Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials\textsuperscript{1–3}

Christa Fischer Walker, Katarzyna Kordas, Rebecca J Stoltzfus, and Robert E Black

ABSTRACT
Iron and zinc are essential micronutrients for human health. Deficiencies in these 2 nutrients remain a global problem, especially among women and children in developing countries. Supplementation with iron and zinc as single micronutrients enhances distinct and unique biochemical and functional outcomes. These micronutrients have the potential to interact when given together; thus, it is important to assess the biochemical and functional evidence from clinical trials before supplementation policies are established. We reviewed randomized trials that assessed the effects of iron and zinc supplementation on iron and zinc status. On the basis of this review, zinc supplementation alone does not appear to have a clinically important negative effect on iron status. However, when zinc is given with iron, iron indicators do not improve as greatly as when iron is given alone. In most of the studies, iron supplementation did not affect the biochemical status of zinc, but the data are not clear regarding morbidity outcomes. Although some trials have shown that joint iron and zinc supplementation has less of an effect on biochemical or functional outcomes than does supplementation with either mineral alone, there is no strong evidence to discourage joint supplementation. Supplementation programs that provide iron and zinc together are an efficient way to provide both micronutrients, provided the benefits of individual supplementation are not lost. Further research is needed before health policies on joint supplementation programs can be established. Am J Clin Nutr 2005;82:5–12.

KEY WORDS
Micronutrients, zinc, iron, supplementation, women, children

INTRODUCTION
Iron and zinc are essential micronutrients for human growth, development, and maintenance of the immune system. Iron is needed for psychomotor development, maintenance of physical activity and work capacity, and resistance to infection (1). Zinc is needed for growth and for maintenance of immune function, which enhances both the prevention of and recovery from infectious diseases (2). Meat products are the best source of both iron and zinc. Consequently, iron and zinc deficiencies may coexist in populations that consume diets with insufficient amounts of animal-source foods. The intake of these 2 micronutrients would ideally be improved through enhanced dietary quality, but food fortification or supplementation programs may also be needed.

If iron and zinc are to be provided together, it is important to determine whether, and if so, how they interact biologically. Because they have chemically similar absorption and transport mechanisms, iron and zinc have been thought to compete for absorptive pathways (3). New evidence based on cell culture studies has shown that iron may inhibit zinc absorption in some cells at very high ratios of iron to zinc, but not vice versa (4). However, evidence of antagonism from studies of low ratios of iron to zinc is needed to assess any biochemical and functional effects of dual supplementation. Assessing the effect of single zinc or iron supplementation on the biochemical indicator of the other (ie, zinc on iron and iron on zinc) may help shed light on whether adverse effects are associated with supplementation with 1–2 times the recommended dietary allowance. Kordas and Stoltzfus (4) recently expounded on the gut interaction theory by reviewing new evidence for potential multiple interaction sites. Briefly, both iron and zinc are functionally important throughout the body and have the potential to interact in many systems, such as the nervous system. Although the separate functions of iron and zinc on brain and neural tissue are well described separately, studies of the possible interactions are scarce. Additional information to better understand the biological basis for potential interactions observed in functional outcomes such as growth, development, and disease resistance is needed.

The present review examines the evidence of iron and zinc interactions provided by placebo-controlled randomized trials of supplementation with iron or zinc alone or in combination in children aged <5 y and in women of child-bearing age. We addressed 4 distinct questions: 1) Does zinc supplementation alone affect iron status?, 2) Does iron supplementation alone affect zinc status?, 3) Does iron supplementation with 1–2 times the recommended dietary allowance of iron affect zinc status?, and 4) Does iron supplementation with 1–2 times the recommended dietary allowance of iron affect zinc status? 1 From the Bloomberg School of Public Health, Department of International Health, Johns Hopkins University, Baltimore, MD (CFW and REB), and the Division of Nutritional Sciences, Cornell University (KK and RJS). 2 Supported by Johns Hopkins Family Health and Child Survival Cooperative Agreement with the US Agency for International Development. 3 Address reprint requests to RE Black, Department of International Health, Johns Hopkins University Bloomberg School of Public Health, 615 North Wolfe Street, Room E8527, Baltimore, MD 21205. E-mail: rblack@jhsph.edu.
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affect zinc status?, 3) Does zinc in combination with iron supplementation have an effect on iron status or functional outcomes?, and 4) Does iron in combination with zinc supplementation have an effect on zinc status or functional outcomes. We conducted a literature review in PubMed (National Library of Medicine, Bethesda, MD) to find randomized trials that were conducted in humans and published in English. We searched under the following phrases: “zinc AND supplementation” and “iron AND supplementation.” Only daily or weekly supplementation trials for which functional or biochemical data from the same population at 2 time points (before and after intervention) were available were included. Nonrandomized studies and trials of short-course supplementation as a treatment for anemia or any infectious disease were excluded. We also excluded studies in which multiple micronutrients were given to all supplementation groups to enable clear comparisons, except for populations supplemented with vitamin A, folic acid, or both as part of a national program policy. For studies with multiple comparison groups, only those with results from groups supplemented with iron or zinc or with both iron and zinc were included.

**CHOICE OF TARGET GROUPS**

Women of childbearing age, both pregnant and nonpregnant, are at high-risk of iron and zinc deficiency in many developing countries (5). As girls approach adolescence, their iron demands to support growth and menstruation often exceed their intakes, which results in widespread iron deficiency anemia (IDA) (6). Daily iron supplementation (60 mg/d) is recommended for all women of reproductive age in countries where the prevalence of anemia is >40% and for pregnant or severely anemic women (hemoglobin < 70 g/L) in all countries (7). Estimating individual zinc deficiency is challenging in a trial setting because no sensitive, simple indicator exists to measure individual zinc status (8). There are no current recommendations for zinc supplementation for pregnant women, but the high nutritional demands of fetal growth have led to a number of trials that have assessed both the biochemical and clinical outcomes of maternal supplementation during pregnancy. Maternal zinc supplementation may be beneficial for neonatal immune status, neonatal morbidity, and infant infections (9), but positive effects on mental development (10, 11) or birth outcomes have not been shown consistently (9).

Children aged <5 y are another group at high-risk of iron and zinc deficiencies. Breastfed infants who do not receive iron-rich complementary foods by 6 mo of age can quickly become iron deficient (12). Iron depletion is more rapid in low-birth-weight infants than in normal-weight infants (6). Because preschool children in developing countries typically consume little meat or animal products, iron and zinc deficiencies in this age group are common. Although there is not yet a global policy for daily supplementation, zinc has been shown to reduce the incidence of diarrhea and pneumonia (13) and is now a component of the World Health Organization’s guidelines for the treatment of diarrhea in children aged <5 y (14).

**EVIDENCE FROM SUPPLEMENTATION TRIALS**

**Does zinc supplementation alone affect iron status?**

There are numerous iron-status indicators, such as hemoglobin, mean cell volume, erythrocyte protoporphyrin, serum iron, total-iron-binding capacity, transferrin saturation, serum ferritin, and serum transferrin receptor. Because many assessment techniques of varying sensitivity exist and because clinical trials often use a combination of different techniques to determine the effect of an intervention, it is often difficult to draw definitive conclusions on the basis of biochemical status alone.

In Nepal, 235 night-blind pregnant women were randomly assigned to receive vitamin A, β-carotene, or placebo and, in addition, daily zinc or placebo (15; Table 1). Zinc-supplemented women had a nonsignificant tendency for a greater decline in hemoglobin and a nonsignificant tendency for an increase in serum ferritin compared with women who received placebo. Osendarp et al (16) randomly assigned pregnant women to receive daily zinc or placebo. There was no difference in hemoglobin concentrations between zinc and placebo after 5 mo of supplementation.

Three studies were conducted in infants and none found effects of zinc supplementation on iron status. Dijikhuizen et al (17) randomized 371 Indonesian infants to receive iron, zinc, both iron and zinc, or placebo, d/wk for 6 mo. After supplementation there were no differences in hemoglobin or serum ferritin concentrations between the zinc- or placebo-supplemented children. In another trial in Indonesia, 680 infants were randomly assigned to receive iron, zinc, both iron and zinc, or placebo daily for 6 mo (18). There were no differences in hemoglobin, serum ferritin, or serum transferrin receptor between children receiving zinc alone and children receiving placebo. Baqui et al (19) randomly assigned 799 Bangladeshi infants to receive iron, zinc, both iron and zinc, a multiple micronutrient mix (not reported here), or a control supplement weekly for 6 mo. The final hemoglobin concentration in the zinc-supplemented children was not significantly different from that in the control subjects after adjustment for age and baseline concentrations.

In Mexico, 219 toddlers were randomly assigned to receive iron, zinc, both iron and zinc, or placebo daily for 6 mo (20, 25). Zinc alone did not have a significant effect on hemoglobin or plasma ferritin concentrations compared with the placebo. Shankar et al (21) randomly assigned young children in Papua New Guinea to receive zinc or placebo daily for 5 mo. Although hemoglobin decreased in both groups, there was no difference between the zinc- and placebo-supplemented children. Children in Chile (22) were randomly assigned to receive zinc or placebo for 6 mo and no difference was observed between groups for either hemoglobin or serum ferritin. A small trial in Belize observed a higher hemoglobin concentration in children supplemented with zinc alone for 6 mo than in children given placebo after adjustment for the pretreatment means (23). Penny et al (24) randomly assigned Peruvian children with persistent diarrhea to receive zinc, multiple micronutrients, or placebo daily for 6 mo after the cessation of the episode. Although no difference in hemoglobin concentration was observed between the zinc- or placebo-supplemented children, there was a 0.1-µg/L increase in plasma ferritin in zinc-supplemented children compared with a 0.5-µg/L decrease in children given placebo (P < 0.0001).

In summary, in trials in which zinc was given at prophylactic doses to pregnant women and children aged <5 y, most of the trials showed no effect of zinc on hemoglobin or serum ferritin. Although one small trial showed a positive effect of zinc on hemoglobin and another trial showed a positive effect on plasma ferritin, it is most important to note that none of the trials showed a negative effect on iron indicators.
TABLE 1
Studies that assessed the effects of zinc supplementation on iron status

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Enrollment criteria</th>
<th>Comparison groups</th>
<th>Duration of study</th>
<th>Iron indicators</th>
<th>Effect on iron status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christian et al, 2001 (15)</td>
<td>Nepal</td>
<td>Pregnant women, night blindness</td>
<td>Vitamin A and B carotene or placebo and 25 mg Zn (n = 84) or placebo (n = 96)</td>
<td>3 wk</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Osendarp et al, 2000 (16)</td>
<td>Bangladesh</td>
<td>Pregnant women at 12–16 wk gestation</td>
<td>30 mg Zn (n = 194) and placebo (n = 216)</td>
<td>5 mo</td>
<td>Hemoglobin</td>
<td>No effect</td>
</tr>
<tr>
<td>Dijkstraen et al, 2001 (17)</td>
<td>Indonesia</td>
<td>4-mo-old infants</td>
<td>10 mg Zn (n = 119) and placebo (n = 119)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Lind et al, 2003 (18)</td>
<td>Indonesia</td>
<td>6-mo-old infants</td>
<td>10 mg Zn (n = 134) and placebo (n = 143)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Baqui et al, 2003 (19)</td>
<td>Bangladesh</td>
<td>6-mo-old infants</td>
<td>1 mg Rb + 20 mg Zn (n = 161) and 1 mg Rb (control; n = 157)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Munoz et al, 2000 (20)</td>
<td>Mexico</td>
<td>18–36-mo-old children</td>
<td>20 mg Zn (n = 54) and placebo (n = 56)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Shankar et al, 2000 (21)</td>
<td>Papau New Guinea</td>
<td>6–60-mo-old children</td>
<td>10 mg Zn (n = 136) and placebo (n = 138)</td>
<td>5 mo</td>
<td>Hemoglobin</td>
<td>No effect</td>
</tr>
<tr>
<td>Ruz et al, 1997 (22)</td>
<td>Chile</td>
<td>27–50-mo-old children</td>
<td>10 mg Zn (n = 49) and placebo (49)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Smith et al, 1999 (23)</td>
<td>Belize</td>
<td>22–66-mo-old children</td>
<td>70 mg Zn (n = 11) and placebo (n = 9)</td>
<td>6 mo</td>
<td>Hemoglobin</td>
<td>Positive effect of zinc on hemoglobin</td>
</tr>
<tr>
<td>Penny et al, 2004 (24)</td>
<td>Peru</td>
<td>6–35-mo-old children</td>
<td>10 mg Zn (n = 80) and placebo (79)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>Positive effect of zinc on plasma ferritin</td>
</tr>
</tbody>
</table>

Rb, riboflavin.

Does iron supplementation alone affect zinc status?

Most studies interested in zinc status use plasma or serum zinc as an indicator of outcome. It is important, however, to recognize the shortcomings of this indicator in reflecting zinc status when interpreting the effects of iron supplementation on zinc status. It may not be sensitive enough to detect antagonisms from iron supplementation, especially if these are relatively small in magnitude.

In the 4 trials in infants or toddlers mentioned previously (17–20), plasma zinc concentrations in children supplemented with iron alone did not differ from those in the control group (Table 2). In another study, 291 infants were randomly assigned to receive daily iron supplements or placebo (26). After 3 mo of supplementation, no difference in serum zinc concentration was observed between the iron and placebo groups. Friel et al (27) randomly assigned breastfed infants to receive iron or zinc for 5 mo and observed no difference in serum zinc concentrations between iron- and placebo-supplemented infants. In a multicountry trial of infant supplementation with multiple micronutrients, daily iron, or placebo, there was no difference between daily iron and placebo in 3 of the 4 study sites (28–30). However, in Indonesian infants, a greater percentage of infants who received daily iron (32.8%) than of those who received placebo (15.6%) were zinc deficient (<10.7 μmol/L) after 6 mo of supplementation (31). In this study there was no significant difference in the change in mean serum zinc after supplementation between the placebo- and iron-supplemented infants.

In 9 of 10 of these studies, iron supplementation did not have an effect on serum zinc status. It is not clear why the negative effect was seen in only one study. Although serum zinc is often criticized for being an unreliable measure of individual zinc status, on a population level the observed lack of effect of iron supplementation on this indicator may still be indicative of overall zinc status (32). These studies do not show evidence of adverse effects of iron on biochemical zinc status.

Does the addition of zinc to iron supplements affect iron status or functional outcomes?

Four trials assessed the effect of adding zinc to iron supplements in women and none found negative effects of combined zinc supplementation on iron status or anemia (Table 3). Kolsteren et al (33) randomly assigned 171 nonpregnant anemic women in Bangladesh to receive 1) iron, 2) iron and vitamin A, or 3) iron, vitamin A, and zinc; the results of a comparison of the groups that received the latter 2 treatments are reported here. Hemoglobin, serum ferritin, serum iron, percentage transferrin saturation (P < 0.0001 for all), and total-iron-binding capacity (P < 0.05) increased after 8 wk of daily supplementation with iron and vitamin A, with or without zinc. The women in this study were asked to take the iron and zinc supplements with separate meals; no attempt was made to ascertain whether the study participants complied with this request.

In Peru (5, 34, 35), 1295 pregnant women (33% anemic) were assigned to daily supplementation with iron and folic acid (IFA),...
with or without zinc. There were no differences between the groups supplemented with IFA alone or iron IFA and zinc on maternal hemoglobin (or proportion anemic) or serum ferritin concentrations at 28–30 or 37–38 wk of gestation or in cord blood hemoglobin or serum ferritin at delivery (35). There were no significant differences between the groups in length of gestation, fetal growth, or birth weight.

In the previous described trial in Nepal (15), night-blind pregnant women were also assessed for anemia and were supplemented with iron if needed. In women given IFA supplements, the addition of zinc resulted in small, nonsignificant differences between the zinc plus IFA and IFA-alone group: increases in hemoglobin (4.8 and 7.8 g/L, respectively, and the increases were significantly greater than in the zinc-supplemented groups by 14.0 g/L and 13.0 g/L, respectively, and the increases were significantly greater than in the zinc-supplemented groups by 14.0 g/L and 13.0 g/L, respectively). In another trial in Nepal, Christian et al (36–38) randomly assigned 4926 pregnant women to 1 of 5 supplement types: 1) folic acid, 2) IFA, 3) IFA and zinc, 4) multiple micronutrients (data not reported), and 5) control (vitamin A, as received by all other women). Birth size was measured in 4130 live-born infants. In all of the groups that received iron, supplementation prevented a decline in hemoglobin from baseline to the third trimester. The addition of zinc did not have a statistically significant effect on the prevalence of severe anemia in the third trimester or on any other iron indicator (38). Birth weight was highest in children born to women supplemented with IFA [37 g (95% CI: 16, 90) higher than in the control group and 53 g (95% CI: 0, 108) higher than in the IFA and zinc-supplemented group]. No statistically significant effect was observed on the number of preterm births (37). In the same trial, fetal loss and infant mortality outcomes were also evaluated (36). No effects on fetal loss were found with any of the supplements. In the first 3 mo of life, infants whose mothers received folic acid, IFA, or IFA plus zinc during pregnancy showed a trend of 15–20% reduction in mortality compared with control infants.

Three previously described studies of infants found mixed results in terms of the effects of zinc plus iron supplementation on iron status. In one Indonesian trial, there was no significant difference in hemoglobin or serum ferritin concentrations; however, more infants remained anemic (<110 g/L) after receiving iron and zinc supplements than after receiving iron alone (46% compared with 28%; P < 0.05) (17). In another Indonesian study (18), supplementation with iron alone increased hemoglobin (119.4 compared with 115.3 g/L; P < 0.05) and serum ferritin (46.5 compared with 32.3 μg/L; P < 0.05) more than did supplementation with iron and zinc. The prevalence of anemia declined more in the iron-supplemented children than in the iron-and zinc-supplemented children (P = 0.026); however, there was no difference in the prevalence of IDA between the 2 groups. In contrast, in Bangladeshi children (19), serum ferritin decreased in all groups after supplementation, but less so in the iron-and zinc-supplemented children (−13.7 μg/L; P < 0.05 compared with control) than in the children supplemented with iron alone (−18.0 μg/L; P < 0.3 compared with control).

In the previously described Mexican trial (20), hemoglobin concentrations increased in both the iron-only and iron- and zinc-supplemented groups by 14.0 g/L and 13.0 g/L, respectively, and the increases were significantly greater than in the placebo group (8.0 g/L; P < 0.05). Both the iron-alone (P < 0.05) and the iron plus zinc (P < 0.0001) groups had significant increases in plasma ferritin concentrations from baseline to post follow-up. Schultink et al (39) randomly assigned 67 anemic Indonesian children to receive iron or iron plus zinc daily for 8

### TABLE 2

Studies that assessed the effect of iron supplementation on zinc-status indicators

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Enrollment criteria</th>
<th>Comparison groups</th>
<th>Duration of study</th>
<th>Zinc indicators</th>
<th>Effect on zinc status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dijkhuizen et al, 2001 (17)</td>
<td>Indonesia</td>
<td>4-mo-old infants</td>
<td>10 mg Fe (n = 120) and placebo (n = 119)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Lind et al, 2003 (18)</td>
<td>Indonesia</td>
<td>6-mo-old infants</td>
<td>10 mg Fe (n = 136) and placebo (n = 143)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Baqui et al, 2003 (19)</td>
<td>Bangladesh</td>
<td>6-mo-old infants</td>
<td>1 mg Rb + 20 mg Fe (n = 165) and 1 mg Rb (control; n = 157)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Munoz et al, 2000 (20)</td>
<td>Mexico</td>
<td>18–36-mo-old children</td>
<td>20 mg Fe (n = 54) and placebo (n = 56)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Yip et al, 1985 (26)</td>
<td>United States</td>
<td>12-mo-old infants</td>
<td>30 mg Fe and placebo (n = 219)</td>
<td>3 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Friel et al, 2003 (27)</td>
<td>Canada</td>
<td>1-mo-old term, breastfed infants</td>
<td>7.5 mg Fe (n = 19) and placebo (n = 19)</td>
<td>5 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Smuts et al, 2005 (28)</td>
<td>South Africa</td>
<td>6–12-mo-old infants</td>
<td>10 mg Fe (n = 68) and placebo (n = 66)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Lopez de Roman et al, 2005 (29)</td>
<td>Peru</td>
<td>6–12-mo-old infants</td>
<td>10 mg Fe (n = 82) and placebo (n = 82)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Hop et al, 2005 (30)</td>
<td>Vietnam</td>
<td>6–12-mo-old infants</td>
<td>10 mg Fe (n = 75) and placebo (n = 73)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>No effect</td>
</tr>
<tr>
<td>Untoro et al, 2005 (31)</td>
<td>Indonesia</td>
<td>6–12-mo-old infants</td>
<td>10 mg Fe (n = 69) and placebo (n = 60)</td>
<td>6 mo</td>
<td>Serum zinc</td>
<td>Negative effect on the proportion of zinc-deficient subjects after supplementation</td>
</tr>
</tbody>
</table>

1 Rb, riboflavin.
wk. Although both groups showed significant increases in hemoglobin after supplementation, the change in the iron-alone group (18 g/L) was significantly greater than the change in the iron-alone wk. Although both groups showed significant increases in he-

TABLE 3
Studies that assessed the effects of the addition of zinc to iron supplementation on iron-status indicators and clinical outcomes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Enrollment criteria</th>
<th>Comparison groups</th>
<th>Duration of study</th>
<th>Iron indicators</th>
<th>Effect on iron indicators and clinical outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kolsteren et al,</td>
<td>Bangladesh</td>
<td>15–45-y-old women, hemoglobin &lt;100 g/L</td>
<td>Vitamin A + 60 mg Fe (n = 57) and vitamin A + Fe + 15 mg Zn (n = 58)</td>
<td>60 d</td>
<td>Hemoglobin, ferritin, transferrin saturation, TIBC, serum iron</td>
<td>No effect</td>
</tr>
<tr>
<td>Caulfield et al,</td>
<td>Peru</td>
<td>Pregnant women, gestation between 10 and 24 wk</td>
<td>IFA [60 mg Fe + 250 µg folic acid (n = 495)] and IFA + 15 mg Zn (n = 521)</td>
<td>Untill 4 wk postpartum</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Christian et al,</td>
<td>Nepal</td>
<td>Pregnant women, night blindness</td>
<td>Vitamin A and β-caro ten or placebo plus 25 mg Zn + IFA [60 mg Fe + 400 µg folic acid (n = 64)] or placebo + IFA (n = 64)</td>
<td>3 wk</td>
<td>Hemoglobin</td>
<td>No effect</td>
</tr>
<tr>
<td>Christian et al,</td>
<td>Nepal</td>
<td>Newly pregnant women</td>
<td>IFA [60 mg Fe + 400 µg folic acid (n = 772)] and IFA + 30 mg Zn (n = 827)</td>
<td>Until 12 wk postpartum</td>
<td>Hemoglobin, serum ferritin, transferrin, serum iron</td>
<td>No effect on biochemical indicators</td>
</tr>
<tr>
<td>Dijkhuizen et al,</td>
<td>Indonesia</td>
<td>4-mo-old infants</td>
<td>10 mg Fe (n = 120) and 10 mg Fe + 10 mg Zn (n = 120)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>No effect of zinc on hemoglobin or serum ferritin; antagonism of zinc on ability of iron to correct anemia</td>
</tr>
<tr>
<td>Lind et al, 2003</td>
<td>Indonesia</td>
<td>6-mo-old infants</td>
<td>10 mg Fe (n = 136) and 10 mg Fe + 10 mg Zn (n = 136)</td>
<td>6 mo</td>
<td>Hemoglobin, serum ferritin</td>
<td>Smaller effect on hemoglobin, ferritin, and transferrin receptor than iron alone</td>
</tr>
<tr>
<td>Baqui et al, 2003</td>
<td>Bangladesh</td>
<td>6-mo-old infants</td>
<td>1 mg Rb + 20 mg Fe (n = 165) and 1 mg Rb + 20 mg Fe + 20 mg Zn (n = 162)</td>
<td>6 mo</td>
<td>Serum ferritin</td>
<td>Positive benefit of the addition of zinc on serum ferritin</td>
</tr>
<tr>
<td>Munoz et al, 2000</td>
<td>Mexico</td>
<td>18–36-mo-old children</td>
<td>20 mg Fe (n = 54) and 20 mg Fe + 20 mg Zn (n = 55)</td>
<td>6 mo</td>
<td>Hemoglobin, plasma ferritin</td>
<td>No effect</td>
</tr>
<tr>
<td>Schultink et al,</td>
<td>Indonesia</td>
<td>30–50-mo-old children, &lt;−2 HAZ, hemoglobin &lt;110 g/L</td>
<td>30 mg Fe (n = 42) and 30 mg Fe + 30 mg Zn (n = 43)</td>
<td>8 wk</td>
<td>Hemoglobin</td>
<td>Antagonism of zinc on hemoglobin</td>
</tr>
</tbody>
</table>

\(^1\) TIBC, total-iron-binding capacity; IFA, iron and folic acid; Rb, riboflavin; HAZ, height-for-age z score.

Does the addition of iron to zinc supplementation affect zinc status or functional outcomes?

In 4 trials among infants and children, there were no adverse effects on plasma zinc concentrations of adding iron to zinc supplementation, and one trial suggested a benefit on morbidity (Table 4). In Indonesian infants (17) there was no difference in plasma zinc concentrations between children who received iron and zinc supplements and children who received zinc alone after 6 mo of supplementation. Growth was also assessed in this study, but no effect of supplementation was observed (17). In the other Indonesian trial (18), there was also no difference in serum zinc concentrations between the iron- and zinc-supplemented children and the children supplemented with zinc alone. In the Bangladeshi trial (19), serum zinc concentration improved in both the zinc-supplemented (0.08 mg/L; P < 0.01) and the iron- and zinc-supplemented children (0.07 mg/L; P < 0.01). The zinc plus iron group had a greater increase in serum zinc (P = 0.05) than
did the control group, after adjustment for age and baseline concentrations. The iron- and zinc-supplemented infants had a 19% lower relative risk of severe diarrhea than did the control infants ($P < 0.05$) during the 6 mo of the study. Zinc alone resulted in a nonsignificant 2% decreased relative risk of severe diarrhea. When malnourished infants ($< -1$ weight-for-age $z$ score) were examined separately, those who were supplemented with iron and zinc had a 30% lower risk of severe diarrhea ($P < 0.01$) and a 40% lower risk of severe acute lower respiratory tract infections ($P < 0.05$) than did the control group.

In a previously described population of Mexican toddlers (20), zinc concentrations increased in both zinc groups with and without iron and were not significantly different from each other. The zinc-supplemented groups (with or without iron) had significantly fewer total illness episodes and diarrhea episodes per child than did the placebo group (25). The coadministration of iron and zinc did not reduce the benefit of zinc.

Iron supplementation, in combination with zinc supplementation, does not appear to have an effect on serum zinc concentrations, but only 2 of the studies reviewed considered morbidity outcomes—an important indicator of subclinical zinc deficiency in populations with low-zinc diets (32). Because the success of zinc-supplementation programs would be measured in improvements in morbidity indicators, not serum zinc status, these clinical outcomes are of great importance.

**Evidence assessing joint supplementation on growth and development indicators**

Iron and zinc are both important components of neural function and are essential for childhood growth and development (4). One study assessed the effect of zinc, iron, or both on infant development. The previously described Bangladeshi infants were also assessed for developmental outcomes (19, 40). At follow-up, the iron- and zinc-supplemented group had a significantly smaller decrease in the Psychomotor Development Index (PDI) than did the control group. The PDI is a motor development score obtained from the Bayley Scales of Infant Development II; higher scores indicate a better outcome. There were no differences in PDI scores between the supplementation groups.

Lind et al (41) assessed 650 Indonesian infants supplemented daily with iron, zinc, both iron and zinc, or placebo. Zinc alone increased weight-for-age $z$ scores and knee-heel length. Iron alone increased knee-heel length. In addition, PDI also improved in the iron-only group compared with the placebo group. Joint supplementation had no effect on either growth or developmental outcomes. Growth was also assessed in the previously described supplementation trial of Mexican children (25). After 12 mo of supplementation with iron, zinc, both iron and zinc, or placebo, growth was not significantly different between supplementation groups.

**SUMMARY AND CONCLUSIONS**

Supplementation with multiple micronutrients would be an appealing strategy for the prevention and treatment of anemia and common morbidities that affect women and young children. However, drawing definitive conclusions regarding the potential benefit or harm of joint supplementation, based on a variety of study designs, target populations, and outcome measures has proven challenging.

This review found that joint supplementation generally does not negatively affect the biochemical outcomes expected from individual supplementation. Three of 9 trials (Table 3) found that zinc may reduce the beneficial effect of iron supplements on iron status, but this negative interaction does not appear to be great enough to discourage joint supplementation. Even in the presence of zinc, the benefit of iron supplementation on iron indicators was significant and important. Iron does not appear to have a negative effect on serum zinc concentrations; if there is an effect, it is small.

Limited data exist on the effect of dual supplementation on infectious disease morbidity, growth, and child development. Many trials have shown a reduction in diarrhea and pneumonia morbidity with zinc supplementation (13, 42). Whereas one study showed that supplementation with both zinc and iron had a slightly greater benefit on diarrhea and respiratory morbidity than did zinc alone (19), more evidence is needed to confirm that the concurrent provision of iron does not reduce the benefits of zinc on infectious morbidity. Finally, additional information is needed to understand the effect of joint zinc and iron supplementation on growth and development.

The currently available data do not allow for firm conclusions on the existence of interactions between iron and zinc, when given together, on biochemical or functional outcomes. Clear programmatic recommendations cannot be made without further studies. Trials in nonanemic pregnant women should be done to ensure that the most beneficial combination of iron and zinc supplementation is promoted to ensure adequate stores before...
pregnancy. Trials in pregnant women are needed to determine whether zinc supplements provide benefits to the mother or infant and to verify that the addition of iron to maternal iron supplements will not diminish the benefits of iron on anaemia or birth weight.

Additional trials in children aged <5 y are needed to ensure that the addition of iron to zinc supplements will not diminish the benefits of zinc supplementation, namely reductions in diarrhea, pneumonia, and other morbidities. Because the iron-regulatory mechanisms of infants may differ before and after 9 mo of age (43), studies in both of these age groups are needed to understand more fully the health effects of micronutrient supplementation. Finally, limited data suggest that supplementation with both iron and zinc may prevent developmental delays that may otherwise occur in the first year of life in vulnerable populations. Future studies need to assess the effect of joint supplementation on growth and child development and not just the global measures of cognitive functions to more specific developmental outcomes, for which the benefits of treatment and interactions might be more easily observed.

Thus far, iron and zinc interaction studies have focused on the effects of antagonisms on biochemical indicators. Although these effects should be measured in future trials, clinically meaningful health outcomes in mothers and infants will provide more useful information than will biochemical outcomes alone.

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