The Plausibility of Micronutrient Deficiencies Being a Significant Contributing Factor to the Occurrence of Pregnancy Complications[1,2]

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ABSTRACT Numerous studies support the concept that a major cause of pregnancy complications can be suboptimal embryonic and fetal nutrition. Although the negative effects of diets low in energy on pregnancy outcome are well documented, less clear are the effects of diets that are low in one or more essential micronutrients. However, several observational and intervention studies suggest that diets low in essential vitamins and minerals can pose a significant reproductive risk in diverse human populations. Although maternal nutritional deficiencies typically occur as a result of low dietary intakes of essential nutrients, nutritional deficiencies at the level of the conceptus can arise through multiple mechanisms. Evidence from experimental animals supports the concept that in addition to primary deficiencies, secondary embryonic and fetal nutritional deficiencies can be caused by diverse factors including genetics, maternal disease, toxicant insults and physiological stressors that can trigger a maternal acute phase response. These secondary responses may be significant contributors to the occurrence of birth defects. An implication of the above is that the frequency and severity of pregnancy complications may be reduced through an improvement in the micronutrient status of the mother. J. Nutr. 133: 1597S–1605S, 2003.

KEY WORDS: • *zinc* • *copper* • *pregnancy* • *nutrition* • *malformations*

Approximately 50% of human concepti are lost before or during early implantation, and of those that implant, an additional 15–20% are lost before delivery. With respect to completed pregnancies, approximately 3% result in a child with one or more severe congenital defects (1–3). Despite intensive investigative efforts, the mechanisms underlying early embryonic loss and those that contribute to the occurrence of congenital malformations are poorly understood. Causative factors for malformations can only be identified for approximately 60% of the developmental defects reported in most populations; genetic defects, multifactorial inheritance, uterine factors and twinning and specific toxicants account for approximately 28%, 23%, 3% and 3% of the defects, respectively (4).

Intrauterine growth retardation (IUGR)[4] is a marker for pregnancy complications. In developed countries the incidence of IUGR is typically about 5% whereas in developing countries it can reach 15–50% (5–7). Although risk factors for IUGR can be identified, including smoking, low maternal energy intake, hypertension and multiple pregnancy (8), in most cases a specific cause cannot be identified. Early postnatal death, another marker of pregnancy complications, ranges from 5 to >100 per 1000 live births in developed and developing countries, respectively (7,9). Maternal deaths associated with pregnancy complications range from 5 to 500 per 100,000 live births in developed and developing countries, respectively (7,10).

The high frequency of maternal deaths in developing countries can be attributed to numerous factors. Reports of marked reductions (>40%) in maternal mortality with vitamin A supplementation (11) and reports of reductions in the incidence of preeclampsia with magnesium supplementation (12) and vitamin C and vitamin E supplementation (13)

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[4] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; IUGR, intrauterine growth retardation; TNF, tumor necrosis factor.

suggest that micronutrient deficiencies can contribute to the risk for maternal mortality. Given that an impaired immune system is a common result of malnutrition (14) and the observation that perinatal infection is a significant contributor to maternal, fetal and infant mortality (15), the observation that micronutrient supplementation in undernourished populations can result in reductions in the frequency of maternal and infant mortality is not surprising. Some repair of the immune defense system should occur with the supplementation if the rate-limiting nutrients are provided. Similarly, given the essential roles that multiple micronutrients have on energy metabolism and anabolic processes, reductions in the frequency and severity of IUGR would be predicted to occur in the above populations. Less predictable is the effect micronutrient supplementation might have on the frequency and spectrum of congenital defects.

Numerous human reproductive insults have been identified and individual susceptibility to these insults may vary considerably. The differential response to reproductive insults can be attributed to a multiplicity of factors, including the genetic background of the mother and conceptus, the physical developmental environment, the timing of the insult and the presence or absence of other interactive stimuli. In this paper, we review the argument that maternal nutritional status is one component of the environment that can modulate the expression of many reproductive insults. In addition, we make the argument that suboptimal embryonic and fetal nutrition are common causes of abnormal development. Two implications can be drawn from the above: first, that the developmental toxicity of some agents and stressors (including certain maternal diseases) will be amplified in women characterized by suboptimal nutritional status, and second, that the correction of maternal nutritional deficits should result in a significant reduction in the frequency of birth defects as well as other pregnancy complications. These implications have significant ramifications for public policy regarding the development of public health programs aimed at improving pregnancy outcome.

Space constraints have led to review articles being cited here rather than primary references in many places. The interested reader is directed to these sources for additional original citations.

Mechanisms underlying the development of essential nutrient deficiencies

Before we discuss the developmental effects associated with deficits of essential nutrients, it is important to recognize the multiple ways by which a nutritional deficiency may arise (**Table 1**). First, a deficiency can occur as a consequence of an inadequate dietary intake of an essential nutrient. An insufficient amount of any essential nutrient in the diet will eventually result in a primary deficiency of the nutrient, with concomitant adverse effects on reproductive capacity of the animal. A secondary, or conditioned, deficiency may occur even if the dietary content of the essential nutrient appears to be adequate (16). Conditioned deficiencies can arise through several mechanisms. Genetic factors may create a higher than normal requirement for a nutrient. For example, individuals with acrodermatitis enteropathica require a large amount of zinc in their diet because of a genetic defect in zinc absorption (17) whereas individuals with Menkes disease suffer from copper deficiency resulting from defects in the intracellular trafficking of this element (18,19). Similar to deficiencies of essential metals, vitamin deficiencies can arise as a consequence of genetic abnormalities. For example, gene polymorphisms associated with folate metabolism may increase the risk for neural tube defects (20). Strain differences resulting from subtle variations in numerous genes can influence an animal's response to specific nutritional deficiencies. For example, the breed of a sheep can affect its susceptibility to the teratogenic effects of copper deficiency (18). Currently there is a paucity of information on the extent to which multiple genes contribute to altered nutritional status in the human population.

TABLE 1

Causative factors in micronutrient deficiencies

Primary deficiency: low dietary intake of a micronutrient
Secondary (conditioned) deficiency
Genetic factors
Mutant genes (e.g., acrodermatitis enteropathica and Menkes disease)
Polymorphisms (e.g., mutations in the methylenetetrahydrofolate reductase gene)
Multiple gene defects
Nutritional interactions
Dietary binding factors (e.g., fiber and phytate)
Micronutrient-micronutrient interactions (e.g., zinc-copper, iron-manganese, cadmium-zinc and zinc-vitamin A interactions)
Physiological stressors
Disease-associated changes in micronutrient metabolism (e.g., diabetes and hypertension-induced changes in mineral metabolism)
Drugs or other chemicals and toxicants
Antimetabolites (e.g., dicumarol)
Metal chelation (e.g., decreased absorption and increased excretion)
Decreased gut absorption and/or increased kidney loss (secondary to tissue damage)
Toxicant-induced changes in tissue pools (secondary to inflammatory or acute phase response)

Second, nutritional interactions can result in conditioned deficiencies. For example, dietary-binding factors, such as fiber and phytate, can form a complex in the gut with essential minerals that limit their absorption. Zinc deficiency syndromes occur in individuals who consume phytate-rich diets (16,21). Mineral-mineral interactions can occur in a variety of ways. If one mineral is involved in the metabolism of another, a deficit of one may influence the other; alterations in iron metabolism are often observed in individuals with copper deficiency (22,23). Another interaction occurs when elements share a common transport site or transport ligand. This often happens when ions have a similar orbital size and coordination number (**Table 2**). Thus, because of their similar physicochemical properties, it can be predicted that zinc and copper will interact, as will iron and manganese. In theory, these interactions can occur at multiple sites, including the gut, within the blood pool and at the placenta. Metal-metal interactions can significantly

TABLE 2

Chemical determinants of potential mineral-mineral interactions

Ion	Orbital	Coordination number	Group
Cu^{+}	d^{10}	4	Ib
Cu^{2+}	d^{9}	4	Ib
Zn^{2+}	d^{10}	4	IIb
Fe^{2+}	d^{6}	6	VIII
Fe^{3+}	d^{5}	6	VIII
Mn^{2+}	d^{5}	6	VIIa
Cd^{2+}	d^{10}	4	IIb

influence the transport of metals into the developing conceptus. Importantly, metal-metal interactions can occur between essential and nonessential metals. For example, exposure to high levels of cadmium can result in a secondary zinc deficiency, which can be teratogenic (16).

A third mechanism by which a conditioned deficiency can arise is through an effect of drugs or chemicals on the metabolism of the nutrient. Broadly speaking, drug-nutrient interactions can be separated into two categories: *1*) drugs that act via the direct chelation of a nutrient, such as the chelation of copper by D-penicillamine and of zinc by ethylenediaminetetraacetate (EDTA), both of which can result in fetal mineral deficiencies (16), and *2*) drugs or chemical nutrients that influence the metabolism of a nutrient, for example, a decreased absorption of some nutrients results from drug-induced reductions in gastrointestinal transit time. Similarly, diuretics increase the urinary loss of some nutrients. The metabolism of other nutrients may also be influenced by the occurrence of an acute phase response. The acute phase response can be triggered by a wide variety of factors, including diverse drugs and toxicants, physical stimuli and several disease conditions. The increased turnover of folic and ascorbic acid, which occurs via oxidative damage secondary to smoking, is an example of how one toxicant can induce essential nutrient deficiencies (24,25).

A fourth mechanism by which a conditioned deficiency can arise is through stressor-induced physiological changes in micronutrient metabolism. For example, diabetes and hypertension alter the metabolism of several minerals, including zinc and copper (16,18), possibly because of the induction by stress of an acute phase response.

Influence of maternal diet on pregnancy outcome

Maternal diet can have a significant effect on pregnancy outcome. On the basis of an early analysis of data on the effects of wartime starvation in Holland on pregnancy outcome, Smith (26) reported that severe overall undernutrition resulted in an increased risk for infertility, intrauterine growth retardation and abortion. Stein et al. (27) extended Smith's finding by noting that prenatal exposure to famine during early gestation was associated with an increased risk of central nervous system abnormalities and increased perinatal mortality. By contrast, exposure to famine during late gestation primarily resulted in IUGR. Individuals born during the Dutch hunger crisis were reported to be characterized by an increased risk for antisocial behaviors when the exposure occurred during the first and second trimester (28). In addition, individuals born during the crisis were reported to have an increased risk for cardiovascular disease (29). The above illustrates the fact that prenatal malnutrition may result in biochemical abnormalities that persist into adulthood.

Maternal nutrition can affect pregnancy outcome even when energy intake is adequate (**Table 3**). In an early study by Ebbs et al. (30), the consumption by women of a poor diet (defined as a diet low in protein, calcium, fruits and vegetables) was correlated with a high incidence of miscarriages, stillbirths and early neonatal mortality compared with the consumption of a good diet. Importantly, a subset of women in the poor diet group given food supplements had a reduced frequency of pregnancy complications. This finding is critical because it suggests that the poor pregnancy outcome observed in the poor diet group was primarily due to nutrition per se rather than other lifestyle factors. Similar observations of an association between poor diets and poor pregnancy outcome have been made by others (31–39). Velie et al. (38) suggested that the low zinc content of the suboptimal diets posed a significant reproductive risk. Numerous investigators have reported that the use of vitamin-mineral supplements during the periconceptional period can result in reductions in the predicted frequencies of birth defects (40–42).

TABLE 3

*Studies reporting an association between the consumption of a poor diet and an increased risk for pregnancy complications**

Investigator	Type of study
Ebbs et al., 1941 (30)	Intervention
Burke et al., 1943 (31)	Observational
Jeans et al., 1955 (32)	Observational
Primrose and Higgins, 1971 (33)	Intervention
Laurence et al., 1983 (34)	Intervention
Friel et al., 1995 (35)	Observational
Wright et al., 1995 (36)	Observational
Torfs et al., 1998 (37)	Observational
Velie et al., 1999 (38)	Observational
DiCintio et al., 1999 (39)	Observational

* Poor diets were typically classified as low in protein, dairy products and fresh fruits and vegetables. Intervention involved the provision of food, vitamin supplements or both.

Several micronutrient deficiencies have been postulated to result in human congenital disorders (**Table 4**), thus it is unlikely that the difference between good and poor diets with respect to pregnancy outcome can be attributed to a single nutrient. Although collectively the above studies support the use of periconceptional vitamin and mineral supplements for the reduction of pregnancy complications, controversy still exists over this issue. A common argument against advocating the use of nutrient supplements or enhanced food fortification programs for the general public is the paucity of studies implicating a maternal deficiency of a specific nutrient in the induction of specific developmental defects. This is an important issue because excessive intakes of essential nutrients during pregnancy can also pose reproductive risks (1). Thus, the potential overuse of supplements might outweigh their benefits.

In contrast to the concept that poor maternal diet represents the sole insult in studies such as those referred to in Table 3, an alternative possibility is that a compromised nutritional status of an individual increases the risk of adverse reactions to other potential reproductive hazards (43). For example, the teratogenicity of many compounds is increased in animals fed marginal protein diets (43), and the teratogenic effects of acetazolamide and methanol are exacerbated by zinc and folate deficiencies (43,44).

Given the above, it is evident that estimates of the nutritional status of women of childbearing potential may be

TABLE 4

Micronutrient deficiencies that have been postulated to contribute to abnormal human prenatal development

Vitamin A*	Copper
Vitamin B-6	Iodine*
Vitamin B-12	Iron*
Vitamin D*	Magnesium
Vitamin K	Zinc
Folate	

* Excessive dietary intakes of these micronutrients during pregnancy have been associated with an increased risk for pregnancy complications.

in considerable error when the estimates are based on a simple inspection of dietary intakes. An individual's nutritional status is influenced by numerous variables including genetics, environment, lifestyle habits, the presence of disease or physiological stressors and drug-toxicant exposures. We suggest that the risk for secondary micronutrient deficiencies is even higher than that for primary deficiencies in many population groups. Below, we will use zinc as an example of a nutrient that may often be suboptimal for the developing conceptus because of either primary or secondary deficiencies.

Essential micronutrient deficiencies and pregnancy

Zinc. There are several reasons for using zinc as a nutrient to illustrate the plausibility of the hypothesis that micronutrient deficiencies are a common cause of abnormal development. A significant proportion of the world's population consumes diets that are low in zinc (21). Although the consumption of a diet low in zinc does not, by definition, translate into a deficiency, it does suggest that a significant proportion of the population is at risk of deficiency.

Severe zinc deficiency during pregnancy is teratogenic. Typical malformations associated with severe zinc deficiency in animal models include cleft lip and palate; brain and eye malformations; and numerous abnormalities of the heart, lung and urogenital systems. Fetuses from zinc-deficient dams also show growth retardation and a high frequency of skeletal abnormalities. Biochemical and functional abnormalities can occur as a result of a zinc deficit. Importantly, even transitory periods of zinc deficiency (5–6 d) can be teratogenic in rodent models (16), and 3 d of periconceptional zinc deficiency can adversely affect embryonic development (45).

Three lines of evidence support the concept that zinc deficiency is a teratogenic risk in humans (**Table 5**). First, women with acrodermatitis enteropathica tend to have complicated pregnancies unless they are given zinc supplements. Second, low plasma zinc concentrations in the first and third trimesters of pregnancy were correlated with an increased risk for malformations and low birth weight, respectively. Third, in several prospective supplementation trials, the use of zinc supplements was associated with increased birth weights and reductions in pregnancy complications. Although the above argues for the concept that zinc deficiency may be a common contributor to pregnancy complications, zinc supplementation has not affected the frequency of pregnancy complications in several trials (60–62). We suggest that one reason for the difference in the results on the efficacy of zinc supplements on pregnancy outcome may be the failure to accurately define the frequency of primary and secondary zinc deficiencies in the populations being studied.

TABLE 5

Evidence that zinc deficiency is teratogenic in humans

Observation	Investigators
High incidence of pregnancy complications in acrodermatitis enteropathica	Hambridge et al., 1975 (46)
Low maternal hair or plasma zinc is correlated with pregnancy complications	Jameson, 1976 (47) Meadows et al., 1981 (48) Simmer and Thompson, 1985 (49) Cavdar et al., 1988 (50) Neggers et al., 1991 (51) Srinivas et al., 2001 (52)
Zinc supplementation and a reduced risk for pregnancy complications	Kynast and Saling, 1986 (53) Cherry et al., 1989 (54) Cavdar et al., 1991 (55) Simmer et al., 1991 (56) Goldenberg et al., 1995 (57) Osendarp et al., 2001 (58) Christian et al., 2001 (59)

Zinc deficiency is thought to influence embryonic and fetal development through various mechanisms, including reduced cell proliferation, reduced protein synthesis, reductions in rates of tubulin polymerization, increased rates of cellular oxidative damage, increased rates of apoptosis and reduced binding of hormones and transcription factors dependent on zinc-finger regions (16,63–65). Illustrative of the above, recent studies with mice show that the zinc-finger transcription factors GATA–6, GATA–4 and Osx are key regulators of lung, heart and bone development, respectively, and that the loss of function of these zinc-finger proteins results in phenotypes similar to those observed with severe zinc deficiency (66–69). As noted, zinc deficiency can affect development within a very short time (4–6 d for rodents) (16,63). This rapid effect is due partly to the fact that maternal plasma zinc concentrations are not under tight homeostasis. Indeed, in rodents the concentration of zinc in plasma can drop by >50% in less than 24 h after the introduction of a zinc-deficient diet (16). The rapid effects on the developing conceptus argues for a lack of substantial zinc stores in the embryo and fetus.

Although the above provides evidence for the hypothesis that maternal zinc status can be an important predictor for pregnancy outcome, this idea has been challenged because it has been difficult to correlate maternal dietary zinc intake to pregnancy outcome in well-nourished populations (60). In the absence of this link, it could be argued that the association between low plasma zinc and poor pregnancy outcome could be spurious if the low zinc concentrations are secondary rather than primary to embryopathy. However, a secondary zinc deficiency and low maternal plasma zinc concentrations can arise through a variety of mechanisms including poor availability of dietary zinc, genetic defects in zinc metabolism (or polymorphisms that increase an individual's need for this nutrient), physiological stress, disease or drug- or toxicant-induced changes in zinc metabolism. That hypozincemia can be a complication of numerous disease states, including diabetes, hypertension, AIDS and alcoholism, is well established (70–74). In the case of diabetes and alcoholism, hypozincemia has been postulated to contribute to the increased risk for pregnancy complications associated with these diseases (75–77).

Zinc deficiency may also be induced during pregnancy as a result of the exposure of the mother to select drugs or chemicals. As discussed, drug-mineral interactions can be separated into two broad categories. In the first, the drug and the trace element interact directly; in the second, the drug indirectly affects the metabolism of the mineral. Numerous compounds can chelate zinc, including EDTA, penicillamine, triethylenetetramine and acetazolamide. The developmental toxicity of each of these compounds has been linked partly to the ability to induce secondary fetal zinc deficiencies (16). The second category of zinc-toxicant interactions is more difficult to predict because it is not based on the ability of the compound to bind the element. A toxicant can indirectly influence zinc metabolism by at least three mechanisms: *1*) by reducing its absorption secondary to gut damage, *2*) by increasing its excretion secondary to kidney damage or a general diuresis, and *3*) by affecting a change in its metabolism secondary to tissue damage. For the third mechanism, induction of an acute phase response may contribute to the developmental toxicity of a wide variety of agents (**Fig. 1**). According to this hypothesis,

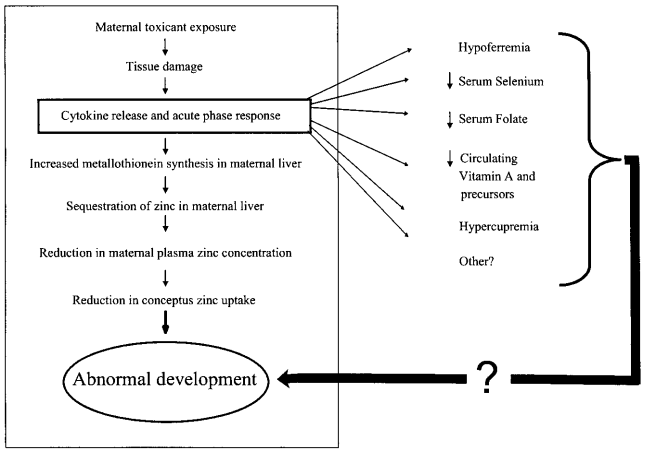

FIGURE 1 Potential effects of the acute phase response on nutrient transfer to the conceptus.

toxicants, which induce maternal tissue damage, are also associated with transitory increases in the production of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin-1 and interleukin-6. Cytokines such as TNF-α can trigger numerous metabolic changes, including fever, shock, hypotension and tissue injury. The intertwined set of biochemical and physiological responses that characterize the above is typically referred to as the acute phase response (78).

Although the term acute phase response accurately reflects the rapid occurrence of a large set of metabolic and physiological changes, these changes can persist from weeks to months (chronic acute phase response) (79). One effect of the TNF-α is a marked stimulation of the synthesis of the acute phase protein metallothionein. Metallothionein is a low-molecular-weight cysteine-rich protein that can avidly bind zinc and other divalent metals. Putative functions of this protein include protection against metal toxicity and short-term storage of zinc and copper and the scavenging of hydroxyl radicals (80–82). One consequence of cytokine-induced increases in liver metallothionein is hypozincemia. In a series of studies, the administration of diverse developmental toxicants, including 6–mercaptopurine, valproic acid, urethane, α-hederin, ethanol and 2–ethylhexanoic acid, resulted in marked elevations in maternal liver metallothionein concentrations. When these toxicants were given during midgestation, the increase in metallothionein resulted in a sequestration of circulating zinc in the maternal liver and a reduction in zinc transport to the conceptus (76,83–87).

The influence of maternal dietary zinc intake on the reproductive toxicity of metallothionein-inducing agents was evaluated to determine whether the toxicant-induced reductions in zinc transport to the conceptus are functionally significant. In a series of studies, pregnant rats or mice were fed diets that contained marginal, adequate or supplemental levels of zinc. For the insults studied to date (TNF-α, 6-mercaptopurine, urethane and 2-ethylhexanoic acid), each agent's developmental toxicity was shown to be inversely related to maternal dietary zinc intake (81,83–86).

Studies conducted using postimplantation rat embryo culture methods support the whole animal work. Control embryos were cultured for 48 h in serum obtained from male rats treated with TNF-α or α-hederin, toxicants that induce hypozincemia. Embryos grown under these conditions are characterized by a high frequency of abnormalities, but when the sera are supplemented with zinc their developmental toxicity is markedly reduced (81,84). These findings support the concept that toxicant-induced reductions in maternal plasma zinc concentrations represent a developmental threat. In further support of this concept, Carey et al. (76) reported that in metallothionein-null mice, the teratogenicity of alcohol (an acute phase inducer) is markedly reduced. Carey et al. suggested that the reduction in the teratogenicity of alcohol was because maternal hypozincemia did not occur in the alcohol-fed metallothionein-null mice.

The observation that the developmental toxicity of select metallothionein-inducing agents can be significantly influenced by maternal dietary zinc intake illustrates the strong influence that maternal nutritional status can have on the teratogenicity of some toxicants. The marginal and supplemental zinc levels used in this experimental animal work were in the range of what has been reported for human dietary zinc intakes. Pregnant women are often exposed to dosages of agents shown to induce metallothionein and alter zinc metabolism. This list of agents includes the known human teratogens valproic acid, ethanol and 6–mercaptopurine and the suspected human teratogen hyperthermia. Infections also typically induce an acute phase response and hypozincemia in human adults.

Although the above provides a rationale for the use of zinc supplements, it must be appreciated that zinc in excess may pose a reproductive risk. In a brief report on four women who took 300 mg zinc daily during the third trimester of pregnancy, it was stated that three gave birth prematurely and a fourth infant was stillborn; an explanation for this high rate of pregnancy complication was not given (88).

The major consequence associated with the long-term ingestion of moderately high amounts of zinc is the induction of a secondary copper deficiency. For humans the chronic intake of zinc supplements as low as 50 mg/d can result in a marginal copper deficiency, as assessed by reductions in plasma copper concentrations and reductions in the activity of erythrocyte copper-zinc superoxide dismutase (89).

In experimental animals, acute zinc toxicity has not been associated with any reproducible pattern of developmental abnormalities (16). When pregnant rats are fed high concentrations of dietary zinc (>1000 μg Zn/g diet compared with average control diet of 50 μg/g) throughout pregnancy, the primary effect is the induction of secondary maternal and fetal copper deficiencies, which can result in the occurrence of fetal abnormalities consistent with those associated with primary copper deficiency (18). The biological importance of mineral interactions has been recognized by the Institute of Medicine (90). Because zinc supplementation has adverse effects on copper metabolism, the Institute of Medicine recommends that an appropriate copper supplement (2 mg copper) should accompany zinc supplement use (90).

Copper. Acute phase responses are also complicated by perturbations in copper and iron metabolism. Hypercupremia is one hallmark of the acute phase response. The extent to which maternal hypercupremia influences embryonic and fetal copper uptake is not clear. Environmental or physiological conditions that perturb copper metabolism and trigger subclinical copper deficiency include exercise, infection, inflammation, diabetes and hypertension, consumption of zinc supplements and consumption of diets high in fructose (89,91–97). A deficit of copper during pregnancy can result in early embryonic death and gross structural abnormalities including skeletal, pulmonary and cardiovascular defects (18,98–100). Persistent biochemical, neurological and immunological abnormalities also occur (101–103). These abnormalities have been shown to occur in humans and animal models. Drugs such as D-penicillamine, triethylenetetramine and meso–2,3 dimercaptosuccinic acid can produce copper deficiency, either directly by chelation or

indirectly by altering copper metabolism. Importantly, their teratogenicity is modulated by maternal copper status (18, 104,105). 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 (106–109).

We (110) have shown that rodent embryos from copper-deficient dams cultured in vitro in low copper serum are characterized by a high incidence of hindbrain swelling and cardiac and vascular abnormalities. The severity and frequency of these defects can be modulated by changes in the dietary or media copper concentrations. Similar to the situation for zinc, embryonic defects can rapidly occur with copper deficiency, with defects occurring 48 h after exposure to a copper-deficient environment, even when embryos are derived from copper-adequate dams (110).

Two mechanisms that may underlie teratogenicity induced by copper deficiency include alterations in extracellular matrix integrity and high oxidative stress. Embryos cultured in copper-deficient media are characterized by heart abnormalities and blood pooling, indicating compromised vessel integrity (18,110). Transverse sections of copper-deficient embryos show that the anterior cardinal veins are enlarged compared with those of controls (110). The incidence of these cardiac and vascular abnormalities (>25%) is markedly lowered when antioxidants, such as superoxide dismutase and glutathione peroxidase, are added to the culture media (110).

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 die in utero (111,112). Mice that are lacking Atox1 generally die after birth. The mice that survive the weaning period are characterized by congenital malformations, hypopigmentation, hemorrhages and seizures (113). These studies demonstrate the critical role that copper chaperones play in embryonic development. The extent to which acute phase–induced changes in maternal copper metabolism influence the conceptus is an issue that merits further study.

Iron. Another consequence of the acute phase response is hypoferremia (Fig. 1). Iron deficiency is a common single-nutrient deficiency, affecting as much as 30% of the world's population (114). Although maternal iron-deficiency anemia, preterm delivery and low birth rate are strongly related (115), it has been difficult to establish a causal relationship because of limitations in study design (116). However, maternal iron deficiency has been shown to affect cognition, behavior and motor development and activity in animal and human offspring (117–119).

Selenium. Selenium metabolism can also be influenced by the acute phase response, with serum concentrations dropping during the early stages (120). The implications of these reductions in maternal serum selenium for the conceptus are not clear, although low blood selenium concentrations have been correlated with an increased risk for spontaneous abortion (121,122). Given the critical roles that selenium plays in many aspects of the oxidative defense system as well as its role in cell signaling and the regulation of cell growth, this is an area that merits further attention.

Vitamins. The acute phase response can affect vitamin as well as mineral metabolism (Fig. 1). For example, serum retinol and several carotenoids decrease during the acute phase response (123–126). In addition, serum folate has been shown to be low in patients undergoing acute phase response resulting from ischemic cardiovascular disease (127). Whether these alterations in vitamin metabolism result in abnormal fetal outcome is unknown. However, in this regard, Botto et al. (42) recently reported that maternal use of multivitamins is associated with a reduced risk for pregnancy complication in women with early pregnancy febrile illness. Although supplementation of vitamins at the level of the Recommended Dietary Allowances may be appropriate, potential risks accompany excessive dietary supplementation. For example, retinoic acid controls numerous genes responsible for the establishment and patterning of the anterior-posterior body axis, but hypervitaminosis A is teratogenic, resulting in craniofacial, limb, neural tube, heart and urogenital system defects (128).

Epigenetic consequences. One question that is increasingly being asked concerns the potential epigenetic consequences associated with micronutrient deficiencies. The study of epigenetics focuses on the change in heritable gene expression that occurs without changes in the DNA sequence (129,130). Numerous studies have shown that maternal exposure to nutritional insults can have persistent effects on the offspring. For example, feeding a diet with marginal amounts of zinc during pregnancy can result in persistent effects on immune function in the offspring even after zinc repletion (131–133). Infants of monkeys fed low-zinc diets are characterized by increased levels of DNA damage, as assessed by increased hepatic DNA strand break levels and 8–hydroxyl–2′deoxyguanosine concentrations (134). These infants can also be characterized by persistent behavior alterations (135).

Similar to zinc deficiency, marginal iron intakes during early development in mice can result in persistent changes in dopamine metabolism, myelin composition, brain iron concentrations and behavioral disturbances (118,136). Persistent effects of perinatal copper deficiency on the auditory startle response, a neurobehavioral index, have also been reported despite copper repletion (137). Collectively, these data show that maternal nutrition influences the programming of certain fetal genes. It can be argued that developmental micronutrient deficiencies may represent significant contributory factors to the Barker effect, which suggests that the intrauterine and postnatal environment can influence the risk for certain diseases such as diabetes, hypertension and coronary heart disease (138).

Summary and conclusions

Embryonic and fetal development are influenced by a multiplicity of factors including nutrition, genetics, environmental toxicants, physiological stressors and maternal health (**Fig. 2**). That deficits in select essential micronutrients during embryonic and fetal development can result in abnormal development is well established. Data reviewed in this paper show that exposure to select toxicants and environmental stressors during pregnancy can result in marked changes in maternal and conceptus mineral metabolism, which in the case of zinc may contribute to the occurrence of developmental abnormalities. The negative effect of toxicants on maternal and conceptus zinc metabolism can be amplified under conditions of marginal zinc intake whereas they can be attenuated by zinc supplementation. Similar to the situation for zinc, changes induced by acute phase responses in the metabolism of other micronutrients, including iron, copper and certain vitamins, may contribute to the occurrence of developmental defects.

The above has three important implications for public health policy: *1*) secondary as well as primary causes of nutritional deficiencies should be considered in population studies, *2*) potential differences in maternal nutritional status

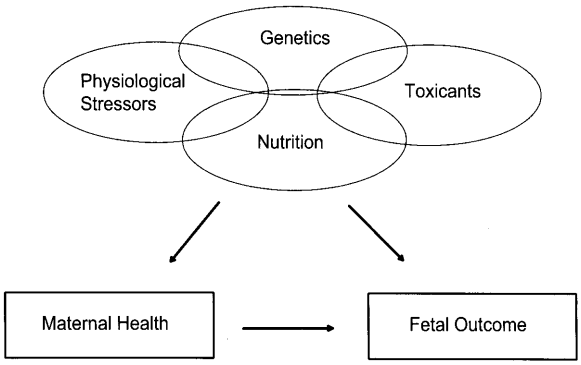

FIGURE 2 Maternal and environmental interactive effects that can influence the developing conceptus.

should be considered in the risk assessment analysis of suspected developmental toxicants, and 3) improvements in the nutritional status of mothers may result in significant reductions in their sensitivity to select reproductive toxicants and insults. Given that improvements can occur through a number of routes, including food fortification programs and provision of supplements to high-risk groups, there is good reason to predict that progress should be made in the future in reducing the frequency of some pregnancy complications. However, several key issues still need to be addressed. For example, at present there is little information concerning what might constitute an optimal micronutrient supplement with respect to which nutrients should be provided and their amount, form and timing.

The evidence that preconception nutrition can influence the early embryo (45,139,140), coupled with the fact that a significant proportion of pregnancy complications occur during the first month of pregnancy argues that fortification and supplementation programs should be aimed at all women of child-bearing potential. However, before the implementation of widespread programs, the risk-benefit ratios of such interventions need to be carefully weighed. Critical to this consideration will be the need for a better understanding of the influence of maternal and conceptus genotype on their susceptibility to the toxicity as well as the deficiency of essential micronutrients. Although it is difficult to predict the extent to which micronutrient supplementation may reduce the overall occurrence of pregnancy complications, the observation of the success that has been achieved with iodine supplementation in the virtual eradication of cretinism in some population groups (141) suggests that the effect of widespread micronutrient supplementation programs will be impressive. The largest effect of micronutrient supplementation on pregnancy outcome will undoubtedly be observed in developing countries, but measurable effects should also occur in developed countries, even in populations characterized by good diets, given that secondary micronutrient deficiencies can arise by multiple means.

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