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ZINC AND MULTIVITAMIN SUPPLEMENTATION HAVE CONTRASTING EFFECTS ON INFANT IRON STATUS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

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Abstract

BACKGROUND—Zinc supplementation adversely affects iron status in animal and adult human studies but few, trials have included young infants.

OBJECTIVE—To determine the effects of zinc and multivitamin supplementation on infant hematologic and iron status.

METHODS—In a double-blind RCT, Tanzanian infants were randomized to daily, oral zinc (Zn), multivitamins (MV), Zn and MV, or placebo treatment arms at age 6 wk. Hemoglobin

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AUTHORS' CONTRIBUTIONS

All authors have read and approved the submitted version of this manuscript. RCC analyzed the data and wrote the manuscript; KM designed the study, supervised data collection, and provided inputs to the manuscript; SA oversaw all laboratory aspects of the study, and reviewed the manuscript; JE performed iron status assays, assisted with results interpretation, and reviewed the manuscript; KG assisted with study design, sample management, and reviewed the manuscript; RK provided inputs to the study design, oversaw data collection, and reviewed the manuscript; EL assisted with statistical analysis and reviewed the manuscript; WFF designed the study, provided inputs to the statistical analysis, and reviewed the manuscript; CM oversaw data management, provided inputs to the statistical analysis, and reviewed the manuscript; CPD designed the study, oversaw study implementation; contributed to the statistical analysis, and provided inputs to the manuscript.

Conflicts of interest: Juergen Erhardt is owner of the company VitMin Lab (Willstaett, Germany), where some of the assays performed in this study were conducted. Roland Kupka is a UNICEF staff member. The opinions and statements in this article are those of the author and may not reflect official UNICEF policies. The other authors have no conflicts of interest to disclose.

concentration (Hb) and red blood cell indices were measured at baseline and at 6, 12, and 18 mo of age. Plasma samples from 589 infants were examined for iron deficiency (ID) at 6 mo.

RESULTS—In logistic regression models, Zn treatment was associated with greater odds of ID (OR 1.8 [95% CI 1.0–3.3]), and MV treatment was associated with lower odds (OR 0.49 [95% CI 0.3–0.9]). In Cox models, MV were associated with a 28% reduction in risk of severe anemia (HR=0.72 [95% Cl 0.56–0.94]) and a 26% reduction in risk of severe microcytic anemia (HR=0.74 [0.56–0.96]) through 18 months. No effects of Zn on risk of anemia were seen. Infants treated with MV alone had higher mean Hb (9.9 g/dL [95% CI 9.7–10.1]) than those given placebo (9.6 g/dL [9.4–9.8]) or Zn alone (9.6 g/dL [9.4–9.7]).

CONCLUSIONS—MV treatment improved iron status in infancy, whereas Zn worsened iron status but without an associated increase in risk for anemia. Infants in long-term zinc supplementation programs at risk for ID may benefit from screening and/or the addition of a multivitamin supplement.

Keywords

Iron supplementation; zinc supplementation; iron deficiency; iron deficiency anemia; anemia; microcytic anemia

INTRODUCTION

Iron deficiency, alone or with resulting anemia, continues to be the most prevalent micronutrient deficiency in the world (1,2). It is associated with poor cognitive function, decreased work performance, and an increase in the frequency of low birth weight, prematurity, and perinatal mortality. Adverse effects of zinc supplementation on iron status have been demonstrated in a number of animal and adult human studies, including lowered iron absorption, hemoglobin and serum ferritin concentrations (3–6). The Dietary Reference Intakes (DRI) lists disruptions in iron metabolism as one of the early signs of excessive zinc intake (7). The impact of zinc supplementation on iron status in children is less clear, as the results of clinical trials examining interactions between iron and zinc have varied widely. Hemoglobin and ferritin improve more when iron is given alone than when paired with zinc supplementation (8,9). However, two meta-analyses have concluded that zinc supplementation has not been shown to decrease hemoglobin or ferritin concentrations in children (10,11). Of note, few trials have included infants age six months or younger.

In contrast to the potential negative effects of zinc on iron status, we and others have shown that multivitamin supplementation has beneficial effects on hemoglobin in young children in resource-limited settings. Two large trials among young infants, one in multiple countries (12) and another in a malaria endemic region (13), found that supplementation with multiple micronutrients was more effective than iron alone in the prevention and treatment of anemia. In secondary analyses of a randomized trial in infants born to HIV-infected women in Tanzania, we found that daily multivitamin supplementation (vitamin B complex, C and E) from ages 6 weeks to 25.5 months led to higher hemoglobin concentrations and lower risk for anemia, compared with placebo (14).

We conducted a trial comparing the effects of daily supplementation with zinc, multivitamin, combination zinc and multivitamin, or placebo from ages 6 weeks to 19.5 months on infectious morbidity in infants born to HIV-negative mothers in Dar es Salaam, Tanzania (15). In secondary analyses of data from this study, we aimed to examine effects of zinc and multivitamin supplementation on infant iron and hematologic status.

METHODS

Study design and participants

This study is a secondary analysis of a randomized, double-blind, 2 × 2 factorial design trial (15). Consenting HIV-negative mothers were enrolled shortly after or before delivery, and their infants were randomized between 5–7 weeks of age. The sample size was chosen based on power calculations for the primary endpoints of the parent study (# lower respiratory illness episodes/year). Exclusion criteria were: multiple births, congenital anomalies, and other conditions that would interfere with the study procedures.

All study procedures were approved by the Harvard T.H. Chan School of Public Health Human Subjects Committee, the Muhimbili University of Health and Allied Science Senate Research and Publications Committee, the Tanzanian National Institute of Medical Research, and the Tanzanian Food and Drugs Authority. All mothers provided written informed consent.

Randomization and masking

Infants were randomly assigned to receive one of four daily oral regimens for 18 months from the date of randomization: 1) Zinc (Zn); 2) Multivitamins (MV); 3) Zn+MV; 4) Placebo. A randomization list (1 to 2400) was created by a biostatistician, using blocks of 20, with stratification by study clinic. Supplements were manufactured by Nutriset (Malaunay, France), prepared as an orange-flavoured powder in opaque gelatinous capsules. In field testing, all four regimens were found to be indistinguishable between groups in taste, smell and appearance. All study personnel and participants remained blinded to treatment assignment for the duration of the study.

Procedures

Infants received one capsule per day from randomization through 6 months of age, and two capsules/day from 7 months of age to end of follow-up. The capsule provided to infants in the Zn group contained 5 mg zinc sulfate ($ZnSO_4$). The capsule provided to the MV group contained 60 mg vitamin C, 8 mg vitamin E, 0.5 mg vitamin B-1, 0.6 mg vitamin B-2, 4 mg niacin, 0.6 mg vitamin B-6, 130 μg folate, and 1 μg vitamin B-12. The MV+Zn group received the micronutrients listed in both the MV and Zn groups in a single capsule. Supplement dosing was chosen to maximize the likelihood of seeing an impact of supplementation (with doses substantially above the RDA), while staying within a range considered safe for young children. From ages 0–6 months, doses were between 150–600% of the Recommended Dietary Allowance (RDA) or Adequate Intake (AI); from 7–12 months of age, 200–400% of the RDA or AI. These doses were consistent with those used in our previous trial of multivitamin supplementation of HIV-exposed children (8). Mothers and

children were followed for 18 months from the date of randomization, or until the child's death or loss to follow-up. Mothers and children returned to the study clinic every four weeks for data collection and standard clinical care. Demographic data were obtained in interviews with the mother at recruitment and monthly follow-up visits. Birth data were collected from medical records.

Iron and hematologic status

Complete blood count assays were performed on venous blood draws at randomization and ages 6, 12, and 18 months using the AcT5 Diff AL hematology analyzer (Beckman Coulter, Jersey City, NJ, USA). Plasma ferritin, alpha-1-acid glycoprotein (AGP), and soluble transferrin receptor (sTfR) were measured using an enzyme-linked immunosorbent assay (ELISA; (16) in samples drawn at age 6 months and frozen at −80 °C from a subset of infants (n = 589) as part of a separate study of enteric dysfunction and growth. To be eligible for those analyses, children were required to have a blood sample available at 6 weeks and 6 months of age, and have a length-for-age Z score (LAZ) >= −2 at 6 weeks of age.

Anemia was defined as hemoglobin concentration (Hb) < 10.0 g/dL at baseline (six weeks of age) and < 11.0 g/dL during follow-up, and severe anemia was defined as hemoglobin < 8.5 g/dL at any age (17). Microcytosis was defined as mean corpuscular volume (MCV) < 70 fL. (17) Iron deficiency was defined as ferritin < 12 μg/L or, in cases where AGP ≥ 1.0 g/L, sTfR > 8.3 mg/L (18).

Statistical Analyses

Children with hemoglobin measured at baseline and at least one follow-up hemoglobin measure were included in analyses (N = 2006). Chi square tests were used to examine the proportion of infants with iron deficiency at age 6 months in each treatment arm. ANOVA was used to compare mean Hb, ferritin, and sTfr between treatment arms at age 6 months. Logistic regression models were used to examine treatment effects on odds of developing iron deficiency at age 6 months. Cox proportional hazard models were used to examine treatment effects on the risk of developing anemia and anemia subtypes (e.g., severe, microcytic) during follow-up. We excluded children who had anemia (remaining n = 1387) or severe anemia (remaining n = 1930) at baseline from the respective analyses conducted to determine the risk of developing anemia and anemia subtypes. ANOVA models with Bonferroni correction for post-hoc comparisons were used to compare hemoglobin among different treatment groups at baseline and ages 6, 12, and 18 months. Analyses were conducted in SAS software Version 9.1 (SAS Institute, Cary, NC, USA). Two-sided p-values < 0.05 were considered statistically significant.

RESULTS

Among infants who met criteria for Hb analyses, sample characteristics were similar between treatment regimen arms (Table 1). Baseline characteristics were similar between infants with (n = 2006) and without (n = 394) sufficient Hb values to be included in Hb analyses and similar between infants selected for iron status assays at age 6 months (n = 589) and the rest of the cohort, with the exception of age at randomization. Infants with 6-

month iron status values were 0.4 (95% CI 0.1 – 0.7) days younger at enrollment than those without iron status values. Baseline demographic and birth characteristics are also shown in Table 1. Baseline hemoglobin values were similar between treatment arms.

Table 2 compares iron status indicators at age 6 months among treatment arms. Infants treated with MV alone had the lowest prevalence of iron deficiency, whereas those treated with Zn alone had the highest. There were no differences among treatment arms in mean ferritin, ferritin adjusted for AGP, and sTfR. In logistic regression models, Zn treatment was associated with greater odds of iron deficiency [OR 1.8 (95% CI 1.0 – 3.3); $p < .05$], and MV treatment was associated with lower odds [OR 0.49 (95% CI 0.3 – 0.9); $p < .05$].

In Cox models, neither MV nor Zn treatment were associated with increased risk of developing anemia or microcytic anemia during the follow up period (Table 3). However, MV use was associated with a 28% reduction in the risk of severe anemia (HR = 0.72 [95%CI 0.56 – 0.94]) and a 26% reduction in the risk of severe microcytic anemia (HR = 0.74 [95% CI 0.56 – 0.96]) through age 18 mo. No effects of Zn on risk of severe anemia or severe microcytic anemia were seen. No interaction effects were seen for sex, low birthweight, preterm delivery, or baseline hemoglobin (data not shown).

Infants treated with MV alone had higher mean Hb than those given Zn alone at 12 mo (9.9 vs. 9.6 g/dL, $p \leq .05$) and 18 mo (9.9 vs. 9.6 g/dL, $p \leq .05$ (Table 4)). Infants treated with MV alone had higher mean Hb than those given placebo at 18 months (9.9 vs 9.6 g/dL, $p \leq .05$). In linear regression models including both MV and Zn treatment, MV treatment was associated with an increase in Hb of 0.2 g/dL ($p \leq .01$) at 12 months and 0.3 g/dL ($p \leq .001$) at 18 months. No differences in Hb values between infants treated with Zn and those with placebo or Zn + MV therapy were observed at any age.

DISCUSSION

In this trial among young infants comparing the effects of daily supplementation with zinc, multivitamin, zinc plus multivitamin, or placebo from ages 6 weeks to 18 months, Zn treatment was associated with a higher risk of iron deficiency at 6 months but no longer-term increase in risk of anemia, while MV treatment was associated with a lower risk of both iron deficiency and anemia. These findings are potentially important as zinc supplementation programs are increasingly implemented in global health initiatives.

Our finding that Zn treatment was not associated with changes in hemoglobin or risk of anemia is consistent with prior studies that have failed to show effects of zinc supplementation on hemoglobin concentrations in children (10,11,19). However, the odds of developing iron deficiency at age 6 months were increased by over 80% in infants treated with Zn, suggesting that Zn treatment for 18 months may worsen iron status but not to a degree severe enough to affect hemoglobin production. Anemia is a late marker of iron deficiency, as iron is preferentially shunted to hemoglobin production from tissues, including the brain, until iron deficiency becomes severe. Furthermore, iron deficiency without anemia during infancy has been associated with delays in cognitive and socioemotional development, some of which are irreversible (20–23). The importance of the

age at which the effect of Zn was seen, 6 months, is underscored by the fact that most public health iron deficiency screening programs begin at age 9 or 12 months. Future studies are needed to determine whether and how the duration and/or dosage level of Zn treatment are needed before the risk of iron deficiency increases.

Previous clinical trials of iron and zinc supplementation in children have also addressed this issue. Among Mexican preschoolers, iron plus zinc increased serum ferritin less than iron alone (9). In Indonesia, the combination of both zinc (10 mg/d) and iron (10 mg/d) in infants was less effective than iron alone in reducing the occurrence of anemia (8). The effects of Zn treatment on iron status may be the result of competitive inhibition of iron absorption or utilization. Evidence suggests that zinc may compete with iron at two levels: the divalent metal transporter-1 (DMT-1) (the protein responsible for importing ferrous iron into the apical membranes of the gastrointestinal epithelial cell) and ferroportin (FPN) (the regulator of iron efflux across the basolateral membrane). Competitive inhibition of iron at these sites would thus reduce transfer of dietary iron to the bloodstream. In adult humans, zinc supplementation has been shown to reduce absorption of radio-labeled iron when the weight ratio of zinc dietary intake to iron dietary intake exceeded 5:1 (6). For infants who were mostly breastfed, this ratio may have been easily exceeded by the zinc supplementation dosage provided in this study given the relatively low iron content of breastmilk. The zinc compound used may impact potential effects on iron absorption. In a study of Indonesian school-aged children, iron absorption from flour fortified with both iron and zinc oxide was superior to flour fortified with iron and zinc sulfate, the compound used in the current study (24).

Although early iron status is largely dependent on fetal iron stores, complementary feeding was started before two months in this cohort on average (with no differences between treatment arms), making potential disruptions in intestinal iron absorption potentially more impactful. Other measured risk factors for iron deficiency, such as premature delivery and low birthweight, were similar between treatment arms. Unmeasured potential factors affecting prenatal and infant iron status include maternal iron status and lack of delayed cord clamping, but given the RCT study design, these factors were likely evenly distributed among treatment arms. Moreover, there were no group differences in baseline hemoglobin concentrations among treatment groups.

MV treatment was associated with a 26% lower risk of developing severe anemia and severe microcytic anemia during the follow up period. The fact that the magnitude of MV-associated risk reduction was similar for severe anemia, which may have many causes, and severe microcytic anemia, which is mainly caused by iron deficiency, suggests that the beneficial effects of MV treatment on hematologic status were largely due to improved iron status. In addition, MV treatment reduced the odds of developing iron deficiency at 6 months by over 50%. There are several potential mechanisms underlying the benefits of MV supplementation on iron status. Vitamin C enhances intestinal iron absorption (25). Vitamin C and E both inhibit oxidative damage of erythrocyte cell membranes (26,27). B vitamins, particularly riboflavin (B-2) and B-6, play crucial roles in hemoglobin synthesis, thereby potentially decreasing anemia (28). These results extend our previous finding of improved hematologic status among infants with vertical HIV exposure treated with the same MV

regimen to children without HIV exposure. Of note, both the prevalence of iron deficiency at age 6 months and hemoglobin concentrations at all ages examined were similar between infants receiving combined MV+Zn treatment and those receiving placebo. Thus, the beneficial effects of MV supplementation on iron status may counterbalance potential detrimental effects of Zn on iron status.

Several strengths of our study deserve comment. To our knowledge, our study is the first to examine the effects of zinc and multivitamin supplementation in African infants from six weeks of age and one of the first to examine hematologic and iron endpoints as early as 6 months of age. We used a 2×2 factorial double-blinded randomized control design with a large number of participants. Serial hematologic measurements at ages 6, 12, and 18 months allowed us to evaluate the effects of supplementation from age 6 months, when iron status is generally reflective of fetal iron stores, through the infancy period, when rates of iron deficiency typically increase. Our examination of iron status measures in addition to hematologic measures enabled us to detect effects on iron status even in the absence of effects on hemoglobin production. Furthermore, we examined both ferritin and soluble transferrin receptor, as well as AGP, which allowed us to detect iron deficiency even in the setting of inflammation.

Limitations of this dataset also exist. After age 6 months, our measures of iron status were limited to hematologic data, with microcytic anemia as a proxy indicator of iron deficiency. Iron deficiency is the most common cause of microcytic anemia, and while environmental lead exposure and hemoglobinopathies may also cause microcytic anemia, these factors were likely distributed evenly among treatment arms, given the randomized trial design. Given our lack of iron status measures at later ages, we were not able to examine how effects of MV and Zn treatment on iron status may have changed over time. It is unclear whether the lack of an association between MV treatment and non-severe anemia was due to a true lack of effect or inadequate power, since the number of subjects in related analyses was smaller than in the number included in analyses examining severe anemia, in which beneficial effects of MV supplementation were seen. This study did not have an iron supplementation arm, and we could thus not examine potential effects of combination therapy with iron and zinc and/or multivitamins. Due to limits on blood volumes obtained from infants, biochemical measures of infant zinc status were not available. Potential effects of maternal micronutrient deficiencies, which are common during lactation in Sub-Saharan Africa (29), were not examined in the current study, but given the RCT study design, such deficiencies were likely evenly distributed among treatment arms.

CONCLUSIONS

In this 2×2 factorial double-blind randomized control trial, treatment with multivitamins was associated with reduced risk of both iron deficiency and severe microcytic anemia, while zinc treatment was associated with increased risk of iron deficiency but no longer term increase in risk of anemia. Given the potential for neurodevelopmental sequelae of iron deficiency during infancy, even in the absence of anemia, infants in long-term zinc supplementation programs who are at risk for iron deficiency may benefit from screening and/or the addition of a multivitamin supplement, such as the one studied in this trial.

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Abbreviations

AGP	α-1-acid glycoprotein
ELISA	enzyme-linked immunosorbent assay
MV	multivitamin
SGA	small for gestational age
sTfR	soluble transferrin receptor
Zn	zinc

References

1. Longo DL, Camaschella C. Iron-Deficiency Anemia. N Engl J Med. 2015; 372:1832–43. [PubMed: 25946282]
2. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr. 2006; 84:1261–76. [PubMed: 17158406]
3. Crofton RW, Gvozdanovic D, Gvozdanovic S, Khin CC, Brunt PW, Mowat NA, et al. Inorganic zinc and the intestinal absorption of ferrous iron. Am J Clin Nutr. 1989; 50:141–4. [PubMed: 2502004]
4. Lim KHC, Riddell LJ, Nowson CA, Booth AO, Szymlek-Gay EA. Iron and zinc nutrition in the economically-developed world: a review. Nutrients. 2013; 5:3184–211. [PubMed: 23945676]
5. Olivares M, Pizarro F, Ruz M. Zinc inhibits nonheme iron bioavailability in humans. Biol Trace Elem Res. 2007; 117:7–14. [PubMed: 17873388]
6. Rossander-Hultén L, Brune M, Sandström B, B L, Hallberg L. Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nutr. 1991; 54:152–6. [PubMed: 2058577]
7. Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academy Press; 2001.
8. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Martuti S. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. J Nutr. 2001; 131:2860–5. [PubMed: 11694609]
9. Rosado JL, López P, Muñoz E, Martinez H, Allen LH. Zinc supplementation reduced morbidity, but neither zinc nor iron supplementation affected growth or body composition of Mexican preschoolers. Am J Clin Nutr. 1997; 65:13–9. [PubMed: 8988907]
10. Brown KH, Peerson JM, Baker SK, Hess SY. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. Food Nutr Bull. 2009; 30:S12–40. [PubMed: 19472600]
11. Dekker LH, Villamor E. Zinc Supplementation in Children Is Not Associated with Decreases in Hemoglobin Concentrations. J Nutr. 2010; 140:1035–40. [PubMed: 20335624]
12. Smuts CM, Lombard CJ, Benadé AJS, Dhansay MA, Berger J, Hop LT, et al. Efficacy of a foodlet-based multiple micronutrient supplement for preventing growth faltering, anemia, and micronutrient deficiency of infants: the four country IRIS trial pooled data analysis. J Nutr. 2005; 135:631S–638S. [PubMed: 15735107]

13. Ouédraogo HZ, Dramaix-Wilmet M, Zeba AN, Hennart P, Donnen P. Effect of iron or multiple micronutrient supplements on the prevalence of anaemia among anaemic young children of a malaria-endemic area: a randomized double-blind trial. Trop Med Int Health. 2008; 13:1257–66. [PubMed: 18764815]
14. Liu E, Duggan C, Manji KP, Kupka R, Aboud S, Bosch RJ, et al. Multivitamin supplementation improves haematologic status in children born to HIV-positive women in Tanzania. JIAS. 2013; 16:180222.
15. McDonald CM, Manji KP, Kisenge R, Aboud S, Spiegelman D, Fawzi WW, et al. Daily zinc, but not multivitamin supplementation, reduces diarrhea and upper respiratory infections in Tanzanian infants: a randomized, double-blind, placebo-controlled clinical trial. J Nutr. 2015; 145:2153–60. [PubMed: 26203094]
16. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr. 2004; 134:3127–32. [PubMed: 15514286]
17. Nathan, DG., Orkin, SH. Nathan and Oski's hematology of infancy and childhood. 7. Philadelphia: Saunders/Elsevier; 2009.
18. UNICEF, United Nations University, World Health Organization. Iron Deficiency Anaemia. 2001
19. Fischer Walker C, Kordas K, Stoltzfus RJ, Black RE. Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. Am J Clin Nutr. 2005; 82:5–12. [PubMed: 16002793]
20. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006; 64:S34–43. discussionS72–91. [PubMed: 16770951]
21. Armony-Sivan R, Angelilli ML, Jacobson SW. Dose-Response Relationships between Iron Deficiency with or without Anemia and Infant Social-Emotional Behavior. J Pediatr. 2008:696–702.
22. Shafir T, Angulo-Barroso R, Jing Y, Angelilli ML, Jacobson SW, Lozoff B. Iron deficiency and infant motor development. Early Hum Dev. 2008; 84:479–85. [PubMed: 18272298]
23. Carter RC, Jacobson JL, Burden MJ, Armony-Sivan R, Dodge NC, Angelilli ML, et al. Iron deficiency anemia and cognitive function in infancy. Pediatrics. 2010; 126:e427–34. [PubMed: 20660551]
24. Herman S, Griffin IJ, Suwarti S, Ernawati F, Permaesih D, Pambudi D, et al. Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. Am J Clin Nutr. 2002; 76:813–7. [PubMed: 12324295]
25. Davidsson L, Walczyk T, Zavaleta N, Hurrell R. Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na2EDTA. Am J Clin Nutr. 2001; 73:283–7. [PubMed: 11157325]
26. Shiva Shankar Reddy CS, Subramanyam MVV, Vani R, Asha Devi S. In vitro models of oxidative stress in rat erythrocytes: effect of antioxidant supplements. Toxicol In Vitro. 2007; 21:1355–64. [PubMed: 17714909]
27. Niki E, Komuro E, Takahashi M, Urano S, Ito E, Terao K. Oxidative hemolysis of erythrocytes and its inhibition by free radical scavengers. J Biol Chem. 1988; 263:19809–14. [PubMed: 3198651]
28. Ma AG, Schouten EG, Zhang FZ, Kok FJ, Yang F, Jiang DC, et al. Retinol and riboflavin supplementation decreases the prevalence of anemia in Chinese pregnant women taking iron and folic Acid supplements. J Nutr. 2008; 138:1946–50. [PubMed: 18806105]
29. Papathakis PC, Rollins NC, Chantry CJ, Bennish ML, Brown KH. Micronutrient status during lactation in HIV-infected and HIV-uninfected South African women during the first 6 mo after delivery. Am J Clin Nutr. 2007; 85:182–92. [PubMed: 17209195]

Table 1

Baseline characteristics of mothers and their children enrolled in the trial[a]

	Placebo (n=508)	Zinc only (n=494)	Multivitamins only (n=507)	Multivitamins plus zinc (n=497)
Maternal characteristics				
Age, y	26.5 ± 5.0	26.9 ± 5.2	26.2 ± 5.1	26.2 ± 5.0
Formal education				
None	6 (1.2)	8 (1.6)	7 (1.4)	7 (1.4)
1–7 y	380 (75.1)	351 (71.3)	354 (70.5)	362 (73.0)
≥ 8 y	120 (23.7)	133 (27.0)	141 (28.1)	127 (25.6)
Employment				
Housewife without income	324 (64.2)	309 (63.7)	289 (57.5)	291 (59.2)
Housewife with income	148 (29.3)	141 (29.1)	178 (35.4)	170 (34.6)
Other	33 (6.5)	35 (7.2)	36 (7.2)	31 (6.3)
Marital status				
Married or cohabitating with partner	450 (89.5)	439 (89.6)	461 (91.8)	446 (90.3)
Prior pregnancies				
None	151 (29.8)	137 (27.9)	176 (35.0)	153 (30.9)
1–4	336 (66.4)	343 (69.9)	317 (63.0)	328 (66.3)
≥ 5	19 (3.8)	11 (2.2)	10 (2.0)	14 (2.8)
Mid-upper arm circumference, cm	27.0 ± 3.1	27.1 ± 3.1	27.0 ± 3.1	26.7 ± 3.3
Daily food expenditure per person in household is < 1000 TSh	129 (26.7)	130 (27.9)	136 (28.0)	148 (31.1)
Household possessions[b]				
None	145 (28.9)	160 (32.8)	143 (28.5)	154 (31.1)
1–3	300 (59.3)	251 (51.4)	278 (55.4)	274 (55.4)
≥ 3	61 (12.1)	77 (15.8)	81 (16.1)	67 (13.5)
Child characteristics				
Age at randomization, wk	5.9 ± 0.4	5.9 ± 0.4	5.9 ± 0.4	5.9 ± 0.4
Male sex	249 (49.0)	252 (51.0)	241 (47.5)	257 (51.7)
Low birth weight, <2500 g	13 (2.59	21 (4.3)	17 (3.4)	16 (3.3)
Born < 37 weeks gestational age	59 (12.8)	72 (15.7)	54 (11.6)	57 (12.5)
Born < 34 weeks gestational age	12 (2.6)	12 (2.6)	12 (2.6)	20 (4.4)
Born small for gestational age, <10th percentile	38 (8.5)	38 (8.4)	44 (9.7)	39 (8.7)
Any breastfeeding, mo	13.3 ± 5.9	13.6 ± 5.7	13.3 ± 5.8	13.0 ± 5.8
Exclusive breastfeeding, mo	1.8 ± 1.5	1.9 ± 1.5	1.9 ± 1.6	1.9 ± 1.6

[a] Values are means ± SD or *n* (%). There were no differences in any baseline characteristics between groups (all *p* values > 0.05).

[b] From a list that includes sofa, television, radio, refrigerator, and fan.

Table 2

Infant iron status indicators by treatment arm at age 6 months[a]

	Placebo (N = 151)	Zinc (N = 151)	Multivitamins (N = 147)	Multivitamins plus zinc (N = 144)
Iron deficiency[b] [N (%)]	13 (8.6)	23 (15.2)	7 (4.8)	11 (7.6)
Ferritin, μg/L	48.1 (40.7, 55.4)	50.7 (43.4, 58.1)	51.3 (43.8, 58.7)	51.7 (44.1, 59.2)
adjusted for α-1-acid glycoprotein	47.8 (40.8, 54.9)	51.5 (43.9, 58.2)	51.3 (44.1, 58.6)	51.3 (44.1, 58.6)
Soluble transferrin receptor, mg/L	1.8 (1.7, 2.0)	1.8 (1.7, 2.0)	1.7 (1.5, 1.8)	1.8 (1.6, 1.9)

[a] Values are N (%) or means with 95%CI from ANOVA models.

[b] Iron deficiency was defined as ferritin < 12 μg/L or, where α-1-acid-glycoprotein ≥ 1.0 g/L, soluble transferrin receptor > 8.3 mg/L.

Comparison of iron deficiency prevalence among treatment arms: $\chi 2(3) = 10.62$, $p = .014$.

Table 3

Zinc and multivitamin (MV) treatment Cox regression hazard ratios for risk of anemia outcomes in infants.

	Non-Zinc	Zinc	*P*	Non-MV	MV	*P*
Baseline hemoglobin (Hb) ≥ 11.0 g/dL						
N	718	669		682	705	
Person-months	2168.2	1983.6		2069.4	2082.4	
Anemia						
No. of cases	598	573		562	609	
HR[a] (95% CI)	1.0 (N/A)	1.05 (0.93, 1.18)	0.47	1.0 (N/A)	1.09 (0.97, 1.22)	0.14
Adjusted HR (95% CI)	1.0 (N/A)	1.05 (0.93, 1.18)	0.43	1.0 (N/A)	1.09 (0.97, 1.23)	0.14
Anemia + microcytosis						
No. of cases	163	137		146	154	
HR (95% CI)	1.0 (N/A)	0.91 (0.72, 1.14)	0.41	1.0 (N/A)	1.07 (0.85, 1.34)	0.57
Adjusted HR (95% CI)	1.0 (N/A)	0.91 (0.73, 1.15)	0.43	1.0 (N/A)	1.06 (0.85, 1.33)	0.61
Baseline hemoglobin ≥ 8.5 g/dL						
N	971	959		960	970	
Person-months	8551.2	8707.2		8581.8	8676.6	
Severe anemia						
No. of cases	119	118		137	100	
HR (95% CI)	1.0 (N/A)	1.00 (0.78, 1.30)	0.97	1.0 (N/A)	0.72 (0.56, 0.94)	0.01
Adjusted HR (95% CI)	1.0 (N/A)	0.99 (0.77, 1.28)	0.95	1.0 (N/A)	0.72 (0.56, 0.94)	0.01
Severe anemia + microcytosis						
No. of cases	112	109		127	94	
HR (95% CI)	1.0 (N/A)	1.05 (0.80, 1.37)	0.73	1.0 (N/A)	0.74 (0.56, 0.96)	0.02
Adjusted HR (95% CI)	1.0 (N/A)	1.04 (0.80, 1.36)	0.76	1.0 (N/A)	0.74 (0.56, 0.96)	0.03

HR = hazard ratio; MV = multivitamin.

Table 4

Infant mean hemoglobin concentrations (g/dL) by treatment arm[a]

	Placebo		Zinc		Multivitamins		Multivitamins plus Zinc	
Baseline	508	10.7 (10.6, 10.9)	497	10.6 (10.5, 10.7)	507	10.7 (10.6, 10.8)	494	10.6 (10.5, 10.8)
6 mo	347	10.0 (9.9, 10.1)	350	10.0 (9.8, 10.1)	354	10.0 (9.9, 10.1)	352	10.0 (9.9, 10.1)
12 mo	272	9.7 (9.5, 9.8)[b]	273	9.6 (9.5, 9.7)[c]	271	9.9 (9.7, 10.0)[b,c]	266	9.7 (9.6, 9.9)
18 mo	168	9.6 (9.4, 9.8)[d]	159	9.6 (9.4, 9.7)[c]	165	9.9 (9.7, 10.1)[c,d]	167	9.9 (9.4, 10.0)

[a] Values are Ns followed by mean haemoglobin (g/dL) with 95%CI from ANOVA models.

[b] ANOVA post-hoc comparison with Bonferroni correction between multivitamin and placebo treatment $P \leq 0.10$.

[c] ANOVA post-hoc comparison with Bonferroni correction between multivitamin and zinc treatment $P \leq 0.05$.

[d] ANOVA post-hoc comparison with Bonferroni correction between multivitamin and placebo treatment $P \leq 0.05$.