ADA AWARD LECTURE

Metabolic Culprits in Obese Pregnancies and Gestational Diabetes Mellitus: Big Babies, Big Twists, Big Picture

The 2018 Norbert Freinkel Award Lecture

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Pregnancy has been equated to a "stress test" in which placental hormones and growth factors expose a mother's predisposition toward metabolic disease, unleashing her previously occult insulin resistance (IR), mild β-cell dysfunction, and glucose and lipid surplus due to the formidable forces of pregnancy-induced IR. Although pregnancy-induced IR is intended to assure adequate nutrition to the fetus and placenta, in mothers with obesity, metabolic syndrome, or those who develop gestational diabetes mellitus, this overnutrition to the fetus carries a lifetime risk for increased metabolic disease. Norbert Freinkel, nearly 40 years ago, coined this excess intrauterine nutrient exposure and subsequent offspring developmental risk "fuel-mediated teratogenesis," not limited to only excess maternal glucose. Our attempts to better elucidate the causes and mechanisms behind this double-edged IR of pregnancy, to metabolically characterize the intrauterine environment that results in changes in newborn body composition and later childhood obesity risk, and to examine potential therapeutic approaches that might target maternal metabolism are the focus of this article. Rapidly advancing technologies in genomics, proteomics, and metabolomics offer us innovative approaches to interrogate these metabolic processes in the mother, her microbiome, the placenta, and her offspring that contribute to a phenotype at risk for future metabolic disease. If we are successful in our efforts, the researcher, endocrinologist, obstetrician, and health care provider fortunate enough to care for pregnant women have the unique opportunity to positively impact health outcomes not only in the short term but in the long run, not just in one life but in two—and possibly, for the next generation.

The power of pregnancy offers the clinician and scientist a unique opportunity to meaningfully affect the outcome of two individuals. Applying observations from the immense field of medicine to optimize the outcome of both mother and her infant, humbled by a placenta that will ultimately determine the exposure to the fetus, is an extraordinary challenge and opportunity. Trained as an internist and inspired by two professors of medicine who were both pioneers in obstetric medicine, I spent my first 12 years pursuing clinical research in thromboembolism in pregnancy, the leading case of maternal mortality at the time. The first major twist along my career path was made possible by two professors of endocrinology who led me to appreciate the profound endocrinologic changes remodeling maternal physiology into an insulin-resistant (IR)

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state. This transformation of maternal metabolism and its effect on pregnancies complicated by obesity and gestational diabetes mellitus (GDM) became my research focus over the next 18 years and is the subject of this article.

This IR state, not only characterized by hyperglycemia, unearths the already present propensity for mothers to develop metabolic disease and may also promote the future development of metabolic disease in her offspring (1,2). In fact, it was indeed an endocrinologist, Norbert Freinkel, who delivered his seminal 1980 Banting lecture (3) the year I started medical school, "Of Pregnancy and Progeny," who is a founding father of the fetal overnutrition hypothesis. In essence, Dr. Freinkel transformed our thinking about the intrauterine environment in mothers with diabetes, overnutrition, and obesity and the future health implications to the offspring. His described that "fuel-mediated teratogenesis"—not limited to only maternal glucose but inclusive of maternal lipids and amino acids—could lead to "alterations occurring subsequent to organogenesis during the differentiation and proliferation of fetal cells," which "could cause long-range effects upon behavioral, anthropometric, and metabolic functions" (3). The work that follows is a direct result of many inspirational collaborators, especially Drs. Jacob (Jed) Friedman and Teresa (Teri) Hernandez (University of Colorado School of Medicine), aiming to shed additional insights on the *1*) causes and mechanisms that underlie the IR of normal pregnancy, obesity, and GDM; *2*) metabolic characterization of the intrauterine metabolic environment that contributes to excess infant adiposity; and *3*) potential targets and timing of pregnancy interventions to promote healthy short- and long-term outcomes for both mother and child.

LESSONS LEARNED ON IR FROM A MOUSE WITH TOO MUCH PLACENTAL GROWTH HORMONE

Endocrinologists spend much of their clinical time attempting to attenuate IR in their patients with obesity, metabolic syndrome, or diabetes. Yet, in the context of pregnancy, IR is a critical normal pathophysiologic mechanism to ensure adequate nutrient substrate provision from mother to a growing fetus (4). Unfortunately, when IR in skeletal muscle, adipose tissue, or liver and/or some degree of compromised β-cell reserve predates the pregnancy, often seen in women with obesity, metabolic syndrome, and in women who develop GDM, the increasing IR demands of normal pregnancy place further burden on the maternal β-cell to step up secretion. The inability to meet this demand results in excess glucose, lipid, and amino acid exposure to the fetal-placental unit (5,6). This often leads to fetal hyperinsulinemia, which can occur by 16 weeks' gestation, enhanced fetal growth, and an overabundance of maternal substrate for excess fetal fat accretion (7).

Textbooks have told us that human placental lactogen (hPL) was the primary cause of this physiologic IR of pregnancy. Yet the data supporting this causation were limited as hPL has both insulin and anti-insulin effects and may actually be more important to stimulate the growth of maternal pancreatic islets and increase lipolysis and free fatty acid (FFA) liberation from adipocytes as an energy substrate (8). The placenta also makes a growth hormone (GH) variant, similar to pituitary GH, that has been known to increase IR in pathologic states such as acromegaly (8). Human placental growth hormone (hPGH) is a product of the human GH variant gene that differs from pituitary GH by 13 amino acids. Its secretion is tonic, increases six- to eightfold throughout gestation, and virtually replaces pituitary GH in the maternal circulation by ~20 weeks (8,9). We set out to prove whether hPGH, never before characterized by its effect on insulin action, also had the capacity to cause IR. Dr. Andrzej Bartke (Southern Illinois University School of Medicine) was generous to share a transgenic mouse that expressed hPGH at levels similar to the third trimester of pregnancy. Simple glucose tolerance testing revealed that fasting insulin levels were ~4 times higher than in control mice and ~7 times higher 30 min after glucose stimulation, confirming pronounced IR. We further showed a marked decrease in insulin sensitivity given that an insulin injection into these hPGH transgenic mice resulted in no significant decline in glucose disposal compared with wild-type mice, who demonstrated >65% decline in glucose levels (10).

How hPGH caused IR at a cellular level was our next puzzle. By focusing on the skeletal muscle of these transgenic hPGH mice, we discovered an unforeseen twist that hPGH selectively increased the expression of the p85 regulatory unit of phosphatidlylinositol 3-kinase (PI 3-kinase), a key effector enzyme necessary for stimulating glucose uptake in insulin-sensitive tissues. In a series of subsequent experiments, the seemingly paradoxical finding of an increase in a subunit of PI 3-kinase being associated with IR was ultimately solved. PI 3-kinase is composed of both a p85 regulatory unit and a catalytic subunit (p110), which must form a p85-p110 heterodimer and bind to insulin receptor substrate 1 (IRS-1) for PI 3-kinase activation to occur. When the p85 monomer is selectively overexpressed by the action of hPGH, it competes with the active p85-p110 heterodimer in a dominant negative fashion for binding to IRS-1, resulting in a decrease in IRS-1–associated PI 3-kinase activity. We confirmed that this decrease in PI 3-kinase activity resulted in the final step of the insulin signaling pathway being attenuated—a reduction in GLUT-4 translocation to the plasma membrane (11) (Fig. 1). Further, the excess GH-induced IR was completely reversible using a GH-releasing antagonist (12).

INSULIN SIGNALING CHANGES IN SKELETAL MUSCLE IN PREGNANT AND POSTPARTUM WOMEN WITH OBESITY OR GDM

Although hPGH mediated the IR in mice that expressed this placental hormone at levels similar to the third trimester of human pregnancy, ascribing this mechanism to the IR in human pregnancy was still a leap. To translate these findings to human pregnancy, we designed a longitudinal study in which we collected four vastus lateralis muscle biopsies from pregnant women who were overweight/obese with BMI-matched diet-controlled GDM mothers before and after a glucose load at ~30–32 weeks gestation and again at 9–10 weeks postpartum. Our intent was to also decipher the dynamic insulin signaling changes in GDM mothers who continued to display impaired glucose tolerance postpartum. Not surprisingly, recruitment for these studies, which involved fasting and insulin-stimulated muscle biopsies in

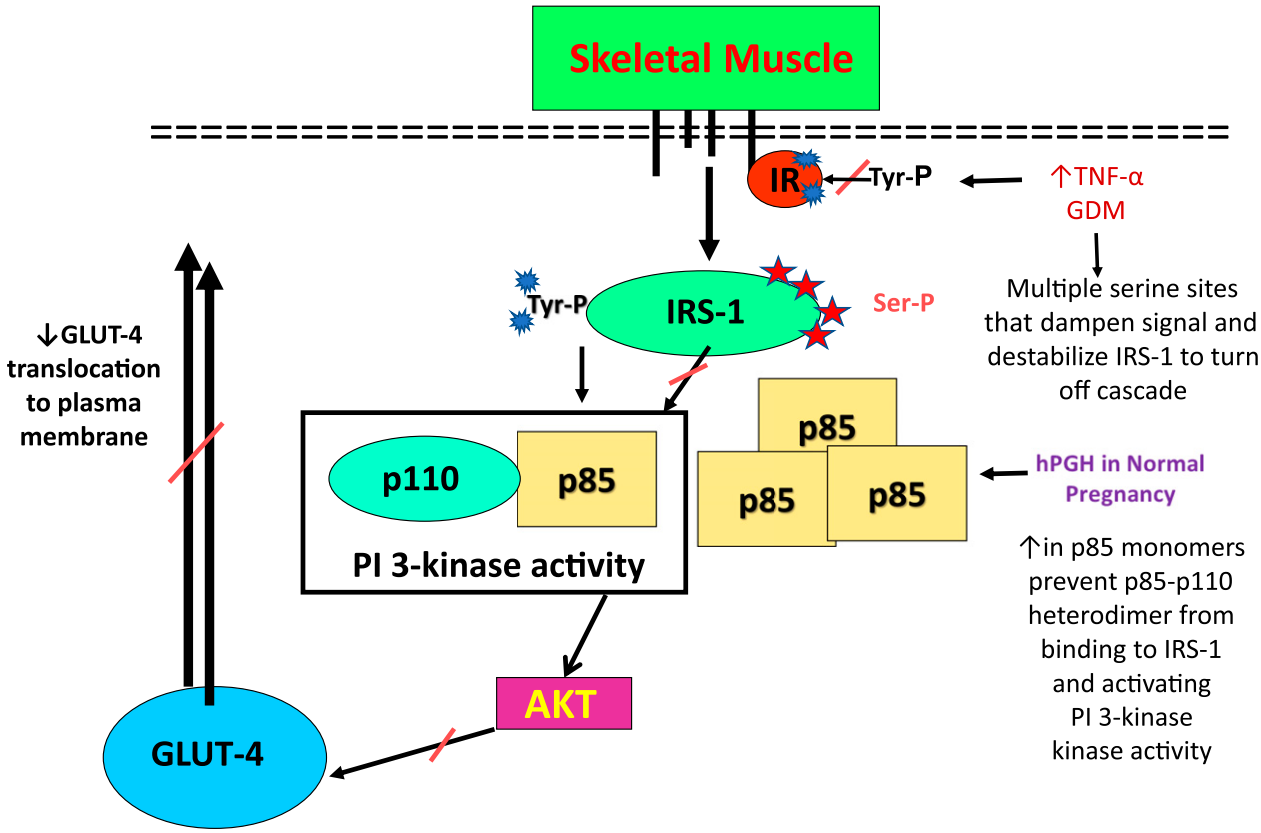

Figure 1—Simple schematic of the proximal insulin signaling pathway in skeletal muscle of normal pregnancy and GDM. Insulin stimulates tyrosine phosphorylation (P) of the insulin receptor (IR), defective in GDM, which activates the insulin receptor to dock IRS-1. After IRS-1 is phosphorylated on tyrosine domains, it triggers the recruitment of PI 3-kinase, a critical enzyme for glucose transport into the cell. GDM women also have decreased tyrosine activation of IRS-1 and reduced IRS-1, in part due to increased serine phosphorylation that increases the degradation of IRS-1 and dampens the insulin signal. The increase in basal IRS-1 serine phosphorylation does not resolve in GDM women who show persistent glucose intolerance postpartum and are at highest risk for type 2 diabetes. PI 3-kinase is composed of a regulatory subunit (p85α) and a catalytic subunit (p110) that must form a heterodimer for PI 3-kinase activation to occur. When excess p85α monomers are stimulated by hPGH in normal pregnancy, it competes with the p85-p110 heterodimer for binding to IRS-1, thereby causing a decrease in the IRS-1–associated PI 3-kinase activity and reduction in GLUT-4 translocation to the plasma membrane. Elevated p85 reverses postpartum with the disappearance of hPGH.

pregnant women both antepartum and postpartum, was painfully slow. However, after ~7 years, our prospective study demonstrated that reduced ability of insulin to phosphorylate the insulin receptor and increased levels of the p85 subunit of PI 3-kinase, similar to our mice, were reversible after delivery, as might be expected given the disappearance of hPGH after pregnancy. However, mothers with GDM also had a decrease in IRS-1 and changes in tyrosine and serine phosphorylation compared with BMI-matched control subjects (13), further dampening signaling. Importantly, some women with GDM had been shown to have a persistent insulin signaling defect with increased skeletal muscle tumor necrosis factor-α (TNF-α) up to 1 year postpartum (14). We also established that GDM women demonstrate impaired IRS-1 signaling–associated increased serine kinase activation, which persisted in women who did not normalize their glucose tolerance postpartum (15) (Fig. 1).

Although we hoped that the physiological changes in hPGH in pregnancy would be a straightforward predictor of the degree of IR in pregnancy, McIntyre et al. (16) found hPGH not to be a simple biomarker of the multiple biologic processes that contribute to the overall IR of pregnancy and excess fetal growth, which also include leptin, adiponectin, TNF-α, and IGFBP1. Catalano and colleagues (17,18) have also shown a reduction in adiponectin and PPARγ in adipose tissue that is also associated with tissue-specific IR. While reducing excessive skeletal muscle or adipose IR in pregnancies complicated by GDM might be a viable target for attenuating IR, the mechanisms driving these changes likely have important roles in normal pregnancy. We anticipate that the insulin signaling defects we observed to persist in skeletal muscle of GDM women, in addition to observations by Boyle et al. (19) that AMPK and oxidative phosphorylation are dramatically reduced in the skeletal muscle of GDM women, might lead to specific targeted therapy or physical activity interventions for prevention of GDM and the development of type 2 diabetes postpartum.

WHY DO WOMEN WITH OBESITY ALONE GIVE BIRTH TO BIG BABIES?

Obesity, which is often associated with IR, is now the leading cause of maternal morbidity and affects nearly 40% of women of child-bearing age (20). In fact, obesity alone accounts for a greater number of large-for-gestational age (LGA) infants than pregnancies complicated by preexisting diabetes or GDM

(6,21,22). Many studies have reported strong associations between intrauterine exposure to maternal obesity, excess adiposity at birth, and offspring development of obesity in childhood (2). Fasting, 1-h, and 2-h glucose on a 75-g oral glucose tolerance test, lower than previously considered as GDM, were correlated with LGA in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study (23) and were recently shown to correlate with glucose intolerance in the 10- to 14-year-old offspring (24). However, Catalano et al. (25) demonstrated in a cohort of 89 offspring from mothers who were normoglycemic or who had GDM that childhood adiposity at 9 years correlated much more strongly with maternal obesity than did GDM, with an OR of ~5.5. In addition to maternal prepregnancy BMI, the degree of gestational weight gain (GWG) also contributes to excess fetal growth, although overall less so than prepregnancy BMI in our review of the literature (26). The association with excess GWG and adiposity at birth appeared strongest in overweight mothers who gained excessive weight compared with mothers with obesity who gave birth to infants with the highest mean fat mass, regardless of GWG.

We hypothesized that given the strong contribution of maternal obesity alone to excess fetal growth independent of GDM (27), women with obesity may have higher fasting and postprandial glucoses than women who are normal weight (NW), although not high enough to meet criteria for GDM. In an extensive review done with Dr. Teri Hernandez of glycemia patterns in normal pregnancy (28), we determined that mean fasting glucose level in NW mothers was 71 mg/dL, 1-h postprandial value was 109 mg/dL, and the 2-h postprandial value was 99 mg/dL with a 24-h mean glucose of 88 mg/dL, all much lower than current therapeutic targets (gestational age 34 ± 2 weeks). In a prospective study, spearheaded by two endocrine fellows, in which we controlled maternal diet while wearing continuous glucose monitors (CGM), women with obesity were shown to have ~8% higher 24-h glycemic profiles compared with NW control subjects (29). We also identified daytime, nocturnal, fasting, and postprandial glycemic profiles that were useful in characterizing fetal growth (30). In a second highly controlled longitudinal study in which NW mothers and those with obesity received controlled eucaloric diets, we documented mean fasting blood glucose values of ~73 vs. ~77 mg/dL at 28 weeks and mean 2-h postprandial glucose values of 87 vs. 96 mg/dL in our NW and obese groups of women, respectively (31). Furthermore, the fasting and repeated measures of postprandial glucose over 4 h both early (~16 weeks) and later (~28 weeks) in pregnancy were also ~10% higher in the mothers with obesity. Women with obesity had larger increases in their 24-h glucose profiles and daytime, nocturnal, and postprandial glucose profiles from early to later pregnancy compared with NW mothers (all $P < 0.05$) when studied on a eucaloric controlled diet (32). In the women with obesity, the postprandial glucose exposure over three meals early in pregnancy was predictive of newborn percent fat mass (NB %fat), and later in pregnancy, the entire 24-h glucose profiles were predictive in women with obesity (all $P < 0.05$) but not in NW women (32). This subclinical pattern of mild hyperglycemia, which remains occult and untreated, may in part explain why the prevalence of LGA and macrosomia is higher in women with obesity (Fig. 2*A*). Interestingly, recent data by a postdoctoral fellow in our group demonstrated that women with obesity who have mild sleep disordered breathing (apnea-hypopnea index >5) have higher 24-h glucose profiles when on a fixed diet than women without obesity. This suggests that mild sleep apnea, common in women with obesity, may be one of the contributors to elevated 24-h glycemic profiles that could be targeted for treatment (33).

In addition to higher glucose levels, our data also showed that fasting and postprandial insulin concentrations, as well as IR estimated by HOMA-IR, are 50–60% higher in women with obesity both early (~16 weeks) and later in pregnancy (28 weeks) as compared with NW women receiving a fixed diet (31) (Fig. 2*B*). The higher degree of IR in women with obesity that predates the pregnancy, as shown by Catalano et al. (34) with hyperinsulinemic euglycemic clamp studies, is clearly present early and worsens later in pregnancy. This additional maternal IR, superimposed on the IR of normal pregnancy, may serve to increase the availability of all nutrients (including lipids and amino acids) to the fetal-placental unit, resulting in excess fetal growth in women with obesity (Fig. 3).

THE UNDERAPPRECIATED ROLE OF MATERNAL LIPIDS AS A CONTRIBUTOR TO INFANT ADIPOSITY

Although much of the focus on fetal growth has been through a glucocentric lens, there is increasing recognition that it is not only maternal glucose that contributes to fetal fat accretion and that triglycerides (TGs) and FFAs also play a significant role (35–39). Another unexpected twist in our earlier CGM data (29), during which women with obesity on a controlled diet demonstrated higher 24-h glycemic profiles, was that a single fasting TG measured once at ~14–16 weeks in pregnancy correlated more strongly with NB %fat by skinfolds than any of the glycemic profiles by CGM. The placenta has lipase activity, and the tireless efforts of another postdoctoral fellow demonstrated that the activity of placental lipoprotein lipase, important in hydrolyzing maternal TGs to FFAs that can be transported across the placenta, is highly correlated with NB %fat by skinfolds at birth ($r = 0.59$; $P = 0.006$) and even more so when corrected for gestational age at delivery ($r = 0.75$; $P = 0.0001$) (40).

Although a number of studies have shown that fasting TGs and FFAs contribute to fetal fat accretion, prospective studies in which maternal diet was controlled and which also examined postprandial FFAs and postprandial TGs were lacking (35). In our prospective study that characterized the maternal metabolic environment in mothers who were NW or obese both early (~16 weeks) and later in pregnancy (28 weeks), we demonstrated that maternal TGs both fasting and postprandially after a controlled diet were a stronger predictor of NB %fat than any of our glucose or insulin measures (31). Fasting TGs and postprandial TGs both at 1 h and 2 h were already ~30–40% higher in mothers with obesity early in pregnancy compared with NW and also 30–40% higher at 28 weeks (Fig. 2*C*). Interestingly, in NW women, it was the increase in TGs from early to later in pregnancy that correlated strongest with NB %fat ($r = 0.57$; $P < 0.01$) by dual-energy X-ray absorptiometry (DXA).

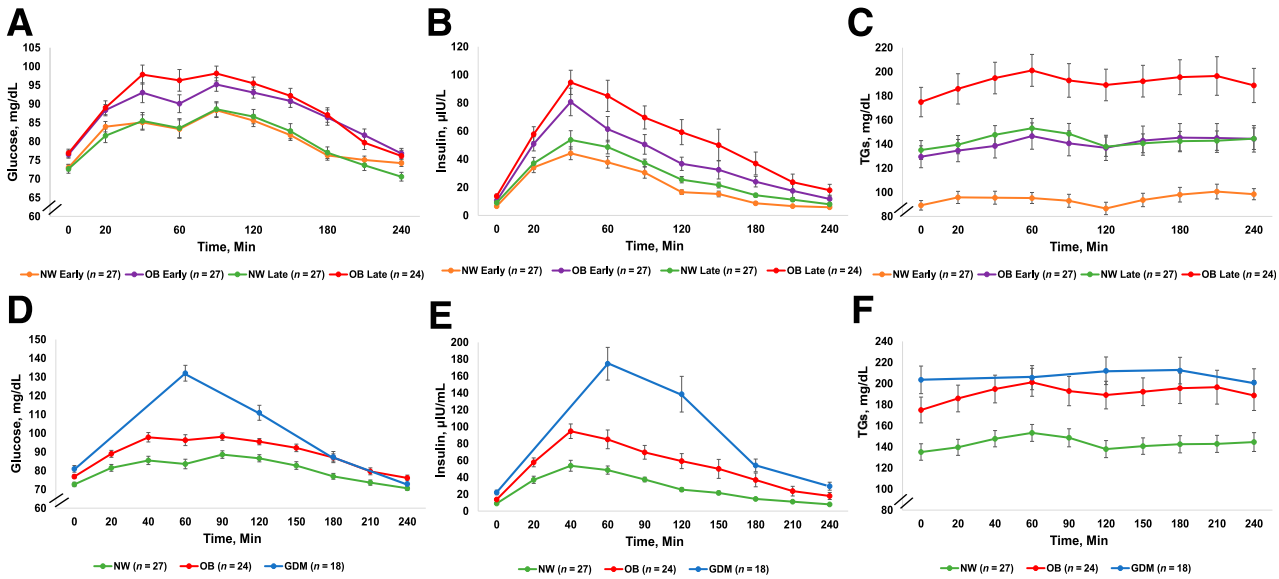

Figure 2—Data from controlled breakfast meal studies showing the 4-h area under the curve of plasma glucose (*A*), insulin (*B*), and TGs (*C*) in NW women vs. women with obesity (OB) early (12–14 weeks) and later (26–28 weeks) in pregnancy and the 4-h area under the curve of plasma glucose (*D*), insulin (*E*), and TGs (*F*) in NW, OB, and diet-controlled GDM at 28–30 weeks.

However, in mothers with obesity, it was 16-week fasting TGs ($r = 0.60$; $P = 0.006$) and 1-h or 2-h postprandial TGs ($r = 0.69$–0.71; $P < 0.01$) in early pregnancy that most strongly correlated with NB %fat, contributed by both VLDL-TG and chylomicron TG (CM-TG) (31). TGs are modestly higher in mothers with GDM compared with mothers with obesity (Fig. 2*E*), and these data, in addition to others, suggest that maternal TGs are a largely unrecognized substrate for fetal fat accretion, especially in mothers with obesity (31). A larger community-based trial in a free-living environment to confirm the contribution and better characterize the relationship of fasting and postprandial TGs to infant adiposity is the focus of a proposed future trial in women who are overweight or obese.

CAN WE INTERVENE WITH DIET? CHALLENGING THE CONVENTIONAL LOWER CARBOHYDRATE DIET IN GDM

Efforts at deciphering the optimal diet for pregnant women have been largely limited to women with GDM given that diet therapy is the first-line therapy and mainstay of treatment. Conventionally, the "GDM diet" was one that limited carbohydrates in an attempt to attenuate the postprandial glycemic response (41). However, many mothers substitute fat for carbohydrate (42,43), which may have unintentional consequences of increasing FFAs, which are a strong contributor to worsening maternal IR, promoting further shunting of all excess nutrients to the growing fetus (Fig. 3). Given the lack of rigorous data supporting the use of one macronutrient over the other, the American Diabetes Association abandoned specific diet recommendations for mothers with GDM in 2005 (44) other than ensuring that women receive, at the minimum, 175 g of carbohydrate/day in the second and third trimester. This amount is critical for meeting the energy demands of the placenta and fetus, of which 80% are dependent on glucose.

My research colleague, Dr. Teri Hernandez, as a former cardiac nurse, was extremely disconcerted about the potential for a low-carbohydrate, higher-fat diet to have unintended consequences and potentially worsen maternal IR, inflammation and actually promote excess fetal fat accretion. We reconstructed the highly quoted ~40% carbohydrate/45% fat/15% protein conventional diet described by Jovanovic-Peterson and Peterson (41) and developed a "challenge" diet precisely matched in calories and types of carbohydrates, fats, and proteins that only differed in the macronutrient percentages (45). This diet, later termed the CHOICE diet (Choosing Healthy Options in Carbohydrate Energy), substituted 20% of the calories from fat with higher-quality, more complex carbohydrates such that the macronutrient composition was instead 60% carbohydrate/25% fat/15% protein. Both diets were matched for types of fats and <18% of total calories from both diets were from simple sugars. Importantly, given that current literature lacks randomized controlled trials (RCTs) comparing diets in which all meals are provided, critical in minimizing overlap between the diets, our design provided all meals to the mothers at the time of GDM diagnosis through the remainder of pregnancy. Data from a randomized controlled crossover trial demonstrated that the higher–complex carbohydrate/lower-fat CHOICE diet achieved similar glycemic profiles using CGM (6% higher on CHOICE) and that the mean 1-h (115 mg/dL) and 2-h (106 mg/dL) postprandial glucoses were still well below the targets for GDM (45). The FFAs were significantly lower postprandially on the CHOICE diet. In a follow-up pilot study in which glucose, insulin, FFAs, adipose tissue lipolysis, and NB %fat were measured after the mothers had been randomized to one of the diets for 6–7 weeks, we found that the fasting blood glucose was actually lower on the CHOICE diet ($P = 0.03$). Furthermore, insulin suppression of lipolysis in maternal adipose tissue biopsies was better on the CHOICE diet, corresponding to lower expression of multiple adipose tissue

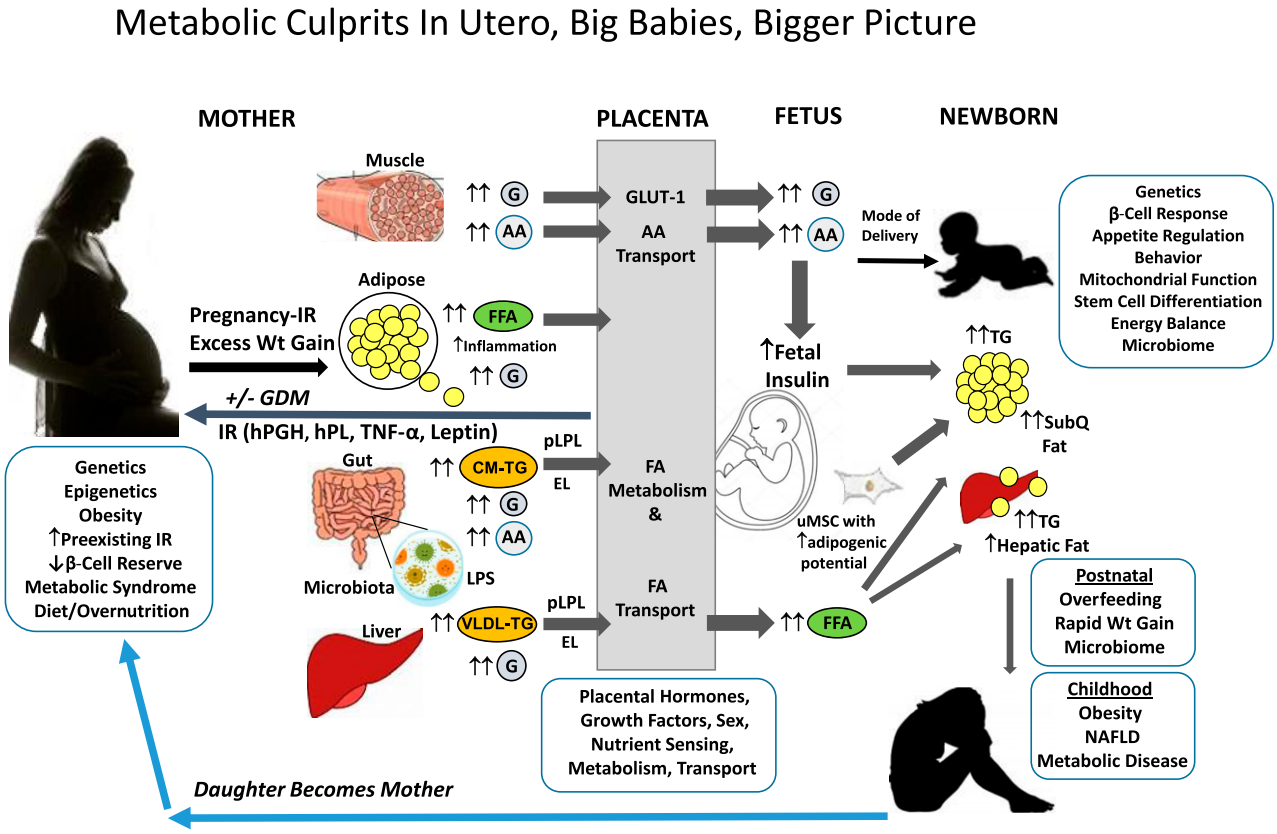

Figure 3—Diagrammatic portrayal of the effects of overnutrition on offspring in pregnancies complicated by obesity, metabolic syndrome, or GDM and the potential life-cycle consequences. A woman becomes pregnant with variable degrees of preexisting metabolic dysfunction; her metabolism is furthered altered by the IR of pregnancy mediated by placental factors. Maternal IR in muscle, adipose tissue, and liver along with dietary nutrient excess results in excess glucose (G), amino acid (AA), FFA, and inflammatory cytokine exposure to the placenta. Placental angiogenesis and the transcriptome are influenced by maternal factors and the sex of the fetus, which determine nutrient sensing, metabolism, transport, and ultimate nutrient exposure to the fetus. The fetus responds to excess glucose and AAs with fetal hyperinsulinemia, a potent growth factor, which also promotes organomegaly, adipogenesis, and lipid storage in subcutaneous (SubQ) and hepatic tissue. Intrauterine overnutrition affects stem cell differentiation, mitochondrial function, and appetite regulation in the offspring, and the maternal microbiome is transmitted at delivery. Postnatal factors such as mode of feeding and rapid weight (Wt) gain along with other early life exposures further promote the development of childhood metabolic disease. The daughter becomes a mother and the cycle perpetuates. EL, endothelial lipase; pLPL, placental lipoprotein lipase; uMSC, umbilical mesenchymal stem cell.

proinflammatory genes (46). Importantly, NB %fat trended lower on the CHOICE higher–complex carbohydrate/low-fat diet in a limited pilot sample of 12 mother/offspring dyads. The findings are consistent with other RCTs where meals were not provided but also support that diets lower in glycemic index, low in saturated fat, and higher in fiber appear to attenuate excess fetal growth (43,47). The final data from the CHOICE RCT powered for a diet difference in NB %fat as the primary outcome should be available within the next year.

THE SIGNIFICANCE AND CHALLENGE IN MEASURING BODY COMPOSITION IN BABIES AND INFANTS

Birth weight is an imperfect proxy for increased infant fat mass given that it includes both lean mass and fat mass. In the cohort of 89 offspring of mothers with normal glucose tolerance or GDM in the study by Catalano et al. (25), increased adiposity at birth (but not birth weight) was correlated to adiposity at 6 to 11 years of age by DXA. Adiposity at birth and during the first 2 years of life when infants triple their fat mass is an important predictor for future obesity risk and metabolic syndrome, much more than birth weight alone. DXA and air displacement plethysmography (PEAPOD) are considered methods with more precision and reproducibility than skinfold measures or bioelectrical impedance analysis and less expensive than MRI techniques (48,49). However, DXA requires infants to be still and exposes them to a very low background level of radiation—a misinterpretation (and unfortunate twist) that halted our recruitment for nearly 1 year. Regrettably, most infants can no longer fit into a PEAPOD by 6 months of life. Given the shortcomings of these different methods and the need to accurately measure fat and fat-free mass throughout infancy, we prospectively measured NB %fat in term infants using DXA, PEAPOD, and skinfolds at ~10–14 days of life (all three measures taken on same day) and again at ~1 year of life by DXA and skinfolds. We evaluated two different Hologic software versions for DXA along with PEAPOD and skinfolds and determined how well they correlated with each other and, more importantly, their degree of agreement (48).

Consistent with our track record of surprising twists, we found that although the Pearson correlations between the our original DXA software version (DXA1) with the newer software version (DXA2) in newborns were strong (r = 0.89), as with DXA1 and PEAPOD (r = 0.74)

and with DXA1 and skinfold estimates ($r = 0.69$), the actual agreement between DXA1 and the other methods was much less impressive (intraclass correlation coefficient [ICC] 0.53 with DXA2; 0.62 for PEAPOD and also 0.62 for skinfolds). By 1 year of age, although the correlation between the two DXA software versions was identical to that seen in the newborn ($r = 0.89$), the agreement was poor (ICC 0.20). The correlation between DXA1 and skinfolds at 1 year was poor ($r = 0.50$), and the agreement was even worse (ICC 0.11) (48). Unfortunately, these data demonstrate that while DXA, PEAPOD, and skinfolds are highly correlated at birth, the agreement is less impressive such that the methodologies cannot be substituted for each other when following offspring longitudinally. Further, at 1 year of life, both the correlation and the agreement with DXA and skinfolds are very poor, with DXA estimating NB %fat at about ~3 times higher than by skinfolds in our study. These data support that the same methodology should be used in longitudinal studies. This is problematic given that MRI is expensive and, like DXA, it is difficult to restrict infant and toddler movement, infants will outgrow the PEAPOD after 6 months, and toddler PEAPOD (TODPOD) measures are less available and challenging.

Another limitation of DXA, PEAPOD, bioelectrical impedance analysis, or skinfolds is that they do not measure liver or visceral fat, likely a more important estimate of metabolic risk (48). Only MRI spectroscopy is able to measure visceral or liver fat, which is expensive and requires that the infant or child lie still. A pediatric gastroenterologist colleague helped to design methodology, with the assistance of radiology experts, to estimate the liver fat in the newborn offspring from a cohort of GDM mothers who were obese compared with newborns from mothers who were NW. It is very concerning that newborns from GDM mothers with obesity already had a 68% increase in intrahepatocellular lipid at ~2 weeks of age (50), and recent pilot data support a strong relationship with fasting TGs and postprandial TGs at GDM diagnosis and newborn liver fat (51). Given the steadily increasing prevalence of childhood nonalcoholic fatty liver disease (NAFLD) that progresses more rapidly to nonalcoholic steatohepatitis (NASH) in childhood, these findings and others in nonhuman primates by McCurdy et al. (52) strongly suggest that an intrauterine environment characterized by excess maternal fuels from obesity, diabetes, or a high-fat diet may result in TG accumulation in the fetal liver. This may be especially important if the exposure is early in pregnancy, before subcutaneous fat stores are developed, and could potentially serve as a "first hit" toward the development of NAFLD (53), which may affect up to 40% of children with obesity.

TARGETING WHO, WHAT, AND WHEN TO PREVENT ADVERSE PREGNANCY OUTCOMES AND METABOLIC DISEASE IN MOTHERS AND OFFSPRING

We and others have demonstrated that the metabolic heterogeneity in human pregnancy, simply based on maternal BMI or the presence or absence of a GDM diagnosis (also influenced by various criteria), is enormous (54). Not all women with normal BMI are insulin sensitive, not all women with a BMI in the obese range are IR (6), and women with "GDM" have highly variable degrees of skeletal muscle, adipose, and hepatic IR predating the pregnancy in addition to the normal IR of pregnancy (4). Further, GDM women have differing degrees of β-cell defects that also predate the pregnancy and some can mount markedly high insulin responses and others cannot (5,6,54) (Fig. 1). Lipid metabolism in pregnancy is also highly variable, partly related to the estrogen-induced influence of VLDL production in pregnancy and CM-TG in the diet, superimposed on the degree of maternal adipose tissue IR (31,39). The degree to which TGs and FFAs may contribute to fetal fat accretion has yet to be fully elucidated, but observations that maternal glucose does not solely explain fetal overgrowth support its future investigation as a therapeutic target.

New technologies and advancements have informed us how maternal diet, maternal body composition, and the maternal response to placental hormones and growth factors, in the context of the mother's own metagenomics and metagenomics of her microbiome, can influence her IR and inflammatory state, β-cell responses, and the levels of glucose, amino acids, and lipids she exposes to the fetal-placental unit (55) (Fig. 3). The placenta is the ultimate gatekeeper to what crosses into the fetal circulation and is affected by placental angiogenesis, its own transcriptome that will influence placental hormone and growth factor production, metabolism, nutrient sensing and transporters, as well as the sex of the placenta (56–58) (Fig. 3). Clearly the timing of these exposures to the fetal-placental unit, very early or later in pregnancy, has immense fetal programming implications (7,59,60). Indeed, most lifestyle interventions to improve pregnancy outcomes at midpregnancy have been largely disappointing (59). How the fetus responds to excess nutrient exposure and growth factors as a result of timing in pregnancy and its own metagenomics will affect its β-cell response, immune and metabolic responses, and fetal fat accretion in subcutaneous versus visceral stores, as well as stem cell differentiation into adipocytes or myocytes (Fig. 3). Notably, Boyle et al. (61) have recently shown that the adipogenic potential of offspring umbilical mesenchymal stem cells is higher in mothers with obesity. Furthermore, Baker et al. (62) demonstrated that metabolic changes in these adipocytes were predictive of adiposity gain in the first 5 months of life. As described cogently by my colleague Dr. Jed Friedman (1), the peripartum and postnatal environment will continue to have a critical effect on childhood developmental programming, partially driven by genetic and epigenetic factors that are influenced by mode of delivery, the transmission of the maternal microbiome (63), feeding practices, antibiotic use, and other environmental exposures and metabolic disruptors (64), the subject of intense investigation in the National Institutes of Health (NIH)-funded Environmental influences on Child Outcomes (ECHO) program (65). Dr. Freinkel (3) set the stage for us to consider far more than simply maternal glucose as the determinant of maternal outcomes, fetal growth, and future maternal and offspring metabolic risk. Technologic advancements in metagenomics, proteomics, metabolomics, and the microbiome have brought previously unimaginable new lenses to our understanding and have humbled what we thought we understood.

Our current ability to identify pregnancies "at risk" for maternal and offspring short- and long-term adverse

outcomes by using only maternal BMI, GWG, or differing diagnostic criteria to draw the line between GDM and no GDM is, unarguably, limited. "Risk engines" (60) that better identify high-risk maternal genetics, new maternal metabolic biomarkers, and that utilize more accurate indicators of diet and lifestyle practices may dramatically alter our definition of at-risk mothers. Personalized medicine may be critical in targeting interventions that are much more likely to be successful during prepregnancy or early gestation that implement personalized nutrition (likely influenced by the microbiome), supplements or prebiotics, and activity prescriptions—all based on nutrigenomics and metabolomics. When it is necessary to prescribe medications, careful consideration of their pharmacokinetic properties (which may differ by the mother's pharmacogenomics), how best to individualize their use to target specific metabolic abnormalities, and evaluation of their potential long-term programming effects for those that cross the placenta argue against a one-size-fits-all interventional approach (66). One thing is clear: our current definition of at-risk women simply based on maternal glucose requires modification, our targeted interventions need to be individualized and occur early, and our efforts cannot be halted at the time of delivery. The challenges are formidable, but the potential for meaningfully impactful consequences on maternal and offspring metabolic health call out loudly to future investigators with new toolboxes and perspectives.

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