Maternal obesity and fetal metabolic programming: a fertile epigenetic soil

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Heerwagen MJR, Miller MR, Barbour LA, Friedman JE. Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. Am J Physiol Regul Integr Comp Physiol 299: R711–R722, 2010. First published July 14, 2010; doi:10.1152/ajpregu.00310.2010.—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. Pregravid 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 proinflammatory pathways, which could impact substrate metabolism and mitochondrial function, as well as stem cell fate, all of which affect organ development and the response to the postnatal environment. Fetal and neonatal life are 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 postnatal energy balance, with an emphasis on epigenetics and the intrauterine environment.

nutrition; pregnancy; epigenetics; inflammation; diabetes; lipids

EMERGENCE OF ADULT METABOLIC disease epidemics in children is an advancing public health concern, with childhood obesity, diabetes, cardiovascular disease, 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 and colleagues (7, 8) has led to extensive research in the effects of fetal undernutrition, low birth weight, and development of chronic metabolic disease in the offspring. However, less is known about the metabolic impact of fetal overnutrition, 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, compared with 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 6 years (14). These findings imply that maternal hyperlipidemia, inflammation, or other metabolic and dietary factors traditionally associated with obesity and the metabolic syndrome may be con-
Review

Obesity, Inflammation, and Insulin Resistance in Nonpregnant Individuals: Fat on Fire

Obesity and pregnancy are independently associated with insulin resistance and inflammatory changes, which may be exacerbated when combined with one another. In the nonpregnant 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 Refs. 74 and 135). Markers of inflammation have been observed in both adipose tissue and liver of obese individuals and rodents, including TNF-α, chemokine receptor-2, monocyte chemotactant protein-1, toll-like receptor-4, and JNK. More than just a passive storage depot, adipocytes can synthesize, store, and secrete multiple proinflammatory cytokines, including IL-6, IL-8, TNF-α, and monocyte chemotactant protein-1, many of which play an important role in obesity-induced insulin resistance (reviewed in Refs. 74 and 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 are associated with an accumulation of adipose tissue macrophages with a proinflammatory phenotype. In the obese state, macrophages appear to become polarized toward 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 proinflammatory 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 antilipolytic 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 proinflammatory signal by binding toll-like receptors expressed on the cell surface of resident macrophages. Toll-like receptor signaling induces NF-κB (95, 100, 117, 140), a proinflammatory transcription factor, leading to perpetual adipose tissue inflammation and increased insulin resistance. Proinflammatory 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 proinflammatory cytokines and FFAs released systemically. Reducing adipose tissue inflammation 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.

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 (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 mellitus (GDM) is a known risk factor for large-for-gestational-age and macrosomic (>4,000 g) births, the majority of large-for-gestational-age 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 prepregnancy body mass index 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 body mass index 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 markedly (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 (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 lipid uptake and de novo lipogenesis (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-TG (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 wk) but net lipolysis in late gestation (34–36 wk). In contrast, in obese women under similar experimental conditions, lipogenesis occurs pregravid but less so in early pregnancy compared with 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 β-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 wk 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 wk of pregnancy, exponentially increasing to 7 g/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 FFAs by diffusion and selectively increases the transport of essential fatty acids and their long-chain polyunsaturated fatty acid derivatives by fatty acid carrier proteins, thereby creating a higher concentration in the fetus than in the mother (65, 92). Furthermore, placental expression of lipoprotein receptors and receptor-related proteins allows maternal lipoproteins such as VLDL and dietary chylomicrons to be taken up by the placenta where they must be hydrolyzed by placental LPL (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 (43), again supporting an enhanced fetal need for maternal FFA in late gestation (Fig. 1).

![Fig. 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 very-low-density lipoprotein (VLDL) secretion. When combined with pregnancy, this leads to an increase in maternal circulating lipids with advancing gestation. Subsequent hydrolysis of maternal triglycerides (TGs) by placental lipoprotein lipase (LPL) and increased free fatty acid (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. MCP-1, monocyte chemotractant protein-1; CM, chylomicron; NAFLD, nonalcoholic liver disease.](https://ajpregu.physiology.org/lookup/doi/10.1152/ajpregu.00398.2009)
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 TGs 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 1 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 adipophilin, 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 upregulate 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 placenta from obese mothers have been shown to have increased expression of proinflammatory cytokines and a marked accumulation of a heterogeneous macrophage population (33). Additionally, placenta from obese (body fat >16%) compared with 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 as leptin and TNF-α (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 TNF-α 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.

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 Ref. 80). Changes in epigenetic marks are associated with multiple human diseases, including many cancers, neurological disorders, and even inflammation (reviewed in Refs. 134, 148, and 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 posttranslational histone modifications. Additionally, microRNAs are emerging as a potential third epigenetic mechanism. While these noncoding RNAs are traditionally associated with regulation of gene expression at the translational level, recent work suggests they may be involved in DNA methylation as well, 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 Ref. 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 leads to recruitment of methyl-CpG binding proteins, which induce transcriptional silencing both by blocking transcription factor binding and by recruiting transcriptional corepressors or histone-modifying complexes, thereby promoting the formation of heterochromatin (reviewed in Refs. 80 and 127). Aberrant DNA methylation in traditionally hypomethylated CpG-rich regions of gene promoters, termed CpG islands (reviewed in Ref. 78), has been associated with inappropriate gene silencing and is known to occur in many cancers (reviewed in Ref. 9). However, a subset of CpG islands 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 Ref. 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 overnutrition; 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. Chroma-
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 postplacental 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-activated 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, a maternal high-fat diet results in persistent lipid accumulation in adult offspring livers, even in the absence of postweaning high-fat diet exposure (20, 38), suggesting a more permanent programming effect by maternal diet. Development of NAFLD in humans is associated with increased expression of genes in de novo lipogenesis, such as SREBP1c, ACC, FAS, SCD1, and LXRα (47, 160), as well as a decrease in expression of genes associated with hepatic fatty acid oxidation such as PPARα, 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 in utero are particularly sensitive to nutrient availability. Finally, both fetal nutrient restriction and nutrient excess have been shown to increase hepatic gluconeogenic pathways, mainly through increased gene expression of phosphoenolpyruvate carboxykinase 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 autophosphorylation of the insulin receptor, allowing insulin receptor substrates (IRS-1, IRS-2) to dock (reviewed in Refs. 41 and 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 proinflammatory cytokines, can all activate serine kinases known to target IRS-1. These include (but are not limited to) JNK, p38, ERK, pPKCs, p70S6K, IKKβ, and, most recently, MAPK4 (16, 18, 48). Studies in sheep and primate models of maternal overnutrition 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 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 toward 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 β-oxidation, including the PPARα coactivator 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 reactive oxygen species 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 was raised, while glutathione was depleted, and thus the redox state became more oxidized, suggesting of oxidative stress. Furthermore, 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 overnutrition, 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-κB activation, decreased AMPK signaling (an activator of lipid oxidation), and increased PPARγ expression (a key adipogenic transcription factor) (171). Maternal overnutrition has also been shown to increase fetal adipose tissue expression of PPARγ, leptin, and adiponectin, suggesting enhanced adipogenesis (116). The use of thiazolidines (PPARγ agonists) greatly improves insulin sensitivity by promoting adipocyte lipid storage and reducing levels of circulating FFAs (141). However, early activation of PPARγ 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. In a recent mouse study, maternal exposure to PPARγ-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 not 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 lead to adaptive regulation of key genes in neuronal pathways associated with appetite (reviewed in Ref. 101). The hypothalamus is an important regulator of appetite and satiety (reviewed in Ref. 17), where
leptin receptor binding activates proopiomelanocortin 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 counterregulated 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 (102), 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 proopiomelanocortin 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, 20 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 nonhuman 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.

Finally, reduced beta cell growth and insulin secretion have 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 (Pdx-1) 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 endoplasmic reticulum stress pathways (99). Interestingly, Pdx1 is protective against pancreatic endoplasmic reticulum 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, only male offspring demonstrated increased incidence of type 2 diabetes with altered hepatic enzyme profiles, including increased phosphoenolpyruvate carboxykinase activity (96). Similarly, perturbations induced by bilateral uterine artery ligation in the rat can induce intrauterine growth restriction, 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 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 beta 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 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 postnatal 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 DNA methyl-transferase, histone acetyl-transferase, 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, fribates, insulin), depending on whether 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 with corn oil (68) and are known to decrease TG in the nonpregnant population. Niacin has been demonstrated to potentially decrease TG in multiple studies involving nonpreg-
nant 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.

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 processes involved in the susceptibility to metabolic diseases.

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DISCLOSURES

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REFERENCES


