Iron and Copper Interactions in Development and the Effect on Pregnancy Outcome\textsuperscript{1,2}

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ABSTRACT During pregnancy, nutrients are transferred from mother to fetus across the placenta. The mechanisms whereby this occurs, and the adaptations that occur in response to deficiency or overload of iron (Fe) and copper (Cu) are examined in this review. Fe deficiency during pregnancy is common and has serious consequences both in the short and the long term such as fetal growth retardation and cardiovascular problems in the adult offspring. Similarly, Cu deficiency, although not so common, also has deleterious effects. The placenta minimizes the effect of the deficiency by up-regulating the proteins involved in Fe transfer. For example, transferrin receptor levels increase inversely to maternal Fe levels. Divalent metal transporter 1 (DMT1) mRNA in the iron-responsive element (IRE) regulated, but not the non-IRE regulated form is increased, as is the placenta Cu oxidase. Conversely, iron-regulated gene 1 (IREG1) expression is not affected. Fe deficiency increases Cu levels in maternal liver, serum and placenta, but has much less effect in the fetal serum and liver. Apart from maternal ceruloplasmin, mRNA levels of Cu-related proteins are not changed. The Cu oxidase, which we suggest fulfils the function of hephaestin in placenta, is regulated by Cu as well as by Fe. Fe deficiency also has marked effects on cytokine levels in the placenta. Tumor necrosis factor \textsuperscript{a} (TNF\textsuperscript{a}) and TNF\textsuperscript{a} receptor 1 (TNF\textsuperscript{a}R1) levels both increase. The data show that altering Fe status has a marked effect on metabolism of other metals and of other important mediators of cell function. This is particularly important during pregnancy, when the developing fetus is very vulnerable to inappropriate micronutrient status. J. Nutr. 133: 1554S–1556S, 2003.

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It has been estimated that more than 50% of human conceptions fail to implant and of those implanting, greater than 30% fail to reach term. About 1–2% of babies that are born suffer from disorders that require them to spend time in an intensive care unit. These babies have a higher risk of neonatal mortality and morbidity. Furthermore, there are many studies showing that the consequences extend into adulthood. For example, small babies have a higher risk of increased cardiovascular disease and stroke as adults, even within the normal range of birthweights. Clearly, we need to understand why these events occur. There are many causes of low birthweight and prematurity, but among them inappropriate nutrition is increasingly being targeted as an important contributor. In this review, we will concentrate on two central micronutrients, copper (Cu) and iron (Fe), and will consider how the developing fetus regulates levels and how these micronutrients interact with each other.

To provide Fe for the developing fetus, the requirements of the mother increase markedly. In total, somewhere between 600 mg and 1 g of Fe is required to provide for the increased maternal red cell mass and the fetal needs (1,2). The increase in maternal dietary Fe absorption that occurs during pregnancy is not enough to provide the extra Fe, and the deficit must be met from maternal Fe stores. Once these stores are depleted, the mother will develop iron deficiency anemia (IDA)\textsuperscript{3}. The frequency of IDA varies throughout the world, but even in developed countries can be as high as 20% of pregnancies, and

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\textsuperscript{4} Abbreviations used: DMT1, divalent metal transporter 1; hCtr, human copper transporter; IDA, iron deficiency anemia.
in developing countries this can increase to 80% of pregnancies. The consequences are serious both for the mother and her developing fetus, and many studies have shown that anemia during pregnancy increases the risk of maternal mortality and morbidity [reviewed in (3)]. Among the consequences of IDA during pregnancy are lower birth weight (3) and an increased risk of cardiovascular disease in adulthood (4). Further, in animal models, Fe deficiency in early life has been demonstrated to cause changes in brain biochemistry and development. These changes persist even after Fe supplementation, suggesting that there are irreversible consequences of developmental Fe restriction (5).

The body of a healthy human contains ~110 mg Cu. The daily intake of Cu from the diet is normally in between 0.6 and 2 mg. However, during pregnancy the need for metabolic micronutrients increases due to the requirement of a growing fetus. In rats and humans, maternal serum Cu and ceruloplasmin concentrations increase throughout pregnancy (6,7). In humans, the maternal sera is reported to have twice the concentration of Cu found in normal healthy adults, which suggests an important role of Cu and ceruloplasmin during pregnancy (8). The relative contributions of ceruloplasmin and other forms of Cu will be examined in more detail below.

Cu deficiency is less common than Fe deficiency during pregnancy. Generally, it is only found under experimental conditions or when the fetus suffers from Menkes disease, a genetic disease resulting in perinatal lethal Cu deficiency. In experimental animals, Cu deficiency during pregnancy may cause a variety of effects such as cardiovascular disease, brain abnormalities and fetal growth retardation (9).

During pregnancy, the amount of metals transferred from mother to fetus is not constant. By far the greatest amount is transferred during the last trimester. All transfer takes place across the placentc. In human placenta, there is a fused layer of the syncytiotrophoblast that forms a selective barrier between the mother and her baby. Nutrients are taken up on the maternal side, transferred across the cell and out into the fetal side. Nutrients must then traverse the fetal endothelium into the circulation. There are no data at present describing how this is accomplished.

The placenta regulates the transfer of micronutrients. In a series of experiments on the effect of Fe deficiency on Fe status in the fetus and offspring, we fed female rats diets with differing amounts of Fe. At the extremes, Fe levels in the maternal liver dropped to about 30% of control. In contrast, fetal liver Fe levels decreased to 54% of control, a significantly lesser degree of deficiency.

To investigate the mechanism of Fe transfer, and the mechanisms of adaptation, we have developed a series of experimental models. Uptake of Fe into the placenta has been studied using rat placental cells in culture, in vivo models and vesicles isolated from the microvillar border of both rat and human placentas. Additionally, we have used human choriocarcinoma cell lines to identify efflux mechanisms.

Uptake of Fe into the placenta is through transferrin receptor mediated endocytosis. The holo-transferrin binds to the receptor and is internalized in clathrin coated pits. The endosomes are acidified and the Fe released. It moves into the cytoplasm, presumably through divalent metal transporter 1 (DMT1), and is carried across the placenta and into the fetal circulation. Efflux is probably through iron-regulated gene 1 (IREG1) and the Fe$^{2+}$ is oxidized to Fe$^{3+}$ before incorporation into fetal transferrin by a ferroxidase protein that is similar, but not identical, to ceruloplasmin.

In Fe deficiency, the expression of the transferrin receptor increases at both mRNA and protein levels. At the same time, expression of the iron-responsive element (IRE)-regulated form of DMT1 increases. Interestingly, expression of the non-IRE regulated form does not change. These adaptations will result in increased uptake by the placenta, in turn ameliorating the deficiency on the fetus. In the efflux pathway, IREG1 expression does not change. In contrast, levels of ferroxidase activity increase in the Fe-deficient animals but, taken together, the results would suggest that regulation occurs primarily at uptake rather than efflux.

Fe deficiency has indirect as well as direct effects on growth. We showed that deficiency resulted in an increase in tumor necrosis factor α (TNFα) levels in the placenta. There was also a rise in TNFα receptor 1 (TNFR1) expression, although the two proteins did not completely overlap. Why this should have occurred is not yet certain, but we are currently investigating the results using our cultured cell systems.

We know much less about the mechanisms of Cu transfer across the placenta. The capacity of the developing fetus to compensate is less than for Fe (10) but there is still some ability, because serum Cu concentrations in the infant do not correlate directly with those of the mother (6). Fetal Cu levels are lower than those of the mother, as are the ceruloplasmin concentrations.

As is the case with Fe, transfer across the placenta develops with gestation, increasing in the last trimester. Uptake is not energy dependent, but is carrier mediated, and specific (11). The placenta can accumulate Cu either from ceruloplasmin, CuHis<sub>2</sub> or from low molecular complexes (12), and some data suggest that ceruloplasmin is a more efficient Cu donor to the fetus than the low molecular weight complexes (13). Similarly, we showed in cultured human placental cells that ceruloplasmin could inhibit Cu uptake from CuHis<sub>2</sub>, and we used these observations to isolate a putative ceruloplasmin receptor (11,14).

In ceruloplasmin knock-out mice, however, Cu metabolism is not affected, suggesting that ceruloplasmin is not essential or a substrate for Cu uptake by the placenta (15). However, it is possible that the CuHis<sub>2</sub> pathway can transfer Cu in the absence of available ceruloplasmin.

In 1997 Zhou and Gitschier identified a putative transmembrane transport protein for Cu uptake in mammalian cells (16). This protein was named human Cu transporter, hCtrl1. Data base searches identified a second Cu transport protein named hCtrl2. Both transporters are expressed in human tissue including the placenta. Early embryonic lethality is observed in Ctrl1−/− homozygous mice as well as deficiency in Cu uptake thus supporting the hypothesis that Ctrl1 is essential for embryonic growth and development and is required for Cu uptake (17,18). Our own data support these observations. As described below, placental cells express both transcripts of Ctrl1, although which specific cell types do is not yet known.

Once Cu is inside the placental cell virtually all further steps of transport are elusive. Presumably the same chaperones are involved as in other cell types, but to date, this has not yet been investigated. We have, however, shown that mRNA for ATX1 is expressed in rat placenta (Gambling, L. and McArdle, H. J., unpublished data).

More importantly, we have also shown, as have others, that mRNA for the Menkes protein, ATP7A, is expressed in placenta. Interestingly, ATP7A is expressed in differentiated, but not undifferentiated, BeWo cells. The role of the protein in Cu transfer has not been elucidated directly, although it presumably fulfills a similar function to that in the intestine. Certainly, the symptoms of Menkes disease are consistent with a role for Cu transfer.

ATP7B, the protein defective in Wilson disease, has been shown to be expressed in mouse placenta at early stages of...
development (19). In rats, null mutation of the ATP7B gene results in intracellular Cu accumulation in the placenta and in lactating mammary glands, whereas the milk is Cu deficient. Also, Cu concentration in the liver of the newborn homozygous null mutants was markedly decreased, indicating that ATP7B plays a role in Cu transport from the mother to the fetus (20). How ATP7B expression is affected in normal Cu deficient animals is, however, less described.

In many tissues, Cu and Fe levels have been shown to be inversely proportional. We have investigated this in the Fe-deficient rat and find similar results, with the notable exception that there is no relation between Fe and Cu in the fetus (L. Gambling, S. Dunford, H.J. McArdle, unpublished results). Why this should be so is not clear, but it may be significant that there is no obvious change in expression of the proteins of Cu metabolism.

In summary, the data discussed in this review point out how important both Cu and Fe are for normal fetal growth and development. Although we are beginning to understand some of the processes, there are still many areas that are unclear. Most significantly, the interactions between the micronutrients and the effect they have on growth and development have not been closely examined, and there is much work yet to be carried out. This work becomes particularly relevant given the high proportion of women who are prescribed Fe supplements during pregnancy and the increasingly common use of prenatal vitamin supplements containing both Fe and Cu.

**LITERATURE CITED**