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DIABETES AND PREGNANCY (MF HIVERT, SECTION EDITOR)

Metabolomics of Diabetes in Pregnancy

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Abstract
Purpose of review The purpose of this review is to describe ways in which metabolomics may enhance understanding of gestational diabetes mellitus (GDM) etiology and refine current diagnostic criteria.
Recent findings Current clinical recommendations suggest screening for GDM between 24 and 28 of gestational weeks using an oral glucose tolerance test. Despite this consensus, there are discrepancies regarding the exact criteria for GDM diagnosis. Further, emerging evidence has unveiled heterogeneous physiological pathways underlying GDM—specifically, GDM with defective insulin secretion vs. sensitivity—that have important implications for disease diagnosis and management.
Summary The objectives of this review are threefold. First, we seek to provide a brief summary of current knowledge regarding GDM pathophysiology. Next, we describe the potential role of metabolomics to refine and improve the prediction, screening, and diagnosis of GDM. Finally, we propose ways in which metabolomics may eventually impact clinical care and risk assessment for GDM and its comorbidities.

Keywords Gestational diabetes · Gestational hyperglycemia · Gestational glucose tolerance · Metabolomics

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Introduction

Gestational diabetes mellitus (GDM)—defined as glucose intolerance with first recognition or onset during pregnancy [1]—is one of the most common pregnancy complications, affecting approximately 10% of the pregnancies in the USA [2]. While the absolute prevalence of GDM is relatively low, it is one of the fastest-growing pregnancy comorbidities in the USA, with an increase of over 50% between 2000 and 2010 [3]. These trends are concerning, because GDM is associated with significant morbidity for both the mother and the offspring [4]. For the mother, GDM is associated with maternal hypertensive disorders during pregnancy, C-section delivery, impaired lactogenesis, and difficulties breastfeeding [5]; future development of overt type 2 diabetes mellitus, cardiovascular disease, and metabolic syndrome [1, 6–8]. For the infant, GDM is a leading risk factor of macrosomia [9], neonatal hypoglycemia, jaundice, polycythemia and hypocalcemia, and preterm birth, which itself is associated with a range of adverse short- and long-term health consequences [10].

The central physiological disturbances of GDM revolve around increased insulin resistance and decreased insulin secretion, with most diagnoses made during the second trimester based on fasting glucose tolerance tests, e.g., the oral glucose tolerance test (OGTT), which is sometimes preceded by non-fasting screening glucose challenge test (GCT). Identification in early pregnancy of women with overt diabetes, as well as those at risk of developing GDM, is of interest to researchers and clinicians alike given the abovementioned morbidities of uncontrolled hyperglycemia during pregnancy. However, gaps and controversies surrounding knowledge of GDM disease etiology (e.g., risk factors, biological mechanisms underlying pathogenesis) and diagnostic criteria (e.g., type of assessment, appropriate cutoffs) present hurdles. In this review, we begin by providing a brief overview of the

pathophysiology of GDM. Next, we introduce the technique of metabolomics and how it could improve current understanding of disease etiology and refine diagnostic criteria. Finally, we end with some suggestions for future directions, including ways in which metabolomics may aid in identification of women who are at risk for development of GDM-related postpartum metabolic conditions and the potential contribution of metabolomics to clinical risk assessment and practice.

Pathophysiology

During pregnancy, a woman's body undergoes profound physiological changes to support fetal development. With respect to glucose metabolism, maternal insulin sensitivity typically decreases towards the end of the first trimester [11, 12]. This phenomenon is thought to favor glucose supply to the fetus, as a result of reduced insulin-mediated utilization of glucose in the mother, which switches her energy metabolism from the pre-dominant use of carbohydrates to lipids [13]. In parallel with the decrease in maternal insulin sensitivity, pancreatic β cell insulin secretion increases steadily from the first trimester, reaching a maximum in the third trimester before returning to normal values after delivery [14, 15].

GDM is caused by an imbalance between insulin resistance and insulin secretion during pregnancy which, historically, has been thought to occur when the pancreatic β cells fail to keep pace with the increasing insulin resistance that occurs during the second half of pregnancy [2]. However, a recent study by Powe et al. brought to light the heterogeneity in GDM pathogenesis [16]. In an analysis of 809 pregnant women, the researchers categorized participants into four subgroups: GDM with an insulin secretion defect (<25th percentile of the Stumvoll first-phase estimate [17, 18]; "GDM-secretion"), GDM with an insulin sensitivity defect (<25th percentile of the Matsuda index [19]; "GDM-sensitivity"), GDM with both defects ("GDM-mixed"), and normal glucose tolerance (NGT) based on results from a fasting 75-g OGTT administered at 24–30 gestational weeks. Compared to the NGT participants, the GDM-sensitivity defect group had greater odds of cesarean delivery and higher offspring birth weight, even after adjustment for maternal BMI (which was higher in GDM-sensitivity defect group). The GDM sensitivity defect group also had higher leptin and lower adiponectin levels. These findings bring to light the physiological heterogeneity within GDM subtypes, a concept that is not addressed by current methods of diagnoses (summarized in Table 1)—a controversial topic that has been reviewed in greater detail elsewhere [20, 21]. Metabolomic profiling could help us to parse out the heterogeneity and better understand the different pathophysiology of GDM subtypes.

Metabolomic Profiles of GDM and GDM-related Maternal/Offspring Outcomes

What is Metabolomics?

In recent years, advancements in high-throughput technologies have made it possible to systematically and comprehensively study associations of various biological conditions with differences in genetics ("genomics"), gene expression ("transcriptomics"), protein structure and function ("proteomics"), and metabolites ("metabolomics"). Of particular interest in this review is metabolomics, as it provides a snapshot of dynamic biochemical processes and, thus, may provide novel insights into disease onset, severity, and progression. In a review published in 2014, Huynh et al. summarized results of 17 studies exploring differences in metabolite profiles associated with GDM, several of which employed conventional methods of biomarker assessment, including the enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) [22]. In the present review, we focus on metabolomic studies that utilize high-throughput platforms—namely, mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy [23]—that allow for the systematic and comprehensive assessment of small molecules in biological tissues and fluids.

Metabolomic studies are broadly categorized as untargeted or targeted. In brief, untargeted assays provide a snapshot of relative concentrations of all measureable analytes within a biological sample. Following spectrographic analyses, a separate labor-intensive step is required to ascertain the chemical identities of statistically significant peaks via cross-reference to a chemical library; this process is described in greater detail elsewhere [24]. On the other hand, targeted assays, which quantify physiological levels of a specific list of compounds of a priori interest, include internal standards in order to derive absolute concentrations of each metabolite. Due to its broad and unbiased coverage, untargeted platforms are often used for biomarker discovery, particularly when there are no pre-existing hypotheses regarding specific biochemical pathways of interest [25], whereas targeted assays are more frequently (but not exclusively) used for hypothesis-driven investigations and/or for confirmatory studies.

Current Knowledge Regarding Metabolomics and GDM

Metabolomic Profiles of GDM

Current knowledge of metabolite patterns associated with GDM have arisen pre-dominantly from case-control studies

Table 1 Commonly used criteria for diagnosis of gestational diabetes mellitus (GDM)

Guideline	Type of test	Method	Timing	No. of abnormal values for diagnosis	Cutoff			
					Baseline	1 h	2 h	3 h
WHO 1998 [56]	75-g OGTT after overnight fast	One-step method	24–28 weeks	1	≥126 mg/dL or ≥7.0 mmol/L		≥200 mg/dL or ≥11.1 mmol/L	
ADA 2016 [57]	75-g OGTT after overnight fast	One-step method (high-risk women only[a])	First prenatal visit	1	>92 mg/dL or >5.1 mmol/L	>180 mg/dL or >10.0 mmol/L	>153 mg/dL or >8.5 mmol/L	
	50-g non-fasting GCT	Two-step method (non-high-risk women)	24–28 weeks	1		>140 mg/dL or >7.8 mmol/L		
	100-g OGTT after overnight fast	Two-step method following abnormal 50-g GCT	Following abnormal 50-g GCT	2 or more	>95–105 mg/dL or >5.3–5.8 mmol/L	>180–190 mg/dL or >10.0–10.6 mmol/L	>155–165 mg/dL or >8.6–9.2 mmol/L	>140–145 mg/dL or >1.8–8.0 mmol/L
IADPSG [58]	75-g OGTT after overnight fast	One-step	24–28 weeks	1	>92 mg/dL or >5.1 mmol/L	>180 mg/dL or >10.0 mmol/L	>153 mg/dL or >8.5 mmol/L	
HAPO 2008 [59]	75-g OGTT after overnight fast	N/A (mean values for study participants)	24–28 weeks	N/A	80.9 mg/dL or 4.5 mmol/L	134.1 mg/dL or 7.4 mmol/L	110 mg/dL or 6.3 mmol/L	

WHO World Health Organization, *ADA* American Diabetes Association, *IADPSG* International Association of Diabetes and Pregnancy Study Groups Consensus Panel, *OGTT* oral glucose tolerance test, *GCT* glucose challenge test

[a] Defined as having a family or personal history of type 2 diabetes, being older than 25 years of age, having had a previous diagnosis of GDM, being overweight or obese, or belonging to a particular ethnic group that has increased risk for developing type 2 diabetes mellitus (e.g., Hispanic, black, Native American, Asian)

comparing metabolite profiles of women with vs. without GDM [26–28], and findings generally indicate altered fatty acid and amino acid metabolism (Table 2). For example, using a targeted MS-based metabolomic approach, Chen et al. investigated the relationship between circulating fatty acids in pregnant women with GDM (failed 50-g GCT followed by ≥2 abnormal glucose values in the subsequent 100-g OGTT; n = 49), women with hyperglycemia (failed 50-g GCT, but fewer than 2 abnormal glucose values in the 100-g OGTT; n = 80), and healthy control gravidas (n = 98) and found a graded increase in fatty acids during the third trimester (e.g., linoleic, linolenic, arachidonic, eicosapentaenoic acid, and docosapentaenoic acid) across the spectrum of GDM severity [27]. Researchers have also found that women with higher fasting glucose levels tend to have higher serum levels of the amino acids alanine, proline, and leucine/isoleucine [28], which have previously been implicated in the pathogenesis of type 2 diabetes in non-pregnant adults [29, 30]. In a study of 400 women in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) cohort, a 5-year prospective observational study of pregnant women in 10 countries, Scholtens et al. carried out both targeted and untargeted metabolomic analyses on a MS-based platform and identified a small but distinct set of metabolites on gluconeogenesis and lipid metabolism pathways that were associated with maternal glycemia during pregnancy [31•]. Specifically, pre-OGTT fasting plasma glucose was positively associated with the gluconeogenic substrates alanine, lactate, hexitol, and fructose; and negatively correlated with medium-chain fatty acids and palmitoleic acid metabolites. One hour following the OGTT, the investigators observed similar positive associations between the plasma glucose and the abovementioned gluconeogenic compounds. Additionally, post-OGTT plasma glucose was also positively associated with non-esterified fatty acids (NEFAs), beta-hydroxybutyrate, triglycerides, glycerol, asparagine/aspartate, glutamine/glutamate, leucine/isoleucine, ornithine, phenylalanine, proline and serine, multiple acylcarnitines, and fatty acids. When the investigators explored associations of change in plasma glucose with change in metabolites, they found that all targeted amino acids, several long- and medium-chain fatty acids, and lipid metabolites, including acylcarnitines, glycerol, and beta-hydroxybutyrate, decreased after the OGTT, whereas triglycerides, carbohydrates, and energy cycle intermediates (e.g., pyruvate, citrate/isocitrate) increased. Together, these results suggest that poor glucose tolerance during pregnancy may be attributable to aberrances in energy and lipid metabolism pathways. On the other hand, in a study of 823 pregnant Norwegian women, Sasche et al. carried out untargeted metabolomic analyses via nuclear magnetic resonance (NMR) spectroscopy in urine collected at 8–20 gestational weeks, ~28 gestational weeks, and 10–16 weeks postpartum found no differences in metabolite profiles of women with GDM as compared to their normoglycemic counterparts at any of the time points [32]. Thus, although current studies shed light on potential mechanisms underlying abnormal glucose tolerance during pregnancy, the inconsistencies in tissue type used for metabolomic analyses (e.g., plasma vs. urine), the laboratory methods employed (MS vs. NMR), and the fundamental differences in the study populations make it challenging to synthesize and interpret findings.

Metabolomic Profiles of GDM in Relation to Infant Outcomes

In attempt to understand the impact of maternal hyperglycemia on the infant, researchers have also compared metabolite concentrations in cord blood of mother-infant dyads with vs. without GDM (Table 2). In a study of 30 term infants born to women with GDM and 40 control newborns, Dani et al. carried out untargeted metabolomic assays in cord artery serum [26] and found that as compared to their healthy counterparts, mother-infant pairs affected by GDM exhibited lower cord artery serum glucose—which the authors posited was due to fetal hyperinsulinemia—in conjunction with higher concentrations of metabolites indicative of defective placental amino acid transportation including pyruvate, histidine, alanine, valine, methionine, arginine, lysine, and hypoxanthine [26]. Similarly, in another study of 400 women in the HAPO study, Scholtens et al. carried out both targeted and untargeted metabolomic analysis in maternal serum collected at ~28 weeks and found disturbances in similar metabolic pathways, namely, those involved in carbohydrate and amino acid metabolism [31•]. The consistency in these findings, despite the fact that Dani et al. analyzed cord serum while Scholtens et al. evaluated maternal serum, points towards the relevance of these two biochemical processes in the etiology of GDM. It is worth noting, however, that in the study by Dani et al., the authors observed no differences in clinical indicators of newborn health (e.g., Apgar score; prevalence of hypocalcemia, hypoglycemia, or hyperbilirubinemia; prolonged hospitalization after birth), which could be related to the fact that cord artery blood represents blood of the fetus directed towards the placenta as opposed to cord vein blood indicative of blood directed towards the fetus, and thus, may not capture the gestational milieu experienced by the infant. Such nuances highlight the complexities of maternal/fetal nutrient exchange and the caution with which metabolomic data must be interpreted.

The Potential Role of Metabolomics in Improving Knowledge of GDM Pathophysiology

Here, we propose that metabolomics offers the potential to improve understanding of GDM pathophysiology, and potentially earlier characterization of pregnancy-associated hyperglycemia. The novelty of capturing the metabolome is in its representation of a "real-time" portrait of a cell or organism, and its functional significance as it reflects an integration of

Table 2 Summary of studies characterizing metabolite patterns associated with gestational glucose tolerance and pregnancy/offspring outcomes

Study	Objective	Study design	GDM diagnosis	GDM criteria	Platform and biospecimen for metabolomic analyses	Main findings
Chen et al. 2010 [27]	To characterize fatty acid metabolite profiles associated with gestational glucose intolerance	Case-control nested within a prospective cohort of low SES, multi-ethnic women in the USA *n* = 49 GDM *n* = 80 IGT *n* = 98 NGT	• Step 1: non-fasting 50-g GCT at 28 weeks' gestation (abnormal value: >140 mg/dL at 1 h) • Step 2: fasting 100-g OGTT (abnormal values: >95, >180, >155, or >140 mg/dL at 1, 2, and 3 h)	• **IGT**: GCT >140 mg/dL and <2 abnormal OGTT values. • **GDM**: GCT >150 mg/dL and ≥2 abnormal OGTT values.	Targeted analysis of fatty acids via GC/MS in fasting maternal serum collected at ~16.5 weeks' gestation	Graded increase across severity of abnormal glucose tolerance (GDM > IGT > NGT) in fatty acids (myristic, palmitic, palmitoleic, oleic, linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexasenoic acids) during the third trimester.
Sachse et al. 2012 [32]	To identify novel biomarkers of GDM in biochemical profile of pregnant women.	Prospective, multiethnic cohort study of 823 healthy, pregnant women in Oslo, Norway (STORK Groruddalen program)	75g OGTT (WHO abnormal value FPG ≥7.0 or 2-h PG ≥7.8 mmol/L)	IADPSG Healthy: FPG <5.1 and 2-h PG <8.5 mmol/L GDM, mild hyperglycemia: above at least one limit GDM, pronounced hyperglycemia: FPG ≥5.8 mmol/L or PG ≥11.1 mmol/L.	NMR spectroscopy of maternal urine collected at three time points (V1: 8–20 weeks' gestation, V2: 28 + 2, and V3: 10–16 weeks postpartum)	No significant changes were observed in GDM women as compared to controls.
Scholtens et al. 2013 [28].	To characterize metabolites across the range of maternal glucose by comparing metabolomic profiles of mothers with high vs. low FPG.	Case-control nested within a prospective cohort study (HAPO) of nearly 25,000 pregnant women across 10 countries. *n* = 50 low FPG *n* = 67 high FPG	75g OGTT between 24 and 32 weeks' gestation. Abnormal values: FPG >5.8 mml/L, 2-h OGTT PG >11.1 mmol/L	Participants, caregivers, and HAPO study staff were blinded to glucose values unless FPG was >5.8 mmol/L, 2-h OGTT PG was >11.1 mmol/L, random PG was ≥8.9 mmol/L, or any PG value was <2.5 mmol/L. Unblinded participants were excluded.	Target and untargeted GC/MS on maternal fasting serum collected between 24–32 weeks' gestation	Among conventional metabolites, high-FPG mothers had higher triglycerides and 3-hydroxybutyrate. Among targeted amino acids, high-FPG mothers to had greater alanine, proline, glutamine/glutamate, arginine, leucine/isoleucine, and asparagine/aspartate.
Dani et al. 2014 [26]	To compare the metabolomic profile of infants of GDM mothers to that of infants of healthy mothers.	Case-control prospective study of women in Firenze, Italy. *n* = 40 control *n* = 30 term infants of GDM mothers	75g OGTT between 28 and 32 weeks' gestation	IADPSG Healthy: FPG <5.1 and 2-h PG <8.5 mmol/L GDM, mild hyperglycemia: above at least one limit GDM, pronounced hyperglycemia: FPG ≥5.8 mmol/L or PG ≥11.1 mmol/L.	NMR spectroscopy of infant cord serum	Infants of GDM mothers had lower levels of glucose and higher levels of pyruvate, histidine, alanine, valine, methionine, arginine, lysine, hypoxanthine, lipoprotein, and lipid as compared to controls.
Scholtens et al. 2016 [31•]	To characterize maternal metabolites and metabolic networks underlying	Studied 400 Northern European women of the HAPO prospective cohort study. Comparison of metabolite profiles at	75g OGTT between 24 and 32 weeks' gestation. Abnormal values: FPG >5.8 mml/L, 2-h	Participants, caregivers, and HAPO study staff were blinded to glucose values unless FPG was >5.8 mmol/L, 2-h OGTT PG was >11.1 mmol/L, random PG was ≥8.9 mmol/L, or any PG value	Targeted and non-targeted GC/MS on maternal serum collected at fasting and 1-h post OGTT at ~28 weeks' gestation	A limited number of fasting metabolites were positively associated with FPG: gluconeogenic substrates alanine and lactate, hexitols, and fructose. Lauric acid and palmitoleic acid

Table 2 (continued)

Study	Objective	Study design	GDM diagnosis	GDM criteria	Platform and biospecimen for metabolomic analyses	Main findings
	maternal glucose during pregnancy.	fasting and 1-h post OGTT	OGTT PG >11.1 mmol/L	was <2.5 mmol/L. Unblinded participants were excluded.		were negatively associated with FPG.

FPG fasting plasma glucose, *GCT* glucose challenge test, *GC/MS* gas chromatography/mass spectrometry, *GDM* gestational diabetes mellitus, *IGT* impaired glucose tolerance, *NGT* normal glucose tolerance, *NMR* nuclear magnetic resonance, *OGTT* oral glucose tolerance test, *PG* plasma glucose, *SES* socioeconomic status

multiple physiological (or pathophysiological) processes [33]. For example, given that defects in insulin secretion vs. defects in insulin sensitivity have different root causes, it is likely that the physiological disturbances may manifest as unique metabolic profiles that, if replicable over time and in multiple populations, could be used to refine the definition, criteria, and methods of treatment for GDM.

While we do not explicitly discuss genetic determinants of GDM in this review, metabolomic analyses may also be useful to identify metabolic signatures associated with genetic variants implicated in GDM risk. In a study of 284 male German participants of the Cooperative Health Research in the Augsburg Region (KORA) study, Gieger et al. [34] examined associations of genetic variants involved in metabolic homeostasis with serum concentrations of metabolites previously implicated in type 2 diabetes pathogenesis [35] quantified by targeted assays. The researchers found that the genetic variants accounted for a significant portion of variance in metabolites of corresponding metabolic pathways, suggesting that common genetic polymorphisms induce major differences in metabolic phenotype. This study points towards the feasibility of metabolomics to unveil differences in metabolism with respect to genetic variants that have been associated with GDM risk [36, 37] for more timely identification of at-risk women.

The Potential Role of Metabolomics in Early Identification and Treatment of GDM

Early Identification

In addition to identifying women with overt GDM, early recognition of those at risk for developing GDM is critical to take advantage of GDM risk-reduction strategies and to minimize the detrimental consequences of this pregnancy complication for mother and offspring. Therefore, although clinical assessment of gestational glycemia typically occurs during the second trimester, research efforts target first trimester detection or prediction, which could eventually be integrated into clinical practice given that blood is collected for other assessments during the first trimester as part of typical clinical practice. For example, in addition to sociodemographic predictors like race/ethnicity, family history, body mass index (BMI), and prior history of GDM, lower levels of adiponectin and sex-hormone-binding globulin and higher circulating C-reactive protein (CRP) during the first trimester have been identified as potential biomarkers of GDM risk [38, 39]. Metabolomics offers a way in which the varying physiological states of hyperglycemia and GDM might be studied, identified, and classified. Outside of pregnancy, researchers are currently using metabolomics to predict and diagnose type 2 diabetes and prediabetes [33]. The BCAAs leucine, isoleucine, and valine have been consistently linked to both conditions, and through

the use of metabolomics, the identification of elevated levels of these compounds has been found nearly 14 years ahead of the clinical manifestation of disease [33]. Because metabolomics can detect relatively small differences in circulating compounds, it could aid in identification of abnormal glucose tolerance or other relevant alterations in metabolism earlier in pregnancy.

So far, a few studies have attempted to characterize metabolite patterns in maternal serum as a potential indicator of GDM risk (Table 3) [40–48]. Despite variability in the type of analytical platform used (e.g., untargeted vs. targeted platforms, NMR vs. MS-based instrumentation), timing of blood collection (first trimester vs. second trimester), study population composition, and the statistical methods employed, current evidence points towards altered amino acid metabolism as a potential predictor of GDM risk. For example, using a case-control design, Pinto et al. carried out untargeted metabolomic assays via NMR spectroscopy in plasma of 32 Portuguese women without clinical signs of GDM at up to 21 gestational weeks, but who developed GDM 2–22 weeks later ("pre-diagnosis group"), 12 pregnant women with a confirmed GDM diagnosis at 18 to 37 gestational weeks ("post-diagnosis group"), and 35 control gravidas [49]. The investigators found that in comparison to controls, the pre-diagnosis group exhibited higher plasma levels of metabolites on amino acid (valine) and glucose (pyruvate, lactate, and glucose) metabolism pathways, and lower levels of glutamine, creatine, dimethyl sulfone, trimethylamine *N*-oxide (TMAO), betaine, proline, methanol, and 1,5-anhydroglucitol [49]. While the difference in concentrations of some of these compounds (e.g., those on amino acid [valine, alanine] and glucose metabolism [glucose, lactate] pathways) between the post-diagnosis and control groups did not align with that of the pre-diagnosis group, these results point towards the relevance of these biochemical pathways in GDM etiology and also highlight their potential to identify apparently healthy women at risk for developing this pregnancy complication [22]. In another study, Bentley-Lewis et al. [41] compared concentrations of amino acids, biogenic amines, and other polar metabolites quantified via MS-based targeted assays in first trimester fasting serum of 96 GDM cases vs. 96 normoglycemic controls selected from a Boston-area cohort of white women. The investigators observed higher levels of several compounds involved in amino acid metabolism, namely alanine and serine, as well as elevated anthranilic acid, glutamate, and allantoin; and lower levels of creatinine in first trimester serum of women who went on to develop GDM, as compared to their normoglycemic counterparts [41]. Although there are discrepancies in the exact compounds identified in these studies, the consistency in the relevance of amino acid pathways point towards the feasibility of using metabolomic technologies for early identification of GDM cases. Additional studies are warranted in larger and more diverse populations.

Treatment

Metabolomics not only offers the opportunity to more accurately characterize and diagnose maternal glucose intolerance and GDM but it may also refine GDM treatment. A large prospective cohort study of ~800 women in the Genetics of Glucose regulation in Gestation and Growth (Gen3G) cohort in Canada found that nearly half of the women who were diagnosed with GDM had an insulin sensitivity defect, 30% had a defect in insulin secretion, and 20% had a mix of both a defect in insulin sensitivity and secretion [16]. In comparison to women who had a normal glucose tolerance and after controlling for BMI, women with GDM with insulin sensitivity defect were at greater risk for complications at delivery. Specifically, women with GDM were more likely to deliver a large-for-gestational age infant, to experience hypoglycemia after birth, and to deliver via cesarean section despite receiving similar clinical care to their normoglycemic counterparts [16]. These results suggest a role for small metabolites in their contribution to these risks and complications (such as inflammatory cytokines, adipokines, or lipid fractions), and the potential for metabolomics to refine current understanding of different GDM subtypes. Ultimately, improvements in this area could lead to more tailored treatment regimens than that of what is currently available.

Future Directions

Advancements in the field of metabolomics have expanded our understanding of the etiology of metabolic disease [50]. In addition to continued research aimed at replicating and validating metabolite patterns reported in the current literature, we envision three key avenues for growth within the realm of metabolomics and GDM.

First, metabolomics offers the potential to identify women afflicted by GDM who are at risk of developing overt type 2 diabetes postpartum—an important research endeavor given that up to 50% of the women affected by GDM progress to type 2 diabetes within 5 years [51, 52]. When reviewing the literature, we identified one study that has attempted to do this. In retrospective cohort study of 1010 women with GDM during pregnancy, Allalou et al. [53•] used targeted metabolomic assays to quantify free fatty acids and amino acids in fasting plasma drawn at 6–9 weeks postpartum and identified elevations in several amino acids, including isoleucine, leucine, threonine, tryptophan, tyrosine, and valine, and proprionylcarnitine (aka acylcarnitine C3) among women who went on to develop type 2 diabetes within the next 2 years, as compared to those who did not. Again, while these seminal findings shed light on etiological underpinnings of the transition from GDM to over type 2

Table 3 Summary of studies characterizing metabolite patterns as potential predictors of GDM risk

Publication	Objective	Study design	Time of blood collection for metabolomics	GDM criteria	Platform	Main findings
Graca et al. 2010 [45]	To characterize metabolite profiles of prenatal disorders in amniotic fluid.	Case-control nested within a cohort of women undergoing diagnostic amniocentesis. *n* = 82 control *n* = 27 pre-diagnostic GDM	Unknown	Unknown	NMR spectroscopy of amniotic fluid collected during the second trimester of pregnancy (14–25 weeks' gestation)	Average increase of 14% in glucose in pre-diagnostic GDM mothers with a slight decrease in amino acids (glutamate, glycine, proline, serine, and taurine), organic acids (acetate and formate), creatinine, and glycerophosphocholine.
Diaz et al. 2011 [46]	To characterize metabolic changes underlying prenatal disorders.	Case-control nested within a cohort of women undergoing diagnostic amniocentesis, >35 years of age. *n* = 20 plasma; 25 urine Control *n* = 14 plasma; 29 urine pre-diagnostic GDM	Unknown	Unknown	NMR spectroscopy of urine and plasma collected during the second trimester of pregnancy (14–25 weeks' gestation)	GDM women demonstrated increases in 3-hydroxyisovalerate, 2-hydroxyisobutyrate, *N*-methylnicotinamide, choline, and *N*-methyl-2-pyridone-5-carboxamide (urine) with decreases in trimethylamine *N*-oxide and betaine (plasma).
Graca et al. 2012 [47]	To investigate the metabolic effects of GDM.	Case-control nested within a cohort of women undergoing amniocentesis, >35 years of age. *n* = 26 AF; 21 urine Control *n* = 23 AF; 20 urine pre-diagnostic GDM	Unknown	Unknown	Untargeted UPLC-MC of 2nd trimester (15–25 weeks' gestation) maternal urine and AF	No significant changes were observed in GDM women as compared to controls.
Diaz et al. 2013 [48]	To characterize the metabolic changes underlying prenatal disorders and identify possible biomarkers.	*n* = 84 control *n* = 42 pre-diagnostic GDM	Unknown	Unknown	NMR spectroscopy of maternal urine collected during the second trimester (14–26 weeks' gestation)	Increases in choline, glucose, *N*-methylnicotinamide, and xylose were observed in GDM mothers as compared to control, while there were decreases in 4-hydroxyphenylacetate, and hippurate.
Bentley-Lewis et al. *Diabetologia* 2015; 58:1329-32. [41]	To determine whether the metabolite profile predictive of type 2 diabetes could identify women who will develop GDM.	Nested case-control study of 18- to 40-year-old women who participated I the Massachusetts General Hospital Obstetrical Maternal study between 1998 and 2007. *n* = 96 NGT *n* = 96 GDM	Fasting blood samples collected during the first trimester.	Women were diagnosed with GDM by a 1-h 50-g GLT value ≥7.8 mmol/L and two abnormal values for a 3-h 100-g OGTT according to Carpenter–Coustan criteria. NGT was defined by a screening 1-h 50-g GLT value <7.8 mmol/L	LC/MS was used to measure the levels of 91 metabolites	Six metabolites (anthranilic acid, alanine, glutamate, creatinine, allantoin, and serine) were identified as having significantly different levels between the two groups in conditional logistic regression analyses ($p < 0.05$). The levels of the BCAAs did not differ significantly between GDM and NGT.
Enquobahrie et al. *J Clin Endocrinol*	To investigate early pregnancy maternal	Case-control nested in a prospective cohort study *n* = 180 control	Maternal serum was collected shortly after	Carpenter and Coustan. Screened: 1-h oral glucose load (50 g). Postload glucose concentrations	Untargeted GC/MS	Seventeen metabolites: (linoleic acid, oleic acid, myristic acid, D-galactose, D-sorbitol, *o*-phosphocolamine,

Table 3 (continued)

Publication	Objective	Study design	Time of blood collection for metabolomics	GDM criteria	Platform	Main findings
Metab 2015; 100 (11):4348-5-6 [43]	serum and subsequent risk of GDM.	n = 178 GDM	enrollment, on average at 16 weeks' gestation.	greater than 140 mg/dL, followed up within 1–2 weeks with a 3-h oral glucose (100 g) tolerance test. Diagnosed with GDM if at least two of the four diagnostic glucose concentration measurements met or exceeded the following: fasting, at least 95 mg/dL; 1-h post-challenge, at least 180 mg/dL; 2-h post-challenge, at least 155 mg/dL; and, 3-h post-challenge, at least 140 mg/dL		L-alanine, L-valine, 5-hydroxy-L-tryptophan, L-serine, sarcosine, L-pyroglutamic acid, L-mimosine, L-lactic acid, glycolic acid, fumaric acid, and urea) differentiated GDM cases from controls. Fold changes of relative abundance of these metabolites among GDM cases compared with controls ranged from 1.47 (linoleic acid) to 0.78 (5-hydroxy-l-tryptophan).
Nevalainen et al. *Rev Diabet Stud* 2016; 13 (4): 236-245 [42]	To evaluate the association between GDM and first–trimester maternal serum concentrations of ten amino acids and 31 acylcarnitines.	Retrospective case-control study n = 295 control n = 69 GDM	Maternal serum was collected during the first–trimester screening.	2-h 75g OGTT (12–16 or 24–28 weeks' gestation) Diagnostic values: ≥5.3 mmol/L (fasting blood glucose), ≥10.0 mmol/L (1-h), and ≥8.6 mmol/L (2-h). GDM diagnosis: one or more abnormal values	Quattro micro mass spectrometry	There were significant differences in the serum levels of arginine, glycine, and 3-hydroxy-isovalerylcarnitine between controls and women who subsequently developed GDM. These differences were already existent in the first trimester of the pregnancy.

BCAA branched chain amino acids, *FPG* fasting plasma glucose, *GCT* glucose challenge test, *GC/MS* gas chromatography/mass spectrometry, *GDM* gestational diabetes mellitus, *GLT* glucose load test, *IGT* impaired glucose tolerance, *LC/MS* liquid chromatography/mass spectrometry, *NGT* normal glucose tolerance, *NMR* nuclear magnetic resonance, *OGTT* oral glucose tolerance test

diabetes and point towards a role for metabolomics in identification of at-risk women, there is need for validation of results in an independent population.

Second, given the rapid advancements in high-throughput technologies, studies that combine metabolomics with other 'omics will provide a more holistic view of complex metabolic phenotypes. For example, proteomic analyses have identified amino acids and low-molecular-weight peptides that are differentially expressed in GDM vs. control patients [54]. Integration of these data with metabolomics could serve as a way to validate biological pathways involved in GDM pathogenesis, while also providing insight into temporality of physiological alterations leading to development of overt disease.

Finally, in the long term, we foresee opportunities for metabolomics in clinical risk assessment and practice. Such applications have already begun for chronic diseases with distinct metabolic characteristics like Alzheimer's disease, hepatocarcinoma, chronic kidney disease, and ovarian endometriosis [55]. Major challenges to achieving clinical impact include accurate identification of perturbed pathways relevant to GDM (e.g., given the current high costs of high-throughput assays, most studies have carried out metabolomic analyses at a single point in time, precluding the ability to evaluate metabolic flux) and replication/validation of not only the utility but also the performance (e.g., sensitivity, specificity, positive predictive value, negative predictive value) of metabolomic biomarkers of GDM in multiple populations.

Conclusions

In the past decade, metabolomics has demonstrated its utility to identify metabolic aberrances, including those associated with GDM, and offers promise as a clinical tool. In the era of personalized medicine characterized by the development of increasingly specific treatment therapies, there is need for reliable and sensitive biomarkers to shed light on disease etiology, monitor disease risk, and develop treatment plans. We envision that collaborative efforts from multiple cohorts and consortia with metabolomic data will improve the power and generalizability of results, eventually leading to a better understanding of risk factors, physiological perturbations, and strategies for management of GDM and its related comorbidities. Ultimately, in addition to improving clinical care, findings from the field of metabolomics have great potential to improve GDM prevention and management, especially because pregnancy is a life stage when women not only have frequent and consistent interaction with the health care system but also because it is a time when a woman may be more receptive to diet and lifestyle changes to reduce the risks posed to her child.

Compliance with Ethical Standards

Conflict of Interest Carolyn F. McCabe and Wei Perng declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent Statement This article does not contain any studies with human or animal subjects performed by any of the authors.

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