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Samantha Bedell

Janine Hutson

Barbra de Vrijer

Genevieve Eastabrook

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Effects of Maternal Obesity and Gestational Diabetes Mellitus on the Placenta: Current Knowledge and Targets for Therapeutic Interventions

Running Title: Placental Consequences of Obesity and Gestational Diabetes

Samantha Bedell[1], Janine Hutson[1], Barbra de Vrijer[1,2], Genevieve Eastabrook[1,2]

[1]Department of Obstetrics and Gynaecology, Schulich School of Medicine and Dentistry, the University of Western Ontario, London, Ontario, Canada; [2]Children's Health Research Institute, London, Ontario, Canada

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Corresponding Author

Barbra de Vrijer MD, FRCSC

Maternal Fetal Medicine Consultant

Associate Professor, Western University

Associate Scientist, Children's Health Research Institute

Division of Maternal Fetal Medicine,

Department of Obstetrics and Gynaecology,

London Health Sciences Centre, Victoria Hospital,

800 Commissioner's Road E, Room B2-412

London, Ontario N6A 3B4

Ph: 519 6858500, ext 64052

Fax: 519 6466213

Abstract

Obesity and gestational diabetes mellitus (GDM) are becoming more common among pregnant women world-wide and are individually associated with a number of placenta-mediated obstetric complications, including preeclampsia, macrosomia, intrauterine growth restriction and stillbirth. The placenta serves several functions throughout pregnancy and is the main exchange site for the transfer of nutrients and gas from mother to fetus. In pregnancies complicated by maternal obesity or GDM, the placenta is exposed to environmental changes, such as increased inflammation and oxidative stress, dyslipidemia, and altered hormone levels. These changes can affect placental development and function and lead to abnormal fetal growth and development as well as metabolic and cardiovascular abnormalities in the offspring. This review aims to summarize current knowledge on the effects of obesity and GDM on placental development and function. Understanding these processes is key in developing therapeutic interventions with the goal of mitigating these effects and preventing future cardiovascular and metabolic pathology in subsequent generations.

Keywords: Placenta, Obesity, Gestational Diabetes Mellitus, Vascular Development, Transport, Metabolism

1. Introduction

The placenta connects the maternal and fetal circulations, facilitating nutrient transfer and regulating the exchange of respiratory gases to promote fetal growth and development [1]. It senses changes in the maternal and fetal environments and responds accordingly [2]. In adverse conditions, the placenta undergoes morphological and functional adaptations to ensure fetal survival, putting the greatest emphasis on sparing fetal brain development and function [2]. The placenta is highly adaptable to environmental changes; however, excessive deviations may alter fetal development and cause lasting metabolic changes resulting in adult disease [1].

Obesity and gestational diabetes mellitus (GDM) are leading contributors of poor reproductive outcomes [3], which is a major concern as approximately two-thirds of women begin their pregnancy either overweight or obese [4] and globally, an estimated 14% of pregnancies are affected by GDM [5, 6]. Both obesity and GDM are independently associated with a number of obstetric complications including preeclampsia, macrosomia, intrauterine growth restriction (IUGR), and stillbirth [6-9], as well as the development of offspring metabolic and cardiovascular anomalies from fetal life through to adulthood [1, 4, 7, 10]. Women with an elevated body mass index (BMI) are at an increased risk of developing GDM [6, 11], and the effects of obesity and GDM are greater when they are combined than if they occur separately [12].

In pregnancies complicated by obesity or GDM, the placenta is exposed to environmental changes, such as increased inflammation and oxidative stress, dyslipidemia, and altered hormone levels [9, 10, 13, 14]. These changes can alter the development and function of the placenta, which can adversely affect the health of both mother and fetus. This review discusses current knowledge on how maternal obesity and GDM affect placental development and function throughout pregnancy and describes possible therapeutic targets for interventions that may prevent adverse pregnancy outcomes and cardiovascular and metabolic aberrations in the offspring.

2. Early Placental Development

Placental development begins in the first few days of gestation with the formation of the blastocyst [15, 16]. The blastocyst is comprised of two compartments: the inner cell mass, which develops into the embryo and later forms the fetal-placental vasculature, and an outer layer of trophoblast cells called the trophectoderm, which eventually gives rise to all placental trophoblast cells [16, 17]. The implantation process is highly organized involving the attachment of the embryo to the endometrial surface of the uterus and the subsequent invasion into the uterine epithelium [18]

(Figure 1). The blastocyst orients itself so that the inner cell mass is facing the uterine attachment site [19] which promotes the interaction between cell adhesion molecules expressed on the surface of the blastocyst trophectoderm and ligands expressed on the endometrial decidual epithelium [18]. Following adhesion, the blastocyst trophoblast cells rapidly proliferate and differentiate into villous and extravillous cytotrophoblasts [20, 21]. Villous cytotrophoblast (VCT) cells fuse together to form a multinucleated syncytiotrophoblast which has endocrine, exchange, and endothelial functions, while extravillous cytotrophoblasts (EVCT) are responsible for invading into maternal tissues [22]. Two types of EVCT cells exist: interstitial EVCT, which migrate into maternal decidua, and endovascular EVCT, which migrate into maternal spiral arteries [23]. The remodeling of the maternal spiral arteries involves the progressive disruption of the surrounding vascular smooth muscle cell layer to decrease resistance in blood vessels and increase blood flow to the placenta [24].

The early stages of pregnancy are sensitive to changes in the maternal environment; even small perturbations can have significant negative effects on placental development and pregnancy outcome. Women with obesity are more likely to be infertile and are less likely to become pregnant even after fertility treatments [25]. For women undergoing *in vitro* fertilization, obesity is associated with a decreased rate of blastocyst formation [26]. The endometrium is only receptive to the blastocyst during a short "window of implantation" [18, 21] and the regulation of growth factors, cytokines and adhesion molecules create the optimal environment for this process to take place [21, 27]. Impaired endometrial receptivity has been seen in women with obesity [28] and in a mouse model of maternal hyperinsulinemia [29]. Additionally, obesity and GDM are associated with altered levels of growth factors, cytokines and adhesion molecules [9, 30, 31], suggesting that there is an adverse environment for placental development.

Pregestational obesity and diabetes mellitus have been linked to impaired trophoblast invasion and spiral artery remodeling. In a rat model of maternal obesity, temporal alterations in trophoblast invasion are associated with increased fetal and neonatal death and decreased birth weight [24]. Hyperglycemia disrupts the invasive profile of human cytotrophoblast cells through the upregulation of stress signaling pathways, leading to dysfunctional angiogenesis and poor placental vascularization [32]. For example, mitogen-activated protein kinase (MAPK) phosphorylation was shown to be upregulated after treatment with 495 mg/dL or more of glucose compared with basal levels (45 mg/dL), and the inhibition of the plasmin pathway, which is involved in facilitating cytotrophoblast invasiveness, occurs following treatment of 135 mg/dL or more glucose compared with basal levels [32]. Furthermore, trophoblast invasion and spiral artery remodeling are reduced in type-1 diabetic rats along with increased uterine

natural killer (uNK) cells and macrophages [33], suggesting an abnormal maternal immune response may alter these processes.

The placenta contributes to the physiological changes that are essential for a normal pregnancy, such as increased oxidative stress and a systemic inflammatory response. Oxygen tension is involved in regulating the proliferation and differentiation of EVCT cells [34]. The placenta is a major source of reactive oxygen species [35] and changes in placental oxygen tension may contribute to the development of pregnancy complications, and both obesity and GDM have been found to induce placental hypoxia [35, 36]. In a mouse model, GDM is associated with increased expression of hypoxia inducible factor-1α (HIF-1α) and lower oxygen tension along with increased expression of TNF-α, IL-1β, and VEGF [35], suggesting GDM may lead to placental hypoxic stress as well as an exaggerated inflammatory response and impaired placental vascular development.

Normal pregnancy is characterized by a tightly regulated systemic inflammatory response [9]. Aberrant maternal inflammation is associated with impaired placental development and is implicated in a number of adverse pregnancy outcomes [9, 37, 38]. Both obesity and GDM induce a state of chronic, low-grade inflammation, affecting both the maternal and placental inflammatory profiles [9]. In a rat model, abnormal maternal inflammation is associated with impaired spiral artery remodeling and restricted fetal growth [39]. Circulating levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are elevated in women with obesity and in GDM [24, 40]. In the placenta, GDM is associated with increased expression of TNF-α, while obesity is associated with increased expression of both TNF-α and IL-6 [41, 42]. Elevated levels of maternal circulating pro-inflammatory cytokines have been associated with increased insulin resistance in the first and second trimesters of pregnancy [43] and many have been found to be involved in implantation and spiral artery remodeling [44]. For example, IL-6 increases migration and invasion of trophoblast cells, whereas TNF-α reduces trophoblast cell invasion and abnormally high levels of TNF-α can lead to impaired spiral artery remodeling [24, 39, 45, 46].

Autophagy, a physiological process responsible for the degradation of damaged cellular components, is necessary for cellular homeostasis, stress response and immune regulation, and is upregulated under physiological hypoxic conditions such as pregnancy [47]. Dysregulations in placental autophagy have been associated with impaired invasion, insufficient vascular remodeling, and the development of pregnancy conditions such as preeclampsia and IUGR [47, 48]. In vitro, first-trimester trophoblast cells that were incubated with 25 mM D-glucose (hyperglycemic) for 24 and 48 h showed reduced proliferation and increased autophagy levels compared with normoglycemic (5.5 mM

D-glucose for 24 and 48 h) controls [49]. In placentas from women with GDM, increased markers of autophagy and abnormal apoptosis have been documented, with a pattern of epigenetic changes distinct from those seen in preeclampsia. In vitro, mmol (24-h incubation with 30 mM D-glucose) induced both autophagy and apoptosis and resulted in a reduced invasive capacity of trophoblast cells compared with physiological blood glucose level (24-h incubation with 5 mM D-glucose) [50].

Placental vasculogenesis, *de novo* formation of a vascular network, and angiogenesis, the formation of new blood vessels from preexisting ones, continue throughout pregnancy to establish a fetomaternal circulation [51]. The maternal-placental arterial circulation forms by the end of the first trimester following invasion and the remodeling of the endometrial spiral arteries [1]. The closed fetoplacental circulation enables a high-volume low-resistance blood flow through the placenta, with a normal placenta containing approximately 40% of the fetal blood volume. Current ultrasound Doppler techniques, although not considered accurate to measure absolute blood flow in the fetoplacental circulation, allow for readily available assessment of the fetoplacental resistance which is positively correlated with maternal BMI [52].

Previous studies have found a linear correlation between placental weight and birth weight [53, 54]. Placental volume in the first trimester has shown to be a good indicator of birth weight [55] and the ratio of birth weight to placental weight is suggestive of placental efficiency [56]. Both maternal obesity and GDM have been associated with increased placental weight [57-60] and decreased placental efficiency [54, 60]. As the size of the placenta increases the surface area for transport may also increase, which can lead to fetal overgrowth [61]. Furthermore, placental weight has shown to be inversely related to placental efficiency [54], suggesting an adaptation to the increased nutrient availability in order to regulate fetal growth. Reduced placental efficiency is associated with changes in placental shape, which is thought to be mainly influenced by the structure of the placental vasculature [62].

Abnormal placental vasculature is the most common placental pathology associated with a multitude of pregnancy complications [51] and has been found in both obese and GDM pregnancies [60, 63, 64]. In a study comparing the placentas of obese and normal weight women, obesity was associated with delayed maturity of the villous tree characterized by villi of larger diameter and reduced number, as well as an increased number of capillaries within the villi [63]. Similarly, in placentas of pregnancies affected by GDM with suboptimal glycaemic control, both villous immaturity and a significant decrease in placental efficiency are observed [64] (Figure 2).

Placental vascular growth is regulated by angiogenic factors, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), transforming growth factor-β (TGF-β), and leptin, as well as anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) [51]. Toward the end of the second trimester, villous blood vessels begin to loop and coil, dramatically increasing the surface area for nutrient and gas exchange [60]. An imbalance between pro- and anti-angiogenic factors is considered to be involved in the pathogenesis of preeclampsia and IUGR [65] and altered levels of these factors have been found in women with obesity and GDM. Obesity has been associated with increased placental expression of VEGF [66] and decreased levels of circulating PlGF and sFlt-1 [67]. First trimester maternal serum levels of PlGF have been found to be elevated in women who go on to develop GDM [68]; while at term, mRNA and protein expression of placental VEGF are reduced in women with GDM [69]. In maternal omental adipose tissue (visceral fat), both obesity and GDM have been associated with increased gene expression of PlGF and sEng, as well as increased secretion of PlGF and sFlt-1 [70]. Altered levels of angiogenic factors in the maternal circulation may affect placental vascular development and lead to impaired fetomaternal circulation.

3. Placental Endocrine Functions

The placenta serves a variety of endocrine functions throughout pregnancy. A number of hormones are produced within the syncytiotrophoblast cell layer, including human chorionic gonadotropin (hCG), chorionic somatomammotropin hormone (CSH, also known as placental lactogen), and placental growth hormone (PGH) [71]. In addition to its role in early pregnancy in stimulating corpus luteal progesterone secretion, hCG also plays a role in trophoblast differentiation and invasion as well as in uterine and placental angiogenesis [72]. Maternal metabolism is regulated by CSH and PGH to ensure optimal nutrient availability and transfer to the developing fetus [73]. PGH is involved in the development of maternal insulin resistance in normal pregnancy, while CSH helps mediate the maternal leptin resistance [74].

Fetal growth is affected by altered levels of placental hormones; low and high expression levels of placental PGH/CSH genes have been associated with small for gestational age (SGA) and large for gestational age (LGA) neonates, respectively [75]. Abnormal placental endocrine functions are seen in maternal obesity and in GDM. As maternal pre-pregnancy BMI increases, serum hCG concentrations decrease, potentially contributing to the increased risk of miscarriage in obese women [72]. Additionally, obesity has been associated with decreased expression of

placental CSH and PGH [76]. Increased PGH has shown to increase insulin resistance in mice, and, in women with GDM, decreased circulating PGH has been associated with increased glycemia following an oral glucose load [77]. In normal pregnancy, leptin resistance increases gradually throughout gestation, peaking in late second or early third trimester [78]. Obesity alone is associated with increased circulating leptin concentration [79], and first and second trimester leptin levels are elevated in pregnant women who later develop GDM [80].

4. Placental Transport and Metabolism

Placental transport has a significant impact on the fetal environment. It acts as a nutrient sensor and is responsible for selectively transporting nutrients and respiratory gases to the developing fetus [81]. The placenta transports a variety of substances from the maternal to fetal circulation, including nutrients such as fatty acids, glucose, oxygen, amino acids, and vitamins. Additionally, the placenta acts as a protective barrier by limiting fetal xenobiotic exposure through selective drug transport. Placental transport proteins localized to the syncytiotrophoblast, the main exchange site of the placenta, can increase or decrease the net transfer of substances. Both facilitative and active transporters have been localized to both the maternal blood-facing microvillous brush-border and the fetal endothelial cell-facing basal membranes of the syncytiotrophoblast. Obesity and GDM are associated with changes in placental transporter expression (Table 1), which can affect fetal nutrient supply and drug exposure. Due to the strong association of GDM with obesity, very few studies have been able to separate specific effects of GDM from those of obesity on placental transporter expression. Reported effects may therefore show a significant overlap and it may remain difficult to provide details on etiology of these effects (e.g. insulin resistance, inflammation or lipotoxicity) from clinical studies alone.

Table 1: Effects of pregestational obesity and GDM on placental transport

	Pregestational Obesity	GDM	References
Fatty Acid Transport			
Fatty acid transport protein 1 (FATP1)	↓	↓	[82]
Fatty acid transport protein 2 (FATP4)	↓	↓	[82]
Fatty acid transport protein 6 (FATP6)	↑	↑	[82]
Fatty acid translocase (FAT/CD36)	↑	↑	[82]
Fatty acid binding protein 4 (FABP4)	↑		[82]
Fatty acid binding protein 7 (FABP7)	↑		[82]
Glucose Transport			

Glucose transporter 1 (GLUT1)	↑	↑*	[83, 84]
Glucose transporter 3 (GLUT3)	↑		[85]
Glucose transporter 4 (GLUT4)		↑/↓	[86, 87]
Amino Acid Transport			
System A	↑	↑*	[88, 89]
Small neutral amino acid transporter 2 (SNAT2)	↑		[88]
System L	↔	↑*/↔	[89-91]
Taurine transporter (TauT)	↓		[92]
Oxygen Diffusion			
Diffusional efficiency†	↓	↓*	[63, 64]
Vitamin/Cofactor Transport			
Folate receptor-α (FRα)	↑		[93]
Reduced folate carrier (RFC)	↓		[93]
Proton-coupled folate transporter (PCFT)	↔	↑	[93, 94]
Low density lipoprotein receptor (LRP2/megalin)	↓		[95]
Vitamin D receptor (VDR)	↓	↑*/↔	[95-97]
Cytochrome P450-27B1 (CYP27B1)	↓	↔	[95, 97]
Cytochrome P450-2J2 (CYP2J2)	↓		[95]
Cytochrome P450-4A1 (CYP4A1)		↑	[97]
Drug Transport			
Breast cancer resistance protein (BCRP)		↔*	[98, 99]
Multidrug resistance protein 2 (MRP2)		↔*	[98, 99]
P-glycoprotein (P-gp)	↓	↓*/↔*	[98-100]

†Diffusional efficiency is defined as reduced villous branching and an increased capillary count per villus.
*Maternal BMI not considered when comparing GDM to control groups.

4.1 Placental nutrient transport

Fatty Acids: The placenta regulates the availability of fatty acids to meet the increasing demands of the developing fetus through lipid transport and metabolism. The maternal surface of the syncytiotrophoblast contains lipases, such as endothelial lipase (EL), which hydrolyze maternal triglycerides (TG) to release non-esterified fatty acids (NEFA) [101]. NEFA can cross the placental membrane either by simple diffusion driven by the concentration gradient from mother to fetus, or facilitated diffusion by means of membrane transport proteins such as fatty acid transport proteins (FATPs), fatty acid translocase (FAT/CD36), and fatty acid binding proteins (FABPs) [101]. Within the placenta, fatty acids are metabolized, stored, or transported across the basal membrane into fetal circulation through facilitated and simple diffusion [101]. Changes in the placental lipid profile have been associated with both obesity and GDM. Obesity is associated with decreased mitochondrial fatty acid oxidation and saturated fatty acid content, as

well as increased placental lipid accumulation and metabolism with increased lipid esterification and storage [101]. Similarly, GDM is associated with decreased mitochondrial fatty acid oxidation, increased placental TG content, and a lower percentage of saturated fatty acids [82, 102]. Maternal obesity and GDM are independently associated with decreased mRNA expression of endothelial lipase, FATP1, and FATP4, as well as increased expression of FATP6 and FAT/CD36 [82]. Additionally, obesity is associated with increased expression of FABP4 and FABP7 [82].

D-glucose: Glucose transport across the placenta is accomplished by facilitated diffusion [103]. Localization of a sodium-independent transport system for D-glucose has been found on both the basal and apical membranes of the syncytiotrophoblast. In the human placenta, three transporter isoforms within the family of the classic glucose carriers (GLUTs) have been identified: GLUT1, GLUT3, and GLUT4 [104]. The basal membrane expression of GLUT1 is increased in obese women delivering macrosomic babies [105], and is positively correlated with birthweight [83]. Expression and activity of GLUT1 are considered rate-limiting steps in transplacental glucose transfer [106, 107] and this overexpression may contribute to increased glucose delivery to the fetus and fetal overgrowth [105]. These findings from human placentae are consistent with a mouse model of diet-induced maternal obesity in which placental transport of glucose is increased and suggested to lead to fetal overgrowth [108]. Similar results have been found in women with GDM, where basal membrane expression of GLUT1 in the placenta increases approximately 2-fold [84]. Furthermore, GDM is associated with a 40% increase in D-glucose uptake across the basal membrane, suggesting an increase in transplacental glucose flux in these pregnancies [84], which may contribute to fetal macrosomia.

There are limited findings on the effects of obesity and GDM on the other GLUT isoforms expressed in the placenta. In rats, diet-induced maternal obesity is associated with increased protein expression of GLUT3, especially in the placentas of male fetuses [85]. Interestingly, insulin-controlled GDM has been found to either increase [86] or decrease [87] GLUT4 protein expression, and these changes are not seen in diet-controlled GDM women, suggesting insulin treatment may alter the expression of glucose carriers.

Amino Acids: The transport of amino acids across the placenta occurs against a concentration gradient across the syncytiotrophoblast, resulting in a 2-fold higher intervillous blood amino acid concentration compared with maternal blood concentration [109]. There are over 20 known amino acid transporters, including 7 neutral amino acid transporters, such as system A and system L. The uptake of nonessential neutral amino acids into the cell is mediated by system A, which is a sodium-dependent transporter. System L is responsible for the transport of large branched and aromatic neutral amino acids independently of sodium [110]. The system A amino acid transporter activity and

protein expression of the small neutral amino acid transporter 2 (SNAT2) isoform within this system are increased, in placentas of obese women giving birth to large babies [88]. In contrast, obesity does not appear to alter system L activity in primary human trophoblast cells [90]. In syncytiotrophoblast microvillous membranes, GDM is associated with increased system A and system L amino acid transport activity; however, this increase is not seen for the transport of all amino acids within these systems [89]. Furthermore, placental perfusion studies have found the GDM does not affect system L transport activity [91].

Taurine is an important amino acid for promoting the development of fetal brain, heart, kidney, pancreas, retina, and skeletal muscle [92]. Taurine in human pregnancy is conditionally essential, as the fetus and placenta lack the enzyme required for taurine synthesis, and thus demand must be supplied through maternal blood [111]. Taurine is transported through the syncytiotrophoblast through the transporter TauT [112]. Activity of TauT in human placenta is negatively correlated to maternal BMI over the range 18-46 kg/m^2 in both the first trimester (7-12 weeks gestation) and at term [92]. This reduction in activity may be a consequence of increased neuropeptide Y, which is elevated in obesity [113], and the reduction of taurine within the placenta and transfer to the fetus may predispose the pregnancy to abnormal placental development and fetal growth restriction [92].

Oxygen: Oxygen diffusion across the placenta is driven by the concentration gradient between oxygenated maternal blood and deoxygenated fetal blood. Factors that can affect oxygen diffusion across the placenta include the position of the villus within the intervillous space, the proximity of surrounding villi, as well as the caliber, position and number of capillaries within each villus [114]. Diffusional efficiency (i.e. oxygen transport per capillary) decreases with increasing number of capillaries within a villus [114]. Histological studies reveal reduced villous branching and a higher capillary count per villus in placenta from women with obesity or GDM [63, 64]. The increased number of capillaries in each villus restricts blood flow within the intervillous space, thus reducing oxygen exchange between mother and fetus [115]. Furthermore, GDM is associated with reduced oxygen content and saturation, as well as increased lactate concentrations in the umbilical vein but not in the umbilical artery, suggesting that GDM alters placental oxygen exchange and/or metabolism [116].

Vitamins and Cofactors: The availability of vitamins and cofactors for the fetus relies on placental transport from the maternal circulation. Obesity and GDM have shown to alter transport of some essential vitamins and cofactors, including folate and vitamin D, which are widely studied in pregnancy. Folate is involved in DNA and RNA biosynthesis and is a cofactor of the vitamin B12-dependent enzyme, methionine synthase, which converts the amino

acid homocysteine to methionine [117]. Transport of folate from mother to fetus is crucial for placental and fetal development as neither can synthesize the vitamin [94]. Obesity is associated with increased expression of folate receptor-α (FRα) in microvillus membranes and decreased reduced folate carrier (RFC); however, obesity does not appear to affect protein expression of proton-coupled folate transporter (PCFT), fetal folate levels, or the activity of these three folate transporters [93]. Additionally, umbilical cord folate levels are unaffected by maternal BMI [118], suggesting that the placenta's capacity to maintain fetal folate transfer is not compromised by obesity. In human cytotrophoblasts, GDM is associated with increased rates of folic acid transport and folic acid uptake is more dependent on PCFT compared with controls [94].

Vitamin D was shown to be involved in a number of processes throughout pregnancy, including conception, implantation, placental development, as well as placental calcium transport and immune function [119], though its main function is to maintain physiological levels of calcium [120]. Obesity and GDM are both associated with vitamin D deficiency, which can result in impaired fetal growth and poor skeletal mineralization due to lack of calcium. In a pregnant baboon model, maternal obesity is associated with the downregulation of the placental vitamin D transporter megalin (LRP2) and the vitamin D receptor (VDR), as well as a reduction in enzymes involved in the activation of vitamin D, including cytochrome P450 27B1 (CYP27B1) and the 25-hydroxylase CYP2J2, which can also lead to suboptimal vitamin D status [95]. In EVCT and fetoplacental endothelial cells, GDM is associated with VDR upregulation, possibly in response to low maternal vitamin D [96], although no change in VDR mRNA expression is seen in placental tissue from GDM women [97]. Additionally, GDM is associated with increased mRNA and protein expression of placental CYP24A1, which catabolizes vitamin D into its biologically inactive form, contributing to the low vitamin D levels seen in GDM patients; however, GDM does not affect expression of CYP27B1 [97].

4.2 Placental drug transport

The transplacental transfer of both endogenous and exogenous substances is mediated by numerous factors, including physiochemical (i.e. size, pKa and lipid solubility) and pharmacokinetic (maternal clearance, protein binding and metabolism) properties of the substrate [121]. The ATP-binding cassette (ABC) drug transporter family plays a key role in important organs, such as the liver and intestine, to protect against toxins, and uses ATP hydrolysis to efflux the substrate bound to the plasma membrane against a concentration gradient [122]. The placenta expresses a number of ABC transporters, including P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), multidrug resistance protein 2 (MRP2), and MRP3, to protect the fetus from overexposure to toxins, xenobiotics, other toxic

metabolites [123]. The metabolic, oxidative and inflammatory stress associated with obesity and GDM can affect the expression of these ABC transporters and lead to changes in fetal development.

The expression of P-gp, which has been localized to the brush-border membrane of the syncytiotrophoblast, is present throughout gestation [124-126] and gradually decreases toward term [126, 127]. Placental expression of P-gp is comparable to that in the intestine and liver [128] and has been shown to mediate fetal exposure to many drug classes, including oral antidiabetic agents such as glyburide, metformin and rosiglitazone [129]. For example, transplacental transfer of digoxin, a treatment of choice for fetal arrhythmia, is significantly controlled by placental P-gp, as it known to efflux this medication back into the maternal circulation [121]. In mice, decreased expression of P-gp is associated with increased digoxin transfer to the fetus [100]. In placental tissue and in C57BL mice, obesity has shown to reduce P-gp mRNA and protein expression, and this corresponded with elevated levels of maternal serum inflammatory markers IL-1β and TNF-α, suggesting the decreased expression of P-gp may be due to an increased inflammatory profile [100]. Studies have found either a slight reduction [98] or no change [99] in P-gp expression levels in GDM placentas.

Although the effect of obesity on placental BCRP expression has not yet been studied, protein and mRNA expression of BCRP are increased in placentas with inflammation [130]. In the intestine, expression of BCRP is decreased in obese compared with normal weight humans [122]. Disruption of the intestinal barrier may contribute to the chronic low-grade inflammation associated with obesity and GDM; however, studies have shown no change in placental BCRP or MRP2 expression in pregnancies affected by GDM [98, 99]; although these studies only looked at insulin-managed GDM patients. Interestingly, one study found a positive correlation between hemoglobin A_{1c} levels and both BCRP protein and mRNA expression in diabetics requiring insulin, suggesting that poorly managed hyperglycemia may be associated with an increase in the expression of placental efflux transporters [99]. Under hypoxic conditions, protein expression levels of BCRP and P-gp are elevated in first trimester human placental villous explants [131]. Thus, consequences of obesity and GDM, such as increased inflammation and hypoxia, may alter placental drug transport and fetal drug exposure, and should be taken into consideration when treating patients during pregnancy.

In summary, there is considerable overlap between obesity and GDM surrounding their impact on placental development and function; common patterns include reduced spiral artery remodeling leading to restricted maternal blood flow, altered nutrient transport and fetal nutrient supply leading to abnormal fetal growth, changes in endocrine

functions leading to further insulin and leptin resistance, and changes in labour patterns. The inflammatory and metabolic abnormalities associated with obesity and GDM are likely to blame for many of these changes; however, there remains many unanswered questions about the interplay between these processes. Nonetheless, as our knowledge of the normal and abnormal formation and function of the placenta has grown, the logical next step is to choose therapeutic targets for the prevention and treatment of obesity- and hyperglycaemia-related complications of pregnancy.

5. Preventive and Therapeutic Interventions

The use of preventive and therapeutic interventions for pregnancies affected by obesity and GDM are mainly based upon retrospective analyses of third-trimester placentas and in vitro and animal models. Multiple medications have been suggested, most of which target the inflammatory or metabolic changes commonly observed in obesity and GDM. However, these studies have primarily focused on the prevention of specific complications which are more common in obesity such as preeclampsia, or targeting specific complications associated with GDM such as insulin resistance, fetal macrosomia and gestational weight gain. A number of anti-hyperglycemic, anti-platelet, and antioxidant agents, commonly used in the treatment or prevention of other disorders, may help to counteract the inflammatory and metabolic changes of obesity and GDM and prevent the development of the associated obstetric complications.

Metformin: Oral anti-hyperglycemic agents such as metformin and glyburide have become increasingly used for pregnant patients with gestational diabetes, as an alternative or adjuvant therapy to insulin. The anti-hyperglycemic actions of metformin include decreasing hepatic glucose production and intestinal absorption of glucose, as well as improving insulin sensitivity by increasing peripheral glucose uptake and utilization [132] without affecting insulin levels. Evidence has accumulated that there may be additional benefits beyond its anti-hyperglycemic effects, decreasing gestational weight gain [133], neonatal hypoglycemia, neonatal intensive care unit admission, and macrosomia [134], and a decrease in the risk of gestational hypertension and preeclampsia [135]. In nondiabetic obese pregnant women, metformin similarly shows a reduced frequency of preeclampsia and gestational weight gain [135, 136], a decreased risk of severe hypoglycemia in the neonate and increased subscapular and biceps skinfolds and upper arm circumferences, while leaving total body fat, blood pressure, and neurodevelopment unchanged at the age of two [137-139]; however, lower doses of metformin have not demonstrated this effect [140]. Small longer term

follow-up studies have indicated that by 8-9 years of age, children who had prenatal exposure to metformin were larger with higher fasting glucose and lower low-density lipoprotein, compared with those who had only been exposed to insulin [138, 141], suggesting there may be long-term metabolic effects on the offspring.

Metformin acts directly on the placenta and its vasculature, and has been shown to reduce endothelial dysfunction, enhance vasodilation in omental arteries, and induce angiogenesis [142]. It reduces sFlT-1 and sEng secretion from primary trophoblasts, possibly by inhibiting the mitochondrial electron transport chain, the activity of which is increased in preterm preeclamptic placenta. Based on these observations, metformin has been suggested to prevent preeclampsia in women with obesity, and although initial studies were promising, a recent meta-analysis failed to demonstrate a beneficial effect and suggests that metformin should be used for the treatment of GDM [136, 143]. Additionally, it has been suggested that metformin treatment should be discontinued if there are signs of placental insufficiency such as IUGR, abnormal dopplers and/or maternal preeclampsia [138]. This practice is primarily based on theoretical concerns that metformin does not only ameliorate the effect of excess fuels but may move the fetal environment into one of inadequate fuel supply. Compared with insulin, metformin treatment of GDM results in greater increases in maternal serum amino acids alanine, isoleucine and lactate [144]. As many amino acids are transported across the placenta [109], higher levels of these amino acids in the maternal circulation may alter placental transport and supply to the fetus, with differential effects on placenta and fetus depending on the amino acid type or function. For example, branch chain amino acids (BCAA), including leucine, isoleucine and valine have been associated with insulin resistance in obesity and levels of BCAA have a positive correlation with pre-pregnancy BMI [145]. These BCAA have shown to reduce insulin resistance, promote fatty oxidation and glucose transport, and improve fetal intrauterine growth [146]. Higher levels of BCAA have also been seen in women with GDM near term and these increased levels correlate with neonatal weight and adiposity as well as childhood obesity risk [145]. Other amino acids have demonstrated beneficial effects on maternal, placental, and fetal health and development. Arginine has shown to decrease adipose tissue deposition in obesity, alleviate vascular insulin resistance in obesity and type 2 diabetes, and lead to improved placental and fetal growth [146]. Pre-pregnancy levels of carnitine, which is synthesized from lysine and methionine, correlates with maternal BMI, and decreased levels of carnitine are associated with maternal fatty acid accumulation, hyperlipidemia and adipose tissue deposition [147]. Glycine improves the maternal cytokine profile and reduces oxidative stress, apoptosis, hypertension, dyslipidemia and insulin resistance, and decreased levels of glycine are associated with adverse fetal growth and development [146]. Thus, targeting

maternal amino acid levels may help to mitigate the negative effects of obesity and GDM and improve pregnancy outcomes.

Myoinositol: A component of the cell membrane and in citrus fruits, vegetables, and seeds, myoinositol is considered to belong to the vitamin B complex. However, in the human body, it is produced from glucose. At the cellular level, myoinositol is converted into D-chiro-inositol phosphoglycan, which acts as a second messenger in the insulin pathway, promoting insulin-like effects and increasing insulin sensitivity [148]. Thus, numerous studies have evaluated myoinositol in the prevention of GDM [149].

Lower levels of maternal myoinositol are detected in a mouse model of diet-induced obesity [150]. In the first randomized controlled trial evaluating the role of myoinositol in GDM prevention, improved insulin resistance and fasting glucose levels was found in women with GDM who were administered myoinositol plus folic acid, compared to folic acid alone [151]. Several subsequent trials in both non-obese and obese women [152-154], as well as a Cochrane review [155], found a lower incidence of GDM in patients treated with myoinositol. Given this evidence, myoinositol is a promising preventive therapy for GDM in high risk populations and can aid in the prevention of negative effects of GDM on the placenta and fetus. Further studies to evaluate to effect of myoinositol on vascular modeling and placenta function are needed.

Choline: Prevention of the negative impact of obesity or GDM on the placenta involves normalizing the changes in placental morphology and transport function [156]. The essential nutrient choline has been investigated as a potential treatment to prevent the effects of obesity on the placenta. Choline has various functions in cellular membrane structure, cellular signaling, epigenetics, and neurotransmission. When demand for choline is high, such as during pregnancy, it is oxidized to betaine [157], and, during this process, methyl groups become available for methylation reactions. In a mouse model, choline and betaine have shown to modify fetal growth as a result of downregulation of the placental growth promoter insulin-like growth factor 2 [158-160]. Additionally, choline decreases fetal adiposity, including normalization of fetal hepatic accumulation of triglycerides in obese mice [159]. In a mouse model of maternal obesity, choline supplementation is associated with decreased placental expression of GLUT1 and FATP1, as well as a lower accumulation of glycogen in the placenta [160]. Furthermore, both choline and betaine supplementation significantly reduce glucose and fatty acid accretion in a human choriocarcinoma cell line, normalize macronutrient transporter expression in human trophoblasts, and mitigates placental morphological changes arising from GDM in mice [156]. Thus, choline treatment may help to improve placental transport that may

be altered in obesity and GDM. In humans, higher maternal choline intake during the third trimester is associated with a decreased expression of placental sFlt-1 [161], suggesting choline treatment may improve placental angiogenesis and help mitigate placental vascular dysfunction in obesity and GDM.

Acetylsalicylic Acid: In North America, obesity is the most significant risk factor for the development of preeclampsia, and GDM further increases this risk and contributes to both maternal and fetal morbidity. To address the endothelial dysfunction and activation of the coagulation system associated with preeclampsia [162], multiple studies have explored anti-platelet agents, in particular low-dose acetylsalicylic acid (ASA), to prevent preeclampsia in low and high-risk populations. The mechanism of action is through the inhibition of cyclooxygenase (COX)-1- and COX-2 [163], expressed in whole placental villi and villous core compartments, but not in the trophoblast itself [164, 165]. COX-1 and COX-2 are essential for prostanoid biosynthesis, and through production of prostaglandin (PG) PGG_2 and PGH_2 affect the production of TXA_2, prostacyclin (PGI_2), and other prostaglandins. These prostaglandins, produced by platelets (TXA_2) and vascular endothelial cells (PGI_2), play a role in inflammation mediated vasoconstriction, vasodilatation, vascular remodeling, platelet aggregation and adhesion and renal function [163]. The affinity of ASA is 10-100 times higher for COX-1 than COX-2 and will only bind to COX-1 when administered at low doses (75-100 mg/day) [163]. However, more recent evidence suggests a greater contribution of COX-2 than COX-1 in the mechanisms implicated in the pathogenesis of preeclampsia. Up-regulation in the placenta of key drivers of inducible COX-2, including hypoxia and inflammatory mediators, likely drives the shift towards vasoconstrictor prostanoids [166]. The restoration of the prostacyclin to thromboxane ratio and amelioration of this vasoconstrictor response to inflammation and hypoxia is the main rationale for using low-dose ASA for the prevention of preeclampsia. There is increasing evidence to support using higher dose regimens (>75-100 mg) in order to exert more of an effect on COX-2 inhibition [163].

Meta-analyses suggest moderate benefits of low-dose ASA with <20% reduction in risk of early preeclampsia, preterm birth, SGA, stillbirth and neonatal death, provided it is started at <16 weeks gestational age and is taken daily at a dose of at least 100 mg/day [167]. Risks of this regimen are considered extremely low; low dose aspirin may only be associated with a marginal increase in risk of placental abruption and postpartum hemorrhage [167-169]. Based on this evidence, the United Kingdom's National Institute of Health and Care Excellence (NICE) guideline [170] recommend prescribing aspirin in a dose of 75-150 mg/day to women with major risk factors such as pre-existent diabetes type 1 or 2, while a pre-pregnancy BMI of 35 kg/m^2 as a moderate risk factor of which 2 need to

be present to advise. This preventive strategy has been more effective in reducing the frequency of preterm preeclampsia [167], associated with shallow trophoblast invasion resulting in placental insufficiency and IUGR in preterm pregnancies, than in late onset preeclampsia, the more prevalent presentation in women with obesity and/or GDM that is thought to be the consequence of a maternal inflammatory response in an otherwise normal or large placenta [171-173].

Most guidelines recognize that obesity is an important player in preeclampsia risk; however, studies have failed to identify obesity as an independent factor affecting the efficacy of low-dose ASA in the prevention of preeclampsia [174]. People with obesity typically have larger blood and tissue distribution volumes, increased liver blood flow and glomerular filtration rates, which may affect drug metabolism and elimination [175]. Additionally, obesity is associated with higher clearances of drugs metabolized through several hepatic and renal drug metabolism pathways, including CYP2C19, a mediator in the metabolism of ASA [175]. Levels of thromboxane B_2, a highly specific marker for the nearly complete suppression of thromboxane A_2 production that is required to have a measurable impact on thromboxane-dependent platelet function and inhibition of platelet-aggregation, are higher in women with elevated BMI, especially in women with class III obesity [176]. These studies suggest that higher doses or frequency of ASA than currently recommended may be required in women who have obesity as an additional risk factor for preeclampsia.

Melatonin and Other Antioxidants: Melatonin is an endogenously produced hormone synthesized from L-tryptophan and is considered to be a highly efficient antioxidant [177]. It has the potential to scavenge free-radicals and reduce oxidative damage in the placenta by increasing antioxidant enzymes and decreasing lipid peroxidation [178]. It is thought to be more potent and have a broader range of efficacy towards different toxins compared with vitamins C or E [177]. Melatonin is important in blood pressure control and in adipose tissue dysfunction through multiple anti-inflammatory/antioxidant actions, including protection against mitochondria-mediated injury in hypertension and obesity [179, 180].

Synthesis of melatonin has been identified in the placenta [181]. Using a human placental explant model, melatonin was shown to reduce oxidative stress and enhance antioxidant markers [182]. It did not, however, affect secretion of sFlt, sEng or activin A. Reduced nocturnal melatonin levels have been found in pregnant women with severe preeclampsia [183]. Furthermore, lower levels of melatonin in pregnancy are associated with a higher risk of developing preeclampsia [184]. In a small phase I study of patients with preeclampsia, melatonin extended the mean

diagnosis to delivery interval by 6 days and reduced the need for increasing antihypertensive medication. Notably, mean BMI in both case and control groups was 29-30 [182].

Testing the antioxidant potential of serotonin (5-hydroxy tryptamine, 5-HT) in pregnancies affected by obesity or GDM has also recently been suggested [185]. Serotonin, similar to melatonin, is also a product of tryptophan. Serotonin has been reported to have significant protective roles against oxidative stress by directly scavenging free-radicals, sequestering metals, and inhibiting free-radical production [186]. Disruption in normal serotonin physiology has been reported in obese women during pregnancy and GDM.

Free levels of 5-HT are reported to be increased in GDM [187] and in obese pregnant women [188] compared to uncomplicated lean pregnant women. Changes in 5-HT levels may lead to the dysregulation of pancreatic glucagon secretion in response to changes in glucose concentrations [188]. Increased maternal free 5-HT levels may increase placental 5-HT levels and potentially lead to preplacental vasoconstriction, elevating vascular resistance and increasing the local blood pressure to the placenta [189]. Placental serotonin transporter (SERT) is increased in GDM pregnancies [190] and SERT mRNA is also increased in obese women with GDM treated with insulin compared with BMI matched controls [190]. A positive correlation was also found between placental SERT mRNA and maternal BMI at 12 weeks gestation and delivery in women with GDM treated with insulin [185]. Expression of the 5-HT receptor (HTR2A) mRNA was decreased by 79% in placental tissue from overweight and obese mothers with GDM [191]. The changes in serotonin are complex in obesity and GDM but may be a target for pharmacotherapies in the future [185].

Other antioxidants, including vitamins C and E, may be useful in reducing the oxidative stress associated with obesity and GDM. Obesity has been associated with lower maternal serum levels of vitamins C and E [192]. In a rat model of maternal obesity, supplementation with an antioxidant cocktail, including vitamins C, E, and A, reduced oxidative stress and prevented the development of adiposity and glucose intolerance in the offspring [193]. Vitamin C supplementation has also shown to reduce maternal and placental oxidative stress and improve neonatal outcomes in women with GDM [194]. However, these vitamins have been trialled as preventive therapies for preeclampsia with disappointing results. Cochrane systematic reviews of vitamin C [195] and E [196] failed to demonstrate prevention of fetal or neonatal death, poor fetal growth, preterm birth or preeclampsia. These vitamins were found to increase the risk of term premature rupture of membranes in this same review.

Exercise: In a mouse model of maternal obesity, exercise has shown to reduce maternal weight gain, lower maternal serum glucose and lipid concentration, improve maternal insulin sensitivity, and prevent fetal macrosomia [197]. In the placenta, a high-fat diet has been found to decrease the area of the junctional zone and increase the labyrinth zone, and this is reversed by exercise training. Furthermore, a maternal high fat diet leads to increased placental lipid accumulation, and this increase is prevented by maternal exercise [197]. Thus, exercise is a potentially inexpensive treatment to mitigate the effects of maternal obesity on the placenta, since even walking has been shown to be beneficial for pregnancy and healthy weight gain [198]. Studies in human pregnancy are needed in order to support the translation of these findings from animals to humans.

6. Conclusion

Changes in the intrauterine environment of women with obesity or GDM affect the development and function of the placenta, are associated with poor pregnancy outcomes and can lead to cardiometabolic abnormalities in the offspring. This is becoming increasingly more important as rates of obesity and GDM continue to rise around the world. Obesity and GDM share similar characteristics, such as increased inflammation and oxidative stress, dyslipidemia, and altered hormone levels, all which contribute to changes in the placenta from implantation through to parturition. As the placenta is constantly adapting to its environment, significant changes in early placental development can modify placental structure and function, which, in turn, affect the developing fetus.

A number of preventive and therapeutic interventions have been studied to combat the effects of obesity and GDM on the placenta, and although many have failed to show a beneficial effect, some may benefit placental function through effects on one or more processes altered by obesity or GDM (Table 2). For example, metformin treatment may reduce insulin resistance in pregnant women with obesity or GDM and may reduce placental endothelial and vascular dysfunction by regulating the secretion of angiogenic factors. Myoinositol is another possible treatment to reduce insulin resistance and fasting glucose levels, especially in women at high-risk for GDM. Choline supplementation may be useful in regulating nutrient and drug transport across the placenta by regulating levels of placental transport proteins that may be altered in obesity or GDM and can improve placental angiogenesis leading to improved vascular function in obesity and GDM. ASA may be used to reduce the risk of preeclampsia and/or IUGR in women with obesity or GDM, by decreasing the systemic inflammatory response or by improving placental vascular health. Melatonin and other antioxidants may be useful in combating the oxidative stress brought on by maternal

obesity or GDM. Individually, these interventions may help to mitigate the consequences of obesity and GDM and prevent the development of pregnancy complications, such as hyperglycemia, excessive gestational weight gain, fetal macrosomia, IUGR, and preeclampsia. However, since so many processes are altered in the placenta affected by obesity and GDM, the treatment of a single metabolic or inflammatory pathway may be less likely to induce an effect on pregnancy complications than a combined approach could be.

Table 2: Summary of potential preventive interventions to combat effects of pregestational obesity and GDM on the placenta

Consequence of Pregestational Obesity or GDM	Intervention	Mechanism of Action	References
Insulin Resistance and Hyperglycemia	Metformin	- Decreasing hepatic glucose production and intestinal absorption of glucose - Increasing peripheral glucose uptake and utilization	[132]
	Myoinositol	- Is converted into D-chiro-inositol phosphoglycan, which acts as a second messenger in the insulin pathway	[148]
Endothelial and Vascular Dysfunction	Metformin	- Improving angiogenesis by regulating expression of placental angiogenic factors (i.e. reducing sFlt-1 and sEng expression)	[142]
	Choline	- Improving angiogenesis by regulating expression of placental angiogenic factors (i.e. reducing sFlt-1 expression)	[161]
	ASA	- Inhibiting the inflammation-mediated vasoconstrictor response driven by COX-1 and COX-2	[163]
Altered Placental Transport	Choline	- Regulating expression of placental glucose and fatty acid transporters - (i.e. decreasing GLUT1 and FATP1 expression)	[160]
	Metformin	- Altering maternal amino acid concentrations (i.e. increasing maternal circulating levels of alanine, isoleucine and lactate)	[144]
Oxidative Stress	Melatonin	- Increasing antioxidant enzymes and decreasing lipid peroxidation	[178, 182]

Very limited literature exists on the differential effects of obesity and GDM on placental development, as most studies on GDM and pregestational diabetes do not take obesity into account. A common thread may be the heightened inflammatory response, which may be a consequence of lipo- or glucotoxicity, regardless of the aetiology. When treatment modalities are considered, differentiating between obesity with and without GDM is important. Furthermore, not all obese women go on to develop metabolic and cardiovascular abnormalities during their reproductive years. More often than not, studies have focused upon the healthy obese population, who may be more

similar to normal weight patients than metabolically unhealthy obese patients. When determining a treatment plan, it is important to consider the overall metabolic and cardiovascular health of the patient, rather than using BMI alone.

It is evident that the placenta plays a major role in fetal programming; however, the placenta is a complex organ, and a number of intricate pathways involved that may be altered by maternal cardiometabolic abnormalities that are not covered in this review as they are not yet fully understood. Future research should aim at unravelling the mechanisms that link maternal cardiometabolic health to placental dysfunction and consequences in the offspring, which would help to improve the prevention and treatment strategies in women with obesity and GDM.

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Figures:

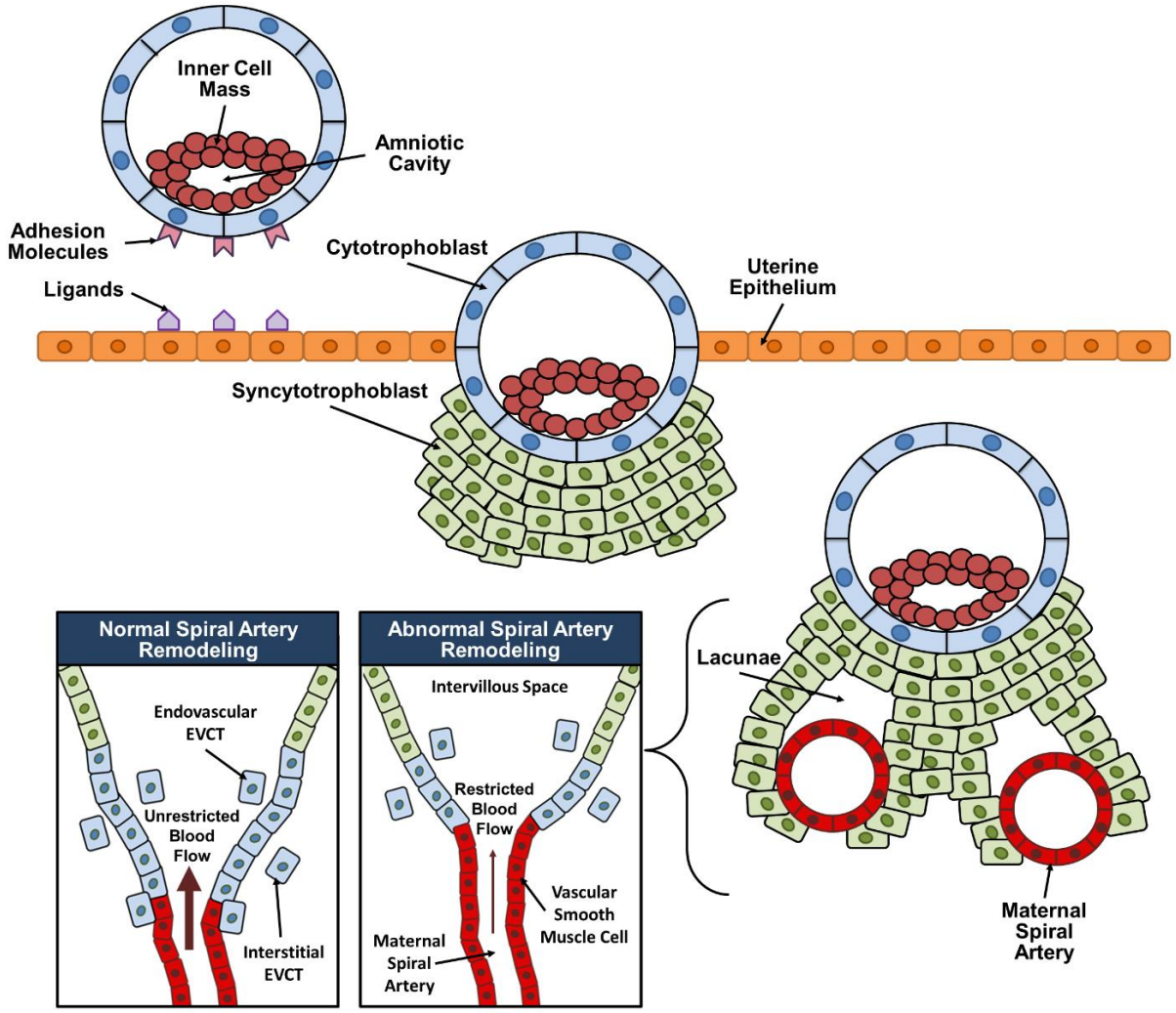

Figure 1: Early placental development

The blastocyst attaches to and invades the maternal uterine epithelium. Once in the blastocyst successfully implants into the uterine endometrium, lacunae, which give rise to the intervillous space, form within the syncytiotrophoblast and the remodeling of the maternal spiral arteries begins. In a normal pregnancy, cytotrophoblast cells disrupt the vascular smooth muscle cells surrounding the maternal spiral arteries, allowing for maternal blood to flow freely into the intervillous space. Insufficient spiral artery remodeling restricts blood flow and has been associated with both obesity and GDM and is a common pathology in preeclampsia and IUGR.

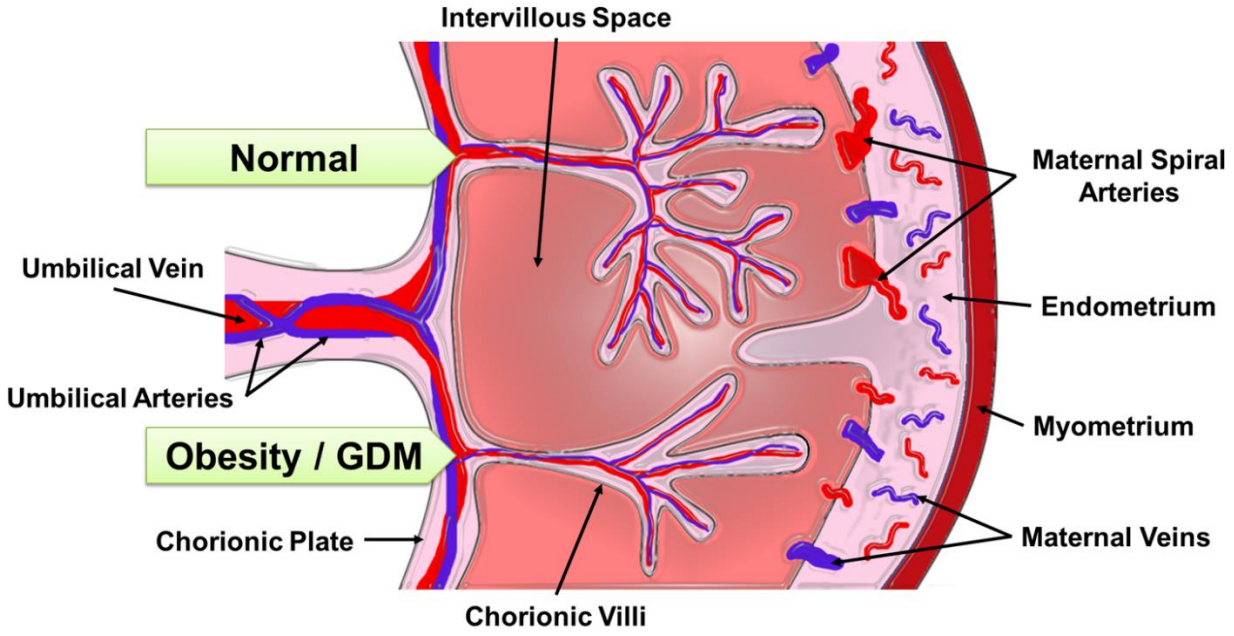

Figure 2: Placental vasculature

Both pregestational obesity and GDM can individually lead to villous immaturity, characterized by fewer branching terminal villi and increased capillary count within each villus. The increased number of capillaries within the villi restricts blood flow within the intervillous space and thus reduces nutrient and gas exchange between the mother and fetus.