Invited Review Article-

Maternal Obesity and Fetal Metabolic Programming: A Fertile Epigenetic Soil

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Abstract:
The incidence of obesity and overweight has reached epidemic levels in the United States and developed countries worldwide. Even more alarming is the increasing prevalence of metabolic diseases in younger children and adolescents. Infants born to obese, overweight and diabetic mothers (even when normal weight) have increased adiposity and are at increased risk of later metabolic disease. In addition to maternal glucose, hyperlipidemia and inflammation may contribute to the childhood obesity epidemic through fetal metabolic programming, the mechanisms of which are not well understood. Pre-gravid obesity, when combined with normal changes in maternal metabolism, may magnify increases in inflammation and blood lipids, which can have profound effects on the developing embryo and the fetus in utero. Fetal exposure to excess blood lipids, particularly saturated fatty acids, can activate pro-inflammatory pathways, which could impact substrate metabolism, mitochondrial function, as well as stem cell fate, all of which affects organ development and the response to the post-natal environment. Fetal and neonatal life is characterized by tremendous plasticity, and the ability to respond to environmental factors (nutrients, oxygen, hormones) by altering gene expression levels via epigenetic modifications. Given that lipids act as both transcriptional activators and signaling molecules, excess fetal lipid exposure may regulate genes involved in lipid sensing and metabolism through epigenetic mechanisms. Epigenetic regulation of gene expression is characterized by covalent modifications to DNA and chromatin that alter gene expression independent of gene sequence. Epigenetic modifications can be maintained through positive and negative feedback loops, thereby creating stable changes in the expression of metabolic genes and their main transcriptional regulators. The purpose of this article is to review current literature on maternal-fetal lipid metabolism and maternal obesity outcomes, and to suggest some potential mechanisms for fetal metabolic programming in key organ systems that regulate post-natal energy balance, with an emphasis on epigenetics and the intrauterine environment.
Introduction

The emergence of adult metabolic disease ‘epidemics’ in children is an advancing public health concern, with childhood obesity, diabetes, cardiovascular disease (CVD) and nonalcoholic fatty liver disease (NAFLD) all increasing at alarming rates. While this is partially due to consumption of calorie-dense, nutrient-low foods (82) and sedentary behaviors (20; 21), an emerging body of evidence also suggests that the ability to respond to metabolic challenges during postnatal life may be linked to environmental influences during fetal development (14; 30; 32; 91; 93; 98; 144). The Developmental Origins of Disease Hypothesis originally posited by Barker et. al. (7; 8) has led to extensive research in the effects of fetal under-nutrition, low birth weight and development of chronic metabolic disease in the offspring. However, less is known about the metabolic impact of fetal over-nutrition, elevated birth weight, and excess adiposity in newborns. Epidemiological studies have revealed strong statistical links between nutritional excess during pregnancy and later development of diseases such as obesity and Type 2 diabetes in adulthood. Most convincing are the studies in Pima Indians showing that, besides a genetic transmission of diabetes, exposure to the diabetic intrauterine milieu during pregnancy can also induce a 10-fold increase in the prevalence of diabetes by early adulthood, in comparison to offspring whose mothers did not develop diabetes until after delivery (43). While maternal hyperglycemia contributes to increased fetal growth and the development of metabolic disorders in offspring (111), recent work suggests that maternal pregravid weight and triglyceride (TG) levels may be a better correlate of excessive fetal growth (46; 59; 84; 85; 131), and in particular, the development of the metabolic syndrome at age six years of age (12). These findings imply that maternal hyperlipidemia, inflammation, or other metabolic and dietary factors traditionally associated with obesity and the metabolic syndrome may be contributing to the childhood obesity epidemic and its associated metabolic disorders. The mechanisms whereby maternal obesity and nutrient excess in utero impart increased risk for future metabolic disease are poorly understood, but likely include changes in fetal nutrient supply in combination with genetic, and epigenetic mechanisms. The in utero environment can substantially modify how the fetal genome is expressed, which can exert stimulatory or inhibitory effects on fetal growth and adiposity. Recent work (63; 81) has shown that DNA methylation, histone modifications, and other epigenetic changes play crucial roles in many biological processes related to
intrauterine development, such as gene expression, chromatin accessibility, DNA replication, imprinting, and human disease patterns. The functional significance of these epigenetic marks and their dynamic and complex interactions regulating gene expression are just beginning to be explored. The purpose of this article is to review current human and animal literature regarding maternal obesity and potential mechanisms for fetal metabolic programming, with an emphasis on maternal-fetal lipid metabolism, epigenetics, and the role of the intrauterine environment.

I. Obesity, Inflammation, and Insulin Resistance in Non-Pregnant Individuals: Fat on Fire

Obesity and pregnancy are independently associated with insulin resistance and inflammatory changes that may be exacerbated when combined with one another. In the non-pregnant state, hypertrophic adipose tissue stores result in reduced uptake and storage of fatty acids along with increased lipolysis, inflammatory cell infiltration and adipokine secretion (reviewed in 71; 131). Markers of inflammation have been observed in both adipose tissue and liver of obese individuals and rodents, including tumor necrosis factor-α (TNF-α), chemokine receptor-2 (CCR2), monocyte chemotactant protein-1 (MCP1), toll-like receptor-4 (TLR-4), and c-Jun N-Terminal Kinase (JNK). More than just a passive storage depot, adipocytes can synthesize, store, and secrete multiple pro-inflammatory cytokines, including interleukin-6 and 8 (IL-6 and IL-8), TNF-α, and MCP-1 (44; 73; 139; 166), many of which play an important role in obesity-induced insulin resistance (reviewed in 74; 135). However, the initiating steps in inflammation, and the mechanisms linking it to insulin resistance are still being investigated.

In both humans and animal models of obesity, adipocyte expansion and hypertrophy is associated with an accumulation of adipose tissue macrophages with a pro-inflammatory phenotype. In the obese state, macrophages appear to become polarized towards a more M1 phenotype, whereas in lean animals the macrophage population expresses greater amounts of M2 markers (58). M1 macrophages are traditionally viewed as being more pro-inflammatory and are important for mounting an immune response, whereas M2
macrophages, or “alternatively activated” macrophages, are important for tissue repair, remodeling and the resolution of inflammation. In inflamed, insulin-resistant adipose tissue, the anti-lipolytic effects of insulin are frequently diminished (35; 147), resulting in elevated levels of free fatty acids (FFAs). This has given rise to the "expandability hypothesis," which postulates that limitations in adipose tissue expandability may govern when lipids are stored in adipose tissue vs. other tissues in the body (155). Inefficient storage of lipids in adipose tissue can suppress insulin sensitivity both locally and systemically. Locally, FFAs released by the adipocyte can produce a strong pro-inflammatory signal by binding Toll-like Receptors (TLRs) expressed on the cell surface of resident macrophages. TLR signaling induces Nuclear Factor kappaB (NF-κB) (95; 100; 117; 140), a pro-inflammatory transcription factor, leading to perpetual adipose tissue inflammation and increased insulin resistance. Pro-inflammatory cytokines and FFAs released systemically can impact other important metabolic tissues, impairing whole-body insulin sensitivity, and promoting disease progression (49).

Preventing the initial recruitment and activation of adipose tissue macrophages appears to be an important step in reducing the downstream consequences of obesity. Targeted deletion of specific inflammatory genes in bone marrow-derived macrophages disrupts the link between dietary/genetic obesity and insulin resistance (129; 149). It has been hypothesized that adipose tissue hypoxia and adipocyte cell death (3; 38) may play important roles in initiating macrophage recruitment; however the exact stimulus has yet to be clarified. There is good evidence that weight loss (27; 40), diet and exercise (23) and treatment with insulin sensitizing drugs (161) can reduce macrophage infiltration of adipose tissue and decrease expression of inflammatory markers. This leads to an overall improvement in whole-body insulin sensitivity due to the reduction of pro-inflammatory cytokines and FFAs released systemically. This may be an important therapeutic target to reduce the negative impact of obesity on maternal metabolism, and ultimately control the type and level of nutrients available for fetal growth and overall development.

II. Maternal Obesity, Fuel Switching, and its Consequences for Fetal Nutrient Supply
Two-thirds of women in the United States are currently overweight or obese at the time of conception (28; 87; 163). Not surprisingly, the increasing prevalence of obesity in pregnant women has led to the suggestion that maternal obesity alone may be a more significant factor than maternal diabetes in perpetuating the overall obesity epidemic (79). Infants born to obese and/or diabetic mothers are often large for gestational age (LGA, defined as ≥90th percentile for gestational age), demonstrate increased adiposity at birth, and are at increased risk for developing obesity and metabolic syndrome in later life (14; 30; 45). While gestational diabetes (GDM) is a known risk factor for LGA and macrosomic (>4000 grams) births, the majority of LGA infants are born to mothers with normal glucose levels. In fact, maternal hyperglycemia only accounts for 25% of the differences in birth weight in multivariate models (97; 120), which suggests that factors other than maternal-fetal glucose may be important. Recent work has demonstrated that maternal pre-pregnancy BMI and TG levels also play a significant role in mediating excessive fetal growth (46; 59; 84; 85; 89; 131; 132). In a prospective study of offspring born to women with either normal glucose tolerance or GDM, maternal BMI was the strongest perinatal predictor for both overweight at 8 years of age and percentage body fat (30). Additionally, in multiple cohorts of GDM women with well controlled glucose levels, elevated maternal fasting serum TG and FFAs were independently associated with increased birth weight and neonatal adiposity (46; 131).

During pregnancy, maternal metabolism undergoes profound adjustments to meet the nutrient needs of the developing fetus. Early in gestation (the first and early second trimester), maternal insulin sensitivity can actually increase modestly (94), leading to increased maternal adipose tissue lipid storage. During this time period, pregnant women are in an anabolic state and accumulate fat as a result of enhanced lipogenesis and increased adipose tissue lipoprotein lipase (LPL) activity, which hydrolyzes circulating TG for tissue uptake and storage (25; 94), resulting in a 3.5-6.0 kilogram increase in fat stores (71). Lean women increase their fat stores more than obese women per kilogram body weight, which is likely due to higher insulin sensitivity in early pregnancy, which promotes lipogenesis and storage (25; 51).
From mid to late gestation, maternal lipid metabolism switches from an anabolic to a catabolic state concomitant with increasing maternal insulin resistance (31; 69). TG stored in adipocyte lipid droplets are hydrolyzed into FFAs through lipolysis (42), which is initiated by hormone sensitive lipase and inhibited by insulin. The insulin resistant state of the third trimester is reflected by a decrease in adipose tissue LPL activity and accelerated lipolysis, leading to high levels of circulating FFAs and glycerol, and a marked increase in hepatic very-low-density lipoprotein-triglyceride (VLDL-TG) synthesis, which is further stimulated by the high estrogen levels of pregnancy (125). The signals responsible for this metabolic switch from lipid storage in early pregnancy to lipid mobilization in late gestation are not well understood; however placental hormones that increase with advancing gestation and are known to induce maternal insulin resistance, may play a major role.

Lipid metabolism differs greatly between lean and obese pregnant women. In lean women, prospective longitudinal studies using hyperinsulinemic-euglycemic clamps and indirect calorimetry demonstrate net lipogenesis pregravid and in early pregnancy (12-14 weeks), but net lipolysis in late gestation (34-36 weeks). In contrast, in obese women under similar experimental conditions, lipogenesis occurs pregravid but less so in early pregnancy compared to lean women, with an earlier shift from the anabolic to catabolic state and a predominance of lipolysis (29). Additionally, a state of inflammation and hyperlipidemia may be present prior to pregnancy. Thus, hormones that have been shown in both human and animal models to promote the insulin resistance of pregnancy, such as placental growth hormone, human placental lactogen, leptin and TNF-α (4-6; 56; 109), may also exacerbate the low-grade inflammation and insulin resistance of obesity (70), leading to greater mobilization of maternal fuel stores earlier in gestation.

Increased lipolysis in late pregnancy was traditionally thought to supply glycerol for maternal hepatic gluconeogenesis and FFAs for skeletal muscle beta-oxidation, allowing glucose and amino acids to be preferentially directed toward the fetal-placental unit. However, recent data suggests that fetal-placental glucose and amino acid utilization rates are highest at 22-26 weeks and decrease near term in contrast to lipid transport which is maximal in the third trimester, coincident with rapid fetal fat accretion (67). Humans are born
with the highest percent fat of any species (12-15%) and 90% of fat deposition occurs in the last 10 weeks of pregnancy, exponentially increasing to 7 grams per day near term (66; 67; 71; 136). Although fatty acids are not readily oxidized in the fetus, essential fatty acids are critical for normal development and the deposition of large amounts of body fat (50). The human placenta is capable of transporting FFA by diffusion, and selectively increases the transport of essential fatty acids (EFA) and their long-chain polyunsaturated fatty acid (LC-PUFA) derivatives by fatty acid carrier proteins (FACPs), thereby creating a higher concentration in the fetus than in the mother (65; 92). Further, placental expression of lipoprotein receptors and receptor-related proteins allows maternal lipoproteins such as VLDL and dietary chylomicrons (CM) to be taken up by the placenta, where they must be hydrolyzed by placental lipoprotein lipase (pLPL), or a second lesser-known placental TG hydrolase (50; 71). Additionally, the placenta expresses phospholipase A2 (PLA2), and other intracellular lipase activities to hydrolyze mono-, di-, and triacylglycerols to FFAs that can be utilized by the placenta or enter into fetal circulation. The activity of placental lipases, especially pLPL, increases from the first to the third trimester (40), again supporting an enhanced fetal need for maternal FFA in late gestation.

It has been shown that high levels of TG in maternal circulation may create a steep concentration gradient across the placenta, which accelerates their transport and deposition in fetal tissues (133). Therefore, the hypertriglyceridemia facilitated by the insulin resistance of obese and diabetic mothers are potential factors to enhance substrate availability to the fetus. Interestingly, in women with well controlled GDM, third trimester FFAs and TG were better predictors of neonatal fat mass than glucose (131). This corresponds with recent data, which demonstrates that 67% of the changes in placental gene expression in women with Gestational or Type I Diabetes relates to lipid transport pathways and only 9% to glucose transport pathways (124). In term human trophoblasts, insulin and fatty acids have been shown to enhance the expression of adipophiliolin, which is associated with cellular lipid droplets and implicated in cellular fatty acid uptake and storage of neutral lipids (53). Additionally, higher circulating levels of insulin may serve to up-regulate placental proteins involved in
lipid transport, thereby increasing availability of fatty acids to both the placenta and the fetus. Consistent with this concept, placental LPL activity has been shown to be stimulated by hyperinsulinemic and hyperglycemic conditions, thus increasing hydrolysis of maternal lipoproteins for transport across the placenta (105).

Interestingly, maternal obesity appears to affect the placenta much as it does adipose tissue, in that placentas from obese mothers have been shown to have increased expression of pro-inflammatory cytokines and a marked accumulation of a heterogeneous macrophage population (33). Additionally, placentas from obese (body fat >16%) compared to lean (body fat <8%) neonates demonstrated a significant increase in expression of the PLA2 genes PLA2G2A and PLA2G5 (the main placenta phospholipases) as well as leptin and TNFα (154). How this might impact placental function is not well established, however activation of phospholipase activity suggests inflammation may be one mechanism by which excess fat accumulates in obese neonates. Further, IL-6 and TNFα are thought to be involved in regulation of fetal growth via modulation of expression and activity of the system A but not L amino acid transporter (83).

In conclusion, fetal lipid supply is regulated by maternal circulating concentrations and by the extent to which they are transported by the placenta. Clearly, maternal obesity can directly impact fetal lipid exposure. However, the mechanisms by which this can alter fetal and later offspring development are still unknown. The ability of lipids to activate cell signaling pathways and serve as ligands for nuclear receptors suggests that aberrant lipid exposure in utero could potentially lead to the alternative regulation of multiple gene expression programs.

III. Epigenetics and Programming of Fetal Metabolism

The emerging field of epigenetics is recognized to have an important but still poorly defined role in fetal metabolic programming. While epigenetic mechanisms have been explored in the context of embryonic development and cancer biology, little has been done to explore their contribution to metabolic diseases. It is well established that poor maternal health affects fetal gene expression, however the precise mechanism by
which differences in nutrient exposure can alter epigenetic programs is not known. Epigenetic regulation of
gene expression is characterized by covalent modifications to DNA and chromatin that alter gene expression
independent of gene sequence. Epigenetic modifications lead to long-standing changes in gene expression
through the complex coordination of multiple binding proteins and enzymes that interact with each other
through positive and negative feedback loops, eventually resulting in the stable alteration of chromatin
structure (reviewed in 74). Changes in epigenetic marks are associated with multiple human diseases,
including many cancers, neurological disorders, and even inflammation (reviewed in 134; 148; 110,
respectively). Given the important role of epigenetic programming during embryonic development and
organogenesis, and the highly plastic nature of such processes, it follows that alterations to the in utero
environment could have powerful epigenetic consequences. Epigenetic alterations typically involve DNA
methylation and post-translational histone modifications. Additionally, Micro-RNAs (miRNAs) are emerging as
a potential third epigenetic mechanism. While these non-coding RNAs are traditionally associated with
regulation of gene expression at the translational level, recent work suggests they may be involved in
regulating DNA methylation, thereby regulating further transcription of their targets (10; 86).

DNA methylation patterns are largely established during embryogenesis and early postnatal life, and are
important for promoting the silencing of specific gene regions, such as imprinted genes and repetitive nucleic
acid sequences. The DNA of the early embryo is hypomethylated, and later organogenesis and tissue
differentiation is traditionally associated with progressive increases in DNA methylation in response to
environmental signals (reviewed in 11). DNA methylation typically occurs on Cytosine bases that are followed
by a Guanine, termed CpG dinucleotides. The covalent attachment of a methyl group by a DNA methyl-
transferase (DNMT) leads to recruitment of methyl-CpG binding proteins, which induce transcriptional silencing
both by blocking transcription factor binding, and by recruiting transcriptional co-repressors or histone-
modifying complexes, thereby promoting the formation of heterochromatin (reviewed in 80; 127). Abberant
DNA methylation in traditionally hypomethylated CpG-rich regions of gene promoters, termed CpG islands
(CGIs, reviewed in 78), has been associated with inappropriate gene silencing, and is known to occur in many
cancers (reviewed in 9). However, a subset of CGIs have been shown to be alternatively methylated in healthy
cells during normal tissue differentiation (62; 77). Previous studies have demonstrated that prenatal conditions
such as growth and nutrient restriction can epigenetically modify gene expression by altering the methylation
level of DNA in gene promoter regions (24; 102; 146; 151 and reviewed in 37). Importantly, this demonstrates
that mechanisms are present in utero to respond to nutritional, hormonal or other metabolic cues by altering
the timing and direction of methylation events during fetal development. Less is known regarding alternative
DNA methylation in the case of fetal over-nutrition; however recent work in a mouse model suggests a role for
both DNA methylation and microRNA regulation of MeCP2 in the alternative expression of IGF-2 in fetal livers
from high-fat fed dams (170).

Differential packaging of chromatin into open (euchromatic) or closed (heterochromatic) states is another
important mechanism of gene expression and silencing (respectively). Chromatin consists of DNA packaged
around histones into a nucleo-protein complex. Post-translational modification of histone tail residues –
including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation, can change the way
histones interact with the DNA, as well as recruit other proteins responsible for altering chromatin conformation
(reviewed in 26; 61). For example, histone tail acetylation by histone acetyl-transferases (HATs) promotes an
open-chromatin conformation, and is associated with regions of active gene expression, while histone tail de-
acetylation by histone de-acetylases (HDACs) promotes a closed-chromatin conformation and is associated
with gene silencing. Histone modifications and DNA methylation patterns are not mutually independent, and it
is believed that both can play roles in regulating the other’s state (36). Indeed, epigenetic regulation of
pancreatic PDX1 in a rodent model of growth-restricted pregnancy, was the result of coordinated histone
deaetylation in utero, and later methylation of the PDX1 promoter region in adulthood (122). Growth restriction
has been shown to alter histone marks of metabolism-related genes in the offspring, including IGF-1 (57) and
Glut-4 (101). Additionally, in a primate model of maternal high-fat diet, fetal livers demonstrated significantly
increased site-specific histone acetylation, which corresponded with gene expression changes (2), suggesting
an important role for histone modifications in addition to DNA methylation in fetal epigenetic programming.
The key challenge in fetal programming research is not only the identification of these epigenetic modifications, but also identification and characterization of specific combinatorial gene expression patterns. Studies in this area are inherently difficult to perform in human subjects. Therefore, the majority of current research has focused on animal models. While inter-species differences, particularly during the establishment of pregnancy and fetal development, have their inherent caveats, the ability to control maternal diet and examine comprehensive outcomes at different developmental stages may further our understanding of the impact of maternal obesity on fetal and offspring development.

**IV. Potential Mechanisms for Fetal Metabolic Programming by Maternal Lipids.**

Previous studies of maternal obesity and high-fat diet in animal models provide evidence of multiple metabolic abnormalities in the fetus, neonate, and adult offspring (52; 64; 108; 137; 158). These include increased adult body weight and fat mass, reduced insulin sensitivity, increased blood glucose and cholesterol levels, increased blood pressure, reduced muscle mass, and increased lipid deposition in the fetal and adult liver. These results are supportive of an early metabolic or a potential epigenetic programming event, but lack a direct gene regulatory pathway. *In utero* exposure to excess maternal lipids could impact a number of pathways in developing organs such as the liver, which is the first to see the majority of post-placental nutrients, as well as other key metabolic organs such as the skeletal muscle, adipose tissue, brain, and pancreas. Lipids and their pro- or anti-inflammatory derivatives can serve as transcriptional activators of multiple nuclear receptors, including the Liver X Receptor (LXR) and Peroxisome Proliferator-Acitvated Receptor (PPAR) families. Interestingly, the main genetic regulators of lipid metabolism are themselves regulated by lipid exposure, as well as by inflammatory cues (39). In the case of LXR, maternal intake of an LXR agonist led to fetal hepatic LXR activation, resulting in increased fetal liver lipogenesis (108; 152; 153). *In utero* exposure to excess maternal lipids could impact a number of gene pathways of metabolic importance,
including those for energy storage, oxidation, growth, death, differentiation, and inflammation. A number of these pathways will be reviewed below.

In rodents, maternal high-fat diet results in persistent lipid accumulation in adult offspring livers, even in the absence of post-weaning high-fat diet exposure (19, 38), suggesting a more permanent programming effect by maternal diet. Development of NAFLD in humans is associated with increased expression of genes associated with \textit{de novo} lipogenesis such as SREBP1c, ACC, FAS, SCD1, and LXR\textsubscript{\(\alpha\)} (47; 160), as well as a decrease in expression of genes associated with hepatic fatty acid oxidation such as PPAR\textsubscript{\(\alpha\}}, CPT-1, and mitochondrial matrix proteins (160). In a rodent model, offspring from high-fat fed dams demonstrated both impaired hepatic mitochondrial metabolism and enhanced lipogenic gene expression concomitant with the development of NAFLD (22). Interestingly, in a mouse model of nutrient restriction, hepatic genes involved in lipid metabolism were again found to be increased in newborn offspring (115), suggesting that early regulatory events \textit{in utero} are particularly sensitive to nutrient availability. Lastly, both fetal nutrient restriction and nutrient excess have been shown to increase hepatic gluconeogenic pathways, mainly through increased gene expression of PEPCK and Glucose-6-Phosphatase gene expression (104; 108; 118). The offspring from both groups demonstrate increased fasting glucose levels (64; 108; 137; 158); however, frequently there is a pancreatic beta cell defect in insulin secretion that reinforces excess hepatic glucose output.

In looking for early origins of insulin resistance, there are a number of potential pathways that dampen the insulin signaling cascade, attenuating insulin action in liver, skeletal muscle, and adipose tissue. In the canonical pathway, insulin binding stimulates auto-phosphorylation of the insulin receptor, allowing insulin receptor substrates (IRS-1, IRS-2) to dock (reviewed in 41; 143). Docking and subsequent IRS-1/IRS-2 tyrosine phosphorylation is necessary for stimulating glucose uptake in muscle and adipose tissue, and suppression of hepatic glucose output in liver. Increased IRS-1 serine phosphorylation is one of the primary mechanisms associated with inhibition of insulin signaling. Circulating FFAs – particularly saturated fats,
intracellular fatty acid intermediates (diacylglycerol, acyl COAs, or ceramides) and pro-inflammatory cytokines can all activate serine kinases known to target IRS-1. These include (but are not limited to) JNK, p38, ERK, aPKCs, p70S6K, IKKβ and most recently MAP4K4 (16; 18; 48). Studies in sheep and primate models of maternal over-nutrition have found increased expression of inflammation-related genes and activation of pJNK and p38 in fetal muscle and liver (162; 103). In humans, umbilical cord blood samples obtained from obese mothers showed increased HOMA-IR (an index of fetal insulin resistance), which was associated with increased fetal adiposity and leptin levels relative to lean control mothers (32). Together, these data suggest a potentially important role for inflammation in the early origins of insulin resistance, but the molecular basis for this has yet to be defined.

In addition to insulin resistance, increased lipid deposition in muscle and liver can lead to mitochondrial dysfunction and an impaired ability to oxidize fatty acids due to an increase in reactive oxygen species (ROS) production and consequent oxidative stress (15; 164). Indeed, skeletal muscle from obese patients shows increased expression and activity of the lipogenic enzyme SCD1, concomitant with a partitioning of fatty acids towards esterification and storage rather than oxidation (75). Additionally, gene profiling of skeletal muscle from both diabetic and lean first-degree relatives of diabetic patients shows a decreased expression of genes associated with fatty acid beta-oxidation, including the PPARα co-activator PGC1α, and mitochondrial genes involved in oxidative phosphorylation (113; 123). Whether early exposure to excess lipids results in an accumulation of fatty acid intermediates, reduced fatty acid oxidation, and/or mitochondrial inflexibility has yet to be investigated in fetal tissues. Currently, very little is known about the control of fatty acid oxidation in fetal mitochondria. There is some evidence that mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and may contribute to the natural history of NAFLD in an obese rodent model (126). Moreover, a recent report in mice demonstrated that maternal diet-Induced obesity increases mitochondrial ROS and oxidative stress in both mouse oocytes and zygotes (76). This study suggests that maternal obesity can potentially increase oocyte and zygote mitochondrial potential, DNA content and biogenesis. Consequently,
generation of reactive oxygen species (ROS) was raised while glutathione was depleted, and thus the redox state became more oxidized, suggestive of oxidative stress. Further, reports have shown that periconceptual exposure to high energy substrates such as fatty acids (156) and proteins (112) results in perturbed oocyte and embryo mitochondrial metabolism, and studies in vitro support this idea that low-level acquired mitochondrial injuries may persist into embryonic life (106; 145). Since mitochondria are affected by maternal nutritional status and are passed on maternally, it suggests that mitochondrial injury due to maternal obesity could compromise metabolism in the developing fetus, and may even impact fetal mitochondrial function prior to conception.

In addition to promoting TG storage and impairing oxidation at the cellular level, increased fetal lipids may favor formation of adipocytes over myocytes or other cell types during early organogenesis. In a sheep model of maternal over-nutrition, fetal skeletal muscle at day 60 (out of 142 days of gestation) showed small but significantly reduced fiber numbers and increased intramuscular adipocyte numbers (162). These changes were associated with increased NF-\(\kappa\)B activation, decreased AMPK signaling – an activator of lipid oxidation, and increased PPAR\(\gamma\) expression – a key adipogenic transcription factor (171). Maternal over-nutrition has also been shown to increase fetal adipose tissue expression of PPAR\(\gamma\), leptin and adiponectin, suggesting enhanced adipogenesis (116). The use of thiazolidines (TZDs, PPAR\(\gamma\) agonists) greatly improves insulin sensitivity by promoting adipocyte lipid storage and reducing levels of circulating free fatty acids (141). However, early activation of PPAR\(\gamma\) or its downstream targets could promote the storage of excess lipids at the expense of oxidative pathways, thereby increasing the risk of developing obesity in cases of nutrient excess. However, in a recent mouse study, maternal exposure to PPAR\(\gamma\)-agonists led to induction of fetal mesenchymal stem cells along the adipocyte lineage, and a reduction in the osteogenic potential in these cells, resulting in greater fat mass in adult offspring (88). The role of stem cell precursor programming in metabolic disease pathways in response to maternal nutrient supply is an area ripe for investigation.
Maternal obesity and high-fat diet also appear to profoundly alter offspring feeding behavior. Epidemiologic studies have demonstrated that maternal macronutrient intake correlates well with offspring macronutrient intake at 10 years of age, both in terms of composition and total caloric energy (19). Interestingly, the strongest correlation was with maternal prenatal diet versus postnatal diet, and the strongest predictor of offspring fat mass was fat intake - rather than protein or carbohydrate. Studies in animal models suggest that maternal obesity and high-fat diet consumption leads to adaptive regulation of key genes in neuronal pathways associated with appetite (reviewed in 101). The hypothalamus is an important regulator of appetite and satiety (reviewed in 17), where leptin receptor binding activates pro-opiomelanocortin (POMC) neurons and anorexigenic downstream pathways. Obesity is often associated with leptin resistance, resulting in an inability to balance food intake with actual energy needs. The leptin pathway is counter-regulated by the orexigenic neuropeptide-Y (NPY). Impaired leptin signaling could result in increased expression of NPY, which would promote increased nutrient intake while decreasing overall physical activity. In a rodent model, maternal high-fat diet led to increased proliferation of orexigenic neurons in the fetus, which closely correlated with circulating fetal lipids (34). In another study (95), offspring from high-fat fed mothers weaned onto a high-fat diet demonstrated increased weight gain, visceral fat deposition, energy intake, and circulating leptin levels. Interestingly, hypothalamic expression of the leptin receptor, POMC, and NPY were all significantly higher than in the control groups, suggesting an overall defect in the leptin signaling pathway, given the inability of elevated leptin to downregulate NPY (121). Alternatively, in a sheep model of maternal over-nutrition, twenty day-old lambs showed an inverse correlation between hypothalamic leptin-pathway gene expression and overall adiposity, demonstrating a disconnect between peripheral signals and central sensors of nutrient homeostasis (116). More recently, chronic consumption of a high-fat diet during pregnancy has been shown to cause perturbations in the serotonergic system, and increase anxiety-like behavior in non-human primate offspring (142), together with a reduction in the melanocortin pathway in the fetal brain (12). This data suggests that maternal high-fat diet has profound effects on fetal brain development, and may impact behaviors beyond appetite control, which warrant further investigation.
Lastly, reduced beta cell growth and insulin secretion has been observed in cases of growth restriction (103; 128; 165), while accelerated beta cell mass and excess insulin secretion was observed in models of obese pregnancy (55). Though apparently opposite, both can lead to later islet cell failure and development of diabetes. The beta cell transcription factor Pancreatic Duodenal Homeobox 1 (Pdx1) is critical for beta cell development, and progressive silencing of PDX-1 expression has been observed in beta cells isolated from growth-restricted rodent models (122). Importantly, this silencing corresponded with altered epigenetic regulation of the PDX-1 gene, which carried through into adulthood. Additionally, increased circulating lipids can induce beta cell apoptosis via ER stress pathways (99). Interestingly, PDX1 is protective against pancreatic ER stress in response to high-fat feeding in rodents (130). While obese pregnancy can increase pancreatic fat deposition in rodent models (119), whether this in turn leads to permanent changes in gene expression as observed with growth-restricted pregnancies remains unknown.

It should be noted that results from programming studies in both human and animal models often show gender specificity in the degree and type of metabolic alteration observed across tissues and species (52; 54; 107; 121; 167; 168). For example, the expression of diabetes in a number of animal models is sexually dimorphic and has been associated with altered hepatic metabolism. In a rat model of maternal protein restriction and intrauterine growth restriction (IUGR), only male offspring demonstrated increased incidence of type 2 DM with altered hepatic enzyme profiles including increased PEPCK activity (96). Similarly, perturbations induced by bilateral uterine artery ligation in the rat can induce IUGR, and these animals undergo a period of normalcy, followed by a male-specific alteration in hepatic fatty acid metabolism and gene expression that contributes to adult dyslipidemia (13). In addition to a gender-specific effect, an additional caveat with the many of these studies is that metabolic programming has global and measured effects across multiple organs. Adult offspring of (streptozocin induced) moderately diabetic mothers have a deficient β-cell response to glucose stimulation, whereas adult offspring from severely diabetic mothers are insulin resistant. (1; 150). When female offspring from these two groups become pregnant, they develop GDM and their fetuses display the same biochemical phenotypes found in the first generation. This transmission occurred only in females of diabetic mothers
suggesting that epigenetic factors, or perhaps an estrogenic environment may be involved. In addition the adult offspring of streptozotocin diabetic mothers are not only insulin resistant but also glucose intolerant, indicating that transmission of hyperglycemia may also occur as a result of exposure to maternal diabetes \textit{in utero} (72). The potential cellular and molecular mechanisms underlying these changes remain unclear. Thus, it becomes difficult to assign the early origins of these disease pathways to a single maternal nutrient and a single organ when examining animal models, particularly during post-natal life. This argues strongly for studies in both genetically defined mice and in large animal models, with an emphasis on maternal and fetal analyses, when looking for early origins of disease.

Given the multiple metabolic gene pathways that may be targeted by excess fetal lipid exposure, the inevitable next question is how do we reverse the program? While epigenetic marks are more plastic during early developmental windows, and are traditionally maintained with differentiation, they are still inherently dynamic. Because of this, treatments designed to alter DNMT, HAT, and HDAC activity are currently being investigated for their utility in correcting epigenetic dysregulation (60). For example, inhibitors of HDAC activity have been shown to promote tumor cell apoptosis (138). In terms of fetal programming, supplementation with folate or choline can promote DNA methylation as they can act as methyl-group donors (169). Studies using mice expressing the Agouti allele, which is known to have variable expression due to differential methylation (114), have described altered gene expression when the maternal diet is supplemented with methyl-donors, leading to differential offspring phenotypes (157; 159). However, given that none of these therapies are targeted, perhaps the most attractive mechanisms to prevent fetal metabolic programming in cases of maternal obesity is at the source; that is, prevention of initial maternal inflammation, insulin resistance, and hyperlipidemia. Interventions to decrease excess maternal lipid availability may be specifically targeted through diet, supplementation (omega-3 fatty acids) or pharmacologic interventions (niacin, fibrates, insulin) depending on if the excess fatty acids are in the form of Chylomicron-TG, VLDL-TG or FFAs. Omega-3 supplements in the form of cod oil were successful in decreasing maternal TG by about 10% compared to corn oil (68) and are known to decrease TG in the non-pregnant population. Niacin has been demonstrated to potently decrease TG
in multiple studies involving non-pregnant individuals by increasing the activity of endothelial LPL and removing chylomicron–TG from plasma, as well as decreasing hepatic TG synthesis and VLDL production. Additionally, fibrates decrease synthesis of VLDL and also increase VLDL clearance, and are currently recommended to be used in pregnant women with severe hypertriglyceridemia due to the risk of TG-induced pancreatitis in pregnancy (90). Finally, insulin is known to suppress lipolysis. The suppression of FFA production by administration of exogenous insulin to pregnant women with well controlled GDM whose fetuses still exhibited excessive growth has been speculated to be the reason why such strategies are effective in decreasing macrosomia (131). Undoubtedly, there is still much to be learned about the role of maternal obesity, diet and lipid metabolism on fetal gene regulation. A better understanding of the mechanisms behind nutrient-gene interactions in the context of fetal development will clearly aid in the development of more targeted and effective means of intervention.

V. Conclusions and Future Directions:

The prevalence of obesity in the developed world has increased markedly over the last 20 years in every country, in each race/ethnic group studied, and in both men and women. Considering the prevalence of obese and overweight adult women, and the fact that pregnancy itself induces a state of insulin resistance and inflammation, maternal obesity may be the most common health risk for the developing fetus. The notion that an abnormal maternal metabolic environment may lead to permanent changes in key organs that underlie fetal/juvenile “programming” of adult disease, is increasingly gaining acceptance. However, the mechanisms involved in generating such responses are far from being understood. One of the most important and challenging goals in this field will be to discover novel ways by which maternal metabolism alters chromatin structure in the fetus through epigenetic events, and to understand how these chromatin dynamics regulate key nuclear processes involved in the susceptibility to metabolic diseases. A persistent change in early gene transcription can change both behavior and organ functioning, and argues for a change in both disease susceptibility as well as age of onset as a result of the in utero insult. Newer techniques such as genome-wide analysis combining chromatin immunoprecipitation and microarray technology (ChIP-chip) may be instrumental
in establishing the roles of many histone modifications. Additionally, with the advent of quantitative high throughput sequencing technology, the generation of reference epigenomes can be created using ChIP-Seq and bisulfite sequencing techniques to further decipher histone codes and DNA methylation patterns, respectively. The proteins and processes that govern these events may be highly conserved in eukaryotic evolution, such that what we learn in cells and genetically malleable animal models may directly relate ultimately to humans. This is clearly an important emerging field, but a challenging research area, that combines fetal physiology, fuel metabolism, and molecular biology. There exists an exciting potential for interdisciplinary investigators to ask mechanistic questions about the interaction between diet and chromatin dynamics to uncover the fundamental mechanisms underlying human fetal metabolic programming. Ultimately, prevention of the juvenile obesity epidemic may begin in the womb.

Reference List


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**Figure 1: Obesity and pregnancy are associated with insulin resistance and inflammatory changes that exacerbate in combination, increasing lipid transfer earlier in gestation.**

Obesity is associated with adipose tissue inflammation and systemic insulin resistance, resulting in increased adipose tissue lipolysis and hepatic VLDL secretion. When combined with pregnancy, this leads to an increase in maternal circulating lipids with advancing gestation. Subsequent hydrolysis of maternal TGs by placental LPL, and increased FFA uptake and transport by the placenta, results in excess lipid transfer to the developing fetus. This increase in fetal lipid exposure may impact the liver, skeletal muscle, adipose tissue, brain, and pancreas to increase the risk for metabolic disease in childhood. Abbreviations: FFA, Free Fatty Acid; VLDL, Very Low Density Lipoprotein; CM, Chylomicron; TG, Triglyceride; LPL, Lipoprotein Lipase.

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**Figure 2: General Example of Epigenetic Regulation of Gene Transcription**

Epigenetic regulation of gene expression is characterized by stable changes to DNA and chromatin structure that alter gene expression independent of gene sequence. The primary forms of epigenetic control involve DNA methylation by DNMTs, and histone tail modifications, such as acetylation/deacetylation, by HAT and
HDAC activities, respectively. Additionally, micro-RNAs have recently been shown to regulate DNA methylation as well. Histone tail acetylation promotes an open-chromatin conformation, and is associated with regions of active gene expression, while histone tail de-acetylation promotes a closed-chromatin conformation and is associated with gene silencing. DNA methylation of CpG dinucleotides in the 5’ promoter region of genes generally induces transcriptional silencing, both by blocking transcription factor binding and by promoting the recruitment of transcriptional co-repressors or histone-modifying complexes. Abbreviations: HDAC, Histone De-Acetylase; HAT, Histone Acetyl-Transferase; MeBP, Methyl-CpG Binding Protein; TF, Transcription Factor; DNMT, DNA Methyl-Transferase; Pol II, DNA Polymerase II.
MATERNAL OBESITY, OVERNUTRITION
↑ INFLAMMATION
↑ INSULIN RESISTANCE

↑ LIPOLYSIS
↑ VLDL SECRETION

FFA

VLDL-TG

CM-TG

LPL

↑ HEPATIC LIPIDS

SKELETAL MUSCLE
ADIPOSE TISSUE
BRAIN
PANCREAS

REPROGRAMMING OF METABOLIC GENE TARGETS:
↑ FETAL INFLAMMATION?

↑ RISK:
- NAFLD
- INSULIN-RESISTANCE
- OBESITY
- HYPERPHAGIA
- DIABETES