Placental Function and the Development of Fetal Overgrowth and Fetal Growth Restriction

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KEYWORDS

• Fetal development • Fetal growth restriction • Fetal overgrowth • Placental transport • Maternal-fetal exchange • Syncytiotrophoblast

KEY POINTS

- Placental signaling and nutrient transport are determinants of fetal growth.
- In fetal growth restriction, placental insulin/insulinlike growth factor-1 (IGF-1) and mechanistic target of rapamycin (mTOR) signaling and nutrient transport are typically inhibited, which may contribute to the restricted fetal growth.
- Activation of placental insulin/IGF-1 and mTOR signaling and nutrient transporters and reduced placental adiponectin signaling may promote fetal overgrowth in some pregnancies complicated by maternal obesity and gestational diabetes mellitus.
- Therapeutic strategies designed to restore normal placental function and fetal growth in high-risk pregnancies are limited and have yielded conflicting results.
- Robust approaches to specifically target the placenta rather than the mother and the fetus and a better understanding of the placental molecular pathways driving fetal growth are required for the development of successful interventions to modulate placental function.

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INTRODUCTION

The developing fetus undergoes a dynamic period of rapid growth that is responsive to changes in the in utero environment that may contribute to the development of fetal growth restriction (FGR) or fetal overgrowth resulting in the delivery of a large-for-gestational age (LGA) infant.[1] Pregnancies complicated by abnormal fetal growth have a major impact on public health because of the increased perinatal morbidity and mortality associated with FGR and LGA and because abnormal fetal growth is strongly associated with long-term health consequences for offspring, including the development of cardiovascular disease, metabolic dysfunction, and obesity later in life.[2–4] An array of conditions, including changes in maternal nutrient, endocrine, and metabolic status and impaired uteroplacental blood flow, are associated with altered fetal growth.[5] However, the precise mechanisms causing changes in the growth trajectory of the fetus remain to be fully established. Accumulating evidence suggests that changes in placental function may contribute to, or mediate, both FGR and fetal overgrowth.

Early clinical studies of fetal growth were instrumental in establishing a link between impaired uteroplacental blood flow and reduced fetal weight as a result of placental insufficiency.[6,7] Although impaired uteroplacental blood flow is an established risk factor for restricted fetal growth, placental insufficiency is more than reduced blood flow and may involve decreased transplacental nutrient transport capacity, altered activity in placental growth factor and inflammatory signaling pathways, and changes in the release of placental extracellular vesicles and their cargo. Placental cell signaling pathways, such as mechanistic target of rapamycin (mTOR), insulinlike growth factor (IGF), adipokine signaling, and nutrient transport are regulated by oxygen and nutrient levels as well as maternal metabolic hormones. Changes in placental function therefore link the availability of oxygen and nutrients for fetal growth to the fetal growth trajectory, in some cases resulting in FGR or, at the opposite end of the growth spectrum, LGA.[8,9]

This article discusses recent work showing compelling associations between changes in placental function and fetal growth. In particular, it focuses on distinct differences in transplacental nutrient transport, cellular signaling pathways, inflammatory markers, and extracellular vesicle regulatory functions that are unique to placentas of LGA and FGR infants. Furthermore, it discusses novel clinical interventions specifically targeting placental function as an avenue to rescue disordered fetal growth patterns. In addition, it speculates on future research and intervention priorities to prevent adverse infant outcomes in pregnancies complicated by altered placental function and fetal growth.

HUMAN PLACENTA

The human placenta is derived from the fetal trophectoderm, the outermost layer of the blastocyst. After implantation, the trophectoderm differentiates into mononuclear villous cytotrophoblasts that can further differentiate into either extravillous trophoblasts or they can fuse to form the multinucleated syncytiotrophoblast (STB). The extravillous trophoblasts proliferate and invade the myometrium to reach the spiral arteries, where they eventually replace smooth muscle cells of the arterial media with eosinophilic materials, resulting in decreased vasoreactivity, allowing the marked increase in uteroplacental blood flow throughout gestation that characterizes a normal pregnancy. During the first weeks after implantation, cytotrophoblasts rapidly proliferate to form the functional unit of the placenta, the trophoblast villous tree, which is lined with a continuous outer layer of STB. Following the onset of the uteroplacental circulation in late first trimester, the STB is directly exposed to maternal blood in the

intervillous space, and serve as the primary barrier between maternal and fetal blood supply as well as the site of placental hormone production and maternal-fetal oxygen, nutrient, and ion transfer.[10,11]

Maternal-fetal exchange occurs across 2 largely continuous cell layers, the fetal capillary endothelium and STB, which separate maternal and fetal blood supplies. Fetal capillary endothelial cells allow largely unrestricted transfer of small molecules such as glucose, amino acids, and ions through intercellular junctions,[12] making STB the limiting factor for maternal-fetal solute exchange. In contrast, although the mechanisms involved remain to be fully established, transplacental transfer of lipids likely requires transport across both STB and fetal capillary endothelium.[13] The STB consists of 2 polarized plasma membranes, the apical or maternal-facing microvillous plasma membrane (MVM), and the fetal-facing basal plasma membrane (BM). The MVM and BM express an array of different transporter proteins critical for mediating vectorial maternal-to-fetal transfer of nutrients[14] and fetal-to-maternal transfer of waste products (**Fig. 1**).

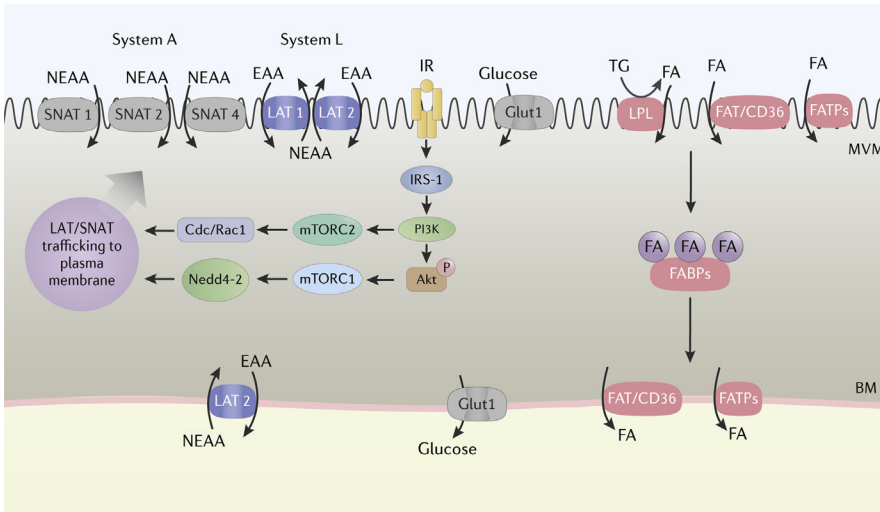

Fig. 1. Some key placental signaling pathways and nutrient transporters. The syncytiotrophoblast consists of 2 polarized plasma membranes, MVM and BM, that express an array of transport proteins that mediate maternal-to-fetal transfer of amino acids, glucose, and fatty acids. The uptake of nonessential and essential amino acids from maternal circulation across the MVM is mediated by system A (SNAT1, 2, 4) and system L (LAT1, 2) transport systems that are trafficked to the plasma membrane as a result of activation of insulin/IGF-1 and mTOR signaling. Glucose transporter-1 (GLUT-1) is highly expressed in the MVM and BM of the syncytiotrophoblast and is considered the primary glucose transporter in the human placenta at term. Maternal triglycerides are hydrolyzed into free fatty acids (FFAs) by membrane-bound lipases and transferred across the MVM by FAT/CD36 and fatty acid transport proteins (FATPs). Internalized FFAs are transferred to the BM by fatty acid binding proteins (FABPs) for export into fetal circulation. Akt, protein kinase B; Cdc/Rac1, cell division control protein/ras-related C3 botulinum toxin substrate 1; EAA, essential amino acids; FA, fatty acids; FAT/CD36, fatty acid translocase/cluster of differentiation 36;IR, insulin receptor; IRS-1, insulin receptor substrate 1; LAT, L-amino acid transporter; LPL, lipoprotein lipase, mTORC, mTOR complex; Nedd4-2, neuronal precursor cell-expressed, developmentally downregulated gene 4 isoform 2; NEAA, nonessential amino acids; SNAT, sodium-coupled neutral amino acid transporter; TG, triglycerides. (*Courtesy of* KIMEN Design4Research, with permission.)

PLACENTAL SIGNALING

Placental receptors for many metabolic hormones and growth factors, including receptors for insulin,[15] IGF-1,[16] and adiponectin,[17] are localized on the MVM of syncytiotrophoblast, mediating regulation of placental function by maternal circulating factors. The coordinated actions of insulin/IGF-1and adiponectin through downstream mTOR signaling act to regulate mitochondrial function, protein synthesis, and the flux of glucose, amino acids, lipids, and folate across the placental barrier. Importantly, these signaling pathways are differentially regulated in pregnancies complicated by fetal overgrowth and FGR (Fig. 2).

Insulin/insulinlike growth factor-1 and mechanistic target of rapamycin Signaling

The insulin/IGF-1 signaling pathway is primarily composed of a system of ligands (IGF-1, IGF-2, and insulin), tyrosine kinase receptors (IGF-1 receptor and insulin receptor isoforms [INSR] A and B), and downstream activation of target proteins insulin receptor substrate 1 (IRS-1), protein kinase B (Akt), and mTOR. Activation of placental insulin/IGF-1 signaling is crucial for normal trophoblast function and fetal growth and development by promoting hormone synthesis, protein synthesis, and nutrient transfer, in part as a result of mTOR activation.[18,19] During pregnancy, IGF-1 availability in the maternal circulation and at the maternal-fetal interface is primarily regulated by IGF binding proteins such as IGFBP-1 synthesized by the decidua.[20] Phosphorylation of IGFBP-1 at serine residues

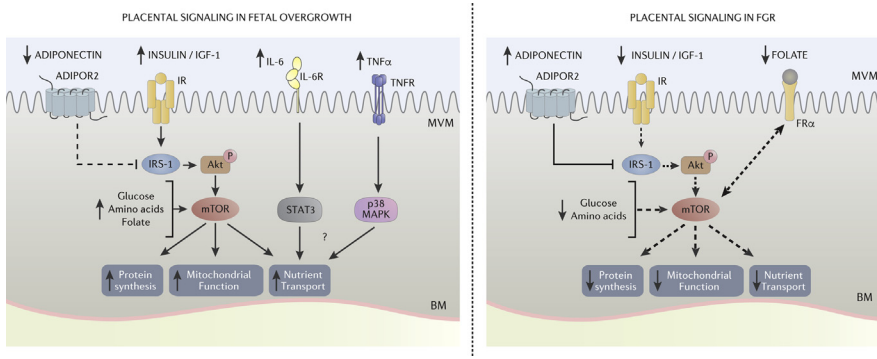

Fig. 2. Placental signaling in fetal overgrowth and FGR. The coordinated actions of placental insulin/IGF-1, mTOR, adiponectin, and inflammatory cytokine signaling pathways act to regulate mitochondrial function, protein synthesis, and the flux of glucose, amino acids, lipids, and folate across the placental barrier. Placental insulin/IGF-1 and mTOR signaling is activated in women with obesity delivering LGA infants likely because of increased circulating maternal insulin/IGF-1 levels and increased availably of nutrients. Moreover, low maternal levels of circulating adiponectin in pregnancies complicated by maternal obesity contribute to enhance placental insulin signaling because of decreased inhibition of insulin receptor substrate (IRS)-1. Levels of maternal circulating proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α are increased in pregnancies with obesity and may contribute to fetal overgrowth by activating signal transducer and activator of transcription 3 (STAT3) and p38 mitogen-activated protein kinase (MAPK) signaling pathways. Conversely, lower levels of maternal circulating insulin/IGF-1 and folate, and increased adiponectin level, contribute to reduced placental insulin/IGF-1 and mTOR signaling in FGR pregnancies. Thus, differential regulation of placental signaling pathways and subsequent impact on placental function and nutrient transfer likely contribute to fetal overgrowth and FGR. ADIPOR2, adiponectin receptor 2; FRα, folate receptor-α; IL-6R, IL-6 receptor; TNFR, tumor necrosis factor-α receptor. (*Courtesy of* KIMEN Design4Research, with permission.)

(Ser101, 119, and 169) markedly increases IGFBP-1 binding affinity for IGF-1, effectively reducing IGF-1 availability and function.[21] FGR pregnancies are associated with reduced maternal serum IGF-1 concentrations and increased abundance[22] and phosphorylation[23,24] of IGFBP-1. In addition, decreased placental IGF-1 expression has been reported in human FGR,[25] which may contribute to the inhibition of placental insulin/IGF-1 signaling pathway in this pregnancy complication.[26–28] In contrast, placental insulin/IGF-1 signaling is enhanced in women with obesity[29] or gestational diabetes mellitus (GDM)[30] delivering a large infant. There are likely multiple factors underlying the activation of placental insulin/IGF-1 signaling in fetal overgrowth, including increased maternal IGF-1 level[22] and lower circulating levels of adiponectin in the mother,[31] as discussed later.

Activation of mTOR complex 1 (mTORC1) occurs by several mechanisms, including insulin/IGF-1 ligand binding and increased availability of ATP, amino acids, fatty acids, folate, and glucose, whereas mTOR complex 2 (mTORC2) primarily responds to insulin/phosphoinositide 3-kinase(Akt) signaling.[32] In the placenta, mTORC1 serves as a positive regulator of amino acid[33] and folate transport,[34] and mitochondrial biogenesis.[35] In contrast, mTORC2 promotes cell proliferation by phosphorylation of Akt, PKCα, and SGK1, which regulate cytoskeletal remodeling and cell migration.[32] Placental mTORC1 activity has been found to be closely related to fetal growth. A consistent decrease in placental mTORC1 activity has been reported in humans[27,36,37] and animal models[38,39] of FGR, whereas human GDM[40] and obesity[29] and rodent models of fetal overgrowth[41,42] are often associated with placental mTORC1 activation. Further, inhibition of mTOR in decidual cells increased the release of hyperphosphorylated IGFBP-1, which decreases IGF-1 bioavailability and is associated with restricted fetal growth.[43] Likewise, mTORC1 is inhibited by adenosine monophosphate-activated protein kinase (AMPK), a critical nutrient sensor that is activated when ATP levels are depleted.[44] Placental mTORC1 activity is positively correlated to infant birthweight, whereas placental AMPK activity shows an inverse relationship, suggesting that the placental mTORC1 signaling pathway constitutes an important link between placental nutrient status and fetal growth.[29]

Adiponectin Signaling

As in nonpregnant individuals, circulating levels of adiponectin are inversely correlated to body mass index (BMI) in pregnant women. Moreover, maternal serum adiponectin level is negatively associated with birth weight.[45] In agreement with these associations, low adiponectin level increases the risk of fetal overgrowth,[31,46–48] whereas maternal adiponectin level tends to be increased in FGR.[45] Adiponectin is the most abundant protein secreted from adipose tissue and has well-documented insulin-sensitizing effects in adipose, liver, and skeletal muscle tissues.[49] Adiponectin binds to adiponectin receptor (AdipoR) 1 and AdipoR2, which activate downstream AMPK, p38 mitogen-activated protein kinase (MAPK), and/or PPARα.[50] Surprisingly, adiponectin blunts insulin signaling in primary human trophoblast cells[51] by PPARα--mediated synthesis of ceramide, which phosphorylates IRS-1 at an inhibitory site.[52] These findings are consistent with the possibility that low circulating adiponectin level, a feature of maternal obesity and GDM, is mechanistically linked to activation of placental insulin/IGF-1/mTOR signaling, stimulating fetal growth. In support of this model, adiponectin supplementation prevented the adverse effects of maternal obesity on placental function and fetal growth in mice.[41] Moreover, normalization of maternal circulating adiponectin levels in obese pregnant mice largely prevented the development of cardiac[53] and metabolic disease[54] in adult progeny, implicating low maternal adiponectin levels as a mechanism underpinning fetal overgrowth and developmental programming of cardiovascular and metabolic disease.

INFLAMMATION

Pregnancy is characterized by a tightly regulated balance between proinflammatory and antiinflammatory cytokines necessary for implantation and placentation. Although results of studies exploring the effect of maternal obesity and GDM on maternal cytokine levels are inconsistent,[55] women with obesity,[56] GDM,[57] or preeclampsia[58] generally are thought to have increased levels of circulating proinflammatory cytokines, such as IL-6 and tumor necrosis factor (TNF)-α. Placental cytokine production, which is critical for the maintenance of pregnancy from implantation to parturition,[59] is altered in pregnancies complicated with obesity and GDM.[55] Maternal BMI is positively associated with activation of distinct placental inflammatory pathways, including p38 MAPK and signal transducer and activator of transcription 3 (STAT3) signaling without changes in classic inflammatory pathways or fetal cytokine profile.[60] These findings suggest that maternal and placental inflammation in maternal obesity and GDM may affect fetal development by altering placental function rather than direct fetal exposure to increased levels of proinflammatory cytokines.

The transcription of placental IL-6 and TNF-α messenger RNA (mRNA) is increased in maternal obesity,[61] which may promote placental lipid and amino acid transfer. In cultured primary human trophoblasts, IL-6 was shown to upregulate STAT3-dependent system A amino acid transport activity through increased sodium-coupled neutral amino acid transporter (SNAT) 2 expression,[62] whereas TNF-α stimulated system A amino acid transport through activation of p38 MAPK signaling.[63] In contrast, IL-1β downregulates insulin-stimulated system A transport but activates system L activity in primary trophoblasts.[64] Collectively, data from in vitro experiments show a mechanistic link between placental response to inflammation and altered nutrient transport. However, the cumulative effects of inflammation on placental nutrient transport are complex and may vary depending on the degree of inflammation and specific cytokine levels that are increased.

NUTRIENT TRANSPORT

Glucose

Glucose is the primary energy substrate for the placenta and the fetus. Fetoplacental glucose needs are met entirely by placental uptake from the maternal circulation via facilitated diffusion and in response to increased postprandial maternal glucose levels. Glucose transporter (GLUT)-1 is highly expressed in the MVM and BM of the STB; however, GLUT-1 localized in the BM is considered the primary glucose transporter in the human placenta at term.[65] BM GLUT-1 expression is associated with birthweight and is increased in pregnancies complicated by obesity and fetal overgrowth.[66] Similarly, GLUT-4 expression and translocation to the BM is upregulated by insulin, which may enhance glucose transport in response to postprandial hyperinsulinemia.[15] MVM and BM GLUT-1 protein expression and activity seem not to be affected in FGR[65]; however, reduced MVM GLUT-1 protein level, activity, and glucose transfer have been reported in preeclampsia.[67] Some FGR fetuses are hypoglycemic in utero, this may be caused by impaired uteroplacental blood flow, increased placental glucose consumption, or altered glycolytic pathways.[21,68]

Amino Acids

Placental uptake of amino acids is mediated by an array of distinct transporter systems. Of these, system A and system L are thought to be some of the most important, in part because these transporters are subjected to regulation. System A is a sodium-dependent transporter mediating the uptake of nonessential neutral amino acids into

the cell. System A comprises 3 isoforms, which are all expressed in the human placenta: SNAT 1 (SLC38A1), SNAT 2 (SLC38A2), and SNAT 4 (SLC38A4).[69] The system L amino acid transporter is a heterodimer, consisting of a light chain, typically L-amino acid transporter (LAT) 1 (SLC7A5) or LAT2 (SLC7A8), and a heavy chain, 4F2hc/CD98 (SLC3A2). System L is a sodium-independent exchanger mediating cellular uptake of essential amino acids, including leucine, in exchange for nonessential system A substrates such as glycine. As a result, the coordinated activity of both system A and system L activity is required for placental transport of nonessential and essential amino acids across pregnancy.[70] Activation of trophoblast system A and system L amino acid transport occurs as a direct result of upstream mTORC1 and mTORC2 activation.[33] mTORC1 modulates SNAT2 and LAT1 trafficking by Nedd4-2-regulated ubiquitination in normal term pregnancies,[69] whereas mTORC2 regulates amino acid transporter trafficking through interactions with Cdc42 and Rac1, which are downregulated in FGR.[71] Placental amino acid transport capacity has been consistently shown to be reduced in human FGR.[37,72–77] The authors have reported that downregulation of key placental amino acid transport systems precedes the development of FGR in rodents[38,78] and nonhuman primates,[39,79,80] supporting the concept that downregulation of placental nutrient transport is a primary event that directly contributes to FGR, rather than a response to a changing fetal demand. In contrast, as previously discussed, activation of placental amino acid transport systems caused by upstream activation of mTOR signaling by increased maternal hormone levels (insulin/IGF, leptin), low adiponectin level, excess nutrients, and a mild proinflammatory activation may contribute to fetal overgrowth in pregnancies complicated by obesity.[29,42,52,62]

Lipids

Placental uptake of lipids from the maternal circulation is mediated by several membrane-bound transport proteins and lipases. Triglycerides (TGs) packaged in circulating lipoproteins (very-low-density lipoprotein, low-density lipoprotein [LDL], chylomicrons) are hydrolyzed into nonesterified fatty acids and glycerol by lipoprotein lipase (LPL) and endothelial lipase (EL) before entering the syncytiotrophoblast. Long-chain fatty acids are transported across the MVM by fatty acid transport proteins (FATPs) and fatty acid translocase/cluster of differentiation 36 (FAT/CD36).[81] Once internalized, free fatty acids are esterified with coenzyme A (CoA) producing acyl-CoAs that are trafficked by cytosolic fatty acid binding proteins for use in placental mitochondrial respiration, incorporated into lipid droplets for placental storage, or transferred to the BM for export.[82] Maternal obesity is generally thought to increase lipid transport, possibly contributing to greater fetal adipogenesis and birthweight.[61] Although the relationship between maternal obesity, placental lipid transport, and fetal growth remains incompletely understood, maternal obesity is associated with decreased placental total FATP1 and FATP4 mRNA and increased FATP6 and FAT/CD36 protein content.[83] FATP2 and FATP4 expression is greater on the BM compared with the MVM in the human placenta, with BM FATP2 expression positively correlated to maternal BMI.[84] In contrast, FAT/CD36 expression is higher in the MVM compared with the BM, but was not associated with maternal BMI.[84] Increased BM FATP2 expression may reflect increased transplacental lipid transfer; however, direct evidence showing an association between increased BM FATP expression and fetal overgrowth is currently lacking. Maternal obesity is also associated with reduced placental EL expression and increased placental storage of long-chain polyunsaturated fatty acids (LCPUFAs), which may limit transplacental transport of LCPUFA species in maternal obesity.[83]

Pregnancies complicated by FGR are also associated with changes in placental lipid transport mechanisms. Placental EL mRNA and MVM LPL activity have been reported to be decreased, but placental LPL gene expression is increased,[85,86] in FGR. The divergence between LPL activity and mRNA expression may reflect that regulation of LPL-mediated lipid transfer does not occur at the transcriptional level.[21] MVM FATP6 and CD36 protein levels are increased in FGR, and LCPUFAs seem to be preferentially routed to storage in placental TG, suggesting a possible defect in intracellular LCPUFA trafficking and export.[87] Further, fetal and placental weights and placental expression of genes related to fatty acid mobilization and TG content were not altered in mice deficient in FATP2 and FATP4.[88] Interestingly, protein expression of placental FATP1, FATP2, FATP4, FATP5, and FATP6 is increased across late gestation in a baboon model of FGR, suggesting a coordinated placental adaptation to facilitate fatty acid transport.[89] However, the potential overlap and redundancy of FATPs involved in placental fatty acid trafficking throughout gestation remains unresolved and warrants further investigation. The expression of LDL receptor (LDLr) mRNA and scavenger receptor class B type I (SR-B1) protein, which are membrane-bound proteins responsible for the uptake of LDL and high-density lipoprotein from maternal circulation, is reduced in FGR placentas. However, whether changes in placental cholesterol transport in FGR influence fetal development is not fully elicited.[90]

Folate

Folate has a well-characterized role in DNA replication, amino acid metabolism, and as a methyl donor. Folate deficiency during pregnancy is associated with FGR and congenital abnormalities such as neural tube defects. Interestingly, mTOR functions as a folate sensor in the trophoblast and other cell types,[34,91] and maternal folate deficiency in mice inhibits placental mTORC1 and mTORC2 signaling and placental amino acid transport contributing to FGR.[91] Furthermore, both mTORC1 and mTORC2 are positive regulators of trophoblast folate uptake by modulating the cell surface expression of folate receptor-α (FR-α) and the reduced folate carrier.[34,92] Placental folate transport capacity is decreased in human FGR,[93] which may contribute to the restricted fetal growth and intrauterine programming of childhood and adult disease.

SMALL EXTRACELLULAR VESICLES AND microRNA

Extracellular vesicles are membrane-bound particles containing bioactive proteins, lipids, DNA, mRNA, and microRNA (miR) that are secreted from most cells and participate in cell-to-cell communication. Small extracellular vesicles (sEVs), commonly referred to as exosomes, are small (<150 nm in diameter) nanovesicles produced from the endosomal pathway and released on fusion of multivesicular bodies with the plasma membrane. Because of a lack of specific subtypes and mixed origins, the International Society of Extracellular Vesicles has recommended the use of size to categorize extracellular vesicles; therefore, this article refers to this heterogenous population as sEVs.[94]

At present, little is known about the relationship between sEVs, placental or originating from other tissues, and fetal growth. However, mice infused with human total and/or placental sEVs from women with preeclampsia developed hypertension,[95] and mice infused with sEVs from women with GDM became glucose intolerant and insulin resistant.[96] Therefore, it is highly plausible that sEVs regulate fetal growth, at least secondarily to changes in maternal physiology (**Fig. 3**). Indirect support of this hypothesis is found in the differential expression of sEV miR isolated from maternal

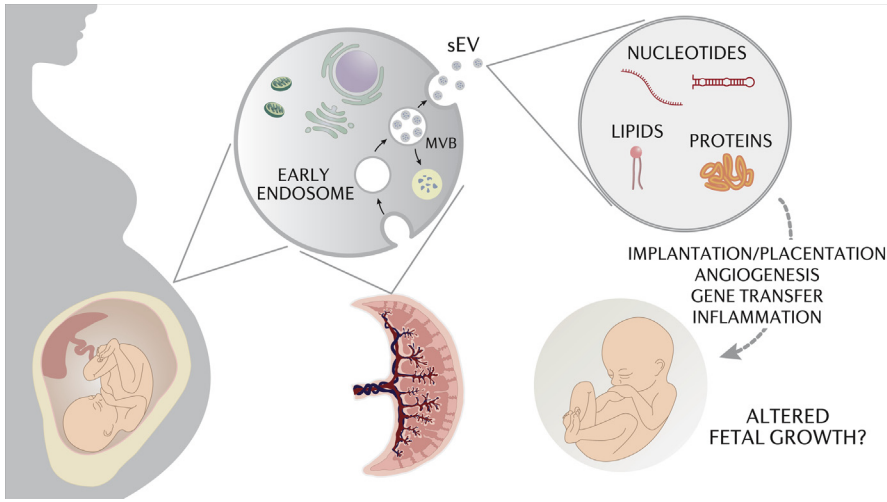

Fig. 3. The influence of small extracellular vesicles on placental function. sEVs are membrane-bound particles containing bioactive proteins, lipids, DNA, mRNA, and miR that are secreted from most cells and participate in cell-to-cell communication. The functions of maternal and placental sEVs remain to be fully established but they are thought to be involved in immune response, angiogenesis, placentation, and the transfer of nucleic acids and proteins important in normal and complicated pregnancies that may influence fetal growth. MVB, multivesicular bodies. (*Courtesy of* KIMEN Design4Research, with permission.)

serum in the second trimester being correlated with birth weight.[97] Further, the fraction of total circulating sEVs that are of placental origin is reduced in FGR, likely because the levels of circulating placental sEVs was correlated with placental weight, suggesting that decreased release of placental sEV may be a result of reduced placental size.[98] The functions of placental sEVs remain to be fully established but they are thought to be involved in immune response, angiogenesis, and the transfer of nucleic acids and proteins important in normal and complicated pregnancies.[99] Placental sEVs containing miR-520c-3p have been reported to inhibit CD44/HA-mediated extravillous trophoblast invasion, suggesting a link between placental sEVs and placentation.[100] sEVs isolated from second trimester cytotrophoblasts contain a significant amount of TNF-α and increase decidual stromal cell transcription and secretion of NF-κB targets, including IL-8.[101] In addition, placental sEVs from women with GDM promote endothelial cytokine release compared with placenta-derived sEVs from normal pregnancies,[102] in agreement with previous studies showing that placental sEVs mediate monocyte recruitment and induce IL-1β production.[103] A direct link between sEVs of maternal or placental origin and abnormal fetal growth remains to be established.

INTERVENTIONS TARGETING PLACENTAL FUNCTION IN FETAL GROWTH RESTRICTION AND FETAL OVERGROWTH

Restoring Uteroplacental Blood Flow in Fetal Growth Restriction

Numerous studies have tested the hypothesis that systemic administration of vasodilators increases uteroplacental blood flow and promotes fetal growth in FGR (**Fig. 4**). Aspirin is a cyclo-oxygenase inhibitor that suppresses the production of thromboxane,

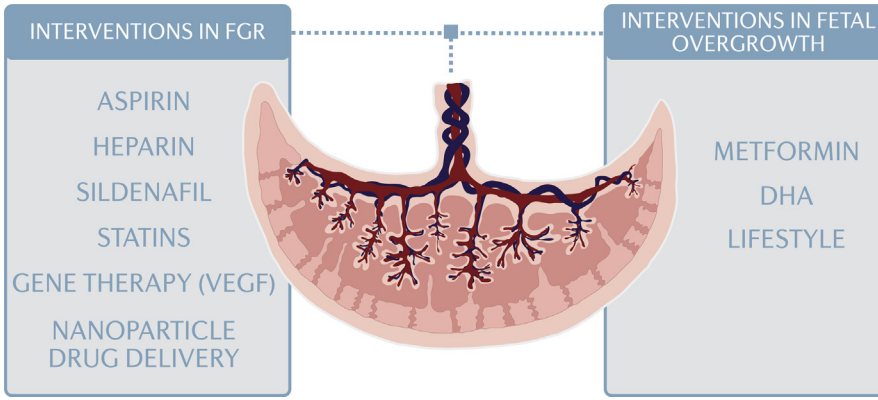

Fig. 4. Clinical interventions targeting placental function to restore fetal growth. Numerous studies have tested the hypothesis that systemic administration of vasodilators such as aspirin, low-molecular-weight heparin, and sildenafil increases uteroplacental blood flow and promotes fetal growth in FGR; however, the results have not been encouraging. Clinical trials to improve fetal growth in FGR pregnancies using statins have been initiated. In addition, gene therapy and nanoparticle drug delivery designed to alter the expression of genes in the uteroplacental circulation and the placenta, including vascular endothelial growth factor (VEGF), is an area of active research. Limited clinical approaches to prevent fetal overgrowth currently exist; however, treatment of GDM and/or maternal obesity with metformin, docosahexaenoic acid (DHA) supplementation, or lifestyle interventions may mitigate fetal overgrowth in high-risk pregnancies. (*Courtesy of* KIMEN Design4Research, with permission.)

which promotes platelet aggregation and functions as a vasoconstrictor. Thus, aspirin has the potential to increase uteroplacental blood flow. Although the evidence on the efficacy of initiating aspirin therapy in early gestation to prevent FGR is conflicting, several meta-analyses suggest that low-dose aspirin (75–100 mg) has a modest effect on reducing the risk for developing preeclampsia and FGR.[104–106] Challenges remain in determining the appropriate timing to initiate treatment (before or after 16 weeks of gestation), defining the optimal dose, and delineating the benefits of aspirin in women with chronic hypertension versus those who develop preeclampsia during late gestation. Further, most of the studies included in the meta-analyses were designed with preeclampsia prevention as a primary end point, with effects on fetal growth as a secondary outcome. In an effort to address these concerns, the Chronic Hypertension and Acetyl Salicylic Acid in Pregnancy (CHASP) trial will compare the efficacy of aspirin (150 mg/d) introduced before 15 weeks of gestation in the prevention of maternal and fetal morbidity and mortality, including FGR, in women with chronic hypertension.[107]

Low-molecular-weight heparin is commonly used in pregnancy as a thromboprophylaxis and for the treatment of venous thromboembolism. Heparin therapy during pregnancy is associated with increased circulating levels of placental growth factor and decreased risk of recurrence of placental-mediated complications in women without thrombophilia.[108] Randomized controlled trials initially showed a potential reduction of the incidence of preeclampsia and FGR; however, recent meta-analyses suggest no benefit.[109,110] Despite early promise in small trials, a recent large trial of the phosphodiesterase type 5 inhibitor sildenafil, Sildenafil TheRapy In Dismal Prognosis Early-onset Fetal Growth Restriction (STIDER), was terminated before completion after several fetal deaths were reported,[111] possibly caused by fetal hypotension as a result of transplacental transfer of sildenafil into fetal circulation.[112] In

contrast, a phase II trial (TADAFER) of tadalafil, a phosphodiesterase 5 inhibitor that does not cross the placenta, decreased fetal and infant deaths associated with FGR, although fetal growth velocity and birthweight were unchanged.[113]

Clinical Interventions Targeting Placental Function in Fetal Overgrowth

Significant challenges exist in designing interventions targeting placental function in pregnancies complicated by maternal obesity. As described in this article, placental dysfunction as a result of increased nutrient availability and transport in response to increased insulin level, low adiponectin level, and enhanced mTOR placental signaling may contribute to fetal overgrowth. However, concerns over safety with the use of pharmaceuticals to reduce circulating maternal nutrients have limited the clinical approaches to prevent fetal overgrowth (see **Fig. 4**). Using metformin for glucose control in GDM pregnancies decreased the risk of fetal overgrowth compared with women receiving insulin or glyburide[114] but was recently shown to increase the proportion of small-for-gestation-age infants in women with type 2 diabetes and obesity.[115] Although the cause of reduced infant birthweights is unknown, it is possible those infants did not experience accelerated growth, highlighting the need for early detection of fetal overgrowth to guide clinical decision making. In contrast, metformin did not prevent fetal overgrowth in obese pregnant women.[116] Because metformin is transported across the placenta into fetal circulation, concerns have been raised that metformin may program the fetus for adverse outcomes, including obesity, later in life.[117] However, a recent report showed that children of obese mothers exposed prenatally to metformin had improved cardiovascular profiles compared with placebo-controlled offspring.[118]

The powerful impact of hormonal regulation on placental function and fetal growth was shown by experimentally increasing adiponectin levels in obese mice compared with those observed in normal-weight pregnant mice. Not only did this treatment improve placental function and prevent fetal overgrowth[41] but the long-term programming effects on metabolism, weight gain, glucose intolerance, and cardiac dysfunction were also corrected.[53,54] In human pregnancy, nutrition and lifestyle interventions and dietary supplements have been explored as treatments to reduce fetal overgrowth in pregnancies complicated by maternal obesity or GDM. The UK Pregnancies Better Eating and Activity Trial (UPBEAT) recently reported that a comprehensive intervention targeting improvements in nutrition and physical activity did not reduce the incidence of LGA infants in pregnant women with obesity,[119] although placental storage of fatty acids in droplets was reported to be modestly decreased in women who received the lifestyle intervention.[120] In addition, supplementation with docosahexaenoic acid (DHA) in pregnancy decreases placental inflammation and amino acid transporter expression in obese pregnancies, which may mitigate fetal overgrowth.[121] However, trials assessing the effect of DHA on clinical outcomes related to fetal growth are lacking.

Future Interventional Approaches and Priorities

Targeting the placenta with pharmaceutical interventions and approaches designed to alter placental gene expression is an area of active research. Injection of adenoviral vectors containing vascular endothelial growth factor (VEGF), a potent angiogenic factor, in the uterine artery of sheep was shown to improve uterine blood flow.[122] Gene therapy targeting maternal VEGF is currently being investigated as a therapeutic intervention in early-onset FGR (EVERREST trial).[123] The use of statins, particularly lipophilic statins that readily cross plasma membranes, is contraindicated during pregnancy because of the risk of congenital malformations.[124] Despite recent interest

in the use of pravastatin to improve outcomes in preeclampsia and FGR mediated by inhibition of placental sFLT1 secretion,[125] the evidence that pravastatin is beneficial in these 2 pregnancy complications is conflicting.[126,127] Large randomized controlled trials are needed to assess the efficacy of statins in preeclampsia and FGR pregnancies. In addition, innovative approaches using nanoparticles for drug delivery or gene targeting to the placenta represents an emerging area of clinical interest and warrants further investigation.[128]

SUMMARY

Placental regulation of fetal growth involves the integration of multiple hormonal, inflammatory, nutrient, oxygen, and energy-sensing signaling pathways that modulate an array of placental functions, including nutrient transport. As a result, the flux of oxygen and nutrients to the fetus is altered, leading to changes in fetal growth. Placental insulin/IGF-1 and mTOR signaling and transport capacity of certain nutrients are inhibited in FGR and activated in some cases of fetal overgrowth, implicating these placental functions in driving fetal growth. Emerging evidence suggests that circulating total and placenta-derived sEVs and miR may modulate placental function; however, it is currently unknown how these novel signaling systems affect fetal growth. Future research priorities include establishing the role of sEVs in regulating fetal growth and determining the mechanistic role of maternal versus placental sEVs in the development of FGR and fetal overgrowth. Despite a considerable body of evidence linking placental function and fetal growth, clinical interventions specifically designed to restore normal placental function in high-risk pregnancies are lacking. Although novel interventions using placental gene targeting and nanoparticle drug delivery are currently being investigated, the development of future clinically useful therapies to alleviate abnormal fetal growth is likely to depend on a better understanding of the specific placental molecular pathways that regulate fetal growth.

CLINICS CARE POINTS

- FGR and fetal overgrowth increase the risk of perinatal complications and the development of obesity, diabetes, and cardiovascular disease in childhood and later in life.
- Changes in placental function contribute to abnormal fetal growth.
- The understanding of the causes of abnormal fetal growth is limited, and no effective treatments are available.
- Therapeutic strategies designed to restore normal placental function in women with an FGR or LGA fetus have largely been unsuccessful and may potentially harm the fetus.
- Novel interventions using placental gene targeting and nanoparticle drug delivery may be effective therapeutic strategies to restore normal fetal growth in high-risk pregnancies but require rigorous research to determine their clinical usefulness.

CONFLICT OF INTERESTS

The authors declare that there are no competing interests associated with this article.

AUTHOR CONTRIBUTIONS

J.H. Dumolt wrote the article. T.L. Powell and T. Jansson were involved in the planning, organization, and revision of the review.

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