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The Placenta: A Maternofetal Interface

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Abstract

The placenta is the gatekeeper between the mother and the fetus. Over the first trimester of pregnancy, the fetus is nourished by uterine gland secretions in a process known as histiotrophic nutrition. During the second trimester of pregnancy, placentation has evolved to the point at which nutrients are delivered to the placenta via maternal blood (hemotrophic nutrition). Over gestation, the placenta must adapt to these variable nutrient supplies, to alterations in maternal physiology and blood flow, and to dynamic changes in fetal growth rates. Numerous questions remain about the mechanisms used to transport nutrients to the fetus and the maternal and fetal determinants of this process. Growing data highlight the ability of the placenta to regulate this process. As new technologies and omics approaches are utilized to study this maternofetal interface, greater insight into this unique organ and its impact on fetal development and long-term health has been obtained.

Contents

INTRODUCTION

The placenta is the first organ to form during pregnancy. This ephemeral organ is responsible for sustaining the pregnancy, protecting the fetus, regulating the delivery of all nutrients and gases, and eliminating fetal waste products across gestation. As data evolve, there is a growing recognition of the regulatory role this organ plays in controlling maternal and fetal metabolism and an increased appreciation of the consequences that manifest when maternal nutrient supply cannot support maternal, fetal, and placental requirements. Advances in research have provided new insights into early placental formation, and a growing body of research links early implantation events to subsequent placental health and function. Despite the key role this organ plays in fetal nutrient and gas exchange, many of the proteins and regulatory processes involved in placental nutrient trafficking remain poorly defined. Nutrients are initially delivered to the placenta via uterine glandular secretions before trophoblast remodeling of the maternal vasculature allows nutrients to be delivered to the placenta from maternal blood. Placental function is now known to be orchestrated by many cell types at the maternofetal interface and by maternal, placental, and fetally derived hormones and signaling molecules. In vitro and in vivo approaches have been employed to characterize nutrient partitioning between the mother, fetus, and placenta, but challenges inherent in undertaking these studies in humans have largely limited studies to those that can be undertaken at term or from pregnancies with complications that resulted in early delivery. Fetal sex is now recognized as an important factor to consider as it is increasingly associated with variability in transporter expression and placental efficiency, and mechanisms responsible for these observations are not well characterized. Finally, technological challenges have limited the ability to investigate the function of this organ in real time, but novel techniques and genomic approaches are producing greater insight into the regulatory roles of the placenta and the mechanisms involved in its ability to nourish the fetus across the gestational period.

PLACENTAL DEVELOPMENT AND NUTRIENT TRANSPORT

Early Placental Development and Histiotrophic Nutrition

DPC: days postconception

TE: trophectoderm

CTB: cytotrophoblast

STB: syncytiotrophoblast

EVT: extravillous trophoblast

T1: first trimester

A fertilized zygote undergoes multiple divisions as it travels down the fallopian tube into the uterine cavity. By 6 days postconception (DPC), the zygote has grown and differentiated into a blastocyst comprised of an outer epithelial layer called the trophectoderm (TE) that will give rise to the placenta and a smaller inner cell mass that will develop into the embryo proper (7). At roughly 6 DPC, the blastocyst reaches the uterine cavity where it comes into contact with, attaches to, and invades the uterine endometrium (99). Cell-to-cell communication between the fetal and maternal tissues begins at this very first interaction. As the blastocyst invades the uterine endothelium to initiate placentation, the TE cells differentiate into cytotrophoblast (CTB) cells, and a primitive syncytium forms that will differentiate into the syncytiotrophoblast (STB). When fully developed, the STB functions as a 5- to 6-μm barrier between maternal and fetal blood and has a total area of approximately 15 m^2 (23). The CTB cellular layer is found under the STB, and these cells serve as a functional stem cell pool that will give rise to extravillous trophoblast (EVT) or STB.

By ~9 DPC, the blastocyst is embedded in the decidua and obtains nutrients from uterine secretions (often referred to as uterine milk) produced by maternal uterine glands. Uterine glands secrete nutrients such as glycogen, lipid droplets, and glycoproteins, which can be utilized by the conceptus (4, 40, 73). This form of nutrient provision, which is not delivered via the maternal vascular supply, is referred to as histiotrophic nutrition. At this stage of development, lacunae begin to form in the syncytium, and these fill with blood from ruptured endometrial capillaries (**Figure 1*a***). The trophoblast itself may help activate the uterine glands by secreting lactogenic hormones (116). The embryonic yolk sac forms between 8 and 14 DPC. In early embryogenesis, the yolk sac likely plays an important role in transport of nutrients to the fetus (4). RNA sequencing (RNA-seq) data from the yolk sac have identified numerous nutrient transporters including transporters for cholesterol, amino acids, glucose, nucleoside sugars, and metals (34). Much can go wrong during these early stages of placental development. As many as 10–40% of detectable conceptions end in an early pregnancy loss (120, 135), and less than 30% of all fertilization events are thought to result in a successful pregnancy (85). Fetal chromosomal abnormalities are thought to be responsible for approximately 50% of the cases of early pregnancy loss (1), but the etiology for the remaining cases remains insufficiently characterized. Multiomics approaches have recently been used to identify differentially expressed genes in villus and decidual tissue from individuals who experience recurrent miscarriage (134), but substantial gaps in knowledge remain about underlying mechanisms responsible for early pregnancy losses that are not due to chromosomal abnormalities.

At approximately 12 DPC, the EVT begins to invade the maternal spiral arteries (endoarterial trophoblast), decidual veins (endovenous trophoblast), uterine glands (endoglandular trophoblast), and lymphatic vessels (endolymphatic trophoblast) (64). The endoarterial trophoblasts remodel the spiral arteries, working to replace the endothelium with trophoblast cells that will dilate these vessels (increasing their radius from 0.25 to 2–3 mm) and leading to a reduction in the perivascular muscular layer (25, 51). This process transforms the maternal spiral arteries from high-resistance, low-capacity arteries into low-resistance, high-capacity arteries, which facilitates release of maternal blood from these arteries at pressures that will not damage the STB. Over the first trimester of pregnancy (T1), these arteries remain largely occluded by the endoarterial trophoblast (**Figure 1*b***). At these early stages of placental maturation, the embryo is developing in a relatively low oxygen environment (~20 mm Hg), and the embryo relies on glycolysis, not oxidative phosphorylation, which is thought to protect the embryo against teratogenic effects caused from

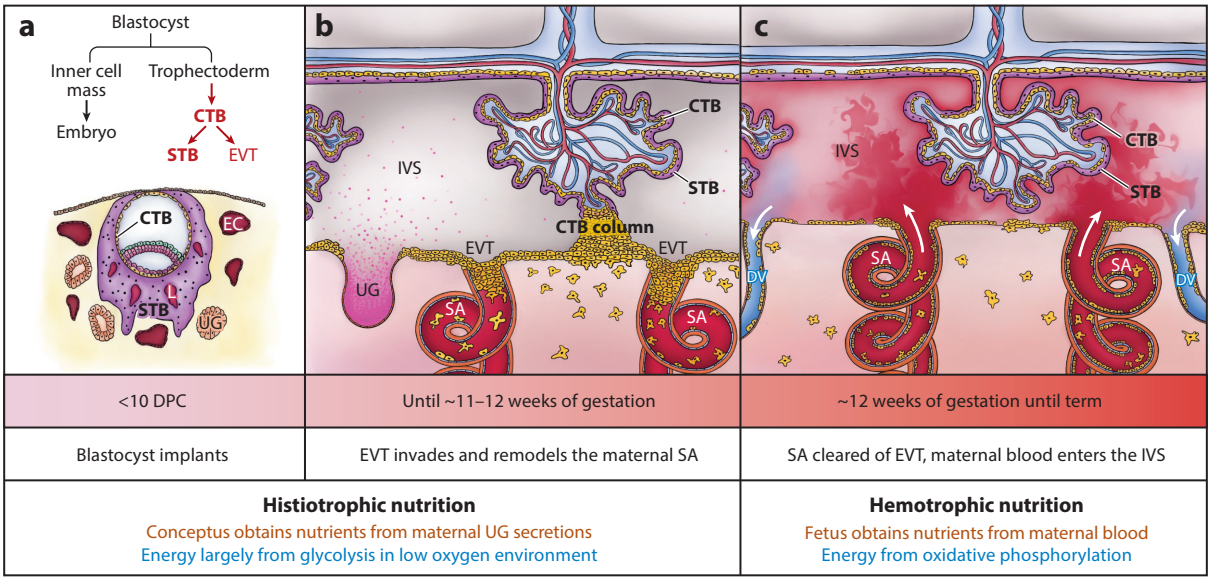

Figure 1

Histiotrophic versus hemotrophic nutrition. (*a*) By ~9 days postconception (DPC), the blastocyst has become fully embedded in decidual tissue and is surrounded by a primitive syncytium, which covers an underlying layer of cytotrophoblast (CTB) cells. Nutrients are obtained from uterine gland (UG) secretions, a process referred to as histiotrophic nutrition. In this lower oxygen environment, glycolysis serves as a predominant energy supply. Endometrial capillaries (ECs) are shown in cross section. Lacunae (L) begin to form in the syncytiotrophoblast (STB) and fill with blood from ruptured endometrial capillaries. (*b*) At ~10 weeks, villous tree structures are covered by the STB and underlying CTB. Fetal capillaries within the chorionic villi deliver nutrients to the fetus and return waste products to maternal circulation. The CTB forms CTB columns that anchor the chorionic villi to the decidual wall. The CTB gives rise to extravillous trophoblast (EVT), which remodels the maternal spiral arteries (SAs), increasing their diameter and decreasing the resistance of these vessels. At this stage of gestation, the EVT occludes the lumen of the SA, limiting maternal blood from being released into the intervillous space (IVS). Nutrients continue to be delivered to the STB via histiotrophic nutrition from the UG. (*c*) Near the end of the first trimester, the EVT is cleared from the SA, allowing maternal blood to be released into the IVS. From this time point onward, nutrients are delivered to the STB via maternal blood, a process referred to as hemotrophic nutrition. Increased perfusion of the placenta facilitates energy production via oxidative phosphorylation. As the pregnancy approaches term, there is less CTB present under the STB, which further facilitates nutrient, waste, and gas transport across the STB. Blood in the IVS is returned into maternal circulation via the decidual veins (DV).

oxygen free radicals (4, 70). Abnormalities in this early vascular remodeling process and premature opening of the spiral arteries are thought to be associated with pregnancy complications, including preeclampsia (PE), fetal growth restriction (FGR), and preterm birth (PTB) (101).

Many gaps in knowledge exist regarding nutrient uptake by the early embryo. This process is difficult to monitor in real time, and consequences of dysregulated implantation, invasion, and early perturbations in nutrient supply often do not become apparent until well into gestation. As an example, while FGR is known to be a leading cause of stillbirth, one-half of FGR cases are not detected prior to birth (45, 64).

PE: preeclampsia

FGR: fetal growth restriction

IVS: intervillous space

Spiral Artery Remodeling and Hemotrophic Nutrition

Human placentas are hemochorial, which means that maternal blood comes into direct contact with the chorion. Near the end of T1 (at approximately 11 weeks of gestation), the endoarterial trophoblast that has occluded the maternal spiral arteries begins to clear, allowing large amounts of maternal blood to be ejected at low pressures to fill the intervillous space (IVS) of the placenta (34).

Maternal blood in the IVS bathes the placental chorionic villi, and at this stage of gestation, the fetus is now nourished by hemotrophic nutrition (**Figure 1*c***). Full transformation of the maternal spiral arteries is a gradual process, and these arteries only become fully patent at ~20 weeks of gestation (64). As these vessels open there is an approximately threefold increase in intraplacental oxygen tension (128).

MVM: microvillus membrane

BM: basal membrane

T2: second trimester

Nutrients in maternal blood cross the maternal-facing microvillus membrane (MVM) of the STB. They are exported across the fetal-facing basal membrane (BM) of the STB and the underlying CTB cells, then travel through the underlying stroma before crossing the fetal endothelium into fetal circulation. The amount of maternal and fetal blood entering the placenta is substantial. At term, the IVS fills with 600–700 mL of maternal blood every minute (65). By late gestation, it has been estimated that approximately 40% of the fetal cardiac output is delivered to the placenta (24). Early problems with placental perfusion and fetoplacental circulation can impair subsequent organ function in the offspring, as shown for cardiac function (24) and by the seminal studies made by David Barker (10).

The transition from histiotrophic to hemotrophic nutrition is accompanied by systemic changes in energy metabolism, hormonal expression, and vasculature formation that are necessary for nutrient and waste exchange in support of fetal growth. The biological mechanisms underlying these changes are complicated, but advances in experimental and computational technologies have begun to provide insights into the molecular landscape of the human placenta through time and space. Comparative RNA-seq analysis of messenger RNA (mRNA) transcripts in the placental villi at 7–8 and 13–14 weeks of gestation showed increased synthesis of peptide hormones and activity of glycolytic pathways in T1. During the second trimester (T2), when the placenta is nourished via hemotrophic nutrition, placental energy metabolism pathways switch to favor mitochondrial respiration, leading to increases in oxidative stress and antioxidant defense and to altered expression of genes regulating glucose, protein, lipid, and ion transport (100). Expression of genes related to vascular formation and angiogenesis are also upregulated in late-stage placentas (100, 118), highlighting the continuous remodeling of placental vasculature that is needed to support the rapidly growing fetus. Moreover, the transition to hemotrophic nutrition may also affect the expression patterns of placental microRNAs (miRNAs). It has been shown that ~60% of miRNAs in the placental villi exhibit a significant differential expression between 6–10 and 11–23 weeks of gestation, some of which are thought to play important roles in mediating the effects of hypoxia, cellular proliferation, and maternal immune suppression (111). Besides RNA profiles, DNA methylation patterns also change after establishment of maternal blood flow in the IVS, which may affect expression of genes known to regulate vitamin A and D metabolism, oxidative stress, ion transport, and progesterone synthesis (100).

Placental Senescence and Nutrient Transfer

The size of the placenta relative to the fetus changes substantially over gestation. Until approximately 15–16 weeks of gestation, the fetus is considerably smaller than the placenta, but by term, the fetus is 5–6 times larger than the placenta, and an ~600-g placenta must support the metabolic and nutritional demands of the ~3,500-g fetus. The placenta can sustain continued fetal growth until approximately 42 weeks of gestation. After 42 weeks of gestation (defined as prolonged pregnancy), fetal weight begins to decline, which some believe is indicative of the placenta no longer being able to support the nutritional and metabolic demands of the fetus. Prolonged pregnancy is more common among individuals who have obesity, those who have previously experienced a prolonged pregnancy, those who are carrying a male fetus, and those who have genetic risk factors (27). Labor is typically induced before 42 weeks of gestation to avoid the increased risks of adverse birth outcomes and perinatal mortality that have been associated with prolonged pregnancy

(89) and that may occur as a consequence of placental senescence. As the placenta ages, structural changes occur including reductions in the size and number of mitochondria, alterations in the Golgi apparatus and endoplasmic reticulum, reduced evidence of pinocytosis on the MVM, visible vacuoles and calcifications in the STB, thickening of the BM, and a reduction in the size of the fetal capillaries. Syncytial knots also increase with placental aging; these reflect aggregations of aged STB nuclei that are removed from metabolically active areas (27). Changes that occur due to placental senescence would be expected to alter placental hormone production, nutrient uptake, nutrient export to the fetus, and fetal oxygenation. Premature placental senescence has been observed in pathological pregnancies and has been postulated to be a contributor to pregnancy complications such as PE (91). Some of the apoptotic material from the placenta is released into maternal circulation and may be useful in diagnosing adverse processes before fetal growth faltering or adverse in utero consequences occur (91).

The Syncytiotrophoblast Is a Unique Nutrient Barrier

Mechanisms of nutrient transport across the STB are frequently assumed to mirror those employed by the enterocyte without accounting for key differences between these cell types. The enterocyte has a finite life span of several days before it is sloughed into the gastrointestinal lumen. This provides these nutrient-trafficking cells a means of eliminating substances that are stored in the enterocyte but not exported into the circulation. The placenta must either restrict uptake of substances, move substances back into maternal circulation, or sequester these substances over gestation if they are not transported to the fetus. Enterocytes are exposed to a variable intraluminal pH—which ranges from approximately 6 in the duodenum to 7.4 in the terminal ileum to 5.7 in the cecum (44)—and to a microbiome composition that changes from the proximal to the distal intestine (122). In contrast, the placenta is bathed in maternal and fetal blood, which has a narrow pH range of 7.35–7.45, and the presence of a low biomass placental microbiome remains controversial (147). The enterocyte is exposed to only one hormonal environment while the placenta is exposed to hormonal signals that are independently produced by the pregnant individual and the fetus and that come into close proximity to the MVM or BM. This complexity is further increased in multiple gestations where independent placentas are formed by dizygotic embryos that may be of different sexes, and fetally derived hormone concentrations may vary in relation to variable fetal nutrient exposures (39, 105). The placenta has also been found to express unique proteins that replace proteins serving the same role in other tissues, such as the placental ferroxidase zyklopen (32). As a syncytium, the STB lacks the paracellular junctions that are found between adjacent enterocytes. This would necessitate that all nutrient transport across the STB must occur via transcellular processes. However, data indicate that mechanical forces or degeneration of the STB as gestation progresses may result in gaps in the STB that provide routes for paracellular maternofetal transfer (48). Small transtrophoblastic channels have also been reported in the STB and are purported to allow molecules with diameters of 20 nm to pass from the basal toward the apical surface of the STB (75). These channels may also allow for fetomaternal fluid shifts to maintain fetal osmoregulation and water balance (48). The unique attributes of the STB highlight the need to characterize nutrient trafficking in placental tissue.

Factors That Influence Placental Nutrient Transport

Movement of nutrients and gases across the placenta into fetal circulation is impacted by the chemical composition of the substance being transported, the chemical gradient between the maternal and fetal compartments, the rate of maternal and fetal blood flow on either side of the placenta, the surface area and thickness of the placenta (distance between the outer surface of the STB

and the fetal capillary endothelium), the nutritional and metabolic demands of the placenta itself, and the presence (number, density, distribution, and activity) of the required receptors, channels, pores, or proteins on placental and endothelial membranes. The umbilical cord insertion site to the placenta may also impact nutrient transfer to the fetus. The umbilical cord insertion site can be located in the center of the placenta (central insertion), slightly off center (eccentric insertion), on the periphery of the placental disk (marginal insertion), or even on the placental membranes themselves (velamentous insertion). Marginal cord insertion has been postulated to promote more heterogeneous transport throughout the villous network (71) while reduced transport efficiency has been observed in placentas with noncentral cord insertion (142).

IDA: iron deficiency anemia

Small hydrophobic compounds and gasses move across the placenta down their diffusion gradients by simple transcellular lipophilic diffusion. Simple diffusion across the placenta is impacted by the placental surface area and thickness and by the blood flow on either side of the membrane. Structural changes that occur as the placenta ages may facilitate diffusion, because as the placenta ages the surface area and number of placental villi increase, the placenta becomes more vascular, and the STB becomes thinner with decreased numbers of persistent CTB cells under the STB (48). In addition to essential nutrients and waste products (such as urea and gases), simple diffusion also allows harmful lipophilic compounds (alcohol, nicotine, gases such as carbon monoxide, and toxins) to cross the STB from maternal into fetal circulation, and placental concentrations of environmental wastes (such as heavy metals) increase with increasing maternal age, presumably due to larger body burden of these contaminants that are released from body stores such as bone during pregnancy (38).

While some fat-soluble nutrients and gases may diffuse across placental membranes, many nutrients require specialized energy-dependent transporters to move across the trophoblast, as recently reviewed (74). The trophoblast expresses two key families of membrane transport proteins: the solute carriers (SLCs) and the ATP-binding cassette (ABC) transporter family. The trophoblast ABC transporters assist with the transport of multiple substrates including steroid hormones, lipids, cholesterol, and inflammatory factors. Trophoblast transport of amino acids, glucose, organic cations and anions, and monoamines is facilitated by SLC transporters. Nutrient transfer across the placenta is also dependent on the placenta's need to utilize this nutrient in support of its own nutritional demands and on the ability of the fetus to signal its nutritional demands to the placenta. Many other factors impact the interpretation of placental nutrient transfer data including variable fetal sizes and numbers (in the case of multiple births), differential gene expression profiles that may occur as a function of stage of gestation, underlying maternal conditions such as obesity, sex of the fetus, and the source of placental sampling on the placental disk. Several processes utilized to transport nutrients to the fetus are highlighted below in the discussion of carbohydrate (glucose), fat (fatty acids), protein (amino acids), and mineral (iron) transport across the placenta. These nutrients provide examples of the range of transport mechanisms utilized; the complexities in balancing maternal, fetal, and placental demands; and the similar challenges that exist when attempting to characterize these pathways in placental tissue. The placenta also needs to sense and respond to alterations in the tempo of fetal nutritional demand, which varies markedly among nutrients, as depicted in **Figure 2**.

MECHANISMS OF PLACENTAL NUTRIENT UPTAKE

Iron Transport—Receptor-Mediated Endocytosis

Maternal iron stores and physiological increases in iron absorption are often not sufficient to supply the nearly 1,000 mg of iron that is needed to support a typical singleton pregnancy (93), and ~50% of pregnant individuals worldwide have iron deficiency anemia (IDA) (14). Maternal IDA

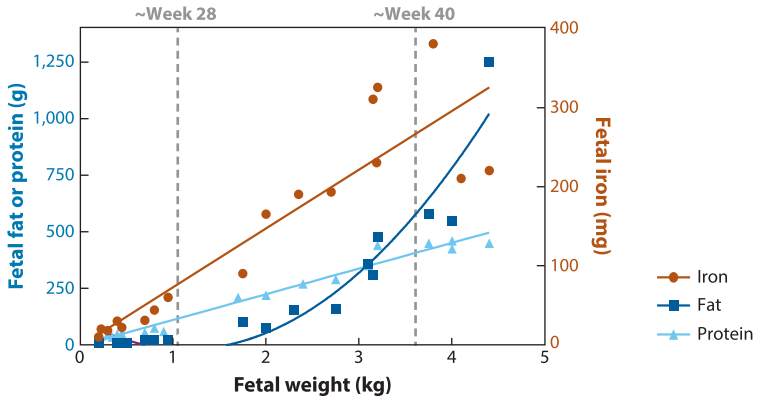

Figure 2

Variable tempo of fetal nutrient accretion. Cross-sectional data on the chemical composition of the human fetus were compiled from early studies carried out by Widdowson & Spray (138). Data were obtained from 13 fetuses (ranging in weight from 225 to 4,375 g). Of these, 6 were stillborn (all weighing more than 3,000 g) and 2 were twins. Causes of death were documented (138) and included demise due to maternal and birth complications (eclampsia, hemorrhage, placenta previa, breech presentation, etc.), habitual or inevitable abortion, or surgical induction. On the basis of this analysis, net accretion of iron, protein, and fat are plotted as a function of fetal weight. Over the last trimester of pregnancy, the average weight of a fetus more than triples from 1.1 kg at 28 weeks to 3.5 kg at term. The average iron content of the fetus nearly triples over this period from ~90 to ~300 mg. Fetal fat accretion shows a nearly exponential increase during the last trimester of pregnancy. In contrast, gains in protein accretion are modest during the final weeks of pregnancy. Mechanisms of placental transport must be differentially regulated to accommodate these highly variable tempos of nutrient accretion. Data from Reference 138.

has been associated with abnormalities in placental physiology including placental hypertrophy (9), placental abruption, and PTB (13). Suboptimal fetal iron exposures are increasingly linked to long-term, irreversible deficits in neurobehavioral outcomes (11).

Iron is a highly reactive metal that is commonly present in the body as either ferric (Fe^{3+}) or ferrous (Fe^{2+}) iron. Iron-trafficking proteins have specific affinities for these oxidation states, making ferroxidases and ferrireductases integral to placental cellular iron transport processes. Nearly all nonheme iron in maternal circulation is transported bound to a specific carrier protein, transferrin (Tf) (encoded by the gene *TF*). Each transferrin molecule can bind two atoms of Fe^{3+}, which when fully loaded is referred to as diferric transferrin. The placenta richly expresses transferrin receptor 1 (TfR1) (encoded by the gene *TFRC*) to capture diferric transferrin from maternal circulation via receptor-mediated endocytosis. The Tf-TfR1 complex is endocytosed in a unidirectional fashion into clathrin-coated pits on the MVM. The resulting endosomes are acidified by proton pumps, releasing the ferric iron from the Tf-TfR1 complex. The ferric iron is reduced by an endosomal ferrireductase (STEAP3 or STEAP4) and exported as ferrous iron into the STB cytosol by divalent metal transporter 1 (DMT1). The lysosome containing the Tf-TfR1 complex is recycled back to the MVM membrane, and the Tf is released back into maternal circulation to bind more iron. Ferrous iron delivered into the cytosol can be transported by an iron chaperone, poly(rC)-binding protein 2 (PCBP2), and iron can be utilized in support of placental mitochondrial energy demands and other cellular functions or stored as ferritin. If not utilized or stored in the STB, iron is exported across the BM by the only nonheme Fe export protein in the body, ferroportin (FPN), and reoxidized to ferric iron by a ferroxidase (zyklopen, hephaestin, or ceruloplasmin) (46, 93, 148) **(Figure 3*a*)**.

Tf: transferrin

TfR1: transferrin receptor 1

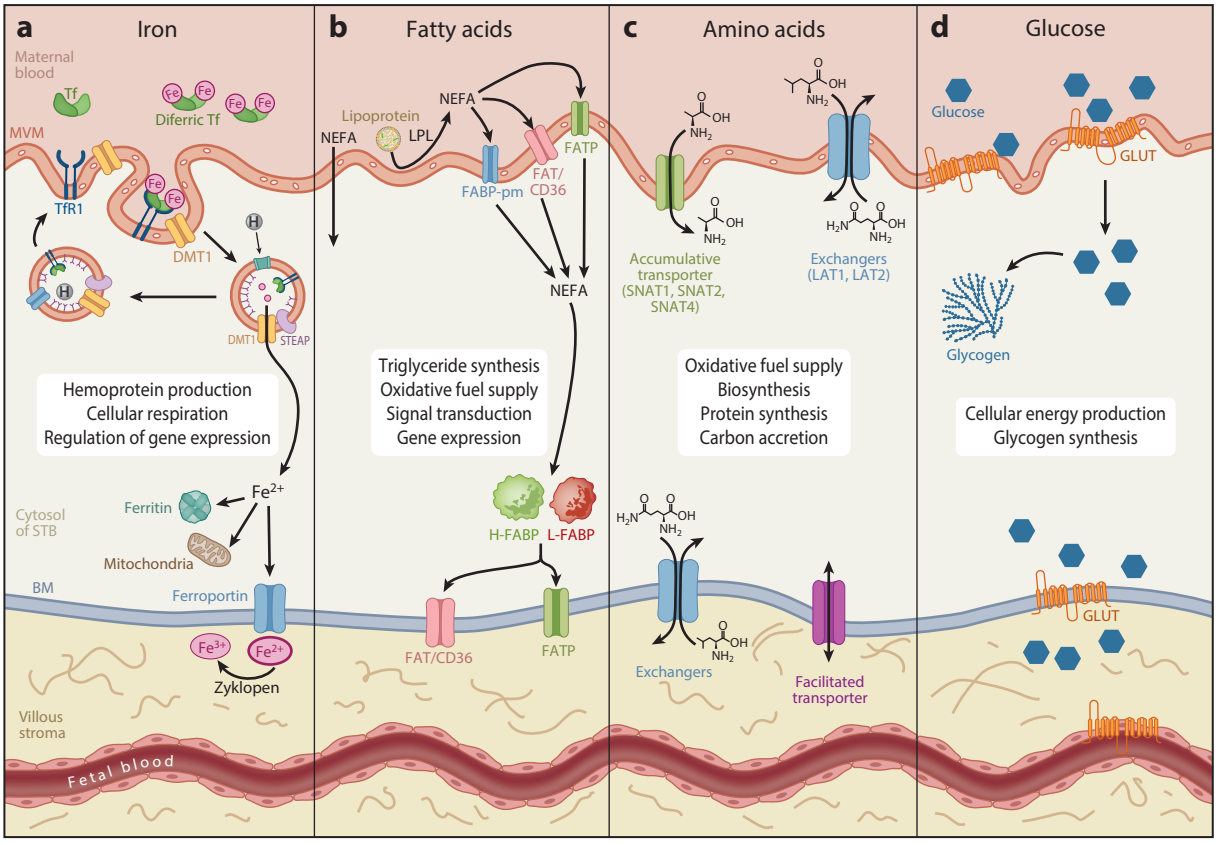

Figure 3

Mechanisms of placental nutrient trafficking. Each of four nutrients (iron, fatty acids, amino acids, and glucose) are labeled at the top; the functions of each are shown in a list below. (*a*) Iron is transported across the placenta via receptor-mediated endocytosis. Transferrin (Tf) binds iron in maternal circulation to produce diferric transferrin. This binds to transferrin receptor 1 (TfR1) on the microvillus membrane (MVM) and is internalized into endosomes that are acidified by proton pumps; the iron is then released from TfR1 and is reduced to ferrous iron by a ferrireductase (STEAP) to be pumped out of the endosome into the cytosol by divalent metal transporter 1 (DMT1). Iron in the syncytiotrophoblast (STB) can be stored as ferritin, used for cellular purposes, or exported by ferroportin across the basal membrane (BM) and oxidized by a ferroxidase (zyklopen) to be exported into fetal circulation. (*b*) Fatty acids are transferred across the placenta by diffusion and active transport. A small amount of nonesterified fatty acids (NEFAs) in maternal circulation can diffuse across the MVM. Most fatty acids are presented to the STB as lipoproteins that are broken down by lipoprotein lipase (LPL) to produce NEFAs. The resulting NEFAs are transported across the MVM using either a plasma membrane fatty acid binding protein (FABP-pm), a fatty acid translocase (FAT/CD36), or a fatty acid transfer protein (FATP). In the cytosol, the NEFAs bind to liver- or heart-type FABPs (L-FABP or H-FABP) and can be utilized by the STB or transported across the BM using FAT/CD36 or FATP. (*c*) Amino acids are transported across the MVM using accumulative transporters (such as the sodium-coupled neutral amino acid transporters SNAT1, SNAT2, and SNAT4) or exchangers (such as the L-type amino acid transporters LAT1 and LAT2). On the BM, amino acids are transported using exchangers or can be transferred across the BM in either direction down the concentration gradient using facilitated transporters. (*d*) Glucose is transported across the MVM down the concentration gradient using facilitated diffusion. A series of glucose transporters (GLUTs) are present on the MVM and move glucose into the cytosol by facilitated diffusion down the concentration gradient. Once in the cytosol, glucose can be used for cellular purposes, stored as glycogen, or exported across the BM by GLUTs to be delivered into fetal circulation.

PIDI: placental iron deficiency index

The majority of research on placental iron trafficking has focused on placental utilization of nonheme iron, but the placenta also richly expresses other transport proteins involved in heme iron utilization including feline leukemia virus subgroup C receptor 1 (FLVCR1), heme-responsive gene 1 (HRG1), and lipoprotein receptor-related protein 1 (LRP1) (15, 62, 148). These proteins may provide the placenta with the capacity to scavenge heme catabolic products that are derived from extravascular red blood cell catabolism. Studies using stable isotopically labeled heme and nonheme iron found a preferential maternal absorption of heme iron, and a significantly greater percentage of maternally absorbed heme iron was transferred to the fetus (144).

The placenta's ability to regulate iron-trafficking proteins in relation to maternal and neonatal iron status and iron regulatory hormones has received much attention. Existing data support an increase in the protein expression of TfR1 on the MVM as maternal Fe stores are depleted, presumably to help the placenta scavenge nonheme iron (15, 106, 145). Fewer data are available on placental FPN protein expression, and the discrepant findings that exist may be driven by methodological differences in the antibody used or the variable iron status of the study population (15, 28, 106, 108). Protein and mRNA expression of the placental ferroxidase zyklopen were recently found to be upregulated in term placenta tissue obtained from individuals with IDA (117). Sangkhae et al. (106) developed a placental iron deficiency index (PIDI), which is based on the ratio of the protein expression of the BM nonheme iron exporter, FPN, to the protein expression of the MVM nonheme importer, TfR1. Significantly lower PIDI ratios have been observed among pregnant individuals with depleted iron stores (serum ferritin) (106) and in placental tissue obtained from individuals with prenatal alcohol-related iron deficiency (28). Animal data have highlighted the role of the placental intracellular iron-regulatory protein and iron-response element system in responding to the cellular labile iron pool to stabilize the mRNA of *TFRC* and repress the translation of FPN. This coordinated regulatory process conserves placental iron in support of placental iron demands (106) but may impair iron transfer to the fetus if placental iron deficits are prolonged.

The fetus has an intact erythropoietin-erythroferrone-hepcidin axis and begins to produce iron regulatory hormones early in gestation (8, 39). Possible associations between neonatal Fe status and regulatory hormones at birth and placental expression of Fe-trafficking proteins in term placental tissue have been explored for a number of heme and nonheme iron-trafficking proteins (15, 127). When evaluating determinants of placental nutrient trafficking, it has been difficult to isolate relative effects that are controlled by the mother, the placenta, or the fetus. A multiple birth model has provided opportunities to examine relative differences in iron partitioning between multiple fetal-placental units by evaluating umbilical cord iron biomarkers at birth. With this approach, highly variable concentrations of the major nonheme iron regulatory hormone (hepcidin) were found between siblings, indicating that the fetus modifies its hormonal milieu in response to the iron it receives (105). However, fetally derived hepcidin does not appear to influence the expression of the BM iron export protein FPN or other nonheme iron-trafficking proteins (15).

Placental Fatty Acid Transport—Diffusion and Active Transport

Maternal lipid sources are needed for fetoplacental development from the earliest stages of placental trophoblast invasion and angiogenesis to the last 10 weeks of pregnancy when approximately 90% of the total fat content of the fetus is accumulated (18, 137). Among mammals, human newborns have the highest percentage of body fat at birth (76). Maternal essential fatty acids (FAs) and long-chain polyunsaturated fatty acids (LC-PUFAs) supply the lipid needed for fetal development. A small amount of nonesterified fatty acids (NEFAs) is found in maternal circulation (~2–3% of circulating FAs), and these can move from maternal circulation into fetal circulation across the STB by diffusion (59). Maternal lipoproteins (as esterified FAs in triglycerides and

phospholipids) are actively transported across the STB after being hydrolyzed into NEFAs by lipases. Lipoprotein lipase on the MVM mainly hydrolyzes triglycerides, while endothelial lipase mainly hydrolyzes phospholipids (77, 78). The free fatty acids (FFAs) released are transported across the MVM into the cytoplasm. FAs that are not used to support key placental metabolic functions are transported through the cytosol and cross the BM and fetal endothelium to enter fetal circulation. Placental transfer of FAs uses a family of transport proteins that include the fatty acid translocase (FAT/CD36), fatty acid transporter proteins 1–6 (FATP1–6), and placenta-specific plasma membrane fatty acid binding protein (FABP-pm), as recently reviewed (42). These lipid handling transporters are not uniformly distributed in the placenta. The maternal MVM of the STB contains FABP-pm as well as FATPs and FAT/CD36. There is some selectivity of the MVM transporters, and FABP-pm binds mainly arachidonic acid and docosahexaenoic acid and some linoleic and oleic acid. In contrast, FATP does not exhibit preferences for FA uptake, and FAT/CD36 interacts with multiple FAs and other ligands (18). Once FFAs enter the cytosol, they are trafficked by two main FABPs, heart-type FABP (H-FABP) and liver-type FABP (L-FABP). Intracellular FAs can be utilized for intracellular biological purposes, oxidized by the placental mitochondria to produce energy, or re-esterified by the endoplasmic reticulum and stored as lipid droplets (18). If not retained or utilized by the placenta, FFAs are transported across the BM using FATPs and FAT/CD36. These exported FFAs must then cross the stroma before entering the fetal endothelium (**Figure 3*b***).

The essential fatty acids, alpha-linolenic acid (C18:3 *n*–3) (ALA) and linoleic acid (C18:2 *n*–6) (LA), are particularly important for fetal neuronal and brain development. Both ALA and LA serve as precursors for the LC-PUFAs arachidonic acid (C20:4 *n*–6) (AA), eicosapentaenoic acid (C20:5 *n*–3) (EPA), and docosahexaenoic acid (C22:6 *n*–3) (DHA). A higher relative amount of DHA and EPA and lower relative proportions of ALA and LA are found in fetal compared with maternal plasma. This ability to preferentially accumulate these FAs is referred to as biomagnification, and it is thought to occur due to enhanced placental transfer of DHA and AA to the fetal circulation (54).

Pregnant individuals with obesity have altered placental expression of many of the FA transport proteins and increased transfer of maternal FFA to the fetus (60). Studies of placental tissue from individuals with obesity have found that lipid storage and esterification are increased, mitochondrial FA oxidation is decreased, and peroxisomal FA oxidation is upregulated to compensate for deficits in mitochondrial function, and together these pathways may work to limit transfer of excess fat to the fetus (26). Several in vivo kinetic studies of maternofetal FFA transport (using stable isotopically labeled FAs) have been undertaken, but conflicting findings on the impact of maternal obesity on FA transport to the fetus have been reported (52, 53). Excess fetal adiposity is often found in both undernourished and overnourished mothers, a process that has been referred to as nutrient-mediated teratogenesis. (141) The role of the placenta in this process requires further attention.

Amino Acid Transport—Accumulative Transporters/Exchangers/Facilitated Transport

Amino acids in the placenta and developing fetus are utilized for biosynthesis of numerous molecules (including nucleotides), for protein synthesis, for carbon accretion, and as an important oxidative fuel substrate. Fetal growth is dependent on maternal supply of amino acids and nutritional or hormonal alterations. The profile and activity of amino acid transporters can contribute to fetal overgrowth or fetal growth restriction. On average, 10–60 g of amino acids are transferred to the fetus each day for every 1 kg of fetal weight (87). While fetal accretion of most nutrients peaks in late gestation, fetal protein accretion slows as fat becomes the dominant contributor to

T3: third trimester

weight gain (113) (**Figure 2**). More than 20 different amino acid transporters supply the placenta and fetus with amino acids (49). Existing amino acid transporters can be classified in multiple ways, including if they are sodium dependent or sodium independent, on the basis of substrate specificity (whether they prefer cationic, zwitterionic, or anionic substrates), and on sequence homology (family). Amino acid transporters are also classified into functional groups on the basis of whether they are accumulative, exchangers, or facilitated transporters. Accumulative transporters mediate syncytiotrophoblast uptake of specific amino acids from maternal or fetal blood. Exchangers mediate efflux of amino acids from the STB in exchange for essential amino acids that are brought into the STB cytosol from maternal or fetal circulation. Exchangers lead to a change in the overall amino acid composition of the cytosol but no change in the overall quantity of amino acids. Facilitated transporters export amino acids down their concentration gradient. The type and location of amino acid transport systems in human placental tissue varies (12, 36), but much of the research to date has focused on system A and system L transporters. System A transporters transport neutral amino acids and are found on both the MVM and BM of the human placenta. The system A transporters are sodium-coupled neutral amino acid transporters (SNATs) that have three isoforms (SNAT1, SNAT2, and SNAT4), all of which are found in the human placenta. System A amino acid transporters mediate the placental uptake of nonessential, neutral amino acids against their concentration gradient, and sodium is cotransported into the cell. Transport of neutral amino acids across the MVM by system A transporters establishes a transmembrane gradient that is utilized to drive the uptake of large essential amino acids via the system L transporters. The system L transporters, L-type amino acid transporters 1 and 2 (LAT1 and LAT2), are sodium-independent exchangers that are heterodimers of a light chain that is covalently linked to a heavy chain. System L transporters exchange nonessential for essential amino acids, and these are transported against their concentration gradient (**Figure 3*c***).

Placental amino acid transport and placental metabolism of amino acids change over the course of gestation. Early in gestation, intact peptides are transferred across the yolk sac to support the amino acid requirements of the conceptus (12). By 12–17 weeks of gestation, the concentration of amino acids in fetal circulation is greater than that measured in maternal circulation, indicative of active transport (30, 69). For some amino acids, the placenta may compete with the fetus, and only when placental protein catabolism exceeds protein synthesis are these amino acids released across the BM to the fetus. Amino acids that are oxidized within the placenta are interconverted into other amino acids, changing the cytosolic amino acid composition, which affects cellular gradients that drive other amino acid transport processes (102). Maternal and fetal metabolism will also alter the total and relative amounts of amino acids being delivered to the placenta in arterial blood (36). Much of what is known about human placental amino acid metabolism has been obtained using in vitro models, which cannot adequately simulate the concentrations and dynamic changes that occur for all amino acids on either side of the MVM or BM; nor do these systems provide data on interorgan cycling between the placenta and fetal liver.

Transplacental flux of amino acids has been evaluated in vivo, in pregnant individuals, using stable isotopically labeled essential and nonessential amino acids. Two main approaches have been used in these studies. The first approach involves administering tracers to women during T2 or the third trimester (T3) to evaluate placental transfer to the fetus using cordocentesis. Individuals in these studies have had medical conditions (thrombocytopenia, seroconversion toxoplasmosis, chicken pox, or late booking for prenatal diagnosis) that necessitated in utero fetal blood sampling. The second approach involves administering amino acid tracers at term shortly before scheduled C-sections and obtaining samples of umbilical blood at birth to evaluate placental transfer.

Examples of studies evaluating fetal uptake of maternally administered amino acids during T2 or T3 include those that infused labeled leucine and glycine (29); labeled leucine, phenylalanine,

glycine, and proline (95); or primed constant infusions of two different leucine tracers with staggered start times, each followed by a constant leucine infusion (94). In all of these studies, umbilical blood was obtained postmaternal dosing by cordocentesis. The fetal-to-maternal (F/M) enrichment of labeled amino acids was evaluated to measure amino acid transfer. These approaches determined that leucine and phenylalanine transfer to the fetus are significantly greater than glycine or proline transfer (29, 95), a finding that has been attributed to the high-affinity exchange transporters used for leucine and phenylalanine. A significantly lower F/M enrichment of leucine and phenylalanine was observed in individuals carrying intrauterine growth retardation (IUGR) fetuses indicative of impaired transplacental flux (95).

IUGR: intrauterine growth retardation

Rapid transplacental flux of nine essential amino acids from maternal to fetal circulation was evaluated at term in individuals scheduled to deliver by C-section. Umbilical blood flow was measured prior to delivery by Doppler ultrasound. Within 10 min of delivery, individuals received one of two infusions that contained either leucine, isoleucine, phenylalanine, lysine, and tryptophan ($n = 5$) or valine, methionine, threonine, histidine, and lysine ($n = 7$). At delivery, the umbilical cord was clamped, and the umbilical arterial and venous F/M enrichment ratios were measured and normalized to the leucine ratio in each study. Four of the amino acids were found to be rapidly transported to the fetus (leucine, isoleucine, methionine, and phenylalanine), with the slowest transfer being evident for lysine. The study also observed a significant linear relationship between the F/M ratio and umbilical blood flow (measured prior to delivery) (50).

The net availability of amino acids for fetal use depends on the concentrations of individual amino acids in maternal and fetal circulation, on the trophoblast permeability, on the placenta's capacity to produce amino acids, on its need to utilize these amino acids for its own metabolic demands, and on the abundance of the necessary transporters on the MVM and BM. Amino acids that are exported across the BM must cross the stroma and fetal endothelium before entering fetal circulation. This process is difficult to study in vivo, but computational modeling data from in vitro human placental perfusions suggest that diffusion through the stroma and into the fetal capillaries is not a rate-limiting step (82). Instead, transfer of amino acids across the BM often appears to be a rate-limiting step in fetal amino acid supply (35). Studies to date have affirmed that the placenta plays an active role in provision of amino acids to the fetus. To move this field forward and address the intricacies of this system, computational modeling approaches are currently being applied to identify rate-limiting steps and factors that impact amino acid partitioning among the mother, placenta, and fetus and to address possible interorgan cycling between the placenta and fetal liver (36).

Glucose Transport Across the Placenta—Facilitated Diffusion

Glucose serves as the primary energy source for the fetus. To help promote fetal glucose availability, pregnant individuals exhibit a decreased insulin sensitivity as pregnancy progresses, a finding that is thought to be mediated, in part, by the placenta (110). Glucose taken up by the placenta can be oxidized by the placenta, stored as glycogen, used to synthesize NEFAs, or transported to the fetus. Glycogen is stored in placental tissue either as a reserve supply of glucose for the fetus or to serve as a source of fuel for the placenta. Glycogen stores in the placenta decrease over the course of gestation (3, 126). Glucose crosses the placenta using facilitated diffusion mediated by a variety of glucose transporter (GLUT) proteins that are differentially expressed throughout the placenta and fetal endothelium. Multiple GLUT isoforms have been identified in placental tissue (GLUT1, 3, 4, 8, 9, and 12), and these vary by location and stage of gestation (115). Research has focused on GLUTs in the MVM (GLUT1, GLUT9, and GLUT3) and BM (GLUT1 and GLUT4) and on the CTB (GLUT1 and GLUT3) and fetal endothelium (GLUT1, GLUT3, and GLUT4), as recently reviewed (109). GLUT1 is found on both the MVM and BM, but given that the surface area of the MVM is approximately fourfold greater than the surface area of the

BM (61), the reduced GLUT1 protein density on the BM is thought to be the rate-limiting step in glucose transfer to the fetus (61, 109) (**Figure 3*d***). GLUT expression changes quantitatively over pregnancy, as shown for GLUT3, which appears to be important in early pregnancy (20).

GDM: gestational diabetes mellitus

In vivo studies of placental glucose transport were completed in healthy individuals undergoing elective C-section deliveries using a unique four-vessel sampling method. This methodology measures the net amount of glucose delivered to the uteroplacental unit (by measuring glucose concentrations in the maternal radial artery and vein) and the net amount of glucose transferred to the fetal circulation (by measuring glucose concentrations in the umbilical artery and vein). Doppler ultrasound measures of uterine and umbilical blood flow are also obtained. Results obtained using this approach in a group of 70 pregnant individuals were recently summarized (58). Modeling of these data concluded that (*a*) the mass of glucose transferred to the fetus is linearly associated with the mass of glucose taken up by the uteroplacental unit; (*b*) uteroplacental utilization of glucose is highly variable (ranging from a net negative to a positive glucose balance); (*c*) the placenta utilizes glucose in support of its own demands, which influences the mass of glucose delivered to the fetus; and (*d*) supply of glucose to the fetus may be supplemented by uteroplacental glucose from placental glycogen stores (58).

Because glucose transport occurs via facilitated diffusion, individuals who develop gestational diabetes mellitus (GDM) or those that enter pregnancy with poorly controlled diabetes mellitus are at risk of excessive placental glucose transfer, resulting in fetal overgrowth (macrosomia) and excess adiposity (86). Excess placental glycogen has been observed in those with GDM. This adaptation is thought to protect the fetus from hyperglycemia (126). Pregnant individuals with obesity have been noted to have alterations in placental glucose transport (109), and increased BM GLUT1 expression has been observed in individuals with obesity who delivered newborns with macrosomia (63). It is difficult to disentangle possible effects that may be driven by obesity from those caused by GDM in individuals who have both GDM and obesity. Altered placental glucose transport has also been noted in individuals carrying monochorionic twins that exhibited hypoperfusion of the placenta, in pregnancies that were established using assisted reproductive technologies, and in pregnant individuals that develop preeclampsia or cholestasis (109). Alterations in placental glucose utilization caused by GDM appear to be impacted more by placental function than by structure, as concluded by a study of transcriptomic alterations in the GDM placenta at the single-cell level that found a similar cell type profile in healthy women and women with GDM (143).

PLACENTAL FUNCTION IN HEALTH AND DISEASE

Fetal Sex as a Modulating Factor of Placental Health

Placental efficiency, a common metric derived using the ratio of fetal or birth weight to placental weight, is often found to be greater in male fetuses (47). The increased placental efficiency in a male fetus may come at a cost, as a smaller placenta has less reserve capacity and is more reliant on a constant maternal nutrient supply. This makes the male fetus more susceptible to nutritional insults in utero and may explain the altered sex ratio that has been observed in newborns from individuals that experience nutrient deprivation during pregnancy or in high-risk pregnancies such as multiple births (66). Metrics of placental efficiency have also been linked to long-term developmental outcomes. A recent study in more than 71,000 pregnant individuals found that risk of neurodevelopmental delay in 3-year-old offspring was significantly higher among toddlers whose placental efficiency values were under the 10th percentile. Of note, this association was only significant among boys (90).

Sexual dimorphism is also evident in placental glucose metabolism. Several studies have noted an elevated prevalence and increased severity of GDM among women carrying males (68). This

increased risk may be a consequence of fetomaternal signaling, with the male fetoplacental unit modifying maternal glucose and insulin metabolism during pregnancy (97, 103). Analyses of placental miRNA in women with diabetes revealed six miRNAs to statistically differ between women with and without diabetes carrying male fetuses, but no differences were observed among women carrying female fetuses (133). Similarly, maternal glycemia was also found to impair mitochondrial biogenesis only in placentas of male fetuses (72). In contrast, other studies have found a significant positive correlation between maternal glucose concentrations and placental weight only in female infants (104), as well as greater effects of maternal glycemia on neonatal subcutaneous and intraabdominal adiposity among female infants (123).

Sexual dimorphism in the placental transcriptome has been explored at varying stages of gestation and in term placentas, as recently reviewed (19). These findings identified extensive sex differences in placental metabolic functions and immune responses that involved changes in a variety of biological pathways related to epigenomic modification, hormonal regulation, and cellular signaling processes (19). Fetal sex–related differences in placental polyamine metabolism have also been studied in detail. Polyamines, such as spermidine and spermine, play diverse roles in cells and have been found to promote longevity in model organisms (43) and human cell lines (149). The gene that controls synthesis of spermine in humans (i.e., *SMS*) is encoded on the X chromosome. In mammals, dosage of X-linked genes is balanced between XY males and XX females by X-chromosome inactivation (XCI), whereby genes from only one copy of the X chromosomes are transcribed in females. However, some X-linked genes, including *SMS*, can escape XCI, resulting in increased gene expression in females relative to males (125). The placental expression of *SMS* and the maternal serum level of spermine metabolites were decreased in women carrying male fetuses relative to those carrying female fetuses (57). One spermine metabolite, diacetylspermine, detected in maternal serum was further associated with an increased risk of PE and a reduced risk of FGR (57). Functional assays of trophoblasts revealed that placentas of male fetuses were more susceptible to polyamine depletion, which was associated with impaired mitochondrial energy metabolism, and decreased histone acetylation and progesterone synthesis (6). Moreover, genes that escape from XCI may be essential for placental function, since monosomy X has been recently shown to impair development and maturation of the STB and EVT and reduce placental hormone production in a trophoblast model derived from human-induced pluripotent stem cells carrying a single copy of the X chromosome (2). Interestingly, variable expression of the paternal allele, maternal allele, or both alleles of an X-linked gene was noted across different locations in term placentas (98) and among single nuclei of isolated trophoblasts (6). How mosaic expression of X-linked genes affects trophoblast phenotypes and contributes to sexual dimorphism in placental function and pregnancy complications are questions that merit further study.

Placental Gene Pathways and Pregnancy Complications

Advances in high-throughput profiling approaches have provided opportunities to identify placental molecular features with an increasingly large sample size. Notably, many disease-associated placental molecular features converge upon important biological pathways that regulate energy and nutrient metabolism and placental development. Deyssenroth et al. (41) examined mRNA profiles in 200 term placental samples and characterized the transcriptome using 17 placental coexpression modules. Perturbation in five modules (implicated in mitochondrial respiration, protein transport, and hormone signaling, among others) showed associations with fetal birth weight and FGR (41). More recently, Gong et al. (56) sequenced both total and small RNA libraries generated from 302 term placentas. By comparing transcriptomic data with other human tissues, the authors highlighted placentally enriched transcripts, ranging from mRNAs of placental hormones to

noncoding RNAs, such as miRNAs, small noncoding RNAs, circular RNAs, and piwi-interacting RNAs, whose functions in the placenta have not been elucidated (56). Coexpression network analysis of mRNA and miRNA transcripts prioritized modules enriched for biological pathways related to angiogenesis and mitochondrial respiration in the term placenta. These mRNA-miRNA modules were further found to be differentially expressed in placentas complicated by FGR and/or PE as compared with placentas from uncomplicated pregnancies (56).

Data from the term placenta have inherent limitations, as observations evident at term do not reveal causality, nor do they offer diagnostic or prognostic abilities to predict pregnancy complications. Therefore, searching for minimally invasive biomarkers of the placenta in early gestational stages remains an important task. The human placenta has been shown to mediate maternal adaptations to pregnancy by secreting multiple hormones, cytokines, and neuropeptides that target key maternal metabolic organs such as the pancreas, liver, skeletal muscle, adipose tissue, and brain (107). Extracellular vesicles (EVs), such as exosomes and microvesicles, are a heterogenous class of lipid-bound particles released by cells into the extracellular environment (17). EVs have been found to mediate intracellular communications in the placenta and between the mother and the fetus. Placenta-derived exosomal miRNAs were found to modulate invasiveness and proliferation of trophoblasts as well as maternal immune responses to the placenta (140). Altered lipid profiles in EVs obtained from trophoblasts of women diagnosed with PE were noted to induce proinflammatory phenotypes in placental macrophages (80). Moreover, signaling molecules delivered by EVs from endometrial stromal cells were shown to regulate endometrial decidualization, placental angiogenesis, and EVT differentiation (84). Placentally derived EVs and EV-encapsulated cargo materials (e.g., nucleotides, proteins, and lipids) as well as other substances from the placenta are detectable in maternal blood and amniotic fluid, which have been investigated in relation to pregnancy complications (17). As our understanding of the placenta accrues, more research is needed to evaluate the sensitivity and specificity of these minimally invasive biomarkers for monitoring placental function during pregnancy and the generalizability of the related findings for disease diagnosis and risk prediction in different populations.

Genetic association is another powerful tool that allows inference of causal relationships between placental phenotypes and pregnancy complications by leveraging existing genetic polymorphisms in human populations (129). Findings from genetic association studies can be further strengthened by integrating placental transcriptome data to pinpoint the potential changes in placental gene expression that predispose an individual to diseases later in life. In studies using multiomics approaches, placental gene expression has been shown to mediate the genetic risks for fetal birth outcomes (121), cardiometabolic traits (16), and autoimmune and neuropsychiatric disorders (16, 130, 131). Moreover, the placenta is in direct contact with maternal nutrients and regulatory signals. Interestingly, of all variable DNA methylation sites in the placental genome, 70% were found to be better explained by the interaction effects between fetal genetics and maternal prenatal environmental factors, such as cardiometabolic traits, than by genetic and environmental factors alone (31). Large-scale population studies that explore the modifying effects of maternal factors (e.g., maternal nutrition) on fetal genetic influence on placental phenotypes and pregnancy complications are still lacking.

Placental Tissue Heterogeneity

Placental function is orchestrated by many cell types at the maternofetal interface. Over the past five years, studies using single-cell technologies such as single-cell RNA-seq (scRNA-seq) have made substantial contributions to the identification of placental cell types, their molecular features and differentiation trajectories, and cell–cell communication networks in the human placenta

(81, 96, 119, 124, 132, 134). scRNA-seq can be performed following cell cytometry to select specific cellular subtypes on the basis of surface antigens or can be applied to all cells to evaluate cellular heterogenicity within a bulk tissue sample. This technology has been utilized to elucidate molecular features underlying the development of trophectoderm cells (79) and trophoblast populations (139) during implantation. The presence of three major trophoblast populations (CTB, EVT, and STB) was confirmed in scRNA-seq studies (81, 96, 119, 124, 132, 134). The EVT pathway is associated with upregulation of genes that affect cellular adhesion or invasion and immunomodulation while the multinucleated STB population is associated with upregulation of genes related to expression of placental hormones and glycoproteins (139). There is also extensive heterogeneity within each trophoblast subpopulation (81, 124, 132, 134). By comparing scRNA-seq profiles of CTB and EVT subpopulations isolated from T1 and T2 placentas (obtained from 8 to 24 weeks of gestation), a continuous differentiation trajectory of CTB into EVT was inferred along with the underlying signaling pathways (81). Moreover, trophoblast subpopulations were found to express a diverse array of polypeptide hormones ($n = 60$ in STB) (81), as well as nutritional SLC family proteins (in both STB and EVT) (119), that are essential to hormonal regulation and nutrient transport in the placenta.

It should be noted that in addition to trophoblasts, the human placenta also contains numerous other cell types. Findings from scRNA-seq studies (119, 124, 132) have verified the presence of fibroblasts, erythroblasts, Hofbauer cells (fetal placental macrophages), stromal cells, smooth muscle cells, decidual cells, epithelial cells, endothelial cells, perivascular cells, and various leukocytes (monocytes, natural killer cells, and T cells) in the placental villi and/or decidual tissue. The maternal and fetal origin of placental cells was further queried using DNA polymorphisms in the corresponding RNA sequences (124, 132, 134). A fundamental question that was addressed was how maternal and fetal cells communicate with each other to ensure successful placentation and pregnancy. To answer this question, several studies systematically investigated receptor–ligand interactions across placental cell types (96, 119, 132, 134). The findings indicated that maternal decidual cells relied on specific G-protein-coupled receptors to augment signal transduction with fetal trophoblasts (96), and maternal natural killer cells exhibited immunomodulatory properties that would suppress maternal immune responses to the fetal trophoblast invasion into endometrial tissue (132).

The diversity of cell types observed in these scRNA-seq studies highlights the possible impact of extensive tissue heterogenicity on interpretation of results observed in studies using bulk tissue samples obtained at variable locations throughout the placenta (67, 112). The total proportion of trophoblasts has been found to vary from 15% to 60% in scRNA-seq data of term placental samples even when collected using a standardized method from different individuals (124). Technologically, availability of RNA-seq (or DNA methylation) data of specific placental cell types enables prediction of cellular compositions in bulk tissue samples by using bioinformatic methods such as cellular deconvolution (5, 146). This approach has been applied to deconvolute bulk placental RNA-seq data obtained at various gestational stages and was able to identify significant increases in Hofbauer cells at T2 and leukocytes at T3 (118).

Trophoblast Cellular Mosaicism

As the placenta ages, differences among trophoblasts increase, as reflected by increased variation in DNA sequences (37) and methylation patterns (92). Placentally derived DNA mutations were once thought to be scarce, as the human placenta has a life span of only ~38 weeks. Recent genomic studies, however, refute this hypothesis by pointing out an extraordinarily high mutation load in term placentas that results in mosaic patches of heterogenous STB differentiated from

independent trophoblast lineages (37). Notably, the rates of nucleotide substitutions and structural variants in placental DNA were close to those in pediatric cancers known to have enhanced mutagenesis. Further analysis of the nucleotide substitution patterns suggested that 43% of placental DNA mutations may be associated with oxidative stress (37). Such a pattern is less frequent in cancers and is rare in healthy tissues. Oxidative stress can impair DNA integrity, shorten telomeres, induce senescence, and promote release of inflammatory cytokines in the placenta (27, 33). These processes are found to be positively correlated with gestational age and may underlie the pathogenesis of many pregnancy complications (27, 33). However, due to small sample sizes and exploratory designs, current studies of placental DNA are insufficient to draw meaningful conclusions on whether DNA mutations have functional consequences in the placenta, such as their influence on placental nutrient trafficking, whether DNA mutation patterns vary among trophoblast populations at different gestational stages with significant changes in oxygen and nutrition utilization, and how evolutionary forces that shape somatic DNA mutations differ between the embryo and the placenta. Larger-scale investigations of placental DNA using improved sequencing technologies are needed to answer these questions.

DNA mutations not only exist in the nuclear genome but also occur in the genome encapsulated in each mitochondrion in the cytoplasm [mitochondrial DNA (mtDNA)]. Human mtDNA encodes 13 protein subunits in four of the five oxidative phosphorylation protein complexes that fuel metabolic reactions critical for nutrient transport, hormone production, and epigenetic modification (83). Compared with nuclear DNA, mtDNA is more susceptible to mutations and oxidative damage, since mtDNA continues to replicate in nonproliferative cells and is in close proximity to reactive oxygen species released from the electron transport chain. This notion has been supported by a recent study showing an average of more than 20 mtDNA heteroplasmic mutations in 365 bulk term placentas using ultradeep mtDNA sequencing (22). The placental mtDNA mutation load was much higher than previously reported in umbilical cord blood or pediatric peripheral blood (136). Interestingly, placental mtDNA mutation load was increased in relation to maternal psychological stress, especially among participants of African descent (22). Maternal stress was also linked to reduced mtDNA content in the placenta in an earlier study of the same cohort (21). Of note, cells may increase mtDNA content in response to mtDNA defects to ensure mitochondrial function (55). This dynamic feature of mtDNA may explain why previous studies found inconsistent results regarding possible associations between changes in mtDNA content in placental tissue obtained from women experiencing pregnancy complications (83). Research that considers both quality and quantity of mtDNA markers as well as other mitochondrial parameters is needed to provide a comprehensive understanding of the role of mtDNA mutagenesis in placental health.

FUTURE DIRECTIONS

Over the past decade, significant advances have been made in our understanding of the molecular underpinnings of placental development, energy utilization, and nutrient trafficking. More research is needed to overcome inherent limitations in establishing the causality of the findings generated and their relationship to long-term health outcomes. Additional tools are needed to characterize nutrient flux across the STB and to identify biomarkers associated with impairments in this process that may increase risk of adverse pregnancy or birth outcomes. Genomic approaches have the potential to identify genes that may predict risk of impaired nutrient partitioning, placental development, and placental function. Marked health disparities in diseases associated with placental function exist, and work is needed to understand and eliminate these disparities. Last but not least, much of the focus on the placenta has centered on how this fetally derived organ

impacts fetal health, but women who experience placental disorders associated with PE, IUGR, or PTB have been found to have a 2- to 10-fold increased risk of developing diabetes mellitus, chronic hypertension, cardiovascular disease, cerebrovascular disease, or metabolic symptoms compared with that of individuals with uncomplicated pregnancy outcomes (88, 114). Integration of multiomics data, data from single-cell experiments, and new technological advances that allow visualization of placental function in real time will provide new avenues to investigate placental responses to nutritional insults and ways to mitigate the impact of nutritional perturbations on health outcomes.

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