

A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc¹⁻³

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ABSTRACT

Background: Combined supplementation with iron and zinc during infancy may be effective in preventing deficiencies of these micronutrients, but knowledge of their potential interactions when given together is insufficient.

Objective: The goal was to compare the effect in infants of combined supplementation with iron and zinc and of supplementation with single micronutrients on iron and zinc status.

Design: Indonesian infants ($n = 680$) were randomly assigned to daily supplementation with 10 mg Fe (Fe group), 10 mg Zn (Zn group), 10 mg Fe + 10 mg Zn (Fe+Zn group), or placebo from 6 to 12 mo of age. Venous blood samples were collected at the start and end of the study. Five hundred forty-nine infants completed the supplementation and had both baseline and follow-up blood samples available for analysis.

Results: Baseline prevalences of anemia, iron deficiency anemia (anemia and low serum ferritin), and low serum zinc ($< 10.7 \mu\text{mol/L}$) were 41%, 8%, and 78%, respectively. After supplementation, the Fe group had higher hemoglobin (119.4 compared with 115.3 g/L; $P < 0.05$) and serum ferritin (46.5 compared with 32.3 $\mu\text{g/L}$; $P < 0.05$) values than did the Fe+Zn group, indicating an effect of zinc on iron absorption. The Zn group had higher serum zinc (11.58 compared with 9.06 $\mu\text{mol/L}$; $P < 0.05$) than did the placebo group. There was a dose effect on serum ferritin in the Fe and Fe+Zn groups, but at different levels. There was a significant dose effect on serum zinc in the Zn group, whereas no dose effect was found in the Fe+Zn group beyond 7 mg Zn/d.

Conclusion: Supplementation with iron and zinc was less efficacious than were single supplements in improving iron and zinc status, with evidence of an interaction between iron and zinc when the combined supplement was given. *Am J Clin Nutr* 2003;77:883-90.

KEY WORDS Iron, zinc, infants, randomized controlled trial, anemia, Indonesia, micronutrient supplementation

INTRODUCTION

Infant and child undernutrition is usually associated with micronutrient deficiencies that significantly contribute to the global burden of disease in childhood. Iron deficiency and iron deficiency anemia have long been a concern (1), and zinc deficiency has become a priority more recently (2). Estimates of the global burden of disease for iron deficiency anemia in children are

based on deaths from severe anemia and on disability related to cognitive impairment (3). A crude estimate of zinc deficiency in children indicates that it ranks before iron deficiency anemia but after vitamin A deficiency in the global burden of diseases and disabilities (4). To date, no programs have been implemented to address iron and zinc deficiencies in children. Such programs can either be food-based, including fortification of commonly used foods, or consist of the provision of supplements. In low-income countries, complementary foods for older infants and young children are rarely manufactured at a central location, making efficient food fortification difficult to achieve. Supplementation may therefore be a more realistic alternative.

Iron deficiency anemia is frequently the result of low intakes of dietary iron, low intakes of meat, and high intakes of phytate, an inhibitor of iron absorption. An iron-deficient infant population commonly also has a low dietary intake of zinc, and the lack of meat and the high phytate intake result in a low bioavailability of zinc (5). It has therefore become evident during the past decade that deficiencies of iron and zinc coexist, and that vulnerable groups may benefit from iron as well as zinc supplementation. Zinc intervention trials in infants and children have shown significant improvement in growth (6) and decreased morbidity, particularly from diarrheal disease (7) and malaria (8), although more recent studies have challenged the latter finding (9, 10).

It is consequently reasonable to believe that supplements containing both iron and zinc would be of value in populations with

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high-phytate diets and low meat intakes. However, it has been shown that a high intake of nonheme iron can inhibit the absorption of zinc (11–13), most likely by competition for absorptive pathways. Similarly, it has been shown that a high ratio of zinc to iron can inhibit iron absorption (14, 15). These interactions were found when the micronutrients were given in a water solution, but not when given in meals or in infant formulas (13, 15–18). Thus, it is possible that iron and zinc, when given together as a supplement, may interact with each other and compete for absorption in the small intestine.

We performed a community-based, randomized, double blind, placebo-controlled trial with a factorial design. Our hypothesis was that daily iron treatment and iron combined with zinc treatment for infants from 6 to 12 mo of age would result in both higher hemoglobin concentrations and higher serum ferritin (SF) values than would treatment with placebo. Furthermore, we postulated that this effect would be stronger in the iron group than in the combined iron and zinc supplementation group. Similarly, we postulated that both zinc treatment and iron combined with zinc treatment would result in higher serum zinc concentrations than would treatment with placebo, and that this effect would be stronger in the zinc only group than in the combined treatment group. Finally, we explored sex differences in the main outcomes.

SUBJECTS AND METHODS

Setting

In Central Java, Indonesia, childhood malnutrition is a public health problem. Stunting reportedly affects some 40% of children under 5 y of age (19), and anemia is prevalent, among both women and children (20). Breast-feeding is common and of long duration, but exclusive breast-feeding is rare and complementary foods are introduced early. The plant-based diet of the area contains little animal protein, and low amounts of iron and zinc together with large amounts of phytate render the diet inadequate in terms of iron and zinc and places the infants at high risk of developing micronutrient deficiencies (21).

Participants

The study was conducted from July 1997 to May 1999 in Purworejo, a predominantly agricultural district in the southern part of Central Java. The district is the location of a health surveillance project run by the Community Health and Nutrition Research Laboratories (CHN-RL) of Gadjah Mada University. Infants were recruited from the surveillance system, with a maximum of 50 infants being assessed for eligibility per month. For feasibility reasons, only infants who lived near the health centers were recruited.

Healthy, singleton infants from the surveillance area who were aged ≤ 6 mo and whose mothers had been monitored during pregnancy and birth were considered eligible for enrollment. Children with metabolic or neurologic disorders; physical handicaps affecting development, feeding, or activity; or severe or protracted illness and infants with hemoglobin concentrations < 90 g/L on assessment of eligibility were excluded after examination by the study physician (RS). Infants in whom initial blood sampling failed and in whom hemoglobin could not be assessed were excluded.

Written informed consent was required for inclusion. The study was approved by the Ethical Committee of Biomedical Research involving Human Subjects, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia, and the Research Ethics Committee, Faculty of Medicine and Odontology, Umeå University, Umeå, Sweden.

Interventions

Infants were randomly assigned to 1 of 4 treatment groups: iron (Fe group), zinc (Zn group), iron + zinc (Fe+Zn group), or placebo. Supplements were administered by the parents or caretakers once daily. Fieldworkers oversaw and administered the daily dose every third day and monitored the intake the previous 2 d by means of parent recall. These fieldworkers also monitored infant morbidity (not reported here). Empty bottles were replaced every 2 wk and the remaining syrup, if any, was measured and registered. The supplementation was initiated at 6 mo of age and continued until the child reached 12 mo of age (180 d of supplementation). Food intake was monitored with monthly 24-h recalls but was not subjected to intervention.

The 4 supplements provided the infants with a daily dose of either 10 mg Fe as ferrous sulfate, 10 mg Zn as zinc sulfate, 10 mg of both Fe and Zn, or placebo in a sweet-tasting syrup. All supplements included, per dose (1.6 mL, ie, 2 measuring pipettes), 30 mg ascorbic acid, sugar, and water. PT Konimex, Solo, Indonesia, manufactured the supplements in association with the Department of Pharmacology, Gadjah Mada University. The supplements were tested for taste acceptance in the study area before initiation of the study.

Outcomes

Primary outcomes for the trial were hemoglobin concentrations and indicators of iron [SF and serum transferrin receptor (sTfR)] and zinc (serum zinc) status at the 12-mo endpoint of the intervention. In addition, serum copper concentrations were analyzed. Other functional outcomes (not reported here) were physical growth, morbidity, and psychomotor development.

Sample size

Sample size calculations were based on the primary outcomes of physical growth (knee-heel difference, 1 mm in 3 mo), psychomotor development (Bayley scales of development, 5-point difference), and diarrheal disease morbidity (relative risk: 0.65). One hundred seventy infants per group would also allow for a dropout rate of 20% and provide 136 infants for analysis. This group size was estimated to allow for detection in differences of hemoglobin of 2.5 g/L ($\alpha = 0.05$, power = 80%), whereas 5 g/L was considered a clinically significant difference. For SF and serum zinc, no judgments about clinically significant differences were made.

Randomization

Randomization was planned and generated by an independent statistician, and was performed in blocks of 20. The pharmaceutical company marked the 4 different supplements with letter codes, blinded to researchers and participants. Information on group assignment was kept in a safe at the administrative offices of Gadjah Mada and Umeå Universities until after the intent-to-treat analysis. Participants were assigned to treatment groups by the recruitment field staff, who strictly followed the randomization list. The laboratory assessing the biochemical outcomes was not aware of the randomization groups.

Baseline and follow-up data collection

Trained fieldworkers collected socioeconomic information in home interviews. Fieldworkers or community midwives measured birth weight at the time of delivery. Anthropometric measurements (nude weight, recumbent length, and knee-heel length) were taken monthly from 4 to 12 mo of age (6- and 12-mo assessments are reported here), as were 24-h diet recalls. After the end of

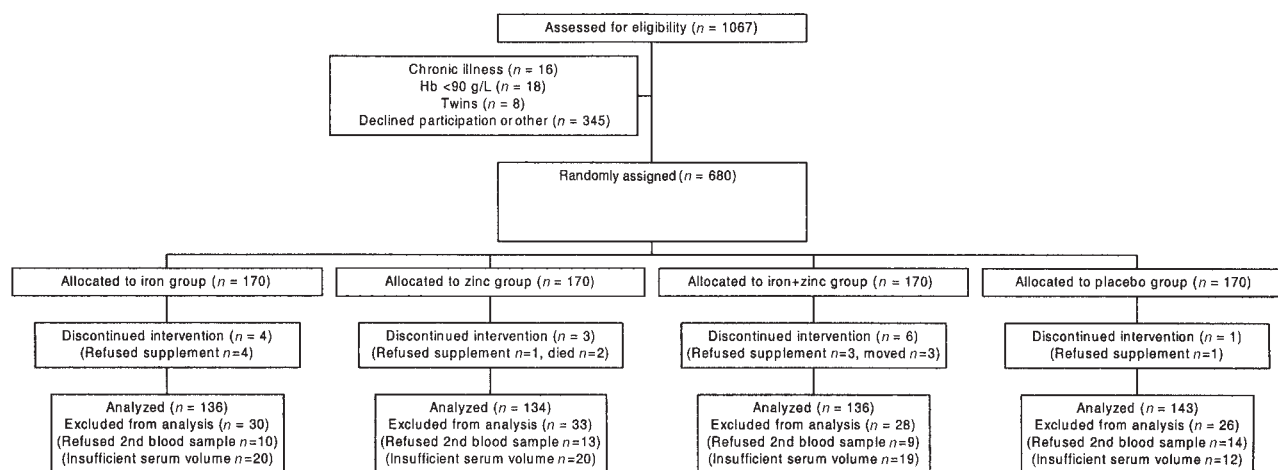


FIGURE 1. Trial profile.

the supplementation period, the families were interviewed on perceived side effects (abdominal pain, decreased appetite, vomiting, diarrhea, constipation, and increased crying or fussiness) as well as the general health condition of the child before and after supplementation. At the time of collection of the endpoint blood samples, body temperature was measured and a 2-wk recall of morbidity was obtained.

Biochemical assays

Venous blood was collected from the study subjects before and after the supplementation period, ie, at 6 and 12 mo of age, with the use of a zinc-free vacuum system. A drop of blood was taken for hemoglobin assessment with a portable Hemocue photometer (Hemocue AB, Ängelholm, Sweden). The remaining blood was immediately transported to the central field laboratory for centrifugation ($1800 \times g$, 10 min, room temperature) and serum separation and then frozen at -20°C until transportation 2 times weekly to the central serum storage location. Serum samples were kept at -70°C until July 1999, when they were transported for further biochemical analyses. Serum zinc and serum copper were assessed in duplicate by atomic spectrometry (22). SF was analyzed by radioimmunoassay (Diagnostic Products, San Diego) and sTfR by enzyme-linked immunosorbent assay (Ramco, Houston). To exclude the possible influence of ongoing inflammation on the hematologic and biochemical indexes, infants with fever or other symptoms of ongoing infection had their blood sampling delayed for 2 wk.

Analytic approach and statistical methods

The analysis aimed at 1) assessment of any differentials in baseline characteristics between treatment groups and between participating and lost-to-follow-up subgroups; 2) intent-to-treat analysis, ie, assessment of the effectiveness of the single or combined iron and zinc treatment regimens on hematologic and biochemical outcomes; 3) assessment of differences in adherence to the treatment regimens between the groups, potential confounding, and adjusted effectiveness analysis; 4) dose-effect analysis for the relevant treatment groups and evaluation of whether a plateau in the dose-effect curve could be observed; and 5) assessment of whether the maximum obtained effect differed between the single and combined supplements.

All statistical computations are based on the 549 infants with both baseline and endpoint hematologic and biochemical results, except for sTfR, for which the analysis is based on 507 infants.

Non-normally-distributed variables, ie SF, sTfR, and serum zinc values, were log-transformed (zero SF values were changed to 0.01). Main outcomes are shown as means and SDs or antilog of mean logarithmic values, when applicable, and proportions. Analysis of variance (ANOVA) was used in the intent-to-treat analysis for the continuous variables comparing the study groups. We also performed factorial analysis (two-factor ANOVA) with supplementation with iron or supplementation with zinc to examine main effects and interactions on main outcomes. In the case of a significant interaction between iron and zinc treatment, subgroup analyses were performed and the one-sided *P* values were Bonferroni corrected. For the iron outcomes (ie, hemoglobin, SF, and sTfR), the Fe group was compared with the Fe+Zn and placebo groups and the Fe+Zn group with the placebo group. For the zinc outcomes (ie, serum Zn), the Zn group was compared with the Fe+Zn and placebo groups and the Fe+Zn group with the placebo group. The intent-to-treat analysis was repeated after stratification for sex. Total supplementation volume was compared between treatment groups, as well as side effects that could influence adherence or outcome. Adjustments were thereafter made for total volume, vomiting, and initial level of the outcome parameter (for hemoglobin, SF, and sTfR but not for serum zinc because of the absence of association between baseline and endpoint values).

Lowess smoothed plots were used to visualize the dose-effect relation between volume taken and outcome for the different treatment groups. This was followed by multivariate linear regression analyses to statistically assess the formulated hypotheses and observations based on the Lowess curves. Adjustments were made for potential confounding of the dose effect. Potential confounding was identified as cofactors with a *P* < 0.20 for any linear or nonlinear association with outcomes, treatment groups, or total volume of treatment. In the regression analysis, presence of vomiting reported as a side effect at the follow-up interview with the mother, educational level of the mother, water source of the household, and birth weight were identified as possible confounders and thus were included in the model. Confounding by the measured variables was discarded as an important contribution to the main effects if the interactions or nonlinear introductions did not change the parameters describing the dose-effect by > 10%. SPSS for WINDOWS 10 (SPSS Inc, Chicago) was used for all statistical computations.

TABLE 1
Baseline characteristics of the participating infants¹

	Fe group (n = 136)	Zn group (n = 134)	Fe+Zn group (n = 136)	Placebo group (n = 143)
Household characteristics				
No. of persons per household ²	4 (1–10)	4 (2–9)	4 (2–10)	4 (1–9)
More than one child aged <5 y (%)	26	25	31	29
Water source outside house (%)	24	31	31	27
Maternal characteristics				
Age (y) ³	29.8 ± 4.8	28.9 ± 4.7	29.5 ± 4.7	29.1 ± 5.1
Elementary education or more (%)	59	50	58	58
Infant characteristics				
Girls [n (%)]	70 (52)	66 (49)	65 (48)	62 (43)
Birth weight (g) ³	3198 ± 440	3210 ± 460	3220 ± 473	3196 ± 477
Age at treatment start (mo) ³	6.1 ± 0.4	6.1 ± 0.5	6.1 ± 0.4	6.1 ± 0.4
Weight at 6 mo (kg) ³	7.17 ± 0.90	7.26 ± 0.91	7.30 ± 0.86	7.29 ± 0.93
Length at 6 mo (cm) ³	65.5 ± 2.4	65.4 ± 2.3	65.5 ± 2.0	65.3 ± 2.8
Breast-fed at 6 mo [n (%)]	133 (98)	127 (95)	134 (99)	136 (96)
Baseline biochemistry indexes				
Hemoglobin (g/L) ³	114.0 ± 13.8	114.1 ± 13.6	112.1 ± 11.5	114.1 ± 14.5
Serum ferritin (μg/L) ⁴	37.2 ± 2.5	31.3 ± 2.7	33.9 ± 2.3	32.2 ± 2.7
sTfR (mg/L) ^{4,5}	6.83 ± 1.44	6.98 ± 1.44	7.28 ± 1.36	7.23 ± 1.33
Serum zinc (μmol/L) ⁴	8.94 ± 1.26	9.11 ± 1.32	8.99 ± 1.28	9.03 ± 1.25
Serum copper (μmol/L) ³	17.2 ± 4.8	17.3 ± 4.3	17.9 ± 4.4	17.2 ± 4.2

¹sTfR, serum transferrin receptor. There were no significant differences between study groups.

²Median; range in parentheses.

³ $\bar{x} \pm$ SD.

⁴Antilog of mean ln value ± SD.

⁵n = 125, 123, 125, and 134 for the Fe, Zn, Fe+Zn, and placebo groups, respectively.

RESULTS

Six hundred eighty eligible infants were randomly assigned to the 4 treatment groups (**Figure 1**); 666 (98%) completed the 6-mo course of supplementation, and 549 (81%) were assessed with the use of both baseline and endpoint blood samples. Baseline characteristics, including hemoglobin concentration and iron and zinc status, were similar in the 4 treatment groups (**Table 1**). Baseline biochemistry (hemoglobin, SF, sTfR, and serum zinc) and sociodemographic characteristics did not differ significantly between those with and without complete biochemical data (data not shown). At baseline, the infants generally had a satisfactory weight and length distribution; only 5% were stunted (length for age < -2 SDs of World Health Organization growth reference values). Anemia (hemoglobin < 110 g/L) was observed in 41%, low SF (< 12 μg/L) in 15%,

iron deficiency anemia (low hemoglobin and SF) in 8%, and low serum zinc (< 10.7 μmol/L) in 78% of the infants.

Trial effectiveness (intent-to-treat analysis)

The Fe group had a significantly higher hemoglobin concentration at the endpoint than did the placebo (difference between means: 5.9 g/L) and Fe+Zn (difference: 4.1 g/L) groups (**Table 2**). The mean hemoglobin concentration in the Fe+Zn group was not significantly different from that in the placebo group. The prevalence of anemia (hemoglobin < 110 g/L) at the endpoint was 34/136 (25%), 48/134 (36%), 51/136 (38%), and 63/143 (44%) in the Fe, Zn, Fe+Zn, and placebo groups, respectively. The prevalence of anemia in the Fe group was significantly different from that in the placebo ($P < 0.001$) and Fe+Zn ($P = 0.026$) groups. However, the prevalence of anemia in the Fe+Zn group was not significantly different from that in the placebo group ($P = 0.266$).

TABLE 2
Outcome of treatment (intent-to-treat analysis) on hemoglobin and measures of iron, zinc, and copper status¹

	Fe group (n = 136)	Zn group (n = 134)	Fe+Zn group (n = 136)	Placebo group (n = 143)	P value ²
Hemoglobin (g/L) ³	119.4 ± 15.3 ^{4,5}	115.7 ± 15.2	115.3 ± 13.9	113.5 ± 16.0	0.012
Serum ferritin (μg/L) ⁶	46.5 ± 2.0 ^{4,5}	13.3 ± 3.6	32.3 ± 2.9 ⁴	12.9 ± 3.7	<0.001
sTfR (mg/L) ^{6,7}	6.71 ± 1.33 ⁴	9.78 ± 1.42	7.56 ± 1.36 ⁴	9.02 ± 1.73	<0.001
Serum zinc (μmol/L) ⁶	8.76 ± 1.24	11.58 ± 1.41 ⁴	10.80 ± 1.34 ⁴	9.06 ± 1.27	<0.001
Serum copper (μmol/L) ³	15.2 ± 4.8	15.0 ± 5.1	14.7 ± 4.5	15.2 ± 5.1	0.814

¹sTfR, serum transferrin receptor.

²ANOVA (on ln values for serum ferritin, sTfR, and serum zinc).

³ $\bar{x} \pm$ SD.

⁴Significantly different from placebo, $P < 0.05$ (Bonferroni corrected).

⁵Significantly different from Fe+Zn group, $P < 0.05$ (Bonferroni corrected).

⁶Antilog of mean ln value ± SD.

⁷n = 125, 123, 125, and 134 for the Fe, Zn, Fe+Zn, and placebo groups, respectively.

TABLE 3

Details of follow-up at the end of supplementation

	Fe group (n = 136)	Zn group (n = 134)	Fe+Zn group (n = 136)	Placebo group (n = 143)
Treatment				
Total supplement volume (mL) ^{1,2}	217 ± 51 ³	236 ± 52	207 ± 67 ³	242 ± 50
Health, growth				
Breast-fed at 12 mo [n (%)]	130 (96)	123 (92)	127 (93)	134 (94)
Weight at 12 mo (kg) ¹	8.33 ± 1.02	8.48 ± 0.97	8.40 ± 0.94	8.31 ± 0.94
Length at 12 mo (cm) ¹	72.5 ± 3.0	72.4 ± 2.8	72.3 ± 2.4	72.4 ± 2.8
Illness 2 wk before endpoint [n (%)]	38 (28)	39 (29)	36 (27)	38 (27)
Body temperature at endpoint (°C) ¹	36.8 ± 0.4	36.8 ± 0.4	36.7 ± 0.4	36.8 ± 0.4
Side effects				
Any perceived side effect [n (%)]	81 (60)	84 (63)	97 (82)	82 (57)
Vomiting [n (%)] ⁴	42 (31)	46 (34)	72 (53) ⁵	39 (27)

¹ $\bar{x} \pm SD$.²ANOVA $P < 0.05$ for difference between study groups.³Significantly different from Zn and placebo groups, $P < 0.05$ (Bonferroni corrected).⁴Defined as vomiting reported as a side effect during supplementation at the follow-up interview.⁵Significantly different from all other groups, $P < 0.05$.

Similarly, the Fe and Fe+Zn groups had significantly higher SF and lower sTfR concentrations than did the placebo group (Table 2). The effect in the Fe group was significantly higher than in the Fe+Zn group regarding SF (difference between geometric means: 14.2 $\mu\text{g/L}$). At the endpoint, the prevalence of low SF (SF < 12 $\mu\text{g/L}$) was 7/136 (5%), 47/134 (35%), 14/136 (10%), and 54/143 (37%) in the Fe, Zn, Fe+Zn, and placebo groups, respectively. The prevalence of iron deficiency anemia (hemoglobin < 110 g/L and SF < 12 $\mu\text{g/L}$) was 3/136 (2%), 21/134 (16%), 5/136 (3%), and 30/143 (21%) in the Fe, Zn, Fe+Zn, and placebo groups, respectively. The Fe and Fe+Zn groups had significantly lower prevalences of iron deficiency anemia than did the placebo group (both $P < 0.001$). Significant differences in iron deficiency anemia prevalence could not be shown between the Zn and placebo groups ($P = 0.255$), nor between the Fe and Fe+Zn groups ($P = 0.473$). The prevalence of iron deficiency anemia increased significantly from 6 to 12 mo of age in the placebo group ($P = 0.002$). Two-factor ANOVA showed a significant interaction between iron and zinc treatment for both hemoglobin ($P = 0.021$) and SF ($P = 0.032$) but not for sTfR.

The Zn and Fe+Zn treatment groups had higher mean serum zinc concentrations at the endpoint than did the placebo group. The proportion of low serum zinc (< 10.7 $\mu\text{mol/L}$) concentrations at the endpoint was 118/136 (87%), 63/134 (47%), 74/136 (54%), and 111/143 (78%) in the Fe, Zn, Fe+Zn, and placebo groups, respectively. The Zn and Fe+Zn groups had significantly fewer infants with low serum zinc concentrations than did the placebo (both $P < 0.001$) or Fe (both $P < 0.001$) group. Two-factor ANOVA showed no significant interaction between iron and zinc treatment on serum zinc levels.

There were no significant differences in treatment effects between boys and girls regarding hemoglobin, SF, or serum zinc outcomes (data not shown). The 4 treatment groups did not differ significantly in mean serum copper concentrations at the endpoint (Table 2).

Deviation from protocol and side effects

The total intake of supplements was lower in the Fe+Zn group than in the Zn (mean difference: 29 mL; $P < 0.001$) and placebo groups but was not significantly different from that in the Fe group

($P = 0.129$; **Table 3**). Vomiting was more common in the Fe+Zn group (Table 3). Other perceived side effects (abdominal pain, diarrhea, constipation, poor appetite, increased crying, and fussiness) did not differ significantly between treatment groups. Vomiting was negatively associated with the total volume of supplement given ($r = -0.387$, $P < 0.001$), which also implies the possibility of loss of supplement after the daily dose.

Baseline and endpoint values were associated for hemoglobin, SF, and sTfR but not for serum zinc. Consumption of supplement and reported vomiting were, as noted, significantly different between treatment groups. Consequently, adjusting for these factors slightly changed the estimated effect size for serum zinc at the endpoint, and the contrast result (Zn compared with Fe+Zn) was no longer significant (geometric mean of serum zinc: 11.51 and 10.99 $\mu\text{mol/L}$, respectively, $P = 0.185$).

Efficacy of the Fe, Zn, and Fe+Zn regimens

Serum ferritin outcome

To evaluate the efficacy of the Fe and Fe+Zn regimens on iron status (SF) at the endpoint, the dose effect over the range of total supplement intake during the treatment period was studied (**Figure 2**). Lowess curves for the Fe and Fe+Zn groups indicated an initial dose effect, followed by a plateau with no further increased effect. In the ordinary least-squares regression model, the dose effect up to 200 mL was compared with that above 200 mL of total supplementation (**Table 4**). Adjustments for potential confounding covariates were made if fulfilling the set criteria. In the Fe group, there was a significant dose effect up to 200 mL (corresponding to a total dose of 1250 mg Fe over a period of 6 mo, or 7 mg/d). After that total dose, no further increase in effect was shown. Above 200 mL of total supplement, the effect in the Fe group was significantly higher than that in the Fe+Zn group ($\beta = 0.171$, $P = 0.003$), but there was no significant interaction between treatment group and volume. Despite the appearance of the Lowess curves, no significant difference in effect for a given dose and no interaction in dose effect between the groups for supplementation amounts < 200 mL could be shown between the Fe and Fe+Zn groups (data not shown).

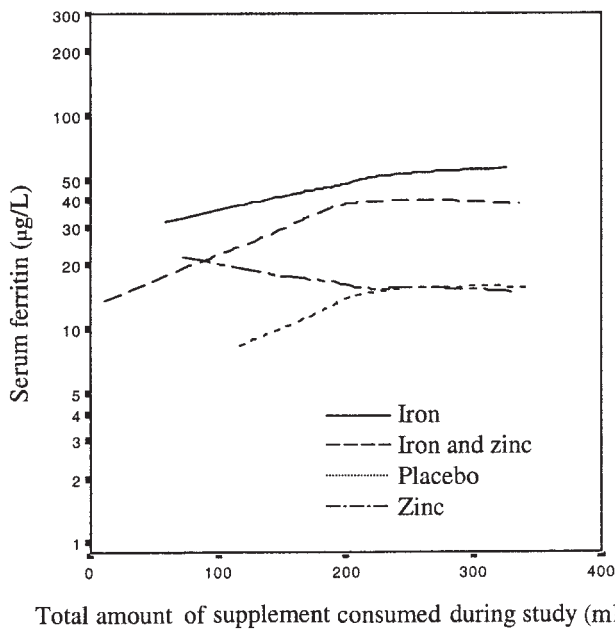


FIGURE 2. Volume of supplement consumed from 6 to 12 mo and serum ferritin concentrations (logarithmic scale) at the endpoint. Shown are Lowess curves for the 4 treatment groups ($n = 549$).

Serum zinc outcome

In the Zn group, a significant dose-effect relation was shown (Figure 3), without any evident plateau and without any identified confounding (β for consumed supplement in mL: 0.0024, $P = 0.001$, $r^2 = 0.15$). In the Fe+Zn treatment group, there was a significant dose effect for supplementation volumes < 200 mL ($\beta = 0.0020$, $P = 0.023$) but not for volumes ≥ 200 mL. A significant interaction between volumes $<$ and ≥ 200 mL was not shown, and the linear regression equation, including an interaction term, was not significant. For supplementation amounts ≥ 200 mL, there was a small but significant difference in effect levels between the Zn and Fe+Zn groups ($\beta = 0.097$, $P = 0.048$).

DISCUSSION

This study of the effect of single or combined iron and zinc supplementation in an iron- and zinc-deficient infant population provides evidence that a combined supplement is less efficacious than are single supplements in improving iron and zinc status. Furthermore, data are presented suggesting a negative effect on zinc

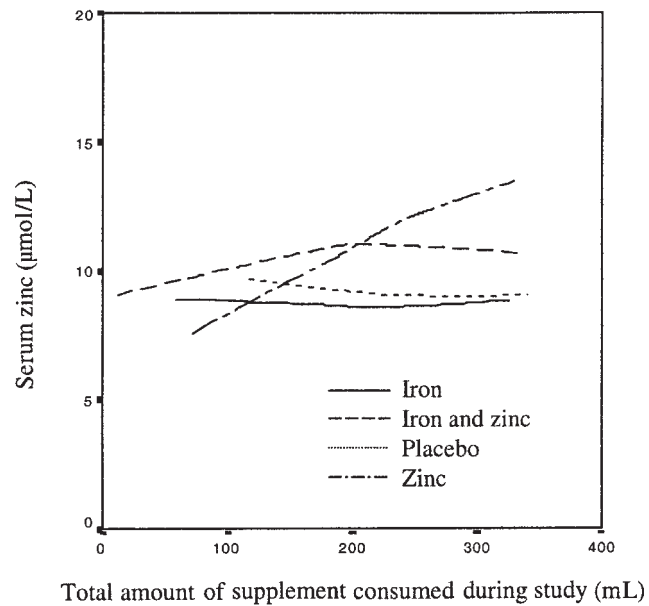


FIGURE 3. Volume of supplement consumed from 6 to 12 mo and serum zinc concentrations at the endpoint. Shown are Lowess curves for the 4 treatment groups ($n = 549$).

status of daily iron supplementation and that a daily dose of 10 mg Fe may be unnecessarily high.

No deviations from the protocol were observed in the allocation of treatment groups and masking. The poorer adherence to the prescribed total dose in the combined supplement group could potentially bias the results, and the higher frequency of vomiting in the same group could spuriously reduce the effect estimate. However, adjustments for these factors modified the results only slightly. Socioeconomic factors related to participation (supplement amount) and biochemical outcomes might bias the dose-effect analysis, and adjustments were made when warranted. No major influence on effect estimates was found.

This group of infants generally had a fair nutritional status—as expressed by anthropometry—at baseline, but had a relatively high frequency of low iron and zinc status and anemia. Absolute levels of effects would be different in other populations, but the observed relative differences in effects between treatment regimens as well as most of the dose-effect observations are most likely relevant for other iron- and zinc-deficient infant populations in the world.

Iron, when given alone, had a significant positive effect on iron status as assessed by higher hemoglobin and SF concentrations,

TABLE 4

Dose effect on iron status (efficacy analysis)

Outcome: In serum ferritin at endpoint	Fe	<i>P</i> value	Fe+Zn ¹	<i>P</i> value
Constant	1.214	0.149	0.409	0.586
Consumed supplement (mL)	0.00975	0.036	0.0144	0.001
In Serum ferritin at baseline	0.210	0.001	0.287	0.006
Volume group (1 = ≥ 200 mL, 0 = < 200 mL)	1.958	0.059	3.364	0.006
Consumed supplement \times volume group	-0.00951	0.078	-0.0189	0.001
<i>F</i>	6.29	< 0.001	7.05	< 0.001
<i>r</i> ²	0.174		0.267	

¹ Adjusted for educational level and educational level \times volume group.

lower sTfR, and a lower prevalence of anemia than in all the other groups. It is apparent that treatment with the combination of iron and zinc was less effective in improving iron status: the high prevalence of anemia at 6 mo of age had not changed by 12 mo, and both hemoglobin and SF were significantly lower than in the Fe group. The combined Fe+Zn supplement resulted in some improvement in iron status, however: SF was higher and sTfR lower than in the placebo group. Thus, the addition of zinc to the iron supplement impaired the utilization of iron but not to the extent that it had no effect on iron status. Finally, in the Zn group the sTfR estimate was higher, although not significantly so ($P = 0.097$); if correct, this may indicate increased intracellular iron needs in that group.

It is likely that zinc interfered with the absorption of iron, as was shown previously for these elements when given in water solution to adults (14, 15). The mechanisms regulating iron absorption have been discovered (23). An iron transporter at the apical side of the enterocyte, the DMT1 (divalent metal transporter 1, also known as Nramp2 or DCT1), has been shown to also transport zinc ions (24). Although this has not yet been shown in mammalian systems, it is possible that high concentrations of zinc may interfere with the transport of iron into the intestinal cell, without completely blocking it. However, the acute effects of zinc on iron transport by DMT1, as well as possible long-term adaptations, need further study.

Serum zinc is not an ideal indicator of zinc status because it is affected by several factors other than zinc intake and zinc status (25). It is generally agreed, however, that serum zinc is of some value as an indicator when used for larger groups in similar settings and when care is taken to standardize conditions for blood sampling. The Zn group had significantly higher serum zinc concentrations than did the placebo group and higher concentrations than did the Fe+Zn group, although this difference was not significant ($P = 0.057$). This may suggest that zinc alone was better utilized than when given in combination with iron. However, zinc in the combined Fe+Zn supplement obviously was utilized to some extent, because serum zinc was higher in that group than in the placebo group, which received no supplemental zinc. Furthermore, the Fe group had a significantly higher proportion of low serum zinc values than did the placebo group. Our findings are thus in agreement with studies in adults showing that high concentrations of inorganic iron inhibit zinc absorption when given in water solution (11–13).

The cellular mechanisms for zinc uptake and transport have been discovered, and some of these transporters, such as ZIP-1, ZnT-1, and ZnT-4, have been found in the small intestine (26, 27). The precise roles of these zinc transporters in the cellular uptake of zinc at the apical side, in intracellular transport, or in efflux at the basolateral side have not been delineated, and it is not known whether they are affected by high amounts of iron. It is obvious that further studies on the interaction between iron and zinc are needed.

High dietary intake of iron has been shown to affect copper status in some studies (17, 28). However, these studies found effects when iron was added to infant formula and not when given as a supplement. We found no negative effects of iron supplements alone or in combination with zinc on serum copper. It is possible that the ratio of iron to dietary copper was not high enough to have an effect or, perhaps more likely, that iron given apart from a meal did not affect copper absorption from the diet. High dietary intakes of zinc have also been shown to affect copper absorption and status in experimental


animals (29), and zinc supplementation in addition to food-based nutrition rehabilitation may reduce plasma copper concentrations in very malnourished children with persistent diarrhea (30). It is likely that these 2 elements interact only when given together in a meal and not, as in our study, when zinc is given apart from meals.

One-half of the parents reported vomiting due to the Fe+Zn supplement. The daily morbidity registration showed that 37% in the Fe+Zn group but only 20% in the placebo group experienced vomiting without concurrent illness. The reason for this increased rate of vomiting without other differences in abdominal symptoms is unclear.

Our results suggest that single supplements of iron and zinc were more effective in improving iron and zinc status, respectively, than was a combination of the 2, even after the analysis was controlled for compliance and possible side effects. We also believe that our data show that this population of infants needs increased intake of both micronutrients. Whether ratios of the 2 elements in a supplement other than the 1:1 we used are more effective in improving both iron and zinc status is not known, and this was not addressed in our study. Note that an increase in the dose of iron and zinc in a combined daily supplement would most likely fail to compensate for the interaction because the dose-effect curve leveled off below the single supplement.

On the basis of the results of animal experiments it has been suggested that intermittent supplementation of iron may be equally as or more effective than daily supplementation in preventing iron deficiency, the so-called mucosal block theory (31). However, experiments in humans have not provided evidence for an increased efficacy with weekly supplementation (32). Thus, the issue is rather whether an intermittent (eg, weekly) iron dose frequency will supply the sufficient total dose. Our results indicate that the total dose provided according to protocol (10 mg Fe/d) was unnecessarily high. No further effect was obtained beyond a total supplementation volume corresponding to 1250 mg Fe (protocol: 1800 mg) over the 6 mo of the study, or 7 mg/d. Whether weekly iron supplements would reduce the inhibitory effect of iron on zinc absorption is not known. It is possible that a bolus dose may inhibit zinc absorption that same day, but it is unlikely to have a long-lasting effect on zinc uptake.

It is highly likely that zinc supplements need to be given every day or most days, because there are no stores of zinc and zinc turnover is rapid. No plateau was observed in the dose-effect analysis of the Zn group. However, the analysis was done with serum zinc as the outcome. Whether the dose-effect pattern is similar in relation to functional outcomes needs to be assessed.

To summarize, our study has provided evidence that combined iron and zinc supplementation is not optimal. The combined supplement showed no effect on hemoglobin, whereas the single iron supplement had a clinically and statistically significant effect. The combined supplement also had a significantly lower effect than did the single supplements on SF. Furthermore, evidence is provided that single daily iron supplementation inhibits zinc absorption from food. In situations in which a supplementation strategy is more feasible than a food-based approach, innovative dose regimens for the provision of these micronutrients are needed. 

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