

# Effects of separate delivery of zinc or zinc and vitamin A on hemoglobin response, growth, and diarrhea in young Peruvian children receiving iron therapy for anemia<sup>1-3</sup>

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## ABSTRACT

**Introduction:** Anemia is the most prevalent nutritional deficiency in the world. Attempts to improve iron indexes are affected by deficiency of and interaction between other micronutrients.

**Objective:** Our goal was to assess whether zinc added to iron treatment alone or with vitamin A improves iron indexes and affects diarrheal episodes.

**Design:** This was a randomized, placebo-controlled, double-blind trial conducted in Peru. Anemic children aged 6–35 mo were assigned to 3 treatment groups: ferrous sulfate (FS;  $n = 104$ ), ferrous sulfate and zinc sulfate (FSZn;  $n = 109$ ), and ferrous sulfate, zinc sulfate, and vitamin A (FSZnA;  $n = 110$ ). Vitamin A or its placebo was supplied only once; iron and zinc were provided under supervision  $\geq 1$  h apart 6 d/wk for 18 wk.

**Results:** The prevalence of anemia was 42.97%. The increase in hemoglobin in the FS group (19.5 g/L) was significantly less than that in the other 2 groups (24.0 and 23.8 g/L in the FSZn and FSZnA groups, respectively). The increase in serum ferritin in the FS group (24.5  $\mu\text{g/L}$ ) was significantly less than that in the other 2 groups (33.0 and 30.8  $\mu\text{g/L}$  in the FSZn and FSZnA groups, respectively). The median duration of diarrhea and the mean number of stools per day was significantly higher in the FS group than in other 2 groups ( $P < 0.005$ ).

**Conclusion:** Adding zinc to iron treatment increases hemoglobin response, improves iron indexes, and has positive effects on diarrhea. No additional effect of vitamin A was found. *Am J Clin Nutr* 2004;80:1276–82.

**KEY WORDS** Anemia, iron, zinc, vitamin A, supplementation, acute diarrhea, children, Peru

## INTRODUCTION

Nutritional disorders, and in particular micronutrient deficiencies, remain important public health problems. Worldwide, 1.5% of deaths are attributable to iron deficiency, and the attributable disability-adjusted life years amount to the loss of 35 million healthy life years (1). Despite available diagnostic tools and a relatively inexpensive treatment, the worldwide prevalence of anemia remains high. Length of treatment, side effects, and compliance affect the success of treatment (2–4). Interactions between iron and zinc and between iron and vitamin A have also been documented to affect recovery from anemia at a metabolic level (5, 6). Intervention studies have confirmed that vitamin A raises hemoglobin concentrations in anemic children and adults

(7, 8). Zinc is thought to increase vitamin A concentrations through the production of retinol binding protein (9), and a direct mechanism has been proposed in children (10). Zinc can also directly affect iron metabolism through competition for absorption and direct interaction at a metabolic level. In the human adult intestine, when ferrous sulfate and zinc sulfate are taken in a 1:1 ratio, the inhibition of zinc absorption is slight or nonexistent, whereas with ratios of 2:1 and 3:1, inhibition increases (11).

Interactions among the 3 micronutrients have been less explored, and the results of such studies are conflicting. In a study of 18–36-mo-old children who were supplemented with zinc, iron, or iron and zinc; only supplementation with zinc and iron was associated with higher plasma retinol concentrations (12). In Indonesia, 4-mo-old children received a supplement for 6 mo of iron, zinc, zinc and  $\beta$ -carotene, iron and zinc,  $\beta$ -carotene, or placebo. A negative effect of zinc supplementation on iron status was observed that was exacerbated by  $\beta$ -carotene (13). In Bangladesh, iron-deficient women who received iron, vitamin A, and zinc had a higher hemoglobin increase than did women who received iron alone. Iron and vitamin A resulted in an intermediate response (14).

In Peru, iron deficiency anemia is the most frequent nutritional disorder, affecting children in particular. A recent national survey documented that 50.4% of children aged  $<5$  y were anemic (15). A national monitoring in 1997 showed that 13.8% of the children below the age of 5 y had serum retinol concentrations  $<20$   $\mu\text{g/dL}$  (16). No data are available about the extent of zinc deficiency in Peru, but a recent food intake study found the average daily zinc intake of pregnant women to be 7 mg/d, which is one-half of the recommended intake of 15 mg/d (N Zavaleta, personal communication, 2000). This could indicate that young children will also be zinc deficient. Given the importance of the deficiencies in the given population and the questions remaining on the additional benefit of combining supplements, a trial was designed to assess to what extent

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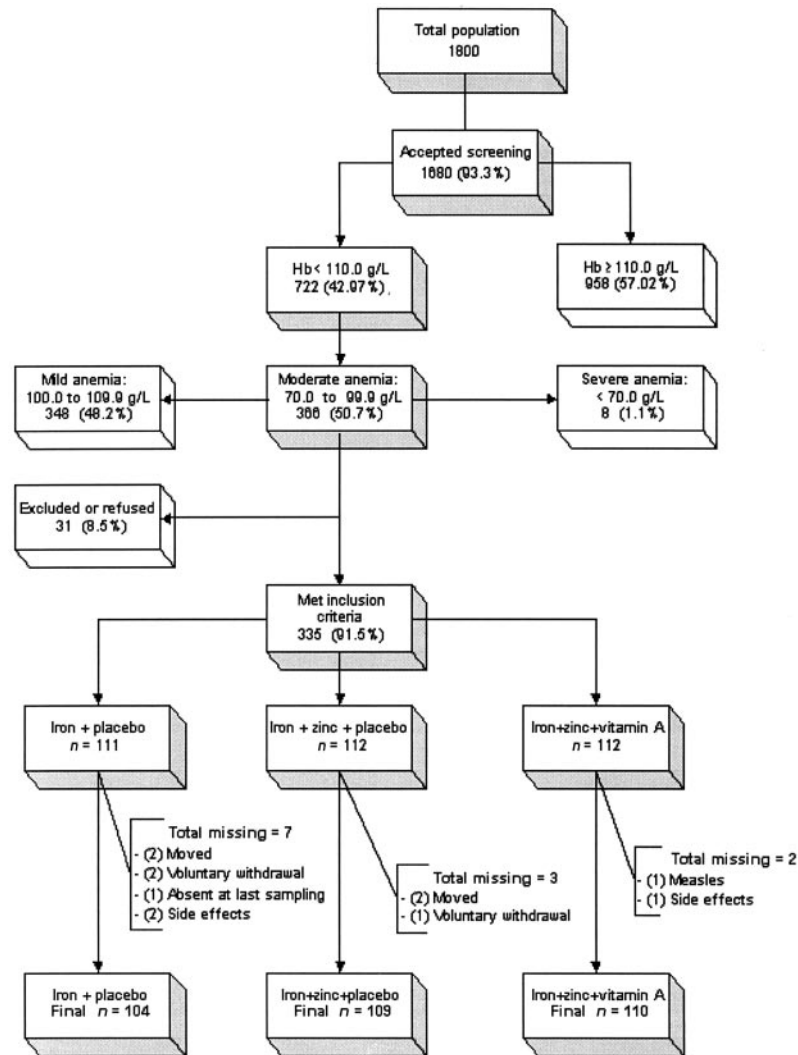


FIGURE 1. Trial profile.

adding zinc alone or in combination with vitamin A to a standard iron treatment would improve iron indexes. In addition, given that in this population diarrhea is an important cause of morbidity and mortality (17), the effect of the micronutrient supplement on episodes of diarrhea was determined.

## SUBJECTS AND METHODS

### Subjects

This was a double-blind, placebo-controlled intervention trial with 3 intervention groups that was carried out between December 2001 and April 2002 in Collique, a peri-urban shanty town of Lima, Peru. We calculated that with a sample of 85 children per group, a difference in hemoglobin concentration of 5 g/L between groups would be detectable at an  $\alpha$  level of 5% and a power of 90%. The SD used in the analysis of variance (ANOVA) sample calculation was 10 g/L. Estimating the loss of cases at a maximum of 25%, the sample size was set at 110 children per group.

A local census carried out previously estimated that 1800 children aged between 6 and 35 mo were living in Collique. The

parents were invited to let their children participate in the study, and 1680 (93.3%) children attended the initial screening for anemia. Of this group, 722 (42.97%) were found to be anemic [hemoglobin concentration < 110 g/L (18)], and 366 (50.7%) of these anemic children were found to be moderately anemic (hemoglobin concentration between 70 and 99.9 g/L).

Three medical doctors who were not part of the research group examined the children with moderate anemia. Children with severe anemia were treated. Trained personnel took anthropometric measurements. Training included standardization exercises. Children with severe anemia (hemoglobin < 70.0 g/L), mild anemia (hemoglobin 100.0–109.9 g/L), chronic disease, or any dietary restrictions; who had received treatment with one of the micronutrients in the study in the previous 6 mo; or who had measles or had received a measles vaccine in the preceding 2 mo (19–23) were excluded. Children with severe malnutrition (defined as weight-for-height < -3 SDs, height-for-age < -3 SDs, or both) were also excluded from the study.

A total of 335 children who met the inclusion criteria and who had parental consent were enrolled in the trial (Figure 1). The children were first stratified by sex, age, and initial hemoglobin

concentration and were then allocated by block randomization to 1 of the 3 groups. One group received iron and a placebo for zinc and vitamin A ( $n = 111$ ), the second group received iron with zinc and a placebo for vitamin A ( $n = 112$ ), and the third group received iron, zinc, and vitamin A ( $n = 112$ ). The Ethics Committee of the Peruvian University Cayetano Heredia approved the study protocol.

## Methods

In all groups, children received  $3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  of elemental iron as ferrous sulfate (24, 25). The dosage of zinc was  $3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  of zinc sulfate providing  $0.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  of elemental zinc (25). In the iron + zinc and iron + zinc + vitamin A groups, the zinc was provided daily, 6 d/wk, for 18 wk. In the iron + zinc + vitamin A group, children between 6 and 12 mo of age received a single dose of 100 000 IU vitamin A as retinol palmitate; those aged  $>1$  y received 200 000 IU (26).

Twenty-six community health workers who were permanent residents of the area were trained for this study. They visited the children twice per day, 6 d/wk, for 18 wk. Each health worker was assigned between 10 and 15 children. Because iron and zinc must be taken  $\geq 1$  h apart and  $\geq 1$  h before or after meals (11, 25, 27, 28), the workers visited every household to give the iron under supervision and then repeated the sequence of visits to administer the zinc. Each round took  $\geq 1$  h, so the doses were given  $>1$  h apart.

Supplements, prepared as syrup, were individually bottled and coded according to treatment group. The code was known only to the pharmacist and was not broken until the data analyses were completed. The placebos were tested before the study started, and no visual or organoleptic differences in the preparations could be detected.

The community health workers also asked daily about the presence of acute diarrhea, defined as  $\geq 3$  liquid or semiliquid stools in 24 h; the final day of diarrhea was defined as the day before which the child was diarrhea free for 48 h (29). The community health workers recorded the length of diarrhea in days and the number of stools per 24 h.

At baseline and after 18 wk, weight, stature or length, hemoglobin concentration, and serum ferritin concentration were measured. Weight was measured with a Salter infant scale to the nearest 0.01 kg (product 0114555; UNICEF, New York). Stature ( $\geq 24$  mo of age) or length ( $<24$  mo of age) was measured by using standard infant length-height measuring boards (product 0114500; UNICEF). A total of 1680 children were screened for anemia. Initial blood samples for the original 1680 children were obtained by finger prick by a trained technician and were measured immediately with a portable hemoglobinometer (Hemocue; Mallinckodt, Courtaboeuf, Canada). A capillary blood sample was taken at the same time. For children participating in the study, this sample was further analyzed for serum ferritin concentration. Blood was centrifuged ( $1098 \times g$ , 10 min, room temperature) to obtain serum and was stored at  $-20^\circ\text{C}$ . Ferritin concentrations were measured by immunoassay with an ELISA kit for ferritin (product OSF200, kit ELISA ferritin; Linear Chemicals, Santiago de Chile, Chile), with a sensitivity of  $2 \mu\text{g/L}$ . A brief socioeconomic questionnaire was completed at the beginning of treatment.

## Statistical analysis

Anthropometric data entry and  $z$  score calculations were done by using EPI INFO 6.0d and EPINUT (Centers for Disease Control and Prevention, Atlanta). All other data were entered in SPSS 10 (SPSS Inc, Chicago). Parametric tests were used for normally distributed variables, according to the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used to compare non-normally distributed variables for data that were too skewed to allow normalization. Differences between treatment groups at baseline and after 18 wk of treatment were examined by ANOVA. Tukey's test was used to detect differences between groups. The McNemar test was applied to compare the change in the proportion of anemia. A general linear model repeated-measures, full factorial type IV model (analysis of covariance) was used to test interactions of baseline characteristics of age, sex, anthropometric measures and indexes, hemoglobin, ferritin, and prevalence of diarrhea with responses by group of treatment in hemoglobin, ferritin, and morbidity data.

## RESULTS

As shown in Figure 1, 722 children (43.0%) in the surveyed population were anemic: 348 had mild anemia and 8 had severe anemia and were excluded from the study. Children with mild anemia were referred for counseling and those with severe anemia were referred for medical treatment. Twelve children did not complete the study: 4 moved out of the trial area, the mothers of 3 children believed after 5–7 wk of treatment that their children were "healthy" and refused further treatment, 1 child developed measles and stopped treatment for  $>2$  wk, 1 subject of the iron only group was not present at the time of the second blood sample, and 3 children stopped treatment for perceived side effects (constipation, stomachaches, and staining of the teeth). The medical doctor at the health center saw these children for further examination. At the end of the trial, there were 104 children in the iron only group, 109 in the iron and zinc group, and 110 in the iron, zinc, and vitamin A group. In all groups,  $>92\%$  of the doses of micronutrients were given.

At baseline, the 3 groups did not differ significantly in socioeconomic factors (Table 1). No significant differences were found in age, weight, height,  $z$  scores, hemoglobin, or serum ferritin. The children who received iron, vitamin A, and zinc had a significantly greater length increment than did the group who received only iron. The  $z$  score increment over this period, however, was not significant (Table 2).

After 18 wk of treatment, the mean ( $\pm$ SD) increase in hemoglobin in the iron only group was  $19.5 \pm 7.31 \text{ g/L}$ , that in the iron and zinc group was  $24.0 \pm 9.95 \text{ g/L}$ , and that in the iron, zinc, and vitamin A group was  $23.8 \pm 7.72 \text{ g/L}$  (Table 3). The hemoglobin increment in the iron and zinc and the iron, zinc, and vitamin A groups was significantly different from that in the iron only group ( $P < 0.001$ ). The median increase in serum ferritin in the group that received iron only was  $24.5 \mu\text{g/L}$ , that in the group treated with iron and zinc was  $33.0 \mu\text{g/L}$ , and that in the group supplemented with iron, zinc, and vitamin A was  $30.8 \mu\text{g/L}$ . The increase in ferritin in the iron and zinc and the iron, zinc, and vitamin A groups was significantly different from that in the iron only group ( $P < 0.001$ ). Hemoglobin response and changes in iron indexes were not modified by baseline variables introduced in the general linear repeated-measures, full factorial type IV

**TABLE 1**  
Comparison of socioeconomic factors at the beginning of the trial<sup>1</sup>

Characteristic	Iron group (n = 104)	Iron and zinc group (n = 109)	Iron, zinc, and vitamin A group (n = 110)
Piped water (%)	33.3	36.8	28.9
Waste system (%)	51.3	52.6	60.0
Mother's education (y)	9.5 (8, 11) <sup>2</sup>	9.0 (9, 11)	10.0 (8, 11)
Family income (US\$/wk) <sup>3</sup>	16.1 (12.3, 21.7)	19.7 (14.1, 27.2)	19.6 (14.1, 22.2)

<sup>1</sup> There were no significant differences between the treatment groups (chi-square or Kruskal-Wallis test).

<sup>2</sup> Median; 25th and 75th percentiles in parentheses (all such values).

<sup>3</sup> Exchange rate in 2002.

model. At the end of the trial, 1% of the children in the iron group, 2.8% of the children in the iron and zinc group, and 0.9% of the children in the iron, vitamin A, and zinc group had ferritin values <12 µg/L. These differences were not significant (McNemar test).

The prevalence of anemia in the 3 groups decreased considerably. By the end of the treatment, 22.5% of the children in the iron only group remained anemic, compared with 3.6% in the iron and zinc group and 9.4% in the iron, zinc, and vitamin A group. The difference between the iron only and the other 2 groups was significant ( $P < 0.005$ ).

The results of the diarrhea episodes are presented in **Table 4**. The incidence of diarrhea in the iron only group was ≈40% greater than that in the other 2 groups. The median duration of diarrhea in the iron only group was 4 d, for a total of 26 diarrhea episodes. The median length in the other groups was 3 d, with 19 and 20 episodes in the iron and vitamin A and iron, vitamin A, and

zinc groups, respectively. The differences in duration were significantly different between the iron only group and the 2 other groups ( $P < 0.005$ ). The mean number of stools per day of the diarrhea episodes was  $3.4 \pm 0.47$  ( $n = 26$ ) in the iron only group,  $3.0 \pm 0.34$  ( $n = 19$ ) in the group supplemented with iron and zinc, and  $2.9 \pm 0.29$  ( $n = 20$ ) in the group that received iron, zinc, and vitamin A, with a significant difference between the iron group and the other 2 groups ( $P < 0.005$ ). No interaction between diarrhea outcomes and anthropometric indexes at the start were found in the general linear repeated-measures, full factorial type IV model.

## DISCUSSION

The results of the present study show that iron deficiency anemia is still a serious problem in young Peruvian children. Forty-three percent of the children in the present study were found to be anemic. This is particularly worrisome given that in 1997 the Peruvian government began a flour fortification program to control iron deficiency (30).

Adding zinc to the iron treatment significantly improved the hematologic response, whereas the addition of vitamin A did not additionally significantly improve the treatment. To our knowledge, this is the first trial to document the effect of a zinc supplement given together with iron treatment  $\geq 1$  h apart in anemic

**TABLE 2**  
Age and anthropometric measures of children at the beginning of the study and after 18 wk by treatment group<sup>1</sup>

Characteristic	Iron group (n = 104)	Iron and zinc group (n = 109)	Iron, zinc, and vitamin A group (n = 110)
Age at start (mo)	17.7 ± 7.6	17.2 ± 7.9	16.9 ± 7.4
Weight (kg)			
Start	10.4 ± 1.7	10.5 ± 1.8	10.4 ± 1.7
End	11.3 ± 1.8	11.5 ± 2.0	11.3 ± 1.8
Change	0.88 ± 0.72 <sup>2</sup>	1.06 ± 0.76 <sup>2</sup>	0.95 ± 0.69 <sup>2</sup>
Height (cm)			
Start	76.9 ± 7.24	76.7 ± 7.23	76.8 ± 7.11
End	81.5 ± 7.17	81.7 ± 7.34	82.1 ± 7.18
Change	4.53 ± 1.58 <sup>2</sup>	5.03 ± 1.80 <sup>2</sup>	5.23 ± 1.77 <sup>2,3</sup>
Weight-age z score			
Start	-0.32 ± 1.09	-0.19 ± 1.12	-0.18 ± 1.18
End	-0.54 ± 1.0	-0.25 ± 1.09	-0.35 ± 1.03
Change	-0.21 ± 0.74 <sup>2</sup>	-0.05 ± 0.73	-0.17 ± 0.63 <sup>2</sup>
Height-age z score			
Start	-1.07 ± 1.13	-1.01 ± 1.07	-0.82 ± 1.19
End	-0.89 ± 1.15	-0.69 ± 1.12	-0.54 ± 1.14
Change	0.17 ± 0.63 <sup>2</sup>	0.32 ± 0.70 <sup>2</sup>	0.28 ± 0.73 <sup>2</sup>
Weight-height z score			
Start	0.47 ± 1.27	0.57 ± 1.18	0.47 ± 1.20
End	0.05 ± 1.09	0.29 ± 1.13	0.04 ± 1.16
Change	-0.43 ± 0.97 <sup>2</sup>	-0.27 ± 0.83 <sup>2</sup>	-0.42 ± 0.86 <sup>2</sup>

<sup>1</sup> All values are  $\bar{x} \pm SD$ .

<sup>2</sup> Significant change from baseline,  $P < 0.05$  ( $t$  test).

<sup>3</sup> Significantly different from the iron group,  $P < 0.05$  (one-way ANOVA and Tukey's test).

**TABLE 3**  
Hemoglobin and serum ferritin concentrations before and after 18 wk of treatment

Characteristic	Iron group (n = 104)	Iron and zinc group (n = 109)	Iron, zinc, and vitamin A group (n = 110)
Hemoglobin (g/L)			
Baseline	95.2 ± 7.10 <sup>1</sup>	95.0 ± 6.89	95.0 ± 7.14
End <sup>2</sup>	114.7 ± 6.19	119.1 ± 7.05	118.8 ± 8.16
Change <sup>2</sup>	19.5 ± 7.31 <sup>3</sup>	24.0 ± 9.95 <sup>3</sup>	23.8 ± 7.72 <sup>3</sup>
Ferritin (µg/L)			
Baseline	3.2 (2.0, 8.1) <sup>4</sup>	2.5 (1.0, 8.5)	2.3 (0.0, 8.9)
End <sup>5</sup>	27.2 (22.0, 34.9)	35.1 (28.7, 48.4)	35.0 (27.9, 46.6)
Change <sup>5</sup>	24.5 (16.9, 29.6) <sup>6</sup>	33.0 (23.9, 41.9) <sup>6</sup>	30.8 (24.4, 40.4) <sup>6</sup>

<sup>1</sup>  $\bar{x} \pm SD$  (all such values).

<sup>2</sup> Significant difference between the iron group and the other 2 groups,  $P < 0.001$  (ANOVA and Tukey's test).

<sup>3</sup> Significant change from baseline,  $P < 0.05$  ( $t$  test).

<sup>4</sup> Median; 25th and 75th percentiles in parentheses (all such values).

<sup>5</sup> Significant difference between the iron group and the other 2 groups,  $P < 0.001$  (Kruskal-Wallis test).

<sup>6</sup> Significant change from baseline,  $P < 0.05$  (Wilcoxon's test).

**TABLE 4**

Duration of diarrhea and number of stools

Characteristic	Iron group	Iron and zinc group	Iron, zinc, and vitamin A group
Prevalence (%)	15.4 (8.4, 22.3) <sup>1</sup>	11.0 (5.1, 16.9)	12.7 (6.4, 18.9)
Incidence in 18 wk	0.25 ± 0.43 <sup>2</sup>	0.17 ± 0.38	0.18 ± 0.43
Median duration of diarrhea (d) <sup>3</sup>	4 (3, 5)	3 (3, 4)	3 (2, 4)
Mean number of stools per day <sup>4</sup>	3.44 ± 0.47	3.01 ± 0.34	2.85 ± 0.29

<sup>1</sup> Median; 25th and 75th percentiles in parentheses (all such values).<sup>2</sup>  $\bar{x} \pm SD$  (all such values).<sup>3</sup> Significant difference among treatment groups,  $P < 0.005$  (Kruskal-Wallis test).<sup>4</sup> Significant difference among treatment groups,  $P < 0.005$  (ANOVA). Significant difference between the iron group and the other 2 groups,  $P < 0.05$  (Tukey's test).

children aged 6–36 mo. In this study, the hemoglobin increase in children who received iron only was not significantly different from the increase observed in assays with  $\geq 8$  wk follow-up (25). The treatment protocol was highly acceptable and participation was very high. Only 3 mothers refused to continue treatment because of perceived side effects. The treatment was supervised and completely blinded. The observed changes can therefore be attributed to the treatment protocol.

There is little information in the literature on the effect of zinc on anemia except for the interaction between zinc and iron at the absorption sites. In a study in children aged 4 mo, iron supplementation did not affect plasma zinc concentrations, and zinc supplementation did not increase the prevalence of iron deficiency anemia (31). Studies in adult women (32) and infants (13) found that the intake of iron and zinc increased only serum ferritin and not hemoglobin. In our study, we found that the combination of iron and zinc improved not only ferritin but also hemoglobin. It is generally accepted that both minerals compete for the same receptors and that high doses of zinc can interfere with iron absorption and vice versa (27, 28, 33, 34). In the present study, we clearly separated in time the intake of zinc and iron and, following the recommendations of Solomons and Jacob (11), used an Fe-to-Zn ratio of 1:1, so that the interference would be as small as possible. Most of the trials mentioned did not separate in time the intake of iron and zinc, and this could be one reason for the response in hemoglobin and ferritin found in the present study.

How zinc can affect iron metabolism has not yet been documented but 3 possible mechanisms exist: 1) modulation of erythropoiesis, 2) modulation of immunity to infectious disease and the anemia of infection, and 3) modulation of iron metabolism (8). Zinc can also indirectly influence vitamin A metabolism, which itself can have a positive influence on iron deficiency. The strongest candidate for explaining this interaction is the immunity-infection pathway. Indeed, zinc deficiency strongly affects immunity. Shankar and Prasad have identified several changes in a zinc deficiency state (35). Zinc deficiency damages the protective epithelial and mucosal barriers and increases susceptibility to infections. Lymphopenia may occur with reduced B and T lymphocytes, which also show reduced function. Antibody production is reduced and the natural antibodies that provide an early line of defense against invasive pathogens are decreased in the neonate. Evidence is also available that the production and secretion of a large number of cytokines is decreased. Dysfunctions of macrophages, natural killer cells, and neutrophils have also been documented. Overall, the evidence is strong that zinc

deficiency can induce immunologic impairment, which consequently increases infection pressure. By this mechanism, zinc deficiency could thus induce anemia of infection.

It has also been proposed that retinoids regulate apoptosis, or programmed cell death, in erythroid progenitor cells and that this may explain the effect of vitamin A on anemia. Zinc-deficient animals exhibit enhanced spontaneous and toxin-induced apoptosis in multiple cell types (35). Whether this also occurs in humans has not been determined (8).

Zinc also regulates intracellular and intercellular vitamin A transport. Zinc deficiency impairs cellular retinol-binding protein synthesis, which regulates mobilization of retinol. Zinc is also an essential co-enzyme for alcohol dehydrogenase and retinal oxidase. In a deficiency state, active retinol metabolites are decreased. Zinc deficiency can, through such an effect on vitamin A, indirectly affect iron metabolism (36).


Except for a small effect on height, no additional benefit was found when adding vitamin A to zinc and iron. A low prevalence of vitamin A deficiency could be one explanation for the lack of effect in the present study. In Peru, vitamin A deficiency in children aged 6–14 y is high in rain forest areas (11.3%) but lower in the Lima region (4.9%) where this trial was done (16, 37). In a controlled trial in nonpregnant women in Bangladesh, the rather weak effect of vitamin A on iron indexes was also attributed to the low prevalence of vitamin A deficiency (14). Positive effects were found only in cases with severely impaired liver function or when the subjects were moderately to severely protein-energy deficient (9, 25, 36). None of the children involved in our study had those conditions. However, the interaction of vitamin A with other micronutrients is still under debate. Most of the effects of vitamin A on zinc have been shown in animal models (38, 39); trials in humans have failed to show a consistent relation between zinc and vitamin A.

Favorable changes regarding the duration of diarrhea and the number of stools per episode were found in the groups who received zinc or zinc plus vitamin A in addition to iron. This is in accordance with previous studies that suggested that zinc supplements may complement the effects of vitamin A (40–43). The small difference of 1 d in duration of diarrhea and less frequent stools could be important in terms of lesser severity, quicker recovery, and maintenance of nutritional status (44, 45).

No side effects of zinc intake were reported. However, there is a need to establish the safety of zinc supplementation in terms of duration of treatment and dosage in communities where other

trace element deficiencies are equally widespread, because prolonged zinc administration is known to interfere with the absorption of copper, iron, and calcium (46). Limited information is available on the bioavailability of different zinc molecules in the presence of dietary inhibitors or on the interaction between other minerals and different formulas of zinc (47). It appears that the interference between zinc and iron for absorption is modulated by the zinc formulation. A recent trial found no competition between zinc oxide and iron, which was not the case for zinc sulfate (48). Hemoglobin, ferritin, and serum iron significantly increased when zinc gluconate (49) or  $\beta$ -alanine-L-histidine zinc (50) was added to iron treatment in Japanese women, even with a zinc-to-iron molecular ratio of 3:1.

No significant differences were found in anthropometric indexes for any treatment group. These findings are similar to trials with even longer follow-up periods (25, 51–55). A meta-analysis pointed out that zinc can have a significant but small effect on length gain in children, and that iron only has an effect in anemic children (51), but the duration of the supplementation must be longer than in the present trial.

We conclude that the addition of a zinc supplement to iron treatment for moderate anemia improves the hemoglobin response and ferritin concentrations. No additional effect of vitamin A was shown. Whether the effect is noticeable only in zinc-deficient children could not be established. Future studies are needed to examine the effects of other zinc formulas on iron indexes to minimize the possible negative effects of zinc on iron metabolism. When these interactions are determined, more severe forms of anemia can be included in clinical trials. 

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