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Genetics, genomics and metabolomics: new insights into maternal metabolism during pregnancy

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Abstract

Maternal glucose metabolism during pregnancy differs from the non-gravid state to allow the mother to meet her own and the growing fetus's energy needs. New insights into the mechanisms underlying maternal metabolism during pregnancy are being gained through the use of new 'omics' technologies. This review focuses on the application of genetics/genomics and metabolomics to the study of maternal metabolism during pregnancy. Following the identification of susceptibility genes for Type 2 diabetes through genome-wide association studies, association has been demonstrated of some Type 2 diabetes susceptibility genes with gestational diabetes mellitus, suggesting that the genetic architecture of Type 2 diabetes and gestational diabetes are, in part, similar. More recent genome-wide association studies examining maternal metabolism during pregnancy have demonstrated overlap of genes associated with metabolic traits in the gravid and non-gravid population, as well as genes that appear to be relatively unique to pregnancy. Metabolomics has also been used to profile the metabolic state of women during pregnancy through the multiplexed measurement of many low molecular weight metabolites. Measurement of amino acids and conventional metabolites have demonstrated changes in mothers with higher insulin resistance and glucose similar to changes in non-gravid, insulin-resistant populations, suggesting similarities in the metabolic profile characteristic of insulin resistance and hyperglycaemia in pregnant and non-pregnant populations. Metabolomics and genomics are but a few of the now available high-throughput 'omics' technologies. Future studies that integrate data from multiple technologies will allow an integrated systems biology approach to maternal metabolism during pregnancy.

Introduction

Maternal glucose metabolism during pregnancy differs from the non-gravid state as the mother must meet both her own and the growing fetus's energy needs [1–3]. As demonstrated by the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study and other population-based studies, maternal glucose levels during pregnancy vary markedly among women, with some women having levels associated with adverse newborn outcomes [3,4]. These same glucose levels may also adversely affect the long-term metabolic health of the offspring [5,6]. Understanding maternal metabolism and factors that account for the observed variability among women is important for ultimately identifying women at risk for hyperglycaemia and developing interventions to prevent it.

While common mechanisms likely underlie differences in metabolism in the gravid compared with non-gravid state, multiple factors likely account for inter-individual

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differences in the levels of glucose and other metabolites, with environmental, lifestyle and genetic factors contributing. While much research has been directed towards characterizing metabolic changes during pregnancy and defining the mechanisms underlying these changes, much remains to be learned. Knowledge of what accounts for individual differences in metabolism also remains limited. With the advent of a variety of 'omics' technologies, new insights are being gained into maternal metabolism during pregnancy and its relationship to outcomes at birth and in childhood. This review will focus on two approaches that have recently been applied to the study of maternal metabolism during pregnancy, genetics/genomics and metabolomics.

Overview of maternal metabolism during pregnancy

Detailed reviews of maternal metabolism during pregnancy have been recently published [1–3,7]. A brief overview is below. Pregnancy is characterized by profound metabolic alterations in carbohydrate, fat and protein metabolism to ensure adequate fetal growth and development and to meet the increased physiological demands of pregnancy, including the need to provide additional energy stores required for labour and lactation.

Insulin is a key regulator of maternal metabolism, and insulin sensitivity is altered substantially in pregnancy. In early gestation, there is a slight enhancement of insulin sensitivity, but a reduction in insulin sensitivity is evident by 12–14 weeks' gestation, with a progressive decline in insulin sensitivity in the second and third trimesters [1,2,7]. During the third trimester of pregnancy, insulin sensitivity has been reported to be 30–70% of pre-pregnant values [1,2]. The fetoplacental unit has been implicated as a major source of maternal insulin resistance during pregnancy as insulin resistance is rapidly reversed upon delivery [8]. Several placental and other hormones, including human placental lactogen, placental growth hormone, progesterone, leptin, cortisol, prolactin, human chorionic gonadotropin and oestradiol, have been speculated to contribute to insulin resistance [1,2,7,9]. Tumour necrosis factor-alpha (TNF-α) and other inflammatory mediators that are produced by placental and other tissues, e.g. adipose tissue, are also thought to be important contributors to insulin resistance during pregnancy [8,9]. To compensate for the insulin resistance, nutrient-stimulated insulin secretion increases during pregnancy, and by the third trimester basal insulin levels typically double compared with the non-gravid state [1]. The mechanisms for enhanced insulin secretion in pregnancy are not completely defined. A pregnancy-induced increase in β-cell mass likely contributes, but the ~1.4-fold increase in β-cell mass does not fully account for enhanced β-cell responsiveness during pregnancy [2,10]. Accompanying the changes in insulin sensitivity are changes in glucose levels. Glucose is the major source of fetal metabolic energy and crosses the placenta by facilitated diffusion. Fasting glucose levels decrease early in pregnancy between 6 and 10 weeks' gestation, with the majority of studies demonstrating a further decrease in fasting glucose levels by week 28 of gestation and a stabilization or slight increase in fasting glucose after that point [1,3,7,11]. Interestingly, obese women do not exhibit a similar decline in glucose levels [11]. The mechanisms underlying the decline in fasting glucose are complex and not well understood, but several potential contributing factors have been proposed [1–3,7]. Among these is the increase in plasma volume characteristic of normal pregnancy, which results in a larger volume of distribution and a dilutional effect. Later in pregnancy, increased fetoplacental glucose utilization also contributes. Hepatic glucose production increases during pregnancy, but does not fully compensate for increased glucose utilization. Beyond the pregnancy-induced decline in fasting glucose, a prolonged fast during pregnancy is associated with hypoglycaemia; reduced availability of alanine as a substrate for gluconeogenesis has been suggested as a possible factor contributing to fasting hypoglycaemia [12].

Normal pancreatic β-cell function is crucial to maternal metabolic adaptations to pregnancy [1–3,7]. Maternal glycaemic control depends on the balance between pancreatic β-cell secretion of insulin, insulin clearance, as well as insulin action in maternal liver, muscle and fat. Women who are unable to respond appropriately to the metabolic changes of pregnancy, including the marked increase in insulin resistance, are at risk for hyperglycaemia and its related risks to fetal growth and adiposity and later metabolic health of the offspring. Inadequate β-cell compensation is also associated with a future risk of Type 2 diabetes in the mother.

Genetics of maternal metabolism during pregnancy

One long-standing question is to what extent genetic variation contributes to variation in maternal glucose levels during pregnancy. The contribution of genetic factors to a phenotype is measured as heritability. To date, the heritability of maternal metabolic traits during pregnancy has not been examined, but studies in non-pregnant twins and families of European ancestry without diabetes have estimated the heritability of fasting plasma glucose and insulin to be ~20–75% and ~20–50%, respectively [13,14]. Based on the likelihood that maternal glucose levels also exhibit significant heritability, a number of studies have sought to identify genes associated with measures of maternal metabolism during pregnancy.

In general, two different approaches have been available to define genetic factors that impact maternal metabolism during pregnancy. Candidate gene studies have examined the association of variants in preselected genes with maternal glucose levels or, as has been more commonly done, with risk of gestational diabetes. Candidate gene studies have inherent limitations as they represent a directed, as opposed to unbiased, approach in the selection of the genes to be studied, which are typically chosen based upon biological plausibility. Unfortunately, this approach has, in general, been of limited utility for identifying genes associated with complex diseases and traits; i.e. traits and diseases for which both genetic and non-genetic factors contribute to the phenotype. As most candidate gene studies were undertaken prior to improved definition of the genetic architecture of complex traits and diseases, they have typically suffered from inherent limitations, including small sample sizes and inclusion of a limited number of variants in the genetic loci being studied [15]. A more recent approach has been to use genome-wide association studies to define genetic variation that contributes to complex traits and diseases [16]. This is an unbiased, hypothesis-free approach in which genetic variants across the entire genome are interrogated for association with a trait or disease. This approach has been relatively successful in beginning to define the genetic architecture of complex traits or diseases. The finding for most complex traits and diseases has been that variants from many different loci are associated with the trait or disease risk, but that each of these variants generally has only a very modest effect on the trait or risk of disease [16]. One consequence of this is the need for a large number of subjects to be studied for significant associations to be demonstrated. To date, studies of the contribution of genetic factors to maternal metabolic traits during pregnancy have focused largely on gestational diabetes mellitus. One limitation in studying gestational diabetes is that, depending on the definition used for a diagnosis of gestational diabetes, it is relatively uncommon, and cohorts available for study have been somewhat limited, thus reducing power. Most studies examining the genetics of gestational diabetes have been candidate gene studies [15,17,18].

Genetics of gestational diabetes

A number of candidate gene studies have been performed to identify genetic variation associated with gestational diabetes [15,17]. Early on, candidate genes were chosen for study based largely on biological plausibility. Given the well-established observation that

women who develop gestational diabetes are at increased risk of developing Type 2 diabetes [19], more recent studies have been based on the underlying assumption that the genetic architecture of Type 2 diabetes and gestational diabetes are similar. A large number of genome-wide association studies, and meta-analyses of these studies with increasingly large numbers of individuals with Type 2 diabetes and control subjects, have identified a large number of genes associated with an increased risk of Type 2 diabetes [20], some of which have been tested for their association with gestational diabetes [17,18].

Many of the candidate gene studies, especially those performed early on, contained a relatively small number of women in the control group and the group with gestational diabetes and, in some cases, groups of mixed ancestry. Thus, robust and reproducible association of many of the genetic variants with gestational diabetes was not demonstrated in individual studies. Recent meta-analyses, which include many of these earlier studies as well as more recent studies, have been successful in demonstrating evidence for association [15,17,18,21]. As shown in Table 1, meta-analyses have demonstrated association of variants within eight different genetic loci, *TCF7L2*, *GCK*, *KCNJ11*, *KCNQ1*, *CDKAL1*, *IGF2BP2*, *MTNR1B* and *IRS1*, with an increased risk of gestational diabetes. All of these loci are also associated with risk of Type 2 diabetes [22]. Multiple additional loci have shown evidence for association in individual studies, but have not been replicated and/or failed to show evidence for association in meta-analyses.

More recently, the results of the first genome-wide association studies for gestational diabetes were reported [23]. This unbiased approach provides an opportunity to determine whether loci different from those associated with Type 2 diabetes also demonstrate association with gestational diabetes. The study was performed in Korean women with a history of gestational diabetes and control subjects. While the number of women in the control group and the group with gestational diabetes was somewhat limited compared with the large meta-analyses performed in populations with Type 2 diabetes, two loci demonstrated genome-wide significant association with gestational diabetes, *CDKAL1* and *MTNR1B*, while a third, *IGF2BP2*, demonstrated nominal association [23]. As noted above, these same loci also demonstrated association with gestational diabetes in candidate gene studies and have also been shown to be associated with risk for Type 2 diabetes [22]. A variant in *CDKAL1* was also associated with decreased fasting insulin level in the group with gestational diabetes, while the variant in *MTNR1B* demonstrated nominal association with fasting insulin levels in this same group [23]. The two next most highly associated loci with gestational diabetes, *FTSJD1/CALB2* and *LBXCOR1*, fell well short of demonstrating evidence for genome-wide significant association, but, of interest, neither has been shown previously to be associated with Type 2 diabetes. Future studies with larger populations and meta-analyses across studies will be needed to determine whether these potentially novel genes are associated with gestational diabetes risk.

Consistent with the candidate gene studies described above, when the association of 34 known Type 2 diabetes genetic risk loci with gestational diabetes was determined in the Korean group with gestational diabetes, an excess of small *P*-values compared with what would be expected under the null hypothesis was observed [23]. However, many of the Type 2 diabetes loci showed no evidence for association with gestational diabetes.

In summary, studies of gestational diabetes genetics still await the large meta-analyses that have been used to successfully identify many of the Type 2 diabetes susceptibility genes. However, even in the absence of these large studies, clear evidence for overlap between the genetic architecture of Type 2 diabetes and gestational diabetes has been demonstrated. When tested, not all Type 2 diabetes susceptibility genes have demonstrated association with gestational diabetes, but whether this relates to differences in the genetic architecture of

Type 2 diabetes and gestational diabetes or the still somewhat limited power of gestational diabetes genetic studies is not clear. Answers to these questions and whether susceptibility genes unique to gestational diabetes also exist await further studies.

Genetics of maternal metabolic traits

A more limited number of studies have sought to identify genetic variation associated with specific metabolic traits during pregnancy. As would be expected based upon earlier heritability studies, genome-wide association studies performed in non-pregnant individuals have identified a large number of variants associated with metabolic traits, including fasting and 2-h glucose levels as well as fasting insulin levels [22,24]. Interestingly, while there is a clear overlap between genetic variants associated with Type 2 diabetes and different metabolic traits, a number of the variants identified have not been shown to be associated with Type 2 diabetes, suggesting the genetic architecture of specific glycaemic traits and Type 2 diabetes are, in part, distinct. Much less information is available about the impact of genetic variation on maternal metabolic traits and whether variants that are associated with an increased risk of gestational diabetes are also associated with maternal metabolic traits. We have addressed these issues using both candidate gene and genome-wide association studies with data from the HAPO Study, a population-based study in which oral glucose tolerance was determined in women from multiple ancestry groups at ~28 weeks' gestation [4]. A previous study had demonstrated association of a variant in the promoter of *GCK* (which encodes glucokinase) with fasting glucose in pregnant women of European ancestry [25]. This same single nucleotide polymorphism was associated with fasting and 1-h glucose in European ancestry HAPO mothers and fasting and 2-h glucose in Thai HAPO mothers [26]. This variant is also associated with gestational diabetes as well as Type 2 diabetes and fasting glucose levels in the general population [18,21,25]. Glucokinase plays a key role in the regulation of insulin secretion through its role in glucose metabolism in pancreatic β-cells. The observed association with glucose levels likely results from altered expression of the glucokinase gene in carriers of the variant, with a subsequent effect on insulin secretion.

TCF7L2, in addition to being associated with Type 2 diabetes and gestational diabetes, is also associated with fasting glucose in the general population [27]. The *TCF7L2* variant (or a variant in high linkage disequilibrium with it) associated with diabetes risk and fasting glucose levels in the general population showed only marginal association with fasting glucose levels in European ancestry HAPO mothers, but strong association with 1- and 2-h glucose levels during an oral glucose tolerance test [26]. This is consistent with an earlier small study that failed to demonstrate association of the *TCF7L2* variant with maternal fasting glucose levels [28]. Of interest, in a large meta-analysis of non-pregnant European ancestry individuals, association of variants in *TCF7L2* with 2-h glucose levels was not reported [22], suggesting, possibly, a more important role in glucose metabolism during pregnancy. Beyond their association with maternal glucose levels, these variants in maternal *GCK* and *TCF7L2* are also associated with offspring birthweight [25,26,28], which is consistent with the known relationship between maternal glucose levels and birthweight [4].

Given the role of inflammatory mediators in insulin resistance during pregnancy and their association with maternal glucose levels during pregnancy [8,29], we used a candidate gene approach to test for association between genetic variation across 31 inflammatory pathway genes and measures of maternal metabolism in HAPO mothers of Northern European and Thai ancestry [30]. Variants in six of these genes, resistin (*RETN*), interleukin-8 (*IL8*), adiponectin receptor 2 (*ADIPOR2*), leptin receptor (*LEPR*), interleukin-6 (*IL6*) and tumour necrosis factor-alpha (*TNF*α), demonstrated evidence for association with maternal metabolic traits and consistency of effect across populations (Table 2).

We have also performed a genome-wide association study to identify genetic variation associated with maternal metabolic traits at ~28 weeks' gestation. This was carried out using ~4500 HAPO mothers from four different ancestry groups (Northern European, Thai, Mexican American and Afro-Caribbean) [31]. Given the large number of single nucleotide polymorphisms tested for association with the trait of interest in a genome-wide association study, strict criteria for evidence of genome-wide association have been established; i.e. $P < 5 \times 10^{-8}$ is required. Using those criteria, we demonstrated association of seven genetic loci with different maternal metabolic traits in a meta-analysis across the four ancestry groups (Table 3). Interestingly, five of these loci, *MTNR1B*, *PPP1R3B*, *PCSK1*, *GCKR* and *G6PC2*, have been shown to be associated with different metabolic traits in non-gravid populations. The association of *MTNR1B* with maternal fasting glucose levels has also been recently demonstrated in pregnant women of East Asian ancestry [23,32].

Two loci that demonstrated genome-wide significant association with maternal metabolic traits in our recent study appear to be relatively specific to pregnancy, *BACE2* and *HKDC1* [31]. *HKDC1* demonstrated association with 2-h glucose levels during the oral glucose tolerance test [31]. It encodes hexokinase domain containing 1, which is a recently identified member of the hexokinase family. *HKDC1* is adjacent to the gene which encodes hexokinase 1 (*HK1*) on chromosome 10, suggesting that *HKDC1* and *HK1* are products of a gene duplication event [33]. *HKDC1* is conserved across multiple species, including mammals, birds, fish and amphibians, and has both a glucose binding domain and ATP binding site in its C-terminal domain, suggesting that it has hexokinase activity, although this is yet to be demonstrated [33]. The biological role of *HKDC1* is unknown, but *HKDC1* mRNA is present in a wide distribution of human tissues [31]. Interestingly, in a large meta-analysis of over 40 000 non-pregnant individuals, only nominal association ($P = 1.2 \times 10^{-4}$) with 2-h glucose levels was observed [22]. This is far smaller than the robust association with 2-h glucose levels ($P = 1.02 \times 10^{-22}$) we observed in 7463 pregnant women [31]. The role that hexokinase domain containing 1 plays in glucose metabolism during pregnancy is not known and an important area for future study.

BACE2, which encodes β-site amyloid polypeptide cleaving enzyme 2, was associated with maternal fasting C-peptide levels. This gene has not been previously associated with metabolic traits in non-gravid populations. *BACE2* is expressed in multiple tissues and is capable of processing amyloid precursor protein [34,35]. In pancreatic islets, *BACE2* is expressed only in β-cells and its protein product is located in endocytic vesicles [36,37]. It is not thought to contribute to amyloid deposition in pancreatic islets, but has been shown to both augment and inhibit insulin secretion and/or production in human islets [36,37]. Thus, *BACE2* appears to be uniquely associated with pregnancy. Alternatively, it may be a newly identified locus specifically associated with insulin processing and C-peptide levels, although a role for *BACE2* in proinsulin processing has not been described.

Beyond determining how maternal genetic variation impacts maternal metabolism, an area of increasing interest is the potential impact of fetal genotype on maternal metabolism [38]. Recently, Petry *et al*. demonstrated an impact of variants of *IGF2*, the gene which encodes insulin-like growth factor-II (IGF-II), on maternal glucose levels [39]. *IGF2* is imprinted, meaning that only one of the two inherited alleles is expressed, which, in the case of *IGF2* is the allele inherited from the father (the paternal allele). What has now been demonstrated is that variation in the copy of the fetal *IGF2* gene inherited from the father, but not that from the mother, is associated with maternal glucose levels [39]. These same variants were associated with increased IGF-II protein content in the placenta. The mechanism by which variation in the paternal allele of the fetal *IGF2* gene impacts maternal metabolism awaits definition, and whether variation in other imprinted fetal genes can also affect maternal metabolism is not known. Thus, progress is being made in defining genetic variation that

contributes to maternal metabolism during pregnancy. However, much remains to be learned. For example, what are the causal variants responsible for the observed associations and what is the mechanism underlying the association with changes in gene expression, protein function, mRNA splicing, etc.? Perhaps, even more importantly, what is the role of the associated gene products in maternal metabolism? To what extent is there overlap between the genetic architecture of metabolism in the gravid and non-gravid state and what gene products are uniquely important for maternal metabolism during pregnancy?

Metabolomics

Metabolomics is another relatively new 'omics' technology that is being applied to better define metabolic changes associated with insulin resistance and hyperglycaemia during pregnancy. The metabolome is the collection of all small molecules, i.e. metabolites, present in a sample of interest, for example blood or other biological fluid, tissue lysate or cells [40]. Thus, metabolomics is able to profile an individual's metabolic state through the multiplexed measurement of many low molecular weight metabolites, over 4000 of which have been identified in human serum [41].

An individual's metabolome provides an integrative readout of inputs from both one's environmental exposures as well as one's underlying genetic make-up [42,43]. The association of genetic variation with metabolite levels is well documented [44]. Many environmental and lifestyle factors, such as diet, physical activity, smoking, etc. also contribute to the metabolome [42,43]. More recently, it has become apparent that an individual's microbiome also contributes [45]. Thus, metabolomics technologies provide an integrative 'omics' perspective into human physiology.

Two of the major technologies used to characterize the metabolome include nuclear magnetic resonance spectroscopy and mass spectrometry [42,43]. The latter technology is typically coupled with a second technology such as gas or liquid chromatography. Nuclear magnetic resonance is able to detect only the more abundant metabolites present at micromolar or greater concentrations, but provides structural information as well as high reproducibility and throughput. Mass spectrometry-based approaches, in contrast, are more sensitive and capable of measuring metabolites present at nanomolar concentrations.

Two complementary approaches, targeted and non-targeted analyses, have been developed for characterizing the metabolome [42]. Targeted analyses involve quantitative measurement of a defined panel of chemically similar metabolites (e.g. amino acids) using stable isotope-labelled internal standards, calibrators and chromatographic separation technologies, together with mass spectrometry-based methods. Targeted analyses are typically sensitive, specific and reproducible, with results expressed in absolute units (e.g. nM) [42]. A drawback of this approach is the relatively limited number of stable isotope-labelled standards that are available for use, thus limiting the number of discrete metabolites that can be easily measured using a targeted approach. In contrast, non-targeted analysis is a more qualitative, shotgun approach that simultaneously measures as many different metabolites as possible in a biological specimen, regardless of the chemical class of metabolites [42]. Unlike targeted assays in which internal standards allow quantification of specific metabolites in absolute units, non-targeted assays provide a more qualitative measure of metabolites present in a specimen. As metabolites of interest are identified using a non-targeted approach, targeted assays need to be developed for absolute quantification of the metabolite in different physiologic states.

Given the relatively recent application of metabolomics technologies to mammalian systems, studies using metabolomics technologies to characterize maternal metabolism during pregnancy are still relatively limited. Two recent metabolomics studies seeking to

define biomarkers of gestational diabetes present early in pregnancy examined amniotic fluid, urine and/or plasma collected from women with gestational diabetes and healthy pregnant control subjects during the second trimester of pregnancy [46,47]. Studies performed using a nuclear magnetic resonance spectroscopy-based approach identified higher levels in gestational diabetes urine of several metabolites, including 3-hydroxyisovalerate, 2-hydroxybutyrate and choline, among others [46]. Lower plasma levels of betaine were also present in the group with gestational diabetes compared with control subjects. Levels of a few unidentified metabolites also differed between groups. Higher levels of 3-hydroxyisovalerate may reflect reduced biotin status [46], while plasma 2-hydroxybutyrate levels have been associated with insulin resistance and proposed as a possible biomarker of Type 2 diabetes [48]. Follow-up studies by this same group using an ultra-high-performance liquid chromatography/mass spectrometry-based approach failed to identify differences in urine or amniotic fluid between women with gestational diabetes and control women [47]. A subsequent study used nuclear magnetic resonance spectroscopy to analyse urines from women with gestational diabetes and healthy pregnant women collected during the first and early third trimester [49]. Use of multivariate analytic methods to analyse the metabolites failed to reliably identify cases of gestational diabetes, whereas univariate analyses demonstrated higher levels of citrate in both the first and third trimester in urine from women with gestational diabetes. The previously reported differences in 3-hydroxyisovalerate, 2-hydroxybutyrate and choline levels were not replicated in this study.

We have also recently undertaken metabolomics analyses using targeted and non-targeted assays comparing fasting serum collected at ~28 weeks' gestation from European ancestry HAPO mothers with comparable BMI from the highest and lowest deciles of fasting plasma glucose [50]. The mothers with high fasting glucose were also significantly more insulin resistant than their low fasting glucose counterparts. These analyses demonstrated metabolic perturbations that distinguished mothers with low and high fasting plasma glucose. Mothers in the group with high fasting plasma glucose had higher levels of triglycerides and 3-hydroxybutyrate, with a similar trend for lactate and glycerol. The finding of higher triglyceride levels in the group with high fasting plasma glucose likely reflects, in part, the greater insulin resistance and probable impaired insulin secretion in this group. Insulin resistance may have also contributed to the higher lactate and 3-hydroxybutyrate levels, as a recent population-based study in non-pregnant individuals reported association of these metabolites with insulin sensitivity [51]. The higher levels of lactate and glycerol in the group with high fasting plasma glucose are consistent with their roles as substrates for gluconeogenesis. Targeted assays also demonstrated higher levels of several amino acids in the group with high fasting plasma glucose, including alanine, proline, glutamine/glutamate, arginine, leucine/isoleucine and asparagine/aspartate, while phenylalanine, citrulline and valine trended higher in mothers with high fasting plasma glucose. Differences in levels of amino acids in mothers with and without gestational diabetes have been reported previously [12,52,53]. The finding of higher alanine levels in the group with high fasting glucose is consistent with some but not all earlier studies [52,53]. Along with lactate and glycerol, alanine is a major substrate for gluconeogenesis. Together, these findings suggest maternal hyperglycaemia is characterized by higher levels of gluconeogenic substrates.

The higher levels of proline, glutamine/glutamate and asparagine/aspartate in the mothers with high fasting plasma glucose are consistent with recent studies in non-pregnant cohorts demonstrating association of higher levels of these amino acids with glucose levels, insulin resistance and/or obesity [51,54,55]. An additional finding of interest was higher levels of branched chain amino acids, including leucine, isoleucine and a trend towards higher valine, in the high fasting glucose group. Higher levels of branched chain amino acids have been demonstrated in some, but not all, earlier studies of pregnant women [12,52,53]. Higher levels of branched chain amino acids are also characteristic of insulin resistance and

hyperglycaemia in non-pregnant populations and are part of a metabolic signature that predicts incident Type 2 diabetes [54,56,57]. Non-targeted analyses were also performed to more fully characterize the metabolic profile of the groups with high compared with low glucose. Not surprisingly, the group with high glucose had a metabolic profile consistent with insulin resistance and, possibly, impaired insulin secretion. This included higher levels of gluconate (perhaps reflecting hyperglycaemic excursions and increased oxidative stress), free fatty acids (indicative of impaired suppression of lipolysis by insulin), by-products of fatty acid oxidation (ketones and citric acid cycle intermediates), multiple amino acids, and fructose, pentitols and hexitols.

Beyond comparison of individual metabolites, the results of the non-targeted analyses can also be used for pathway analyses to determine if specific pathways exhibited collective differences in metabolite levels between groups. Consistent with the differences in amino acid levels identified by the targeted analyses, the pathway analyses suggested differences in amino acid metabolism between the groups with high and low fasting plasma glucose, including altered degradation of proline, phenylalanine, lysine, leucine and alanine in the group with high compared with low fasting plasma glucose.

In summary, targeted analyses demonstrated metabolite changes in the more insulin-resistant mothers with high glucose similar to changes that have been observed in non-gravid, insulin-resistant populations, suggesting similarities in the metabolic profile characteristic of insulin resistance and hyperglycaemia in pregnant and non-pregnant populations. More limited data are available from non-targeted assays in pregnant and non-pregnant populations. Our initial findings suggest differences in the metabolic profiles of mothers with high and low fasting plasma glucose, but determining whether hyperglycaemia in pregnancy is accompanied by a distinct metabolic profile awaits further studies in larger populations. Moreover, the association of maternal metabolites and metabolic profiles with fetal outcomes also awaits future studies.

Conclusion

Maternal metabolism during pregnancy is a major determinant of the intrauterine environment and fetal outcomes. Differences in metabolism in the gravid and non-gravid state and the profound insulin resistance that occurs during pregnancy have long been recognized. While progress has been made in defining the mechanisms underlying these changes and their impact on fetal outcome, much remains to be learned. New technologies are becoming available to better define the mechanisms. These new technologies are allowing for easier identification of genetic variation that contributes to individual differences in metabolism and may help identify gene products that play a more important role in the gravid compared with non-gravid state. Similarly, new metabolomics technologies are allowing for more detailed characterization of metabolic profiles during pregnancy and will assist in identifying metabolites that impact fetal outcomes. These represent but a few of the high-throughput ‘omics’ technologies becoming available. The real power of these technologies will be realized as the results obtained using individual ‘omics’ technologies are combined so as to take an integrated systems biology approach to maternal metabolism during pregnancy and its impact on both newborn outcomes and long-term health outcomes of the offspring.

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Table 1

Candidate genes demonstrating association with gestational diabetes in meta-analyses

Gene	Chromosome	Encoded protein	Protein function
IRS1	2	Insulin receptor substrate 1	Substrate of insulin receptor tyrosine kinase; key molecule in the insulin signalling pathway
IGF2BP2	3	Insulin-like growth factor 2 mRNA-binding protein 2	Binds insulin-like growth factor 2 mRNA and may regulate protein translation; risk allele associated with decreased insulin secretion
CDKAL1	6	CDK5 regulatory subunit associated protein 1 like-1	Function of the protein is not known but non- pregnant carriers of the risk alleles have impaired oral and intravenous glucose stimulated insulin secretion
GCK	7	Glucokinase	Phosphorylates glucose in pancreatic β-cells and hepatocytes; involved in the regulation of insulin secretion
TCF7L2	10	Transcription factor 7-like 2	Transcription factor and member of the Wnt signalling pathway; risk allele associated with reduced insulin secretion
MTNR1B	11	Melatonin receptor 1B	G-protein coupled receptor that is expressed on β-cells, binds melatonin and may antagonize insulin release
KCNJ11	11	Potassium inwardly rectifying channel, subfamily J, member 11	Integral membrane protein and inward-rectifier type potassium channel that is controlled by G- proteins and associated with the sulphonylurea receptor; involved in the regulation of insulin secretion
KCNQ1	11	Potassium voltage-gated channel, KQT-like subfamily, member 1	Voltage-gated potassium channel; involved in the regulation of insulin secretion

Table 2

Inflammatory pathway genes associated with maternal metabolic traits

Gene	Chromosome	Trait	Populations
LEPR	1	1-h C-peptide	Thai, all
IL8	4	1-h plasma glucose	European, all
TNFα	6	HbA_{1c}	European, Thai, all
IL6	7	1-h plasma glucose	European, all
ADIPOR2	12	Fasting C-peptide	European
RETN	19	Fasting plasma glucose	European, all

Table 3

Genetic loci demonstrating genome-wide association with maternal metabolic traits

Gene	Chromosome	Encoded protein	Protein function	Associated maternal trait	Associated metabolic traits in non-gravid populations
G6PC2	2	Glucose-6-phosphatase, catalytic, 2	Component of an integral membrane system that catalyses glucose-6-phosphate hydrolysis	Fasting glucose	Fasting glucose
GCKR	2	Glucokinase regulator	Regulatory protein that inhibits glucokinase by forming an inactive complex with the enzyme	Fasting C-peptide, fasting glucose	Lipid phenotypes, fasting glucose, 2-h glucose, fasting insulin, Type 2 diabetes
PCSK1	5	Proprotein convertase subtilisin/kexin type 1	Calcium-dependent serine endoprotease involved in proteolytic activation of several precursor proteins including proinsulin, pro- glucagon-like peptide 1 and pro-opiomelancortin, among others	Fasting glucose	Findings not consistent but associated with obesity-related traits, fasting plasma glucose, 2-h plasma glucose, and fasting and post-glucose proinsulin levels
PPP1R3B	8	Protein phosphatase 1, regulatory subunit 3B	Facilitates interaction of protein phosphatase 1 with enzymes of glycogen metabolism	Fasting C-peptide, fasting glucose	Lipid phenotypes, fasting glucose, C-reactive protein, Type 2 diabetes
HKDC1	10	Hexokinase domain containing 1	Structure consistent with a hexokinase, but function not yet defined	2-h glucose	Nominal association with 2-h glucose in a meta-analysis of over 40 000 individuals
MTNR1B	11	Melatonin receptor 1B	G-protein coupled receptor that is expressed on β-cells, binds melatonin and may antagonize insulin release	Fasting glucose, 1-h glucose	Fasting glucose, impaired fasting glucose, Type 2 diabetes, altered β-cell function, including decreased insulin release following oral and intravenous glucose
BACE2	21	β-site amyloid polypeptide cleaving enzyme 2	Not defined	Fasting C-peptide	None reported