Developmental Consequences of Trace Mineral Deficiencies in Rodents: Acute and Long-Term Effects\(^1,2\)

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ABSTRACT  Approximately 3% of infants born have at least one serious congenital malformation. In the U.S., an average of 10 infants per thousand die before 1 y of life; about half of these deaths can be attributed to birth defects, low birth weight or prematurity. Although the causes of developmental abnormalities are clearly multifactorial in nature, we suggest that a common factor contributing to the occurrence of developmental abnormalities is suboptimal mineral nutrition during embryonic and fetal development. Using zinc and copper as examples, evidence is presented that nutritional deficiencies can rapidly affect the developing conceptus and result in gross structural abnormalities. Deficits of zinc or copper can result in rapid changes in cellular redox balance, tissue oxidative stress, inappropriate patterns of cell death, alterations in the migration of neural crest cells and changes in the expression of key patterning genes. In addition to well-recognized malformations, mineral deficiencies during perinatal development can result in behavioral, immunological and biochemical abnormalities that persist into adulthood. Although these persistent defects in part are attributed to subtle morphological abnormalities, in other cases they may be secondary to epigenetic or developmental changes in DNA methylation patterns. Epigenetic defects combined with subtle morphological abnormalities can influence an individual’s risk for certain chronic diseases and thus influence his or her risk for morbidity and mortality later in life. J. Nutr. 133: 1477S–1480S, 2003.

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It is estimated that ~50% of human concepti are lost before or during implantation, and of those that successfully implant, an additional 15–20% are lost before delivery. With respect to completed pregnancies, ~3% result in a child with one or more severe malformations. Although numerous factors can contribute to the occurrence of fetal complications, it is now well established that human pregnancy outcome can be compromised by suboptimal maternal nutritional status [reviewed in (1)]. Consistent with this, it was demonstrated that the occurrence of birth defects in certain at-risk populations can be markedly reduced if dietary supplements are provided to the mother during the periconceptional period. Illustrative of this is the use of iodine and folic acid supplements to reduce the occurrence of cretinism and spina bifida, respectively [reviewed in (2,3)]. Although more controversial, the use of multivitamin/mineral supplements during pregnancy is also reported to reduce the frequency of some pregnancy complications in the general population (4–6). One argument made for the use of supplements in addition to food-fortification programs such as those developed for iodine and folic acid is that individuals can suffer from a wide range of micronutrient deficiencies (1,7). In this article, we present evidence for the hypothesis that maternal zinc and copper deficiencies can rapidly affect the developing conceptus. Potential mechanisms that underlie the teratogenicity of zinc and copper deficiency are considered. In addition to a higher risk for gross malformations that are evident during the neonatal period, we also discuss the concept that deficits of zinc or copper during the perinatal period may result in health complications that persist into adulthood. Owing to space constraints, review articles are cited in several instances, and the reader is directed to them for additional references.

Zinc deficiency and abnormal development

Dietary zinc deficiency during gestation is recognized to be teratogenic in many species including rats, mice, sheep, chickens and Xenopus (8,9). A zinc-deficient diet given to rats

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throughout pregnancy results in offspring that are characterized by anomalies affecting nearly every organ system (8). When rat dams are fed a zinc-deficient diet during discrete periods of pregnancy, the malformations that are induced reflect the period of organogenesis during the time the deficient diet was fed (8). Severe zinc deficiency for a week before mating can result in anestrous in the rat or a lack of fertilization. Preimplantation mouse embryos have also been shown to be influenced by zinc deficiency. For example, two-cell embryos obtained from dams that were fed a zinc-deficient diet for 6 d during oocyte maturation and fertilization were characterized by altered development even when they were cultured in zinc-adequate media. Under these conditions, the preimplantation embryos from the dams fed zinc-deficient diets show reduced cell proliferation and blastocyst formation, which are indicative of defects in differentiation (10). Similar to preimplantation embryos, peri-implantation embryos are sensitive to zinc deficiency (11). Our group has reported that whereas peri-implantation mouse embryos cultured in control medium (3.5 μM Zn) develop normal egg-cylinder morphology, embryos grown in a zinc-deficient medium (0.5 μM Zn) show impaired morphological and cellular development after 144 h. The embryos in the zinc-deficient medium frequently fail to develop egg-cylinder morphology and do not differentiate visceral and parietal endoderm. Based on Hoescht labeling, the embryos grown in the zinc-deficient medium have a markedly lower cell number than embryos grown in control medium. Confocal imaging of TUNEL labeled cells (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) reveals that the embryos grown in the deficient medium are characterized by a higher incidence of apoptosis. These in vitro observations of abnormal differentiation and cell death are consistent with the finding of a high frequency of early embryonic loss in rats and mice fed zinc-deficient diets (8). Similar to peri-implantation embryos, the development of postimplantation embryos in vitro can be influenced by zinc deficiency. Record and co-workers (12) demonstrated that embryos obtained from zinc-deficient dams grow abnormally when cultured in serum from zinc-deficient rats. Similarly, Meiden et al. (13) and Taubeneck et al. (14) reported that gestation day-9 embryos from zinc-adequate dams develop abnormally when cultured in serum that is low in zinc (either due to a dietary zinc deficiency or an acute phase-response–induced hypozincemia). Importantly, in both studies, when the deficient serum is supplemented with zinc, its teratogenicity is significantly ameliorated. This observation supports the concept that a lack of zinc per se can result in abnormal development.

The rapidity of the effects of zinc deficiency on developmental processes and the severity of the defects that result suggest two points. First, with respect to in vivo model systems, these results show that in the face of an acute dietary deficiency, maternal mobilization of zinc stores is inadequate to supply the needs of the conceptus (at least in rodent models). Consistent with this, it was reported that plasma zinc concentrations can decrease up to 40% in pregnant rats after the consumption of a zinc-deficient diet, and that this reduction can be correlated with a higher incidence of apoptosis in the embryo (15).

The second conclusion that can be drawn from the rapid effects of zinc deficiency on the conceptus that are observed in both in vivo and in vitro models is that cellular metabolism can be rapidly altered by changes in cellular zinc concentrations. Illustrative of this, we have shown that there is a marked rise in cellular oxidative stress, a shift in the redox state to a more oxidized environment, a marked increase in activator protein-1 (AP-1)4 DNA binding, a reduction in nuclear factor (NF)-κB nuclear binding activity, abnormal mitochondrial function and finally apoptosis (16,17). The finding of a reduction in NF-κB binding is surprising given that in many cases, NF-κB binding increases with oxidative stress. Given that NF-κB represents a survival pathway in many cell types, lack of this binding activity may predispose certain cell types to enhanced cell death. The fact that cytosolic NF-κB accumulates in the cytosol during zinc deficiency suggests that its nuclear transport may be impaired. Recently we demonstrated that this impairment may partly be explained by zinc-deficiency–associated impairment in tubulin polymerization (18).

Mechanistically, zinc deficiency is thought to influence embryonic and fetal development through several mechanisms including abnormal nucleic acid metabolism, reduced protein metabolism, reduced rates of tubulin polymerization, higher rates of cellular oxidative damage, higher rates of apoptosis, impaired cell migration and reduced binding of transcription factors and hormones that are dependent on zinc-finger regions (8,15,18).

**Copper deficiency and abnormal development**

Similar to zinc, a severe deficit of copper during pregnancy can represent a significant reproductive insult. A deficit of copper during pregnancy can result in early embryonic death and gross structural abnormalities including skeletal, pulmonary, central nervous system and cardiovascular defects (19–21). Copper deficiency can result from low dietary intake and can occur secondarily to drugs and genetic abnormalities that alter copper metabolism. Human infants with Menkes syndrome, an X-linked defect in the copper transporter ATP7A, are characterized by hypothermia, neuronal degeneration, abnormalities in hair, skin and connective tissue, bone fractures and widespread vascular abnormalities with tortuosity and fragmentation of the elastic fibers of the aorta and other major arteries of the heart (22–26). The intracellular trafficking of copper is mediated by copper chaperones such as Ctr1 and Atox1. Heterozygote null mutants for Ctr1 are growth retarded, have open neural tubes, fail to rotate and therefore die in utero (27,28). Mice that lack the metallochaperone ATOX1 gene generally die after birth; those that survive the weaning period are characterized by congenital malformations, hypopigmentation, hemorrhages and seizures (29). Female mice with a targeted disruption of copper, zinc–superoxide dismutase (CuZn–SOD) ovulate and conceive normally but are characterized by high embryonic lethality (30,31). These studies that involve genetic mutations in copper transporters and copper enzymes demonstrate the critical role that copper has in embryonic development.

Severe copper deficiency in mice has been shown to reduce fertilization rates and ova recovery rates (32). When pre-implantation embryos were cultured in vitro, embryos from dams fed diets that contain 1 μg of copper/g of diet had a lower incidence of blastocyst formation, and blastocysts failed to hatch from the zona pellucida (32). We have shown that compared with control embryos, postimplantation rat embryos from copper-deficient dams have a high incidence of heart and brain malformations (swollen hindbrain and forebrain, cardiac abnormalities including distention of the anterior cardinal veins, blisters, blood pooling and hemorrhaging) after 48 h of

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4 Abbreviations used: AP-1, activator protein-1; CuZn–SOD, copper, zinc–superoxide dismutase; ECM, extracellular matrix; NF-κB, nuclear factor-κB.
culture in copper-deficient serum, which indicates that the heart and brain are particularly susceptible to copper deficiency (33). Transmission electron microscopy reveals that copper-deficient embryos are characterized by disruption of mitochondrial cristae, enlarged myofibrils that are poorly organized and lack alignment, focal disruption of internal elastic lamina of major arteries and a higher accumulation of lipid in the endothelium and myocardia. The addition of copper or the oxidant-defense enzymes CuZn–SOD or glutathione peroxidase to the culture media reduces the incidence of malformations (13,33). SOD activity is lower and superoxide anion concentrations are higher in copper-deficient embryos (34). Superoxide anions are localized to the forebrain, heart, forelimb and somites, which are areas characterized by malformations (34). Although whole-body lipid oxidative damage (assessed by conjugated dienes) was similar between groups, there was a tendency for higher whole-body DNA oxidative damage (assessed by 8-hydroxy-2'-deoxyguanosine) in the copper-deficient compared with the copper-adequate embryos.

Our recent work with a copper-deficient mouse model suggests that there is a species-dependent susceptibility to copper deficiency. In addition to the heart and brain defects noted for the rat, copper-deficient mouse embryos cultured in copper-deficient serum also exhibit a high incidence of yolk-sac defects including abnormal vascularization and blebbing (Lanoue, Beckers, Keen, Rucker and Uriu-Adams, unpublished data). We are currently investigating whether the yolk-sac abnormalities are due to abnormal cross linking of extracellular matrix (ECM) proteins as a result of low l-lysyl oxidase activity, altered composition of ECM proteins or altered angiogenesis. Similar to rats, copper-deficient mouse embryos cultured in copper-deficient sera have higher levels of superoxide anions. The superoxide anions, likely due to the low CuZn–SOD activity, can react with nitric oxide to form peroxynitrite, a strongly oxidizing long-lived reactive oxygen species that can nitrate the protein-bound tyrosines and permanently modify protein function or activity. Using immunohistochemistry, our preliminary data show that copper-deficient embryos have higher staining for 3-nitrotyrosine compared to controls, indicating protein oxidative damage with copper deficiency (Lanoue, Beckers, Keen, Rucker and Uriu-Adams, unpublished data). These data from rat and mouse models support the concept that a copper-deficiency–induced compromised oxidant-defense system can lead to oxidative damage and contribute to the occurrence of developmental defects.

Similar to zinc deficiency, embryonic defects can rapidly occur with copper deficiency. Developmental defects are observed 48 h after exposure to a copper-deficient environment even in embryos derived from copper-adequate dams (33). Thus, even a transitory copper deficiency can result in developmental defects. Mechanistically, copper deficiency is thought to influence embryonic and fetal development through several mechanisms including excessive oxidative damage that is secondary to a compromised oxidant-defense system, altered angiogenesis, compromised energy production, altered ECM composition and integrity secondary to a reduction in lysyl oxidase activity.

Epigenetic consequences associated with micronutrient deficiencies during early development

The study of epigenetics focuses on the change in heritable gene expression that occurs without changes in the DNA sequence (35,36). Various diverse chemicals and environmental stressors can result in the transmission of genes with altered gene activity (37,38). Numerous studies have shown that maternal exposure to nutritional insults can also have significant and persistent effects on the offspring. For example, feeding a marginal zinc diet during pregnancy can result in persistent deleterious effects on immune function in the offspring even after zinc repletion (39–41). The state of immunodeficiency can span two generations, which indicates that subsequent adequate-zinc intakes may not rapidly reverse the deleterious effects of an in utero zinc insufficiency (39). Infants of monkeys fed low-zinc diets are characterized by higher levels of DNA damage as assessed by hepatic DNA strand breaks and 8-hydroxy-2'-deoxyguanosine concentrations (42). DNA damage can result in loss of methylation that may be transmitted to the offspring (43). These infants can also be characterized by persistent alterations in behavior (44); the mechanism(s) that underlie these behavioral abnormalities have not been identified.

Similar to zinc deficiency, persistent deleterious effects of perinatal copper deficiency have been reported. The feeding of a copper-deficient diet beginning in midgestation through lactation results in a diminished auditory startle response, which indicates persistent neurobehavioral abnormalities despite copper repletion (45). Marginal copper-deficient diets during the perinatal period have also been shown to result in long-term abnormalities in cardiac ultrastructure (46) and suppression in the response of immune cells to in vitro stimuli (47). In a study by Arce and Keen (48), dams were fed a copper-deficient diet from the beginning of gestation through postnatal day 18 and then switched to a copper-adequate diet. The offspring were assessed on postnatal day 42 after the animals were exposed to endotoxin, an oxidative stressor. Offspring exposed to copper deficiency in utero had persistent alterations in metallothionein concentrations, lipid peroxidation parameters and cytochrome P450 activity despite copper repletion after weaning.

When micro- and macronutrients together are restricted during pregnancy as in prolonged partial restriction of caloric intake, antibody formation is impaired even in second-generation offspring (49). DNA methylation is one mechanism of epigenetic modification of DNA (50). Maternal diets supplemented with cofactors and methyl donors for methyl metabolism such as choline, betaine, folic acid, vitamin B-12, methionine and zinc were shown to epigenetically regulate the expression of the agouti gene in their offspring (51), and these epigenetic phenotypes are strongly correlated with methylation of the proximal intracisternal A-particle long-terminal repeat of the agouti locus (52). Interestingly, the maternal diets that contain the highest concentrations of methyl supplements with the addition of methionine and zinc have the highest proportions of offspring with high degrees of eumelanic motting; these Avy/a mice generally do not become obese, have normal insulin metabolism and have a lower risk of tumorigenesis compared with obese Avy/a mouse offspring with no eumelanic motting (51). Taken together, these data indicate that maternal nutrition can alter the programming of certain fetal genes, and that the intrauterine and postnatal environment may affect health and longevity in adulthood.

In summary, collectively these data support the concept that maternal deficits of essential nutrients can rapidly influence a developing conceptus. Nutritional deficiencies during early development can result in malformations that are evident at birth and in biochemical abnormalities that persist into adulthood. Therefore, it is reasonable to suggest that improvements in the intake of essential micronutrients during pregnancy could result in marked reductions in the occurrence of pregnancy complications in at-risk populations.
LITERATURE CITED


