Iron and copper, and their interactions during development

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Abstract
During development, the fetus is entirely dependent on the mother for its nutrient requirements. Subsequently, it is a period when both are vulnerable to changes in dietary supply, especially of those nutrients that are marginal under normal circumstances. In developed countries, this applies mainly to micronutrients. Even now, iron deficiency is a common disorder, especially in pregnancy. Similarly, copper intake in the U.K. population is rarely above adequate levels. It is now becoming clear that nutrient deficiencies during pregnancy can result in problems for the offspring, in both the short- and long-term. Early studies showed that lambs born to mothers on copper-deficient pastures developed ‘swayback’, with neurological and muscular symptoms that could not be reversed by postnatal supplementation. Our own findings have shown that prenatal iron deficiency results in increased postnatal blood pressure, even though the offspring have normal dietary iron levels from birth. These observations emphasize the importance of iron and copper in growth and development. Complicating the situation further is the fact that copper and iron are known to interact with each other in many ways, including absorption and intracellular transport. However, their interactions during the pregnancy appear to be more complex than during the non-pregnant state. In the present review, we examine the importance of these metals and their interactions, the consequences, both short- and long-term, of deficiency and consider some possible mechanisms whereby these effects may be generated.

Introduction
The impact of inappropriate maternal nutrition on pregnancy outcome has been known for decades [1]. Now, 70 years later, it is clear that nutrition in utero affects not only fetal and neonatal health, but also adult well-being [2]. It is unlikely that the differences between good and poor diets with respect to fetal development can be attributed to a single nutrient. Deficiencies in several essential micronutrients have been implicated in problems with development and with birth defects [3]. In the U.K., two micronutrients which continue to cause concern are copper and iron. It is estimated that the average intake of both copper and iron by women of childbearing age is lower than the current estimated safe and adequate daily intake for adults [4].

The role of copper and iron
The nutritional need for iron and copper in living organisms is derived from their role in the metabolism of living cells. Their ability to accept or donate electrons makes them central to the catalytic activity of many redox-active enzymes. However, these same characteristics may cause cellular toxicity if the metals are not confined in compartments or bound to molecular chaperones. Copper is a central component of many enzymes involved in metabolic reactions, including angiogenesis and oxygen transport [5]. Iron is an integral part of several classes of enzymes, including cytochromes, enzymes involved in the synthesis of steroid hormones, detoxification of foreign substances in the liver, synthesis of neurotransmitters and DNA synthesis and breakdown. Owing to these vital roles in many of the body’s biochemical processes, it is clear that deficiencies in copper and iron would lead to widespread problems, particularly if these deficiencies were to occur during times of rapid growth and development, such as pregnancy, infancy and puberty.

Copper in fetal development
Copper is essential for fetal development, and maternal dietary copper deficiency can have both short- and long-term consequences (for a review, see [6]). Evidence for the importance of copper in fetal development itself first arose from studies of sheep that grazed on copper-deficient pasture. Lambs were born with a disease called enzootic ataxia, more commonly known as ‘swayback’ [7]. This disease is characterized by spastic paralysis, especially of the hind limbs, severe uncoordination, convulsions and blindness. Supplementing the animals postnatally did not reverse the neurological and muscular symptoms [7], but copper supplementation of the mothers during gestation could prevent the disease [8]. The extent to which copper deficiency affects pregnancy outcome is very much dependent on the degree of copper limitation. Severe copper deficiency can lead to reproductive failure, early embryonic death and gross structural abnormalities in the fetuses [9], whereas moderate or mild copper deficiency has little effect on either the number of live births or neonatal weight [10]. It is now
becoming clear that not only are there short-term pregnancy outcome consequences of maternal copper deficiency, but also, in animal models, there is now evidence for long-term consequences, including abnormal motor function [11], compromised immune response [12], altered cardiac ultrastructure [13] and abnormal neurobehaviour [14].

In humans, nutritionally induced clinical copper deficiency is rare, but moderate or mild copper deficiency may occur more widely than is appreciated currently. Copper deficiency can also occur as a secondary deficiency even if the dietary content is adequate, e.g. by interaction with drugs or other nutrients. Copper metabolism can also be altered in disease states such as diabetes, and it has been suggested that these alterations may contribute to diabetes-associated teratogenicity [15]. Congenital abnormalities are also observed in infants born to mothers who received a copper chelator, penicillamine, as a treatment for Wilson disease [16].

The most devastating impact of copper status on human fetal development is seen in the X-linked disorder Menkes disease [17]. Menkes disease originates in utero and manifests full symptoms during the perinatal period. These symptoms include hypothermia, neuronal degeneration, abnormalities of the hair, skin and connective tissue, bone fractures and widespread vascular abnormalities. Menkes disease is caused by a mutation in the gene encoding the copper ATPase, ATP7A. Mutations in this gene lead to defective cellular export of copper [18]. Although this disease has been recognized to be a disorder of copper metabolism for over 20 years, the prognosis for infants with this disorder is still poor, with death typically occurring by 3 years of age [19].

Iron in fetal development
The World Health Organization considers iron deficiency the number one nutritional disorder in the world [20]. It is prevalent in most of the developing world. In industrialized countries, the occurrence of iron deficiency is highest among young children and women of childbearing age, particularly pregnant women. Several studies indicate that, in Europe, the level of maternal iron deficiency during pregnancy is in the region of 20–40%, significant cause for concern (see, e.g., [21,22]).

The consequences of maternal iron deficiency are serious, for both the mother and her developing fetus. Many studies have shown that mothers suffering from iron deficiency anaemia are at increased risk of mortality and morbidity (reviewed in [23]). Babies have an increased risk of being born prematurely and/or smaller [24]. Several studies have shown that iron deficiency during pregnancy and/or lactation, both in humans and in animal models, also results in problems postnatally for the offspring. In the neonatal period, there is an increased risk of impaired motor development and co-ordination. In children, language development and scholastic achievement can be affected, there are significant psychological and behavioural effects and decreased physical activity (reviewed in [25], and see also the review by Professor Georgieff on pp. 1267–1271 of this issue [25a]).

As adults, the effects persist and can result in elevated blood pressure and cardiovascular problems [26–28].

Interactions
The fact that the metabolism of copper and iron are interlinked has been known for some 150 years. The initial observation came in the middle of the 19th Century, from physicians studying the causes and cures of chlorosis. These physicians discovered that taking copper sulfate led to an improvement in symptoms [29]. However, it was not until the seminal study of Hart et al. in 1928 [30] that the link was demonstrated in a study conforming to more rigorous scientific standards. This now classic study showed that, although iron supplementation failed to resolve anaemia in rats, administration of copper in the form of either ashed food or acid extracts of the ashes restored haemoglobin levels.

Several studies have now confirmed that iron deficiency has secondary effects on copper metabolism and that the inverse relationship also occurs. In general, iron deficiency results in increased copper levels in the liver [31–33], whereas severe copper deficiency causes changes in iron metabolism, leading to anaemia and liver iron accumulation [34,35].

Ceruloplasmin
The mechanisms behind the copper and iron interactions are largely unknown; however, the discovery of Cp (ceruloplasmin) gave the first direct link. Copper- and iron-deficiency-induced anaemias exhibit similar haematological features, which led to the initial suggestions that a common pathway was behind the symptoms. The search for commonality led to the discovery and isolation of a multi-copper protein with oxidase activity; this protein was named ceruloplasmin [36]. Cp was shown to act as a ferrioxidase, converting Fe$^{2+}$ into Fe$^{3+}$, and was capable of rapidly stimulating iron efflux from perfused liver [37]. Cp is now considered to be the critical factor in the mobilization of iron from body stores. This pivotal role has been confirmed in both human and animal studies. In patients with aceruloplasminaemia, an autosomal recessive disorder leading to the absence of Cp, iron accumulates within specific tissues [38]. A knockout mouse model of the human disease confirmed the lack of Cp as the cause of the symptoms ([39], and see also the review by Professor Harris on pp. 1277–1281 of this issue [39a]).

Intestinal absorption
Copper–iron interactions also occur at the level of intestinal absorption and, again, a multi-copper ferrioxidase plays a pivotal role. In copper-deficient pigs, studies revealed that intestinal iron absorption was normal, but release from the enterocytes was impaired [40]. This failure of the intestinal epithelium iron release during copper deficiency was better understood following studies of sex-linked anaemic (sla) mice. These also absorbed normal amounts of iron from the diet, but failed to release the metal into the portal circulation from the enterocytes. It has now been shown that the sla mice have a mutation in the gene coding for the hephaestin protein.
Hephaestin is a homologue of Cp, widely expressed in intestinal tissue. Iron leaves the enterocyte in the form of Fe$^{2+}$. In order for it to be bound by transferrin, it must first be oxidized to Fe$^{3+}$, which is accomplished by the ferrioxidase activity of hephaestin. Clearly, the failure of iron release from the enterocyte in the sla mice is linked directly to the mutated hephaestin protein [41]. Whether hephaestin expression and function could also be modulated by nutritional treatments has now been examined, and it is clear that nutritional copper deficiency results in decreased expression/function of hephaestin, in turn leading to systemic iron deficiency [42].

Pregnancy

The interactions are also important during pregnancy. In the early 1930s, it was reported that, as well as iron, copper needed to be added to a milk diet in order for pregnancy to be successfully established in female rats [43]. However, it is only recently that the interactions have started to be investigated further.

Effect of iron deficiency on copper metabolism

In the 1980s, Sherman and colleagues studied the effect of iron deficiency on copper metabolism during pregnancy and the interaction of these metals in the pregnant animal or her offspring [44,45]. In a more recent study, we have examined the effect of iron deficiency on copper levels in maternal and fetal tissue. Results highlighted the fact that maternal iron deficiency had a differential effect on copper metabolism in the mother and fetus [46]. In the mother, iron deficiency results in an increase in liver copper levels, inversely correlating with iron levels, as has been reported previously. A corresponding increase in copper levels and Cp activity were also seen in the maternal serum [46]. However, and importantly, in the fetus, as the iron content of the maternal diet decreased, so did the iron and copper liver levels. This is in direct contrast with the effects in the mother. At present, the mechanism underlying this paradox is not clear. The changes in copper levels all occur without any change in transcription levels of genes involved in copper transport, a fact which could simply be explained by the fact that most of the genes involved in copper transport are at least partly regulated at the post-translational level [46].

Effect of copper deficiency on iron metabolism

Present data on the effect of mild copper deficiency on maternal to fetal transfer of iron are contradictory. A study by Wapnir et al. [47] showed that fetal liver iron levels increased in copper deficiency, whereas another study reported a decrease [48]. Our own data supported the latter result [49]. With induction of mild dietary copper deficiency, copper levels and Cp activity decrease in maternal serum, as would be expected. Secondary effects were seen in iron metabolism, with maternal liver iron inversely correlated with liver copper levels; in addition, maternal serum iron levels decreased [49]. Therefore, in maternal copper deficiency, delivery of iron, as well as copper, to the fetus is compromised. Fetal liver copper and iron levels decrease.

Interestingly, levels of iron within the placenta do not change, suggesting that the conceptus may protect the placenta at the expense of the developing embryo and fetus.

These changes in copper metabolism again occurred without any alteration in transcript levels of Ctrl1, Cu-ATPase7A or 7B and Atox1 [49] in the placentas of copper-deficient rats. In contrast, expression data in the placenta suggest that it is responding to the indirectly induced iron deficiency in the same way as it does to iron deficiency itself [49]. mRNA levels of both transferrin receptor and the IRE (iron regulatory element)-regulated form of DMT1 (divalent metal transporter 1) rise as copper decreases, whereas mRNA of the non-IRE-regulated form and of ferroportin do not change [49]. These results indicate that both primary and secondary iron deficiency lead to the induction of a placental-mediated protection mechanism.

Conclusions

In conclusion, copper and iron are essential for normal fetal development. Maternal copper and/or iron deficiency during pregnancy has serious consequences for the offspring. These range from direct effects of a decreased enzyme activity to indirect results of changed activities of signalling pathways. Although most data have been obtained in animal models, the extreme examples of Menkes disease and the milder effects of maternal iron deficiency on infant cognitive ability provide strong supporting evidence for humans being equally vulnerable. It has been clearly established that deficiencies of either iron or copper alter the distribution of the other mineral, although the mechanisms are not yet understood. However, given the high incidence of maternal anaemia during pregnancy, and that many women who are prescribed iron supplements show no significant improvement, it is tempting to conclude that at least some of the cases are as a consequence of low copper. This is, in our opinion, an area of considerable public health significance, and one worthy of further investigation.

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References


