



A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development¹⁻³

Torbjörn Lind, Bo Lönnerdal, Hans Stenlund, Indria L Gamayanti, Djauhar Ismail, Rosadi Seswandhana, and Lars-Åke Persson

ABSTRACT

Background: Deficiencies of iron and zinc are associated with delayed development, growth faltering, and increased infectious-disease morbidity during infancy and childhood. Combined iron and zinc supplementation may therefore be a logical preventive strategy.

Objective: The objective of the study was to compare the effects of combined iron and zinc supplementation in infancy with the effects of iron and zinc as single micronutrients on growth, psychomotor development, and incidence of infectious disease.

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 and 10 mg Zn (Fe+Zn group), or placebo from 6 to 12 mo of age. Anthropometric indexes, developmental indexes (Bayley Scales of Infant Development; BSID), and morbidity were recorded.

Results: At 12 mo, two-factor analysis of variance showed a significant interaction between iron and zinc for weight-for-age z score, knee-heel length, and BSID psychomotor development. Weight-for-age z score was higher in the Zn group than in the placebo and Fe+Zn groups, knee-heel length was higher in the Zn and Fe groups than in the placebo group, and the BSID psychomotor development index was higher in the Fe group than in the placebo group. No significant effect on morbidity was found.

Conclusions: Single supplementation with zinc significantly improved growth, and single supplementation with iron significantly improved growth and psychomotor development, but combined supplementation with iron and zinc had no significant effect on growth or development. Combined, simultaneous supplementation with iron and zinc to infants cannot be routinely recommended at the iron-to-zinc ratio used in this study. *Am J Clin Nutr* 2004;80:729-36.

KEY WORDS Infants, growth, knee-heel length, development, iron, zinc, randomized controlled trial, Indonesia

INTRODUCTION

Deficiencies of iron and zinc often coexist and cause growth faltering (1), delayed development (2), and increased morbidity due to infectious disease (3) that affect the health, development, and well-being of millions of infants and children. Combined supplementation with both iron and zinc in vulnerable populations may therefore be a logical preventive strategy when iron and zinc are low in complementary foods or when iron and zinc

have low bioavailability. However, iron and zinc may compete for absorptive pathways (4, 5). We showed that simultaneous supplementation with iron and zinc was less efficacious in improving iron and zinc status in Indonesian infants than was supplementation with iron or zinc alone (6). Antagonistic interactions between the 2 minerals were not previously described for functional outcomes such as growth, development, or incidence of common infectious diseases, but they may have far-reaching implications with regard to supplementation programs.

We conducted a community-based, randomized, double-blind, placebo-controlled trial with a factorial design to investigate the hypothesis that daily supplementation with zinc alone or with zinc in combination with iron in infants from the age of 6 mo to 12 mo would improve linear growth (knee-heel length) and weight gain and reduce morbidity from diarrhea as compared with those in a placebo group. We also hypothesized that, in comparison with placebo, daily supplementation with iron alone or iron in combination with zinc would improve development, measured by the Bayley Scales of Infant Development (BSID), and that combined supplementation would improve growth as well as development and reduce diarrheal morbidity. The biochemical and hematologic outcomes of this randomized trial were reported elsewhere (6). We now report on the effects on growth, infant development, and infectious disease morbidity.

¹ From the Department of Public Health and Clinical Medicine, Epidemiology and Public Health Sciences, Umeå University, Umeå, Sweden (TL and HS); the Department of Nutrition, University of California, Davis, CA (BL); the Community Health and Nutrition Research Laboratories, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia (ILG, DI, and RS); and the International Maternal and Child Health, Uppsala University, Uppsala, Sweden (L-ÅP).

² Supported by grants from the Swedish Agency for Research Cooperation with Developing Countries, the Swedish Medical Research Council, the Swedish Foundation for International Co-operation in Research and Education, the Swedish Medical Society, the Maud and Birger Gustavsson Foundation, and the Umeå University Foundation. None of the funding sources had any role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

³ Reprints not available. Address correspondence to T Lind, Department of Public Health and Clinical Medicine, Epidemiology and Public Health Sciences, Umeå University, SE-901 87 Umeå, Sweden. E-mail: torbjorn.lind@epiph.umu.se.

Received September 18, 2003.

Accepted for publication April 4, 2004.

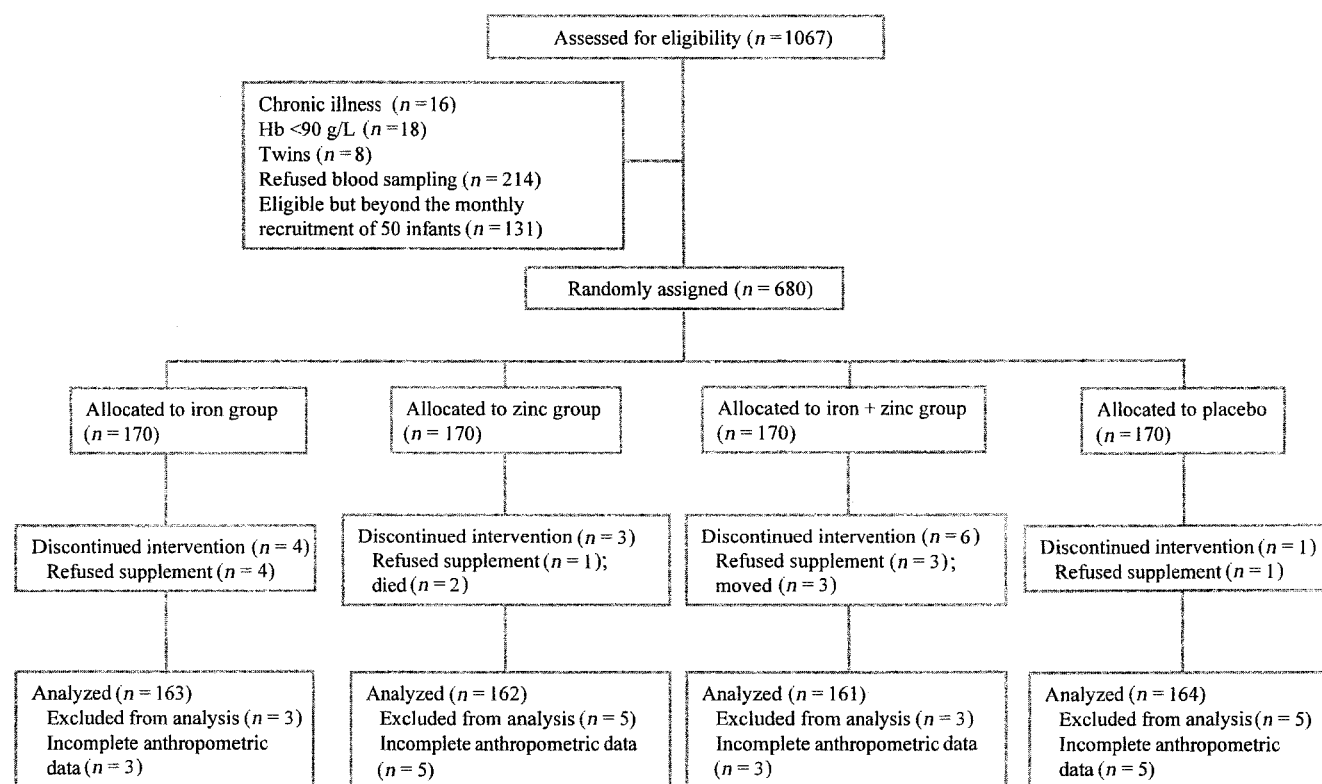


FIGURE 1. Trial profile. Hb, hemoglobin.

SUBJECTS AND METHODS

Setting

Childhood malnutrition is a public health problem in Central Java, Indonesia. Stunting reportedly affects $\approx 40\%$ of children < 5 y old (7), and micronutrient deficiencies are prevalent among infants and children (8, 9). Breastfeeding is common and of long duration, but exclusive breastfeeding is rare, and complementary foods are introduced early. Vitamin A deficiency among infants is reportedly common in the area (10), and the Indonesian national health program provides vitamin A from 12 mo of age. The diet is plant-based and contains little animal protein and low amounts of iron and zinc with low bioavailability, which places the infants at high risk of developing micronutrient deficiencies (11).

Participants

The study was conducted from July 1997 to May 1999 in Purworejo, Central Java, where the Community Health and Nutrition Research Laboratories (CHN-RL) of Gadjah Mada University run a health and demographic surveillance project. Mothers in the surveillance area were monitored during pregnancy and birth. Healthy singleton infants from the surveillance system were recruited (a maximum of 50 infants/mo were assessed for eligibility) at < 6 mo of age after written informed consent was obtained from the parents. Children with metabolic or neurologic disorders; handicaps affecting development, feeding, or activity; or severe or protracted illness, as well as infants with hemoglobin < 90 g/L, were excluded (Figure 1). The ethics committees of Gadjah Mada University and Umeå University (Sweden) approved the study.

Interventions

Infants were randomly assigned to one of 4 treatment groups—iron, zinc, iron + zinc, or placebo—from 6 to 12 mo of age (180 d of supplementation). The 4 supplements provided the infants with a daily dose of either 10 mg Fe as ferrous sulfate (Fe group), 10 mg Zn as zinc sulfate (Zn group), 10 mg Fe and 10 mg Zn (Fe + Zn group), or placebo in a sweet-tasting syrup. The iron:zinc molar ratio in the combined Fe + Zn supplement was 1.17:1. The dosages were chosen to be close to the recommended daily intakes of iron and zinc in 6–12-mo-old infants consuming a low-bioavailability diet (12, 13). Each dose (1.6 mL; ie, 2 measuring pipettes) of all supplements (PT Konimex, Solo, Indonesia) included 30 mg ascorbic acid, sugar, and water. Supplements were administered once a day by the parents or caretakers. Fieldworkers oversaw and administered the daily dose every third day and monitored the intake on the other 2 d by means of parent recall. Bottles were replaced every 2 wk, and the remaining syrup, if any, was measured and registered.

Outcomes

Major outcomes were changes in weight, length, knee-heel length, and infant development, as measured by using the Bayley Scales of Infant Development (BSID; 14) at 12 mo of age and the incidence of diarrheal disease and lower respiratory infections during the 6 mo of the study (from 6 mo to 12 mo of age). A detailed description of the effect of the intervention on the hematologic and biochemical status of the infants was published elsewhere (6).

Sample size

Sample size calculations were based on the major outcomes of physical growth (knee-heel length), psychomotor development (BSID psychomotor development index, PDI), and diarrheal disease morbidity. For the detection with $\alpha = 0.05$ and a power of 80% of a difference from the placebo group of 5 points in BSID PDI, 71 infants per group were needed; for that of 2 mm/6mo in knee-heel length growth velocity, 96 infants per group were needed; and for that of a 30% reduction in the incidence of diarrheal disease, 140 infants per group were needed. To allow for a 20% dropout rate, 170 infants per group were invited, which provided 136 infants for analysis.

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 to which the researchers and participants were blinded. Participants were assigned to treatment groups by the recruitment field staff in strict accordance with the randomization list. Researchers and field staff were blinded to the information on group assignment, because this information was kept in safes at the administrative offices of Gadjah Mada and Umeå universities until after the intent-to-treat analysis.

Baseline and follow-up data collection

Socioeconomic information was collected in home interviews. Fieldworkers or community midwives measured birth weight within 72 h of delivery. After the end of the supplementation, the families were interviewed on perceived side effects (abdominal pain, decreased appetite, vomiting, diarrhea, constipation, and increased crying or fussiness).

Biochemical and anthropometric measurements

Venous blood was collected from the study infants at 6 and 12 mo of age and analyzed as described previously (6). Anthropometric measurements were performed on a monthly basis from 6 to 12 mo of age by a team of 2 anthropometrists at home visits. Naked weight was measured to the nearest 0.02 kg with the use of a Seca 835 digital baby scale (Seca, Hamburg, Germany). Recumbent length was measured to the nearest 1 mm with the use of a locally produced wooden board. Knee-heel length was measured to the nearest 0.1 mm by using an infant knemometer (Infant Knemometer BK5; FORCE Instituttet, Brøndby, Denmark). Head circumference and midupper arm circumference was measured to the nearest 1 mm by using a nonstretchable plastic measuring tape. All measurements were done in triplicate, and the mean value was used in the analysis.

Infant development

Infant development was tested with BSID (14) at 6 and 12 mo of age. A team of 8 psychologists from the Department of Psychology, Gadjah Mada University, administered the tests at village health posts close to the infants' homes. All of the psychologists had experience in testing infants and extensive training in the use of BSID. Three facets of the test were recorded: the mental development index (MDI), the PDI, and the behavioral rating scale (BRS). In 81 infants (12%), we assessed interrater agreement. Correlation between testers was $r = 0.93$ for MDI

(Pearson's $P < 0.001$), $r = 0.95$ for PDI ($P < 0.001$), and $r = 0.70$ for BRS ($P < 0.001$).

Morbidity registration

Fieldworkers visited the families every third day to record compliance with supplementation as well as symptoms of illness for the day of visit and by parental recall for the 2 days preceding fieldworker visits. Symptoms of fever (mother's own definition); coryza, cough, difficult or rapid breathing or both, ear discharge, diarrhea, and vomiting were recorded. Diarrhea was defined as ≥ 3 loose or watery stools on any single day, with or without fever. The fieldworker referred infants showing signs of severe or protracted illness to the nearest health center, and transportation assistance was provided if needed.

Statistical analysis

For statistical computations, SPSS for WINDOWS software (version 10; SPSS Inc, Chicago) was used. Anthropometric data are shown as mean ($\pm$ SD) z scores compared with the World Health Organization/National Center for Health Statistics reference population (15). Before analysis, the anthropometric data were interpolated to correspond to each completed month of age. Conversion to anthropometrical z scores was done by using EPI INFO 2000 software (version 1.1.1; Centers for Disease Control and Prevention, Atlanta) and the 2000 Centers for Disease Control and Prevention reference growth data (15). Development is shown as mean ($\pm$ SD) MDI and PDI and median BRS with interquartile range. The BRS was severely skewed, and thus ranks were used as outcome in the analysis of variance (see below). Morbidity was analyzed with Poisson's regression by using STATA software (version 6.0; Stata Corp, College Station, TX) and is shown as incidence and incidence rate ratios (95% CI) for diarrhea and lower respiratory infections (LRIs) with the placebo group as reference. LRI was defined as fever in combination with cough, difficult or fast breathing, or both. An episode of either diarrhea or LRI was defined as 3 disease-free days followed by the symptom for ≥ 1 d. Chi-square test (2) or Fisher's exact test was used to test associations between categorical variables.

Two-factor analysis of variance was performed to examine main effects and interactions between iron and zinc supplementation. When significant interaction was found, follow-up test using Bonferroni's adjustment was performed. To adjust for possible confounding, the covariates sex, birth weight, initial values for the main outcomes, mother's education, and (as a proxy for socioeconomic status) the location of the household's water source were included in the analysis.

RESULTS

Of the 680 recruited infants, 666 completed supplementation, 662 had complete morbidity data, 655 had complete BSID data, and 650 had complete anthropometric data (Figure 1). The basic characteristics of the study population are shown in **Table 1**. There were no significant differences in any of the background or baseline variables among the treatment groups (**Table 2**) or between the group that completed the trial and the groups that did not. At baseline, the overall prevalence of stunting [height-for-age z score (HAZ) < -2 SD] was 3.5%, that of underweight [weight-for-age z score (WAZ) < -2 SD] was 4%, and that of wasting [weight-for-height z score (WHZ) < -2 SD] was 4.5%.

TABLE 1Baseline characteristics of participating infants¹

	Study group			
	Placebo (n = 169)	Fe (n = 166)	Zn (n = 167)	Fe+Zn (n = 164)
Household characteristics				
Persons per household (n)	4 (1–9) ²	4 (1–10)	4 (2–9)	4 (2–10)
>1 Child <5 y old (%)	29	26	22	29
Water source outside house (%)	25	23	31	32
Mother characteristics				
Age (y)	29.1 ± 5.2 ³	29.4 ± 4.7	29.0 ± 4.7	29.6 ± 4.7
No formal education (%)	3.6	2.4	1.8	1.8
> 6 Y of formal education (%)	44	43	46	39
Infant characteristics				
Girls [n (%)]	75 (44)	84 (51)	83 (50)	78 (48)
Birth weight (g) ⁴	3200 ± 479	3190 ± 436	3198 ± 498	3204 ± 474
Age at treatment start (mo)	6.2 ± 0.4	6.2 ± 0.4	6.1 ± 0.5	6.1 ± 0.5
Breastfed at 6 mo [n (%)]	162 (96)	163 (98)	160 (96)	162 (99)

¹ Fe group received 10 mg Fe as ferrous sulfate; Zn group received 10 mg Zn as zinc sulfate; Fe+Zn group received 10 mg Fe and 10 mg Zn. There were no significant differences between the study groups.

² Median; range in parentheses (all such values).

³ $\bar{x} \pm SD$ (all such values).

⁴ n = 157, 154, 155, and 146 in the placebo, Fe, Zn, and Fe+Zn groups, respectively.

Anemia (hemoglobin < 110 g/L) was observed in 41%, iron deficiency anemia (IDA: hemoglobin < 110 g/L and serum ferritin < 12 μ g/L) in 8%, and low serum zinc (<10.7 μ mol/L) in 78% of the infants; there were no significant differences between treatment groups.

Deviation from protocol and side effects

Vomiting, both reported by the parents as a side effect to supplementation and measured in the day-to-day morbidity recording, was more common in the Zn and Fe+Zn groups than in the other 2 groups (Table 3). For consumption of supplement, the

main effects for both iron and zinc were significant, but interaction was not (Table 3). Adjusting for difference in side effects (eg, vomiting) and amount of supplement consumed did not change the main outcome effects (data not shown).

Intent-to-treat analysis

Growth

At 12 mo of age, two-factor analysis of variance showed significant interaction between iron and zinc treatment for WAZ. WAZ was significantly higher in the Zn group than in the placebo

TABLE 2Baseline values for anthropometric and developmental indexes¹

	Study group			
	Placebo	Fe	Zn	Fe+Zn
Anthropometry (n = 650)				
WAZ	−0.42 ± 0.99 ²	−0.40 ± 0.98	−0.36 ± 1.06	−0.38 ± 0.93
WAZ <−2 SD [n (%)]	7 (4)	8 (5)	4 (2)	7 (4)
HAZ	−0.41 ± 0.96	−0.28 ± 0.81	−0.33 ± 0.84	−0.36 ± 0.83
HAZ <−2 SD [n (%)]	10 (6)	2 (1)	7 (4)	4 (2)
WHZ	0.02 ± 1.03	−0.12 ± 1.11	−0.01 ± 1.19	−0.00 ± 1.17
WHZ <−2 SD [n (%)]	8 (5)	7 (4)	8 (5)	6 (4)
Midarm circumference (cm)	14.6 ± 1.15	14.6 ± 1.15	14.7 ± 1.19	14.6 ± 1.11
Knee-heel length (cm)	17.34 ± 9.67	17.27 ± 9.81	17.32 ± 9.06	17.37 ± 8.25
Development (n = 655)				
Mental development index	98 ± 7.7	98 ± 10.1	100 ± 9.4	99 ± 8.8
Psychomotor development index	94 ± 11.2	94 ± 12.0	96 ± 11.4	95 ± 13.3
Behavioral rating scale	35 (14–67) ³	29 (12–63)	35 (16–73)	32 (16–68)

¹ n for placebo, Fe, Zn, and Fe+Zn groups was 164 and 165, 163 and 163, 162 and 167, and 161 and 160 for anthropometric and developmental indexes, respectively. Fe group received 10 mg Fe as ferrous sulfate; Zn group received 10 mg Zn as zinc sulfate; and Fe+Zn group received 10 mg Fe and 10 mg Zn. WAZ, weight-for-age z score; HAZ, height-for-age z score; WHZ, weight-for-height z score. There were no significant differences between the groups at baseline.

² $\bar{x} \pm SD$ (all such values).

³ Median; 25th–75th percentiles in parentheses (all such values).



TABLE 3

Details of follow-up at the end of supplementation¹

	Study group				P for main effect		P for Fe × Zn interaction
	Placebo (n = 164)	Fe (n = 163)	Zn (n = 162)	Fe+Zn (n = 161)	Fe	Zn	
Treatment							
Total supplement volume (mL)	242 ± 51 ²	218 ± 54	232 ± 57	202 ± 70	<0.001	0.004	0.60
Health							
Breastfed at 12 mo [n (%)]	134 (94)	154 (94)	155 (95)	148 (92)	0.12	0.04	0.99
Illness ≤ 2 wk before endpoint [n (%)]	40 (26)	43 (28)	43 (29)	40 (26)	0.82	0.90	0.53
Side effects							
Any perceived side effect [n (%)]	94 (57)	96 (59)	102 (63)	112 (70)	0.001	0.34	0.88
Vomiting ³ [n (%)]	44 (27) ^a	49 (30) ^a	56 (35) ^a	84 (53) ^b	0.004	<0.001	0.043
Vomiting as only symptom ⁴	1.0	1.0 (0.7, −1.2) ⁵	1.9 (1.5, −2.3)	4.1 (3.4, −4.9)			

¹ Fe group received 10 mg Fe as ferrous sulfate; Zn group received 10 mg Zn as zinc sulfate; and Fe+Zn group received 10 mg Fe and 10 mg Zn. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (two-factor ANOVA with Bonferroni adjustment).

² $\bar{x} \pm$ SD (all such values).

³ As side effect during supplementation at follow-up interview.

⁴ Vomiting without any other symptoms (from daily morbidity registration).

⁵ Relative risk; 95% CI in parentheses (all such values).

and Fe+Zn groups (Table 4). For WHZ, two-factor analysis of variance showed a significant main effect of zinc treatment but no significant effect of iron treatment and no interaction. Furthermore, two-factor analysis of variance showed a significant interaction between iron and zinc treatment for knee-heel length. Knee-heel length was higher in the Zn and Fe groups than in the placebo group. For midupper arm circumference, interaction was significant (Table 4). However, there were no significant differences among groups. Control for potential intervening variables, such as consumption of supplement and occurrence of vomiting, did not significantly change the result. There were no statistically significant differences among the groups in height-for-age or head circumference.

The proportion of wasting at 12 mo was significantly ($P < 0.05$; chi-square test; 2) higher in the Fe group (21.5%) than in the

Zn group (11.1%); the values in the placebo and Fe+Zn groups were 18.3% and 13.0%, respectively. There were no significant differences in stunting—8.5%, 7.4%, 10.5%, and 11.8%—or low weight—39.6%, 39.3%, 32.7%, and 39.8%—among the placebo, Fe, Zn, and Fe+Zn groups, respectively, at 12 mo of age.

Overall, anthropometric status deteriorated significantly from 6 to 12 mo of age: the prevalence of wasting increased from 4% to 16%, that of low weight increased from 4% to 38%, and that of stunting increased from 4% to 10% (all: $P < 0.001$; Fisher's exact test). The prevalence of low weight increased significantly in all groups.

Development

Two-factor ANOVA showed significant interaction between iron and zinc treatment for BSID PDI at 12 mo. The PDI attained

TABLE 4

Outcome of treatment on anthropometric and developmental indexes at 12 mo of age¹

	Study group				P for main effect		P for Fe × Zn interaction
	Placebo	Fe	Zn	Fe+Zn	Fe	Zn	
Anthropometry (n = 650)							
WAZ	−1.72 ± 1.00 ^{a2}	−1.65 ± 1.08 ^a	−1.46 ± 1.08 ^b	−1.68 ± 1.02 ^a	0.092	0.079	0.004
HAZ	−0.81 ± 0.86	−0.66 ± 0.91	−0.77 ± 0.92	−0.90 ± 0.90	0.84	0.16	0.16
WHZ	−1.01 ± 1.16	−1.07 ± 1.23	−0.70 ± 1.06	−0.86 ± 1.06	0.26	0.004	0.14
Midarm circumference (cm)	14.7 ± 1.18	14.7 ± 1.12	14.8 ± 1.14	14.6 ± 1.04	0.36	0.77	0.039
Knee-heel length (cm)	19.30 ± 0.95 ^a	19.45 ± 0.96 ^b	19.50 ± 0.99 ^b	19.40 ± 0.94 ^{a,b}	0.46	0.62	0.003
Development (n = 655)							
Mental development index	99 ± 10.0	101 ± 9.7	101 ± 9.3	100 ± 9.8	0.76	0.63	0.069
Psychomotor development index	103 ± 10.8 ^a	106 ± 11.0 ^b	105 ± 10.6 ^{a,b}	103 ± 10.3 ^{a,b}	0.82	0.39	0.009
Behavioral rating scale ³	42 (20–62) ⁴	42 (22–69)	39 (20–66)	35 (19–53)	0.55	0.062	0.091

¹ n for placebo, Fe, Zn, and Fe+Zn groups was 164 and 165, 163 and 163, 162 and 167, and 161 and 160 for anthropometry and development, respectively. Fe group received 10 mg Fe as ferrous sulfate; Zn group received 10 mg Zn as zinc sulfate; and Fe+Zn group received 10 mg Fe and 10 mg Zn. WAZ, weight-for-age z score; HAZ, height-for-age z score; WHZ, weight-for-height z score. Outcomes are adjusted for sex, birth weight, initial values for the main outcomes, mother's education, and location of the household's water source. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (two-factor ANOVA with Bonferroni adjustment).

² $\bar{x} \pm$ SD (all such values).

³ Main effect and interaction calculated from group ranks.

⁴ Median; 25th–75th percentiles in parentheses (all such values).

at 12 mo in the Fe group was significantly higher than that in the placebo group ($P = 0.042$; Table 4). Control for potential confounders such as volume of supplement consumed, initial iron status, vomiting, and mother's education did not significantly change the result. There was no significant difference in MDI or BRS between the groups.

Morbidity

The incidence of diarrhea (2.9, 3.0, 2.7, and 2.8 episodes/person-year for the Fe, Zn, Fe+Zn, and placebo groups, respectively) and LRI (3.5, 3.6, 3.4, and 3.7 episodes/person-year for the Fe, Zn, Fe+Zn, and placebo groups, respectively) did not differ between treatment groups, and neither did duration of diarrhea or LRI (data not shown). Anthropometric status was not associated with incidence of infectious disease in this population, nor was there any significant interaction between treatment group and nutritional status with respect to morbidity.

DISCUSSION

The analysis showed significant interaction between iron and zinc treatment for WAZ, knee-heel length, and psychomotor development. Zinc supplementation significantly improved growth (WAZ and knee-heel length), and iron supplementation significantly improved knee-heel length and psychomotor development compared with placebo. However, combined supplementation with iron and zinc did not have a significant effect on either growth or development. These differences between Zn and Fe+Zn or between Fe and Fe+Zn could not be explained by differences in compliance or side effects (eg, vomiting from the supplement), because adjustment for these variables changed treatment effects only marginally. None of the supplements could halt the deterioration in anthropometric status as such, and the prevalence of stunting and wasting increased significantly in all groups. This suggests that zinc and iron are not the only factors limiting growth in this population.

Perrone et al (16) reported an effect on growth of children when the administration of iron and zinc was separated in time by 12 h. However, the subjects were older (4–11 y) and all were more stunted ($HAZ < -2$ SD) than were those in the present study. Our results are in line with those of several other studies that found no significant effect on growth when combining iron and zinc supplements (17–19).

However, as reported earlier, the combined iron and zinc supplement did have some effects on iron and zinc status in these infants (6), inasmuch as it increased serum ferritin and serum zinc and reduced the proportion of infants with IDA and low serum zinc. Supplementation with iron alone had a significantly stronger effect on iron status than did the combined supplement, although the former did result in a significantly larger improvement in both hemoglobin and serum ferritin and a decrease in the proportion of infants with anemia.

A small but significant effect of iron supplementation on PDI was seen, but there was no significant effect on cognitive development or behavior. This difference in PDI persisted after control for initial iron status, amount of supplement consumed, vomiting, and mother's education. The difference between groups was small (3 points) and possibly not of significance to public health. However, our results are in line with those published by Idjradinata and Pollitt (2) and by Moffatt et al (20), which showed

significant effects of iron supplementation on psychomotor development in iron-deficient children or infants at high risk of developing IDA. In the present study, the overall prevalence of anemia at baseline was very high at 40%, but the prevalence of IDA was moderate at 8%, although it increased significantly to 12 mo of age in the groups not treated with iron [9% compared with 18%; $P = 0.001$ (chi-square test; 2)]. The low occurrence of IDA at baseline may have diminished the differences in psychomotor development between the iron treatment and placebo groups. In addition, we found no decline in psychomotor development with age in the infants not treated with iron, although their anthropometric status declined and the prevalence of IDA increased significantly, which is contrary to other studies (20, 21). This finding may imply that other environmental or nutritional factors, eg, long breastfeeding duration and a high level of formal education among mothers, may have moderated the effects of iron deficiency in the non-iron-treated groups, as has been described in the case of protein-energy malnutrition and psychomotor development (22). The effects of zinc supplementation on infant development and activity have been inconclusive (23–28). In the present study, we found no effect of zinc supplementation on infant development or behavior.

Negative biochemical interactions between iron and zinc have been described, whereas no previous study reports interaction between iron and zinc that affects functional outcomes such as growth and infant development. There are several possible aspects of iron-zinc interactions. First, reported side effects of the various supplements were different: ie, vomiting in relation to the supplements was reported most often in the combined Fe+Zn group. We adjusted for this variable and consumption of supplement in the analysis, and the interactions remained significant. However, we do not know what proportion of the different supplements was lost through vomiting, and thus we cannot rule out the possibility that vomiting (ie, losing an unknown proportion of the ingested supplement before absorption) may have affected the results. In similar studies, Dijkhuizen et al (17) reported a higher dropout rate in a combined Fe+Zn group, and Penny et al (29) reported that vomiting within 30 min of receiving supplement was significantly more common in a combined zinc, iron, and vitamins group. Finally, Baqui et al (30) reported higher dropout rates among the infants given a weekly mix of iron, zinc, and vitamins as well as in both the group given the mineral and vitamin mix and in the Fe+Zn group (because of vomiting). A second aspect of interaction is intestinal absorption. It has been shown that high concentrations of inorganic iron inhibit zinc absorption (31–33) and that zinc given in water inhibits iron absorption (5, 34). Solomons and Jacob (31) found that 25 mg Fe added to a water solution with 25 mg Zn decreased plasma zinc, whereas lower total amounts of minerals (10 mg Fe and 5 mg Zn) and an iron:zinc ratio of 2:1 had no effect on zinc concentrations (35). However, Sandström et al (33) found no effect on zinc absorption when the iron:zinc ratio was 1:1 or 2.5:1 in a water solution, but they found decreased zinc absorption when iron:zinc was 25:1. When the micronutrients were given as infant foods, no significant effect of a high iron:zinc molar ratio on zinc absorption was seen (36–38). Crofton et al showed that iron and zinc given in a 1:1 ratio significantly reduced iron absorption (34) but that iron:zinc at a 2:1 ratio had no significant effect on iron absorption. Rossander-Hultén et al (5) found that iron:zinc at a ratio of 1:4 significantly reduced iron absorption when given as a water solution but had no significant effect on iron absorption



when given as a meal. Iron is transported from the intestinal lumen through the apical membrane of the enterocyte by divalent metal transporter 1 (DMT1, also known as Nramp2 or DCT1), which has been shown also to transport zinc ions (39), but other common iron-zinc absorption pathways have been suggested as well (40). The substrate specificity and the actions of these transporters in vivo, particularly in human infants, are not known. A third possible aspect of interaction is the effects and counter-effects of the 2 minerals on the functional outcomes. Zinc supplementation in zinc-deficient infants has been shown to improve growth (1), whereas iron supplementation to iron-replete infants has been shown to negatively affect growth (41–43). These mechanisms may also have been present in the present study.

Unlike previous studies (3), we found no effect of zinc supplementation on either diarrheal or respiratory infections. One possible reason for this lack of effect may be that the preventive effect of zinc on diarrheal disease was shown to be more pronounced in children aged ≥ 12 mo than in younger children (3), although Baqui et al (30) showed that weekly supplementation with iron and zinc lowered the incidence of severe diarrhea and acute respiratory infections in infants from age 6 mo. Furthermore, the diarrheal incidence in this study was lower than that in some other studies (30, 44, 45). The high proportion of infants in this study who were still being breastfed, together with high accessibility to safe water and family factors such as the relatively high educational level of the mothers, might have added a protective effect. Taken together, these factors may have made it difficult to achieve further reductions in the morbidity incidences through zinc supplementation in this population.

In conclusion, this study in infants, which found significant deterioration of nutritional status during the first year of life, indicates that both zinc and iron are growth-limiting nutrients and that significant interactions exist between iron and zinc, not only in measurements of iron and zinc status but also in assessments of functional outcomes such as weight, knee-heel length, and psychomotor development. The consequence for micronutrient deficiency-prevention programs is that combined, simultaneous supplementation with iron and zinc cannot be routinely recommended at the iron:zinc molar ratio used. Instead, when planning simultaneous interventions with iron and zinc in vulnerable populations, the use of innovative regimens in the provision of the minerals is deemed necessary. These may include intermittent supplementation with iron and zinc (eg, weekly dosage of the 2 minerals), separating the supplements in time (eg, zinc supplementation only during diarrheal episodes), the use of iron and zinc compounds with absorptive properties different from those of iron sulfate and zinc sulfate, administration of iron and zinc at molar ratios other than those applied here, or other, alternative strategies. Before the interaction of iron and zinc can be properly interpreted from a public health point of view, the efficacy of these regimens must be assessed in randomized trials of sufficient size, preferably across populations with different nutritional status and infection loads.



We are grateful to the families in Purworejo who participated in the trial. We are also greatly obliged to the dedicated field staffs of CHN-RL at Purworejo and Yogyakarta, without whom this effort would have been impossible. Lennarth Nyström of Umeå University generated the randomization list, and Stig Uhlin of the Umeå University Computer Centre contributed significantly in the preparation of the longitudinal data files.

TL was the main author of the paper and also participated in the planning and performance of the trial and in data analysis. TL had full access to all the

data in the study and had final responsibility for the decision to submit for publication. BL participated in the study design, data analysis, and writing of the manuscript. HS contributed to the data analysis and writing of the manuscript. ILG and DI took part in the study design and data collection. RS assisted in the data collection. L-ÅP participated in designing the study, analyzing the data, and writing the manuscript. None of the authors had any financial or personal interests in any of the bodies sponsoring this research.

REFERENCES

1. Brown KH, Pearson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2002;75:1062–71.
2. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* 1993;341:1–4.
3. Bhutta ZA, Black RE, Brown KH, et al. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. *J Pediatr* 1999;135:689–97.
4. Sandström B. Micronutrient interactions: effects on absorption and bio-availability. *Br J Nutr* 2001;85:S181–5.
5. Rossander-Hultén L, Brune M, Sandström B, Lönnnerdal B, Hallberg L. Competitive inhibition of iron absorption by manganese and zinc in humans. *Am J Clin Nutr* 1991;54:152–6.
6. Lind T, Lönnnerdal B, Stenlund H, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. *Am J Clin Nutr* 2003;77:883–90.
7. Dibley MJ, Sadjimin T, Kjolhede CL, Moulton LH. Vitamin A supplementation fails to reduce incidence of acute respiratory illness and diarrhea in preschool-age Indonesian children. *J Nutr* 1996;126:434–42.
8. Kodyat B, Kosen S, de Pee S. Iron deficiency in Indonesia: current situation and intervention. *Nutr Res* 1998;18:1953–63.
9. Dijkhuizen MA, Wieringa FT, West CE, Muherdiyantiningsih, Muhilal. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. *Am J Clin Nutr* 2001;73:786–91.
10. Kjolhede CL, Stallings RY, Dibley MJ, Sadjimin T, Dawiesah S, Padamawati S. Serum retinol levels among preschool children in Central Java: demographic and socioeconomic determinants. *Int J Epidemiol* 1995;24:399–403.
11. Ninuk TSH, Dibley MJ, Sadjimin T, Serdula M. Food and nutrient intakes of infants and young children in Central Java, Indonesia. Yogyakarta, Indonesia: University of Gadjah Mada; 1997.
12. World Health Organization. Iron deficiency anaemia. Assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization, 2001.
13. World Health Organization. Trace elements in human nutrition and health. Geneva: World Health Organization, 1996.
14. Bayley N. Bayley Scales of Infant Development. 2nd ed. San Antonio: The Psychological Corporation, 1993.
15. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2002 CDC growth charts for the United States: methods and development. *Vital Health Stat* 11 2002(246):1–190.
16. Perrone L, Salerno M, Gialanella G, et al. Long-term zinc and iron supplementation in children of short stature: effect of growth and on trace element content in tissues. *J Trace Elem Med Biol* 1999;13:51–6.
17. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Muhilal. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. *J Nutr* 2001;131:2860–5.
18. Rosado JL, Lopez P, Munoz 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.
19. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekars A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. *J Nutr* 2003;133:1075–80.
20. Moffatt ME, Longstaffe S, Besant J, Dureski C. Prevention of iron deficiency and psychomotor decline in high-risk infants through use of iron-fortified infant formula: a randomized clinical trial. *J Pediatr* 1994;125:527–34.
21. Williams J, Wolff A, Daly A, MacDonald A, Aukett A, Booth IW. Iron supplemented formula milk related to reduction in psychomotor decline

- in infants from inner city areas: randomised study. *BMJ* 1999;318:693–7.
22. Pollitt E. Developmental sequel from early nutritional deficiencies: conclusive and probability judgements. *J Nutr* 2000;130:350S–3S.
 23. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. *Am J Clin Nutr* 1993;57:344–52.
 24. Sazawal S, Bentley M, Black RE, Dhingra P, George S, Bhan MK. Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. *Pediatrics* 1996;98:1132–7.
 25. Bentley ME, Caulfield LE, Ram M, et al. Zinc supplementation affects the activity patterns of rural Guatemalan infants. *J Nutr* 1997;127:1333–8.
 26. Ashworth A, Morris SS, Lira PI, Grantham-McGregor SM. Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. *Eur J Clin Nutr* 1998;52:223–7.
 27. Castillo-Duran C, Perales CG, Hertrampf ED, Marin VB, Rivera FA, Icaza G. Effect of zinc supplementation on development and growth of Chilean infants. *J Pediatr* 2001;138:229–35.
 28. Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *Am J Clin Nutr* 2001;74:381–6.
 29. Penny ME, Peerson JM, Marin RM, et al. Randomized, community-based trial of the effect of zinc supplementation, with and without other micronutrients, on the duration of persistent childhood diarrhea in Lima, Peru. *J Pediatr* 1999;135:208–17.
 30. Baqui AH, Zaman K, Persson LA, et al. Simultaneous weekly supplementation of iron and zinc is associated with lower morbidity due to diarrhea and acute lower respiratory infection in Bangladeshi infants. *J Nutr* 2003;133:4150–7.
 31. Solomons NW, Jacob RA. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. *Am J Clin Nutr* 1981;34:475–82.
 32. Valberg LS, Flanagan PR, Chamberlain MJ. Effects of iron, tin, and copper on zinc absorption in humans. *Am J Clin Nutr* 1984;40:536–41.
 33. Sandström B, Davidsson L, Cederblad A, Lönnerdal B. Oral iron, dietary ligands and zinc absorption. *J Nutr* 1985;115:411–4.
 34. Crofton RW, Gvozdanovic D, Gvozdanovic S, et al. Inorganic zinc and the intestinal absorption of ferrous iron. *Am J Clin Nutr* 1989;50:141–4.
 35. Solomons NW. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *J Nutr* 1986;116:927–35.
 36. Haschke F, Ziegler EE, Edwards BB, Fomon SJ. Effect of iron fortification of infant formula on trace mineral absorption. *J Pediatr Gastroenterol Nutr* 1986;5:768–73.
 37. Fairweather-Tait SJ, Wharf SG, Fox TE. Zinc absorption in infants fed iron-fortified weaning food. *Am J Clin Nutr* 1995;62:785–9.
 38. Davidsson L, Almgren A, Sandström B, Hurrell RF. Zinc absorption in adult humans: the effect of iron fortification. *Br J Nutr* 1995;74:417–25.
 39. Gunshin H, Mackenzie B, Berger UV, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997;388:482–8.
 40. Yamaji S, Tennant J, Tandy S, Williams M, Singh Srai SK, Sharp P. Zinc regulates the function and expression of the iron transporters DMT1 and IREG1 in human intestinal Caco-2 cells. *FEBS Lett* 2001;507:137–41.
 41. Idjradinata P, Watkins WE, Pollitt E. Adverse effect of iron supplementation on weight gain of iron-replete young children. *Lancet* 1994;343:1252–4.
 42. Dewey KG, Domellöf M, Cohen RJ, Landa Rivera L, Hernell O, Lönnerdal B. Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. *J Nutr* 2002;132:3249–55.
 43. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. *J Trop Pediatr* 2003;49:84–8.
 44. Sazawal S, Black RE, Bhan MK, Jalla S, Sinha A, Bhandari N. Efficacy of zinc supplementation in reducing the incidence and prevalence of acute diarrhea—a community-based, double-blind, controlled trial. *Am J Clin Nutr* 1997;66:413–8.
 45. Bhandari N, Bahl R, Taneja S, et al. Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young north Indian children. *Pediatrics* 2002;109:E86.

