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ORIGINAL ARTICLE

Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants

J Berger[1], NX Ninh[2], NC Khan[2], NV Nhien[2], DK Lien[2], NQ Trung[2] and HH Khoi[2]

[1]*Institut de Recherche pour le Développement (IRD), UR 106 'Nutrition, Alimentation, Sociétés', Montpellier Cedex, France and* [2]*National Institute of Nutrition, Hanoi, Vietnam*

Objective: To evaluate the effect of combined iron–zinc supplementation on micronutrient status, growth and morbidity.
Design: Randomized, double-masked, placebo-controlled supplementation trial.
Setting: Rural district of Que Vo, in the Red River Delta in Vietnam.
Subjects: A total of 915 breast-fed infants aged 4–7 months were included and 784 completed the study.
Interventions: The Fe-group received daily and for a 6-month period 10 mg of iron, the Zn-group 10 mg zinc, the Fe–Zn group 10 mg iron + 10 mg zinc and the placebo group a placebo. Hemoglobin (Hb), serum ferritin (SF) and zinc (SZn), and anthropometry were measured before and at the end of the intervention. Morbidity was recorded daily.
Results: Changes of Hb and SF were higher in both Fe and Fe + Zn groups (respectively 22.6 and 20.6 g/l for Hb; 36.0 and 24.8 μg/l for SF) compared to Zn and placebo groups (Hb: 6.4 and 9.8 g/l; SF: −18.2 and −16.9 μg/l, $P<0.0001$). SZn increased more in Zn group (10.3 μmol/l) than in Fe + Zn group (8.0 μmol/l, $P=0.03$) and more in these groups compared to Fe and placebo groups (1.6 and 1.2 μmol/l, $P<0.0001$). Weight gain was higher in the Zn group. No significant effects of supplementations on growth in length or morbidity.
Conclusions: Combined iron–zinc supplementation had a positive effect on iron and zinc status in infants. However, the positive effect of zinc alone on SZn and weight would indicate a negative interaction of iron when added to zinc supplements.
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Keywords: iron supplementation; zinc supplementation; infant; Vietnam

Introduction

In most developing countries, infant diets are not adequate to meet the high iron (Dallman *et al*., 1980) and zinc (Hotz and Brown, 2001) requirements related to rapid growth. As a result, most infants develop iron deficiency anemia (IDA) by 1 year of age (Fairweather-Tait, 1992) and suffer from growth retardation, delayed development and cognitive function and increased morbidity related to infections (Bhutta *et al*., 1999; Beard and Stoltzfus, 2001).

Iron supplementation in infants has shown positive effects on iron deficiency (ID), anemia and cognitive development (Lozoff *et al*., 1987). Zinc supplementation is associated with improvement of growth (Brown *et al*., 2002) and has beneficial effects on diarrhea and pneumonia morbidity (Bhutta *et al*., 1999). The importance of these two micronutrient deficiencies in public nutrition and their likely coexistence in infants in developing countries support that combined supplementation with both iron and zinc would be appropriate and would improve the cost-effectiveness of interventions. However, recent trials show inconsistent

Correspondence: Dr J Berger, Institut de Recherche pour le Développement (IRD), French Embassy, 57 Tran Hung Dao, Hanoi, Vietnam.
E-mail: j.berger@fpt.vn
Guarantor: J Berger.
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results of the impact of association of iron and zinc supplements on micronutrient status (Rosado *et al*., 1997; Dijkhuizen *et al*., 2001; Lind *et al*., 2003) probably related to the nutritional status and the environmental conditions that differs between countries.

In Vietnam, the 1995 national survey (NIN/UNICEF/CDC/PAMM, 1995) showed that about 60% of the infants and children under 2 years of age suffer from IDA and a second national survey conducted in 2000 showed little improvement (Khoi *et al*., 2003). A 6-month daily iron supplementation of infants is associated with a significant improvement of hemoglobin (Hb) concentration and disappearance of anemia that was present in 81% of infants at the beginning of the intervention (Ninh *et al*., 2002). Moreover, about 40% of Vietnamese children also suffer of growth retardation (Khoi *et al*., 2003). A daily zinc supplementation in stunted Vietnamese young children improves growth in weight and height and reduces the frequency of infectious diseases (Ninh *et al*., 1996). These results suggest that iron and zinc deficiencies are significant public health problems among Vietnamese children and that interventions associating iron and zinc supply should be considered. The aim of this study was therefore to investigate the effects of a 6-month iron and zinc supplementation on iron status, zinc status, growth and morbidity in Vietnamese infants and to compare with supplementation with zinc or iron alone.

Subjects and methods

Study design and subjects

The study was a randomized, double-masked, placebo-controlled supplementation trial. It was conducted from March to November 1998 in 24 communes of Que Vo, a rural and poor district, 50 km northwest of Hanoi in the Red River Delta in Vietnam.

The sample size was estimated to be about 140 subjects per group to detect a difference of 0.5 s.d. in height-for-age (HAZ) or weight-for-age (WAZ) with a confidence level of 95% and a power of 0.90. Anticipating 30% dropouts a sample size of at least 200 subjects per group was required at baseline.

Subjects were singleton breast-fed infants, between 4 and 7 months of age. All the parents were informed orally and in writing about the aims and procedures of the study and written informed consents were obtained from at least one parent before enrollment into the study. At baseline, the infants were examined by a physician and assessed for anthropometry. Only infants free from chronic or acute illness, severe malnutrition or congenital abnormality were included. Included infants were randomly assigned, following a computer-generated block randomized group allocation, to four groups: the Fe-group received a daily dose of 10 mg of iron as ferrous sulfate, the Zn-group a daily dose of 10 mg zinc as zinc sulfate, the Fe–Zn group a daily dose of 10 mg iron + 10 mg zinc and the placebo group a placebo.

The four types of supplement were included in a sweet-tasting syrup and provided free of charge by the UNICEF Supply Division in Copenhagen. Supplements were presented in similar coded bottles avoiding participants and health workers to differentiate between treatments. The supplements were coded with a letter at production and the code-allocation kept secret until the end of the statistical analysis. To avoid vitamin A deficiency becoming a limiting factor to infant health during the study period, a dose of 100 000 IU of vitamin A was given to all infants at the start of the study.

The supplements were given 7 days/week during 6 months by trained field workers, one in each village, recruited for the study. The supplementation was given in the early morning, in a central place of each village or in the house of the field worker. The parents were taught to bring their infant between 0700 and 0900 The field workers were provided with a list of infants and the corresponding treatment identified by a letter. Two milliliters of syrup were given directly into the mouth of the infant using a small plastic syringe. No food or beverage were given to the infant with the supplement and the parents were instructed to wait 2 hs after the intake of syrup before giving any food or beverage. The presence of infants was noted daily. The quality of the work of the each field worker was checked all along the study by supervisors chosen in the commune and by members of the research team. New bottles of syrup were given each month to the field workers. The last distribution of syrups to the infants occurred the day before the final blood sampling.

Methods

Trained assistants measured weight and height monthly from baseline to the end of the study. Each assistant was responsible for the same infants all along the study. Length was measured to a precision of 1 mm using a Harpenden Infant Measuring Table (CMS Weighing Equipment Ltd, UK) with a resolution of 1 mm. Each measure was taken in triplicate and length calculated as the mean of the three measures. If two measures were different by more than 3 mm, length was again measured in triplicate. Infants were weighed naked on a baby scale with a precision of 10 g (Seca, Germany). The anthropometric indicators, WAZ, HAZ and weight-for-height (WHZ) were expressed in *z*-scores according to the National Center for Health Statistics reference using EPI-INFO 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Stunting was defined by HAZ < -2 *z*-scores, wasting by WHZ < -2 *z*-scores and underweight by HAZ < -2 *z*-scores.

Nonfasting blood samples were collected in the morning between 0800 and 0010, at baseline and after 26 weeks, at the end of the intervention period. At each sampling, 3 ml venous blood was drawn using a zinc-free vacuum system; 20 μl of whole blood were immediately mixed in 5 ml of Drabkin's solution for assessment of Hb concentration. All tubes were kept cool and transported to the laboratory at the

National Institute of Nutrition in Hanoi within 4 h. Serum was separated by centrifugation at 5000 *g* for 10 min at 4°C. Aliquots of serum were stored at −30°C until analysis of serum ferritin (SF) and zinc (SZn).

Hb was measured by the cyanomethemoglobin method within 12 h (Boehringher kits). SZn was assessed in duplicate by flame atomic absorption spectrometry (Perkin-Elmer Model 5000 spectrophotometer, Norwalk, USA). Before analysis, the serum samples were diluted (1:4) with de-ionized water. A standard curve was established by using commercial zinc standards (Wako Puro Chemical Industry Ltd, Japan) in concentrations of 0.1, 0.2, 0.4 and 0.5 mg/l. The within-run and between-run analytical precision for 10 replicate analyses of test samples ranged respectively from 2.1 to 3.5% and from 2.5 to 5.2%. Two standard points were repeated after measuring 10 samples. Hemolyzed serum samples were discarded to avoid interference of high zinc content from red blood cells. SF was measured by a two-site enzyme-linked immunosorbent assay (Ramco, Houston, TX, USA) using monoclonal reagents for both the capture and indicator antibodies as described previously (Flowers *et al*., 1986).

Anemia was defined by Hb less than 110 g/l, low iron stores by SF less than 12 μg/l (INACG, 1985) and low SZn by SZn less than 9.9 μmol/l (Hotz *et al*., 2003). IDA was defined by simultaneous low SF value and anemia.

Morbidity

The field workers who gave the supplements recorded morbidity daily. Diarrhea was defined by three or more unformed stools per day and diarrhea recovery by a 48-h diarrhea-free period. Acute respiratory infection (ARI) was defined by presence of cough or/and difficulty to breathe and/or elevated respiratory rate (RR). An elevated RR was higher than 50/min in infant and higher than 40/min in children (>1 year of age).

Ethics

The Scientific Committees of the National Institute of Nutrition, and of the Ministry of Health in Hanoi reviewed and approved the study protocol. Infants with Hb <70 g/l at baseline were excluded from the study and referred to the health center of the district for treatment. At the end of the intervention period, all infants still anemic were referred to the health center to received iron supplements.

Statistical analysis

The principal objective of the analysis was to assess the efficacy of the single or combined iron and zinc treatment on biochemical and anthropometrical outcomes and changes between baseline and end point. In addition, we evaluated the effect of treatments on the prevalence of anemia and IDA during the intervention study.

When data were not normally distributed, statistical analysis was carried out after log transformation (SF, SZn). For continuous response variables (Hb, SF, SZn, HAZ, WHZ, WAZ), results are presented as means and standard deviation or geometric means with confidence interval for log-transformed data. Prevalence data are given for binary indicators (anemia, IDA, low SF values and elevated SZn values, stunting and wasting).

Comparison of treatment groups (Fe, Zn, Fe + Zn, placebo) for end point values and/or changes between baseline and end point was performed by analysis of variance (ANOVA) for continuous outcomes and by logistic regression for binary responses variables. When the overall treatment F or LR test was significant, differences among groups were further investigated with conservative multiple comparison tests (Bonferroni's *post hoc* comparison).

Factorial analysis (two-factor ANOVA) was also performed to assess the interaction of combined iron and zinc supplementation on main outcomes.

The experimental design (randomization of treatment group at the subject level) allows getting groups with similar initial iron, zinc and nutritional status. However, possible interaction between the effect of treatments and infant's sex, baseline anemia, iron status, zinc status or nutritional status was evaluated by introducing treatment group × baseline status interaction terms in the linear regression models with changes between baseline and final evaluation for each outcome as dependent variable. No significant interactions were found.

For all outcomes variables, statistical analyses were repeated after adjustments were made for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ to control for the initial nutritional status of the infants. Changes from baseline to end point between groups were compared before and after adjustment with the same initial six variables excluding the corresponding initial variable of the measured outcome (e.g. changes in SF were analyzed after adjustment for initial Hb, log SZn HAZ, WAZ and WHZ but not for initial log SF).

Results

A total of 988 infants were eligible to participate in the study and randomly assigned to the four groups (Figure 1). However, at baseline the parents of 55 infants refused the blood sampling and these infants were excluded from the study. Eighteen infants with Hb less than 70 g/l were also excluded and referred to the health center of the district for treatment. Thus, 915 infants were included in the study and 784 infants completed the 6-month supplementation period. The rate of dropouts ($n = 131$, 14.3%) was similar in each group. The main reasons for dropouts were the difficulty of parents to bring their infant every day to the syrup distribution place (93 infants stopped the treatment) or absence at the end point of intervention mainly because of

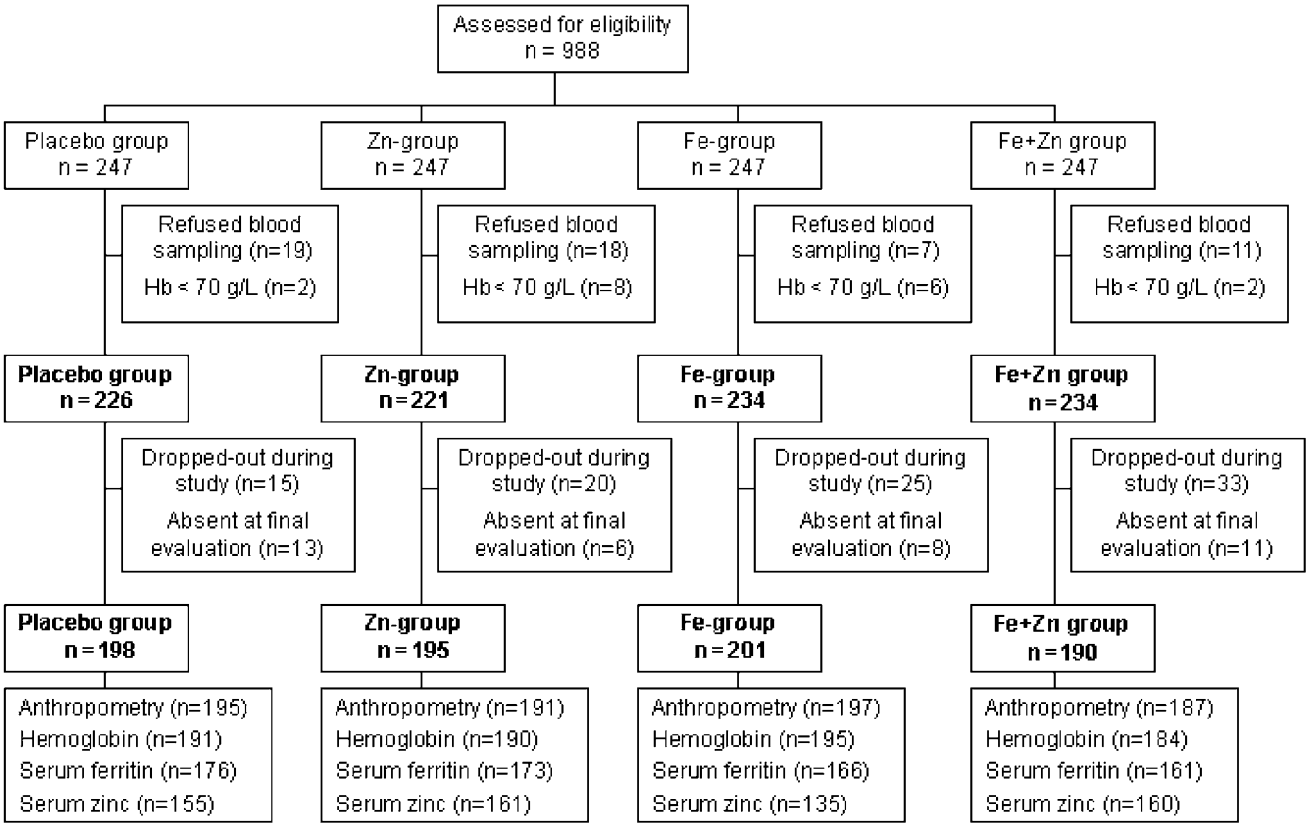

Figure 1 Flow diagram of study participants. Zn-group: zinc supplements; Fe-group: iron supplements; Fe + Zn group: combined iron and zinc supplements.

refusal from parents of the second blood sampling (38). Of the 784 infants who completed the study, 770 had final anthropometry measurements and 760 end point blood samples. Owing to low serum volume, end point SF was measured in 676 infants whereas SZn was measured in 611 infants.

The mean values of initial anthropometrical indexes and indicators of micronutrient status as well as prevalence of stunting, wasting and anemia of dropouts were not statistically different compared to infants who completed the study (data not shown).

At baseline, the initial mean age of the infants who completed the study was 5.9 ± 0.7months; most of them (676 infants, 86.2%) were 5.0–6.9 months old whereas 71 (9.1%) were 4.0–4.9 months old and 37 (4.7%) 7.0–7.9 months old. The age distribution was not different between the four groups ($P = 0.42$). Mean HAZ was −1.03 ± 0.80 *z*-scores and mean WHZ was 0.29 ± 0.77 *z*-scores. About 11.4% (89) of the infants had HAZ < −2 *z*-scores but only one infant had WHZ < −2.0 *z*-scores. Anemia was observed in 54.1%, low SF in 16.9%, IDA in 12.5% and low SZn in 1.0% of the infants. Baseline characteristics, including mean age, anthropometry, Hb concentration, iron and zinc status were not significantly different between the four groups (Table 1).

Indicators of micronutrient status at outcome

At the end of the supplementation period, the Fe group and the Fe + Zn group had significantly higher Hb than the placebo and Zn groups ($P < 0.0001$. for all *post hoc* comparison tests) (Table 2). Final Hb was not significantly different between Fe and Fe + Zn groups ($P = 0.83$) and between the placebo and Zn groups ($P = 0.99$). The final prevalence of anemia was significantly lower in both the Fe and Fe + Zn groups compared to the placebo and Zn groups ($P < 0.0001$ for all *post hoc* tests). The prevalence of anemia was not significantly different between the Fe and Fe + Zn groups ($P = 0.98$) and between the placebo and Zn groups ($P = 0.83$). The factorial analysis indicated that iron supplementation significantly increased Hb ($P < 0.0001$) whereas the effect of zinc supplementation was not significant ($P = 0.21$). The interaction between iron and zinc treatment was not significant ($P = 0.41$).

Similar inferences were obtained for Hb when statistical analyses (ANOVA, factorial analysis) were performed after adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ. Moreover, there were no significant differences in treatment effects between boys and girls (data not shown).

Table 1 Baseline characteristics of infants who completed the study

	Placebo 198	*Zn 195*	*Fe 201*	*Fe + Zn 190*	P[a]
Girls (*n* (%))	97 (49.0)	98 (50.3)	114 (56.7)	91 (47.6)	0.29
Age (months)[b]	5.8 (0.7)	5.9 (0.7)	5.9 (0.6)	5.9 (0.7)	0.83
Height (cm)	63.9 (2.5)	63.9 (2.5)	63.8 (2.6)	63.7 (2.4)	0.83
Weight (kg)	6.9 (0.8)	6.9 (0.9)	6.9 (0.9)	6.9 (0.9)	0.90
HAZ (*z*-scores)	−1.01 (0.79)	−1.00 (0.80)	−1.03 (0.85)	−1.10 (0.75)	0.59
WAZ (*z*-scores)	−0.56 (0.76)	−0.57 (0.89)	−0.60 (0.90)	−0.56 (0.82)	0.96
WHZ (*z*-scores)	0.27 (0.75)	0.25 (0.80)	0.26 (0.74)	0.36 (0.78)	0.50
Stunting (%)	9.1	10.3	10.0	15.8	0.15
Hb (g/l)	108.2 (15.5)	109.0 (13.9)	111.2 (116.7)	109.1 (13.6)	0.23
Anemia (%)	56.1	48.2	56.7	55.3	0.30
SF (μg/l)[c]	27.1 (23.8–30.9)	27.5 (24.1–31.4)	25.3 (22.2–28.9)	26.6 (23.2–30.5)	0.83
Low ferritin values (%)	16.6	17.2	17.9	16.0	0.99
Zinc (μmol/l)[c]	14.59 (14.16–15.03)	14.41 (13.99–14.85)	14.25 (13.86–14.66)	14.10 (13.68–14.52)	0.43
Low SZn values (%)	0.5	1.5	1.5	0.5	0.99

[a]*P*-value of ANOVA.
[b]Mean (s.d.);
[c]Geometric mean (confidence interval).

Table 2 Outcome of treatments on indicators of micronutrient status

	Placebo	*Zn*	*Fe*	*Fe + Zn*	P[1]
Hemoglobin[2] (g/l)	118.3 (15.7)[a]	117.8 (16.1)[a]	131.8 (16.7)[b]	129.3 (15.9)[b]	<0.0001
Anemia (%)	30.4[a]	31.0[a]	8.2[b]	8.2[b]	<0.0001
Ferritin[3] (μg/l)	16.5 (14.7–18.4)[a]	16.5 (14.7–18.4)[a]	58.0 (51.7–65.0)[b]	52.3 (46.5–58.7)[b]	<0.0001
Low ferritin values (%)	33.5[a]	32.4[a]	1.2[b]	1.2[b]	<0.0001
IDA (%)	16.6[a]	14.0[a]	0.6[b]	0[b]	<0.0001
Zinc (μmol/l)[3,4]	15.79 (15.20–16.40)[a]	23.07 (22.23–23.95)[b]	15.21 (14.61–15.84)[a]	21.54 (20.75–22.36)[b]	<0.0001
Low serum zinc values (%)	0.6	0	3.0	0.6	—

[1]ANOVA for continuous variable (Hb, SF, SZn), logistic regression for categorical variable (anemia, low SF, low SZn): significant difference between groups with different letters.
[2]Mean (s.d.),
[3]Geometric mean (confidence interval).
[4]After adjustments for initial Hb, log SF, log SZn, HAZ, WAZ and WHZ, end point serum zinc was also significantly higher in the Zn group compared to the Fe + Zn group ($P = 0.02$).
Sample size was respectively for the placebo group/Zn-group/Fe-group/Fe + Zn group; Hb: 195/191/197/187; SF: 176/173/195/184 and SZn: 155/161/135/160.

Like for Hb, the Fe group and the Fe + Zn group had significantly higher final SF than the placebo and Zn groups ($P < 0.0001$ for all *post hoc* tests) (Table 2). SF was not significantly different between the Fe and Fe + Zn groups ($P = 0.97$) and between the placebo and Zn groups ($P = 0.98$). At the end of the intervention period, the prevalence of low SF values (SF < 12 μg/l) was very low in both the Fe and Fe + Zn groups and significantly lower than in the placebo and Zn group ($P < 0.0001$ for all *post hoc* tests). The final prevalence of low SF values was not significantly different between the Fe and Fe + Zn groups ($P = 0.95$) and between the placebo and Zn groups ($P = 0.81$). At baseline the mean prevalence of IDA was not significantly different between the groups ($P = 0.24$). At end point, only one child in the Fe group and no child in the Fe + Zn group had IDA but 29 (16.6%) in the placebo group and 24 (14.0%) in the Zn group.

The factorial analysis indicated that iron supplementation significantly increased SF ($P < 0.0001$) whereas the effect of zinc supplementation was not significant ($P = 0.38$). The interaction between iron and zinc treatments was not significant ($P = 0.37$).

Adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ did not change the results and there were no significant differences in treatment effects between boys and girls (data not shown).

The Zn and Fer + Zn groups had significantly higher final SZn than the placebo and Fe groups ($P < 0.0001$ for all *post hoc* tests) (Table 2). SZn was not significantly different between the Zn and Fe + Zn groups ($P = 0.07$) and between

the placebo and Fe groups ($P=0.99$). However, after adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ, SZn was significantly higher in the Zn compared to the Fe + Zn group ($P=0.02$). At the end of the intervention period, the prevalence of low SZn values (SZn < 10.7 μmol/l) was very low in all groups and no infants in Zn group had low SZn values. The factorial analysis indicated that both zinc and iron supplementation significantly and independently affected SZn ($P<0.0001$ and $P=0.007$ for zinc and iron respectively) but the interaction between iron and zinc treatment was not significant ($P=0.42$). Adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ did not change the results of the factorial analysis.

Changes of micronutrient indicators over the study period

The changes of the indicators of micronutrients status were calculated in subjects who had both baseline and end point values for all main variables (Figure 2). Hb changes between baseline and end point were not significantly different between the Fe group (22.6 ± 19.4 g/l) and the Fe + Zn group (20.6 ± 19.4 g/l, $P=0.99$) and significantly higher compared to the Zn group (6.4 ± 20.6 g/l) and the placebo group (9.8 ± 19.3 g/l, $P<0.0001$ for all comparisons). Changes were not significantly different ($P=0.59$) between the Zn and placebo groups.

Similar results were obtained for SF. SF changes were not significantly different between the Fe group (36.0 ± 47.1 μg/l) and the Fe + Zn group (24.8 ± 50.0 μg/l, $P=0.30$) but significantly higher than SF changes in the placebo (−16.9 ± 42.6 μg/l) and in the Zn group (−18.2 ± 56.5 μg/l, $P<0.0001$ for all comparisons). Changes were not significantly different ($P=0.99$) between the Zn and placebo groups.

Serum Zn increased 10.3 ± 8.2 μmol/l in the Zn group and 8.0 ± 6.7 μmol/l in the Fe + Zn group ($P=0.03$ between the two groups). These changes were significantly higher than changes in the placebo and Fe groups (respectively, 1.6 ± 4.0 and 1.2 ± 3.0 μmol/l, $P<0.0001$ for all comparisons). Changes in the Fe and placebo groups were not significantly different ($P=0.98$). Adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ, WHZ (excluding the initial variable corresponding to the measured change) did not change the results.

Figure 3 shows the changes of the prevalence of anemia, ID and IDA over the study period. By the end of the intervention, the prevalence of anemia and ID had decreased significantly in both the Fe and Fe + Zn groups ($P<0.0001$ for anemia and $P=0.001$ for ID in both groups). In the placebo and Zn groups, the prevalence of anemia (with and without ID) had decreased significantly ($P<0.001$ and $P=0.04$, respectively) whereas the prevalence of iron deficiency (ID + IDA) had increased significantly ($P<0.0001$ in the placebo group and $P=0.004$ in the Zn group).

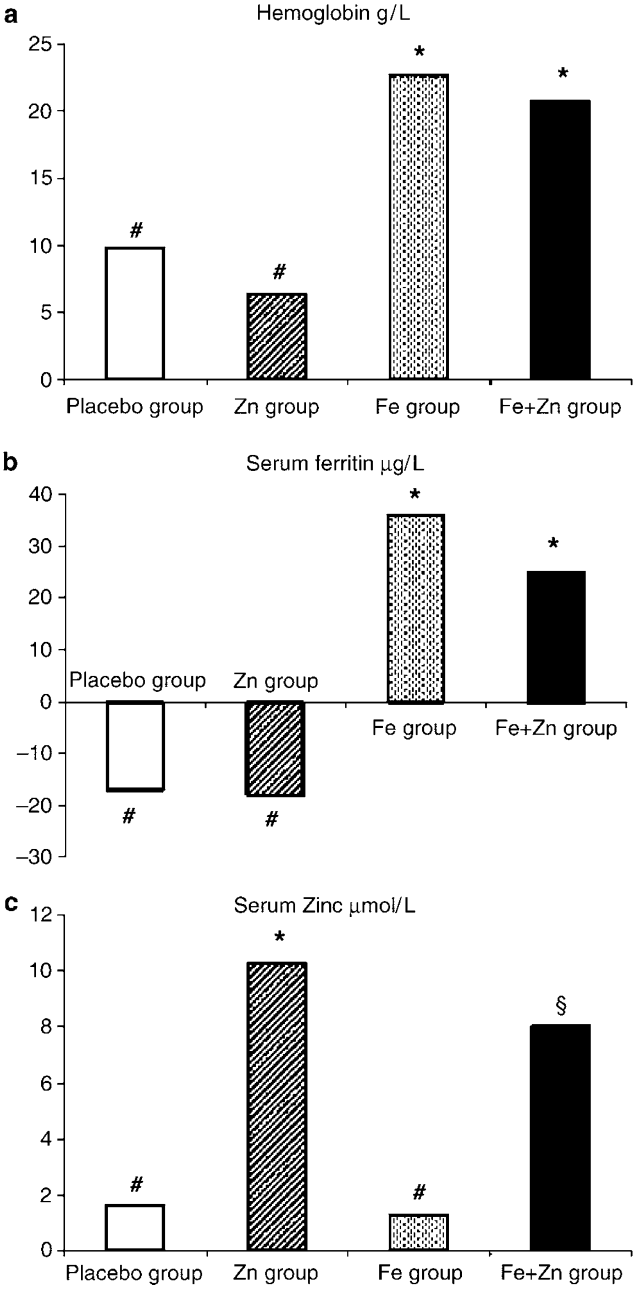

Figure 2 Changes of Hb concentration (**a**), serum ferritin (**b**) and SZn (**c**) between baseline and end of the 6-month intervention period. Zn-group: zinc supplements; Fe-group: iron supplements; Fe + Zn group: combined iron and zinc supplements. $^{*,\#,\S}$Significant differences between groups with different superscripts.

Anthropometry

During the study, HAZ, WAZ and WHZ decreased significantly in all groups. At the end of the intervention, 25.3% (195) of the infants had HAZ < −2 *z*-scores and 4.0% (31) had WHZ < −2 *z*-scores. The prevalence of stunting and wasting was not significantly different between the groups. End

point HAZ was not significantly different between groups (Table 3). The factorial analysis indicated no effect of either zinc or iron supplementation on HAZ *z*-scores and no significant interaction. Inclusion of baseline Hb, log SF, log SZn, HAZ, WAZ and WHZ as covariates did not change the conclusion.

End point WAZ and WHZ were not significantly different between groups but WHZ just failed to reach significance (Table 3). However, after adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ, WAZ and WHZ end points were significantly better in the Zn group compared to the Fe group ($P=0.001$ for WAZ and $P=0.008$ for WHZ) whereas only WAZ was also better in the Zn group compared to both the placebo and the Fe + Zn groups. The factorial analysis indicated no significant differences for zinc or iron supplementation on WAZ *z*-scores. However, after

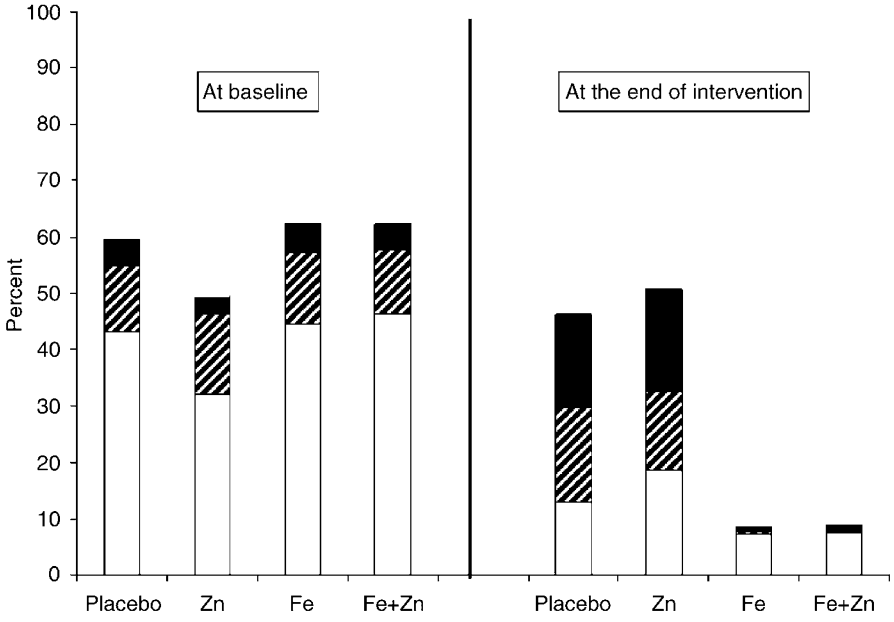

Figure 3 Prevalence of anemia □, iron deficiency (ID ■) and iron deficiency anemia (IDA ▨) at baseline and after 6 months of intervention. Zn-group: zinc supplements; Fe-group: iron supplements; Fe + Zn group: combined iron and zinc supplements.

Table 3 Outcome of treatments and changes between baseline and endpoint of height, weight and nutritional indicators

	Placebo 195	*Zn 191*	*Fe 197*	*Fe + Zn 187*	P[1]
Outcomes[2,3]					
Height (cm)	71.01 (2.41)	71.15 (2.53)	70.99 (2.51)	70.84 (2.47)	0.69
Weight (Kg)	8.05 (0.80)	8.19 (0.92)	7.98 (0.90)	8.05 (0.90)	0.13
HAZ (*z*-scores)	−1.42 (0.82)	−1.37 (0.83)	−1.39 (0.84)	−1.51 (0.79)	0.36
WAZ (*z*-scores)	−1.68 (0.75)	−1.54 (0.85)	−1.71 (0.81)	−1.71 (0.80)	0.13
WHZ (*z*-scores)	−0.85 (0.69)	−0.71 (0.70)	−0.90 (0.68)	−0.81 (0.70)	0.052
Stunting (%) HAZ ⩽ 2 *z*-scores	25.6	20.4	26.5	27.3	0.40
Wasting (%) WHZ ⩽ 2 *z*-scores	4.1	3.1	5.6	3.2	0.58
Changes[2,4]					
HAZ (Z-scores)	−0.42 (0.46)	−0.37 (0.39)	−0.38 (0.39)	−0.41 (0.47)	0.60
WAZ (Z-scores)	−1.13 (0.46)[a]	−0.97 (0.41)[b]	−1.13 (0.40)[a]	−1.14 (0.48)[a]	0.0004
WHZ (Z-scores)	−1.13 (0.57)[a]	−0.96 (0.51)[b]	−1.18 (0.51)[a]	−1.16 (0.56)[a]	0.001

[1]ANOVA for continuous variable (height, weight, HAZ, WAZ, WHZ), logistic regression for categorical variable (stunting and wasting): significant difference between groups with different letters.
[2]Mean ± s.d.
[3]After adjustment for initial Hb, log SF, log SZn, HAZ, WAZ and WHZ, *P*-values of the variance analysis were significant for weight ($P=0.001$), WAZ ($P=0.001$) and WHZ ($P=0.008$). Weight and WAZ end points were significantly higher in the Zn group compared to the three other groups whereas WHZ end point was significantly higher in the Zn group compared to the Fe group.
[4]After adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ (excluding the initial variable corresponding to the measured change) *P*-values of the variance analysis were still significant for WAZ changes ($P=0.001$) and WHZ ($P=0.007$). Changes of WAZ were significantly lower in the Zn group compared to the three other groups whereas WHZ changes was significantly lower in the Zn group compared to the Fe group.

adjustment WAZ indicated a significant effect of both the iron ($P=0.006$) and zinc supplementation ($P=0.03$) and a significant interaction ($P=0.02$). This indicated that the Zn group performed better than other three groups on WAZ.

Concerning WHZ, only zinc supplementation had a significant effect ($P=0.02$). However, after adjustment, both iron and zinc supplementations had a significant effect (respectively, $P=0.04$ and $P=0.02$) on WHZ but the interaction between the two nutrients was not significant ($P=0.26$).

HAZ changes between baseline and end of intervention period were not different between groups whereas changes of WAZ and WHZ were significantly different. Decreases of WAZ and WHZ were significantly lower in the Zn group compared to the placebo group ($P=0.002$ for WAZ and $P=0.01$ for WHZ), to the Fe + Zn group ($P=0.002$ for WAZ and WHZ) and to the Fe group ($P=0.004$ for WAZ and $P<0.001$ for WHZ). After adjustment for baseline values of Hb, log SF, log SZn, HAZ, WAZ and WHZ (excluding the initial variable corresponding to the measured change), the decrease of WHZ was no more significantly different between the Zn and the placebo group ($P=0.10$) or the Fe + Zn group ($P=0.20$) but still lower compared to the Fe group ($P=0.03$). WAZ and WHZ changes were not significantly different between placebo, Fe and Fe + Zn groups without or after adjustment.

The mean number of doses of syrup consumed during the study period was 173.9 ± 11.9 similar in each groups ($P=0.97$). This corresponds to a mean quantity of 1739 mg of iron or zinc over the 6-month period. Only 10 infants (3, 3, 2 and 2, respectively in the placebo, Zn, Fe and Fe + Zn groups, respectively) consumed less than 145 doses (about 80% of maximum doses).

The effects of the supplements on morbidity are presented in Table 4. The cumulative incidence represents the ratio of number of new cases during the 6-month study period/at-risk total population. The mean number of episodes and the mean total number of days of illness were reported for ARI, cough, fever and diarrhea. Neither the cumulative incidence nor the number of episodes or days of any illness were significantly different between the groups. To take into account the potential interaction of the initial iron status of the infants on the effect of the treatments on morbidity, we compared the effects of treatments in anemic and non-anemic infants as well in infants with low initial SF ($<12\,\mu$g/l) and normal initial SF ($\geqslant 12\,\mu$g/l). The number of episodes and the number of days of any illness were not significantly different between the treatments in anemic or non-anemic infants and in infants with low or normal initial SF (data no showed). Owing to the low prevalence of zinc-deficient infants (SZn $<9.9\,\mu$mole/l), we compared morbidity in infants with low SZn (first quartile or the SZn distribution) with other infants. No significant differences between treatments were found in both subgroups.

Discussion

This study provides the evidence that a 6-month daily combined iron–zinc supplementation was effective in im-

Table 4 Incidence of morbidity over the study period

	Placebo 197	*Zn 195*	*Fe 200*	*Fe + Zn 189*
Cumulative incidence[a]				
ARI	53.3 (105)	52.3 (102)	56.0 (112)	55.0 (104)
Cough	42.1 (83)	42.6 (83)	39.5 (79)	40.2 (76)
Fever	15.2 (30)	11.3 (22)	14.5 (29)	14.8 (28)
Diarrhea	25.9 (51)	26.7 (52)	23.0 (46)	26.5 (50)
Number of episodes and total number of days of illness among positive cases[b]				
ARI				
Number of episodes	1.87 (1.01)	1.83 (1.13)	1.91 (1.14)	1.85 (0.88)
Number of days	9.64 (7.75)	9.12 (6.18)	9.86 (9.09)	9.44 (6.75)
Cough				
Number of episodes	1.31 (0.60)	1.51 (0.76)	1.44 (0.73)	1.28 (0.60)
Number of days	6.62 (5.97)	6.83 (5.20)	6.84 (6.02)	6.34 (4.69)
Fever				
Number of episodes	1.00 (0.00)	1.09 (0.29)	1.03 (0.19)	1.14 (0.36)
Number of days	8.17 (7.02)	7.23 (6.58)	6.38 (4.95)	8.11 (6.38)
Diarrhea				
Number of episodes	1.33 (0.59)	1.42 (0.75)	1.41 (0.78)	1.42 (0.76)
Number of days	5.25 (3.57)	6.54 (5.06)	5.83 (5.05)	6.08 (4.78)

Cumulative incidence over 6 months intervention: number of new cases during the study period/total population at risk.
No significant differences.
[a]Cumulative incidence (number of cases).
[b]Mean (s.d.).

proving significantly the iron and zinc status and in decreasing significantly the prevalence of anemia and ID in infants living in a rural area in Vietnam. The combined zinc and iron supplementation had the same effect on iron status than supplementation with iron alone but a lower effect on the increase of SZn concentration than supplementation with zinc alone. No treatment had a significantly effect on HAZ or on morbidity whereas zinc supplementation alone had a significant better effect on WAZ index than the other three treatments and on WHZ index compared to the Fe group.

In the two groups supplemented with iron, alone or combined with zinc, iron stores increased twice and ID was almost cleared whereas the prevalence of ID doubled in the infants who did not receive iron. This indicates that the diets provided by the mothers to their infants in their second semester of life did not cover their iron needs and that infants in this rural area should receive either iron supplements or iron-fortified foods. The combined iron–zinc supplementation was as effective as iron supplements to control ID and anemia. Similar efficiency of combined iron–zinc (with 20 mg iron and 20 mg zinc) and of supplementation with iron alone on ferritin concentrations is also shown in a study conducted in older Mexican children (Rosado *et al*., 1997; Allen *et al*., 2000; Munoz *et al*., 2000). In contrast, in two recent studies carried out in Indonesian infants with the same doses and duration of zinc and iron supplementation than in our study, the combined iron–zinc supplementation also resulted in improvement of iron status but less effectively than supplementation with iron alone (Dijkhuizen *et al*., 2001; Lind *et al*., 2003). The differences in nutritional status of infants, especially in iron status, between the studies may account for the different results. In our study, the mean initial Hb concentration was lower and the initial prevalence of anemia and IDA were higher than in Lind's study (baseline data were not reported in the other study). Moreover, the prevalence of IDA measured with the SF, a highly sensitive but low specific indicator of iron status, was probably underestimated at baseline. With a cutoff of 41 μg/l that would provide optimal diagnostic efficiency in anemic subjects (Punnonen *et al*., 1997), 74% of anemic infants would have been iron deficient. Thus, iron requirements were higher in infants in this study that may have favored iron absorption from both the iron and iron + zinc supplements. It is also worthwhile to note that the final Hb and ferritin concentrations were higher in both the groups that received iron in our study compared to both other studies. However, by the end of the study, about 8% of infants in both iron-supplemented groups still suffered from anemia but only one infant had IDA suggesting that ID was probably the main, but not the only cause of anemia. Other nutrient deficiencies, such as folate, vitamin B_{12} or vitamin A, as well as intestinal parasitic infection, but not malaria that did not occur in this area, may have contributed to anemia. The decrease of the prevalence of anemia in all groups could result in part from the vitamin A supplements given to all infants just before the start of the intervention to comply with the Vietnamese policy. Indeed, daily vitamin A supplementation (Mejia and Chew, 1988) and high single vitamin A doses (Bloem *et al*., 1990) improve Hb concentration but had no effect on plasma ferritin. Supplementation with zinc alone had no effect on iron status as demonstrated by similar Hb and ferritin concentrations in the Zn and the placebo groups. The positive effect of daily zinc supplements on Hb concentration after a massive single dose of vitamin A as described in non-pregnant women in Bangladesh (Kolsteren *et al*., 1999) was not found here.

Zinc supplementation, alone or combined with iron, increased significantly the SZn concentration. However, the final SZn and the increases of SZn were significantly higher with zinc alone and the factorial analysis indicated that SZn was lower when iron was supplied. It is, however, important to mention that the negative impact of the supplementation with iron on zinc status was very limited and that the increase of SZn with the iron–zinc supplements was significantly higher compared to the control group.

SZn is not considered as an ideal indicator of individual zinc status. However, Zn supplementation causes a large increase in SZn concentrations and population mean zinc concentration is a useful indicator of the successful delivery and absorption of zinc supplements in children (Brown *et al*., 2002), even in developing countries with high prevalence of common childhood infections (Brown, 1998). Interaction of iron with zinc absorption has been widely studied but results show discrepancies (Solomons and Ruz, 1997; Whittaker 1998). Most studies would agree upon a negative effect of iron on zinc absorption when both micronutrients are given together as supplements but no effect when both micronutrients or iron alone are added to foods (Whittaker, 1998). Our study is in agreement with the study carried out in Indonesian infants (Lind *et al*., 2003) that concludes that combined iron–zinc supplementation is significantly less effective to improving zinc status than zinc supplementation alone, whereas two studies conducted in Mexican children (Rosado *et al*., 1997) and Indonesian infants (Dijkhuizen *et al*., 2001) did not find any negative impact of zinc supplementation on iron status.

In the group who received only the zinc supplements, SZn increase was about 3 times higher compared to changes in Indonesian (Lind *et al*., 2003) and Mexican infants (Munoz *et al*., 2000) despite a double zinc dose in Mexican infants and a lower initial SZn concentration in Indonesian infants. Zinc measurement would not account for increases in SZn because zinc measurements in sera from baseline and end of intervention were performed at the same time after the end of intervention. Lower increase in SZn in Mexican and Indonesian children could be related to other micronutrient deficiencies. Indeed, a 10-week-daily micronutrient supplements containing 20 mg zinc increases mean SZn by about 11 μmol/l in 6 to 9-year-old Chinese children whereas zinc alone increased SZn five times less (Sandstead *et al*., 1998).

Although the supplementation with iron and zinc was effective to improve the iron and zinc status, the

supplementation with zinc or iron alone or with zinc and iron had no effect on linear growth. However, zinc supplementation alone was associated with higher weight gain.

The lack of effect of any supplement on linear growth is consistent with the studies carried out in low birth weight full-term Brazilian infants supplemented with zinc during the 6 first months of life (Lira *et al*., 1998) and in 4 months-old Indonesian infant supplemented with iron, zinc or iron + zinc for 6 months (Dijkhuizen *et al*., 2001). A recent study also conducted in Indonesian infants with a similar design shows no impact of zinc or iron or combined iron–zinc supplementation on HAZ but improvement of knee-heel length with zinc or iron supplementation alone (Lind *et al*., 2004). This positive effect of zinc or iron supplements on knee-heel length is not shown in the former study carried out in the same country (Dijkhuizen *et al*., 2001). A study conducted in 6 to 24-month-old Vietnamese children concludes that 3-month daily micronutrient supplementation containing zinc had no effect on linear growth of the complete sample of subjects but a positive impact on HAZ of children who were stunted before supplementation (Thu *et al*., 1999). Another study shows the positive effect of zinc supplementation on plasma IGF-1, an indicator of growth activity, and on linear growth in stunted 4 to 36-month-old Vietnamese children (Ninh *et al*., 1996) whereas the study conducted in 4-month-old Indonesian infants shows no effect of zinc or iron supplementation on plasma IGF-1. Both the older age of children and the higher prevalence of stunting in the two studies carried out in Vietnam would account for difference compared to studies carried out in younger infants. Indeed, a recent meta-analysis of randomized-controlled trials indicates that zinc supplementation results in a highly significant increase in the linear growth and that both the mean initial HAZ and WAZ predict the magnitude of linear growth response (Brown *et al*., 2002). Moreover, the impact of zinc supplementation on linear growth is greater in stunted infants aged more than 6 months but this is not true for younger infants (Brown *et al*., 2002).

The positive effect of zinc supplementation on weight gain has been already demonstrated in malnourished children (Golden and Golden, 1981), in Guatemalan (Rivera *et al*., 1998), Brazilian (Lira *et al*., 1998) and Indonesian infants (Lind *et al*., 2004) and in Zimbabwean schoolchildren (Friis *et al*., 1997) and was attributed to an increase synthesis of the lean body mass (Golden and Golden, 1981; Friis *et al*., 1997; Rivera *et al*., 1998). In contrast, another study concludes that zinc has no effect on height or weight gain but on body composition increasing the fat status of Guatemalan (Cavan *et al*., 1993). However, a recent meta-analysis of 32 studies indicates that zinc supplementation results in a highly significant increase in weight gain of prepubertal children but has no detectable effect on children's WHZ index and that the mean WAZ is a predictor of weight gain (Brown *et al*., 2002).

The interesting result in this study was the loss of the positive effect of zinc supplementation on weight gain when iron was added in the supplement, an effect that was also found in one study in Indonesian infants (Lind *et al*., 2004). And, despite the fact that weight gain in the placebo group was not significantly different from the iron group, the factorial analysis indicated that iron supplementation was associated with lower WAZ and weight-for-height indexes. An adverse effect of the iron supplementation on weight-gain is not usual despite it has been documented in iron-replete children (Idjradinata *et al*., 1994). Our study would support that the slight but significant negative effect of iron on SZn, when zinc and iron were given together, would have a stronger negative effect on weight gain. Because no treatment had a significant different effect on morbidity, the effect of zinc on weight gain could not be explained by a beneficial effect of zinc on morbidity as demonstrated in children in developing countries (Bhutta *et al*., 1999). And iron supplementation was not associated with increase susceptibility to infection that confirms previous observations (Berger *et al*., 2000; Gera and Sachdev, 2002). One has to keep in mind that all infants received vitamin A capsules before the beginning of intervention that may have also benefited to all infants.

Conclusions

Iron and zinc deficiencies, and probably other micronutrient deficiencies such as vitamin A, were prevalent nutritional problems in the Vietnamese infants included in this study. Therefore, these micronutrients should be supplied from the beginning of the second semester of life. Our study has provided the evidence that combined daily iron–zinc supplementation had a significant positive effect on iron and zinc status and that single zinc supplements increased weight gain. Zinc supplements had no negative impact on iron status when given alone or combined with iron. In contrast, iron supplements were associated with lower mean SZn and adding iron to zinc in supplements cancelled the positive effect of zinc on weight gain. This would suggest that iron should be given apart from zinc. Weekly iron supplementation that improves the iron status of infants (Ninh *et al*., 2002) and young children (Liu and Liu, 1996; Berger *et al*., 1997) should be considered. Weekly supplementation of other micronutrients has been investigated (Hop and Berger, 2005; Smuts *et al*., 2005) but more information is needed about alternative supplementation schemes and appropriate doses of micronutrients. The decrease of HAZ and WHZ indexes during the study in all groups and the lack of interaction between zinc and iron in foods (Whittaker, 1998) suggest that adequate complementary foods bringing energy, macro and micronutrients should also be considered.

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