Don't sugar coat it: the effects of gestational diabetes on the placental vasculature

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The placenta is a temporary organ, which facilitates the exchange of nutrients, waste and gases between the maternal and fetal circulatory systems. To perform its role, the placenta is a villous structure, which branches to cover a large surface area. In gestational diabetes (GDM), a major complication that affects otherwise healthy pregnancies, the placenta displays aberrant vasculature, including altered vascularization, villous immaturity, and endothelial dysfunction. Several contributors including reactive oxygen species (ROS), advanced glycation end products (AGEs) and the dysregulation of key angiogenic factors have been attributed to vascular dysfunction in GDM.

The placenta

Within the vascular structure of the placenta, the chorionic villi contain the fetal capillaries. These villi are bathed in maternal blood, within the intervillous space, and are the site of maternal/fetal exchange. In addition to vascular cells, syncytiotrophoblast and cytotrophoblast cells make up the trophoblast compartment of the placenta (see Table 1 for definitions). The syncytiotrophoblast is a multinucleated syncytium, in contact with the maternal blood. The syncytium has several functions: it not only serves as the transporting epithelium of the placenta but is also responsible for protecting the fetus from the maternal immune system and for the endocrine functions of the placenta, releasing hormones and growth factors important for placental development and maintaining pregnancy. Maintenance and expansion of the syncytiotrophoblast over pregnancy is provided by continuous proliferation, differentiation and fusion of the underlying cytotrophoblasts. As the trophoblast stem cell population of the placenta, cytotrophoblasts also differentiate into extravillous trophoblasts which migrate into the maternal decidua to remodel the maternal spiral arteries, regulating the maternal blood flow to the placenta. Beneath the cytotrophoblasts, within the villous core, lie several stromal cells, including fibroblasts, placental macrophages, known as Hofbauer cells, and placental mesenchymal stem cells which differentiate into many cell lineages, including endothelial and smooth muscle cells which form the blood vessels in the placenta (Figure 1).

Placental vascular development

Villous development relies heavily on vasculogenesis, the *de novo* formation of blood vessels, and angiogenesis, the branching of existing vessels. The process begins with the primitive mesenchymal villi that promote villous proliferation, mediated by the syncytiotrophoblast. These villi become tertiary villi through the growth of endothelial cell cords, via vasculogenesis. These cell cords develop a lumen and give rise to the first fetal capillaries. Once vasculogenesis has progressed and a primitive capillary network has formed, shown in Figure 2, the process of angiogenesis can begin, usually around 32 days after conception. Angiogenesis is dependent on trophoblast sprouting and proliferation allowing existing vessels to branch or elongate. This vast network of capillaries developed by these processes forms the chorionic villi.

Several pro- and anti-angiogenic factors are responsible for regulating angiogenesis and vascular development in the placenta. VEGF is one of the most essential growth factors responsible for regulating angiogenesis, and there are several forms of the VEGF receptor including VEGFR1, VEGFR2 and VEGFR3. Placental growth factor (PlGF) is also a member of the VEGF family and controls trophoblast proliferation and differentiation. It is also thought to initiate angiogenesis by either displacing VEGF from the VEGFR1, increasing the availability of VEGF that can bind to VEGFR2 or acting on VEGFR1 receptors and eliciting intracellular signals through this receptor. While VEGF and PlGF elicit pro-angiogenic responses in the placenta, the soluble form of VEGFR1, sFlt1, is anti-angiogenic,

Table 1.

Placental structure/cell type	Definition
Chorionic villi	Functional units of the placenta, which contain the fetal capillaries and are bathed in maternal blood
Cytotrophoblast	Proliferate, differentiate and fuse to form the overlying syncytiotrophoblast. Also differentiate into extravillous trophoblasts
Extravillous trophoblast	Invasive cytotrophoblasts, which remodel the maternal spiral arteries to regulate maternal blood flow to the placenta
Hofbauer cells	Placental macrophages
Intervillous space	The space between the chorionic villi, which contains maternal blood
Maternal decidua	The maternal part of the placenta
Maternal spiral arteries	Provide maternal blood to the placenta in the intervillous space
Placental mesenchymal stem cells	Mesenchymal stem cells of the placenta, which are multipotent and can differentiate into many cell lineages, including endothelial and smooth muscle cells
Syncytiotrophoblast	Multi-nucleated syncytium (single-cell layer). It is the outer layer of the chorionic villi in contact with the maternal blood. Releases hormones and growth factors during pregnancy

acting as a VEGF and PlGF antagonist by blocking their binding to their receptors. Similarly, fibroblast growth factors (FGFs) are highly abundant in the placenta and are known to be important in trophoblast migration, self-renewal, and invasion. Particularly, FGF2 is known to play a significant role in placental angiogenesis and placental vascular growth throughout gestation.

In several complications of pregnancy, including gestational diabetes (GDM), there is villous immaturity and changes to placental vascularization and endothelial dysfunction are observed. This placental vascular dysfunction can compromise nutrient and waste exchange between the mother and the fetus, leading to placental insufficiency, where the placenta cannot fully support the developing fetus.

Gestational diabetes

GDM is one of the most common complications of otherwise healthy pregnancies, in which women without pre-existing diabetes develop a state of chronic hyperglycaemia during pregnancy. This metabolic condition occurs as a result of 'diabetogenic' changes in pregnancy, in which an array of contributory mechanisms elevate maternal glucose levels for fetal consumption. In the UK alone, GDM is diagnosed in up to 16% of pregnancies and prevalence is increasing in association with the rising prevalence of obesity. However, this worldwide problem has even higher rates of prevalence in developing countries, such that in the Punjab region of India, GDM has a reported prevalence of up to 35%.

There are a plethora of peri-natal complications that arise in poorly controlled GDM; most notably, infants can be born large for gestational age (LGA), predisposing to multiple difficulties during birth including shoulder dystocia, newborn asphyxia, and requirement for an emergency Caesarean section. Furthermore, infants born to GDM mothers also have a higher prevalence of respiratory distress syndrome due to hyperglycaemia-induced delay in fetal lung maturation. There is also the risk of neonatal hypoglycaemia, which can be easily rectified, but failure to diagnose promptly can produce seizures and rarely fatality. In addition to the short-term consequences, exposure to a GDM environment *in utero* is associated with several biochemical alterations in the fetus that elevates long-term cardiometabolic risks for baby, indicating the public health burden of this condition may extend beyond our initial expectations.

Complications are not only restricted to the fetus, with maternal consequences at birth from delivering LGA infants, an elevated risk of pre-eclampsia and meta-analysis data indicating the mother's lifetime risk of a type 2 diabetes mellitus diagnosis was above 50%. Aside from this, these women are also at a twofold

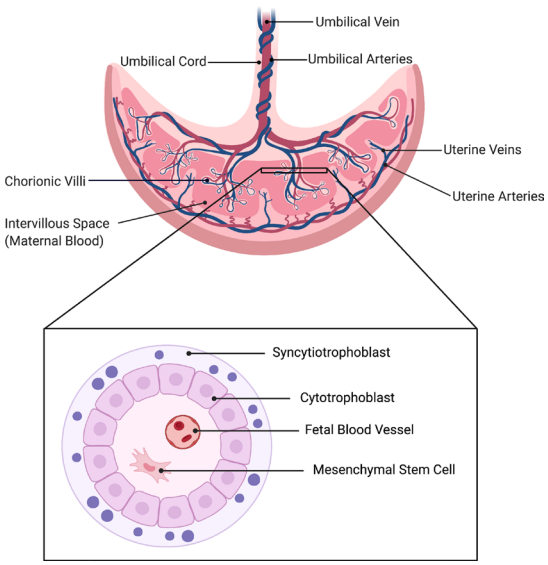

Figure 1. Structure of the placenta and its vasculature. Maternal uterine arteries supply oxygenated blood to the intervillous space, for the transport of oxygen and nutrients to the fetal circulation. The umbilical cord connects the placenta to the fetus, with the umbilical vein delivering the oxygenated and nutrient-rich blood to the fetus. The umbilical arteries carry deoxygenated blood to the placenta, for waste and carbon dioxide to be removed by the maternal veins. **Inset:** Schematic representing a cross section of the placental chorionic villi, containing the trophoblast layers (syncytiotrophoblast and cytotrophoblast), fetal blood vessels and placental mesenchymal stem cells. Other stromal cells are also present within the placental villi, including Hofbauer cells (placental macrophages) and fibroblasts. Figure created with Biorender.com.

high risk of cardiovascular events post-partum than mothers without GDM, regardless of their diabetes status. Comparative to the available information on peri-natal complications, the mechanisms by which these long-term maternal and fetal cardiometabolic complications arise are not fully understood. However, it is thought to link to biochemical alterations in the placenta. The distinct association between GDM pregnancies and transgenerational metabolic disease proposes that the epigenetic alterations observed in type 2 diabetes mellitus may occur in mothers with GDM. In turn, alterations may cross the placenta and induce fetal epigenetic changes.

Placental vascular dysfunction in GDM

Due to the constituently active placental growth and the mediation of angiogenesis by multiple complex biological processes, the placenta is very vulnerable to alteration in maternal and fetal metabolic function. Principally, in women with poorly controlled GDM the placenta has several changes, particularly in the vasculature. Microscopic analysis revealed vascular morphological changes of fibrinoid necrosis and villous oedema. Furthermore, several studies have reported hypervascularization in GDM placentas, whereas others have reported hypovascularization. These contradicting findings on placental vascularization in GDM are likely a result of other underlying factors, such as maternal obesity or levels of glycaemic control achieved or, of particular note, the degree of endothelial dysfunction.

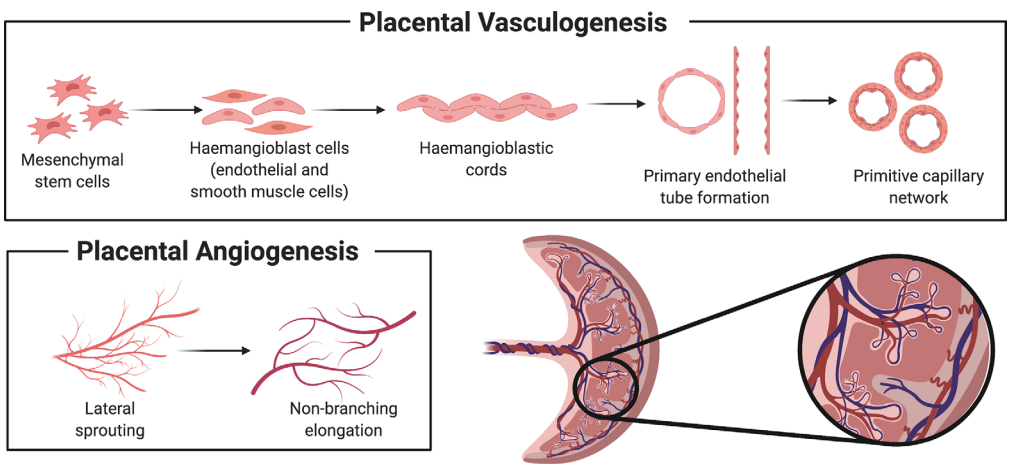

Figure 2. Development of the placental vasculature. Villous development is dependent on the processes of vasculogenesis and angiogenesis. During vasculogenesis, mesenchymal stem cells differentiate and form endothelial cell cords, which develop into fetal capillaries. Once the capillary network has formed, the sprouting and elongation of existing vessels occurs via angiogenesis. Figure created with Biorender.com.

Molecular and biochemical pathways responsible for endothelial dysfunction in GDM

Whilst the exact underlying molecular pathways of hyperglycaemia-induced complications in GDM are unclear, patients with diabetes outside of pregnancy display endothelial dysfunction which is partially responsible for elevating their cardiometabolic disease risk. Endothelial dysfunction is characterized by an imbalance in the endothelium-derived vasodilating and vasoconstricting factors, thus predisposing to hypertension and atherosclerosis. To determine the presence of this association in GDM, human umbilical vein endothelial cells (HUVECs) were exposed to the chronic hyperglycaemia of GDM and have been seen to consequently display insulin insensitivity. As insulin acts as an activator of nitric oxide (NO) release, a potent vasodilator, this identifies one contributor pathway to the endothelial dysfunction of GDM. Similarly, insulin is required for the uptake of L-arginine, the substrate for NO synthase, and therefore insulin insensitivity impedes not only NO release but also NO synthesis. Upon further analysis, the protein kinase C (PKC) pathway has also been linked to deleterious changes in NO production in the placentas of GDM pregnancies, supported further by surrounding literature that found pathological vascular changes in GDM can be reversed with inhibition of the PKC pathway. Identifying therapeutic targets for this endothelial dysfunction may be essential to reducing placental changes and subsequent pathological fetal growth.

A secondary pathway to endothelial dysfunction in GDM is mediated by hyperglycaemia-induced changes including reactive oxygen species (ROS). Elevated ROS production is theorized to be of particular importance in vascular remodelling, and through epigenetic activity enhances the transcription of a multitude of pro-inflammatory mediators, including intracellular adhesion molecule 1, interleukin-6, and tumour necrosis factor-α, which collectively promote endothelial dysfunction. Alongside this, ROS can increase mitochondrial membrane permeability which facilitates the release of further ROS from the mitochondria into the cytosol. Release of mitochondrial ROS is accompanied by mitochondrial DNA, leaving it vulnerable to damage that can produce further mitochondrial dysfunction. Hyperglycaemia is responsible not only for ROS production, but also for advanced glycation end products (AGEs). These compounds have been observed to induce endothelial cell toxicity, and association between progression of cardiovascular disease and AGEs is evident in the existing literature. An overview of the plethora of contributors to endothelial dysfunction in GDM is outlined in Figure 3.

The imbalance between pro- and anti-angiogenic factors in GDM

Several of the angiogenic factors, responsible for regulating placental vascular development, have been reported to be altered in GDM, within the maternal circulation, cord blood and placenta. For example,

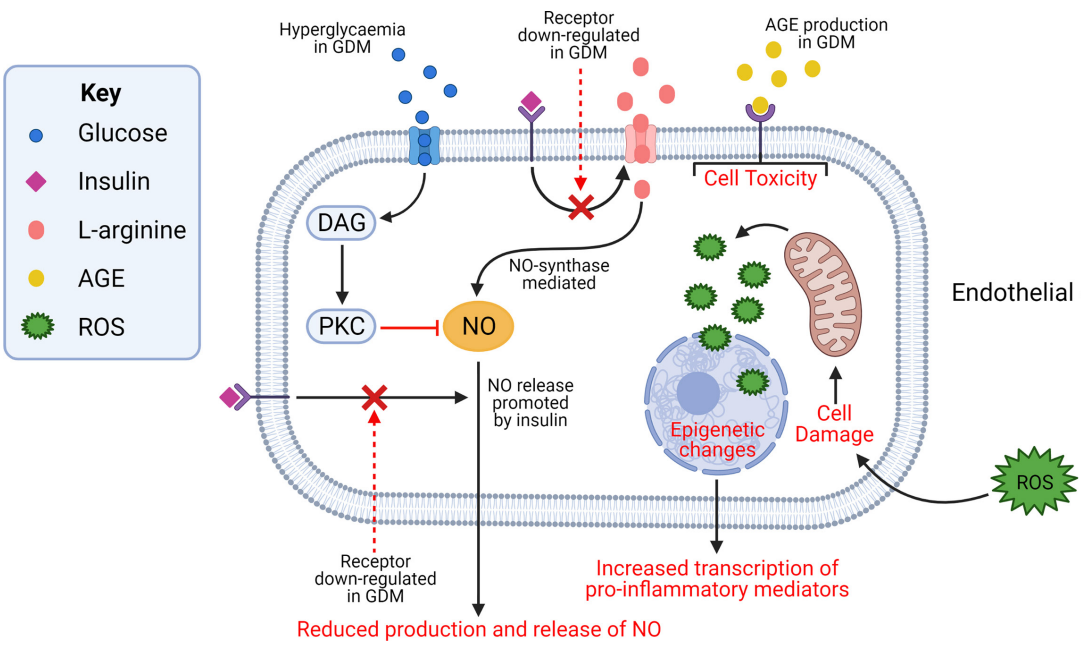

Figure 3. Diagrammatic representation of a plethora of contributors to endothelial dysfunction in gestational diabetes mellitus. GDM = gestational diabetes mellitus. DAG = diacylglycerol. PKC = protein kinase C. NO = nitric oxide. AGE = advanced glycation end products. ROS = reactive oxygen species. Figure created with BioRender.com

concentrations of the anti-angiogenic factor sFlt1 are known to be increased in the maternal circulation in GDM. The anti-angiogenic effect elicited by sFlt1 can alter endothelial integrity and increase vascular permeability, which parallels the endothelial dysfunction observed in GDM.

In one study an increase in FGF2 was observed in maternal serum, cord serum and amniotic fluid in pregnancies complicated by maternal diabetes, including GDM. Interestingly, the FGF2 concentrations were correlated with fetal and placental weight, proposing that FGF2 may also contribute to the development of LGA and abnormal fetal growth in GDM pregnancies. In one *in vitro* study, FGF2 and VEGF significantly increased the proliferation of HUVECs from normal and GDM pregnancies. However, in GDM HUVECs, at higher concentrations of FGF2, cell proliferation was significantly lower than in HUVECs from normal pregnancies, further demonstrating potential endothelial dysfunction in GDM. Similar results were observed when HUVECs from normal pregnancies were treated with high glucose, proposing maternal hyperglycaemia as a causal factor. In this study, both FGF2 and VEGF were shown to induce ERK1/2 phosphorylation, and high glucose treatment inhibited FGF2-induced phosphorylation. This suggests a role of FGF2 and ERK1/2 in endothelial dysfunction induced by hyperglycaemia in women with GDM.

These aforementioned angiogenic factors can be released from the villous trophoblast of the placenta and elicit a paracrine function on neighbouring endothelial cells. Term placental trophoblasts isolated from GDM placentas were shown to have increased expression levels of anti-angiogenic, Angiopoietin-2 (ANGPT2) and KISS1 metastasis suppressor (KISS1), as well as pro-angiogenic, hepatocyte growth factor (HGF) and PlGF. Many *in vitro* studies have also demonstrated that glucose can alter the trophoblast secretome, demonstrating the contribution of maternal hyperglycaemia to placental vascular dysfunction in GDM. High glucose treatment in cultured trophoblast cells has been found to down-regulate pro-angiogenic factors, VEGF and PlGF, and upregulate anti-angiogenic factors, sEndoglin (sEng) and sFlt1, in addition to altered levels of inflammatory cytokines and chemokines. In other studies, PKC was shown to increase sFlt1 secretion from trophoblasts, suggesting the PKC pathway is responsible for this alteration in sFlt1 and in turn impaired angiogenesis in the placenta. Overall, the altered levels of these angiogenic factors in GDM demonstrate how an imbalance may lead to altered pathways and placental vascular and endothelial dysfunction in this disease.

Conclusions and future directions

Complications associated with GDM have been linked to aberrant placental vasculature, and particularly the increased long-term risk of cardiometabolic disease for the mother and baby has been attributed to endothelial dysfunction. As a myriad of factors are thought to contribute to this placental vascular dysfunction in GDM, including angiogenic factors, ROS, pro-inflammatory cytokines and AGEs, understanding the altered vasculature in GDM is incredibly complex, and the interaction between these factors needs to be investigated further. Additionally, other studies have reported the potential contribution of extracellular vesicles, and non-coding RNAs, such as micro-RNAs (miRNAs). Collectively, these markers may also pose as potential biomarkers for GDM diagnosis and its associated complications. For example, identifying markers of persistent endothelial dysfunction may in future be utilized to screen women with a history of GDM and identify those in the early stages on the pathway to cardiometabolic disorders. With this in mind, optimal follow-up for those deemed most at risk, including infants, may allow for prevention of future disease to minimize the burden of GDM. ■

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