

# Obesity and Placental Function

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## Abstract

An increasing number of women of reproductive age are obese which affects the continuum of pregnancy and is associated with an increased incidence of adverse maternal and fetal outcomes, including preeclampsia, preterm birth, stillbirth, congenital anomalies, and macrosomia. Maternal obesity is associated with an increased incidence of metabolic and cardiovascular disease later in life in the mother and in the offspring who are developmentally programmed by the obese pregnancy environment. The placenta transduces and mediates the effect of the adverse maternal environment to the fetus. The obese maternal environment is characterized by hyperlipidemia and an exaggerated state of inflammation and oxidative stress compared with normal pregnancy. Heightened inflammation and oxidative/nitrative stress are found in the placenta in association with placental dysfunction. We have described reduced mitochondrial respiration and ATP generation in trophoblast isolated from placentas of obese compared with lean women, again suggesting compromised placental function. In utero development exhibits sexual dimorphism with the male fetus at greater risk of poor outcome. We have shown dimorphism in inflammation-mediated regulation of trophoblast mitochondrial respiration. There is also increasing evidence that the obese in utero environment may cause epigenetic changes in placenta leading to altered function.

## Keywords

- ▶ placenta
- ▶ obesity
- ▶ inflammation
- ▶ mitochondria
- ▶ oxidative stress
- ▶ epigenetics

Obesity is a global health concern among adults and children of both sexes and has major societal and economic costs. The number of women of reproductive age who are overweight (body mass index [BMI] = 25–30 kg/m<sup>2</sup>) or obese (BMI > 30 kg/m<sup>2</sup>) continues to increase, with the incidence of obesity among pregnant women now estimated at between 18.5 and 38.3%.<sup>1</sup> The economic cost of obesity in pregnancy is greater than \$100 million annually.<sup>2</sup> Maternal obesity affects the continuum of pregnancy. Fertility and fecundity rates are lower among overweight and obese women, in spontaneous conception as well as in artificial reproductive techniques.<sup>1</sup> During pregnancy, these women are more susceptible to hypertensive disorders, gestational diabetes, respiratory complications, and thromboembolic events.<sup>1,3–6</sup> While unknown cause remains the most common contributor to stillbirth with

40% of these cases occurring in late gestation,<sup>7</sup> up to 25% of stillbirths between 37 and 42 weeks of gestation are perhaps due to obesity.<sup>8</sup> Indeed obesity may explain the increased morbidity including the increased risk (2.8 fold) of late stillbirth, particularly in males, in such pregnancies.<sup>9</sup> Overweight women also have a slower labor progression rate; higher rates of cesarean deliveries<sup>10</sup>; and more surgery-related complications such as difficult spinal, epidural, or general anesthesia, wound infection, and endometritis.<sup>1,3</sup> From the fetal and newborn perspective, complications include congenital malformations,<sup>11</sup> large-for-gestational-age infants,<sup>12</sup> intrauterine growth restriction, stillbirth, and shoulder dystocia. Finally, the effects are not confined to pregnancy alone. Obese and also gestational diabetic women have greater rates of type 2 diabetes<sup>13</sup> and cardiovascular disease later in life.

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## Obesity and Developmental Programing

By virtue of its location and roles at the interface between the mother and fetus, the placenta is the key regulator of fetal growth and development.<sup>14</sup> The placenta not only conveys the maternal metabolic environment to the fetus but can also become both a target and a source of pathogenic factors affecting the fetus.<sup>15</sup> Developmental programing occurs when the normal developmental pattern is disrupted by inappropriate or ill-timed signals reaching the fetus or neonate which is then set on an altered developmental trajectory that can lead to disease in adult life.<sup>16</sup> A large body of evidence<sup>17</sup> shows that an adverse or altered intrauterine or early postnatal environment, including obesity, can program for disease in adult life including cardiovascular disease, obesity and metabolic syndrome, diabetes, osteoporosis, cancer, and disorders of the hypothalamic/pituitary/adrenal axis.<sup>1,3,18–20</sup> Hence, while obese women may have babies within the normal birth weight range, with normal sized placentas and apparently a normal outcome there may be a programing effect on the fetus that is only revealed subsequently. While it is clear that maternal obesity programs the fetus, the mechanism and physiological consequences of the adverse metabolic and inflammatory environment of obesity for placental function and fetal development are just now being elucidated.

## Sexual Dimorphism and Developmental Programing

It is clear that male and female fetuses respond differently to the adverse intrauterine environment. This may then relate to their risk of developing disease in adult life where differences in incidence of various diseases are clearly documented. Even in “normal” pregnancy and development, there is a sexually dimorphic effect. Male fetuses grow faster and are usually larger than females.<sup>21</sup> However, male fetuses are at much higher risk during pregnancy and show greater incidences of preterm birth, preterm premature rupture of membranes, placenta previa, lagging lung development, greater incidence of macrosomia with maternal glucose intolerance, and more late stillbirths associated with pregestational diabetes.<sup>22</sup> The female neonate can more readily adapt to ex utero life even when delivered in a highly immature state at midgestation, an effect possibly mediated by in utero adaptations to an adverse environment prior to delivery.<sup>23</sup> The male fetus is claimed “to live dangerously in the womb” to maximize its growth potential but with consequent high risk when faced with additional adverse events.<sup>22</sup> It is likely that there is a complex interaction between the adverse environment of obesity and fetal sex.

## Sexual Dimorphism and the Placenta

The placenta is a fetal tissue that shows sexual dimorphism. Microarray analysis revealed distinct sexually dimorphic profiles of gene expression in the human placenta; in particular immune genes were expressed at higher level in female placenta compared with male.<sup>24</sup> Gene expression in the placenta also responds to maternal inflammatory status in sex-dependent manner.<sup>25</sup> Expression of 59 genes was

changed in the placenta of women with asthma versus no asthma with a female fetus compared with only 6 genes changed in those with asthma with a male fetus.<sup>26</sup> Some of these genes were associated with growth, inflammatory, and immune pathways. Changes in diet provide distinctive signature of sexually dimorphic genes in placenta with expression generally higher in genes in female than in male placenta.<sup>27</sup> The male placenta has higher toll-like receptor 4 (TLR4) expression and a greater production of tumor necrosis factor (TNF)- $\alpha$  in response to lipopolysaccharide (LPS) than the female placenta, which can underlie the propensity to preterm birth in males.<sup>28</sup> The mechanisms of sexual dimorphism in placenta with obesity remain unstudied; however, evidence from other complicated pregnancies links sex differences to gonadal steroids. Women with preeclampsia have increased plasma testosterone levels compared with those of healthy pregnant women, with significantly higher levels in male- than in female-bearing preeclamptic pregnancies.<sup>29</sup> At the same time, the placental levels of aromatase, a rate-limiting enzyme converting androgens to estrogens, varied depending on fetal sex: it was much higher in the preeclamptic placentas with female than male fetuses.<sup>30</sup> Interestingly, aromatase can be downregulated by TNF $\alpha$ , hypoxia, insulin, and leptin, which mirror the actual conditions of the placenta in the context of maternal obesity.<sup>31–34</sup>

## Inflammation in Pregnancy with Obesity

Pregnancy per se is an inflammatory state.<sup>35</sup> This is enhanced in pregnancies complicated by obesity,<sup>36</sup> where increased concentrations of inflammatory cytokines can be seen in maternal plasma<sup>37</sup> and the placenta.<sup>38</sup> The increased placental inflammation in obese pregnancy may be stimulated by endotoxin,<sup>36</sup> lipids,<sup>39</sup> reactive oxygen species (ROS),<sup>40</sup> or oxidized lipids.<sup>41</sup> Chronic low-grade inflammation in obese women prior to pregnancy initiates a cascade of events which translate into an inflammatory in utero environment. Significant accumulation of subsets of macrophages has been shown in placentas from obese patients resulting in production of proinflammatory cytokines and adipokines including interleukin-6 (IL-6), leptin, TNF- $\alpha$ , monocyte chemoattractant protein 1, and TLR4.<sup>42–44</sup> Uncontrolled placental inflammation leads to the impairment of overall placental function such as increased free fatty acid (FFA) delivery to the fetal circulation, which is expected to alter fetal growth and development.<sup>45</sup> We found that TNF- $\alpha$ , used to simulate the inflammatory milieu of obesity, decreases trophoblast mitochondrial respiration but in a sexually dimorphic manner. The effect is seen only in trophoblasts of a female placenta and is mediated by the transcription factor NF $\kappa$ B1.<sup>46</sup>

## Effect of Obesity on Maternal and Placental Metabolism in Pregnancy

Pregnancy is a state of profound metabolic changes characterized by increased fat mass, insulin resistance, low-grade inflammation,<sup>35</sup> and mild hyperlipidemia,<sup>47</sup> where phospholipids, total LDL and HDL cholesterol, and triglycerides all

increase. The metabolic changes become exacerbated with pregravid obesity.<sup>48</sup> Obese pregnant women are characterized by high levels of FFA, higher circulating levels of leptin, TNF- $\alpha$ , IL-1, IL-6, IL-8, oxidative stress, and reduced levels of adiponectin.<sup>49</sup>

The placenta, particularly syncytiotrophoblast, has tremendous oxygen consumption<sup>50</sup> and metabolic activity, the energy for which is derived from ATP mainly generated by oxidative phosphorylation in mitochondria. Glucose was traditionally thought of as the major (if not sole) substrate for energy generation in fetus and placenta.<sup>51</sup> However, the placenta does not appear to utilize anaerobic glycolysis to generate energy during periods of anoxia.<sup>52</sup> Work in the past 10 years has shown that the placenta can generate energy from fatty acids<sup>53</sup> via fatty acid oxidation (FAO).<sup>54</sup> Long chain fatty acids necessary for placental FAO are abundant in maternal plasma in late gestation but are markedly increased with obesity and thought to play a role in insulin resistance.<sup>55</sup> Deficiencies in placenta of enzymes involved in FAO lead to accumulation of toxic long chain metabolites and are associated with maternal HELLP syndrome and with preeclampsia.<sup>56</sup> Saturated fatty acids, palmitate and stearate, activate inflammatory signaling pathways via interaction with TLRs and via secretion of cytokines including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6.<sup>57</sup> We find significantly increased level of TNF- $\alpha$  in the placenta of female fetuses of obese women.<sup>46</sup> Fatty acids also reduce mitochondrial function through induction of proinflammatory cytokines, and chronically elevated fatty acids are associated with increased production of reactive oxygen and nitrogen species.<sup>58</sup> There has not been an investigation of FAO in the placenta of obese pregnancies, nor has it been studied in relation to circulating maternal saturated or unsaturated fatty acids, inflammatory cytokines, or oxidative stress or to fetal outcomes.

### Free Fatty Acids and Lipid Transport in Placenta during Maternal Obesity

Although maternal hyperglycemia has long been associated with increased fetal growth,<sup>59</sup> maternal triglycerides also contribute with aberrant fetal growth seen with gestational diabetes mellitus (GDM) despite good glucose control. Indeed in multivariate analysis, increased birth weight positively correlates only with hypertriglyceridemia in women with GDM.<sup>60</sup> However, such studies have not been performed in pregnancies complicated by obesity alone, nor in relation to fetal adiposity.

Placental uptake of FFAs from the maternal circulation provides fatty acids both for placental metabolism and delivery to the fetus.<sup>61</sup> Cells involved in active lipid trafficking express discrete fatty acid binding proteins (FABP), implicated in cellular uptake and transport of fatty acids and coordination of metabolic and inflammatory pathways.<sup>62,63</sup> FABP1, FABP3, FABP4, FABP5, and FABP plasma membranes are expressed in human trophoblasts.<sup>64</sup> Maternal obesity can alter lipid content and increase the expression of FABP4 in trophoblasts.<sup>65</sup> An ovine model of maternal obesity showed

significantly higher concentration of FFA in the fetal circulation of obese ewes at midgestation than in control ewes.<sup>45</sup> In addition, the level of peroxisome proliferator-activated receptor gamma which is known to be essential for placental development and placental uptake of fatty acids was found to be activated in the placenta of obese ewes.<sup>61</sup> As fatty acids are ligands for TLR4, which drives the inflammatory response,<sup>66</sup> it was postulated that excessive fatty acids in the fetal circulation in the setting of maternal obesity would activate TLR4 signaling, resulting in inflammation of fetal tissues.

### Placental Oxidative/Nitrative Stress and Obesity

Pregnancy is a state of oxidative stress. Mitochondria are the major source of ROS under physiologic conditions. Increased metabolic activity in placental mitochondria and the reduced scavenging power of antioxidants may be responsible for rapid ROS generation by different placental cell types.<sup>67,68</sup> At the same time, mitochondrial function itself can be compromised by severe and/or prolonged oxidative stress. Oxidative inactivation of mitochondrial DNA polymerase gamma could slow down mitochondrial DNA (mtDNA) replication and eventually lead to inhibition of oxidative phosphorylation.<sup>69</sup> The placenta can also produce nitric oxide (NO) and this molecule in combination with excess superoxide can result in the production of peroxynitrite (ONOO<sup>-</sup>), leading to nitrative stress. Peroxynitrite is a powerful prooxidant that can modify tyrosine residues within a protein sequence to give nitrotyrosine, or protein nitration. Covalent modification of proteins by nitration may be a physiologic regulatory mechanism in redox regulation for signaling pathways.<sup>70</sup> Nitrotyrosine residues have been demonstrated in the placenta of pregnancies complicated by preeclampsia,<sup>71</sup> pregestational diabetes,<sup>72</sup> and chronic hypoxia at high altitude.<sup>73</sup> We have previously shown nitration of several proteins in the human placenta, and demonstrated that the extent of nitration is increased in obese compared with lean and overweight placentas.<sup>40</sup> Koeck et al<sup>74</sup> provided evidence for rapid and selective oxygen-regulated protein tyrosine denitration/nitration in the mitochondria. Nitrated proteins can be eliminated from mitochondria during hypoxia/anoxia and regenerated during reoxygenation. This nitration/denitration in mitochondria may affect cellular energy and redox homeostasis and therefore cell and tissue viability.

### Placental Mitochondrial Energetics and Obesity

As stated previously, the placenta can generate energy from fatty acids<sup>53</sup> following FAO and generation of acetyl CoA. We have shown that with increasing maternal adiposity, there is a significant fall in mitochondrial respiration by oxidative phosphorylation and in ATP generation in the placenta<sup>75</sup> that is not compensated for by glycolysis. In galactose-containing medium, the trophoblast from obese pregnancies cannot increase oxidative phosphorylation, that is, they show metabolic inflexibility. This would suggest that with obesity,

the generation of acetyl CoA by FAO is compromised. Fatty acids also reduce mitochondrial function perhaps via proinflammatory cytokines and/or increased production of reactive oxygen and nitrogen species.<sup>58</sup> In turn, mitochondrial dysfunction can lead to a reduction in mitochondrial FAO.<sup>76</sup> Saturated fatty acids (palmitate, stearate) may be more damaging while unsaturated fatty acids (oleic, DHA) may be beneficial.

Mitochondria generate most of the cell's supply of ATP, used as a source of chemical energy which are also involved in a range of other processes, such as signaling, cellular differentiation, apoptosis and programmed cell death, control of the cell cycle and cell growth, regulation of the membrane potential, regulation of cellular metabolism, and steroid synthesis. Damage, reduced content, and functional capacity of mitochondria are involved in neurodegenerative and cardiovascular diseases,<sup>77</sup> obesity and diabetes.<sup>78,79</sup> Diminished FAO and greater dependence on glucose for ATP synthesis,<sup>80</sup> ectopic lipid accumulation in skeletal muscle, the liver, and other cells<sup>81</sup> and low basal ATP concentrations<sup>43</sup> are seen with obesity. Mitochondrial oxidative capacity is decreased in skeletal muscle of obese individuals,<sup>76</sup> in the kidney of high-fat diet (HFD)-fed mice,<sup>82</sup> as well as in the liver and the heart of ob/ob mice.<sup>83,84</sup> An isoenergetic HFD in healthy young men for only 3 days was sufficient to reduce the expression of genes involved in mitochondrial complexes I and II, and mitochondrial carriers.<sup>85</sup> While oxidative stress and mitochondrial dysfunction are often proposed as mechanisms mediating dysfunction in various organs in obesity models, little data are available for the placenta.

### Sexual Dimorphism in the Effect of Inflammation on Placental Mitochondria

MicroRNAs (miRNAs) are conserved, regulatory molecules that have an important role in the posttranscriptional regulation of target gene expression by promoting mRNA instability or translational inhibition.<sup>86</sup> MicroRNA-210, which has been traditionally linked to hypoxia,<sup>87</sup> targets and decreases activity of mitochondrial subunits in placenta,<sup>88</sup> hence reducing cellular respiration. We have shown that expression of miR-210 was significantly increased in placentas of obese and overweight women conceived with female, but not male, fetuses compared with female placentas of lean women.<sup>46</sup> We also demonstrated increased TNF- $\alpha$  in female but not male placentas of overweight and obese women, and that via NF $\kappa$ B1 (p50) signaling this resulted in activation of miR-210 expression. Chromatin immunoprecipitation assay showed that NF $\kappa$ B1 binds to placental miR-210 promoter in a fetal sex-dependent manner such that female but not male trophoblast treated with TNF- $\alpha$  showed overexpression of miR-210, reduction of mitochondrial target genes, and decreased mitochondrial respiration. Overall, our data suggest that the inflammatory intrauterine environment associated with maternal obesity induces an NF $\kappa$ B1-mediated increase in miR-210 in a fetal sex-dependent manner, leading to inhibition of mitochondrial respiration and placental dysfunction in the placentas of female fetuses.

We propose that impaired mitochondrial function in placenta and hence altered placental metabolism can evoke changes in the fetus and may potentially link maternal obesity to metabolic and cardiovascular disease in the offspring.

We have recently shown that increasing maternal adiposity is associated with increased generation of ROS and decreased mitochondrial respiration in the placenta.<sup>75</sup> Total antioxidant capacity and activity of superoxide dismutase are significantly greater in the lean male placentas than in lean female placentas or placentas of either sex from an obese mother (unpublished data, L. Myatt PhD, 2015), that is, there is sexual dimorphism and an effect of obesity. The connection of oxidative stress to mitochondrial dysfunction has refocused use of antioxidants in pregnancy toward alleviation of mitochondrial dysfunction. Selenium is a trace element necessary for normal cellular function and which protects trophoblast mitochondria against oxidative stress<sup>89</sup> by upregulating activity of antioxidant enzymes glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases.<sup>90</sup>

### Obesity and Epigenetics in the Placenta

Epigenetics describes heritable changes in gene expression that are not mediated by DNA sequence alterations<sup>91</sup> but are susceptible to environmental influences.<sup>92</sup> Several diverse factors epigenetically regulate genes, including age, lifestyle, inflammation, gender, genotype, stress, nutrition, metabolism, drugs, and infection.<sup>93</sup> Epigenetic information is conveyed in mammals via a synergistic interaction between mitotically heritable patterns of DNA methylation<sup>94</sup> and chromatin structure.<sup>95</sup> Local chromatin conformation regulates specific methylation patterns to control gene transcription.<sup>96</sup> Epigenetic mechanisms have been postulated to have a role in developmental programming of obesity and type 2 diabetes in offspring by the intrauterine environment<sup>97</sup> and may therefore also regulate placental function. There are several mechanisms that regulate epigenetic changes.

Gene expression can be altered via posttranslational covalent modifications of chromatin by histone methylation or acetylation which determines accessibility to transcription factors<sup>98</sup> leading to transcriptionally repressive or permissive chromatin structures.<sup>99,100</sup> Repressive histone modifications seem to confer short-term, flexible silencing important for developmental plasticity, whereas DNA methylation is believed to be a more stable, long-term silencing mechanism.<sup>101</sup> Differential histone modification occurs in a gender-specific manner,<sup>102</sup> and in primates<sup>103</sup> and rats,<sup>104</sup> consumption of a maternal HFD gave altered histone modifications of fetal hepatic genes accompanied by alterations in hepatic gene expression. There is, however, relatively little data<sup>105</sup> on histone modification in the human placenta with pregnancy complications.

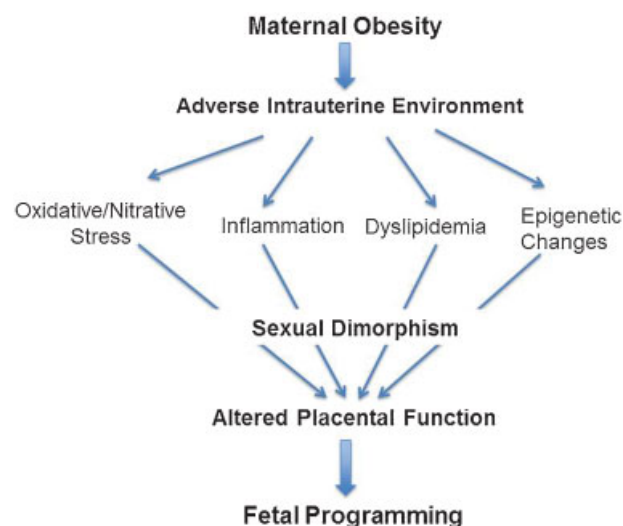
Hypermethylation of DNA in promoter regions typically is associated with transcriptional repression of genes, whereas hypomethylation leads to gene activity.<sup>106</sup> Global DNA methylation in the placenta increases with advancing gestational age,<sup>107</sup> but with greater interindividual variation in the third

trimester suggesting environmental factors may influence methylation, gene expression, and function of the placenta. Variations in DNA methylation profiles in the term placenta are seen in relation to pregnancy outcome (reviewed in Koukoura et al<sup>108</sup>). Recently, a novel modification DNA hydroxymethylation has been described.<sup>109</sup> Ten-eleven translocase (TET) enzymes convert 5mC to 5hmC. Although 5mC is repressive, 5hmC is permissive for gene expression. Therefore, the balance of 5mC to 5hmC at particular CpGs may control gene expression. Alpha ketoglutarate ( $\alpha$ KG) and ascorbate are cofactors for TET enzymes, suggesting a link between cellular metabolism and epigenetic regulation of cellular activity as  $\alpha$ KG is produced in the citric acid cycle. Maternal nutritional status may alter the epigenetic state of the fetal genome and imprinted gene expression.<sup>110</sup> Hyperglycemia induces demethylation of specific cytosines throughout the genome<sup>111</sup> with altered gene expression.

In mammalian genomes, DNA methyltransferase (DNMT) enzymes mediate the transfer of methyl groups from S-adenosylmethionine to cytosine,<sup>112</sup> establish and maintain DNA methylation patterns at specific regions of the genome, and contribute to gene regulation. DNMT1 is primarily a maintenance methyltransferase preserving methylation patterns during cell division, while DNMT3 enzymes are responsible for de novo methylation. The metabolic/inflammatory milieu of obesity increases DNMT3a expression of DNMT3a in adipose tissue of obese mice<sup>113</sup> and correlates with gene suppression. There is little data available on DNMTs in human placenta.

### The Influence of Nutrition and the Metabolic Environment on Epigenetic Modifications

While there is increasing evidence from other tissues that metabolic regulation of epigenetic mechanisms occurs, it is relatively unstudied in placenta. Tight regulation of epigenetic changes is essential especially in the early phase of gestation where global DNA demethylation in the zygote is seen but may subsequently be influenced by the maternal metabolic environment. Chromatin-modifying enzymes including DNMTs can sense and respond to alterations to the nutritional environment through their effects on intermediary metabolites.<sup>114</sup> Differences in DNA methylation have been reported in individuals exposed to famine during the Dutch Hunger Winter.<sup>115,116</sup> In later life, the epigenome appears to be capable of responding to changes in nutrients including deficiencies in methyl donors,<sup>117</sup> folic acid supplementation,<sup>118</sup> as well as fat<sup>119</sup> and caloric restriction.<sup>120</sup> The dramatic changes in methylation seen in early gestation and the relative hypomethylation of the placenta suggest it to be susceptible to dietary influences. Recently, intrauterine caloric restriction in mice, which programs male offspring for glucose intolerance, increased fat mass, and hypercholesterolemia, gave a significant decrease in overall methylation throughout the placental genome.<sup>121</sup> The level of demethylation was greater in placentas of male mice than in placentas of female mice and imprinted genes appeared to be more susceptible to methylation changes.



**Fig. 1** Mechanisms linking maternal obesity to placental dysfunction and developmental programming.

### Conclusion

The intrauterine environment found in the obese women is associated with poor pregnancy outcomes and importantly with programming the fetus for disease in later life. This effect is mediated via the placenta (→ Fig. 1), which displays altered function and compromised energetics related to the obese environment of hyperlipidemia, heightened inflammation, and oxidative stress. Evidence that the metabolic environment of obesity causes epigenetic changes is accumulating and needs to be studied in the placenta to link cellular metabolism to changes in gene expression and cellular function. There is also an overarching effect of fetal and placental sex, which now needs to be considered when studying placental function.

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