

Anthony R. Angueira,¹ Anton E. Ludvik,¹ Timothy E. Reddy,^{2,3} Barton Wicksteed,⁴ William L. Lowe Jr.,¹ and Brian T. Layden^{1,5}



New Insights Into Gestational Glucose Metabolism: Lessons Learned From 21st Century Approaches

Diabetes 2015;64:327–334 | DOI: 10.2337/db14-0877

Pregnancy presents a unique physiological challenge that requires changes coordinated by placentally and non-placentally derived hormones to prepare the mother for the metabolic stress presented by fetal development and to ensure appropriate nutrient allocation between mother and fetus. Of particular importance is the maintenance of normal glucose metabolism during pregnancy. Here, we describe physiological changes in glucose metabolism during pregnancy and highlight new insights into these adaptations that have emerged over the past decade using novel methodologies, specifically genome-wide association studies (GWAS) and metabolomics. While GWAS have identified some novel associations with metabolic traits during pregnancy, the majority of the findings overlap with those observed in nonpregnant populations and individuals with type 2 diabetes (T2D). Metabolomics studies have provided new insight into key metabolites involved in gestational diabetes mellitus (GDM). Both of these approaches have suggested that a strong link exists between GDM and T2D. Most recently, a role of the gut microbiome in pregnancy has been observed, with changes in the microbiome during the third trimester having metabolic consequences for the mother. In this *Perspectives in Diabetes* article, we highlight how these new data have broadened our understanding of gestational metabolism, and emphasize the importance of future studies to elucidate differences between GDM and T2D.

A major challenge in maternal fetal medicine over the past few decades has been the increasing prevalence of

gestational diabetes mellitus (GDM) (i.e., new-onset hyperglycemia that presents during pregnancy) (1). Exemplifying the importance of studying GDM is that hyperglycemia during pregnancy not only increases the risk of maternal type 2 diabetes (T2D), but also predisposes the developing fetus to poor metabolic health later in life (2). In this *Perspectives in Diabetes* article, we first highlight key aspects of normal gestational glucose metabolism. We then describe new findings that have emerged in recent years spurred by new technologies (genome-wide association studies [GWAS], metabolomics, and gut microbiota investigations). Finally, we place these findings in context with current knowledge in the field and emphasize new directions emerging from these investigations.

GESTATIONAL GLUCOSE METABOLISM

Maternal adaptations occur in multiple systems, including cardiovascular, respiratory, and metabolic, throughout pregnancy. These maternal adaptations aim to maintain a healthy balance between the mother and fetus while ensuring proper fetal development. In the context of glucose metabolism, these adaptations occur to ensure adequate shunting of glucose to promote fetal development while maintaining adequate maternal nutrition. This balance in glucose regulation is paramount to maternal-fetal health during all trimesters of gestation. Initially during gestation, fasting blood glucose levels drop due, in part, to dilutional effects as maternal blood volume increases, remain constant in the second trimester, and

¹Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

²Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC

³Institute of Genome Science and Policy, Duke University, Durham, NC

⁴Kovler Diabetes Center, The University of Chicago, Chicago, IL

⁵Jesse Brown Veterans Affairs Medical Center, Chicago, IL

Corresponding author: Brian T. Layden, b-layden@northwestern.edu.

Received 3 June 2014 and accepted 2 September 2014.

© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

further decrease during the third trimester (3,4). Increased glucose utilization by the fetal-placental unit throughout pregnancy, removing glucose from the maternal circulation, also contributes to the decline (3). During this period of increased glucose utilization by the fetal-placental unit, maternal insulin sensitivity decreases. To compensate for these changes, both maternal hepatic gluconeogenesis and fatty acid levels increase (3). While gravid fasting blood glucose levels remain lower than pregravid fasted levels, postprandial glucose levels are elevated relative to the pregravid state (5). This elevation is likely a result of impaired insulin action, leading to diminished postprandial glucose utilization by the mother (3). Other contributing factors may include altered pancreatic β -cell-mediated insulin secretion and hepatic gluconeogenesis (3).

Insulin Sensitivity

As one of the key determinants of glucose homeostasis, peripheral insulin sensitivity is dynamically altered throughout pregnancy, initially increasing following embryonic implantation and decreasing markedly later in pregnancy. The mechanisms underlying the changes in insulin sensitivity have been detailed previously (6).

In brief, during the first weeks of pregnancy, the presence of the fetal-placental unit causes a drop in growth hormone levels, resulting in enhanced insulin sensitivity (6). After this period of increased sensitivity to insulin, circulating levels of human placental lactogen, placentally derived human growth hormone (GH-V), progesterone, cortisol, prolactin, and other hormones increase and contribute to decreasing insulin sensitivity in peripheral tissues such as adipocytes and skeletal muscle by interfering with insulin receptor signaling (6). Elevated levels of these placentally and non-placentally derived hormones, particularly progesterone, cortisol, and GH-V, lead to markedly decreased insulin sensitivity during the second and third trimesters of pregnancy, with the highest levels of insulin resistance occurring during the third trimester (3). The role of placentally derived hormones in mediating insulin resistance is made evident by the marked decrease in insulin resistance immediately postpartum (7).

In addition to maternally and placentally derived hormones, changes in the production of inflammatory mediators by the placenta (e.g., tumor necrosis factor- α), and cytokines produced by adipose tissue also contribute to the decrease in insulin sensitivity in peripheral tissues (3,8,9). The role of cytokines during pregnancy has been extensively reviewed previously (10). The levels of the adipocyte-derived hormone leptin, which acts as a sensor of nutrient storage, also increases during late gestation (6). Interestingly, lactogens such as prolactin lead to central leptin resistance by decreasing leptin transport across the blood-brain barrier despite increased circulating levels of leptin. Central leptin resistance has been implicated in contributing to increased feeding behavior and maintenance of body weight despite the catabolic state characteristic of late gestation (6). The end result of all these

changes is decreased insulin sensitivity, which helps to maintain normal glucose homeostasis in a manner that is suitable for both mother and offspring. One direct consequence of the marked decline in insulin sensitivity is that circulating insulin levels, and consequently the secretory capacity of pancreatic β -cells, are increased as gestation progresses to maintain adequate maternal and fetal nutrition (9).

Pancreatic β -Cell Adaptations

Pancreatic β -cell adaptation is critical for the response to the decline in maternal insulin sensitivity. This response is mediated, at least in part, by maternal and placental hormones such as prolactin and human placental lactogens, which have been shown to enhance insulin secretion and also increase the size and number of pancreatic β -cells (11,12). Additionally, the activity and levels of glucokinase, the primary glucose sensor in β -cells, are increased in pancreatic β -cells during this insulin-resistant phase of pregnancy, thus enhancing glucose-stimulated insulin secretion at lower than normal blood glucose levels (11). Interestingly, in addition to placental lactogens and glucokinase, paracrine and autocrine signaling by serotonin may also contribute to β -cell adaptations to pregnancy (13,14). Recently, the importance of microRNAs in regulating β -cell mass and function during pregnancy has been described. Specifically, miR-338-3p has been shown to play a role in regulating β -cell proliferation during gestation and is regulated by hormones such as estradiol (15). The end result of these adaptations is increased pancreatic β -cell mass and a lower threshold for glucose-stimulated insulin secretion.

Of importance, the research described above has been performed almost exclusively in rodent models, and there are likely differences in β -cell adaptations during pregnancy between humans and rodents, as has been previously reported (12). For example, prolactin appears to have a similar role *in vitro* in regulating pancreatic β -cell function in humans and mice, but it is not clear whether the function of prolactin *in vivo* is similar (16). Likewise, it is not clear whether the action of other hormones and receptors (e.g., GH-V and the prolactin receptor) differs between rodents and humans in mediating pregnancy-induced changes in β -cells (16). Additionally, the focus of much of this past work has been on β -cell adaptation during the insulin-resistant phase, whereas, the mechanisms underlying the increase in insulin sensitivity during early gestation as well as the return of pancreatic β -cell mass to prepregnancy levels during the postpartum period remain less well defined.

Hepatic Gluconeogenesis

Along with changes in insulin sensitivity and the subsequent response of pancreatic β -cells, hepatic gluconeogenesis contributes to glucose homeostasis during pregnancy. During pregnancy, rates of hepatic gluconeogenesis increase in women both with and without GDM (5). The rise in gluconeogenesis despite higher insulin

levels reflects a decrease in insulin sensitivity by the third trimester (5). Thus, during late gestation, in the background of increased circulating insulin levels and decreased insulin sensitivity, hepatic gluconeogenesis increases as a mechanism to maintain euglycemia in the face of greater fetal glucose utilization.

Metabolic Changes Characteristic of GDM

During pregnancy, a host of environmental and genetic factors influence the extent to which a mother can properly compensate for increased insulin resistance. In GDM, although insulin sensitivity in peripheral tissues is only slightly decreased compared with pregnant mothers without GDM, insulin secretion by mothers with GDM is significantly decreased (3,9). Together with the impaired insulin secretion, higher levels of hepatic gluconeogenesis result in the elevated glycemia observed in mothers with GDM (5).

NEW INSIGHTS INTO GESTATIONAL GLUCOSE METABOLISM

New insights into maternal glucose metabolism during pregnancy have emerged in the past few years, due in large part to the advent of new technologies. Here we place these insights in the context of what has been traditionally known about gestational metabolism with the new concepts that have emerged, and suggest new directions for research.

GWAS

Advances in high-throughput genotyping platforms have led to an explosion in GWAS, which have helped to reveal

the genetic architecture of polygenic diseases and traits (17). Because only a small number of large cohorts of pregnant subjects exist, a limited number of studies exploring gestational metabolism have occurred (see Table 1 for a summary of results). Some of these initial studies centered on probing known T2D risk alleles in ethnically homogenous cohorts (18,19). These studies successfully identified genes previously implicated in T2D that are associated with glucose metabolism during pregnancy and/or GDM, such as *IGF2BP2*, *MTNR1B*, *TCF7L2*, *INSR*, *IRS1*, *HHEK*, *CDKAL1*, *GCK*, *KCNQ1*, and other genes (18,20–22). Interestingly, loci identified in these studies include genes that are important for peripheral insulin sensitivity (*INSR*, *IRS1*) as well as β -cell function (*CDKAL1*, *KCNQ1*, and *GCK*) (19). While here we have focused on shared genetic loci between T2D and glucose metabolism during pregnancy and GDM, it is noteworthy that genetic loci shared between type 1 diabetes and glucose metabolism during pregnancy or GDM have not been reported (23). Data from these initial studies clearly reaffirm the genetic link between GDM and T2D, and highlight key genes in glucose homeostasis. However, whether genes different from those associated with T2D are important for GDM risk could not be determined from these studies, as single nucleotide polymorphism (SNP) selection was based on previous T2D studies.

More recently, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study has examined maternal glycemic traits during pregnancy (1). Using a multinational, multiethnic cohort studied at ~28 weeks of gestation,

Table 1—Notable genetic loci associated with glycemic traits identified in recent studies using cohorts of pregnant subjects

Gene	Associated trait in gravid cohort	Brief description	Reference
<i>CDK5 Regulatory Subunit Associated Protein 1-like 1 (CDKAL1)</i>	Elevated GDM risk	<i>CDK5</i> , the target of <i>CDKAL1</i> , has been demonstrated to play a role in β -cell regulation, particularly in the areas of β -cell survival as well as insulin production. Loci within <i>CDKAL1</i> have been previously identified in studies of nongravid cohorts.	18 22 48
<i>Glucokinase (GCK)</i>	Elevated FBG, GDM risk	<i>GCK</i> has been well studied, serving as a primary glucose sensor of pancreatic β -cells, regulating insulin secretion, among other important functions. <i>GCK</i> levels and activity are increased in β -cells during gestation. Additionally, SNPs in <i>GCK</i> are associated with FBG in nongravid populations.	21
<i>Glucokinase Regulator (GCKR)</i>	FBG, FCP	<i>GCKR</i> is a well-known inhibitor of <i>GCK</i> in the liver as well as in pancreatic β -cells. SNPs located in <i>GCKR</i> have also been associated with glycemic traits in T2D populations.	24
<i>Hexokinase Domain Containing 1 (HKDC1)</i>	Elevated 2-h postchallenge BG	<i>HKDC1</i> is a putative hexokinase, situated on chromosome 10, just upstream of Hexokinase 1. The function of the product of this gene is not fully understood.	24,25
<i>Beta-site APP-Cleaving Enzyme 2 (BACE2)</i>	FCP	<i>BACE2</i> is an enzyme whose main function is to cleave β -secretase in a variety of tissues. In pancreatic β -cells, this protein has been implicated in playing a role in regulating insulin secretion and β -cell function.	24,26

BG, blood glucose; FBG, fasting blood glucose; FCP, fasting C-peptide.

investigators used an unbiased genome-wide approach to identify loci associated with maternal glucose metabolism (1). As expected, known T2D loci, such as *GCK* and *TCF7L2*, were associated with higher glucose levels during pregnancy (1,21). Other loci previously shown to be associated with metabolic traits and/or T2D in nonpregnant populations included *GCKR* and *PP1R3B*, which were associated with fasting C-peptide levels, and *G6PC2*, *GCK*, *PCKS1*, and *MTNR1B*, which were associated with fasting glucose levels (24). In addition, two novel genes were identified: Hexokinase Domain-Containing 1 (*HKDC1*), which was associated with 2-h glucose levels, and Beta-site Amyloid Cleaving Enzyme 2 (*BACE2*), which was associated with fasting C-peptide levels (24). *HKDC1* encodes a putative fifth hexokinase that is widely expressed, most prominently in the colon, kidney, and thymus (24,25). Though predicted to be a functional hexokinase, the role of *HKDC1* in glucose homeostasis is unknown (25). The second gene, *BACE2*, encodes a protein whose function is to cleave the amyloid precursor protein. Interestingly, *BACE2* is expressed in pancreatic β -cells, and has been reported to play a role in regulating β -cell mass and insulin secretion (26). Neither gene has been definitively associated with glycemic traits in nonpregnant populations.

Given the overlap in genes associated with both GDM and T2D, a clear shared genetic architecture exists, which is further substantiated by the observation that mothers are at an increased risk for the development of T2D following GDM (27). However, to date, not all loci associated with T2D have demonstrated association with GDM. This may be a function of studies probing particular glycemic traits rather than testing the association with a disease such as T2D or GDM, or could be attributable to the lack of statistical power necessary to determine the association of certain T2D genes with GDM.

Interestingly, there are many loci now associated with T2D, and, while some appear to impact peripheral insulin resistance, the majority are important for β -cell function. Likewise, the genes associated with gestational glycemia are mainly implicated in β -cell function, consistent with impaired β -cell function being a primary driver of diabetes. Failure to identify genes important for peripheral insulin resistance during pregnancy that have been previously identified in studies of T2D may reflect the increased importance of pancreatic β -cell compensation in glucose homeostasis during pregnancy relative to the role played by peripheral tissues in mediating insulin sensitivity during pregnancy. Additionally, an increased environmental component associated with insulin resistance during pregnancy may decrease the portion of variation explained by genetic influences. While it is apparent that genetic factors that are important for pancreatic β -cell function may be identified in the background of increased insulin resistance during pregnancy, the identification of potentially unique loci offers another avenue of investigation specific to gestational metabolism. If such loci are ultimately identified, they may provide a view into the potential differences

in the mechanisms underlying insulin resistance that are characteristic of gestation and obesity. Moreover, the fact that the increased prevalence of GDM has occurred in the face of little to no shift in the genetic composition of the population suggests that environmental factors also play an important role in GDM. Regardless, to date, no unique (non-T2D) genetic locus associated with insulin resistance during gestation has been reported.

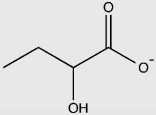
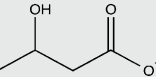
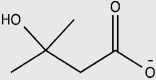
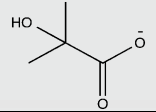
An additional consideration not fully appreciated in GWAS is the influence of inherited fetal genotype. Until recently, GWAS have accounted only for the maternal genotype and have not considered the influence of the fetal factors on maternal glucose homeostasis. Of relevance here is a mouse study, which demonstrated that a maternally transmitted disruption of *H19* correlated with elevated maternal blood glucose levels (28). The potential interaction between maternal and fetal genotype presents a new and unexplored area of investigation into the genetics of GDM.

Taken together, we expect that the genetic loci associated with gestational glycemia and GDM-specific glycemic dysregulation to be more precisely defined through follow-up studies with increased cohort sizes. Along with larger studies and deeper genetic sequencing approaches, a more complete understanding of glucose homeostasis during pregnancy as well as similarities and differences between GDM and T2D are likely to emerge.

Metabolomics

Recent advances in metabolomics have enabled high-throughput identification and quantification of large classes of metabolites in a tissue or body fluid, revealing disease-specific changes in cell and tissue function (29) (see the study by Bain et al. [29] for recent reviews). As with genetic studies, metabolomics has been used to study T2D more extensively than GDM (30–32) (see Table 2 for a summary of related findings between T2D and gestational studies). It has been observed in several studies (30,31,33) that circulating levels of branched-chain amino acids (BCAAs [valine, leucine, and isoleucine]) and their related metabolites are higher in individuals with T2D and insulin resistance. One popular hypothesis suggests that increased circulating levels of BCAAs interfere with fatty acid oxidation, resulting in decreased insulin sensitivity (33). An alternative theory suggests that the increased catabolism of these BCAAs, in conjunction with the higher circulating levels of these metabolites, is responsible for the decrease in insulin sensitivity (31). Supporting these two prevailing hypotheses, many of the studies using a metabolomics approach to study T2D have observed changes in BCAAs and their related metabolites. Expanding on initial studies that characterized the impact of BCAA metabolic dysregulation on increased insulin resistance, some recent studies have identified that BCAAs and their metabolites play a role in pancreatic β -cell function. For example, levels of α -hydroxybutyrate, a metabolite that shares a common intermediate, propionyl-CoA, with catabolized BCAAs, were found to be positively

Table 2—Common metabolites associated with glycemic traits, T2D, and GDM discovered in recent studies

Metabolite	Structure	Brief description	Reference
α -Hydroxybutyrate (2-hydroxybutyrate; α -HB)		α -HB is produced by the reduction of α -ketobutyrate, an intermediate in threonine and methionine catabolism. α -HB is elevated in populations with T2D as well as GDM.	32,35
β -Hydroxybutyrate (3-hydroxybutyrate; β -HB)		3-HB is a hydroxyacid that is positively associated with insulin resistance during pregnancy. 3-HB levels are elevated in the cord blood of mothers with high FBG levels.	31,35
3-Hydroxyisovalerate		This compound is an intermediate in biotin metabolism. Elevated levels of this metabolite were demonstrated in the urine of pre-GDM mothers.	37
2-Hydroxyisobutyrate		Higher concentrations of this compound in urine have been associated with insulin resistance. This compound was shown to be higher in the urine of pre-GDM mothers.	37

Structures were generated using ChemDraw version 13.0. FBG, fasting blood glucose.

associated with insulin resistance, and follow-up studies (32,34) showed a role in inhibiting insulin secretion in mouse islets. As is apparent, metabolomics has offered investigators a window into the metabolic changes that occur during T2D and, in particular, into the role of novel metabolites in insulin resistance and β -cell function.

Metabolomics methodologies have more recently been applied to studying maternal glycemia during pregnancy (35). Early metabolic studies (36) focused on changes in amino acid and circulating triglyceride levels during pregnancy. Many of the amino acids assayed by these groups were not perturbed during GDM; however, one study (36) found that levels of β -hydroxybutyrate, a circulating ketone body, were increased in the plasma of mothers with GDM. Recently, a combination of targeted and nontargeted approaches was used to quantify metabolites in the blood of pregnant women with either low or high fasting plasma glucose levels between weeks 24 and 32 of pregnancy (35). The targeted analysis indicated that mothers with high fasting plasma glucose levels had higher levels of triglycerides, 3-hydroxybutyrate (β -hydroxybutyrate), and select amino acids, including alanine, leucine, and isoleucine (35). Nontargeted analysis also found 2-hydroxybutyric acid levels, the acid analog of α -hydroxybutyrate, were higher in mothers with high fasting plasma glucose levels compared with those with low fasting plasma glucose levels (35). A study (37) using nuclear magnetic resonance to study metabolites in the urine of pregnant mothers found significantly higher levels of 3-hydroxyisovalerate and 2-hydroxyisobutyrate during the second trimester in women who eventually presented with GDM over those who had normal pregnancies. In contrast, a mass spectrometry-based assessment (38) of metabolites in both the urine and amniotic fluid of pregnant mothers at a similar time point in pregnancy did not identify any metabolites that were significantly higher between mothers in whom GDM did and did not develop in the third trimester. Of note,

3-hydroxyisovalerate and 2-hydroxyisobutyrate levels were not evaluated in this latter study. Taken together, the metabolic profile of pregnant women with high fasting plasma glucose levels shared many features of the metabolic profile observed in T2D subjects, including perturbations in BCAA metabolism, resulting in higher levels of metabolites that decrease insulin sensitivity and impact β -cell function (30,32,34,35).

These T2D studies noted above suggest that the metabolic signature of T2D involves perturbations in BCAA levels and metabolism (30). Importantly, some of these metabolites have been found to have a role in aspects of metabolism such as insulin sensitivity and pancreatic β -cell function. While a limited number of GDM metabolomics studies have been performed, evidence suggests that the metabolic signatures of T2D and GDM overlap. Future studies in this area should focus on the extent to which the metabolic profiles of insulin resistance produced by normal pregnancy, obesity, and clinically diagnosed GDM differ, if at all. These experiments may provide insight into whether insulin resistance during pregnancy and obesity arise from unique pathways, and the extent to which perturbations in these pathways influence the development of GDM and T2D. Information gained from these studies could identify a metabolic signature characteristic of GDM that is distinct from the metabolic profile of pregnancy-induced insulin resistance (but is not associated with GDM) and, thus, suggest new biomarkers for GDM.

The Gut Microbiome

The emergence of the gut microbiome as a novel environmental factor that directly impacts metabolism has been an exciting new area of research that has stimulated interest in how gut bacterial commensals may influence host metabolism and vice versa (39). The emerging and prevailing notion is that the microbiome functions not only to harvest untapped energy from undigested food,

but to also feed back onto the host, potentially serving as a nutrient sensor or regulator of nutrient sensors (39). The ability to investigate this recently characterized factor in the areas of obesity and T2D has been enhanced by advances in next-generation sequencing of bacterial genes encoding the 16S rRNA ribosomal subunit (see the study by Karlsson et al. [40] for a recent review). Thus far, perturbations in the microbiome, particularly in the ratio of *Bacteroidetes* to *Firmicutes*, have been implicated in mouse studies to impart a phenotype reminiscent of the metabolic syndrome, including increased weight gain, adiposity, and peripheral insulin resistance (41). However, it remains hotly debated whether the *Bacteroidetes*-to-*Firmicutes* ratio is positively or negatively correlated with obesity and whether perturbations in this ratio are causal in establishing insulin resistance or are secondary to obesity-induced insulin resistance (42,43). Some of the work in this now maturing field has transitioned from focusing on how the microbiome composition contributes to host metabolism, to how the microbiome metagenome—the genetic material of the microbiome—is altered during disease states (40,44). The analysis of the microbial metagenome has allowed investigators to ask questions about what types of bacterial genes are enriched or depleted during different states, offering species-independent investigations into the impact of gut bacteria upon host health.

While a majority of the research conducted on the microbiome has attempted to assess its role in influencing energy harvest and nutrient sensing during obese states relevant to T2D, some recent studies have sought to characterize its changes during pregnancy (45). One early study using flow cytometry–coupled fluorescent in situ hybridization concluded that the amount of bacteria that compose the microbiota increases throughout pregnancy (46). Koren et al. (45), using a Finnish cohort, determined that bacterial β -diversity—that is diversity in the bacterial population between mothers—increases dramatically between trimesters 1 and 3, with observed increases in *Proteobacteria* as well as *Actinobacteria* (45). Transplants of fecal material obtained during different trimesters were sufficient to confer different phenotypes in mouse models, with third-trimester fecal matter leading to increased adiposity and inflammation, similar to the phenotype observed during pregnancy (45). Additionally, in third-trimester samples, these investigators observed higher levels of bacteria belonging to the family *Enterobacteriaceae* and the genus *Streptococcus*, albeit with no difference in levels between GDM and non-GDM mothers (45). These studies suggest that changes in the gut microbiome occur during pregnancy and may play a role in the observed increase in gestational inflammation (45).

More studies tracking the changes that occur in the microbiome during pregnancy and pathologies that occur during pregnancy such as GDM in various ethnic backgrounds are required to fully determine the extent to which the microbiome influences and/or responds to the maternal metabolic phenotype. Given the dramatic

reorganization of maternal metabolism that occurs during pregnancy, it seems logical that the microbiome is altered during pregnancy. However, what needs to be studied is whether changes in the microbiome mediate some of the observed changes in insulin resistance and β -cell function observed during pregnancy, or whether metabolic changes in these tissues cause reorganization of the microbiome during pregnancy. Moreover, it remains to be determined whether the relationship of the gut microbiome is simply a response to or is an active participant in generating metabolic changes such as the decreased insulin sensitivity and even hyperglycemia present during GDM. Interestingly, the microbial signature of pregnancy, which is characterized by an increase in diversity as well as an increase in bacteria belonging to the phyla *Actinobacteria* and *Proteobacteria*, is remarkably different from that of obesity, which is characterized by a decrease in bacteria belonging to the phylum *Firmicutes* (43,45). Here, future studies will be enhanced by complementary metagenomic and metabolomics analyses of the microbiome during pregnancy, and will improve our understanding of how the gut microbiome changes in response to increased metabolic demands, placental hormones, and, in the case of GDM, hyperglycemia during pregnancy.

NEW DIRECTIONS

Taken together, the past decade has seen some great advances in our understanding of maternal metabolism during pregnancy. Genetic studies and GWAS have revealed that, while the genetic bases for the development of GDM and T2D may have considerable overlap, there may also be unique genetic factors important in maternal metabolism during pregnancy. Metabolomics studies in the area of maternal-fetal medicine have begun to identify metabolic signatures of pregnancy during dysglycemia as well as normal pregnancy. These results suggest that the metabolic signatures of hyperglycemia in T2D and GDM are, in part, similar. Microbial studies have established that the gut microbial profile changes during pregnancy and is involved in inducing inflammation and increased adiposity in mice. These data raise some exciting new questions that future studies may answer.

In the coming years, there lies a great opportunity in using genetics, metabolomics, and gut microbiota to determine whether and how glucose regulation differs in pregnant and nonpregnant states, and likewise in GDM and T2D. Of note, the role of the epigenome in mediating metabolic changes during pregnancy is just now being studied using next-generation technologies. Learning about the role of microRNAs and histone deacetylases in regulating gene expression in the β -cell as well as other metabolically relevant tissues shows great promise in shedding light on the poorly understood areas of gestational glycemia. Moreover, recent genomic profiling studies have identified unique microRNAs that may potentially serve as new biomarkers for GDM, which, to date, the limited metabolomics studies have not yet succeeded in doing

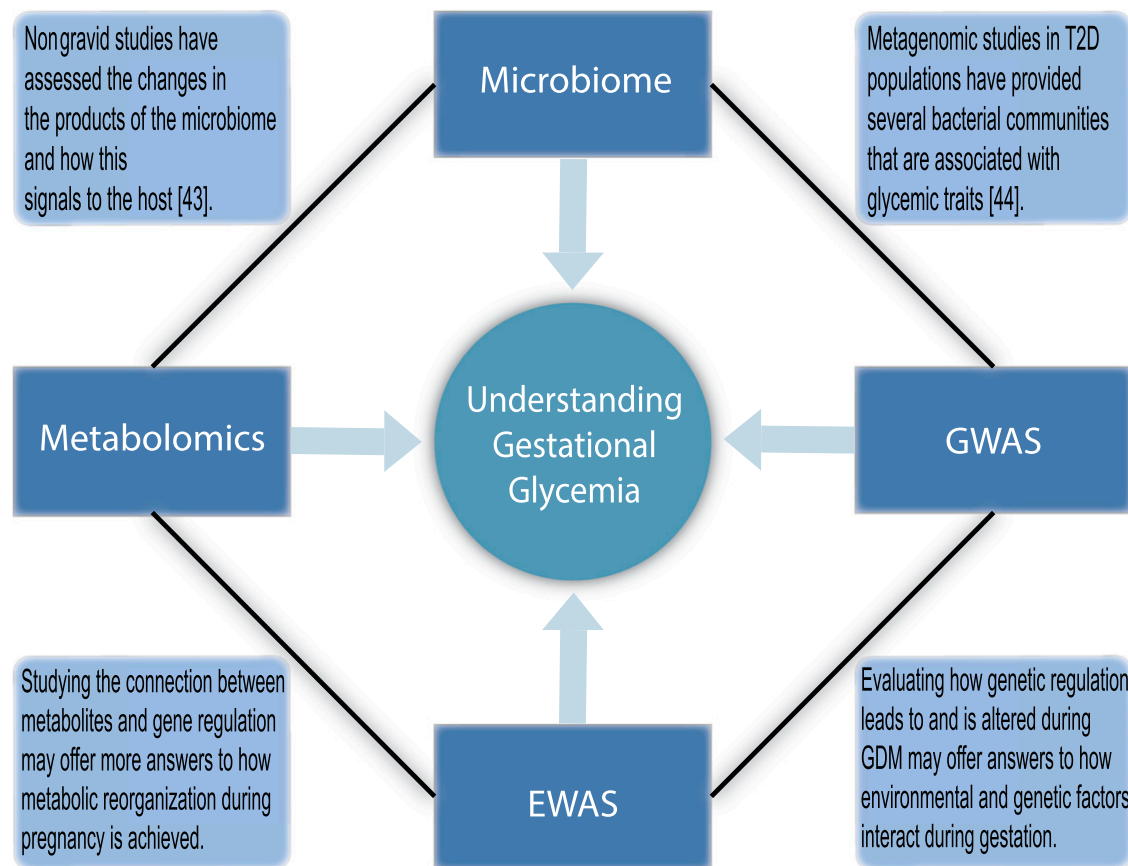


Figure 1—A depiction of the some of the observations from recent studies, and current new directions that have emerged from GWA, metabolomics, and gut microbiome studies. EWAS, epigenetic-wide association studies.

(47). Going forward, future studies should combine these technologies to increase their power and subsequently the number of scientific questions that they can answer (see Fig. 1 for a summary of new directions). However, with these new powerful approaches, challenges in managing and drawing conclusions from large and complex systems, such as the human and microbial genome, will arise.

An important focus in this area of research should be to identify differences and similarities in GDM and T2D. Of particular importance should be the examination of mechanisms underlying the shared risk between GDM and T2D, and, more precisely, the genetic and environmental factors associated with GDM that lead to T2D. This will not only improve our ability to identify and treat mothers during pregnancy but also after pregnancy. Similarly, these data will provide mechanistic insight regarding the mechanisms shared between GDM and T2D. Ultimately, these new data will likely be translated into the clinic, leading to improvements in maternal and fetal outcomes.

Funding. B.T.L. is supported by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Career Development (grant 1-1K2-BX-001587-01).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.R.A. and B.T.L. conceived, designed, and wrote the manuscript. A.E.L., T.E.R., B.W., and W.L.L. edited the manuscript. B.T.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Metzger BE, Lowe LP, Dyer AR, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; 358:1991–2002
2. Ruchat SM, Hivert MF, Bouchard L. Epigenetic programming of obesity and diabetes by in utero exposure to gestational diabetes mellitus. *Nutr Rev* 2013;71 (Suppl. 1):S88–S94
3. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Del Prato S. Intermediate metabolism in normal pregnancy and in gestational diabetes. *Diabetes Metab Res Rev* 2003;19:259–270
4. Hadden DR, McLaughlin C. Normal and abnormal maternal metabolism during pregnancy. *Semin Fetal Neonatal Med* 2009;14:66–71
5. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr* 2000;71(Suppl.):1256S–1261S
6. Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. *Curr Opin Endocrinol Diabetes Obes* 2011;18:409–416
7. Mazaki-Tovi S, Kanety H, Pariente C, et al. Insulin sensitivity in late gestation and early postpartum period: the role of circulating maternal adipokines. *Gynecol Endocrinol* 2011;27:725–731

8. Desoye G, Hauguel-de Mouzon S. The human placenta in gestational diabetes mellitus. The insulin and cytokine network. *Diabetes Care* 2007;30(Suppl. 2):S120–S126
9. Lain KY, Catalano PM. Metabolic changes in pregnancy. *Clin Obstet Gynecol* 2007;50:938–948
10. Romero R, Gotsch F, Pineles B, Kusanovic JP. Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. *Nutr Rev* 2007;65:S194–S202
11. Sorenson RL, Brelje TC. Prolactin receptors are critical to the adaptation of islets to pregnancy. *Endocrinology* 2009;150:1566–1569
12. Butler AE, Cao-Minh L, Galasso R, et al. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia* 2010;53:2167–2176
13. Pasek RC, Gannon M. Advancements and challenges in generating accurate animal models of gestational diabetes mellitus. *Am J Physiol Endocrinol Metab* 2013;305:E1327–E1338
14. Kim H, Toyofuku Y, Lynn FC, et al. Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat Med* 2010;16:804–808
15. Jacovetti C, Abderrahmani A, Parnaud G, et al. MicroRNAs contribute to compensatory β cell expansion during pregnancy and obesity. *J Clin Invest* 2012;122:3541–3551
16. Ben-Jonathan N, LaPensee CR, LaPensee EW. What can we learn from rodents about prolactin in humans? *Endocr Rev* 2008;29:1–41
17. Welter D, MacArthur J, Morales J, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 2014;42:D1001–D1006
18. Cho YM, Kim TH, Lim S, et al. Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population. *Diabetologia* 2009;52:253–261
19. Kwak SH, Kim SH, Cho YM, et al. A genome-wide association study of gestational diabetes mellitus in Korean women. *Diabetes* 2012;61:531–541
20. Robitaille J, Grant AM. The genetics of gestational diabetes mellitus: evidence for relationship with type 2 diabetes mellitus. *Genet Med* 2008;10:240–250
21. Feathy RM, Hayes MG, Urbanek M, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: common genetic variants in GCK and TCF7L2 are associated with fasting and postchallenge glucose levels in pregnancy and with the new consensus definition of gestational diabetes mellitus from the International Association of Diabetes and Pregnancy Study Groups. *Diabetes* 2010;59:2682–2689
22. Zhang C, Bao W, Rong Y, et al. Genetic variants and the risk of gestational diabetes mellitus: a systematic review. *Hum Reprod Update* 2013;19:376–390
23. Polychronakos C, Li Q. Understanding type 1 diabetes through genetics: advances and prospects. *Nat Rev Genet* 2011;12:781–792
24. Hayes MG, Urbanek M, Hivert MF, et al.; HAPO Study Cooperative Research Group. Identification of HKDC1 and BACE2 as genes influencing glycemic traits during pregnancy through genome-wide association studies. *Diabetes* 2013;62:3282–3291
25. Irwin DM, Tan H. Molecular evolution of the vertebrate hexokinase gene family: identification of a conserved fifth vertebrate hexokinase gene. *Comp Biochem Physiol Part D Genomics Proteomics* 2008;3:96–107
26. Esterházy D, Stützer I, Wang H, et al. Bace2 is a β cell-enriched protease that regulates pancreatic β cell function and mass. *Cell Metab* 2011;14:365–377
27. Lee AJ, Hiscock RJ, Wein P, Walker SP, Permezel M. Gestational diabetes mellitus: clinical predictors and long-term risk of developing type 2 diabetes: a retrospective cohort study using survival analysis. *Diabetes Care* 2007;30:878–883
28. Petry CJ, Evans ML, Wingate DL, et al. Raised late pregnancy glucose concentrations in mice carrying pups with targeted disruption of H19delta13. *Diabetes* 2010;59:282–286
29. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics applied to diabetes research: moving from information to knowledge. *Diabetes* 2009;58:2429–2443
30. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 2009;9:311–326
31. Menni C, Fauman E, Erte I, et al. Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. *Diabetes* 2013;62:4270–4276
32. Gall WE, Beebe K, Lawton KA, et al.; RISC Study Group. alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PLoS One* 2010;5:e10883
33. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 2012;15:606–614
34. Ferrannini E, Natali A, Camastra S, et al. Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. *Diabetes* 2013;62:1730–1737
35. Scholtens DM, Muehlbauer MJ, Daya NR, et al.; HAPO Study Cooperative Research Group. Metabolomics reveals broad-scale metabolic perturbations in hyperglycemic mothers during pregnancy. *Diabetes Care* 2014;37:158–166
36. Pappa KI, Vlachos G, Theodora M, Roubelaki M, Angelidou K, Antsaklis A. Intermediate metabolism in association with the amino acid profile during the third trimester of normal pregnancy and diet-controlled gestational diabetes. *Am J Obstet Gynecol* 2007;196:65.e1–5.
37. Diaz SO, Pinto J, Graça G, et al. Metabolic biomarkers of prenatal disorders: an exploratory NMR metabolomics study of second trimester maternal urine and blood plasma. *J Proteome Res* 2011;10:3732–3742
38. Graça G, Goodfellow BJ, Barros AS, et al. UPLC-MS metabolic profiling of second trimester amniotic fluid and maternal urine and comparison with NMR spectral profiling for the identification of pregnancy disorder biomarkers. *Mol Biosyst* 2012;8:1243–1254
39. Bäckhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004;101:15718–15723
40. Karlsson F, Tremaroli V, Nielsen J, Bäckhed F. Assessing the human gut microbiota in metabolic diseases. *Diabetes* 2013;62:3341–3349
41. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JL. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 2005;102:11070–11075
42. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012;143:913–916.e7
43. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013;341:1241214
44. Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013;498:99–103
45. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;150:470–480
46. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008;88:894–899
47. Zhao C, Dong J, Jiang T, et al. Early second-trimester serum miRNA profiling predicts gestational diabetes mellitus. *PLoS One* 2011;6:e23925
48. Palmer ND, Goodarzi MO, Langefeld CD, et al. Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the Insulin Resistance Atherosclerosis Family Study. *Diabetes* 2008;57:1093–1100