and serotonin metabolism may be fundamental in the pathogenesis of GDM, which requires confirmation by further studies analyzing larger cohorts.

Indoleamine 2,3-dioxygenase 1 (IDO1), the rate-limiting enzyme of the Trp-KYN pathway, has been confirmed to cause dysregulation of Trp-KYN pathway and mediate pregnancy IR. K.P. Law et al. investigated the dynamic change of the urine metabolome in a longitudinal cohort of thirty-four healthy women and twenty-seven women diagnosed with GDM. The researchers conducted ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) analysis on urine samples collected at 11~14 weeks, 23~27 weeks and 29~33 weeks of gestation. The trajectories of the urine metabolome illustrated that the majority of tryptophan metabolites were upregulated in women with GDM at all stages of gestation, especially the metabolites in the Trp-KYN pathway. 5-Hydroxykynurenamine, serotonin, indoleacetic acid, indoleacetaldehyde, oxitriptan and indole-3-acetamide demonstrated good potential as GDM biomarkers, with an AUC value > 0.7 in the first and second trimesters.[20] A conventional view of the pathogenesis of GDM is that hormones secreted by the placenta act as the dominant factor for IR in GDM. In contrast, this study found that activation of the Trp-KYN pathway in GDM occurred before the physiological effects of placental hormones were exerted, offering support for the hypothesis that GDM may be a predisposed condition in which a prediabetic state is completely expressed under stress due to alteration of metabolism in

pregnancy. Chronic low-grade inflammation in women with GDM activates IDO1, which upregulates the Trp-KYN pathway and ultimately mediates IR.[47] Genetic knockout or pharmacological inhibition of IDO1 in pregnant female mice demonstrate a reduced kynurenine to tryptophan ratio (K/T), decreased intestinal inflammation and improved IR.[52] Catabolism of tryptophan along the Trp-KYN pathway represents a promising opportunity for management of IR and GDM.[53]

BCAA

BCAAs are amino acids with a branched side chain structure consisting of leucine, valine and isoleucine. These amino acids are crucial for maintaining energy homeostasis, nutritional metabolism and immunity.[54] The positive relationship between the circulating (serum or plasma) levels of BCAAs and the risk of IR, T2DM and GDM has been reported by many studies in recent years.[55] One possible mechanism is that elevated levels of BCAAs activate mammalian targets of rapamycin complex 1 (mTORC1), ultimately contributing to IR via phosphorylation of insulin receptor substrate 1 (IRS-1).[56] Another mechanism is that toxic BCAA metabolites accumulate, leading to oxidative stress, mitochondrial malfunction and ultimately impaired insulin function.[57] The abundance of valine in the blood of GDM women is increased,[58–60] and similar results were obtained for valine in feces in Wang's study. Considering that the gut microbiota can regulate host metabolic homeostasis, this study explored interaction between the gut microbiota and relevant stool and urine metabolites in GDM.[23] Compared to healthy controls, three upregulated metabolites and twelve downregulated metabolites were observed in the urine of GDM subjects. These urinary metabolites are products of amino acid and carbohydrate metabolism according to functional clustering analysis. Maternal microbial composition was altered in feces from GDM patients, as characterized by increased abundance of the family Lachnospiraceae and decreased abundance of the families Enterobacteriaceae and Ruminococcaceae. Importantly, Lachnospiraceae and Enterobacteriaceae showed significant co-occurring relationships with metabolites related to amino acid and carbohydrate metabolism in GDM subjects. Hence, a novel combinatorial marker panel including bacteria (Enterobacteriaceae and Enterococcaceae), urine metabolites (trehalose and 3-dehydroshikimic acid) and fecal metabolites (5-hydroxyindoleacetic acid and valine) effectively distinguished GDM from healthy women, with an AUC value of 0.957. The strategy of integrating metagenomics and metabolomics provides a new perspective for understanding the pathogenesis and pathophysiology of GDM that alteration of gut microbiota may initiate GDM by disrupting the host's amino acid and carbohydrate metabolism.[23]

Lipid metabolites

Lipids, which are divided into eight principal categories, fatty acids (FAs), glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides, play a significant part in energy metabolism and cell signaling.[61] They also constitute crucial elements of hormones, cell membranes and lipid particles. Lipid homeostasis represents a unique readout for health conditions due to the dependence of cell and organ functions on lipids. Dysfunction of lipid metabolism can initiate or aggravate a variety of diseases, including atherosclerosis and stroke, diabetes and obesity, neurodegenerative and neurological disorders, autoimmune disorders, and even cancers.[62] Lipidomics, a branch of metabolomics, differs from general metabolomics because of the particular physicochemical properties of lipids, contrary to other water-soluble cellular metabolites.[63] Some lipid metabolites are strongly associated with the risk of T2DM, extending the current spectrum of predictors of T2DM in normoglycemic populations.[64] There are also some lipid metabolites that can be used as biomarkers of diabetes complications, including cardiovascular events[65] and polyneuropathy.[66] The main lipid differences closely associated with GDM are FAs, phospholipids, glycerolipids, glycerophospholipids, sphingolipids and steroids.[67–69] Due to their complex structural composition and multiple functionalities, urine lipid metabolites of GDM will be discussed separately below.

FA

FAs are the simplest lipid molecules and act as regulators of membrane properties, energy suppliers and storage materials.[70] FAs can be classified based on the saturation degree of the hydrocarbon chain into three groups: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs).[61] Qiu et al. utilized LC-MS/MS technology to measure variation in organic acid biomarkers in urine samples collected on average at 16 weeks of pregnancy. These organic acid metabolites are characterized by altered fatty acid metabolism (ethylmalonate and adipate) and carbohydrate metabolism (pyruvate). The risk of subsequent GDM correlated positively with the concentration of ethylmalonate and negatively with the concentration of adipate in maternal urine during early pregnancy.[24] The profile of organic acids in urine can provide important clues to maternal fatty acid and carbohydrate metabolism during pregnancy and may help to understand the pathogenesis of GDM. Total plasma SFAs levels are higher before the onset of GDM. Evidence from in vitro and animal models demonstrate that SFAs suppress the insulin signaling pathway and cause IR by reducing phosphorylation of insulin receptors and IRS-1. SFAs may also exert lipotoxic effects by increasing inflammation, oxidative stress and endoplasmic reticulum stress, leading to pancreatic β-cell

apoptosis and dysfunction.[71] However, most studies about circulating and urine levels of fatty acids in gestational diabetes lack consistent results, and different fatty acid subtypes play different roles in GDM risk and require further validation.

Carnitine

Carnitine, which is synthesized from lysine and methionine, is combined with long-chain acyl-CoA to form acylcarnitine. This process aids delivery of long-chain FAs across the inner mitochondrial membrane during β-oxidation of FAs.[72] The effects of several acylcarnitine species on GDM vary depending on chain length, and medium-chain acylcarnitine may induce pancreatic β-cell dysfunction.[73,74] By regulating phosphorylation of mTOR, acylcarnitine dysfunction can interfere with insulin signaling and result in IR.[38] The urine metabolic fingerprint of GDM in Dudzik's study demonstrated not only changes in amino acids but also abnormalities in carnitine metabolism. The level of carnitine in the urine of GDM patients at 22∼28 weeks of gestation is apparently decreased.[21] Similarly, low plasma carnitine concentration has been significantly associated with abnormal glucose and lipid metabolism in GDM patients during the second trimester of gestation. Based on prepregnancy BMI, weight gain, and three plasma carnitine metabolites, a prediction model for macrosomia in GDM offspring was established, with an AUC value of 0.88.[75] Carnitine supplementation boosts the ability of resting and exercising muscles to form acylcarnitine, which contributes to the beneficial effects on metabolic flexibility in individuals with impaired glucose tolerance.[76] Carnitine is expected to become a potential target for future research in diagnosis and treatment of GDM.

Glycerophospholipid

Glycerophospholipids play a role in biomembrane formation and cell signal transduction and can be further subdivided into phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, and cardiolipin, among others.[77] Many studies have reported glycerophospholipid perturbation in disease states, including prediabetes, T2DM and GDM.[78–80] Sakurai et al. carried out a case-control study in a cohort investigating predictive metabolic biomarkers for GDM in a Japanese population. Urine metabolites were analyzed by hydrophilic interaction chromatography tandem mass spectrometry (HILIC-MS/MS). In urine samples obtained during the first or early second trimester of gestation, ethanolamine, one of the metabolites in glycerophospholipid metabolism, showed an AUC value > 0.8 in the prediction model for discriminating GDM patients from others.[25] The glycerophospholipid molecules PC (32:1) and choline ether phospholipid (PCae) (40:4) in serum correlate significantly with 1-h postload glucose (1 h-PG) in the 75 g oral glucose tolerance test (OGTT) and the occurrence of GDM, which also supports involvement of glycerophospholipid metabolism in the pathogenesis of GDM. Together with triglycerides (51:1) and triglycerides (48:1), these four lipid biomarkers raised the AUC value of a predictive model based on maternal age and BMI from 0.69 to 0.74.[81] However, the effect of ethanolamine itself on GDM is not clear and needs further study.

Sphingolipid

Sphingolipids are involved in formation of membrane domains, exosomes and endosomes and modulate cell-cell interactions and cell recognition, such as cell adhesion and migration, cell senescence and death.[82] Sphingolipids affect pregnancy status, with implications for preeclampsia, intrahepatic cholestasis of pregnancy, GDM and intrauterine growth restriction.[83] By adopting the UPLC-MS method for untargeted metabolomics analysis, Yamilé López-Hernández et al. discovered fourteen metabolites that increased dramatically in the urine from GDM subjects during the third trimester, including ceramide (Cer) (d18:0/23:0) and sphingomyelin (SM) (d18:0/22:0).[22] Ceramide, which belongs to the sphingolipid family, has been implicated in IR, T2DM and GDM.[84] Serum Cer (d18:1/24:0) in early pregnancy was higher among women with GDM compared to women without GDM and identified as an independent predictor of GDM.[85] Increased de novo ceramide synthesis in the endoplasmic reticulum can activate protein phosphatase 2A (PPA2), inactivate protein kinase B (Akt) in the insulin signaling pathway and ultimately inhibit insulin sensitivity and β-cell function.[86] As one of the most prominent phospholipid components in animal cell membranes, sphingomyelin has been observed to correlate inversely with GDM in several studies.[68] Furthermore, downregulated sphingolipid metabolism may be an early predictor of the transition from GDM to T2DM. In vivo and in vitro experiments have confirmed that insulin secretion stimulated by glucose is damaged by inhibition of SM metabolism.[87] The chain length and degree of desaturation of the FA moieties in lipid molecules increase the complexity of their biological roles. In addition, lipids that are endogenously synthesized or obtained from the diet influence their accumulation and/or metabolism and subsequent biological roles.[37] Therefore, it is not surprising that there are conflicting views on the pathogenicity and mechanisms of glycerophospholipids and sphingolipids in the development of GDM. New high-resolution metabolomics technology offers broad prospects for solving this problem.

Steroid

Steroid hormones, including estrogen, progestin, androgen and corticosteroid, form a metabolic network that exerts powerful biological activity within the body

through specific receptors.[88] Abnormal steroid hormone metabolism can contribute to various gynecology and obstetrics diseases, such as preeclampsia, polycystic ovary syndrome (PCOS) and GDM.[89,90] Steroid hormones regulate pancreatic function and induce IR, mainly including diabetogenic progesterone, antidiabetic estradiol, androgens related to IR and hyperinsulinemia, neuroactive steroids that affect pituitary functioning, and cortisol, which inhibits the proinflammatory effects of corticotropin-releasing hormone (CRH).[91,92] In the study of López-Hernández, seven steroid derivatives were significantly elevated in GDM patient urine. The dysregulation of the metabolic pathway in steroid hormone biosynthesis that was identified in the pathway topology analysis and pathway enrichment analysis had a close relationship with development of GDM.[22] The total metabolic pathway in serum from cholesterol to downstream steroid hormones was increased in GDM condition, especially the estrogen metabolites via 16-pathway. 16α-hydroxyestrone achieved the highest diagnostic performance with an AUC value of 0.85.[88] Steroid metabolites have the potential to improve GDM diagnosis and further in vitro and in vivo studies on the role of steroid metabolites in pathological process of GDM should be conducted.

Purine metabolites

Purines are among the biomolecules essential for sustaining life and are involved in DNA/RNA synthesis, energy production and signaling transmission. Dysregulation and malfunction of purine and purinergic signaling are involved in the pathophysiology of gout, diabetes mellitus, nervous system diseases, cancers and other diseases.[93] Purines and their derivatives in urine are closely related to the etiology of GDM.

Uric acid

Hypoxanthine is a degradation product of ATP and can be converted to xanthine and uric acid by xanthine oxidase (XO). In the aforementioned Law's study, tracking of urine metabolites in the three trimesters of pregnancy also revealed a significant change in purine metabolism in GDM patients. Urine hypoxanthine, xanthine, xanthosine and 1-methylhypoxanthine increased in GDM subjects. As an end product of purine catabolism, uric acid could distinguish GDM patients from healthy pregnant women. Receiver operating characteristic (ROC) analysis of key purine metabolites showed that xanthine had a particularly good capacity to diagnose GDM, with an AUC value > 0.8, in each trimester.[20] In some studies on plasma purine degradation metabolites, similar results have shown that xanthine, hypoxanthine, and inosine monophosphate (IMP) are elevated in GDM patients.[94,95] A meta-analysis including 11 observational studies found that the level of serum uric acid in GDM patients was higher and correlated positively with an increased risk of GDM, especially in the first trimester.[96] Therefore, a model to elucidate the role of tryptophan and purine metabolism in the pathogenesis and pathophysiology of GDM is hypothesized. Imbalance of proinflammatory and anti-inflammatory cytokines induces chronic low-grade inflammation in GDM and upregulates the Trp-KYN pathway by activating IDO1. Increased synthesis of xanthurenic acid in the Trp-KYN pathway, which has diabetogenic effects, leads to pancreatic β-cell dysfunction and hyperglycemia. Furthermore, hyperglycemia accelerates purine nucleotide synthesis, thereby stimulating nucleotide breakdown and increasing the concentration of nucleotide degradation products, including superoxide molecules and uric acid. Reactive oxygen species (ROS) and uric acid lead to further deterioration of the condition. Excessive uric acid induces oxidative stress, ROS accumulation and mitochondrial injury in hepatocytes. Moreover, a high concentration of uric acid also contributes to local inflammation in adipocytes, with a reduction in adiponectin and induces IR.[47]

8-Hydroxy-2-deoxyguanosine

Oxidative stress is a state of imbalance between overproduction of ROS and inadequate antioxidant defense mechanisms. Accumulation of ROS, including superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, leads to oxidative damage to proteins, lipids, and DNA. 8-Hydroxy-2-deoxyguanosine (8-OHdG) is generated by interaction of hydroxyl radicals with guanine on DNA strands and is stably excreted in the urine; it is one of the most extensively studied biomarkers of oxidative damage of DNA.[97] Several diseases have been reported to correlate with high urine concentrations of 8-OHdG, such as diabetes, hypertension, CVD and chronic obstructive pulmonary disease (COPD). Aging, smoking, or occupational exposure to physical, chemical or biological substances may also increase the level of 8-OHdG.[98] Qiu et al. conducted a case-control study comparing the urine 8-OHdG level of GDM and non-GDM pregnant women at 16 weeks of pregnancy. The risk of developing GDM in patients with a urine 8-OHdG concentration >8.01 ng/mg creatinine was 3.79-fold higher than that in patients with a urine 8-OHdG concentration <4.23 ng/mg creatinine.[26] This result supports that oxidative stress is a contributor to GDM and needs to be kept under strict control.[99] Furthermore, some studies have found that iron overload is associated with an increased risk of GDM, with possible mechanisms including increased oxidative stress, which contribute to IR and inadequate insulin secretion.[100] In addition to collecting urine to assess changes in 8-OHdG, Tuna et al. collected blood to measure indicators related to blood glucose and iron status at 11~14 weeks of gestation and 24~28 weeks of gestation, including fasting blood glucose (FBG), HbA1c, ferritin and hemoglobin levels. Compared with the control group, urine 8-OHdG levels were higher in

the GDM group in the first trimester. After stratification by serum ferritin levels in the first trimester, women in the ≥50th percentile (≥13 ng/mL) had higher 8-OHdG levels than women in the <50th percentile. Notably, a significant positive association between 8-OHdG and ferritin and 1 h-PG in the OGTT was observed in the second trimester.[27] Overall, interaction between hyperglycemia, iron status and DNA oxidative damage may play a role in the pathogenesis of GDM and is a direction that needs to be highlighted in future research.

Urine proteomics

The urine proteome originates from the urogenital tract and glomerular plasma filtration and thus contains potential biomarkers associated with general health and specific urogenital diseases.[101] A proteomic biomarker is defined as a specific peptide or protein related to a specific state, for example, initiation, feature or progression of a disease or response to treatment.[102] In several diseases, abnormal proteins have been found in urine, mainly focusing on urogenital system disorders.[103,104] Urine biomarkers subtype 22 of the family of nuclear matrix proteins and human complement factor H-related protein have been approved by FDA for diagnosis and surveillance of bladder cancer.[105] MS-based urine proteomics facilitates biomarker discovery. Differences in protein or peptide concentrations between a control group and disease group are compared by means of label-free or label-based relative quantification methods and lead to selection of candidate biomarkers. Confirmation of these biomarkers by targeted absolute quantitative analysis is needed prior to clinical validation and application.[102] In the biomarker discovery phase, two-dimensional electrophoresis (2DE), LC and CE coupled to MS platforms are common technologies; in the validation phase, both traditional immunological assays such as ELISA and novel techniques such as selected/multiple reaction monitoring (SRM/MRM) technology are frequently used.[106] ELISA has high sensitivity and specificity, but is also challenged by antibody availability and the cost of assay development for new target analytes. MS-based absolute quantification has emerged as a successful alternative to immunoassays, offering lower cost, shorter turnaround time, and greatly improved throughput.[107] With the application of urine proteomics, a number of potential protein biomarkers for GDM have been identified in recent years.

Liver-type fatty acid-binding protein

A member of the fatty acid-binding protein superfamily, liver-type fatty acid-binding protein (L-FABP) is synthesized in the proximal renal tubules and is involved in FA metabolism by binding and transporting FAs to mitochondria and peroxisomes, where they are oxidized to produce energy.[108] Elevated levels of L-FABP in urine can identify diabetic nephropathy in patients with T1DM and T2DM and can also predict progression and severity of diabetic nephropathy.[109] Because GDM and diabetes have similar pathogeneses, kidney damage, a common complication of T2DM, also occurs in GDM. Some studies have shown that women with GDM have an increased risk of kidney disease after delivery, including CKD and end-stage renal disease.[110,111] Urine L-FABP measured by the ELISA kit was higher in pregnant women with GDM than in healthy pregnant women and healthy nonpregnant women and correlated positively with HbA1c and FBG.[28] Urine L-FABP may become an indicator of renal tubular injury under the stress of hyperglycemia during pregnancy.

Inter-alpha-trypsin inhibitor heavy chain H4

Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) is a glycoprotein synthesized in the liver and belongs to the inter-alpha-inhibitor (ITI) family.[112] It has been implicated in the pathogenesis of several diseases, such as various cancers, preeclampsia and recurrent pregnancy loss.[112–118] Hu et al. identified two urine peptides that exhibited noteworthy differences between the healthy pregnancy group and GDM group through utilization of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS.[29] One of the two urine peptides was ITIH4, which was detected at higher levels in GDM patients. Tracking of the dynamic changes in urine ITIH4 levels demonstrated no significant difference between the second and third trimesters, providing a basis to study the alterations in secretion patterns of urine peptides during GDM. In the GDM group, the subgroup with high fasting plasma glucose (FPG) had higher urine ITIH4 levels than the subgroup with low FPG. Secretion of ITIH4, an acute-phase inflammation-associated protein, is increased with GDM exacerbation, vascular inflammation, and immune dysfunction. Moreover, the trend of urine ITIH4 levels in GDM patients is in parallel with the reciprocal of neonatal fasting plasma glucose (NFPG) and neonatal weight (NW), indicating that it might be a predictor not only of the severity of glucose metabolism dysfunction in patients with GDM but also of neonatal hypoglycemia and macrosomia.

Coagulation factor IX

Coagulation factor IX is a vitamin K-dependent glycoprotein that mediates the coagulation response by receiving both intrinsic and extrinsic activation signals.[119] In Hu's study, peptides in urine collected during the second and third trimesters were analyzed by MALDI-TOF MS. Coagulation factor IX, TBC1 family member 5 isoform a (TBC1D5a) and immunoglobulin kappa constant were higher in GDM patients than in nonpregnant women and healthy pregnant women. A model containing FPG, HbA1c and three urine peptides can diagnose GDM, with an AUC value of 0.785.[30]

Pregnancy is often in a physiologic hypercoagulable state. However, under the burden of hyperglycemia, it becomes a pathological coagulation/fibrinolysis disorder.[120] Proteomics analysis of serum, blood exosomes and placental villi also led to similar results, namely, that the imbalance of coagulation and fibrinolysis is involved in the pathophysiology of GDM.[121–123] Hyperglycemia-induced endothelial damage upregulates the coagulation system in pregnant women with GDM, and GDM patients have a higher risk of arteriovenous thrombosis.[124] In the future, urine peptide detection kits based on urine coagulation-related peptides may be employed for diagnosis of GDM.

Conclusion

Metabolomics and proteomics related to GDM study is in a period of vigorous development. Urine amino acid metabolites, lipid metabolites, purine metabolites, key proteins and peptides show good potential in predicting and diagnosing GDM and are associated with the etiology of GDM (Fig. 1). Further large-scale and standardized longitudinal research is necessary to validate and extend current findings. Urine biomarkers of GDM will be a powerful tool in improving maternal and fetal health and interrupting the intergenerational transmission of metabolic diseases.

Outstanding questions

Although numerous studies have identified changes in urine components in pregnant women with GDM that are expected to serve as predictive or diagnostic biomarkers, there are several questions that need to be addressed before translating these findings from the laboratory to clinical application.

Metabolomics and proteomics techniques have been employed in studies about urine biomarkers of GDM, yielding a substantial quantity of data. However, data repeatability remains low, with certain outcomes appearing to be contradictory. Disparities among such findings may arise from variations in GDM diagnostic criteria, ethnicity, population size, urine sample preparation and analysis platforms. Most of the studies on urine biomarkers of GDM that have been conducted are small in scale and only detected urine in a certain period during pregnancy, without whole-pregnancy tracking. In general, the composition of urine is susceptible to interference from various factors, such as diet, exercise, and medication, which may hinder the interpretation of research results.

Therefore, to validate these identified urine biomarkers of GDM and discover new biomarkers, large-scale, multicenter, multiethnic, dynamic surveillance and prospective cohort studies are necessary in the future. Although spot or morning urine collection is a convenient approach to sample collection, 24-h urine collection is recommended because it provides a more precise and accurate measurement of urine components. Combining changes in GDM biomarkers in urine with their corresponding changes in blood or feces provides insight into their metabolic trajectories in the body and well reflects their potential for predicting or diagnosing GDM. Participant recruitment, sample preparation and storage procedures, biomarker

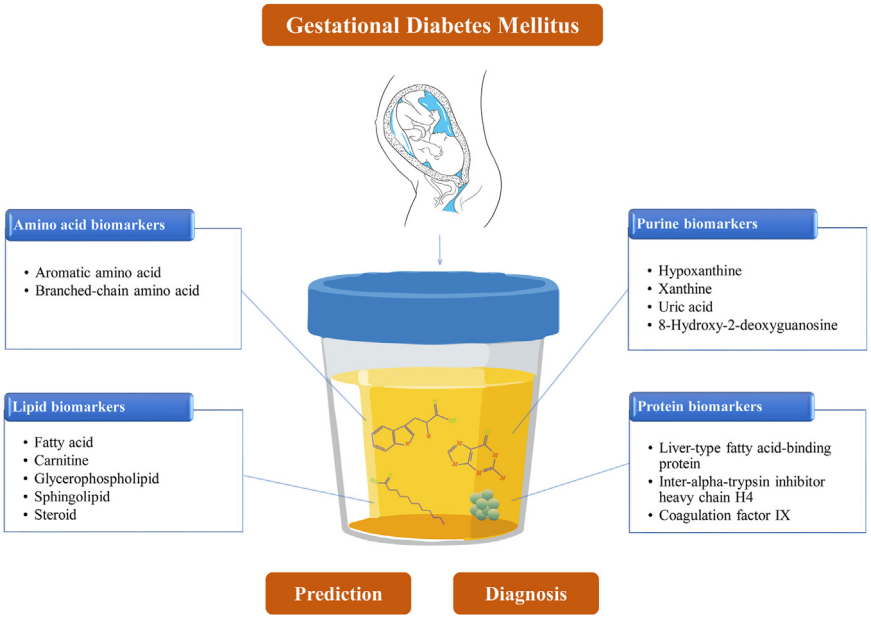

Fig. 1: **Potential urine biomarkers for prediction and diagnosis of GDM by using metabolomics and proteomics.**

Search strategy and selection criteria

Data for this review were identified by searches of PubMed and references from relevant articles using the search terms: "gestational diabetes mellitus" and "urine" cross-referenced with "biomarker", "prediction", "diagnosis", "metabolite", "metabolomics", "lipid", "protein" and "proteomics". Only articles published in English up to Oct 1, 2023 were included.

measurement and analysis, and elaboration and evaluation of results all require standardized designs to collect meaningful and comparable data. Rapid advancement in analytical technology and the upgrade of metabolic databases, bioinformatics and artificial intelligence have also provided assistance in further searching for urine biomarkers of GDM. By using sophisticated biology computational tools to integrate urine metabolomics, proteomics, genomics and transcriptomics data, such powerful integrated analysis will elucidate the pathogenesis and pathophysiology of GDM and provide targets for precise therapy.

Contributors

Jie Yu, Xinhua Xiao and Qian Zhang conceived the paper. Jie Yu, Jing Ren and Yaolin Ren wrote the original draft. Jie Yu, Yifan Wu and Yuan Zeng polished the manuscript and prepared tables and figures. Xinhua Xiao and Qian Zhang contributed to the final revision and editing of this manuscript. All authors have read and approved the published version of manuscript.

Declaration of interests

The authors declare no conflicts of interest.

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References

1 ElSayed NA, Aleppo G, Aroda VR, et al. 2. Classification and diagnosis of diabetes: standards of care in diabetes-2023. *Diabetes Care*. 2023;46:S19–S40.
2 Jiang L, Tang K, Magee LA, et al. A global view of hypertensive disorders and diabetes mellitus during pregnancy. *Nat Rev Endocrinol*. 2022;18:760–775.
3 Alejandro EU, Mamerto TP, Chung G, et al. Gestational diabetes mellitus: a harbinger of the vicious cycle of diabetes. *Int J Mol Sci*. 2020;21:5003.
4 McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. *Nat Rev Dis Primers*. 2019;5:47.
5 Ye W, Luo C, Huang J, Li C, Liu Z, Liu F. Gestational diabetes mellitus and adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ*. 2022;377:e067946.
6 ElSayed NA, Aleppo G, Aroda VR, et al. 15. Management of diabetes in pregnancy: standards of care in diabetes-2023. *Diabetes Care*. 2023;46:S254–S266.
7 ACOG practice bulletin no. 190: gestational diabetes mellitus. *Obstet Gynecol*. 2018;131:e49–e64.
8 Metzger BE, Gabbe SG, Persson B, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. 2010;33:676–682.
9 American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2011;34(Suppl 1):S62–S69.
10 Vandorsten JP, Dodson WC, Espeland MA, et al. NIH consensus development conference: diagnosing gestational diabetes mellitus. *NIH Consens State Sci Statements*. 2013;29:1–31.
11 Lorenzo-Almoros A, Hang T, Peiro C, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. *Cardiovasc Diabetol*. 2019;18:140.
12 Shepherd E, Gomersall JC, Tieu J, Han S, Crowther CA, Middleton P. Combined diet and exercise interventions for preventing gestational diabetes mellitus. *Cochrane Database Syst Rev*. 2017;11:CD010443.
13 Davenport MH, Ruchat SM, Poitras VJ, et al. Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *Br J Sports Med*. 2018;52:1367–1375.
14 Brown J, Ceysens G, Boulvain M. Exercise for pregnant women with gestational diabetes for improving maternal and fetal outcomes. *Cochrane Database Syst Rev*. 2017;6:CD012202.
15 Egan AM, Dow ML, Vella A. A review of the pathophysiology and management of diabetes in pregnancy. *Mayo Clin Proc*. 2020;95:2734–2746.
16 Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69:89–95.
17 Roverso M, Dogra R, Visentin S, et al. Mass spectrometry-based "omics" technologies for the study of gestational diabetes and the discovery of new biomarkers. *Mass Spectrom Rev*. 2023;42:1424–1461.
18 Zhang T, Watson DG. A short review of applications of liquid chromatography mass spectrometry based metabolomics techniques to the analysis of human urine. *Analyst*. 2015;140:2907–2915.
19 Leitner M, Fragner L, Danner S, et al. Combined metabolomic analysis of plasma and urine reveals AHBA, tryptophan and serotonin metabolism as potential risk factors in gestational diabetes mellitus (GDM). *Front Mol Biosci*. 2017;4:84.
20 Law KP, Han TL, Mao X, Zhang H. Tryptophan and purine metabolites are consistently upregulated in the urinary metabolome of patients diagnosed with gestational diabetes mellitus throughout pregnancy: a longitudinal metabolomics study of Chinese pregnant women part 2. *Clin Chim Acta*. 2017;468:126–139.
21 Dudzik D, Zorawski M, Skotnicki M, et al. Metabolic fingerprint of gestational diabetes mellitus. *J Proteomics*. 2014;103:57–71.
22 López-Hernandez Y, Herrera-Van Oostdam AS, Toro-Ortiz JC, et al. Urinary metabolites altered during the third trimester in pregnancies complicated by gestational diabetes mellitus: relationship with potential upcoming metabolic disorders. *Int J Mol Sci*. 2019;20:1186.
23 Wang X, Liu HL, Li YF, et al. Altered gut bacterial and metabolic signatures and their interaction in gestational diabetes mellitus. *Gut Microbes*. 2020;12:1.
24 Qiu C, Enquobahrie DA, Frederick IO, et al. Early pregnancy urinary biomarkers of fatty acid and carbohydrate metabolism in pregnancies complicated by gestational diabetes. *Diabetes Res Clin Pract*. 2014;104:393–400.
25 Sakurai K, Eguchi A, Watanabe M, Yamamoto M, Ishikawa K, Mori C. Exploration of predictive metabolic factors for gestational diabetes mellitus in Japanese women using metabolomic analysis. *J Diabetes Investig*. 2019;10:513–520.

26 Qiu C, Hevner K, Abetew D, Enquobahrie DA, Williams MA. Oxidative DNA damage in early pregnancy and risk of gestational diabetes mellitus: a pilot study. *Clin Biochem*. 2011;44:804–808.
27 Erbagci MO, Tuna G, Kose S, et al. Association between early oxidative DNA damage and iron status in women with gestational diabetes mellitus. *Reprod Toxicol*. 2021;103:171–180.
28 Fu WJ, Wang DJ, Deng RT, et al. Urinary liver-type fatty acid-binding protein change in gestational diabetes mellitus. *Diabetes Res Clin Pract*. 2015;109:e36–e38.
29 Hu Z, Hou J, Zhang M. Levels of inter-alpha-trypsin inhibitor heavy chain H4 urinary polypeptide in gestational diabetes mellitus. *Syst Biol Reprod Med*. 2021;67:428–437.
30 Hu Z, Zhang M, Tian Y. Screening and analysis of small molecular peptides in urine of gestational diabetes mellitus. *Clin Chim Acta*. 2020;502:174–182.
31 Koos BJ, Gornbein JA. Early pregnancy metabolites predict gestational diabetes mellitus: implications for fetal programming. *Am J Obstet Gynecol*. 2021;224:215.e211–215.e217.
32 Pinto J, Diaz SO, Aguiar E, et al. Metabolic profiling of maternal urine can aid clinical management of gestational diabetes mellitus. *Metabolomics*. 2016;12:48.
33 Miller IJ, Peters SR, Overmyer KA, Paulson BR, Westphall MS, Coon JJ. Real-time health monitoring through urine metabolomics. *NPJ Digit Med*. 2019;2:109.
34 Dinges SS, Hohm A, Vandergrift LA, et al. Cancer metabolomic markers in urine: evidence, techniques and recommendations. *Nat Rev Urol*. 2019;16:339–362.
35 Gao H, Lin J, Xiong F, Yu Z, Pan S, Huang Y. Urinary microbial and metabolomic profiles in kidney stone disease. *Front Cell Infect Microbiol*. 2022;12:953392.
36 Salek RM, Maguire ML, Bentley E, et al. A metabolomic comparison of urinary changes in type 2 diabetes in mouse, rat, and human. *Physiol Genomics*. 2007;29:99–108.
37 Wang QY, You LH, Xiang LL, Zhu YT, Zeng Y. Current progress in metabolomics of gestational diabetes mellitus. *World J Diabetes*. 2021;12:1164–1186.
38 Xie JW, Li L, Xing HY. Metabolomics in gestational diabetes mellitus: a review. *Clin Chim Acta*. 2023;539:134–143.
39 Zhang MQ, Yang HX. Perspectives from metabolomics in the early diagnosis and prognosis of gestational diabetes mellitus. *Front Endocrinol*. 2022;13:967191.
40 Mao X, Chen XY, Chen C, Zhang H, Lawa KP. Metabolomics in gestational diabetes. *Clin Chim Acta*. 2017;475:116–127.
41 Alesi S, Ghelani D, Rassie K, Mousa A. Metabolomic biomarkers in gestational diabetes mellitus: a review of the evidence. *Int J Mol Sci*. 2021;22:5512.
42 Lu WQ, Hu C. Molecular biomarkers for gestational diabetes mellitus and postpartum diabetes. *Chinese Med J*. 2022;135:1940–1951.
43 Du C, Liu WJ, Yang J, Zhao SS, Liu HX. The role of branched-chain amino acids and branched-chain alpha-Keto acid dehydrogenase kinase in metabolic disorders. *Front Nutr*. 2022;9:932670.
44 Sunny NE, Kalavalapalli S, Bril F, et al. Cross-talk between branched-chain amino acids and hepatic mitochondria is compromised in nonalcoholic fatty liver disease. *Am J Physiol Endocrinol Metab*. 2015;309:E311–E319.
45 Yoon MS. The emerging role of branched-chain amino acids in insulin resistance and metabolism. *Nutrients*. 2016;8:405.
46 Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe*. 2018;23:716–724.
47 Law KP, Zhang H. The pathogenesis and pathophysiology of gestational diabetes mellitus: deductions from a three-part longitudinal metabolomics study in China. *Clin Chim Acta*. 2017;468:60–70.
48 Gao JL, Yang T, Song BH, et al. Abnormal tryptophan catabolism in diabetes mellitus and its complications: opportunities and challenges. *Biomed Pharmacother*. 2023;166:115395.
49 Choi WG, Choi W, Oh TJ, et al. Inhibiting serotonin signaling through HTR2B in visceral adipose tissue improves obesity-related insulin resistance. *J Clin Invest*. 2021;131:e145331.
50 Kim H, Toyofuku Y, Lynn FC, et al. Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat Med*. 2010;16:804–808.
51 Paulmann N, Grohmann M, Voigt JP, et al. Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol*. 2009;7:e1000229.
52 Priyadarshini M, Navarro G, Reiman DJ, et al. Gestational insulin resistance is mediated by the gut microbiome-indoleamine 2,3-dioxygenase Axis. *Gastroenterology*. 2022;162:1675.
53 Koziel K, Urbanska EM. Kynurenine pathway in diabetes mellitus-novel pharmacological target? *Cells*. 2023;12:460.
54 Nie CX, He T, Zhang WJ, Zhang GL, Ma X. Branched chain amino acids: beyond nutrition metabolism. *Int J Mol Sci*. 2018;19:954.
55 Wang XX, Zhang Y, Zheng W, et al. Dynamic changes and early predictive value of branched-chain amino acids in gestational diabetes mellitus during pregnancy. *Front Endocrinol*. 2022;13:1000296.
56 Neinast M, Murashige D, Arany Z. Branched chain amino acids. *Annu Rev Physiol*. 2019;81:139–164.
57 Li NH, Li J, Wang H, et al. Branched-chain amino acids and their interactions with lipid metabolites for increased risk of gestational diabetes. *J Clin Endocrinol Metab*. 2022;107:E3058–E3065.
58 Hou WL, Meng XY, Zhao AH, et al. Development of multimarker diagnostic models from metabolomics analysis for gestational diabetes mellitus (GDM). *Mol Cell Proteomics*. 2018;17:431–441.
59 Enquobahrie DA, Denis M, Tadesse MG, Gelaye B, Ressom HW, Williams MA. Maternal early pregnancy serum metabolites and risk of gestational diabetes mellitus. *J Clin Endocr Metab*. 2015;100:4348–4356.
60 White SL, Pasupathy D, Sattar N, et al. Metabolic profiling of gestational diabetes in obese women during pregnancy. *Diabetologia*. 2017;60:1903–1912.
61 Wu Z, Bagarolo GI, Thoroe-Boveleth S, Jankowski J. "Lipidomics": mass spectrometric and chemometric analyses of lipids. *Adv Drug Deliv Rev*. 2020;159:294–307.
62 Meikle TG, Huynh K, Giles C, Meikle PJ. Clinical lipidomics: realizing the potential of lipid profiling. *J Lipid Res*. 2021;62:100127.
63 Han X, Gross RW. The foundations and development of lipidomics. *J Lipid Res*. 2022;63:100164.
64 Lu J, Lam SM, Wan Q, et al. High-coverage targeted lipidomics reveals novel serum lipid predictors and lipid pathway dysregulation antecedent to type 2 diabetes onset in normoglycemic Chinese adults. *Diabetes Care*. 2019;42:2117–2126.
65 Ferreira-Divino LF, Suvitaival T, Rotbain Curovic V, et al. Circulating metabolites and molecular lipid species are associated with future cardiovascular morbidity and mortality in type 1 diabetes. *Cardiovasc Diabetol*. 2022;21:135.
66 Rumora AE, Guo K, Alakwaa FM, et al. Plasma lipid metabolites associate with diabetic polyneuropathy in a cohort with type 2 diabetes. *Ann Clin Transl Neurol*. 2021;8:1292–1307.
67 Zhan Y, Wang J, He X, et al. Plasma metabolites, especially lipid metabolites, are altered in pregnant women with gestational diabetes mellitus. *Clin Chim Acta*. 2021;517:139–148.
68 Wang Y, Wu P, Huang Y, et al. BMI and lipidomic biomarkers with risk of gestational diabetes in pregnant women. *Obesity (Silver Spring)*. 2022;30:2044–2054.
69 Wu P, Wang Y, Ye Y, et al. Liver biomarkers, lipid metabolites, and risk of gestational diabetes mellitus in a prospective study among Chinese pregnant women. *BMC Med*. 2023;21:150.
70 de Carvalho C, Caramujo MJ. The various roles of fatty acids. *Molecules*. 2018;23:2583.
71 Wang Y, Pan XF, Pan A. Lipidomics in gestational diabetes mellitus. *Curr Opin Lipidol*. 2023;34:1–11.
72 Virmani MA, Cirulli M. The role of l-carnitine in mitochondria, prevention of metabolic inflexibility and disease initiation. *Int J Mol Sci*. 2022;23:2717.
73 Lin Y, Wu J, Zhu Y, et al. A longitudinal study of plasma acylcarnitines throughout pregnancy and associations with risk of gestational diabetes mellitus. *Clin Nutr*. 2021;40:4863–4870.
74 Batchuluun B, Al Rijjal D, Prentice KJ, et al. Elevated medium-chain acylcarnitines are associated with gestational diabetes mellitus and early progression to type 2 diabetes and induce pancreatic beta-cell dysfunction. *Diabetes*. 2018;67:885–897.
75 Sun M, Zhao B, He S, et al. The alteration of carnitine metabolism in second trimester in GDM and a nomogram for predicting macrosomia. *J Diabetes Res*. 2020;2020:4085757.
76 Bruls YM, de Ligt M, Lindeboom L, et al. Carnitine supplementation improves metabolic flexibility and skeletal muscle acetylcarnitine formation in volunteers with impaired glucose tolerance: a randomised controlled trial. *eBioMedicine*. 2019;49:318–330.
77 Wang R, Li B, Lam SM, Shui G. Integration of lipidomics and metabolomics for in-depth understanding of cellular mechanism and disease progression. *J Genet Genomics*. 2020;47:69–83.

78 Guasch-Ferré MHA, Toledo E, Clish CB, et al. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. *Diabetes Care*. 2016;39:833–846.
79 Zeng H, Tong R, Tong W, et al. Metabolic biomarkers for prognostic prediction of pre-diabetes: results from a longitudinal cohort study. *Sci Rep*. 2017;7:6575.
80 Zhao H, Li H, Chung ACK, et al. Large-scale longitudinal metabolomics study reveals different trimester-specific alterations of metabolites in relation to gestational diabetes mellitus. *J Proteome Res*. 2019;18:292–300.
81 Lu L, Koulman A, Petry CJ, et al. An unbiased lipidomics approach identifies early second trimester lipids predictive of maternal glycemic traits and gestational diabetes mellitus. *Diabetes Care*. 2016;39:2232–2239.
82 Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. *Nat Rev Mol Cell Biol*. 2018;19:175–191.
83 Enthoven LF, Shi Y, Fay E, et al. Effects of pregnancy on plasma sphingolipids using a metabolomic and quantitative analysis approach. *Metabolites*. 2023;13:1026.
84 Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. *Nat Rev Endocrinol*. 2017;13:79–91.
85 Mustaniemi S, Keikkala E, Kajantie E, et al. Serum ceramides in early pregnancy as predictors of gestational diabetes. *Sci Rep*. 2023;13:13274.
86 Yang J, Wang M, Yang D, et al. Integrated lipids biomarker of the prediabetes and type 2 diabetes mellitus Chinese patients. *Front Endocrinol (Lausanne)*. 2022;13:1065665.
87 Khan SR, Mohan H, Liu Y, et al. The discovery of novel predictive biomarkers and early-stage pathophysiology for the transition from gestational diabetes to type 2 diabetes. *Diabetologia*. 2019;62:687–703.
88 Yang N, Zhang W, Ji C, et al. Metabolic alteration of circulating steroid hormones in women with gestational diabetes mellitus and the related risk factors. *Front Endocrinol (Lausanne)*. 2023;14:1196935.
89 Altinkilic EM, du Toit T, Sakin O, Attar R, Groessl M, Fluck CE. The serum steroid signature of PCOS hints at the involvement of novel pathways for excess androgen biosynthesis. *J Steroid Biochem Mol Biol*. 2023;233:106366.
90 Shu C, Han S, Xu P, Wang Y, Cheng T, Hu C. Estrogen and preeclampsia: potential of estrogens as therapeutic agents in preeclampsia. *Drug Des Devel Ther*. 2021;15:2543–2550.
91 Hill M, Parizek A, Simjak P, et al. Steroids, steroid associated substances and gestational diabetes mellitus. *Physiol Res*. 2021;70:S617–S634.
92 Sun Y, Zhu B, Meng X, et al. Effect of maternal body mass index on the steroid profile in women with gestational diabetes mellitus. *Front Endocrinol (Lausanne)*. 2022;13:999154.
93 Huang Z, Xie N, Illes P, et al. From purines to purinergic signalling: molecular functions and human diseases. *Signal Transduct Target Ther*. 2021;6:162.
94 Heath H, Rosario R, McMichael LE, et al. Gestational diabetes is characterized by decreased medium-chain acylcarnitines and elevated purine degradation metabolites across pregnancy: a case-control time-course analysis. *J Proteome Res*. 2023;22:1603–1613.
95 McMichael LE, Heath H, Johnson CM, et al. Metabolites involved in purine degradation, insulin resistance, and fatty acid oxidation are associated with prediction of Gestational diabetes in plasma. *Metabolomics*. 2021;17:105.
96 Su S, Zhang E, Gao S, et al. Serum uric acid and the risk of gestational diabetes mellitus: a systematic review and meta-analysis. *Gynecol Endocrinol*. 2023;39:2231101.
97 Di Minno A, Turnu L, Porro B, et al. 8-Hydroxy-2-Deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature. *Antioxid Redox Signal*. 2016;24:548–555.
98 Graille M, Wild P, Sauvain JJ, Hemmendinger M, Guseva Canu I, Hopf NB. Urinary 8-OHdG as a biomarker for oxidative stress: a systematic literature review and meta-analysis. *Int J Mol Sci*. 2020;21:3743.
99 Urbaniak SK, Boguszewska K, Szewczuk M, Kazmierczak-Baranska J, Karwowski BT. 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine (8-oxodG) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a potential biomarker for gestational diabetes mellitus (GDM) development. *Molecules*. 2020;25:202.
100 Petry CJ. Iron supplementation in pregnancy and risk of gestational diabetes: a narrative review. *Nutrients*. 2022;14:4791.
101 Harpole M, Davis J, Espina V. Current state of the art for enhancing urine biomarker discovery. *Expert Rev Proteomics*. 2016;13:609–626.
102 Thomas S, Hao L, Ricke WA, Li L. Biomarker discovery in mass spectrometry-based urinary proteomics. *Proteomics Clin Appl*. 2016;10:358–370.
103 Chebotareva NV, Vinogradov A, Brzhozovskiy AG, et al. Potential urine proteomic biomarkers for focal segmental glomerulosclerosis and minimal change disease. *Int J Mol Sci*. 2022;23.
104 Khoo A, Liu LY, Nyalwidhe JO, et al. Proteomic discovery of non-invasive biomarkers of localized prostate cancer using mass spectrometry. *Nat Rev Urol*. 2021;18:707–724.
105 Maas M, Todenhofer T, Black PC. Urine biomarkers in bladder cancer–current status and future perspectives. *Nat Rev Urol*. 2023;20:597–614.
106 Thomas CE, Sexton W, Benson K, Sutphen R, Koomen J. Urine collection and processing for protein biomarker discovery and quantification. *Cancer Epidemiol Biomarkers Prev*. 2010;19:953–959.
107 Chantada-Vazquez MDP, Bravo SB, Barbosa-Gouveia S, Alvarez JV, Couce ML. Proteomics in inherited metabolic disorders. *Int J Mol Sci*. 2022;23:14744.
108 Schrezenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM. Biomarkers in acute kidney injury–pathophysiological basis and clinical performance. *Acta Physiol (Oxf)*. 2017;219:554–572.
109 Zhang L, Xue S, Wu M, Dong D. Performance of urinary liver-type fatty acid-binding protein in diabetic nephropathy: a meta-analysis. *Front Med (Lausanne)*. 2022;9:914587.
110 Hare MJL, Maple-Brown LJ, Shaw JE, et al. Risk of kidney disease following a pregnancy complicated by diabetes: a longitudinal, population-based data-linkage study among Aboriginal women in the Northern Territory, Australia. *Diabetologia*. 2023;66:837–846.
111 Bomback AS, Rekhtman Y, Whaley-Connell AT, et al. Gestational diabetes mellitus alone in the absence of subsequent diabetes is associated with microalbuminuria: results from the Kidney Early Evaluation Program (KEEP). *Diabetes Care*. 2010;33:2586–2591.
112 Sun Y, Jin J, Jing H, et al. ITIH4 is a novel serum biomarker for early gastric cancer diagnosis. *Clin Chim Acta*. 2021;523:365–373.
113 Wen Q, Liu LY, Yang T, et al. Peptidomic identification of serum peptides diagnosing preeclampsia. *PLoS One*. 2013;8:e65571.
114 Li L, Choi B-C, Ryoo JE, et al. Opposing roles of inter-α-trypsin inhibitor heavy chain 4 in recurrent pregnancy loss. *eBioMedicine*. 2018;37:535–546.
115 Larsen JB, Pihl R, Aggerbeck MA, et al. Inter-alpha-inhibitor heavy chain H4 and sepsis-related coagulation disturbances: Another link between innate immunity and coagulation. *Res Pract Thromb Haemost*. 2023;7:100078.
116 Laursen TL, Bossen L, Pihl R, et al. Highly increased levels of inter-alpha-inhibitor heavy chain 4 (ITIH4) in autoimmune cholestatic liver diseases. *J Clin Transl Hepatol*. 2022;10:796–802.
117 Noh CK, Kim SS, Kim DK, et al. Inter-alpha-trypsin inhibitor heavy chain H4 as a diagnostic and prognostic indicator in patients with hepatitis B virus-associated hepatocellular carcinoma. *Clin Biochem*. 2014;47:1257–1261.
118 Huang M, Zhang W, Zhao B, Li L. Relationship between inter-alpha-trypsin inhibitor heavy chain 4 and ovarian cancer. *Chin J Cancer Res*. 2019;31:955–964.
119 Yin R, Liu C. Human coagulation factor IX: a systematic review of its characteristics. *Blood Coagul Fibrinolysis*. 2022;33:1–7.
120 Shen L, Zhao D, Chen Y, et al. Comparative proteomics analysis of serum proteins in gestational diabetes during early and middle stages of pregnancy. *Proteomics Clin Appl*. 2019;13:e1800060.
121 Zhao D, Shen L, Wei Y, et al. Identification of candidate biomarkers for the prediction of gestational diabetes mellitus in the early stages of pregnancy using iTRAQ quantitative proteomics. *Proteomics Clin Appl*. 2017;11:7.
122 Liu B, Xu Y, Voss C, et al. Altered protein expression in gestational diabetes mellitus placentas provides insight into insulin resistance and coagulation/fibrinolysis pathways. *PLoS One*. 2012;7:e44701.
123 Bernea EG, Suica VI, Uyy E, et al. Exosome proteomics reveals the deregulation of coagulation, complement and lipid metabolism proteins in gestational diabetes mellitus. *Molecules*. 2022;27:5502.
124 Zheng Y, Hou W, Xiao J, Huang H, Quan W, Chen Y. Application value of predictive model based on maternal coagulation function and glycolipid metabolism indicators in early diagnosis of gestational diabetes mellitus. *Front Public Health*. 2022;10:850191.