

# Micronutrients in fetal growth and development

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The roles that the different vitamins and minerals play in fetal growth and development are reviewed, primarily with respect to growth and differentiation in humans; but, as appropriate, data provided from animal and cellular studies are also considered.

Pregnancy is a period of rapid growth and cell differentiation, both for the mother and the fetus she carries. Consequently, it is a period when both are very susceptible to alterations in dietary supply, especially of nutrients which are marginal under normal circumstances. Inappropriate nutrition leads not only to an increased risk of death *in utero*, but also to alterations in birth weight and functional changes in the neonatal organs. These changes can have far-reaching consequences. For example, babies who are small at birth are at an increased risk of cardiovascular disease and diabetes as adults<sup>1</sup>, while animals who are born to zinc deficient mothers have a compromised immune system<sup>2</sup>. The underlying mechanisms relate to nutrition effects on gene expression in the fetus. There is an argument, therefore, for supplementation in the diet, in order to avoid the consequences of deficiency during pregnancy.

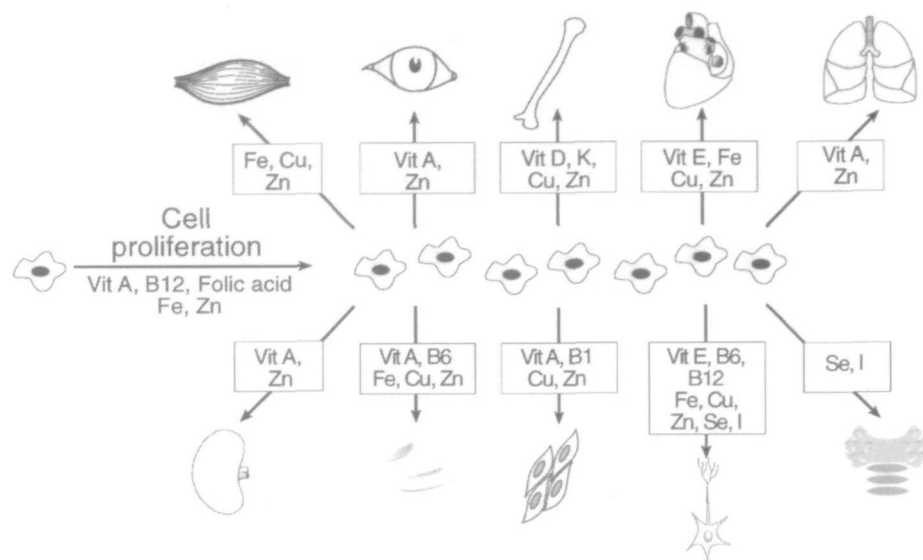
Supplementation with one specific micronutrient is not straightforward, however, since there are many interactions between them, summarised below. Further, other dietary components are also important. For example, phytates inhibit calcium and other metal absorption and the n-3/n-6 ratio in fatty acids alters fat-soluble vitamin absorption.

Up until now, excess intake has been an uncommon problem. However, with the increasing practice of fortifying foods, especially in developing countries, the possibility of micronutrient toxicity has increased<sup>3</sup>.

Micronutrient deficiencies have such varied effects because of the diverse roles they play. During the process of cell growth, DNA is transcribed to RNA, which is then translated to proteins, which provide the enzymes and structures of the cell. At every stage in the process, micronutrients are essential, either as signals (retinoic acid, for example), or structural (zinc in transcription factors) or catalytic (*e.g.* copper) elements. Different organs develop at different times in pregnancy. This gives rise to

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**Fig. 1** Tissues affected by micronutrient deficiencies. Cell growth and development is divided into two parts; proliferation, in the middle of the diagram, followed by differentiation into the different organs. The deficiencies which exert most effect on that proliferation or differentiation pathway are given in the box associated with each organ. This is, obviously, a simplification of the actual effects and does not take into account any interactions between nutrients, which are likely also to be critically important.

‘critical windows’ – periods when particular organs, because of the developmental stage they have reached, are more susceptible to alterations in supply. This is critically important when considering supplementation or fortification strategies and there are clear lessons to be drawn from agriculture, where feeding during pregnancy is strictly controlled and monitored. A very simplified diagram of the stages in cell proliferation and differentiation where micronutrients can exert their effect is given in Figure 1.

## Vitamins

Vitamins play important roles in cellular metabolism, maintenance and growth to such an extent that even before clinical symptoms are apparent, marginal deficiencies may be manifest as impaired fertility or reduced fetal and neonatal viability<sup>4</sup>.

Vitamin deficiencies arise as a consequence of socio-economic factors, inappropriate dietary habits, a failure of complete absorption and smoking. Smoking during pregnancy is associated with poor outcomes of

pregnancy such as reduced birthweight and an increased risk of intra-uterine growth retardation. Smokers generally have a poorer dietary intake of micronutrients than non-smokers and, interestingly, even when intakes are equivalent, circulatory concentrations of antioxidant nutrients, including vitamins A, E and C, of smokers are lower than non-smokers. Antioxidant nutrients are believed to defend the body against attacks from free radicals produced by tobacco smoke. The use of these vitamins in this way would mean that less are available to perform other vital functions in the body.

Many human problems associated with vitamin deficiencies are difficult to attribute to a particular vitamin as vitamin deficiencies rarely occur in isolation. Furthermore, many studies report beneficial effects of multi-vitamin supplements on pregnancy outcome, for example periconceptual multivitamin use is associated with a reduced risk of conotruncal defects in human infants<sup>5</sup>, although it is not clear which vitamin is the effective agent. It is, therefore, important to refer to controlled animal studies to understand the consequences of specific vitamin deficiencies during pregnancy and the fetal organ systems primarily affected.

### *Fat-soluble vitamins*

#### **Vitamin A**

Retinoids are essential for growth, development and reproduction with demonstrated roles in vision, embryogenesis, spermatogenesis, skin development and in the maintenance of differentiated epithelial cells. Vitamin A is the parent compound of biologically active retinoids such as retinaldehyde (or retinal), the active element of visual pigment, and retinoic acid, an intracellular messenger which modulates cellular differentiation.

In many areas of the world, vitamin A intakes are below recommended daily levels, due either to limited and seasonal availability of foods rich in retinol or its precursor  $\beta$ -carotene, lack of nutritional awareness or inappropriate dietary choices. The World Health Organization estimates that up to 50% of pregnant women have daily intakes below defined minimum levels. Worryingly, 51% of pregnant women in rural Nepal suffer from night blindness<sup>6</sup>. Vitamin A deficiency during pregnancy has been associated with increased vertical transmission of the human immunodeficiency virus<sup>7</sup> and has particularly serious effects on fetal lung development<sup>8</sup>. Damage caused to the lungs by such deficiency can be irreversible, affecting lung function throughout adult life.

Animal models have shown that vitamin A deficiency leads to placental dysfunction, fetal loss and congenital malformations. However, many of these studies use models of severe vitamin A deficiency. Recent studies using more modest (~50%) reductions in maternal retinol concentrations

during late pregnancy in the rat have shown reductions in neonatal survival, by about 50% and the relative weights of fetal lungs, liver and heart<sup>9</sup>. Only in the case of the lungs did such differences persist into post-natal life. Day 20 fetal lungs were morphologically immature with less well developed bronchial passageways and fewer centrifugal branches and elastic fibres and reduced expression of the key developmental genes growth arrest specific 6 (Gas6)<sup>10</sup>.

High dietary intake of preformed vitamin A (retinol and retinyl esters) during pregnancy is associated with birth defects originating in the cranial neural crest. Rothman and colleagues<sup>11</sup> reported that the incidence of malformations in babies born to women who took more than 10,000 IU of vitamin A per day (RDA = 2670 IU/day) was 1 baby in 57. Interestingly, neither teratogenicity nor vitamin A toxicity has been observed in multiple species exposed to high doses of  $\beta$ -carotene<sup>12</sup>.

### Vitamin D

In addition to the factors associated with inadequate vitamin status described above, lack of exposure to sunlight and excess clothing can predispose individuals to vitamin D deficiency. Maternal serum parathyroid hormone levels at term are inversely related to neonatal crown-heel length<sup>13</sup>, suggesting that vitamin D deficiency affects fetal growth through an effect on maternal calcium homeostasis. In a study comparing bone mineral content and calcium status of summer born and winter born babies, Namgung<sup>14</sup> observed that babies born between January and March had a higher bone mineral content, lower serum osteocalcin and higher serum calcium than babies born between July and September. These data suggest that the vitamin D status of the mother 6 months prior to parturition affects the calcium status of her offspring.

### Vitamin E

The term vitamin E is used as a generic designation for 8 compounds, primarily tocopherols, synthesised by plants which function as antioxidants protecting cells against damage induced by free radicals. In addition to dietary intake, other factors can either increase (deficiencies of sulphur-containing amino acids, riboflavin, copper, zinc and/or manganese) or decrease (selenium) vitamin E requirements<sup>15</sup>.

Vitamin E deficiency during pregnancy in experimental animals results in malformed embryos and fetal death<sup>16</sup>, but in a recent study of 289 women, no relationship was found between serum vitamin E concentrations at 18 and 30 weeks of gestation and birth weight or the Apgar score of infants<sup>17</sup>. Maternal vitamin E deficiency may, however, be associated with pre-eclampsia and accumulation of lipid peroxidase products in vitamin E deficient mothers causes vasoconstriction and consequent pregnancy induced hypertension<sup>18</sup>.

### Vitamin K

Developing teeth and bone contain two vitamin K dependent proteins; matrix gla protein, necessary to maintain growing cartilage in a non-calcified state and bone gla protein which is important for tooth mineralisation. Maternal dietary deficiency or the use of a number of therapeutic drugs during pregnancy, may result in frank vitamin K deficiency in the embryo (reviewed by Howe & Webster<sup>19</sup>). The effects of deficiency are reflected in its function in teeth and bone mineralisation. First trimester deficiencies result in maxillofacial hypoplasia in the neonate with subsequent facial and orthodontic implications while infants born to mothers with vitamin K malabsorption suffer from abnormalities of the spine and abnormal calcifications<sup>20</sup>.

## Water-soluble vitamins

### B vitamins

Vitamin B1 (thiamin) may have a role in prevention of teratogenesis<sup>21</sup> and vitamin B2 (riboflavin) intake may be positively correlated with fetal growth<sup>22</sup>.

Pregnant women may have dietary vitamin B6 intakes that are well below the recommended dietary allowance, which may affect the vitamin status of their offspring. Vitamin B6 deficiency impairs pancreatic insulin production and supplementation during the second and third trimester of pregnancy may improve glucose intolerance in women with gestational diabetes<sup>23</sup>. It is also an essential cofactor in the developing central nervous system and may influence brain development and cognitive function. Recent work in rats suggests that vitamin B6 deficiency during gestation and lactation alters the function of N-methyl-D-aspartate receptors, a subtype of receptors of the glutamatergic neurotransmitter system thought to play an important role in learning and memory<sup>24</sup>.

Because of its role in nucleic acid synthesis, the need for folate increases during times of rapid tissue growth, including pregnancy. It is now well established that periconceptual use of folate-containing vitamin and mineral supplements reduces the incidence of neural tube defects<sup>25</sup>. Studies of folate-related enzymes have, however, failed to identify the metabolic defect in the neurulation-stage embryo that is corrected by folic acid. Approximately 30% of neural tube defects appear resistant to folic acid but recent work in a mouse model suggests that administration of *myo*-inositol may be a complementary therapeutic option<sup>26</sup>.

The beneficial effects of folate are not confined to events in early pregnancy. Marginal levels of maternal folate throughout gestation impairs cellular growth and replication, while mothers with low folate levels at 28 weeks of gestation have an approximately 2-fold greater risk of preterm delivery and low infant weight at birth<sup>27</sup>.

Lack of maternal vitamin B12 during pregnancy retards myelination of the fetal nervous system<sup>28</sup>. However, these effects can be counteracted by post-natal administration of vitamin B12.

### Vitamin C

There has been little work on the relationship between maternal vitamin C status and pregnancy outcome despite the key role of this vitamin in collagen synthesis and bone formation. Extensive studies to examine possible relationships between dietary vitamin C intake and birth weight did not find evidence of significant associations (reviewed by Matthews<sup>29</sup>).

## Minerals

Minerals are important either as central components of the catalytic sites of enzymes (Cu and Fe, for example) or as stabilising factors in enzymes and transcription factors (Zn, for example). Generally, the problems that are associated with deficiencies can be related back to these functions. However, alterations in dietary levels of one mineral can alter transfer across the gut or the placenta of another mineral, which may have an important bearing on physiological effects of deficiencies.

Frank mineral deficiencies are rare in the western world, but intake is often marginal especially in adolescents and in women from poor socio-economic backgrounds. These more subtle aberrations are not usually recognised and could be the cause of many problems of unknown aetiology.

### Iron

Iron deficiency is associated with increased risk of maternal haemorrhage, and peri-partum blood loss has more severe consequences for an anaemic mother (see Woolf<sup>30,31</sup> for reviews and policy statements of the American Medical Association). Significantly, Barker's group have found that low iron status during pregnancy leads to an increased placental:fetal ratio, in turn a good predictor of cardiovascular disease later in life<sup>1</sup>. Similarly, rat pups born to iron deficient mothers have increased heart weights and increased blood pressure by early adulthood<sup>32</sup>.

Anaemia (defined as blood haemoglobin concentration less than 11.0 g/dl) is fairly common in women, especially in those from a poor socio-economic background. It is commonly assumed that it is as a result of iron deficiency<sup>3</sup>. However, this may not be the case. For example, in several studies reviewed by Walker<sup>33</sup>, anaemia was relatively common

but about 75% of these women did not have iron-deficiency anaemia when defined as serum ferritin < 12 µg/ml. This does, of course, beg the question as to the cause of the remaining anaemias<sup>34</sup>.

## Copper

Severe copper deficiency causes fetal resorption while milder deficiencies result in skin, neuronal and hair abnormalities. The lesions underlying the alterations presumably occur as a result of reduced cupro-enzyme activity. For example, cytochrome c oxidase activity is reduced in the brain of affected lambs<sup>35</sup>. More recently, Prohaska and colleagues have correlated altered psychological responses (the startle response) to changing levels of peptidylglycine mono-oxygenase (PAM) in the brain of copper deficient neonatal rats<sup>36</sup> and further showed that restoration of serum Cu levels did not restore the reduced response, indicating that deficiency *in utero* caused unalterable changes post-natally. In sheep, copper deficiency during pregnancy causes a disorder called 'swayback', which is manifest as muscular weakness and ataxia and it, too, cannot be reversed post-natally.

It has been suggested that the problems that arise during development may be related to excessive oxidative damage to the cell<sup>37</sup>. There are several reports showing reduced Cu Zn superoxide activity in rats born with neonatal copper deficiency and reductions of the enzyme are associated with excessive lipid and protein damage and cell death. However, the levels of peroxidation are not noticeably increased. To test the hypothesis, Keen and colleagues cultured rat embryos in normal and Cu deficient serum and added reactive oxygen scavengers to the medium. They found that Cu Zn SOD activity was reduced in Cu deficient medium, that there was a higher incidence of abnormality in these embryos and that addition of the scavengers reversed this effect (reviewed in Keen *et al*<sup>37</sup>).

However, other problems associated with copper deficiency *in utero* cannot be ignored. For example, deficiencies in collagen and elastin cross linking, due to a fall in lysyl oxidase activity, lead to problems with lung development and with aortic elasticity. These in turn lead to pulmonary difficulties, such as persistent respiratory distress syndrome, and to an increased risk of aortic aneurysm in early life<sup>37</sup>.

## Zinc

Zinc is a central part of more than 300 enzymes and proteins. Consequently maternal deficiency results in a wide spectrum of extremely severe problems exacerbated by the fact that the first symptom is loss of appetite.

Zinc deficiency is teratogenic and even marginal changes can cause long-term problems. For example, mild deficiency during pregnancy in monkeys causes immunodeficiency in the offspring, which does not improve post-natally<sup>38</sup>. Deficiency also causes complications of labour, premature rupture of membranes (PROM) (summarised in Jameson<sup>39</sup>) and an increased risk of pre-term delivery which may be related to problems with oestrogen receptor expression (see Caulfield *et al*<sup>40</sup> for a review).

In a recent review, Caulfield and colleagues<sup>40</sup> clearly showed that zinc supplementation of pregnant women has maternal and fetal benefits. Most of the unequivocal data come from animal experiments, but there are also human studies which show supplementation increasing gestation length, decreasing risk of PROM and decreases in vaginal bleeding<sup>41</sup>.

An intriguing hypothesis linking zinc metabolism with teratogenicity has recently been advanced by Keen and colleagues. Any factors which lower plasma zinc can result in a functional zinc deficiency. If this occurs during the critical periods of organogenesis, teratogenicity can result, an observation which has important implications for strategies of supplementation. They have now identified several teratogens which act through this pathway<sup>42,43</sup>.

## Iodine

During pregnancy, three factors act to increase iodine requirements. Firstly, human chorionic gonadotrophins and oestrogens are thyrotropic hormones, and increase thyroid activity. Second, there is an increase in circulating levels of thyroxine binding globulin (TBG) which decreases the amount of free circulating hormone, and thirdly, the placenta has a deiodinase activity. As long as dietary intake of iodine is adequate, this is not a problem, but there are several geographical areas where intake can be below or at the minimum limit of the needs for healthy non-pregnant humans. Glinioer and colleagues have conducted extensive studies in Brussels, where the levels are at the lower limit (100 µg/day) and have defined the limits and importance of iodine in relation to development of goitre (about 10% of patients)<sup>44,45</sup> in one study, but higher (16%) in another<sup>46</sup>. Iodine deficiencies are especially common in developing countries and there are, as described further below, indications that it is associated with an increased incidence of cretinism.

## Selenium

In both humans and animals, final selenium status of the offspring reflects that of the mother<sup>47-49</sup>. Selenium absorption in the diet is high



(about 70%), so there is little scope for regulation at gut level and consequently, the developing fetus is very dependent on dietary supply or maternal stores.

The dietary supply may be the more important factor. In women with a high selenium status, there was an increase in blood glutathione peroxidase activity during pregnancy of about 25% (glutathione peroxidase is a seleno-enzyme). In contrast, in women from a low selenium environment, there was a decrease in glutathione peroxidase levels<sup>49</sup>.

Generally, it is thought that selenium deficiency does not cause serious problems in the fetus. However, recent data suggest that endemic cretinism may result from combined selenium and iodine deficiency, rather than iodine alone (see Arthur *et al*<sup>50</sup> for a recent review).

## Interactions between micronutrients

### *Vitamin A and zinc*

Several studies suggest that zinc deficiency can alter vitamin A metabolism and *vice versa*. The data are equivocal, however. At least some of the studies have been confounded by the fact that altering zinc changes appetite and in humans zinc deficiency is often secondary to some other problem – liver cirrhosis, for example. A recent summary by Christian and West<sup>51</sup> concluded that there were grounds for investigation, but that it was not clear that there was a public health problem, except in relation to moderate-to-severe nutritional deficiencies.

### *Vitamin A and iron*

There are several studies in non-pregnant animals showing an interaction between vitamin A status and iron metabolism. Whether the interaction is important during pregnancy is not so clearly established.

### *Vitamin A and copper*

Ceruloplasmin levels in plasma are increased by giving vitamin A and a recent report has suggested that it acts at the post-transcriptional level<sup>52</sup>. However, these are studies where animals were given injections of vitamin A. Data from our laboratory (Finch A, Ashworth CJ, Antipatis C and McArdle HJ, unpublished data) have suggested that retinol regulates ceruloplasmin tonically. The significance of this preliminary observation remains to be established.

### *Iron and zinc*

Some studies have shown that high iron can inhibit zinc absorption when the two minerals are given together in solution. In contrast, when given in a food, the inhibition is not so apparent. The studies' results have been summarised by Whittaker<sup>53</sup> who concluded that there is probably little physiologically significant interaction at the gut level. In cells and tissues, zinc can affect iron metabolism, again through the alteration of zinc and protein production.

### *Copper and iron*

Copper deficiency results in accumulation of iron in certain specific tissues together with anaemia. The anaemia is not caused by a down-regulation of haem synthesis enzymes, but rather by a functional iron deficiency in the erythron. Whether this plays a role in pregnancy is not clear.

Iron deficiency results in copper deficiency. Iron overload can also cause a decrease in copper levels in the liver – possibly by down regulation of a common uptake pathway<sup>54</sup>.

## **Key points for clinical practice**

With the exception of folic acid, there is a dearth of scientific evidence describing beneficial effects of micronutrient supplements on pregnancy outcome in humans. Deficiencies of micronutrients and minerals may be more prevalent than previously thought. Apart from the well known deficiencies, iron and vitamin A, for example, changes in copper, selenium and zinc should be considered. If iron supplementation is given, additional micronutrient supplementation should be considered. Further, attention must be paid as to the stage during pregnancy when the supplements are given. The 'critical windows' of development mean that if supplements are given at an inappropriate time, they will be ineffective and may even be harmful. Clearly, more research is needed to clarify these windows and interactions.

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