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The role of the placenta in iron transfer from mother to fetus and the relationship between iron status and fetal outcome

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Abstract

During pregnancy, iron is transferred from the mother to the fetus across the placenta. The mechanism has been extensively studied. Altered iron metabolism changes transfer, but also has other consequences. In this review, we examine how the placenta adapts to altered iron supply, both in terms of changing cytokine expression and in relation to the proteins of iron transfer. Changing iron levels alters the levels of other metals, especially copper, and we review how this is related to changing function. There are also consequences to the placenta itself, to vascularisation and other aspects of the physiology. In turn, this has effects on the fetus and we review how growth and development are modified. Finally, we examine in more detail the efflux process, how it is regulated and, especially, the putative role of the placental Cu oxidase in the efflux process. As appropriate, we draw on data from humans, from animal models and from cell culture systems to illustrate the information.

Iron is an essential nutrient, required for a large number of biological processes. It is also toxic, and sophisticated mechanisms have evolved to maintain iron levels within very narrow constraints. There are specific times, however, when requirements change markedly, and pregnancy represents the most significant of these. The iron requirement during pregnancy is significantly increased. Although there is a respite from losses through menstruation, the total requirement for a 55-kg mother is about 1 g (Bothwell 2000). The absorption pattern through pregnancy is not uniform – probably actually decreasing in the first trimester, rising to maximum levels later in gestation (Svanberg *et al.* 1975). There are many calculations that show that the increase in absorption is not sufficient to provide the Fe required by the fetus and stores of approximately 300 mg are necessary (Beard 1994).

It is not surprising, therefore, that anaemia during pregnancy is relatively common. There is, of course, a normal expansion of blood volume, but in a surprisingly high proportion of women, the accompanying decrease in haematocrit and haemoglobin concentration is higher than considered safe. In developing countries, the proportion of women suffering from clinical anaemia can be as high as 80%, and even in developed countries can still be 20% (WHO 2001).

Anaemia during pregnancy can result in serious problems both for the mother and for her developing fetus. Many studies have shown that it induces fetal growth retardation and that the effects generated in utero and in early development can persist into adulthood (e.g., Crowe *et al.* 1995; Godfrey *et al.* 1996; Godfrey & Barker 1995; Kwik-Uribe *et al.* 2000). Godfrey and colleagues have shown that maternal Fe status may be a risk factor for adult disease (Godfrey *et al.* 1991). Several studies have shown that Fe deficiency during pregnancy, both in humans and in animal models, results in long-term problems for the offspring. For example, there are increases in blood pressure (Crowe *et al.* 1995) diminished brain function (Kwik-Uribe *et al.* 2000; Rao & Jagadeesan 1996; Rao *et al.* 1999; Soewondo 1995; Walters *et al.* 1973) and compromised immune system development (Hallquist *et al.* 1992; Lockwood & Sherman 1988). The mechanisms underlying these effects have not been clarified, but it is clear that growth and development *in utero* may play an important part.

Despite the resistance of the fetus to maternal deficiency, there are still serious consequences, both for the mother and her baby. The mother is at greater risk of requiring transfusion during parturition (Singh *et al.* 1998), and babies that are born to iron deficient women have a higher risk of developmental delay, neonatal mortality and morbidity (Hall *et al.* 1979; Scholl & Hediger 1994; Scholl *et al.* 1992; Scholl & Reilly 2000). Furthermore, there are some data suggesting that consequences can be long lasting, with an increased risk of cardiovascular problems in adulthood (Godfrey *et al.* 1991). Treatment with iron supplements does not always resolve the anaemia (Gofin *et al.* 1989). Consequently it is important to try and understand why anaemia can occur during pregnancy, precisely what is the molecular basis of the deficiency and to try and optimise conditions to alleviate the problems.

There have also been many studies examining Fe supplementation during pregnancy. Generally, the consensus appears to be that, under experimental or trial conditions, where the women are supervised and factors are controlled, supplementation can be beneficial. Outside of these trials, however, success is more limited (see the World Health Organisation web site for more information (WHO 2001)) and due care must be taken to ensure good compliance (Yip 1996). There have also been suggestions that supplementation is more effective if given weekly rather than daily (Ridwan *et al.* 1996). Furthermore, in an extensive review of the literature, Rasmussen came to the conclusion that ... *the currently available evidence from studies with designs appropriate to establish a causal relationship is insufficient to support or reject this practice for the specific purpose of raising birth weight or lowering the rate of premature birth.* (Rasmussen 2001) – a conclusion specifically contradicted by Allen (2000).

How maternal and fetal iron status are related is not entirely clear. Early studies suggested that there was no effect of iron supplementation on fetal serum levels in pregnant women (Arnaud *et al.* 1993), but these workers used classical indicators of iron status, such as haemoglobin, serum iron and transferrin, which may not give a reliable estimate of iron status. Other studies also demonstrated no correlation (reviewed in (Gaspar *et al.* 1993)), even when iron supplements are given (De Benaze *et al.* 1989; Milman *et al.* 1994). In contrast, there does seem to be a correlation between serum ferritin in some studies but not others (reviewed in Allen 2000). A second study (Burns & Paterson 1993) suggested there may be links between fetal iron status and iron-folate supplementation, but could not exclude the possibility that the effect was due to folate rather than iron itself.

In more recent studies, the value of the serum transferrin receptor (sTfR) as an indicator of iron status has been examined. Many studies in non-pregnant humans have demonstrated that it is a more sensitive and accurate indicator of status than haemoglobin or haematocrit or even ferritin concentration, not affected by factors such as inflammation, for example (Ahluwalia 1998; Akesson *et al.* 1998; Skikne *et al.* 1990). Several studies have also suggested that sTfR is a good indicator of iron status in pregnancy (Akesson *et al.* 1998; Carriaga *et al.* 1991; Ponka & Lok 1999; van den Broek *et al.* 1998). For example, in a study of 81 women, Rusia and colleagues showed that this is a more sensitive indicator of status than ferritin or any other aspect of the haemogram (Rusia *et al.* 1999). These data confirm our own which show an increase in levels (decrease in Fe status) in each subsequent pregnancy (C. Fosset, D. Abramovich and H.J. McArdle, unpublished results).

There are more limited data on the effect of iron status or supplementation in pregnancy on other metabolic processes in humans. This is, in our opinion, a serious oversight, especially since, as discussed above, the value of supplementation is not always obvious. We have shown a relationship between the serum ceruloplasmin and sTfR levels, suggesting that Fe in the mother may regulate either production or secretion of ceruloplasmin, as has been suggested in cell culture models (Mukhopadhyay *et al.* 1998). There is no correlation to serum diamine oxidase, which is thought to be an indicator of copper status. This result is one to which we shall return in a later section.

In contrast to the situation in humans, there have been several studies examining the effect of Fe deficiency during pregnancy in animal models. In the rat, Lewis and colleagues showed that maternal iron deficiency resulted in decreases in capillary surface area in the placentas supplying the fetus (Lewis *et al.* 2001a). Although they did not demonstrate it directly, their data may imply that there could be reduced nutrient transfer capacity, resulting in the decreased size of the fetuses that they observed (Lewis *et al.* 2001a). Further studies supported the hypothesis, showing decreased levels of fetal amino acids in deficient animals while glucose and butyrate levels were not changed (Lewis *et al.* 2001b).

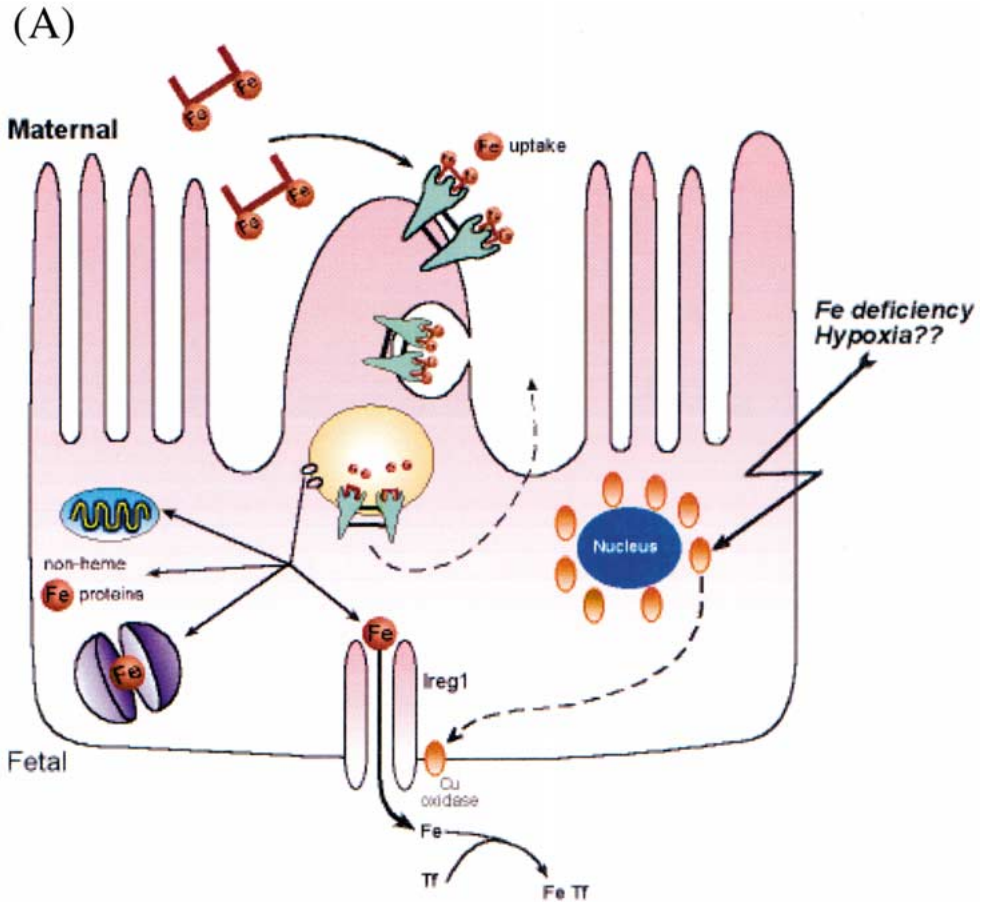

Fig. 1. Two possible models for iron transfer and efflux from the placenta. (A) The oxidase is normally located in a peri-nuclear compartment and moves to the basolateral membrane in response to Fe deficiency or perhaps hypoxia and (B) Efflux is into a vesicular compartment followed by movement of the vesicle to the basolateral membrane.

The placenta is the pathway for delivery of the majority of nutrients to the developing fetus. Consequently, any stress that alters placental development or function is likely to have consequences for the developing fetus. Placental function is regulated, at least in part, by a wide spectrum of cytokines, produced both locally and distally. Elevated levels of TNFα at the maternal fetal interface are associated with early and mid-pregnancy failure in rodents and with premature labour in humans (reviewed in Lea & Calder 1997). However, TNFα is also produced at low levels in placental and decidual immune cells in normal healthy pregnancies, and is therefore thought to be beneficial for pregnancy. TNFα is reported to induce apoptosis of placental cells and may therefore be important in trophoblast turnover and re-modelling (Lea *et al.* 1999). There are also data to suggest that TNFα may regulate placental steroid production by the placenta and may down-regulate amino acid transfer. Since the suggested beneficial and detrimental roles of TNFα are concentration dependent, the regulation of TNFα expression at the maternal fetal interface must be crucial for successful placental development and function.

The relationship between Fe status and cytokines has been the subject of considerable study. Most studies have concentrated on the effect of cytokines on Fe uptake or metabolism. Recently, however, several groups have examined the effect of Fe status on TNFα production. Scaccabarozzi and co-workers (Scaccabarozzi *et al.* 2000) have shown that Fe supplementation increased, and desferrioxamine, an iron chelator, decreased the production of TNFα by monocytic cells. The same effect was obtained in the leukemic cell line THP-1 (Silver *et al.* 1997) and in mice treated with DFO (Vulcano *et al.* 2000). Similar data were also obtained in Kupffer cells, where load-

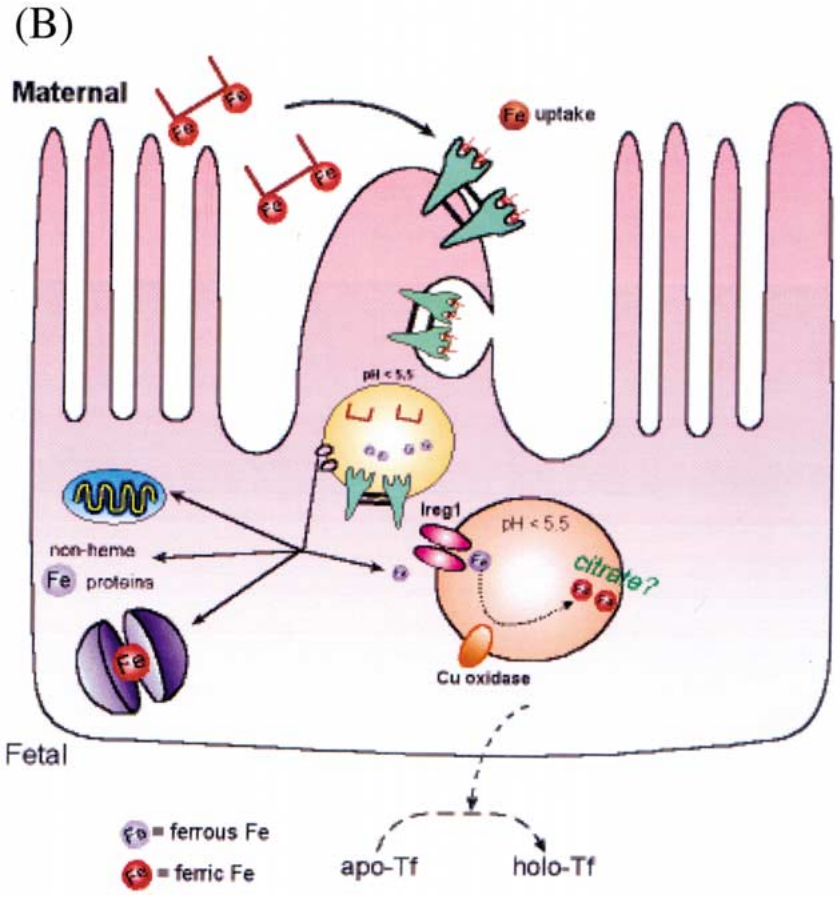

Fig. 1. Continued.

ing with Fe reduced the sensitivity to lipopolysaccharide (Olynyk & Clarke 2001). How this relationship operates in the placenta has not been examined, but, given the association between elevated concentrations of TNFα and problems with pregnancy, it is clearly of considerable importance.

Our data show a clear correlation between maternal Fe status, placental cytokine levels and fetal development, For example, there is a marked increase in TNFα and TNFαR1 levels in the placentas of iron deficient rats, coupled with a decrease in fetal size. Although we cannot demonstrate causality, the data suggest a relationship that may explain at least some of the effects of maternal Fe deficiency on her offspring (Gambling *et al.* 2001).

The relationship between Fe levels and Cu has been studied by several groups. In animal studies, (Yu *et al.* 1994, 1995a, it was shown that increased Fe intake resulted in decreased Cu status, with a concomitant decrease in biliary excretion of Cu. Our own data in cell culture suggested that this occurred as a result of down-regulation of a surface membrane reductase, involved in reduction of Cu(II) to Cu(I) as a pre-requisite to Cu uptake (Whitaker & McArdle 1996). More recently, data have been presented suggesting that SFT, a stimulator of Fe transport, may be dependent on Cu for optimal function (Yu & Wessling-Resnick 1998). Our own preliminary data suggest that the changes in Cu levels that occur do not alter expression of proteins of Cu metabolism (L. Gambling, S. Gair and H.J. McArdle unpublished data)

The results in relation to the Cu-oxidase are of particular interest, since we believe it has a central role to play in the efflux of iron into the fetal circulation. We have studied the protein in BeWo cells, a placental cell line, and have examined expression under a variety of different conditions to test the hypothesis.

The role of ceruloplasmin in iron efflux has been studied for many years. Cartwright and colleagues showed that Cu deficient pigs accumulated iron in spe-

cific tissues (Ragan *et al.* 1969). They then went on to show that perfusion with ceruloplasmin resulted in rapid release of the iron from the liver (Osaki *et al.* 1971). More recently, several groups have examined families with hereditary loss of ceruloplasmin and have also shown that iron accumulates in specific tissues (Harris *et al.* 1998; Harris *et al.* 1995; Okamoto *et al.* 1996; Takahashi *et al.* 1996, Yazaki *et al.* 1998). The data suggest that the protein acts as a ferroxidase and facilitates the incorporation of Fe(II) into transferrin as Fe(III).

Recently, Vulpe and co-workers identified the mutation in the *sla* mouse (Vulpe *et al.* 1999). They named the putative protein product 'hephaestin', and genetic analysis suggested that it, too, could be a ceruloplasmin homologue, with oxidase characteristics. Our own data showed that extracellular ceruloplasmin did not facilitate Fe efflux and that, instead, there was an endogenous oxidase which may play the same role (Danzeisen & McArdle 2000).

We found some unexpected results, however. The protein was not located on the basolateral membrane, as might have been expected. Instead, it was found in an intracellular compartment. The protein did not co-localise with any of the usual markers for trans-Golgi apparatus, endoplasmic reticulum or cytoskeleton. Similarly, it was not in association with mitochondria or with ferritin (Danzeisen & McArdle 2000). Interestingly, similar data were published recently in an abstract examining distribution of hephaestin in gut (Kuo *et al.* 1999).

As mentioned previously, the copper oxidase expression is increased in iron deficiency. We have examined the function of this protein extensively using a placental cell line (BeWo cells). We have demonstrated that the oxidase expression is regulated by iron. Increasing concentrations of desferrioxamine (which decrease intracellular iron) give a marked increased of expression of the protein. As iron concentrations within the cell increase, levels of copper oxidase activity decrease. Similarly expression is increased by increased copper and decreased by copper chelators. These results are seen at both the protein and enzyme levels.

This observation gave us the opportunity to manipulate expression and to examine the effect on Fe efflux. Consequently, we made cells Cu-deficient and measured Fe accumulation and efflux.

At normal oxygen concentrations there was no effect on iron accumulation or efflux. This was, initially, a surprising finding, but on reflection, we considered the fact that the O_2 partial pressure in the culture medium is much higher than in the placenta (Jauniaux *et al.* 1999; Watson *et al.* 1998). We hypothesised, therefore, that under experimental conditions there was sufficient oxygen within the medium to allow for spontaneous oxidation of the Fe from Fe(II) to Fe(III), rendering the oxidase superfluous. We repeated the experiments, therefore, at low oxygen concentration (5% oxygen) (Danzeisen *et al.* 2000). The data clearly showed an increase in the levels of iron within the cells. This increase appears to be caused by a decrease in the efflux, rather than an increase in uptake of iron when Cu-oxidase levels are decreased by Cu chelators (Danzeisen *et al.* 2000). Although we cannot say for certain that these events are causally related, they strongly support the hypothesis that the copper oxidase is involved in the efflux of iron from the placenta and into the fetal circulation. There are two possible ways we hypothesise the process operates. Either the oxidase traffics to the basolateral surface under conditions of iron deficiency, or possibly hypoxia, or release of iron occurs into a vesicular compartment, which then fuses with the basolateral membrane, releasing its contents into the fetal circulation (see Figure 1) (Danzeisen 2001).

The nature of the oxidase remains uncertain. Hephaestin codes for a protein showing similarities to ceruloplasmin and the hypothesis is that Fe efflux in the *sla* mouse is defective from gut as a consequence of decreased oxidase activity (Vulpe *et al.* 1999) – the same situation as occurs in the BeWo cells deprived of copper. It seemed most likely, therefore, that the oxidase we had identified was hephaestin. However, at this stage we cannot identify mRNA for hephaestin in BeWo cells, using either Northern blotting or PCR.

Other ceruloplasmin homologues have been identified. For example, David and co-workers have shown that a GPI anchored ceruloplasmin homologue is located in astrocytes in the brain (Patel & David 1997) and then demonstrated that it was an alternatively spliced product of the ceruloplasmin gene (Patel *et al.* 2000). The placental Cu oxidase cross reacts with monoclonal antibody to this protein (Danzeisen *et al.* 2001; Danzeisen & McArdle 2000), but at present we have not identified the epitope to which the antibody is raised.

The placental protein is also not identical to serum ceruloplasmin. Polyclonal antibodies raised to serum ceruloplasmin inhibit oxidase activity of both the serum protein and the placental oxidase, but with different sensitivity. There have been reports of cerulo-

plasmin mRNA being identified in BeWo cells (Yang *et al.* 1990), but these have not been confirmed by either other groups or by our own work.

In this review, we have examined the relationship between iron metabolism and pregnancy. We have identified some of the pathways involved in transfer across the placenta and shown how the proteins of iron metabolism are regulated. It is clear that there are sophisticated mechanisms evolved to try |to maintain homeostasis during pregnancy, but it is also clear that the regulation still remains largely to be discovered. Finally, we have hopefully demonstrated that pregnancy, as a time when normal metabolism of iron is changing dramatically, is a period which will help yield fruitful and valuable information on the relationship between iron metabolism and the metabolism of other compounds and nutrients.

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