Iron and zinc supplementation improves indicators of vitamin A status of Mexican preschoolers[1,2]

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ABSTRACT

Background: The coexistence of multiple micronutrient deficiencies is a widespread public health problem in many regions of the world. Interactions between zinc deficiency and vitamin A metabolism have been reported but no longitudinal studies have evaluated the effect of iron deficiency on vitamin A.

Objective: The objective of this study was to investigate the effect of supplementation with iron, zinc, or both on vitamin A and its metabolically related proteins retinol binding protein (RBP) and transthyretin.

Design: The study was a longitudinal, double-blind, placebo-controlled trial in which 219 rural Mexican children aged 18–36 mo were randomly assigned to receive 20 mg Zn/d, 20 mg Fe/d, 20 mg Zn/d plus 20 mg Fe/d, or placebo.

Results: Six months after supplementation, plasma retinol increased in all supplemented groups. Compared with placebo, zinc supplementation was associated with significantly higher plasma retinol and transthyretin but the increase in RBP was not significant. Iron supplementation significantly increased plasma retinol, RBP, and transthyretin. Supplementation with zinc plus iron significantly increased plasma retinol but not RBP or transthyretin. Children deficient in zinc, iron, or vitamin A (as indicated by nutrient plasma concentration) at the beginning of the study had a significantly greater increase in retinol than did children with adequate nutrient status.

Conclusions: Supplementation with zinc, iron, or both improved indicators of vitamin A status. The results of this study agree with previous observations of a metabolic interaction between zinc and vitamin A and suggest an interaction between iron and vitamin A metabolism.   *Am J Clin Nutr* 2000;71:789–94.

KEY WORDS   Zinc deficiency, iron deficiency, vitamin A deficiency, retinol binding protein, RBP, transthyretin, nutrient interactions, preschoolers

INTRODUCTION

The coexistence of multiple micronutrient deficiencies is increasingly recognized as a widespread public health problem in developing countries (1–4). In Mexico, iron deficiency is highly prevalent (1, 5) because of the low bioavailability of iron in the plant-based, high-phytate diets consumed habitually in rural areas. We showed previously that the rural Mexican diet significantly impairs absorption of both iron and zinc (6) and has a low vitamin A content (7). A deficiency in one or more of these nutrients may result in growth stunting (8–10), increased morbidity (11, 12), or delayed cognitive function (13, 14).

Interactions between zinc and vitamin A were reported in animals (15–22) and humans (23–27). Zinc deficiency is commonly associated with low plasma concentrations of vitamin A, even when hepatic vitamin A stores are normal, suggesting that there is a defect in mobilization of vitamin A rather than in its absorption or transport to the liver. With zinc deficiency there is impaired synthesis of proteins that turnover rapidly, such as retinol binding protein (RBP). This impairment affects retinol transport from the liver to the circulation and other tissues because retinol is transported as a retinol-RBP complex in association with transthyretin. Previous reports indicated beneficial effects of zinc supplementation on vitamin A metabolism in malnourished children (23), preterm infants (24), and adults with alcoholic cirrhosis (25). Other studies showed no such effect of zinc on serum indicators of vitamin A metabolism (26, 27). However, a functional interaction between zinc and vitamin A was suggested in that there was significantly less abnormal conjunctival impression cytology in subjects receiving both zinc and vitamin A than in subjects receiving a placebo or zinc alone. These conflicting results may be explained by differences in the subjects' nutritional status for zinc, vitamin A, and perhaps other nutrients (23).

Studies in humans (28, 29) and animals (30–32) showed that vitamin A deficiency causes abnormalities in iron metabolism and that supplementation with vitamin A improves iron status as measured by hematologic indexes (33–40). No longitudinal studies have evaluated the effect of iron supplementation on vitamin A status. In the present study we investigated the effect of supplementation with iron, zinc, or both on plasma concentrations of retinol, RBP, and transthyretin.

SUBJECTS AND METHODS

Subjects

A longitudinal, double-blind, placebo-controlled supplementation trial was conducted in 5 rural communities in the Solis Valley, located in the central highland plateau of Mexico, ≈150 km northwest of Mexico City. The communities ranged in size from 700 to 1500 persons (≈100–214 households). All children aged 18–36 mo were considered as potential participants. According to a baseline census there were 290 children in this age group in the study area. After they learned about the design and potential risks and benefits of the study, the mothers of all of these children were invited to allow their children to participate. The mothers of 219 children agreed to their children's participation and signed consent forms. The protocol was approved by the Committee on Biomedical Research in Human Subjects of the National Institute of Nutrition. The children were assigned to 1 of 4 groups depending on their age, sex, and height-for-age deficit. Birth dates were obtained from birth certificates.

Zinc and iron supplementation

Children in each of the 4 groups received 20 mL/d of a beverage containing 20 mg Fe as ferrous sulfate, 20 mg Zn as zinc methionine, 20 mg Zn plus 20 mg Fe, or placebo. Zinc methionine was chosen after we showed that postconsumption plasma zinc concentrations were higher with this form of zinc than with zinc sulfate or zinc polyascorbate (41). Ferrous sulfate is the most commonly used form of supplemental iron. To improve the taste of the mineral solutions and to ensure that they were similar in appearance, texture, and taste to each other and to the placebo, all beverages contained sugar, citric acid, water, and artificial orange or lemon flavor. The acceptability of the beverage was ensured by testing it before the trial in a sample of children of the same age as the study subjects.

Children in each group were visited at home from Monday through Saturday each week by a fieldworker who gave the beverage to each child and ensured that it was consumed completely. The flavor of the supplement was changed weekly to improve compliance. The supplements were consumed on 97% of the days on average and only 15 children dropped out of the study before the end of the 6 mo.

Indicators of iron, zinc, and vitamin A status

A 2-mL sample of fasting venous blood was collected from each preschooler at baseline and after 6 mo of supplementation. Blood was collected in a mineral-free evacuated tube and transferred to an acid-washed tube containing 0.05 mL sodium citrate as an anticoagulant. Hemoglobin was measured within 3 h (Coulter Electronics, Hialeah, FL). Plasma was separated by centrifugation at $1000 \times g$ for 10 min at 20°C. Portions of plasma were frozen immediately and maintained at −70°C until analyzed. For plasma zinc measurements, samples were diluted 1:10 with deionized water and measured by atomic absorption spectrophotometry against a zinc reference (Sigma Chemical Co, St Louis) in 5% glycerol (42). Plasma ferritin was measured with a solid-phase immunoradioassay kit (Coat-A-Count Ferritin IRMA; Diagnostic Products Corp, Los Angeles). Vitamin A was extracted from plasma after the addition of retinyl myristate as an internal standard and was analyzed by isocratic reversed-phase HPLC using the method of Barua et al (43), with slight adaptations; the column was a Waters Resolve C_{18}

(3.9 × 150 mm, 5-μm particle size; Millipore Corp, Milford, MA); the mobile phase consisted of acetonitrile, dichloroethane, methanol, and N-butanol (90:15:10:0.1); and the flow rate was 1 mL/min. The samples were analyzed at 300 nm. Plasma RBP and transthyretin were measured by immunoassay and laser nephelometry (Behring Diagnostics Inc, Somerville, NJ). C-reactive protein (CRP) was measured with the NA-Latex-CRP kit (Behring Diagnostics Inc). Analyses of all samples were performed in duplicate and were accompanied by standards and certified control sera. Control serum for vitamin A analysis was obtained from the National Institute of Standards and Technology (Standard Reference Materials; Gaithersburg, MD), control serum for zinc analysis was obtained from the Centers for Disease Control and Prevention (US Department of Health and Human Services, Atlanta), and control serum for ferritin, RBP, and transthyretin analyses were obtained from Bio-Rad (Anaheim, CA).

Statistical analysis

Biochemical data were analyzed as changes between basal and 6-mo values by using SAS (44). Group differences were analyzed by two-way analysis of variance using a Latin-square repeated-measures design that considers unequal numbers of subjects among treatment groups (Proc-GLM). Means were compared by using Tukey's range test. Group, sex, and initial vitamin A, iron, and zinc status (deficient or adequate) were used as independent variables. Cuttoff values for deficiency were 0.70 μmol/L for retinol, 12 μg/L for ferritin, and 10.7 μmol/L for zinc. Children with plasma CRP concentrations >5.0 mg/L were excluded from the statistical analyses because plasma retinol, RBP, transthyretin, and ferritin concentrations are altered by acute infection or inflammatory processes. Each dependent variable was tested for homogeneity of its variance by one-way analysis of variance and Bartlett's test. Values of $P \leq 0.05$ were considered to be significant.

RESULTS

Subject characteristics and baseline nutritional status

The characteristics and nutritional status of the children at the beginning of the study are shown in **Table 1**. There were no significant intergroup differences in the number of subjects, mean age, sex distribution, or weight and height deficits. The mean height-for-age deficit of the children was 1.6 (z score). Mean hemoglobin concentrations were below normal, whereas mean ferritin, zinc, and retinol concentrations were within the normal range. At baseline, the mean prevalence of anemia in all groups was 73%, that of low plasma ferritin was 51%, that of low plasma zinc was 25%, and that of low plasma retinol was 29%.

Effect of iron and zinc supplementation on iron and zinc status

Hemoglobin, plasma ferritin, and plasma zinc concentrations at baseline and after 6 mo of supplementation are shown in **Table 2**. As expected, supplementation with iron alone or in combination with zinc resulted in significantly higher hemoglobin and ferritin concentrations after 6 mo than those in the placebo group, whereas supplementation with zinc alone or in combination with iron resulted in significantly higher plasma zinc concentrations.

TABLE 1
Characteristics of subjects in each group at the beginning of the study[1]

	Placebo (n = 30 F, 26 M)	Zinc (n = 27 F, 27 M)	Iron (n = 30 F, 24 M)	Zinc plus iron (n = 30 F, 25 M)	All groups (n = 117 F, 102 M)
Age (mo)	28.9 ± 7.9[2]	28.4 ± 7.5	27.5 ± 6.9	28.8 ± 8.9	28.4 ± 7.8
Anthropometric measurements (z score)					
Weight-for-age	−1.4 ± 0.6	−1.4 ± 0.8	−1.6 ± 0.9	−1.2 ± 0.9	−1.4 ± 0.8
Height-for-age	−1.8 ± 0.9	−1.6 ± 1.0	−1.6 ± 1.2	−1.5 ± 1.0	−1.6 ± 1.0
Weight-for-height	−0.4 ± 0.08	−0.4 ± 0.08	−0.7 ± 0.08	−0.3 ± 0.12	−0.5 ± 0.09
Biochemical indicators (% deficient)[3]					
Hemoglobin	71	70	67	82	73
Plasma ferritin	57	43	49	55	51
Plasma zinc	27	26	28	18	25
Plasma retinol	28	23	34	33	29

[1] There were no significant differences among groups.

[2] $\bar{x}$ ± SD.

[3] Deficiency defined as <117.0 g/L for hemoglobin, <12 μg/L for ferritin, <10.7 μmol/L (<70 μg/dL) for zinc, and <0.70 μmol/L (<20 μg/dL) for retinol.

Effect of iron and zinc supplementation on vitamin A status

The changes in plasma retinol, RBP, and transthyretin after 6 mo of supplementation with zinc, iron, or both are shown in **Table 3**. The increase in plasma retinol and TTR, but not in RBP, was significantly higher in the zinc group than in the placebo group. Supplementation with iron alone significantly increased retinol, RBP, and transthyretin. Supplementation with zinc plus iron significantly increased retinol but had no significant effect on RBP or transthyretin. Iron supplementation was associated with a higher increase in retinol and RBP than supplementation with zinc or zinc plus iron.

Effect of baseline iron, zinc, and vitamin A status on changes in retinol

The effect of baseline zinc, iron, and vitamin A status on changes in plasma retinol after supplementation with zinc, iron, or both is shown in **Table 4**. Supplementation with zinc or zinc plus iron in children with zinc deficiency at baseline resulted in a higher mean change in plasma retinol than that in children with adequate plasma zinc at baseline, in whom there was a very small decrease. Similarly, the effect of supplementation with iron or iron plus zinc on plasma retinol was significantly greater in the iron-deficient children than in the children with adequate iron status. Vitamin A–deficient children had a higher increase in plasma retinol than did children with adequate vitamin A status in groups supplemented with zinc, iron, or both.

DISCUSSION

In this longitudinal, placebo-controlled community trial, supplementation with iron, zinc, or both was associated with a significant increase in the plasma retinol concentrations of Mexican preschoolers. This effect was much more evident in children who were initially deficient in zinc, iron, or vitamin A. Iron supplementation also produced a significant increase in the vitamin A–associated proteins RBP and transthyretin, and zinc supplementation increased transthyretin concentrations.

Previous studies showed a positive effect of zinc supplementation on vitamin A nutritional status (23–25), suggesting a

TABLE 2
Biochemical indicators of zinc and iron status in preschool children at baseline and after 6 mo of supplementation with zinc, iron, or both[1]

	Placebo	Zinc	Iron	Zinc plus iron
Hemoglobin (g/L)[2]				
Baseline	108 ± 14	109 ± 11	108 ± 13	107 ± 10
Posttreatment	116 ± 10	118 ± 9	122 ± 9	119 ± 10
Change	8.0 ± 16.3	8.0 ± 12.9	14.0 ± 14.0[3]	13.0 ± 13.0[3]
Plasma ferritin (μg/L)[4]				
Baseline	20.0 ± 44.6	18.9 ± 15.8	21.2 ± 38.2	14.7 ± 15.6
Posttreatment	16.2 ± 13.8	16.6 ± 15.4	37.3 ± 17.2	33.6 ± 18.7
Change	−4.6 ± 41.6	−2.3 ± 15.6	16.0 ± 38.7[5]	18.6 ± 20.5[5]
Plasma zinc (μmol/L)[6]				
Baseline	14.2 ± 4.8	13.2 ± 4.2	15.2 ± 4.4	16.5 ± 4.7
Posttreatment	14.3 ± 4.7	16.8 ± 5.6	14.9 ± 4.5	18.1 ± 5.0
Change	0.18 ± 3.2	3.63 ± 4.7[5]	0.28 ± 3.4	1.62 ± 4.9[5]

[1] $\bar{x}$ ± SD.

[2] n = 50 in the placebo group, 49 in the zinc group, 52 in the iron group, and 51 in the zinc plus iron group.

[3,5] Significantly different from placebo group (two-way ANOVA): [3] $P < 0.05$, [5] $P < 0.0001$.

[4] n = 48 in the placebo group, 48 in the zinc group, 49 in the iron group, and 49 in the zinc plus iron group.

[6] n = 54 in the placebo group, 47 in the zinc group, 45 in the iron group, and 48 in the zinc plus iron group.

TABLE 3
Biochemical indicators of vitamin A status in preschool children at baseline and after 6 mo of supplementation with zinc, iron, or both[1]

	Placebo	Zinc	Iron	Zinc plus iron
Retinol (μmol/L)[2]				
Baseline	1.19 ± 0.4	1.02 ± 0.4	0.98 ± 0.5	1.02 ± 0.5
Posttreatment	1.14 ± 0.3	1.10 ± 0.2	1.26 ± 0.3	1.10 ± 0.3
Change	−0.05 ± 0.3	0.08 ± 0.4[a,3]	0.27 ± 0.5[b,3]	0.08 ± 0.5[a,3]
Retinol binding protein (mg/L)[4]				
Baseline	21.5 ± 8.1	24.2 ± 7.7	22.9 ± 8.7	22.8 ± 10.0
Posttreatment	22.4 ± 7.2	26.2 ± 8.7	28.4 ± 10.7	24.8 ± 7.2
Change	0.9 ± 8.6	1.9 ± 10.2	5.4 ± 7.8[a,3]	2.0 ± 9.9
Transthyretin (mg/L)[5]				
Baseline	192.0 ± 41.0	199.0 ± 50.0	205.0 ± 53.0	208.0 ± 63.0
Posttreatment	207.0 ± 50.0	241.0 ± 56.0	239.0 ± 49.0	230.0 ± 59.0
Change	14.0 ± 45.0	42.0 ± 66.0[a,3]	33.0 ± 56.0[a,3]	23.0 ± 59.0

[1] $\bar{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] $n = 38$ in the placebo group, 37 in the zinc group, 43 in the iron group, and 42 in the zinc plus iron group.
[3] Significantly different from placebo group, $P < 0.05$ (two-way ANOVA).
[4] $n = 45$ in the placebo group, 42 in the zinc group, 46 in the iron group, and 45 in the zinc plus iron group.
[5] $n = 43$ in the placebo group, 41 in the zinc group, 45 in the iron group, and 44 in the zinc plus iron group.

metabolic interaction between the 2 nutrients. Shingwekar et al (23) found a highly significant increase in plasma retinol and RBP after 40 mg Zn/d was given for 5 d to zinc-deficient, vitamin A–deficient Indian children with protein-energy malnutrition (PEM). The effect was not found in children without PEM who were less zinc deficient. Mean plasma concentrations of zinc, retinol, and RBP before supplementation were 8.7 μmol/L, 0.44 μmol/L, and 20 mg/L, respectively, in children with PEM and 11.1 μmol/L, 0.53 μmol/L, and 21.9 mg/L, respectively, in children without PEM.

Studies that showed no effect of zinc supplementation on vitamin A status were carried out in populations with no clear evidence of zinc deficiency (26, 27). Udomkesmalee et al (27) studied the effect of 6 mo of supplementation with 25 mg Zn/d on the vitamin A status of preschoolers in Thailand. They found no effect of zinc supplementation on plasma retinol or RBP. At baseline, children in that study had mean (±SD) plasma zinc and retinol concentrations of 13.2 ± 1.4 and 1.0 ± 0.2 μmol/L, respectively. Palin et al (26) found no effect of zinc supplementation on biochemical indicators of vitamin A status in patients with cystic fibrosis whose plasma zinc concentrations were within the normal range. The results of these studies are consistent with our observation that zinc supplementation benefits the metabolism of vitamin A when zinc status is poor.

In this study, baseline plasma concentrations of zinc and vitamin A predicted the response of vitamin A to zinc supplementation. The children were from a poor rural community at risk of marginal deficiency of several nutrients. On average, plasma zinc and retinol concentrations were within the normal range at baseline (14.7 and 1.05 μmol/L, respectively). Nevertheless, 29% of children had low plasma retinol (<0.70 μmol/L) and 25% had low plasma zinc (<10.7 μmol/L). Mean plasma concentrations of RBP and transthyretin were lower (22.8 ± 8.6 and 201.0 ± 51.0 mg/L, respectively) than those normally found in children with adequate vitamin A status (26–76 and 250–450 mg/L, respectively) (45). The effect of supplementation on plasma retinol concentrations was greater in the children deficient in zinc or vitamin A, strengthening the theory that zinc interactions with vitamin A metabolism are dependent on both zinc and vitamin A status. The

effect of zinc supplementation on plasma retinol concentrations was greater in children who were vitamin A deficient at baseline than in children with adequate vitamin A status.

Studies in humans (28, 29, 35–39, 46–49) and animals (31, 50) showed associations between vitamin A deficiency and iron deficiency anemia. Vitamin A supplementation improves indicators of iron nutritional status, such as serum iron, transferrin, transferrin saturation, hematocrit, and hemoglobin, suggesting that vitamin A affects iron metabolism (35, 36, 39). In children (29, 37–39, 46–47) and in pregnant women (48), plasma hemoglobin and ferritin concentrations were correlated with plasma retinol and in some instances with RBP and transthyretin (36).

To our knowledge, only Mejía and Chew (35) studied the effect of iron supplementation on vitamin A status. These authors supplemented anemic children aged 1–8 y for 2 mo with either 3 mg elemental Fe·kg·d^{-1} or iron plus vitamin A and evaluated the effect on

TABLE 4
Change in plasma retinol concentrations in preschool children after 6 mo of supplementation with zinc, iron, or both according to nutritional status of children at baseline[1]

	Zinc	Iron	Zinc plus iron
		μmol/L	
Zinc status			
Deficient[2]	0.26 ± 0.1[3]	0.05 ± 0.8	0.38 ± 0.2[3]
Adequate	−0.05 ± 0.6	0.31 ± 0.5	−0.007 ± 0.3
Iron status			
Deficient[4]	−0.15 ± 0.5	0.41 ± 0.4[3]	0.29 ± 0.4[3]
Adequate	0.24 ± 0.5	0.18 ± 0.6	−0.26 ± 0.6
Vitamin A status			
Deficient[5]	0.65 ± 0.3[3]	0.60 ± 0.3[3]	0.53 ± 0.2[3]
Adequate	−0.09 ± 0.4	0.15 ± 0.5	−0.19 ± 0.5

[1] $\bar{x}$ ± SD.
[2] Defined as a plasma zinc concentration <10.7 μmol/L (<70 μg/dL).
[3] Significantly different from children with adequate status, $P < 0.05$ (two-way ANOVA).
[4] Defined as a plasma ferritin concentration <12 μg/L.
[5] Defined as a plasma retinol concentration <0.70 μmol/L (<20 μg/dL).

plasma retinol and hematologic indicators. They did not find a beneficial effect of iron supplementation on serum retinol concentrations, but children in that study were not vitamin A deficient and were only marginally iron deficient. There are no reports of the effect of iron supplementation on plasma transport proteins of vitamin A. However, preliminary research with experimental iron-deficient animals showed a reduction in plasma concentrations of retinol even though the concentration of vitamin A in the liver was normal or higher than normal (F Rosales, J Beard, unpublished observations, 1999), suggesting that vitamin A use during iron deficiency is abnormal.

In this prospective mineral-supplementation study of free-living children with a high incidence of iron deficiency (51% with low ferritin) and anemia (72% with low hemoglobin), we found that supplementation with iron alone or in combination with zinc improved vitamin A status. The effect was much stronger in children deficient in either iron or vitamin A than in children with adequate iron and vitamin A status. Moreover, the subjects who were zinc or iron deficient at baseline had lower retinol concentrations than did subjects with adequate zinc or iron status, although the difference was not significant for iron deficiency.

The fact that the children in our study had a higher incidence of low hemoglobin than of low plasma ferritin could be at least partially explained by the high incidence of other nutrient deficiencies. About 33% of our subjects had low vitamin B-12 concentrations, 29% were vitamin A deficient, and 32% had low riboflavin concentrations.

This study showed that supplementation for 6 mo with 2 times the recommended daily allowance (51) of iron and zinc improved vitamin A status as assessed by plasma concentrations of retinol, RBP, and transthyretin in children with a high risk of marginal deficiency of zinc, iron, and vitamin A. In developing populations, the coexistence of marginal vitamin A deficiency with zinc and iron deficiency is common. Attention to nutritional status for a single nutrient might not be appropriate (9, 10). We showed that vitamin A status is affected by zinc and iron deficiency, which could explain the failure of vitamin A status to improve in some vitamin A supplementation trials and programs (52). The precise mechanism by which these interactions occur requires further investigation.

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