Zinc Needs During Pregnancy

Noel W. Solomons, M.D.*, **
Deborah L. Helitzer-Allen***
José Villar, M.D., M.P.H.*, ***
Guatemala City, Guatemala, and Baltimore, Maryland

Zinc is an essential nutrient and is involved in many processes affecting cellular growth and development. Dietary requirements for this mineral are increased during pregnancy. Depending on the quality of the diet it may be difficult for a pregnant woman to meet intake needs. Consumption of large amounts of dietary iron may reduce the bioavailability of zinc. The processes of pregnancy itself—plasma volume expansion, placental hormone influences, and fetal uptake—have a profound influence on zinc metabolism. Zinc deficiency can produce severe birth defects in experimental animals. Human zinc deficiency—the condition known as acrodermatitis enteropathica—has been associated with early abortion and various teratologic abnormalities. Zinc status, or the pattern of change in zinc metabolism during pregnancy, has also been associated with the outcome of pregnancy. Amniotic fluid zinc levels are reduced in infections of the fetus or placenta. Whether this is a primary or secondary response has not been clarified for all situations. The clinical and laboratory assessment of zinc nutriture is not well developed for humans, and this is more compelling in the case of human pregnancy. Continued research will be required to determine the true magnitude of inadequate zinc status, its consequences for mother and fetus, and sound and sure public health measures to reduce the impact. (CLIN NUTR 1986;5:63-71)

Zinc is a trace element and an essential nutrient. There are 2 to 3 gm of zinc in the adult body, and it is present in almost all human tissues.[1] It is necessary for normal growth and development. It is a component of more than 400 enzyme systems throughout nature, and more than 70 zinc metalloenzymes have been identified in higher organisms. DNA and RNA polymerases are two such zinc metalloenzymes.[2] Thus it is not surprising that zinc is recognized as essential for cellular growth and development, division, and differentiation. Zinc is also important for the stabilization of membranes,[3] for immune host defenses,[4] and in antioxidant protection. Human requirements for zinc increase during periods of rapid tissue growth, such as wound healing or fetal growth. Fetal requirements for zinc increase rapidly during the first trimester of pregnancy and undergo more than a fiftyfold increase during the second and third trimesters.[5]

From the *Perinatal Nutrition Program, Division of Nutrition and Health, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, C.A.; **The Center for Studies of Sensory Impairment, Aging and Metabolism, Guatemala City, Guatemala, C.A.; and ***Departments of International Health and Maternal and Child Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

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Reprint requests: José Villar, M.D., Perinatal Nutrition Program, Division of Nutrition and Health, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, C.A.

ZINC METABOLISM

Zinc is not efficiently absorbed from the intestinal lumen. Fractional absorption of zinc from food sources and meals ranges from 14% to 41%.[6] Foods high in fiber and phytates reduce the absorption of zinc. Dietary iron in large amounts competes with zinc for absorption.[7] Once absorbed, zinc is transported to the liver on albumin. About 60% of zinc in the peripheral circulation is carried by albumin; the remainder is linked to an α_2-macroglobulin. Endogenous zinc is excreted primarily in pancreatic and intestinal secretions. Only small amounts—500 μg—are excreted by the kidneys. A similar amount can be desquamated from the skin. Up to 1 mg of zinc is lost per liter of sweat.

ZINC REQUIREMENTS DURING PREGNANCY

The Food and Nutrition Board[8] has established 20 mg/day as the recommended dietary allowance (RDA) for zinc during pregnancy. This represents 5 mg more than the RDA for the nonpregnant adult woman. Both recommendations assume the consumption of a typical mixed North American diet by a *healthy* individual. The World Health Organization[9] derived a more intricate estimation of zinc requirements for pregnancy based on a *factorial approach*. They assumed that a daily accretion of zinc in the standard pregnant woman varies with the stage of pregnancy. From 0 to 20 weeks it is 2.55 mg; from 20 to 30 weeks, 2.9 mg; and from 30 to 40 weeks, 3 mg/day. *Absorption* of this amount of zinc from the diet depends on the biologic availability of the mineral. For instance, if the bioavailability of zinc in a diet is 40% in late pregnancy, as little as 7.5 mg of the ingested mineral will satisfy absorption requirements; if the bioavailability is 10% a woman would need to consume 30 mg of zinc daily. Studies in experimental animals suggest that the intestinal efficiency of zinc absorption increases during gestation.[10,11] Observations during human pregnancy have not confirmed this enhanced uptake of zinc.[12]

DIETARY SOURCES OF ZINC

Zinc is widely distributed in a variety of foods. The zinc content of seafood—

Table I. Representative zinc contents of various foods and food groups*

Food	Zinc content (μg/gm)
Oysters	1000
Other shellfish	300
Meats	22
Eggs	15
Cereals and grains	9
Green vegetables	6
Dairy products	5
Fish	5
Roots and tubers	2

*Modified after Hazell T. Minerals and foods: dietary sources, chemical forms, interactions, bioavailability. World Rev Nutr Diet 1985;46:1-123.

especially Atlantic oysters—is high. The zinc content of selected foods is shown in Table I.[18] The zinc content of most municipal drinking water is negligible.[8] Foods rich in protein tend to have higher zinc content. Foods with a high energy density from fat or starch are poor zinc sources. Thus, to meet the increased zinc requirements of pregnancy from diet *alone*, greater intakes of protein-rich foods are needed as the time of delivery approaches.

ZINC AND PREGNANCY

Four issues regarding zinc and pregnancy predominate. The first is the effect of pregnancy on zinc metabolism and on circulating zinc levels. The second is the teratologic effects of zinc deficiency. The third relates to zinc status and other aspects of pregnancy outcome, such as birth weight, gestational age, and birth complications. Finally, the zinc content of amniotic fluid may be relevant to fetal health and outcome of pregnancy. We shall endeavor to provide an updated discussion of these four issues.

Zinc Metabolism During Pregnancy

It has been the common experience of many observers over decades that circulating zinc levels tend to decline over the course of pregnancy. The extent to which this reflects the nutritional status of the individual is problematic, since the use of circulating zinc levels as the sole index of zinc nutriture is not generally acceptable.[11] Among the factors that intercede in determining the pattern of plasma or serum zinc during pregnancy is the expansion of plasma volume associated with pregnancy. Then there are the hormonal influences on zinc levels. Estrogens and progesterone tend to reduce circulating zinc concentrations,[15] and both of these steroid hormones are greatly elevated in pregnancy. Finally, the nutritional transfer of zinc from the maternal to the fetal compartment might produce a shift in zinc distribution, as reflected in the mother's serum zinc levels.

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A significant decrease in circulating zinc levels has been demonstrated in many studies of either a longitudinal or cross-sectional nature.[16-26] In Australian women an average reduction of 16% in zinc levels was observed between the nonpregnant state and the eighteenth week of gestation. This same degree of plasma zinc reduction was seen at the thirty-second week as well.[18] Similar results were found by other investigators. For instance, Hambidge et al.[20] observed a 17% reduction is plasma zinc concentration by the second month of gestation, and the levels continued to fall until delivery. Hunt et al.[19,21] found levels of serum zinc on average to be 63 μg/dl at 18 weeks, with a slight further drop (2 μg/dl) through the thirty-second week. Mukjerjee et al.,[22] in a *cross-sectional* survey of 455 women, found a decrease in zinc levels of about 10 μg/dl from the second to the seventh month of gestation. Breskin et al.,[23] in Seattle, followed women who had normal infants and found a drop from 97 μg/dl at 20 days to 42 μg/dl at 240 days after the last ovulation.

Three recent studies of circulating zinc levels during the pregnancies of a total of 610 women from England, the Indian subcontinent (resident in England), and Hong Kong, published in a single issue of the *British Journal of Obstetrics and Gynecology*,[24-26] contributed new, but largely confirmatory, observations for a description of zinc metabolism during normal pregnancy. Two were longitudinal in design[24,26] and one cross-sectional.[25] In the cross-sectional study of Chinese women in Hong Kong,[25] a 55% fall in mean serum zinc levels from 123 to 68 μg/dl, similar in magnitude to that of the Seattle women,[23] was observed. The 25 women from Newcastle upon Tyne[26] showed an average 35% decline in serum levels at 36 weeks as compared to prepregnancy levels, while in Hindu Asian and European women in Britain, the decrease in circulating zinc from time of enrollment in clinic to 36 weeks of gestation ranged from 10.8% to 11.7%.[24] Interestingly, in this latter report zinc concentrations were consistently higher at each sampling interval for European women as compared to the subjects of Asian extraction ($p < 0.05$), but no differences existed between the strictly vegetarian Hindu women and those who consumed meat in their diets.

In three studies[19,21,23] the effects of *zinc supplementation* on circulating zinc values during pregnancy were examined. In the respective reports the supplemented groups received 20 mg of zinc daily,[19] 15 mg daily,[21] or variable amounts (15 to 25 mg).[23] In none of the studies was any impact on zinc concentration attributable to the level of supplemental zinc, as is true with placebo (or nonsupplemented regimens). Thus it would seem that the aforementioned physiologic factors are dominant determinants of zinc redistribution within the body of the pregnant woman.

The nutritional interpretation of this decline in zinc values is elusive. However, two observations are revealing. It is known from studies in laboratory animals that zinc deprivation reduces circulating serum alkaline phosphatase activity (a zinc metalloenzyme) and raises levels of ribonuclease. Despite no effect on plasma zinc, zinc supplementation increased alkaline phosphatase activity in the serum of the Denver, Colorado, subjects studied by Hambidge et al.[20] In the Los Angeles study of Mexican-American women, Hunt et al.[21] found a significantly greater level

of ribonuclease activity in women who had low levels of serum zinc (< 53 μg/dl) at the end of pregnancy; this effect was independent of the zinc supplementation status. Thus, at certain levels of circulating zinc a risk of physiologic dysfunction, reminiscent of zinc deficiency found in experimental animals, is developed in pregnant women.

Changes in other indirect indices of zinc status—notably hair and urinary zinc levels—have been reported in pregnancy, but their interpretation is subject to the same (or greater) uncertainty as the serum or plasma zinc alterations. In the cross-sectional study in Hong Kong[25] mean hair zinc levels declined from 182 μg/gm in nonpregnant controls (n = 25) to 158 μg/gm in 38 women at delivery. However, the longitudinal follow-up of European and Asian women[24] revealed no differences between hair zinc levels at booking in the clinic and at 36 weeks of gestation. Interesting in this latter study is the fact that urinary zinc excretion was lower in Hindu women throughout pregnancy and the increased renal zinc output after 20 weeks of gestation was of a lesser magnitude in the Asian women. In the subsample of women in whom plasma, urinary, and dietary zinc were measured, a positive correlation was found among all three indices.

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Zinc Deficiency and Birth Defects

The broad background of findings in experimental animals and domestic livestock that zinc deficiency produces congenital malformations has been succinctly reviewed in two recent reviews.[27,28] Obviously the roles of zinc in cellular proliferation and protein synthesis make its inadequacy a likely situation for teratogenesis. Hurley and Swenerton,[29] in 1966, were the first to demonstrate the teratogenic potential of zinc deficiency in mammals. They fed young, adult female rats a diet marginal in zinc (9 ppm) before pregnancy occurred, switched them to a deficient ration (< 1 ppm) after mating, and delivered the pups by cesarean section on day 21 of gestation. Inspection of the placentas revealed a high percentage of fetal resorption, and 98% of the fetuses surviving to term were grossly malformed. In the rat model of zinc deficiency in pregnancy, defects have included abnormalities of the skeletal system (e.g., clubfoot, kyphoscoliosis, fused or missing digits, tail malformations),[29-33] nervous system (e.g., brain, eye, spinal tract),[31,35] pulmonary system (e.g., trachea, lung),[29,36] and urogenital system.[29] Gestational zinc deficiency in sows[37] and ewes[38] also leads to a substantial incidence of congenital defects in the piglets and lambs. Mild (marginal) zinc deficiency has been produced in female rhesus monkeys during gestation, but birth defects of the kind seen in the other mammalian species with more drastic zinc restriction have not been observed.[39]

The critical timing for the imposition of a severe dietary restriction of zinc has been determined in a series of elegant experiments in Hurley's laboratory.[40] If the 1 ppm diet was introduced only from the day of mating to day 10, with an adequate ration given thereafter, 22% of the rat pups were born with malformations; if this period was extended for 2 days the malformation rate rose to 56%; if the zinc-deficient regimen was only imposed from days 6 through 14, 49% of the young had defects. Moreover, as expected, there was a correspondence between the type of malformation and the organogenesis occurring during the period of transient zinc deprivation.

A molecular mechanism for teratogenesis is based on disruption of DNA and RNA synthesis. As Hurley and Baly[27] state:

> "Impaired synthesis of nucleic acids caused by zinc deficiency may thus bring about alterations in the differential rates of growth that are necessary for normal morphogenesis. The resulting asynchrony in histogenesis and organogenesis would then lead to malformations. Such a hypothesis is also consistent with the development of abnormalities in the nervous system of zinc deficient fetuses."

The evidence for this conclusion derives from several quarters. Bell et al.[41] found chromosomal abnormalities in liver cells from fetuses of zinc-deficient males. Also, ^{3}H-thymidine incorporation into DNA was reduced in fetuses from zinc-deficient dams as compared to appropriate zinc-adequate controls, with the greatest relative reduction of cell division in the head region. The activity of thymidine kinase and DNA polymerase (two zinc-dependent enzymes) was lower in embryos of zinc-deficient dams as compared to controls on days 9 through 12 of gestation.[42,43] Thus the rapidly growing and differentiating tissues of the fetus appear to be limited by zinc restriction. However, it must be borne in mind that the degree of zinc deprivation that can be imposed experimentally in laboratory animals is far greater than that which occurs in humans, especially in relation to women healthy enough to conceive in the first place.

Although evidence for teratogenesis produced by a range of *experimental* zinc deficiency states in animals is clear and compelling, its reflection in human pregnancy remains uncertain. Breskin et al.,[23] in Seattle, for instance, found a precipitous drop in serum zinc levels even in the first trimester, with a nadir some 25% below initial levels between 30 and 45 days after ovulation. Referring this to the pattern of organogenesis in the human fetus,[44] they postulated that this low-zinc period corresponds to the time of greatest susceptibility to teratogenesis for human embryos. However, not all groups have found the sudden and dramatic decline in circulating zinc levels that was seen in Seattle. In terms of observational evidence only disparate and fragmentary data can be mobilized to argue for a teratogenesis effect of human zinc deficiency. The problems with the literature on humans accumulated to date derive from the underlying uncertainties regarding the clinical assessment of zinc status in pregnancy and from the inferential nature of much of the epidemiologic data, which at time borders on the anecdotal.

The clearest example of uncontestable zinc depletion in human pregnancy probably is to be found in acrodermatitis enteropathica (AE). This is a disorder of zinc absorption that manifests clinically as a zinc deficiency syndrome. Before the recognition of the role of zinc

in the pathogenesis of AE, it was usually fatal in childhood. However, an occasional woman survived to childbearing age, and in early reports a frequency of teratogenic pregnancy is evident.[45,46] Hambidge et al.[47] noted the striking number of aborted and malformed fetuses that resulted from pregnancies of women with AE under their care in Denver just in the year that the zinc connection in this disorder was recognized. Subsequently, prospective zinc supplementation to two women with treated AE, who consistently maintained adequate circulating zinc levels, resulted in normal births and deliveries.[48]

Eckhert[49] reported the anecdote of a young woman who gave birth to an anophthalmic child. In her history, during the critical first months of pregnancy,[50] she had a week-long period of vomiting and anorexia, followed by a fairly uneventful subsequent course. He argues that the critical deprivation of a zinc supply during the period of ocular formation could have been a factor in the anophthalmia, and he supported his argument with the finding of a segmental decline in zinc along the shaft of maternal hair at the point in time corresponding to early gestation.

Since defects of the neural tube figure prominently in zinc deprivation of laboratory animals,[27] it is of interest that some evidence for an association of nervous system birth defects with human maternal zinc deficiency has been advanced.[50-56] Sever and Emanuel[50] proposed that maternal zinc inadequacy conditioned neural tube defects by pointing to the geographic association between diets low in available zinc and prevalence of this abnormality. The correspondence of low maternal serum zinc levels and infants born with anencephaly was reported from Turkey,[51,52] a nation with several cultural and environmental factors conditioning poor zinc nutriture. Buamah et al.[55] screened 259 pregnant women during the second trimester who had been referred for routine screening by amniocentesis to rule out fetal malformation and chromosomal abnormalities. Two hundred forty-four women had normal infants, and nine produced anencephalic fetuses. The mean zinc concentration in the normal group was 80 μg/dl as compared to 67 μg/dl for the mothers of the children with the neural tube defect. The association of zinc status and spina bifida, another neural tube defect, was evaluated by a group in Frankfort, Germany.[53] They compared the hair zinc levels of 30 unselected healthy mothers and their offspring and 17 mother-child dyads with spina bifida in the infant. Hair zinc content was higher (216 μg/gm versus 182 μg/gm) in the hair of the mothers of affected children, and levels correlated positively with levels in their infants. These investigators speculated that some abnormality of zinc availability or metabolism was present in the mothers of malformed infants. On the other hand, in their prospective analysis of women in Hong Kong, Ghosh et al.[25] were unable to detect any difference in serum or hair zinc for the six mothers of anencephalic newborns or the 14 mothers with offspring with other types of malformations, as compared to the control pattern.

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Fetal alcohol syndrome represents a constellation of anatomic and functional disorders characteristic of newborns of some women with severe ethanol abuse during pregnancy. The zinc-depleting effects of alcohol are well documented, which lead Flynn et al.[54] to question whether altered zinc metabolism is a factor in the pathogenesis. Plasma zinc levels were lower in alcoholic as opposed to nonalcoholic mothers as were fetal-cord zinc concentrations in the offspring of the abusers of ethanol ($p < 0.05$). An increased number of birth defects per infant was associated with lower zinc levels in both maternal and cord blood. However, as pointed out by Kiely[57] no greater number of congenital defects were observed in the newborns of alcoholic mothers (18/25 children affected) as compared to nonalcoholic mothers (14/25 affected). A recent experimental study in rats, however, lends support to the notion of a synergistic influence of ethanol intake and zinc deprivation.[58] More research is needed along these avenues.

Zinc Status and the Outcome of Pregnancy

Other aspects of pregnancy besides the malformation of the fetus are relevant to obstetric and neonatal biology and should be related to zinc status. One such additional feature is the intrauterine growth and birth size of the fetus. In the initial studies of Hurley and Swenerton[29] the average weight of zinc-deprived pups was less than that of controls. This has generally been confirmed by subsequent studies, but in some, the loss of fetuses in utero has been such that the *few* born to the zinc-deficient dams had the same per-individual body mass as the *many* pups born to the well-fed controls.

Also, outcome features such as maternal health (hypertension, edema), the delivery (duration and stress of labor; hemorrhage and tearing), and the offspring (aborted, stillborn, maturity, Apgar score) are also of interest. As with teratology, a series of observations in laboratory animals and livestock bear on the issue of zinc and pregnancy outcome. The article by Apgar[28] provides a good review of the effects of experimental zinc deficiency on pregnancy outcome. Prolonged labor, hemorrhage, and intrapartum death occur in zinc-deficient rat dams.[59-62] There appears to be an inverse relationship between malformations and stressful parturition in zinc-deprived rats studied in Apgar's laboratory,[28] possibly due to the reduced litter size associated with teratologic pregnancies. Ewes on zinc-reduced diets who delivered normal-weight lambs also had more difficult deliveries as compared to controls.[63]

The relationship between circulating zinc and birth weight has been a major focus of investigators. They carried over the concern fostered by animal experiments in which low maternal zinc status was associated with maternal growth failure. Several human studies tend to confirm this relationship. A significant correlation between birth weight and maternal zinc levels was reported by Jameson[64] and Crosby et al.[65] However, the associations were not overwhelming in their strength. Another pattern advanced by Metcoff et

al.[66] in Oklahoma and McMichael et al.[67,68] in Adelaide, Australia, was the association of a high midpregnancy circulating zinc level with small babies and, conversely, of a lower midpregnancy zinc concentration in the mother with a normal-weight offspring. The explanation for this phenomenon is the maternal-to-fetal transfer of the circulating zinc. The interpretations of this phenomenon have varied[67] in terms of whether this movement of zinc from the maternal to the fetal compartment represents "push" or "pull." The former suggests that those mothers *capable of* mobilizing their zinc reserves to the benefit of their fetuses will have larger babies; in this explanation the lowering of zinc is seen to influence birth size. The latter suggests that fetuses that are growing vigorously in utero will *demand* more of the maternal zinc to sustain this normal growth; in this explanation the lowering of zinc is a consequence of fetal health. Observations, to date, do not allow one to judge whether the availability of zinc in the mother can be limiting to fetal growth in otherwise healthy pregnant women, but they underscore an apparently clear example of an additional physiologic change in pregnancy related to zinc metabolism.

Intriguing findings from London relate to zinc content in white blood cells.[69,71] In the first study, samples of leukocytes were taken from 17 mothers of preterm infants, 44 mothers of small-for-gestational-age (intrauterine growth-retarded or IUGR) infants, and 150 mothers of normal term infants.[69] The white cell zinc content of mothers of offspring with IUGR (43.0 ng/mg) was significantly lower than that in the control group (52.3 ng/mg). Mothers of preterm infants had a mean level of 49 ng/mg, indistinguishable from controls. In a subsequent study[70] from the same laboratory, cord blood was collected from newborns of 110 mothers and the fetal leukocyte zinc levels were determined. Levels for normal births, which numbered 63, averaged 54.2 ng/mg, and preterm infants (n 20) had a mean level of 53.9 ng/mg. By contrast, however, the 19 children who were born small for gestational age with the growth failure of recent origin ("acute" IUGR) showed a mean leukocytic zinc content of 50.7 ng/mg, significantly less than the control values ($p < 0.05$), and the eight infants with "chronic" IUGR had a mean zinc content of 42.4 ng/mg in the white cells of their umbilical cords ($p < 0.01$). As in the previous study[69] the *maternal* leukocyte levels showed a similar and parallel association, with mothers from both groups of IUGR babies having significantly lower leukocyte zinc levels in their peripheral white cells. Strongly supporting these findings of Meadows' group are the results obtained in the same laboratory in London. Simmer and Thompson[71] measured plasma, red cell, polymorphonuclear leukocyte, and mononuclear leukocyte zinc levels in 79 mothers within 24 to 48 hours post partum. Forty-three of the women had normal-size babies and 36 had small-for-dates newborns (IUGR). Levels of plasma and red cell zinc did not correlate with the birth weights of the infants, but the levels for both types of white cells were significantly lower ($p < 0.001$) for the mothers with small-for-gestational-age infants.

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On the other hand, a number of investigators have found less than clear-cut relationships between indicators of zinc status and birth weight, or no relationship at all. Campbell-Brown et al.,[24] in their study of Asian and European women, found no association between crude or adjusted birth weight and hair zinc, or serum zinc, or urinary zinc output. Tuttle et al.,[71a] in Aberdeen, Scotland, studied 33 healthy primigravid women and 31 primigravidas with a high risk of giving birth to a low-birth-weight child. No differences in plasma zinc *concentrations* at 30 and 35 weeks of gestation were found, but plasma volumes were considerably lower (by 600 ml per individual, on average) in the high-risk group of "mothers to be" ($p < 0.001$). Thus, when the mass of zinc in the intravascular compartment was computed for the two groups, there was 12% to 20% less circulating zinc in the high-risk women ($p < 0.001$). However, no causal relationship of zinc mass influencing the process of IUGR in the small babies of the high-risk mothers was imputed by the authors. Finally, the cross-sectional survey of pregnant women in Hong Kong[25] found a confusing constellation of a positive correlation of infant birth weight with serum zinc and a negative correlation with hair zinc levels. This points to zinc *redistribution* as a potential factor, but a clear interpretation of these findings in nutritional terms is elusive.

Researchers have also tried to focus on the other outcome variables of pregnancy besides birth weight and to relate these to indices of zinc status. Although fetal resorption was clearly a feature of experimental zinc deprivation, no association between hair or serum zinc levels and miscarriages was found by Ghosh et al.[25] in Hong Kong. On the other hand, of the 25 women who aborted in the Seattle study of Breskin et al.,[21] seven had zinc levels outside the confidence limits of the groups as a whole.

Studies in New Orleans[72] and in Los Angeles[21] bear on the issue of whether or not zinc status is related to hypertension and the risk of toxemia of pregnancy. Cherry et al.[72] studied 272 adolescent pregnant women at the Charity Hospital. Forty-nine (19%) had hypertension/toxemia as a complication. The average mean zinc levels were 54 μg/dl in the women with this condition and 59 μg/dl in nontoxemic subjects ($p < 0.009$). Hunt et al.,[21] studying Mexican-American women, found that zinc supplementation (20 mg/day) reduced the incidence of pregnancy-induced hypertension. The interpretation of this preventive effect is difficult, since the initial assignment of treatment or placebo was not based on any selection for "low" zinc status.

McMichael et al,[67,68] studied 878 pregnant women in South Australia who registered in their hospitals during calendar year 1979. The 18-week (midpregnancy) zinc levels for mothers of small-for-dates babies, stillborn infants, and neonates with congential anomalies were not different from those of the group as a whole.[68] The seven mothers who had excessive intrapartum hemorrhage, however, had a mean serum zinc level of 67 μg/dl, significantly below that of the whole cohort

($p < 0.05$). The greatest risk of having some complication or abnormality at delivery was observed in those Australian women who (1) had an initial (18-week) zinc level below the mean of the group *and* (2) showed a *further* relative decline in zinc at the second (32-week) examination.

Mukherjee et al.,[22] in their cross-sectional study of women in Ohio, found no difference between the upper and lower quartiles of plasma zinc concentration distribution with respect to pregnancy complications. They examined this relationship for maternal infection, hemorrhage, fetal distress, prematurity, stillbirth, abortion, maternal tear of the birth canal, and toxemia. However, the group of mothers with infants having the lowest 1-minute Apgar scores did have significantly lower preterm zinc concentrations than mothers with more animate newborns. A similar association between low maternal zinc levels during gestation and poor Apgar scores was also documented in the adolescent mothers of Cherry et al.[72]

Jameson[14,73] conducted studies in selected pregnant Swedish women. If woman had serum zinc levels above 65 μg/dl on entry into the study, they were assigned to the normal-zinc group (n = 179). The 133 women with zinc levels below 65 μg/dl were randomly assigned to a no-treatment group (n = 69, low-zinc controls) or to receive 90 mg of zinc daily (n = 64, zinc-supplemented low-zinc group). Fifty-four percent of the normal-zinc controls had a normal birth and delivery as compared to 62% of the zinc-supplemented women and 48% of the low-zinc women who received no supplements. For postmaturity, prolonged labor, and cesarean section rates—but not for gestation exceeding 42 weeks—a tendency toward a better performance in the zinc-supplemented women, as compared to the untreated women with low initial zinc levels, was observed. Unfortunately, formal comparative statistical tests were not applied to these data.

The Zinc-iron Interaction in Pregnancy

Given the controversy over pregnancy outcome and the patterns of alteration of circulating zinc concentration reviewed herein, it is not surprising that a modifier of zinc levels might be linked to the issues previously raised. Such is the case with oral iron. It is obvious that vigorous supplementation of pregnant women with iron pills is a common practice. Evidence from many quarters—including observations in pregnant subjects themselves—suggests that excessive oral iron intake during pregnancy has negative implications for the biological availability of dietary zinc.

The biologic bases for this concern were elegantly laid out by Hill and Matrone,[71] who provided the theoretic and empiric bases for a competitive biologic interaction between transition elements of similar chemical configuration. A competitive interaction of iron and zinc, and evidence for a common intestinal pathway for the lumen-to-bloodstream movement of these two metals was suggested by studies in experimental animals.[76-78] Then, in healthy, nonpregnant human subjects undergoing a zinc tolerance test,* Solomons et al.[79,80] demonstrated that the simultaneous presence of inorganic, ferrous iron interfered with the uptake of zinc into plasma. Interestingly, *heme* iron (the form of iron found in muscle and blood in dietary meats such as beef or pork) did not interfere with zinc uptake.[79] In a subsequent study of explicit relevance to pregnancy, Meadows et al.,[81] in London, showed that *pretreatment* of normal subjects for 14 consecutive days with 100 mg of iron as an iron- and folate-containing prenatal supplement reduced the uptake of zinc even when no iron was administered during the test. This "action at a distance" suggests some effect of iron in "saturating" the channels for the admission of zinc through the intestinal membrane.

Observations in pregnant women in several recent series tend to suggest a true biologic relevance of the amount of oral iron consumed from supplements for the pattern of zinc metabolism during pregnancy. Two strategies have been used to determine whether oral iron exerts an independent effect on circulating zinc. The first, employed by Breskin et al.[23] in Seattle, in their study of 37 diabetic women and 69 control women, was to compare levels of zinc for women with higher or lower daily intakes of iron. Irrespective of the amount of *zinc* taken by the subject, if her daily iron intake was from 30 to 60 mg, zinc levels were lower than in women receiving a lesser amount of iron. The findings of Sheldon et al.,[26] in their longitudinal study of 25 women from early pregnancy to 12 weeks post partum—15 women without iron supplementation and 10 women receiving 480 mg of ferrous fumarate daily (160 mg of iron)—conducted in England, failed to confirm an iron-related effect on plasma zinc. No differences in zinc concentrations were found at any interval. When analyzed in a similar way the data for plasma zinc in 91 Hindu Asian women and 51 European women were not different between women who reported having taken iron supplements for at least 3 weeks before examination and those whose duration of supplementation was short or who had taken no oral iron at all (average amounts of iron per group not reported).[24] However, these authors noted that three specific women reported an intake in the prior 3 weeks of more than 100 mg of iron daily. Their mean plasma zinc level, 46 μg/dl, was well below the overall group mean of 56 μg/dl. This finding is in striking agreement with the findings in Denver of Hambidge et al.[20] They had 45 women who took some prenatal iron up to the third trimester. Their range of daily iron intake was 40 to 260 mg. A clear, inverse association was demonstrable between the amount of iron in the regimen and their zinc levels in late pregnancy. This effect was also observed in the first- and second-trimester examinations.*

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*A zinc tolerance test is a procedure in which from 5 to 50 mg of zinc as a zinc salt is given orally in aqueous solution and the serial increments in circulating zinc concentration are determined at regular intervals over the subsequent 4 to 7 hours.

*Hambidges KM. Unpublished personal communication.

In summary, animal data and human physiologic experiments point to a competitive interaction between zinc and iron at the level of intestinal uptake. In the one human study of pregnant women in which a *fixed* dose of iron was assigned by random allocation,[21] no iron effect was observed. In each of the other pregnancy studies,[20,23,24] however, in which iron dosages were not manipulated by the investigators, and in which a wide range of iron intake existed across the population, an effect of level of oral iron intake on circulating zinc was seen, accentuated toward the upper end of the iron-intake distribution. If the absolute circulating level of zinc at any point in human gestation truly governs outcome, high daily intakes of oral iron from prenatal supplements stand a more than even chance of being an aggravating factor for lowering zinc concentrations. More research into the threshold dosages for significant zinc depression is needed, as is a clarification (see previous discussion) of the specific risks conveyed by alterations of zinc metabolism beyond the usual limits for pregnancy.

Zinc in Amniotic Fluid

Amniotic fluid contains zinc. Its exact state of protein binding has been widely discussed.[84] A number of studies on the concentration of zinc in amniotic fluid from human pregnancies, specifically in pathologic states, have been reported.[85-92] Normally, zinc levels in amniotic fluid are less than 2 μg/dl before 12 weeks of pregnancy. They rise to the 5 to 25 μg/dl range from 12 to 20 weeks and exceed these levels in late pregnancy.[26,86,87] Amnoitic fluid levels are decreased with intrauterine infections. Whether this is a phenomenon of redistribution of zinc in the face of infection—a situation analogous to the fall in serum zinc levels during the acute-phase response—or whether low zinc concentrations in amniotic fluid condition an increased risk of infection in utero has been debated without resolution. Intrauterine growth retardation, diabetes, toxemia, and prolonged gestation have also been associated with decreased amniotic fluid zinc levels.[84,86,90] A number of investigators have found that pregnancies at high fetal risk are associated with *increased* concentrations of amniotic fluid zinc.[85,86,92] However, in a recent prospective study of more than 200 pregnancies—both normal and pathologic—amniotic fluid zinc levels did not prove to be predictive of pregnancy outcome.[91]

CONCLUSIONS

It is clear that the physiologic changes of pregnancy exercise an effect on the metabolism of zinc. It is also clear that zinc is so essential a nutrient to various functions of the mammalian organism that its deficiency during pregnancy would be expected to have assorted consequences for the mother, the fetus, and the delivery. Elegant animal experiments demonstrate the aforementioned relationships, but extreme caution is warranted in using these experimental findings to model the hypotheses about zinc in *human* pregnancy: caution because of the severe degree of deficiency imposed in the laboratory (quite different from the human dietary situation) and because primary animal models have been rats and mice with multiple (litter) births, rather than the singlet offspring of humans. Pigs, sheep, and monkeys—species in which only a limited exploration of the topic has been undertaken to date—may ultimately serve as better models than rodents for women's pregnancies.

One encounters an array of observations relating human gestational zinc status to teratology or pregnancy outcome. These observations, however, are characterized by great variability in sample size, in design (cross-sectional versus longitudinal; duration of gestation covered by observations) and in data analysis strategies (highest quartile versus lowest quartile; at-risk subjects versus controls; "normal" zinc status versus "inadequate" zinc status; regressional analyses of zinc indices on outcome variables; selected outcomes of the extremes of the distribution, etc.). It often appears that the analysis is chosen a posteriori to see which findings would reach an acceptable level of statistical significance. Such an approach is justified at this early stage in our understanding of human pregnancy and zinc to *generate hypotheses;* deriving biologic conclusions from the present data, however, should be undertaken with the utmost caution. Needed now is the testing in prospective studies with impeccable epidemiologic design, of the hypotheses generated about zinc-pregnancy relationships. How the paradigm of zinc supplementation can be used in advancing our knowledge is problematic. Random supplementation of the whole group or of only the selected "low-zinc status" group has been variously employed in the past. Given the uncertainties about the interpretation of clinical indices of zinc status, both approaches have their strengths and weaknesses in providing interpretable results. Perhaps carefully planned intergestational and intergenerational studies in human populations will allow us to further our understanding of the relevance of the variability of zinc nutriture within pregnant women for the outcome of their pregnancies and the health of their offspring.

Evidence that oral iron exerts an effect on zinc bioavailability is strong and is reflected in a number of studies of iron-supplemented pregnant women. The practical application of this finding, however, is not yet clear. Moreover, present data do not support routine zinc supplementation during human pregnancy. With the rapid emergence of new data in this field, however, practitioners must continue to follow the literature for results in ongoing studies that should improve our ability to make policy decisions in the area of prenatal diet and health in relation to zinc intake.

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