The Need for Maternal Zinc Supplementation in Developing Countries: An Unresolved Issue 1–3

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on behalf of the Maternal Zinc Supplementation Study Group

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ABSTRACT Maternal zinc deficiency during pregnancy has been related to adverse pregnancy outcomes. Most studies in which pregnant women have been supplemented with zinc to examine effects on pregnancy outcome have been carried out in industrialized countries and the results have been inconclusive. This review discusses preliminary findings of eight randomized, controlled intervention trials performed recently in less-developed countries. It is based on evidence presented by investigators and discussed during a workshop held in Wageningen, The Netherlands in June 2001. Preliminary findings from these studies indicate maternal zinc supplementation has a beneficial effect on neonatal immune status, early neonatal morbidity and infant infections. With respect to labor and delivery complications, gestational age at birth, maternal zinc status and health and fetal neurobehavioral development, evidence is conflicting and more research is required. Data currently available do not support the hypothesis that maternal zinc supplementation promotes intrauterine growth. Thus despite the emerging evidence for a positive effect of zinc on some outcomes of pregnancy, the workshop concluded that the full results of studies carried out need to be known and that more research is required to determine the benefits of large-scale introduction of zinc supplementation of pregnant women in less-developed countries. J. Nutr. 133: 817S–827S, 2003.

KEY WORDS: • zinc • supplementation • pregnancy • developing countries • birth weight

Zinc has been known as an essential trace element for humans and animals since the 1930s (1). In a study carried out in the 1960s in Iran, Prasad et al. (2) identified zinc deficiency as an underlying cause of stunting and delayed sexual maturation in humans. More recently, moderate zinc deficiency in infants and children has been found to be associated not only with reduced growth and development, but also with impaired immunity and increased morbidity and mortality from infectious diseases (3–5). Because zinc is essential for the activity of over 300 enzymes, it is involved in processes such as mitosis; synthesis of RNA and protein; and gene expression and activation (6). The physiological role of zinc during periods of rapid growth and development emphasizes its importance during periods of gestation and fetal growth.

Despite difficulties in measuring zinc status validly (7), it
has been estimated using the probability approach that about 82% of all pregnant women worldwide are likely to suffer from zinc deficiency (8). During the last two trimesters of pregnancy, it is recommended that women should absorb 3 mg elemental zinc/d assuming a bioavailability of 20% (9). Median dietary zinc intakes of 8–14 mg/d have been reported from pregnant women in developed countries (8), whereas intakes of 6.2–7.0 mg/d have been observed in studies in Malawi (10) and Brazil (11). These figures are well below the recommended intakes for pregnant women and support the hypothesis that zinc deficiency is widely prevalent in pregnant women, especially among those from less-developed countries.

The possible mechanisms and pathways of maternal zinc deficiency and adverse health effects to the mother and fetus were previously reviewed (8). Maternal zinc status during pregnancy may have a direct effect on fetal growth and infant birth weight. Moderate maternal zinc deficiency has also been related to high, the positive effects of maternal zinc supplementation in turn affect pregnancy outcome. It is further possible that maternal zinc status during pregnancy influences infant growth and morbidity beyond the neonatal period through its effect on intrauterine growth and development of the immune system (12) and possibly through an interaction with vitamin A metabolism (13). This theoretical framework has been supported by the results of many observational studies, suggesting that low dietary zinc intake or low maternal plasma zinc is associated with an increased risk of low birth weight and preterm delivery (14–16), although other studies did not find evidence for such an association (17).

Low plasma zinc concentrations have also been reported to correlate with pregnancy complications such as prolonged labor, hypertension, and postpartum hemorrhage, spontaneous abortion, and congenital malformation (18). Despite these associations, the evidence from trials that pregnancy outcome is improved by zinc supplementation has been less convincing.

Up until now, most randomized, controlled trials have been performed in developed countries, and the results have been described and summarized (19–22). The studies differed substantially in number of participants, duration of supplementation and dosages used and their results have been inconsistent. A clinically and statistically significant increase of 126 g in birth weight and 0.4 cm in head circumference after zinc supplementation was observed in the United States among thin (body mass index < 26) African-American women, in which only women with low plasma zinc concentrations at enrollment were included (23).

Based on the results of this study it was hypothesized that in less-developed countries where diets low in zinc are likely to result in zinc deficiency and where the prevalence of low birth weight is high, the positive effects of maternal zinc supplementation on pregnancy outcome would be even greater. In the past 10 y eight maternal zinc supplementation trials have been performed in less-developed countries. Although some results have already been published (24–30), most trials are still in analysis and reviewing results. To review and discuss the evidence base for improving the zinc status of women during pregnancy, a workshop was held June 19–21, 2001 in Wageningen, the Netherlands, organized jointly by Wageningen University and the Johns Hopkins Bloomberg School of Public Health under the auspices of the International Zinc Nutrition Consultative Group (IZiNCG). Researchers from each of the eight randomized, controlled supplementation trials and a selected group of scientists and policy makers discussed the results from these trials as well as other relevant trials in industrialized countries. The following is a review of the preliminary results of maternal zinc supplementation trials conducted in less-developed countries.

**Previous studies in less-developed countries**

Before 1993 only two maternal zinc supplementation trials in less-developed countries had been published (31,32). Unfortunately, both studies suffered from methodological flaws in that they did not use a double-blind design or were not properly placebo-controlled. Ross et al. (31) assigned 127 Zulu women in South Africa before the 20th wk of gestation randomly to receive 30–90 mg zinc gluconate daily (no further details were provided), a placebo and a high bulk or a low bulk food supplement containing enough energy, protein and vitamins/minerals, not including zinc, to bring their daily intake to recommended intake levels. Despite the randomization, women in the zinc-supplemented group had significantly lower body weights at the onset of the study compared to women in the control group, but the difference being greatest when supplementation was started in the third trimester of pregnancy. Infants of mothers in the zinc-treated group had higher gestational ages than those of mothers in the control group, but the difference reached statistical significance only when the supplement had been given for > 3 mo (39.4 ± 0.1 wk vs. 38.5 ± 0.3 wk in zinc vs. control group, respectively). Newborns from mothers in the zinc-supplemented group also had significantly higher Apgar scores at birth compared to those of mothers in the control group.

**Randomized, controlled maternal zinc supplementation trials in less-developed countries**

In this review an attempt was made to include all randomized, controlled trials performed in less-developed countries in the last 10 y that investigated the effect of maternal zinc supplementation. The study settings and study designs of eight intervention studies are summarized in Table 1 and Table 2.

**Study site and population.** All studies were carried out in countries where zinc deficiency is thought to be common, mainly based on the consumption of diets low in dietary zinc and/or with zinc of low bioavailability. The mean daily intake of dietary zinc as estimated from repeated dietary recalls was known for only three studies (studies 2, 6 and 7) and median intakes ranged from 6.5 to 7 mg/d. In contrast to the recent dietary reference intakes for zinc in the United States by the Institute of Medicine (33), the WHO guidelines (9) provide estimates for recommended zinc intakes for diets with different levels of bioavailability. Bioavailability of zinc in the diet is influenced by the food source as well as other components of the diet that inhibit or promote absorption of zinc. The primary inhibitor of zinc absorption is phytic acid, which is...
present in significant amounts in staple foods such as cereals, corn and rice. Unfortunately, none of the studies included in this review collected data on the intake of phytic acid.

Serum or plasma zinc concentrations at entry in the study were known for three studies and ranged from 9.8 ± 1.7 μmol/L in Peru to 15.3 ± 4.3 μmol/L in Bangladesh. In adults, a normal range for serum zinc is considered to be between 10.7 and 15.3 μmol/L (34), similar to values observed in children, and these values usually tend to decline during pregnancy. Lower plasma zinc concentrations in pregnant women compared to nonpregnant controls of comparable age have been observed as early as 2 mo gestation (35). At 19 wk of gestation, serum zinc concentrations of 9.7 (SD 1.5) μmol/L were observed in low income women in the United States (36), whereas average plasma concentration at 24 wk gestation was 8.1 (SD 2.3) μmol/L in Malawi (37). The higher serum and plasma zinc concentrations, notably in the study in Bangladesh, might suggest that the populations studied were probably not zinc-deficient. However, the higher zinc concentrations in Bangladesh could also in part have been caused by the use of nonfasting blood samples and the delay of a maximum of 6 h between sampling and separation of the samples (27).

The concentration of zinc in plasma or serum is still considered to be a useful indicator for assessing the zinc status of the total body zinc (9). Furthermore, plasma/serum levels are considered to be a useful indicator for assessing the zinc status of the populations studied varied widely. The nutritional status of the populations studied varied widely. The

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**TABLE 1**

Randomized double-blind trials on maternal zinc supplementation in developing countries: (1) study setting

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Country/region</th>
<th>Authors</th>
<th>Description of study site</th>
<th>Recruitment</th>
<th>Dietary characterization population</th>
<th>Baseline plasma/serum zinc concentrations (μmol/L)</th>
<th>Baseline nutritional status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ecuador</td>
<td>Dirren et al. (np)²</td>
<td>Peri-urban communities, hospital delivery</td>
<td>Community-based</td>
<td>Not available</td>
<td>Not available</td>
<td>Body Mass Index: 25.5 ± 4.5 (c)², 25.5 ± 4.2 (c)², 27.5 ± 5.6 (fs)</td>
</tr>
<tr>
<td>2</td>
<td>Peru</td>
<td>Caulfield et al.² (24-26)</td>
<td>Urban shanty town, hospital deliveries</td>
<td>Hospital-based using LMP² and ultrasound</td>
<td>Zinc intake, 7 mg/d; low to moderate bioavailability</td>
<td>10.6 ± 2.1 μmol/L; no differences between groups</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Chile</td>
<td>Castillo-Duran et al. (np)</td>
<td>Urban slums, teenagers, hospital deliveries</td>
<td>Hospital-based</td>
<td>Zinc intake, 40–50% RDA; low bioavailability</td>
<td>Not available</td>
<td>Body Mass Index: 24.0 ± 3.2 (z), 24.1 ± 3.3 (c)</td>
</tr>
<tr>
<td>4</td>
<td>Indonesia, West Java</td>
<td>Dijkhuizen et al. (30)</td>
<td>Rural area, home deliveries</td>
<td>Community-based using detailed amenorrhea history and physical examination</td>
<td>Not available</td>
<td>11.8 ± 4.1 μmol/L; no differences between groups</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Indonesia, East Java</td>
<td>Hakimi &amp; Dibley (np)</td>
<td>Rural area</td>
<td>Community-based, using LMP²</td>
<td>Energy intake, 6073 kJ/d</td>
<td>15.3 ± 4.3 μmol/L; no differences between groups</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bangladesh</td>
<td>Osendarp et al. (27-29)</td>
<td>Urban slums, home deliveries</td>
<td>Community-based, using LMP</td>
<td>Zinc intake, 6.5 mg/d; low to moderate bioavailability</td>
<td>Body Mass Index: 18.9 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Peru</td>
<td>Merialdi et al. (np)</td>
<td>Urban shanty town, hospital deliveries</td>
<td>Hospital-based using LMP and ultrasound</td>
<td>Zinc intake, 7 mg/d; low to moderate bioavailability</td>
<td>9.8 ± 1.7 μmol/L; no differences between groups</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Indonesia, East Timor</td>
<td>Hidayat et al. (np)</td>
<td>Rural, malaria zone, home deliveries</td>
<td>Community-based</td>
<td>Zinc intake animal products, staple food is local corn. Habit of earth eating</td>
<td>Not available</td>
<td>Body Mass Index: 24.1 ± 5.0 (z), 22.9 ± 3.0 (c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Weight: 152.5 ± 5.0 (z), 152.6 ± 5.2 (c)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Height: 45.5 ± 1.0 kg (z), 45.1 ± 1.0 kg (c)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Baseline concentrations at entry in the study.
² np, not yet published. Numbers in parentheses refer to numbers in reference list.
³ z, zinc-supplemented group; c, control or placebo group; fs, food-supplemented group.
⁴ LMP, last menstrual period.
populations in South America were relatively well nourished or even overnourished, with body mass indices before pregnancy being lower than in populations in Asia studies were mostly malnourished, with mean body mass indices at 6 mo postpartum as low as 18 in Bangladesh (study 6). Maternal nutritional status is one of the most important predictors of pregnancy outcome (42), and the populations of the eight studies were therefore thought to be at varying degrees of risk of poor pregnancy outcomes including prematurity and low birth weight. The study in Chile (study 3) was performed among adolescents (age, < 19 y), a group known to be at risk of having poor pregnancy outcomes.

All studies started the intervention during gestation, mainly during the second trimester of pregnancy (studies 2, 3, 6 and 7). It is difficult to identify pregnant women earlier in gestation in many developing country settings. Only the studies in Ecuador (study 1) and East Timor (study 8) included women from (the end of) the first trimester (wk 8–14 of gestation). Duration of gestation was assessed by ultrasound in the studies in Peru (studies 2 and 7) and Chile (study 3) and by recalled last menstrual period in all other studies sometimes combined with a physical examination (studies 4 and 5).

**Design.** All studies were randomized, controlled, double-blind zinc supplementation trials. Six studies followed a two-cell design with an intervention group and a control group. The control groups received a placebo (studies 3 and 6), a regular iron/folate supplement without zinc (studies 2, 7 and 8) or iron in combination with vitamin A without zinc (study 5). The trials that provided other micronutrients did so in both zinc and control groups. The study in Ecuador (study 1) had a three-cell design with two intervention groups receiving either a zinc supplement containing 30 mg elemental zinc/d or a food supplement containing 28 mg elemental zinc/d in combination with 300 kcal energy and other micronutrients. The control group received a corn starch placebo. The study in West Java (study 4) followed a 2 × 2-cell factorial design with 30 mg elemental zinc/d and/or 4.5 mg β-carotene/d. All treatment groups in this study received standard supplementation with iron and folic acid. Study subjects were individually randomized to the different intervention groups in all studies. In study 6 the subjects were stratified by parity before randomization. The study in East Timor (study 8) included five different intervention groups receiving zinc (as zinc sulfate), zinc and a micronutrient mix, the micronutrient mix only, a placebo group receiving a placebo supplement and a control group receiving no intervention.

For the purpose of this review only comparisons between the zinc only and placebo group of studies 4 and 8 were considered. The study in East Timor thus differed quite substantially from the other seven studies included in this review. Because information from this study was available on only one outcome variable (i.e., cleft lip/palate) and because this outcome was not considered in any of the other studies, we excluded the East Timor study from the tables comparing outcomes between studies (Tables 3–6).

**Exclusion criteria.** Most studies excluded women with complicated pregnancies and/or medical risks for reduced or excessive birth weights, such as chronic diseases, hypertension, renal disease or diabetes (studies 1, 2, 4, 6 and 7). Most studies also used exclusion criteria based on gestational age and/or place of residence (studies 1, 2, 3, 4, 6 and 7). The study in Ecuador (study 1) in addition excluded nulli- and primi-parae,
supplementation in developing countries: (2) study design

<table>
<thead>
<tr>
<th>Compliance</th>
<th>Duration of supplementation until delivery</th>
<th>Other interventions</th>
<th>Duration of postnatal follow-up</th>
<th>Major outcome variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplements administered by health workers (6 d/wk)</td>
<td>&gt; 8–14 wk gestation</td>
<td>105 mg Fe + 800 µg folic acid weekly (z + p)</td>
<td>9 mo</td>
<td>Child growth</td>
</tr>
<tr>
<td>85% (based on number of tablets)</td>
<td>From 10 to 24 wk gestation</td>
<td>60 mg Fe + 250 µg folic acid (z + c)</td>
<td>12 mo</td>
<td>Nutritional status at birth</td>
</tr>
<tr>
<td>Capsules provided monthly; those with &lt; 50% consumption were excluded (n = 297)</td>
<td>Before 20 wk gestation</td>
<td>Usual Fe care. 40 mg daily (as sulfate) (z + p)</td>
<td>6 mo</td>
<td>Pregnancy complications, length of gestation, anthropometry at birth and serum zinc status</td>
</tr>
<tr>
<td>85%, no differences between groups</td>
<td>&gt; 10–20 wk gestation</td>
<td>Fe: 30 mg + 0.4 mg folic acid (z + c)</td>
<td>6 mo</td>
<td>Complications during pregnancy and delivery, nutritional status at birth, vitamin A status at 6 mo postpartum</td>
</tr>
<tr>
<td>Not available</td>
<td>Before 120 d, since 6–17 wk</td>
<td>Vitamin A (2400 RE/d)</td>
<td>12 mo</td>
<td>Nutritional status at birth</td>
</tr>
<tr>
<td>86% (based on number of days)</td>
<td>&gt; 12–16 wk gestation</td>
<td>Usual Fe care: if Hb &lt; 90 g/L 60 mg Fe (as sulfate) + 200 µg folic acid/d (z + p)</td>
<td>6 mo</td>
<td>Birth weight</td>
</tr>
<tr>
<td>90% consumed tablets at least 5 d/wk</td>
<td>From 10 to 16 wk gestation</td>
<td>60 mg Fe + 250 µg folic acid (z + c)</td>
<td>1 mo</td>
<td>Fetal growth (ultrasonography) and fetal neurobehavioral development</td>
</tr>
<tr>
<td>78% (based on number of days or tablets??)</td>
<td>First trimester (98.5%) Second trimester (1.5%)</td>
<td>Fe + folic acid (z + c)</td>
<td>1 wk</td>
<td>Obstetric complications and congenital malformations</td>
</tr>
</tbody>
</table>

women aged < 18 and > 40 y, as well as heavy smokers, alcoholics and drug addicts. The Chilean study (study 3) was performed in adolescents and excluded women ≥ 19 y of age.

Sample sizes and dropout. In most studies, sample size calculations were performed based on expected differences in the main outcome variables, and the number of women enrolled ranged from 58 and 57 in zinc and control groups, respectively, in West Java up to > 1050 per group in East Java. The dropout rates were substantial in some of the studies. For example, 14, 22 and 27% of the women were lost to follow-up before delivery in Peru (study 7), West Java (study 4) and Bangladesh (study 6), respectively. An additional 6% dropout was observed postpartum in the study in Bangladesh, whereas in Peru an additional 9% of the women and infants enrolled in the postpartum follow-up were lost before 6 mo of age. However, when comparisons of baseline characteristics were performed for women who were lost to follow-up and women who completed the follow-up in the study in Bangladesh, no major differences were observed. The studies in Ecuador, East Java and East Timor did not provide information on characteristics of cases lost to follow-up and for these studies it cannot be assessed whether the final study population differed from the population as enrolled.

Intervention and compliance. Women in the intervention groups received tablets containing elemental zinc in the form of zinc sulfate or zinc acetate, sometimes in addition to an iron/folate or multi-micronutrient supplement. Dosages ranged from 15 mg/d in the first study in Peru to 22.5 mg/twice a day in East Timor, with most studies providing once or twice the recommended daily intakes for zinc during pregnancy as per WHO guidelines (9).

Information on compliance with the study regimen was available for seven studies (all except study 5). Compliance was usually expressed as the proportion of total days that women took a supplement or proportion of tablets that were consumed and ranged from 78% in the study in East Timor to 90% in Peru (study 7). In Chile (study 3) 115 women were excluded from further participation in the study or from the final analysis because they had consumed < 50% of the tablets. None of the other studies excluded women for reasons of low compliance and statistical analyses in most of the other studies were performed on an intention-to-treat basis.

Maternal health outcomes

All studies examined the effect of maternal zinc supplementation on gestational age, although data were not yet available for two studies (Table 3). In general no effects were observed on gestational age or on the incidence of prematurity, with the exception of the study in Chile (study 3) in which the incidence of prematurity decreased after zinc supplementation. The study in Ecuador (study 1) was the only one to examine the effect of maternal supplementation on maternal immune response and did not observe differences either between zinc and control groups for antibody responses to the pneumococcal vaccine given during pregnancy or to the cholera vaccine given at 3 mo of lactation. However, higher tetanus toxoid antibody titers were observed in women who had been supplemented with zinc compared to placebo during pregnancy for a subgroup of women with intermediate tetanus toxoid antibody titers at baseline. Assessment of maternal serum or plasma zinc status was done in six studies; maternal
serum zinc increased after supplementation in some (study 1) but not all (studies 2, 3, 4 and 6) studies. Very few studies presented findings on pregnancy and postpartum complications and puerperal morbidity. Significantly, more delivery complications in the zinc-supplemented (n = 12) vs. control (n = 3) group were observed in the study in West Java (study 4), emphasizing the need for further research in this area.

**Fetal health and birth outcomes**

None of the studies provided evidence for an effect of maternal zinc supplementation alone on fetal weight at delivery, except for a small effect on the incidence of low birth weight in Chile (study 3) that was most likely explained by the effect on gestational age (Table 4). Data from one study (study 7, Peru) indicated that more subtle effects of zinc supplementation on fetal bone growth can be detected with fetal ultrasound (26,43). In West Java (study 4), a lower proportion of low birth weight (<3000 g) male infants were born to women supplemented with zinc plus β-carotene but not to those supplemented with zinc alone or with β-carotene alone. None of the trials commenced supplementation sufficiently early or were of sufficient magnitude to effectively address the outcome of congenital malformations apart from cleft lip/palate. In the study in East Timor (study 8), a significantly lower incidence of cleft lip/palate was reported from children of mothers in the zinc group compared to those of mothers in the placebo group. The two studies in Peru presented findings indicative of improved neurobehavioral development of fetuses after maternal zinc supplementation, suggesting that further research including these outcomes is a high priority (26,44).

**Infant immunity**

Five studies examined various aspects of infant immunity (Table 5). Zinc supplementation during pregnancy resulted in higher IgG levels in cord blood in Peru (study 2). Fewer anergic reactions to the tetanus skin test were observed after maternal zinc supplementation in infants born with low birth weight in Bangladesh (study 6), but no effect on PPD response was observed in Ecuador (study 1). The study in Ecuador found no evidence for an effect on the transfer of antibodies to the
### TABLE 4

**Fetal health and birth outcomes**

<table>
<thead>
<tr>
<th>Outcomes studied</th>
<th>Study 1: Ecuador</th>
<th>Study 2: Peru</th>
<th>Study 3: Chile</th>
<th>Study 4: Indonesia, West Java</th>
<th>Study 5: Indonesia, East Java</th>
<th>Study 6: Bangladesh</th>
<th>Study 7: Peru</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal growth (birthweight, IUGR)</td>
<td>No effect</td>
<td>3267 ± 461 g (2) vs. 3300 ± 498 g (c)</td>
<td>3.319 ± 460 g (2); 3.250 ± 514 g (p) (NS)</td>
<td>No differences in birth weight between z and c group</td>
<td>Birth weight of male infants in zinc + β-c group (3600 ± 300 g) higher than male infants in z group (3000 ± 600 g; P = 0.036)</td>
<td>No effect</td>
<td>2513 ± 390 g (z); 2554 ± 393 g (p) NS</td>
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<td></td>
</tr>
<tr>
<td>% LBW</td>
<td>2.4% (z); 6.2% (p); (P = 0.036)</td>
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<td></td>
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</tr>
<tr>
<td>% IUGR</td>
<td>74.7 (z); 74.5 (p) NS</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

1 The study in East Timor (study 8) collected data only on congenital malformations and a significantly lower incidence of cleft lip/palate in the zinc group compared to the placebo group (0 vs. 1.18%) was observed in this study (see text). This study was excluded from the table.

2 Data on fetal neurobehavioral development have been collected in the two studies in Peru (studies 2 and 7); In general, fetuses in (z) group had steeper declines in heart rate (FHR), more variability (P = 0.08), more accelerations, no difference in movement (FM), trend for more coupling of FHR and FM in (z) group (see text).

3 Data on cord blood immune function have been collected in studies 1 and 2. In study 2 higher Ig concentrations in cord blood of zinc treated group were observed. Data were not yet released for study 1.

4 Data on perinatal morbidity or mortality have been collected in studies 1, 2, 3, 4 and 7. In study 4 significantly higher perinatal complications, in particular neonatal hypoxia (6 (z) vs. 1 (p)) were observed in the zinc group. Data were not yet analyzed or released for studies 1, 2, 3 and 7.

5 See Tables 1 and 2 for abbreviations; IUGR, intrauterine growth retardation; LBW, low birth weight.

### TABLE 5

**Infant immunity including response to vaccines**

<table>
<thead>
<tr>
<th>Outcomes studied</th>
<th>Study 1: Ecuador</th>
<th>Study 2: Peru</th>
<th>Study 4: Indonesia, West Java</th>
<th>Study 5: Indonesia, East Java</th>
<th>Study 6: Bangladesh</th>
<th>Study 7: Peru</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific immunity: Maternally transmitted antibodies</td>
<td>No differences between groups</td>
<td>Cord blood: 35% increase in IgA 15% increase in IgM 25% increase in IgG2 40% increase in IgG3 20% increase in IgG4 in zinc group</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
<td></td>
</tr>
<tr>
<td>Vaccine immunogenicity</td>
<td>No effect on PPD response at 6 mo of age</td>
<td>Samples not fully analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not released</td>
<td>In LBW infants only: differences in % anergic (no response) tuberculin skin test at 6 mo of age: 66.2 (z), 78.5 (p) P = 0.07 No differences in NBW infants No differences in Hib (PRP) antibody concentrations at 1 and 6 mo of age</td>
<td></td>
</tr>
</tbody>
</table>

1 The studies in Chile (study 3) and East Timor (study 8) did not measure infants' immune response. The study in Peru (study 7) did not follow infants beyond the age of 7 d. These studies were excluded from the table.

2 Data on breastmilk immunologic functions have been collected in studies 1, 2, 4 and 5. In study 1 no differences between groups were observed in breastmilk cholera vaccine antibodies at 3 mo lactation. Samples have not yet been analyzed in studies 2, 4 and 5.

3 Data on nonspecific immunity have been collected in studies 1, 2, 4 and 5 but samples have not yet been analyzed.

4 See Table 1 for abbreviations; NBW, normal birth weight; Hib (PRP), *Haemophilus influenzae* type b polysaccharide (polyribosylribitol phosphate).
### Infant health outcomes

<table>
<thead>
<tr>
<th>Outcomes studied</th>
<th>Study 1: Ecuador</th>
<th>Study 2: Peru</th>
<th>Study 4: Indonesia, West Java</th>
<th>Study 5: Bangladesh</th>
<th>Study 6: Indonesia, East Java</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical growth:</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>No differences between groups</td>
<td>Data not yet analyzed</td>
<td>No differences between groups</td>
</tr>
<tr>
<td>0–6 mo</td>
<td>No differences between groups</td>
<td>Evidence that Zn group had less growth faltering (0.2 Z-scores) between 6 and 12 mo</td>
<td>No differences between groups</td>
<td>Not measured</td>
<td>No differences in mean weight and length at 13 mo of age</td>
</tr>
<tr>
<td>6+ months</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
</tr>
<tr>
<td>Infant morbidity:</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
<td>Data not yet analyzed</td>
</tr>
<tr>
<td>Diarrheal incidence</td>
<td>Mucous diarrhea: 0–12 mo RR 0.86 (NS)</td>
<td>8–12 mo RR 0.69 (p &lt; 0.05) vs. p group</td>
<td>Data not yet analyzed</td>
<td>Acute diarrhea</td>
<td>All infants RR 0.84 (p &lt; 0.05); LBW infants RR 0.68 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>8–12 mo RR 0.71 (p &lt; 0.05)</td>
<td></td>
<td></td>
<td>NBW infants RR 1.04 (NS)</td>
<td></td>
</tr>
<tr>
<td>Diarrheal prevalence</td>
<td>Total diarrhea: 0–12 mo RR 0.94 (NS)</td>
<td>8–12 mo RR 0.73 (p &lt; 0.05)</td>
<td>8–12 mo RR 0.64 (p &lt; 0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucous diarrhea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease incidence</td>
<td>ALRI: 0–12 mo RR 0.90 (NS)</td>
<td>8–12 mo RR 0.65 (NS)</td>
<td></td>
<td>ALRI</td>
<td>All infants RR 0.89 (NS); LBW infants RR 0.97 (NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NBW infants RR 0.87 (NS)</td>
<td></td>
</tr>
</tbody>
</table>

1 The study in East Timor (study 8) did not collect data on infant health outcomes. The study in Chile (study 3) assessed data on infant’s growth up to 6 mo only and did not observe differences between treatment groups. The study in Peru (study 7) did not follow infants beyond the age of 7 d. These studies have been excluded from the table.

2 Data on infant’s neurobehavioral development have been collected in the studies in Peru (study 2), West Java (study 4) and Bangladesh (study 6). In the study in Peru (study 2) no differences between treatment groups in NBAS at 3 d and BSID II at 6 mo were observed, whereas a slightly better novelty preference assessed by FTII at 6 mo of age was observed in the zinc group compared to placebo group. In Bangladesh (study 6) significantly lower scores at the Bailey’s mental and motor development index were observed at the age of 13 mo in the zinc group compared to placebo group (see text). Data were not yet analyzed in study 4.

3 See Tables 1 and 2 for abbreviations.

Infant health outcomes

Six studies assessed infant growth during the first 6 mo of life and no differences were observed between zinc and control groups for the four studies that had results available (Table 6). Only two studies (studies 1 and 2) continued their growth assessments beyond the age of 6 mo and data were available from one of these studies. In Peru (study 2) there seems to be some evidence for less growth faltering (0.2 Z-scores) after maternal zinc supplementation between 6 and 12 mo of age. In contrast, at 13 mo of age, no differences in nutritional status were observed between zinc and control groups in Bangladesh (study 6), although no longitudinal observations had been performed between 6 and 13 mo of age.

Five trials examined the effect of zinc supplementation in pregnancy on infant morbidity from infectious diseases but results were available only for the trials in Peru and Bangladesh (Table 6). Reduced rates after zinc supplementation for the incidence of diarrhea (total acute diarrhea, dysentery) were observed in both studies, although in Peru (study 2) the difference was statistically significant only during the period of 8 to 12 mo of age. In Bangladesh (study 6), lower rates of infectious diseases in the zinc-supplemented compared with the placebo-treated group were found in infants born with low birth weight, whereas no differences were observed in infants with normal birth weight. Finally, in Peru improvements in some aspects of infant neurobehavioral development (novelty preference) were observed in the zinc-supplemented group at 6 months.
Discussion and research implications

This paper reviews the preliminary findings of all randomized, controlled intervention trials performed in less-developed countries on the effect of maternal zinc supplementation. Although some of the results available are still preliminary or incomplete, it was felt that such a review was timely, to direct future research toward specific outcomes that may be influenced by zinc supplementation.

Taking this into consideration we can conclude that the preliminary findings indicate potential benefits with regard to neonatal immune status, early neonatal morbidity and infant infections. Data are conflicting, suggesting that more research is required with respect to the following outcomes: certain labor and delivery complications, maternal zinc status, gestational age at birth and fetal neurobehavioral development. Current data do not suggest that maternal zinc supplementation alone stimulates intrauterine growth. Other potential outcomes of interest for which data are currently not available or very limited include: reproductive tract and urinary infections as it relate to preterm delivery and intrauterine infections, pregnancy-induced hypertension, postpartum hemorrhage, maternal and perinatal mortality and malaria in pregnancy. The lack of effect on fetal and early infant growth as observed in most of these studies is disappointing and in contrast with the small, but statistically significant effect of zinc on growth in children where effect size on height and weight increments of 0.350 (95% CI: 0.189, 0.511) and 0.309 (0.178, 0.439) respectively have been observed (3). The differences in morbidity observed in these studies were expected to make a substantial contribution to the usual dietary intake, although, unfortunately, data on dietary intakes of zinc were known for only a limited number of studies. The dietary zinc intakes in these studies were low compared to recommended intakes and, in addition, zinc was assumed to be poorly bioavailable from most of the diets that were described.

The adequacy of zinc intakes during pregnancy to meet increased physiologic demands are influenced by usual diets and possible changes in fractional zinc absorption and/or endogenous zinc excretion during pregnancy. Regulation of zinc absorption and endogenous intestinal excretion are the primary means for maintaining zinc homeostasis at varying levels of zinc intakes (40). In populations with habitually low zinc intakes, conservation of endogenous zinc is thought to be more critical in maintenance of zinc homeostasis than changes in absorption, but there are important limitations of adaptation mechanisms in situations of extremely low or chronically low intakes (45).

The increased zinc requirements during pregnancy may be partly met by an increased fractional absorption (9), as has been described in detail for iron (46). However, the changes in fractional absorption during human gestation are modest and less than the significant increases that occur during early human lactation (9,47). Estimates of zinc requirements during pregnancy do not take into account these possible metabolic adaptations (9,33). The concurrent existence of multiple micronutrient deficiencies in the populations studied, as has been shown in the study in West Java (48), could also provide a possible explanation for the lack of effect on fetal growth. However, very little is known about the individual and combined contribution of multiple micronutrient deficiencies to fetal growth, and the effect of zinc supplementation on other micronutrients has not been adequately studied. There seems to be evidence for an enhancing effect of zinc on bioconversion of β-carotene into vitamin A, thus improving vitamin A status (30). In the study in Peru, prenatal iron supplements impaired zinc absorption in pregnant women, but the inclusion of zinc in the supplements reduced the potential inhibitory effects of iron (49). Adding zinc to the iron/folate supplement did not affect hemoglobin concentrations (50) and led to higher zinc concentrations in the women (24).

Although there is evidence for both additive effects of micronutrients and interactions among various micronutrients at the metabolic level (51), very little is known about the significance of such effects on pregnancy outcomes. The higher birth weight of male infants of mothers supplemented with zinc and β-carotene compared with those mothers supplementation alone suggests that zinc supplementation in addition to the multivitamin-mineral prenatal supplement beginning in the first or second trimester of pregnancy was shown to be associated with twofold reductions in the risk of low birth weight and preterm delivery in low income U.S. women (52). The supplement used in this study contained, among other ingredients, 25 mg zinc, 1 mg folic acid and 65 mg iron. On the other hand, preliminary findings from an intervention study in Nepal suggest that the addition of 30 mg zinc to an iron/folate supplement appears to block the beneficial effect of an iron/folate supplement on birth weight and reduced hemoglobin levels, but not on mortality (53). The results of other multiple micronutrient supplementation trials in pregnant women in developing countries, including a multicountry trial coordinated by UNICEF (54), are awaited, given that such results may allow more definitive conclusions to be drawn.

More research is required in areas where HIV and malaria are prevalent, given that none of the studies was performed in such areas. There is evidence that zinc supplementation in children reduces malaria infection (55,56), although a recent study in West African children could not confirm these findings (57). Earlier studies showed adverse effects of oral iron supplementation in subjects exposed to malaria but a recent study in Kenya showed that iron supplementation gives substantial health benefits that outweigh possible inherent risks caused by malaria (58). The reduced risks after maternal zinc supplementation in the incidence of diarrhea as observed in some subgroups in the studies in Peru and Bangladesh are promising findings but results of the other studies are needed before any definitive conclusion can be drawn. The differences in morbidity observed are consistent with findings of clinical trials in infants and children showing beneficial effects of zinc supplementation on both prevalence and incidence of diseases (4,56) and are thought to reflect the prominent role of zinc in both immune and nonimmune host defense mechanisms (4). Work with animal models indicates a role of perinatal zinc status in optimal development of the fetal immune system. Reduced lymphoid organ size, gamma globulin concentrations and blood lymphocyte numbers have been observed in offspring born to zinc-deficient mice and rhesus monkeys. Antenatal zinc deprivation in rats resulted in lower serum immunoglobulin concentrations, in particular IgA, IgG2 and IgM, in the offspring at 6 mo of age. These abnormalities may persist into adulthood (12). Maternal zinc status during pregnancy may also affect in utero acquisition of antibodies ascribed to the
role of zinc in placental transport of immunoglobulins (8). It is therefore conceivable that the reduction in morbidity observed in the studies in Peru and Bangladesh are the result of improved immune development during gestation. However, the findings of these studies do not provide clear evidence for a biological pathway through improved immunity.

In view of the serious consequences of zinc deficiency on immunity (12), it is surprising that the effect of supplementing zinc-deficient women with zinc is not more pronounced. One explanation may be that the outcomes chosen may not reflect the most important effects of zinc supplementation. Another explanation may be that the effects of maternal zinc supplementation on infant immunity and health become evident only after some months, perhaps when maternally transferred immunity begins to wane and the infants’ own immune system develops. Another explanation may be that zinc deficiency is only a marker of other micronutrient deficiencies or problems, which influence infant immunity and which are not relieved by zinc supplementation alone. There are a number of studies, including some of those reported here, that have investigated the effect of supplementing infants with zinc. Data from such studies help to clarify the role of zinc in infant immunity. However, more research on the effect of supplementation of mothers and infants on infant immunity is warranted.

The conflicting results of these studies suggest that future work should pay particular attention to study design. This may best be achieved by carrying out a series of studies in populations at high risk for adverse pregnancy outcomes with a range of dietary zinc intakes based on a common protocol using standardized outcome definitions. Measurement of intake of zinc and of dietary constituents affecting zinc absorption should also be made, although analysis of such data would require further development of data on the composition of foods consumed. Interventions should begin not only during gestation but also before conception, and effects on infant health, growth and cognitive development beyond the first 6 mo of life should be evaluated.

In conclusion, in many countries zinc intake of pregnant women is low and needs to be optimized. Data are emerging that indicate that increasing zinc intake and status improves health. Despite the emerging evidence for a positive effect of zinc on some outcomes of pregnancy, the workshop concluded that the full results of studies carried out need to be known and that more research is required to determine the benefits of large-scale introduction of zinc supplementation of pregnant women in less-developed countries.

LITERATURE CITED

EFFECTS OF MATERNAL ZINC SUPPLEMENTATION


