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Iron, copper and fetal development

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Pregnancy is a period of rapid growth and cell differentiation for both the mother and fetus. Consequently, 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 vulnerability applies mainly to micronutrients. Even now, Fe deficiency is a common disorder, especially in pregnancy. Similarly, Cu intake in the UK population is rarely above adequate levels, which is a matter of some concern, both in terms of public health and possible clinical consequences. In early studies it was shown that lambs born to mothers on Cu-deficient pastures develop 'swayback,' with neurological and muscular symptoms that cannot be reversed by postnatal supplementation. More recently, rat studies have shown that responses such as the 'startle' response are lost in offspring of Cu-deficient mothers. Data have shown that prenatal Fe deficiency results in increased postnatal blood pressure, even though the offspring have normal dietary Fe levels from birth. These observations emphasise the importance of Fe and Cu in growth and development. In the present review the importance of these metals and the consequences, both short term and long term, of deficiency will be discussed and some possible mechanisms whereby these effects may be generated will be considered.

Iron: Copper: Pregnancy

The impact of inappropriate maternal nutrition on pregnancy outcome has been known for decades. The now classic study by Ebbs *et al*. (1941) showed an association between the consumption of a diet low in protein, Ca, fruit and vegetables, i.e. a 'poor' diet, and an increased risk of pregnancy complications when compared with women who eat a 'good' diet. Now, >70 years later, it is clear that nutrition *in utero* affects not only fetal and neonatal health but also adult well-being. Many studies have demonstrated a link between fetal nutrition, low birth weight and CHD, hypertension and impaired glucose tolerance in adults (Barker *et al*. 1993*a*; Ravelli *et al*. 1998; Law *et al*. 2000). It has been proposed that adaptations made by the fetus to cope with inappropriate nutrition may lead to morphological and physiological changes that persist into postnatal life. These changes, although ensuring fetal survival, may have detrimental affects in later life (Barker, 1995).

It is unlikely that the differences between good and poor diets with regard to fetal development can be attributed to a single nutrient. Deficiencies in several essential micronutrients, such as folate, vitamins K and A, I, Mn, Zn, Cu and Fe (Hurley, 1981; Castillo-Duran & Cassoria, 1999; McArdle & Ashworth, 1999; Black, 2001), have been implicated in problems with development and with birth defects. Clearly, discussing the impact of all these micronutrients on fetal development is beyond the scope of one review, hence only the role of Cu and Fe will be examined. The consequences, both short term and long term, of deficiency *in utero* will be discussed and some possible mechanisms whereby these effects may be generated will be considered.

Copper in fetal development

Enzootic ataxia

In the early 1930s it was reported that Cu, as well as Fe, needed to be added to a milk diet in order for pregnancy to be successfully established in female rats (Keil & Nelson, 1931). Evidence for the importance of Cu in fetal development arose from studies of sheep that grazed on Cu-deficient pasture. Lambs were born with a disease termed enzootic ataxia, more commonly known as swayback (Bennetts & Chaoman, 1937). This disease is characterised by spastic paralysis, especially of the hind limbs, severe incoordination, convulsions and blindness. Other

Abbreviation: BP, systolic blood pressure.
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abnormalities include aneurysms of the aortic arch, anaemia, osteoporosis and other skeletal lesions, and abnormalities of the skin and hair. These studies have been expanded to identify neonatal ataxia in other species (Bennetts *et al*. 1941; Joyce, 1955; Wilkie, 1959; O'Sullivan, 1977). Supplementing the animals postnatally does not reverse the neurological and muscular symptoms (Bennetts & Chaoman, 1937), but it has been demonstrated that Cu supplementation of the mothers during gestation can prevent this disease (Allcroft *et al*. 1959). Importantly, whilst the cause of enzootic ataxia is clearly embryonic–fetal Cu deficiency, the pregnant animals often appear quite healthy and show no obvious signs of Cu deficiency (Bennetts *et al*. 1948).

Copper deficiency in man

In man nutritionally-induced clinical Cu deficiency is rare; however, moderate or mild Cu deficiency may occur more widely than is currently appreciated. It is estimated that the average intake of Cu by women of childbearing age is lower than the current estimated safe and adequate daily intake for adults, which is 0·9–1·2 mg Cu/d (Department of Health, 1991; Food and Nutrition Board and Institute of Medicine, 2001). Cu deficiency can also occur as a secondary deficiency even if the dietary content is adequate; for example, by interaction with drugs or other nutrients. Cu 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 (Uriu-Hare *et al*. 1985; Jankowski *et al*. 1993).

The most devastating impact of Cu status on human fetal development is seen in the X-linked disorder Menkes disease (Danks *et al*. 1972). 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 for the Cu ATPase, ATP7A; mutations in this gene lead to defective cellular export of Cu (Vulpe *et al*. 1993). Although this disease has been recognised to be a disorder of Cu metabolism for >20 years, the prognosis for infants with this disorder is still poor, with death typically occurring by 3 years of age (Menkes, 1999).

Effect of maternal copper deficiency on fetal development

The extent to which Cu deficiency affects pregnancy outcome is very much dependent on both the severity and timing of the deficiency. If deficiency occurs before mating it may lead to reproductive failure and early embryonic death (Dutt & Mills, 1960; Hall & Howell, 1969). Maternal Cu deficiency during pregnancy has been shown to be teratogenic in many species, including man, cattle, sheep and rats (Hurley, 1981). Fetuses are found to suffer from gross structural abnormalities, including skeletal, pulmonary and cardiovascular defects (Fields *et al*. 1990; Jankowski *et al*. 1993; Sarricolea *et al*. 1993; Keen *et al*. 1998; Table 1); symptoms that clearly mirror those described for Menkes disease.

Table 1. Consequences of maternal copper deficiency during pregnancy for the offspring

Short term	Long term
Connective tissue abnormalities	Cardiac ultrastructure abnormalities
Pulmonary insufficiency	Immune suppression
Neuronal degeneration	Impaired cognitive and behavioural function
Skeletal defects	
Lower survival rate	

Short-term consequences of maternal copper deficiency

The effect of maternal Cu deficiency on embryo development has been studied through the use of post-implantation embryo culture systems. In these systems embryos are removed at approximately 8–10 d gestation from rats or mice fed a control or Cu-deficient diet. They are then cultured for ≤48 h in serum obtained from control or Cu-deficient animals. Embryos from Cu-deficient mothers cultured in Cu-deficient serum have reduced crown–rump lengths, head:crown–rump length and protein content (Mieden *et al*. 1986). These embryos also have a marked increase in brain and cardiac abnormalities (Hawk *et al*. 1998).

In contrast, moderate Cu deficiency, resulting in a 30 and 68% drop in maternal and neonatal Cu levels respectively, has little effect on either the number of live births or neonatal weight (Masters *et al*. 1983; Ebesh *et al*. 1999). However, when born the offspring of the Cu-deficient dams do suffer from gross structural abnormalities. Cu-deficient neonates are typically characterised by severe connective tissue abnormalities. Cardiac haemorrhages are a frequent finding in Cu-deficient sheep, rats, guinea-pigs and mice (Tinker & Rucker, 1985; Rucker *et al*. 1998). Carotid and cerebral arteries of Cu-deficient neonates tend to have sparse poorly-developed elastin that lacks the concise fibril arrangements seen in control animals. Skeletal defects also often occur as a result of prenatal Cu deficiency. Lambs with enzootic ataxia may have poorly-developed light brittle bones and frequent fractures. Lung abnormalities are also a frequent consequence of prenatal Cu deficiency (Abdel-Mageed *et al*. 1994). After birth 35% of the newborn Cu-deficient rats show respiratory distress syndrome (Sarricolea *et al*. 1993). Brain neurochemistry is also affected; offspring of Cu-deficient dams show reduced noradrenaline levels in specific brain regions and there is also an increase in regional dopamine levels. (Prohaska & Bailey, 1994).

The timing of the onset of Cu deficiency is critical to the survival of the offspring. In their established murine model Prohaska & Brokate (2002) have shown that if Cu deficiency is induced 8 d before birth there is no effect on pregnancy outcome or birth weight, but none of the offspring survives beyond postnatal day 13. When the deficiency is induced 2 d before birth all offspring survive through to weaning.

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Table 2. Consequences of maternal iron deficiency during pregnancy for the offspring

Short term	Long term
Reduced fetal weight	Increased blood pressure
Asymmetric organ growth	Increased glucose tolerance
Fe deficiency	Impaired cognitive and behavioural function
Lower survival rate	

Long-term consequences of maternal copper deficiency

The effect of maternal Cu deficiency on postnatal gene expression and function has been assessed in both rat and murine models. Effects have been noted in both brain and liver gene expression. In the mouse model higher brain Cu–Zn superoxide dismutase activity and higher levels of brain thiobarbituric acid-reactive substances (an index of lipid peroxidation) are seen in response to oxidative stress (Arce & Keen, 1992). In addition, the restoration of liver Cu levels as a result of being weaned onto control diets does not lead to a recovery in metallothionein expression, indicating that prenatal Cu deficiency could have permanently altered the expression of proteins involved in Cu metabolism (Arce & Keen, 1992).

Marginal-Cu-deficient diets beginning during the perinatal period and continued following weaning have been shown to lead to long-term abnormalities in cardiac ultrastructure and a suppression in the response of immune cells to *in vitro* stimuli at 6 months of age (Hopkins & Failla, 1995; Wildman *et al*. 1995; Table 2).

The clearest evidence for long-term functional consequences of maternal Cu deficiency *in utero* and during lactation comes from studies of the Sprague Dawley rat. The rats were fed a Cu-deficient diet from mid pregnancy and through lactation, the offspring were weaned onto a control diet and the long-term effects on brain function were investigated. Whilst the offspring show normal responses to tactile startle, the auditory startle response of those offspring born to Cu-deficient dams is markedly decreased (Prohaska & Hoffman, 1996). These neurobehavioural abnormalities occur in both genders, and there is no difference in the Cu status of the control and experimental groups at the time of testing.

Possible mechanisms of action

One major area for discussion is the means by which these deficiencies exert their effects. Are the effects seen in micronutrient deficiencies the result of a direct effect on the embryo–fetus or a result of indirect effects via alterations in maternal metabolism? In the case of Cu deficiency *in utero* there is substantial evidence to indicate that the developmental abnormalities, both pre- and postnatal, are a result of direct effects. Mieden *et al*. (1986), using the embryo culture system, have reported that embryos cultured in Cu-deficient serum show developmental abnormalities. However, when Cu is added to the same serum all embryos subsequently cultured develop normally. In addition, the clinical features of both enzootic ataxia and Menkes disease can be explained by deficiencies in the activities of Cu-containing enzymes.

Cytochrome c *oxidase*

Cytochrome *c* oxidase is an inner-mitochondrial-membrane protein complex that catalyses the reduction of O_2 to water and utilises the free energy of this reaction to generate a transmembrane proton gradient during respiration. A decrease in the activity of this enzyme would affect the offspring's ability to carry out oxidative phosphorylation, which may in turn lead to lower levels of ATP. When brain tissue from lambs suffering from enzootic ataxia is analysed for cytochrome *c* oxidase activity, there is a marked reduction in activity compared with that of normal lambs (Howell & Davison, 1959). Subsequent studies have supported this finding, indicating that enzootic ataxia is caused by low brain Cu concentrations, leading to a deficiency in cytochrome *c* oxidase activity in the motor neurons and aplasia of the myelin surrounding these neurons (Mills & Williams, 1962; Fell *et al*. 1965). The effect of maternal Cu deficiency on cytochrome *c* activity has also been confirmed in the murine model (Prohaska & Bailey, 1993).

Dopamine-β-monooxygenase

Murine models have also established that the effect of maternal Cu deficiency on the activity of Cu-containing enzymes in the brain may not be restricted to cytochrome *c* oxidase. When compared with normal offspring of the same age the offspring of Cu-deficient mothers have altered brain catecholamines pools. A reduction in noradrenaline levels in the brain occurs in parallel with an increase in dopamine levels, indicating that the activity of dopamine-β-monooxygenase, which is responsible for conversion of dopamine to noradrenaline in noradrenergic neurons, is reduced in the offspring of Cu-deficient mothers (Prohaska & Bailey, 1993).

Lysyl oxidase

In addition to the effect on neuronal development, abnormalities in both connective tissue and lung development (O'Dell *et al*. 1978; Tinker *et al*. 1985; Harris, 1986; Abdel-Mageed *et al*. 1994; Rucker *et al*. 1998) can be linked to impairment of the activity of one particular enzyme, lysyl oxidase. Lysyl oxidase is an extracellular Cu-containing enzyme that is responsible for the formation of lysine-derived cross-links in connective tissue, particularly in collagen and elastin. It is this cross-linking that is required for connective tissue stability. Studies on rat heart (Farquharson & Robins, 1991) and chick lung (Harris, 1986) show that Cu deficiency adversely influences the production of mature elastin and collagen. Carotid and cerebral arteries of Cu-deficient offspring have sparse poorly-developed elastin, and a reduction in the activity of lysyl oxidase has been implicated (Tinker & Rucker, 1985; Rucker *et al*. 1998). The impairment in lysyl oxidase activity is also thought to be the cause of the increased bone fragility observed in Cu-deficient neonates (Tinker & Rucker, 1985). The impact of maternal Cu deficiency on the activity of lysyl oxidase has been directly examined in the lungs of offspring born to Cu-deficient rabbits

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(Abdel-Mageed *et al*. 1994). Offspring born to the Cu-deficient mothers have a markedly reduced 24 h survival rate. Analysis of the activity of lysyl oxidase in the lungs of these Cu-deficient offspring indicates that it is half that of control animals (Abdel-Mageed *et al*. 1994).

Superoxide dismutase

Superoxide dismutase activity in embryos cultured in Cu-deficient serum is markedly reduced when compared with that of their control counterparts (Hawk *et al*. 1998). Embryo abnormalities are reduced on the addition of reactive oxygen scavengers to the culture medium, clearly supporting a functional role for the reduction in superoxide dismutase activity (Hawk *et al*. 1998). These results suggest that free radical-induced damage is involved in the induction of embryo abnormalities. Further to this finding, the embryo culture system has been used to establish that not only are superoxide anion concentrations higher in the Cu-deficient embryos, but that these anions are localised in areas of the embryos that are characterised by abnormalities, e.g. forebrain and heart (Hawk *et al*. 2003).

These data clearly support the hypothesis that maternal Cu deficiency impacts on embryo and fetal development through several direct mechanisms. Brain development and function are directly affected by the reduction in the activity of a number of key enzymes, and other teratogenic effects occur via a compromised oxidant defence system and extracellular matrix integrity.

Iron in fetal development

Iron deficiency during pregnancy

The World Health Organization (2003) considers Fe deficiency to be the primary nutritional disorder in the world, second only to tuberculosis as the world's most common and costly health problem. It is prevalent in most of the developing world. In industrialised countries the occurrence of Fe deficiency is highest among young children and women of childbearing age, particularly pregnant women (see Fairweather-Tait, 2004).

In most species maternal blood volume increases and the packed cell volume and Hb concentration fall during pregnancy; this condition is known as the anaemia of pregnancy. However, in a high percentage of women the fall in Hb levels is greater than that regarded as both physiological and safe (World Health Organization, 1992). A high proportion of these types of anaemia arise as a result of Fe deficiency (World Health Organization, 2003). Several recently-completed studies indicate that currently in Europe the level of maternal Fe deficiency during pregnancy is a major cause for concern (Hercberg *et al*. 2001). These investigations have studied pregnant women in Germany, Belgium and Scotland, and they indicate that the incidence of Fe deficiency during pregnancy is in the region of 20–40 % (Bergmann *et al*. 2002; Fosset *et al*. 2003; Massot & Vanderpas, 2003).

Inadequate intake of Fe related to diets poor in bioavailable Fe is thought to be responsible for the majority of Fe deficiency both before and during pregnancy (Bothwell, 2000). In one recent survey carried out in France 93 % of women of childbearing age were reported to have dietary Fe intakes lower than the recommended daily allowance (Galan *et al*. 1998). In the UK >40 % of women between the ages of 19–34 years had total Fe intakes below the lower reference nutrient intake level (Henderson *et al*. 2003). An increase of 50 % is recommended in the daily dietary intake of Fe during pregnancy (Galan *et al*. 1998). An additional risk factor for Fe deficiency during pregnancy is multiparity, which can lead to subsequent successive deficits without sufficient repletion of reserves (Hindmarsh *et al*. 2000).

The consequences of maternal Fe deficiency are serious, both for the mother and her developing fetus. Many studies have shown that mothers suffering from Fe-deficiency anaemia are at increased risk of mortality and morbidity (for review, see Rush, 2000). Babies have an increased risk of being born premature and/or smaller (Allen, 2000). Several studies have shown that Fe deficiency during pregnancy, both in man and in animal models, results in both short- and long-term problems for the offspring.

Short-term consequences of maternal iron deficiency

In order to investigate the effects of maternal Fe deficiency on fetal growth and development, as well as the long-term health and well-being of the offspring, several rodent models have been established (Crowe *et al*. 1995; Kwik-Uribe *et al*. 1999; Lewis *et al*. 2001*b*; Gambling *et al*. 2002). As would be expected the fetuses from the Fe-deficient dams are Fe deficient, with lower packed cell volume and lower liver Fe levels (Gambling *et al*. 2002). However, the level of Fe deficiency seen in the fetus is markedly less than that seen in the mother. Maternal Fe status also has a direct impact on the Fe stores for early neonatal life; consistent with fetal findings, neonates are also Fe deficient (Gambling *et al*. 2003). Early studies in man have established a direct relationship between the concentration, as well as the total content, of storage Fe in the fetal liver and maternal plasma Fe levels, suggesting that babies born to Fe-deficient mothers would have poor Fe stores (Singla *et al*. 1985). More recent studies have now established that Fe deficiency in the mother is a risk factor for Fe deficiency in the young infant (Preziosi *et al*. 1997; Singla *et al*. 1997; Allen, 2000; Halvorsen, 2000; Harthoorn-Lasthuizen *et al*. 2001), with consequent more pronounced anaemia at approximately 10–12 weeks of age.

While maternal Fe deficiency has no effect on the fertility and growth of the dams or the viability and number of fetuses, it does markedly reduce fetal weight (Gambling *et al*. 2002). In addition to changes in total fetal weight, fetal liver weights (expressed as a proportion of fetal size) are decreased, indicating disproportionate fetal growth. The reduced fetal weight seen in the Rowett Hooded Lister model (Gambling *et al*. 2002) is consistent with that seen in other rat models (Tojyo, 1983; Crowe *et al*. 1995). As with maternal Cu deficiency, the severity of the Fe deficiency induced substantially affects the outcomes. While milder maternal Fe deficiency has no

marked effect on fetal number, fetal weight or placental weight (Sherman & Moran, 1984), severe Fe deficiency during pregnancy can lead to a fall in maternal weight, fertility and fetal viability (Tojyo, 1983).

Increased placental weight:birth weight is one of the indicators for the development of adult diseases, such as cardiovascular problems and diabetes (Barker *et al*. 1993*b*; Phipps *et al*. 1993). In the early 1990s maternal anaemia and Fe deficiency were linked to an increase in placental weight : birth weight (Godfrey *et al*. 1991). An increase in placental weight and placental weight : birth weight in anaemic and Fe-deficient pregnancies has since been confirmed in several studies (Williams *et al*. 1997; Lao & Wong, 1997; Hindmarsh *et al*. 2000). An increase in placental weight:fetal weight is also seen in rodents (Lewis *et al*. 2001*b*; Gambling *et al*. 2002).

Using the Rowett rat model these studies have been expanded to investigate the early postnatal period. The survival rate of the offspring born to Fe-deficient mothers is reduced, with 25% of the pups born to Fe-deficient mothers dying within the first 24 h after birth (Gambling *et al*. 2003). The pups that survive are smaller and have larger hearts and smaller kidneys than their control counterparts. Lower birth weight is a consistent finding in several rodent models (Crowe *et al*. 1995; Lewis *et al*. 2001*b*) and, of course, man (Allen, 2000). The effects on the offspring of maternal Fe deficiency during pregnancy are summarised in Table 2.

Long-term consequences of maternal iron deficiency

Blood pressure

Long-term effects of maternal Fe deficiency on the health and well-being of the offspring have been predicted by animal models since the mid 1990s. Crowe *et al*. (1995) have examined the effects of maternal Fe deficiency on the systolic blood pressure (BP) of the offspring. Before weaning the BP of the offspring of Fe-deficient mothers is lower than that of controls. This reduction is followed by a pronounced post-weaning rise in BP when compared with controls. This raised BP has since been shown to occur in both male and female offspring of Fe-deficient mothers (Lewis *et al*. 2001*c*). The study into the effect of maternal Fe deficiency on BP in the offspring has been further expanded using the Rowett model (Gambling *et al*. 2003). At birth both the control and the Fe-deficient litters were cross-fostered to control dams and the BP of the offspring measured at 6, 10 and 16 weeks. Results show that the BP of the male offspring born to Fe-deficient dams is raised at all time points, while the BP of the corresponding female offspring is lower at 6 weeks, but at 10 and 16 weeks it is higher than that of the controls. There are no differences in growth rate, body or organ weight between the two groups of offspring at any of the time points. Measurement of the total liver Fe levels of the rats at each of the time points shows that at no time is there a difference between the levels for the offspring born to control and Fe-deficient mothers. Thus, the alterations in BP occur despite the offspring being of normal Fe status (Gambling *et al*. 2003).

Glucose tolerance

As well as increased BP the Wistar rat model of maternal Fe deficiency studied by the Hales group in Cambridge (Lewis *et al*. 2001*c*) has also shown an effect on glucose tolerance in offspring of Fe-deficient mothers. At 3 months of age offspring of Fe-deficient mothers display an improvement in glucose tolerance. However, unlike the effect on BP the effect of maternal Fe deficiency on glucose tolerance in the offspring does not appear consistent across time and models. When the experimental time point is extended in the Wistar rat model to 14 months an increase in BP in the offspring of Fe-deficient mothers is still present; however, there is no longer a difference in glucose tolerance between the offspring (Lewis *et al*. 2002). Glucose tolerance and insulin levels have been investigated in the Rowett model (Gambling *et al*. 2003). However, in these rats there are no apparent differences at 10 weeks of age between the offspring born to control or Fe-deficient mothers.

Neuronal development

In man the clearest evidence for a long-term effect of maternal Fe deficiency on postnatal development relates to brain development and function (Deregnier *et al*. 2000; Nelson *et al*. 2000; Tamura *et al*. 2002). It is believed that the effects of Fe deficiency in early development lead to later problems in cognitive and behavioural functions (Lozoff, 2000). Poor fetal Fe status is associated with diminished language ability, fine motor skills and tractability, as assessed at 5 years of age (Tamura *et al*. 2002). These findings in the human population are supported by extensive studies in animal models. Offspring born to rats fed an Fe-deficient diet either in early or late gestation show reduced activity and homing ability (Felt & Lozoff, 1996). Investigations carried out in a murine model provide evidence that maternal Fe deficiency leads to persistent alterations in behaviour and cognitive function that cannot be reversed by postnatal Fe supplementation (Kwik-Uribe *et al*. 2000).

Possible mechanisms of action

Direct effects

The investigations into the mechanisms by which maternal Fe deficiency exerts its effect on fetal growth and development are only in the early stages compared with those for Cu. Again possible mechanisms can be subdivided into direct and indirect effects. As with Cu, Fe deficiency may exert effects directly by reducing the activity of the enzymes that use Fe as a cofactor. Rodent models have indicated that enzymes involved in neurotransmitter synthesis and neuronal energy may be perturbed in maternal Fe deficiency. For example, Taneja *et al*. (1990) have demonstrated that maternal Fe deficiency leads to a reduction in γ-aminobutyric acid metabolism that is not reversible by postnatal Fe supplementation. In addition, areas of the brain that are involved in higher cognitive functions have lower cytochrome *c* oxidase activity in

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neonatal rats born to mothers who were Fe deficient during pregnancy (Deungria *et al*. 2000).

Indirect effects

Most of the abnormalities and developmental problems associated with maternal Cu deficiency can be directly related to the perturbation of the activity of a particular enzyme. With the exception of brain development and function there does not appear to be a similar relationship with maternal Fe deficiency. In fact, data from recent studies (Gambling *et al*. 2002; L Gambling, A Czopek, HS Andersen, R Wojak, Z Krejpcio and HJ McArdle, unpublished results) indicate that birth weight is dependent on the mother's Fe status and not that of the neonate. Thus, maternal Fe deficiency may also affect fetal development by more indirect mechanisms. Fe-deficiency-induced changes in maternal metabolism may have downstream effects on placental structure, endocrine and transport functions, nutrient interactions and fetal organ development.

Placental function. 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. TNFα has been suggested to play an important role in pregnancy (Hunt *et al*. 1996). Elevated levels of TNFα are associated with early to mid-pregnancy failure and premature labour in man (Silen *et al*. 1989; Chaouat *et al*. 1990; Tangri & Raghupathy, 1993). 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. It may also be important in trophoblast turnover and re-modelling (Yui *et al*. 1996). Leptin has also been suggested to be important in the maintenance of pregnancy (for review, see Ashworth *et al*. 2000) and is thought to be a growth factor for the fetus. Studies on TNFα and leptin expression in placentas from control and Fe-deficient litters have been carried out (Gambling *et al*. 2002). Maternal Fe deficiency increases levels of TNFα in the trophoblast giant cells of the placenta. Levels of the type 1 TNFα receptor are increased in giant cells, cytotrophoblasts, the labyrinth and fetal vessels. Leptin is also increased in the labyrinth and marginally in trophoblast giant cells. There is no change in leptin receptor levels in any region of the placentas from Fe-deficient litters. Growth and development are clearly dependent not on a single cytokine but on the presence of an appropriate profile of factors, so that here the increased leptin may be acting to counter some of the worst effects of the increased TNFα levels.

In human pregnancy the placental structure is also altered in maternal anaemia (Mayet, 1985). Maternal anaemia has been shown to be associated with increased placental weight and fetal weight:placental weight (Beischer *et al*. 1970; Godfrey *et al*. 1991). This increase in placental weight has been interpreted as compensatory placental hypertrophy. The effect of maternal Fe deficiency on placental structure has been investigated in both rodent and human pregnancies. The Wistar rat model has been used to investigate the effect of maternal Fe deficiency on placental structure. This study has shown that there is decreased capillary length and surface area in the placentals from Fe-deficient litters (Lewis *et al*. 2001*a*). Low ferritin concentrations in early human pregnancy are associated with increased placental vascularisation at term. The surface area of capillaries involved in gas exchange is strongly and inversely related to serum ferritin concentrations (Hindmarsh *et al*. 2000). The relationship between maternal Fe deficiency and placental size and birth weight exists across the normal range for these measures and is not just restricted to severely-anaemic mothers. Alteration in placental structure would clearly have an impact on its ability to transport nutrients to the fetus. Maternal Fe deficiency has been shown to cause fetal plasma amino acid and cholesterol and triacylglycerol levels to be decreased, clearly suggesting decreased placental transport of amino acid and NEFA to the fetus (Lewis *et al*. 2001*b*).

Nutrient interactions. The initial observation of a link between Fe and Cu metabolism came with a study that showed that while Fe supplementation fails to resolve anaemia in rats, administration of Cu in the form of either ashed food or acid extracts of the ashes restores Hb levels (Waddell *et al*. 1927; Hart *et al*. 1928). Several subsequent studies have now confirmed that Fe deficiency has secondary effects on Cu metabolism. Generally, Fe deficiency results in increased Cu levels in the liver and rises in serum caeruloplasmin concentrations (Sourkes *et al*. 1968; Evans & Abraham, 1973; Owen, 1973; Sherman *et al*. 1977). Few studies have investigated the effect of Fe deficiency on Cu metabolism during pregnancy and the interaction of these metals in the pregnant animal or her offspring (Sherman & Tissue, 1981; Sherman & Moran, 1984). Thus, in a recent study the Rowett model has been used to examine the effect of Fe deficiency on Cu levels in maternal and fetal tissue (Gambling *et al*. 2004). Results have highlighted the fact that maternal Fe deficiency has a differential effect on Cu metabolism in the mother and fetus. In the maternal liver Cu levels are inversely correlated with those of Fe, while in the fetus both Fe and Cu levels are reduced. A similar differential effect between mother and fetus is also seen in vitamin A metabolism. Maternal liver retinol levels are reduced in maternal Fe deficiency, while in the fetus the opposite is seen, as the level of Fe decreases the levels of retinol in the fetal liver increase (Gambling *et al*. 2001). Although the reduction in Cu and vitamin A levels seen in the Fe-deficient fetuses is not as great as that seen in their respective deficiencies, this further restriction in nutrient supply may have an impact on fetal development.

Fetal organ development. It has been proposed that one possible mechanism for the increased BP in the offspring born to Fe-deficient mothers is that maternal Fe deficiency may interfere with normal kidney development. Kidney nephron number is an important determinant of BP. Low nephron number reduces the surface area available for filtration (Brenner *et al*. 1988), and therefore limits the ability of the kidney to excrete Na and maintain normal extracellular fluid volume and BP (Brenner *et al*. 1988).

Nephron number is established during kidney development, beyond which point the number cannot be increased (Wintour, 1997). Nephron number is related to birth weight over the normal range (Merlet-Bencichou *et al*. 1994), and intrauterine growth retardation has been associated with low nephron number (Merlet-Bencichou *et al*. 1994; Bassan *et al*. 2000; Bauer *et al*. 2002). Fe deficiency during gestation appears to affect the cellular development of the kidney, in which DNA concentration has been found to be lower in 2-d-old neonates (Kochanowski & Sherman, 1985). Expanding on their earlier work with the Wistar Fe-deficiency model, Hales and colleagues (Lisle *et al*. 2003) have recently studied the effect of maternal Fe deficiency on the renal morphology of the adult offspring. Their results show a reduction in the number of glomeruli in the kidney of offspring born to Fe-deficient mothers. Offspring from both control and Fe-deficient mothers also show an inverse relationship between glomerular number and BP. It is suggested that the reduction in nephron number induced by maternal Fe deficiency may be partly responsible for the increase in BP seen in these animals.

Conclusions

In conclusion, it is clear that maternal Cu and/or Fe deficiency during pregnancy has serious consequences for the offspring. These consequences range from direct effects of a decreased enzyme activity to indirect results of changed activities of signalling pathways. Although most of the data has been obtained in animal models, the extreme examples of Menkes disease and the milder effects of maternal Fe deficiency on infant cognitive ability provide strong supportive evidence for similar vulnerability in man. The animal models discussed in the present review have considerable heuristic value, and will provide the data underpinning the design of therapeutic strategies in the treatment of human maternal micronutrient deficiencies.

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