Lead Article

The placenta: the forgotten essential organ of iron transport

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Optimal iron nutrition in utero is essential for development of the fetus and helps establish birth iron stores adequate to sustain growth in early infancy. In species with hemochorial placentas, such as humans and rodents, iron in the maternal circulation is transferred to the fetus by directly contacting placental syncytiotrophoblasts. Early kinetic studies provided valuable data on the initial uptake of maternal transferrin, an iron-binding protein, by the placenta. However, the remaining steps of iron trafficking across syncytiotrophoblasts and through the fetal endothelium into the fetal blood remain poorly characterized. Over the last 20 years, identification of transmembrane iron transporters and the iron regulatory hormone hepcidin has greatly expanded the knowledge of cellular iron transport and its regulation by systemic iron status. In addition, emerging human and animal data demonstrating comprised fetal iron stores in severe maternal iron deficiency challenge the classic dogma of exclusive fetal control over the transfer process and indicate that maternal and local signals may play a role in regulating this process. This review compiles current data on the kinetic, molecular, and regulatory aspects of placental iron transport and considers new questions and knowledge gaps raised by these advances.

INTRODUCTION

During pregnancy, the placenta actively transports iron from the mother to the fetus. Iron is an essential component of many enzymes and hemoproteins vital for normal function of all cells, and iron demand increases during rapid growth and development. Iron deficiency in human infants is associated with a number of short- and long-term neurodevelopmental deficits that persist even after iron repletion. Animal data have attributed these effects to neural processes most vulnerable to iron deficiency in early life, including neurotransmitter production, neuronal energy metabolism, and myelination.[1] Recent work in rodents has further implicated epigenetic changes and abnormal gene expression in the brain in the pathophysiology of long-term neurological dysfunction associated with early iron deficiency.[1,2] In addition to impaired brain function, emerging human and animal data have demonstrated other developmental consequences of prenatal iron deficiency, including elevated blood pressure, altered nephron morphology, and an increased risk of iron deficiency during infancy.[3–6] The identification of gestational windows of organ development most profoundly impacted by iron deficiency further underlines the importance of adequate iron supply throughout fetal life.[7,8] Finally, the relatively frequent occurrence of iron deficiency in breastfed infants before 6 months of age, both in industrialized (6%–15%)[9–11] and in developing (12%–37%)[10] countries, suggests that a considerable number of newborns may not have adequate iron stores at birth.

The importance of iron nutrition in utero has prompted sustained interest in research on placental iron transport since the early 20th century. While early studies provided valuable data on the kinetics and chemical nature of placental iron transfer, it was not

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Key words: iron, fetal development, metabolism, placenta, regulation.

doi: 10.1093/nutrit/nuw009

until the introduction of modern biochemical and molecular genetic tools that a detailed characterization of the transport process became possible. Even so, fundamental questions remain regarding the roles of key cellular iron transporters and the systemic iron regulatory hormone hepcidin in iron transport across the placenta. This review summarizes decades of human and animal data on the mechanism and regulation of placental iron transport and identifies important gaps to address in future research.

NONHEME IRON TRANSPORT

Iron uptake

In humans, approximately 80% of fetal iron accrues in the last trimester of pregnancy,[12] with the peak transfer rate estimated to be as high as 7 mg/d.[13] Expressed as a proportion of the basal plasma iron turnover in nonpregnant adults, this represents 23% to 35% of plasma iron flux, second only to iron directed to the bone marrow.

In species with hemochorial placentas such as humans and most primates and rodents, maternal blood is in direct contact with fetal tissue, namely the trophoblasts of the placental villi, to promote efficient exchange of gas, nutrients, and waste. Transferrin-iron is the major, if not only, maternal iron source for placental transfer, and its uptake is mediated by transferrin receptor 1 (TFR1) (Figure 1). Transferrin receptor 1 is a homodimeric transmembrane protein with high affinity for diferric transferrin and is a part of the principal mechanism of cellular iron acquisition in vertebrates.[14] Expressed in virtually all cells except mature erythrocytes, TFR1 is found at uniquely high levels in nucleated erythroid precursors and placental syncytiotrophoblasts, two cell types that transport iron for distinctly different purposes: hemoglobin synthesis and fetal iron acquisition, respectively.[15] In fact, the placenta was one of the first tissues from which TFR1 was isolated and characterized[16,17] and has been the major source of TFR1 standards used in serum TFR1 immunoassays.[18–20]

The strong placental expression of TFR1 during peak fetal demand,[21,22] the efficient transfer of maternally injected ferric iron to the fetus,[23] and the profoundly anemic phenotype of *Tfr1*-null mouse embryos[24] suggest a potential role of TFR1 in placental iron transport. Despite the likely importance of TFR1, the mechanism of iron transport in the placenta remains poorly characterized, partly because of the lack of in vitro systems that mimic the polar iron physiology of the placental syncytiotrophoblast and because placental iron transport has not been studied with modern molecular genetic techniques, including tissue-specific gene targeting in vivo.

Certain aspects of the transferrin cycle have, however, been studied in isolated trophoblasts and the BeWo human choriocarcinoma cell line; they have yielded results similar to those found in other tissues that express high levels of TFR1, such as erythroblasts. Immunohistochemical studies have localized TFR1 and transferrin to the apical membrane of syncytiotrophoblasts.[25,26] Concordantly, immunoelectron microscopy demonstrated the presence of transferrin[26–28] and TFR1[29] on the membrane of cellular invaginations and intracellular vesicles, likely representing clathrin-coated endosomes that contain transferrin,[30,31] TFR1,[32] and TFR1-transferrin complexes.[30,32] Kinetic studies in BeWo cells[33] and placental microvillous membrane preparations[17,34,35] showed that diferric transferrin uptake is a specific and saturable process, with binding affinities similar to values reported in K562 erythroleukemia cells[36] and reticulocytes.[37,38] The transferrin cycle time in BeWo cells[33] is also comparable to that in HepG2[39] and K562 cells.[40] Finally, the weak base chloroquine, which disrupts endosomal acidification, inhibits placental accumulation and fetal transfer of transferrin-iron in a dose-dependent manner.[41] Studies in rat placenta suggest that the inhibitory effect of high pH is due to reduced iron release from internalized transferrin,[42] also consistent with the requirement of acidification in transferrin-iron utilization in the classic transferrin cycle. Collectively, these data suggest that syncytiotrophoblasts utilize a mechanism of transferrin-iron endocytosis similar to that observed in other cells.

Questions remain regarding the metabolic fate of transferrin-iron following uptake. It is unclear how transferrin-iron in endosomes enters the cytoplasm and reaches the basal membrane for export. In erythroid endosomes, ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) by the ferrireductase STEAP3 (six-transmembrane epithelial antigen of the prostate 3) and is subsequently transported across the endosomal membrane by divalent metal transporter 1 (DMT1, solute carrier family 11 member 2 [SLC11A2]). Whether STEAP3 and DMT1 play similar roles in the placenta is unclear, but strongly suspected. STEAP3 is highly expressed in mouse and human placenta. The STEAP3 homolog, STEAP4 (six-transmembrane epithelial antigen of the prostate 4), which likewise exhibits ferrireductase activity,[43] is also highly expressed in the human placenta.[43,44] Iron reduction in the placenta might also involve a cytochrome B561 isoform expressed on the late endosomal/lysosomal membranes.[45]

Similar to the uncertainty regarding the requirement for and the identity of a placental endosomal reductase, the protein responsible for endosomal iron

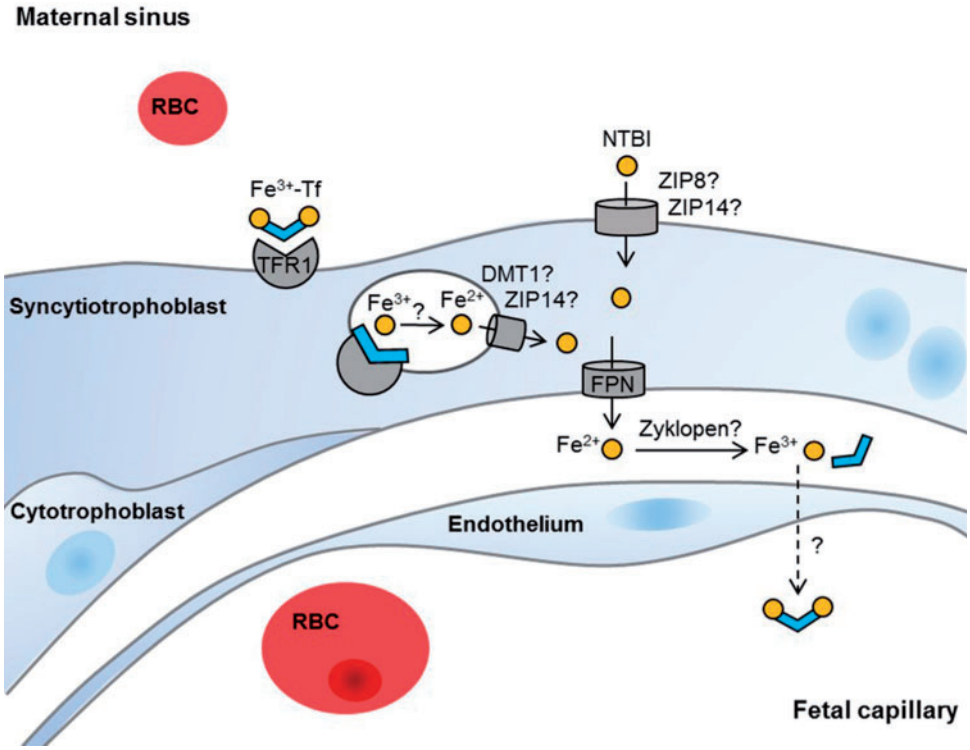

Figure 1 **Mechanisms of iron transport across human placenta.** Cross-section of a placental villus bathed in maternal blood and surrounded by the multinucleated syncytiotrophoblast. The basal side of the syncytiotrophoblast is in contact with a discontinuous layer of mononucleated cytotrophoblasts or the basement membrane. In terminal villi, fetal capillaries are in close vicinity to the syncytiotrophoblast and are separated only by fetal endothelium. Maternal diferric transferrin (Tf) binds to transferrin receptor 1 (TFR1) on the apical side of the syncytiotrophoblast and is internalized by receptor-mediated endocytosis. Within the endosomes, iron dissociates from Tf and is reduced and released into the cytoplasm via an unknown mechanism that may involve ferrous iron transporters divalent metal transporter 1 (DMT1) and/or Zrt and Irt-like protein 14 (ZIP14). Iron is exported from the syncytiotrophoblast by the iron exporter ferroportin (FPN) and is oxidized to a ferric state by ferroxidases with uncertain identities. Ferric iron likely binds to fetal Tf after exiting the syncytiotrophoblast and is subsequently transported across the fetal endothelium through unknown mechanisms. The placenta may also take up non-Tf-bound iron from the maternal circulation by ZIP8 or ZIP14, expressed on apical side of the syncytiotrophoblast. *Abbreviations*: DMT1, divalent metal transporter 1; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; FPN, ferroportin; NTBI, non-transferrin-bound iron; RBC, red blood cell; Tf, transferrin; TFR1, transferrin receptor 1; ZIP, Zrt- and Irt-like protein

export has been presumed to be DMT1. DMT1 is critical for luminal iron uptake in enterocytes and for endosomal iron release in erythroid cells.[46] In human syncytiotrophoblasts, DMT1 has variably been reported to have a punctate cytoplasmic distribution and to be localized predominantly to the apical[47,48] or basal membranes.[25,49] (Table 1) This dispersed pattern of DMT1 distribution may suggest multiple sites of action. The necessity of DMT1 in placental iron transport requires experimental confirmation, especially in light of the normal body iron concentration in neonatal *Dmt1*-null mice.[50] Another potential endosomal iron transporter is ZIP14. ZIP14 is a member of the SLC39A (solute carrier family 39 A) zinc transporter family that also transports Fe^{2+}.[51] ZIP14 is highly expressed in mouse placenta[52] and has been shown to mediate plasma membrane uptake of non-transferrin-bound iron[53] as well as transferrin-iron assimilation from endosomes.[54] Targeted *Zip14* mutants have no abnormal birth phenotype except lower birth weight, which might be attributed to abnormal bone morphogenesis and endocrine abnormalities.[55] Another member of the SLC39A family, ZIP8, which transports Fe^{2+}, is also highly expressed in placenta.[56] Targeted deletion of *Zip8* in mice leads to complete preweaning mortality.[57] Finally, mucolipin 1, a ubiquitous membrane protein, was recently found to function as an Fe^{2+}-permeable channel in late endosomes and lysosomes.[58] The role of ZIP14, ZIP8, and mucolipin 1 in placental iron transport is unknown, and it is possible that these three, together with DMT1, play redundant roles in endosomal iron export.

Radioisotope studies in perfused human placentas,[41] cultured trophoblasts,[35] and BeWo cells[33] showed that the majority of iron derived from transferrin-iron is incorporated into ferritin, with a small fraction found in a low-molecular-weight cellular fraction[35]

Table 1 **Function and localization of iron transporter proteins in human and mouse placentae**[a]

Proposed function	Protein	Localization in human placenta	Localization in mouse placenta
Apical iron uptake			
	Transferrin	Syncytiotrophoblast: strong, continuous staining on apical membrane[23] Other locations: fetal stroma[23]	No localization data
	TFR1	Syncytiotrophoblast: strong, continuous staining on apical membrane[23,47,48] Other locations: cytotrophoblasts, fetal stromal macrophages, fetal endothelium[48]	Strong, predominant staining on apical membrane of labyrinth trophoblasts; also stains fetal endothelium at E10[20]
	HFE	Syncytiotrophoblast: staining confined to basal[23] or apical membrane[49] Other locations: fetal stromal macrophages[23]	No localization data
	SCARA5	Syncytiotrophoblast: weak cytoplasmic and membranous staining[50] Other locations: fetal endothelium[50]	No localization data
	ZIP8	Syncytiotrophoblast: strong, continuous staining on apical membrane; weak cytoplasmic staining[50] Other locations: fetal endothelium[50]	No localization data
Intracellular iron trafficking			
	ZIP14	Syncytiotrophoblast: strong cytoplasmic and membranous staining[50]	No localization data
	DMT1	Syncytiotrophoblast[b]: punctuate and diffused staining in cytoplasm and basal membrane[23,47]; continuous staining in cytoplasm and apical membrane[45,46] Other locations: fetal stroma,[23,51] fetal stromal macrophages[47]	No localization data
	Ferritin	Syncytiotrophoblast: very weak/negative staining[23,50,51]; predominant staining toward apical side[52] Other locations: fetal stroma,[23,52] fetal endothelium[51]	No localization data
	STEAP3	Syncytiotrophoblast: weak and diffuse cytoplasmic staining; intense and granular staining along basal membrane[50]	No localization data
	STEAP4	No localization data	No localization data
Basolateral iron export			
	FPN1	Syncytiotrophoblast: strong, continuous staining on basal side[23,53] Other locations: fetal stromal macrophages[23]	Strong, continuous staining in labyrinth trophoblasts at E16.5[54]
Ceruloplasmin	Syncytiotrophoblast[b]: intense[55] or weak diffuse staining[56] Other locations: fetal stroma,[55] intervillous space, fetal endothelium[56]	No localization data	
	Hephaestin	Syncytiotrophoblast: membranous and cytoplasmic staining [50]	No localization data
	Zyklopen	No localization data	Labyrinth trophoblasts and spongiotrophoblasts at E15.5[57]
Heme iron metabolism			
	HRG1	No localization data	No localization data
	LRP1	Syncytiotrophoblast: strong cytoplasmic staining, more intense on apical side[58] Other locations: fetal stromal macrophages[58]	Strong staining in ectoplacental cone at E7.5[59]
	PCFT	Syncytiotrophoblast: cytoplasmic and membranous staining, more intense on apical side[60] Other locations: fetal endothelium[60]	Weak staining in labyrinth trophoblasts, spongiotrophoblasts, trophoblast giant cells, and fetal endothelium at E12.5–18.5[61]

(continued)

Table 1 **Continued**

Proposed function	Protein	Localization in human placenta	Localization in mouse placenta
	FLVCR1	Syncytiotrophoblast: cytoplasmic and membranous staining, more intense on apical side[50] Other locations: fetal stromal macrophages	No protein localization data High mRNA expression in labyrinth trophoblasts, spongiotrophoblasts, and trophoblast giant cells from E9.5 to term[62]
	FLVCR2	Syncytiotrophoblast: cytoplasmic and membranous staining, very intense on apical side[50] Other locations: cytotrophoblasts[50]	No localization data
	HO1	Syncytiotrophoblast[b]: intense[63,64] or negative/very weak staining[65,66] Other locations: fetal endothelium,[63,64] cytotrophoblasts[63]	Labyrinth trophoblasts[2]: positive staining at E13[67]; negative staining at E14.5[68]; strong staining in spongiotrophoblasts at E13 and E14.5[67,68]
	HO2	Syncytiotrophoblast[b]: strong,[63] weak,[65,66] or negative staining[64] Other locations: fetal endothelium[63–66]	Moderate staining in labyrinth trophoblasts, spongiotrophoblasts, and trophoblast giant cells at E13; weak staining in fetal endothelium[67]

[a]All human data are from studies in term healthy placentae; mouse studies represent expression after the formation of mature placenta at E10.5, if data are available. Subcellular locations of some proteins are not described because the immunohistochemistry images are at low resolution.
[b]Denotes inconsistent findings.
Abbreviations: DMT1, divalent metal transporter 1; E, embryonic day; FLVCR, feline leukemia virus subgroup C cellular receptor; FPN, ferroportin; HO, heme oxygenase; HRG, heme-responsive gene; LRP, low-density lipoprotein receptor-related protein; PCFT, protein-coupled folate transporter; SCARA5, scavenger receptor class A member 5; STEAP, six-transmembrane epithelial antigen of the prostate; TF, transferrin; TFR1, transferrin receptor 1; ZIP, Zrt- and Irt-like protein.

and the reminder recovered in the extracellular media. Ferritins in the placenta contain mainly the heavy chain subunit.[59] There is also evidence for a placental-specific ferritin heavy chain homolog with immunosuppressive activity.[60] Immunohistochemical studies of ferritin expression in syncytiotrophoblasts are conflicting, with the majority of reports showing a lack of expression,[25,61–63] while others demonstrate some staining.[64,65] (Table 1) This discrepancy may be due to the use of antibodies that react differentially with each isoferritin. In contrast to the syncytiotrophoblast, fetal villous stromal cells consistently show pronounced ferritin staining.[25,61,62,64,65] This raises the possibility that villous stroma may serve as a buffer between the syncytiotrophoblast and fetal circulation to ensure adequate, but not excessive, iron supply. Whether synthesis and degradation of ferritin in the stromal cells respond to fetal iron demand is unknown. Thus, although it can be inferred that TFR1, DMT1, STEAP3, and possibly other proteins are essential for iron uptake in the placenta, experiments designed to directly test these hypotheses have not been performed.

Basal transport of iron

Iron export from the syncytiotrophoblast to the fetal stroma is likely mediated by the iron exporter ferroportin 1 (FPN1), also known as SLC40A1 (solute carrier family 40 member A1). FPN1 is abundantly expressed along the basal membrane of human syncytiotrophoblasts[25,66] and mouse labyrinthine trophoblasts.[67] Studies in transgenic animals with aberrant *Fpn1* expression provide further evidence for a role of *Fpn1* in maternal–fetal iron transport. Mouse embryos with a hypomorphic mutation in *Fpn1* are severely iron deficient at E12.5 and exhibit defects in neural tube closure and forebrain patterning.[68] Iron-chelating experiments suggest that the developmental abnormalities in the *Fpn1* hypomorphs are due to impaired iron delivery from the visceral endoderm to the embryo.[68] In addition, a deletion of the iron-responsive element in the *Fpn1* 5′ untranslated region results in dysregulation of *Fpn1* in multiple organs and markedly reduced FPN1 protein expression in the placenta, causing severe anemia and tissue iron deficiency at birth.[67]

In contrast to the global *Fpn1* knockout mice that die early in gestation, animals with selective retention of *Fpn1* in primitive endoderm (the precursor of the visceral yolk sac) and trophoectoderm (the precursor of the placenta) survived to term and were indistinguishable from their wild-type littermates at birth,[69] suggesting that *Fpn1* expression in the maternal–fetal interface is essential for normal embryonic development. However, it is not clear whether *Fpn1* expression in the visceral endoderm, the placenta, or both conferred embryonic viability in the conditional mutants. While all *Fpn1*

transgenic mouse studies suggest a role for *Fpn1* in placental iron transfer, not one directly address its role in mediating iron efflux from the syncytiotrophoblast.

Following export by FPN1, iron must be oxidized to the ferric state before binding to transferrin. Three multicopper ferroxidases have been identified, all of which can be found in the placenta: ceruloplasmin, hephaestin, and zyklopen). Ceruloplasmin is a soluble copper-dependent ferroxidase that facilitates iron efflux from macrophages and hepatocytes.[70] Immunohistochemical staining in human placenta demonstrated ceruloplasmin in syncytiotrophoblasts[71,72] and fetal capillaries[71]; however, whether the former is due to local production is uncertain, since ceruloplasmin gene (*Cp*) mRNA is largely restricted to the liver, retina, and endothelial cells, although mRNA expression has been detected in cultured human syncytiotrophoblasts.[71] *Cp*-null animals exhibit a normal phenotype at birth, suggesting that *Cp* is not essential for placental iron transport.[70] On the basis of the phenotype of sex-linked anemia (*sla*) mice that carry a mutation, the membrane-bound *Cp* homolog *Heph* appears to be important for iron egress from enterocytes.[73] *Heph* has not been localized to human placenta, but expression of *Heph* mRNA has been demonstrated in rat placenta[74] and human BeWo choriocarcinomas.[75] Hemoglobin levels in *sla* pups is usually decreased compared with that in wild-type pups, though there is some overlap in the phenotypes.[76] Placental transfer of maternally injected radioiron in the second half of pregnancy was not different between *sla* and wild-type mice, although *sla* pups accumulated less radioiron given in the maternal diet throughout pregnancy.[76] It is worth pointing out that the *sla* allele of *Heph* retains partial ferroxidase activity,[77] so the minimally perturbed placental iron transfer in *sla* pups may underrepresent the degree to which *Heph* is important for placental iron transport. A third ferroxidase in the placenta has been described by Danzeisen et al.,[78] who detected an intracellular, membrane-bound protein in BeWo cells that exhibited ceruloplasmin-like oxidase activity and reacted with a ceruloplasmin antibody.[72] Recently, this group identified zyklopen as a placenta-specific ferroxidase[79] that has approximately 50% protein identity with ceruloplasmin and hephaestin. Zyklopen contains a transmembrane domain and an extracellular ferroxidase domain with appropriate topology to interact with FPN1.[79] Absent in liver and intestine, zyklopen is abundantly expressed in the placenta and has been localized to the labyrinth, spongiotrophoblasts, and yolk sac of mouse placenta.[79] Thus, there is evidence that all 3 mammalian multicopper ferroxidases are expressed in the placenta, but little is known definitively regarding their functions in placental iron transport. Research is needed to determine the subcellular locations of the ferroxidases in placental tissue and to elucidate the function and possible interaction of the ferroxidases with one another and/or with FPN1 in facilitating iron export from the syncytiotrophoblast.

The specific events following iron exit from syncytiotrophoblasts, like those following iron exit from enterocytes, the intestinal counterparts of syncytiotrophoblasts, are obscure. In terminal placental villi, the syncytiotrophoblast comes in close contact with fetal capillaries to facilitate gas, nutrient, and waste exchange.[80] It is possible that a fraction of iron released from syncytiotrophoblasts is not bound by transferrin and is utilized by fetal tissues through non-transferrin-mediated pathways, but the majority likely binds to transferrin in the extracellular space[25] before traversing the fetal endothelium into the fetal circulation. How iron is trafficked across the endothelial layer is unknown. The weak cytoplasmic staining of TFR1[81] and the nondetectable expression of FPN1[25] in placental fetal endothelium suggest a mechanism of transport that is different from the syncytiotrophoblast. Large molecules such as immunoglobulin G traffic across fetal endothelium by vesicular transport,[82] but it is unclear if this type of mechanism applies to iron or iron chelates. Thus, it seems likely that some unique mechanisms and regulatory processes are involved in iron transport across the fetal vascular endothelium. It should be noted that, in contrast to the nonfenestrated endothelium in human placenta, the villous endothelium in rodents has numerous fenestrae that confer greater permeability to small solutes such as glucose.[83,84] It is unclear whether larger molecules such as transferrin can move across fetal endothelium through fenestrations, but this may represent an additional route in addition to vesicular or receptor-mediated pathways. Future research to examine the possible role of fetal endothelium in placental iron transport should be of interest.

Other cells in the placental stroma that may participate in iron transfer are the villous macrophages known as Hofbauer cells. The function of Hofbauer cells is not well defined but may include the support of trophoblast differentiation, stromal development, angiogenesis, and erythroid cell maturation.[85,86] Located in close vicinity to fetal capillaries, Hofbauer cells express most of the major heme and nonheme iron transporters and storage proteins,[25,49] suggesting a role in iron transport and/or regulation.[86,87] It is tempting to speculate that Hofbauer cells may function as the temporary iron storage site in villous stroma, storing iron when maternal supply exceeds fetal demands and releasing iron when supply is insufficient.

NON-TRANSFERRIN-BOUND IRON TRANSPORT

In addition to maternal transferrin-iron, other circulating forms of iron such as non-transferrin-bound iron and heme may also be taken up by the placenta. There

is no definitive data on whether transferrin-iron is the exclusive source of iron for fetal growth. Although global deletion of *Tfr1* in mice leads to embryonic lethality by E12.5, some $Tfr1^{-/-}$ embryos have appreciable numbers of hemoglobinized red blood cells as late as E10.5, and cultured yolk sac hematopoietic progenitors from these embryos stained positively for hemoglobin,[24] suggesting that the transferrin cycle may not be essential for erythropoiesis during early development. Furthermore, it is unclear whether anemia in *Tfr1*-null embryos is due to insufficient placental iron transport or to defects in erythroid iron uptake, or both. Targeted deletion of *Tfr1* in the placenta is needed to resolve this controversy.

Although its physiological relevance is unclear, there is some evidence of ferritin transport in the placenta. Radioisotope studies in rabbits and guinea pigs demonstrated transfer of maternally injected ferritin to the fetus.[88,89] Electron microscopy of placental villi from ferritin-injected animals showed ferritin-containing endocytic vesicles[88] and ferritin accumulation in the basement membranes,[89] suggestive of ferritin endocytosis. This is consistent with human data showing specific ferritin binding by placental villous membranes[90,91] and appreciable amounts of ferritin-containing vesicles in the placenta.[31] The molecular mechanism of ferritin uptake by the placenta is unknown and may involve TFR1[92] and/or the renal ferritin receptor SCARA5 (scavenger receptor class A member 5).[93] Furthermore, the relevance of this process to maternal delivery of iron to the fetus is uncertain, as plasma ferritin is a processed and glycosylated form of L-ferritin that does not bind iron.

In addition to utilizing nonheme iron, the placenta may be able to utilize heme iron sources, as suggested by its high expression of heme iron transporters and catabolic enzymes (Table 1). Several of these proteins, including lipoprotein receptor-related protein 1, proton-coupled folate transporter, heme oxygenase 1, and heme oxygenase 2, have been localized to the syncytiotrophoblast and/or stroma (Table 1), but it is unknown whether there is co-localization at the subcellular level. Data are lacking on placental localization of the more recently identified heme transporters such as feline leukemia virus subgroup C receptor 1 and heme-responsive gene 1. Research is needed to characterize the function of these heme-transport proteins in the placenta and to elucidate the placental heme metabolic pathway, especially in light of the recent recognition of placenta as a site for hematopoietic stem cell production and erythroid differentiation.[86,94,95]

REGULATION OF IRON TRANSPORT ACROSS THE PLACENTA

Placental iron transfer can be viewed as a balancing act between the mother and the fetus for a limited iron supply and reflects the capacity of the regulatory mechanism to maintain normal fetal iron content. The significance of maternal and fetal factors in regulating placental–fetal iron transfer has been the focus of many scientific inquiries since the early 20th century, and yet fundamental questions regarding the mechanisms behind this regulation remain to this day.

The observation that infants of anemic women are generally born with normal hemoglobin status forms the basis of the widely accepted notion that the fetus is a perfect parasite for maternal iron, able to acquire adequate iron irrespective of the mother's iron status.[96] The strong linear correlation between iron content and body weight in human fetuses provides further evidence that fetal need drives placental iron transport.[12] Fetal signals, such as iron status[97,98] and gestational age,[99] have been shown to impact the expression of placental iron transporters.

At this time, it is unclear how the placenta senses fetal demand, but this is likely mediated by hormonal action. Hepcidin, the iron regulatory "hormone" expressed by the liver, negatively regulates cellular iron transport via an FPN1-dependent mechanism and has been detected in mouse embryos at midgestation.[21] It may fulfill the role of relaying information on fetal iron status to the placenta. Whether fetal hepcidin inhibits placental iron transport has not been shown conclusively, but the anemic, iron-deficient phenotype of transgenic mouse embryos overexpressing hepcidin[100] suggests this is likely the case. Interestingly, the reduced iron level in hepcidin transgenic embryos was associated with lower placental *Tfr1*, with no change in *Fpn1* mRNA.[101] Likewise, studies in rats showed that fetal liver hepcidin correlates negatively with placental *Tfr1* mRNA but not with Fpn1 protein.[102] Thus, available evidence suggests that TFR1-mediated iron uptake may be the primary target of hepcidin action in the placenta, although it cannot be ruled out that hepcidin also affects iron efflux by regulating subcellular localization of FPN1.[102] Interestingly, recent human studies failed to find significant relationships between cord hepcidin levels and either placental TFR1 expression[103] or placental transfer of maternal dietary iron,[104] suggesting differences between humans and rodents in hepcidin regulation of placental iron transfer. More research is needed to clarify the role of hepcidin in regulating iron homeostasis during the prenatal period and to identify other fetal factors regulating placental iron transport, such as those related to growth and development.

While the fetus may provide the driving force for iron transfer across the placenta, there is ample evidence that maternal iron status also impacts the transport process. The relative immunity of fetal hemoglobin to mild maternal anemia highlights the ability of the

placenta to respond to variations in maternal supply. Stable isotope data in human pregnancies has shown that more iron in the maternal diet is transferred to the fetus when the maternal stores are low.[104,105] This is likely accomplished by upregulation of intestinal and placental iron transporters. Elevated placental TFR1 expression has been consistently observed in human and animal models of gestational iron deficiency[97,103,106,107] and is perhaps the best-known compensatory change in maternal deficiency. The mechanisms underlying this regulation is not well characterized and may involve placental iron regulatory protein 1 and intracellular iron.[98] Data are limited regarding how other placental iron transporters respond to maternal iron deficiency. Cell culture and rat studies show that iron deficiency increases placental DMT1 expression but has little effect on FPN1 expression.[75,106,107] A study in 40 healthy pregnant women found no difference in placental FPN1 expression between anemic and iron-replete women.[108] These data echo the observations between fetal iron status and placental iron transporter expression and again suggest that placental TFR1 is the major target of regulation by systemic iron status.

In addition to affecting the expression of placental iron transporters, maternal iron status appears to play a role in determining the maximum iron available for placental transfer. This is not surprising, considering the competition between the fetal–placental unit and the maternal bone marrow for the same limited supply of iron from maternal diet and liver stores. As discussed above, the placenta exhibits great capacity to mobilize iron for fetal use over a wide range of maternal statuses. However, there is evidence that the regulatory system can no longer sustain transfer with increasing severity of maternal deficiency. Several studies showed significantly lower cord hemoglobin levels in the infants of severely anemic women compared with infants born to iron-sufficient mothers.[109–112] Likewise, diet-induced maternal anemia in rhesus monkeys caused a significant reduction in hemoglobin, mean corpuscular volume, and bone marrow colony-forming units in the newborns,[113] suggesting a limited capacity of the placenta to support fetal erythroid needs in the face of severe maternal deficiency.

The relationship between maternal and fetal iron stores mirrors that of maternal and fetal hemoglobin levels, with an interdependence becoming evident when maternal iron reserves are depleted. Maternal iron stores are generally improved by iron supplementation, with no resultant changes observed in cord ferritin,[114,115] and maternal ferritin exhibits a weak or no relationship with neonatal ferritin levels in small observational studies. In contrast, animal studies consistently show compromised fetal liver iron status in maternal iron deficiency,[102,116,117] suggesting an interdependence between maternal and fetal stores. A recent study in China, which included a large sample of mother–child pairs (n = 3702), detected a strong correlation between maternal and neonatal ferritin in women whose plasma ferritin levels fell below a threshold of depletion, with every unit of decrease in maternal serum ferritin corresponding to a 2.4-unit drop in cord ferritin.[118] This finding is consistent with rodent data indicating a "broken stick" relationship between maternal and fetal iron parameters, suggesting a critical point below which the two become dependent.[102]

While most mechanistic studies are modeled on healthy pregnancy, it is worth noting that conditions with underlying placental abnormalities, such as intrauterine growth restriction and gestational diabetes, may disrupt the normal regulatory mechanism of the placenta and negatively affect fetal iron transfer. For example, despite the presence of fetal hypoxia and low fetal iron stores,[119] expression of placental TFR1 in intrauterine growth restriction is significantly lower compared with that in normal term pregnancies,[81] indicating impaired ability of TFR1 to sense and/or respond to its classic stimuli. Gestational diabetes is another common pregnancy complication associated with decreased infant iron stores at birth.[119] Unlike expression of placental TFR1 in intrauterine growth restriction, expression of placental TFR1 in gestational diabetes shows expected relationships with fetal iron stores and placental iron regulatory protein 1.[98] The defect appears to be a reduced affinity of placental TFR1 to bind transferrin.[120] Studies are needed to determine the factors underlying the regulatory and functional aberrations of placental TFR1 in intrauterine growth restriction and gestational diabetes and to identify other components of the placental iron transport mechanism that may contribute to low fetal iron levels associated with these pregnancy complications.

Taken together, these data indicate that, under most circumstances, maternal iron status modulates the expression of placental iron transporters in favor of fetal demands. However, there appears to be a breakpoint in maternal iron status below which the mother can no longer maintain supply, resulting in a disruption in the normal hierarchy between the fetal–maternal partitioning.

Despite years of public health efforts, maternal iron deficiency remains prevalent, accounting for half of the anemia burden (38.2%) in pregnant women worldwide[121] and affecting 29.5% of pregnant women in the third trimester in the United States.[122] Besides reflecting the markedly increased requirement for iron during pregnancy, the high prevalence of maternal iron deficiency also indicates that most women do not have sufficient iron reserves (≈300 mg) at the start of

pregnancy[123] and that maternal dietary iron intakes consistently fall below recommended levels, even in developed countries.[124] As discussed above, adequate maternal iron status is needed for sustained placental iron transfer to support fetal demands. Thus, it is important to improve iron nutrition in women of reproductive age to build adequate iron stores prior to pregnancy and to target women with suboptimal iron status early in pregnancy for dietary interventions. It is worth pointing out that a large portion of the interindividual variation in placental iron transfer and transporter expression cannot be explained by maternal and fetal factors alone. The continued placental accumulation of iron in fetectomized rat dams[125] and the absence of a relationship between placental transfer of an intravenous iron isotope and either maternal or neonatal iron status in pregnant women[105] suggest that other factors, such as local regulation by the placenta, play important roles in the transfer process.

CONCLUSION

Optimal transfer of iron across the placenta is essential for fetal development in utero and for the establishment of adequate birth iron stores to sustain growth in early infancy. This review summarizes decades of research on placental iron transport and regulation, identifies major knowledge gaps, and proposes directions for future research. With the exception of the initial step of iron uptake, the molecular details of iron trafficking in the placenta remain poorly characterized. Many cellular iron transporters are expressed at very high levels in the placenta, but very few have been definitively localized to the syncytiotrophoblast, and their functions are largely inferred from studies in other tissues and global knockout mice. Thus, there is a compelling need to generate placental-specific deletions of these proteins to conclusively study their physiological significance in placental iron transport. Furthermore, there is virtually no information on the movement of iron after it exits the syncytiotrophoblast and before it enters the fetal circulation. Research is needed to better characterize iron passage through villous stroma and to determine whether this process is subject to regulation. In addition, there is still very little known about the systemic and local mechanisms that regulate placental iron transport. How the placenta senses and integrates signals from maternal and fetal compartments and whether hepcidin is the mediator of this regulation are both important questions to address. Finally, it may be of interest to assess the potential contribution of non-transferrin-bound iron to placental iron transport in light of recent data showing appreciable levels of plasma non-transferrin-bound iron in pregnant women receiving intravenous iron supplementation.[126]

Acknowledgments

Funding. This research is supported by grant no. 5T32HL110852 from the National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA.

Author contributions. C.C. and M.D.F. conceived the review topic and wrote the manuscript. All authors approved the final draft. C.C. and M.D.F. had responsibility for the final content.

Declaration of interest. The authors have no relevant interests to declare.

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