

Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children¹⁻³

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ABSTRACT

Background: Zinc deficiency limits the bioavailability of vitamin A. Because zinc and vitamin A deficiency often coexist in malnourished children, simultaneous zinc and vitamin A supplementation may improve the vitamin A deficiency in these children.

Objective: A randomized, double-blind, placebo-controlled intervention trial was conducted to evaluate whether combining zinc and vitamin A supplementation would improve the biochemical indexes of vitamin A nutriture.

Design: Children aged 12–35 mo were randomly assigned to 1 of 4 intervention groups: 20 mg Zn/d for 14 d (Z group), 60000 retinol equivalents (200000 IU) vitamin A on day 14 (A group), zinc plus vitamin A (ZA group), or placebo syrup and placebo capsule (placebo group). Venous blood was drawn at enrollment and on day 21.

Results: Mean serum retinol concentrations were not significantly different between the A and ZA groups. Among vitamin A-deficient children, the proportion of children who remained vitamin A deficient (serum retinol <0.7 $\mu\text{mol/L}$) after supplementation was 40.6% in the Z group, 37.5% in the A group, and 47.0% in the placebo group; only 13.3% in the ZA group remained vitamin A deficient ($P < 0.05$ compared with the placebo group). The proportion of children whose retinol binding protein concentrations remained low was significantly lower in the ZA group than in the other groups ($P < 0.05$).

Conclusion: Combined zinc and vitamin A supplementation improves vitamin A nutriture in vitamin A-deficient children. *Am J Clin Nutr* 2002;75:92–8.

KEY WORDS Zinc, vitamin A, malnutrition, supplementation, retinol binding protein, retinol, children

INTRODUCTION

The results of several experimental and clinical studies suggest an interaction between zinc and vitamin A (1–3). Zinc deficiency is accompanied by a reduction in circulating retinol concentrations in animals (4–10), and vitamin A supplementation alone fails to revert this vitamin A deficiency. After the animals are given either zinc supplements or zinc-containing diets, however, their serum retinol concentrations improve, suggesting that the low serum retinol concentrations are related to zinc deficiency (4, 5, 7, 10). The association of zinc deficiency and vita-

min A metabolism is further supported by the simultaneous reduction in retinol and retinol binding protein (RBP) in zinc-deficient rats (11), which suggests that the low plasma retinol concentrations in zinc deficiency might be caused by an impaired ability of the deficient animals to mobilize hepatic retinol. Another study (12) showed that zinc deficiency reduces hepatic cellular RBP (cRBP), which is essential for the intracellular transport of vitamin A in addition to its well-established role in intercellular transport.

Data on the interaction between zinc and vitamin A in humans are more limited and the results of such studies are inconclusive. Most studies of the interaction have been conducted in individuals with severe disease conditions, such as cystic fibrosis (13) and cirrhosis of the liver (14–18). Some studies showed an association between low zinc status and reduced retinol concentrations (19–21) and others did not (22–24). Studies in preterm infants (25) and in children with severe protein-energy malnutrition (26) showed improvement in serum RBP and retinol with zinc supplementation. In contrast, a population-based study in Thailand failed to show an effect of zinc supplementation on retinol or RBP concentrations in children (27).

In developing countries where protein-energy malnutrition is highly prevalent, children usually have multiple micronutrient deficiencies, particularly of zinc (28, 29) and vitamin A (30). Reports from Bangladesh showed that >60% of children remain vitamin A deficient despite supplementation with large doses of vitamin A (31–33). Simultaneous zinc and vitamin A deficiency may be one of the explanations for the failure of vitamin A supplementation in these children. In this report, we study the effect of simultaneous zinc and vitamin A supplementation on vitamin A and RBP status in deficient children.

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SUBJECTS AND METHODS

Study population and sample

This study was conducted in urban slums in the older part of Dhaka City, the capital of Bangladesh. These slums were clusters of households with very high housing density and overall poor facilities (lacking an adequate water source, garbage removal, paved streets, street lighting, and gas supply). Most of the households (>90%) were constructed of poor, nondurable materials, and 82% had only one small room. One-third of the households had access to supplies of cooking gas, but these were usually shared. About two-thirds of the households had electricity and almost all households had access to safe drinking water through either pipes or tube wells. Most of the latrines (92%) were shared among multiple households and were often unhygienic (34). The infant mortality rate in the slums was \approx 138 per 1000 live births (35).

Children aged 12–35 mo of either sex who had not received any vitamin A supplementation within the past 4 mo were included in the study. However, children with severe malnutrition (weight-for-age <60% of the National Center for Health Statistics median), with signs or symptoms of vitamin A or zinc deficiency, or with any systemic illness such as diarrhea, respiratory infection, fever, or any other illness that warranted medical intervention at the time of enrollment were excluded. Informed consent was obtained from the parents for their children to participate in the study. The study was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh, and the Committee of Human Research, The University of Alabama at Birmingham.

Study design, randomization, and supplementation

This was a randomized, double-blind, placebo-controlled intervention trial. Children were randomly assigned to receive either zinc (Z group), vitamin A (A group), zinc plus vitamin A (ZA group), or placebo. The Z group received 5 mL (1 tsp) zinc syrup containing 20 mg elemental Zn/d for 14 d and a placebo capsule on day 14. The A group received 5 mL placebo syrup/d for 14 d and a 60000–retinol equivalents (RE) (200000 IU; 60-mg) vitamin A capsule on day 14. The ZA group received 5 mL (1 tsp) zinc syrup containing 20 mg elemental Zn/d for 14 d and a 60000-RE vitamin A capsule on day 14. The placebo group received 5 mL placebo syrup/d for 14 d and a placebo capsule on day 14.

A local pharmaceutical company (ACME Laboratories Ltd, Dhaka, Bangladesh) prepared the study syrups (zinc and placebo), which were supplied in identical 50-mL bottles. Each 50 mL placebo syrup contained 25 g sucrose, 5 g sorbitol, 50 mg methyl hydroxy benzoate, 15 mg propyl hydroxy benzoate, 5 mg lemon yellow color, 0.1 mL orange flavor, and 50 mL water. The zinc syrup contained all of the above plus 555.77 mg Zn as zinc sulfate (equivalent to 200 mg elemental Zn).

F Hoffmann-La Roche Ltd (Basel, Switzerland) manufactured and supplied the vitamin A and placebo capsules. The vitamin A capsules contained 60 mg (60000 RE) vitamin A as retinyl palmitate and 26.8 mg α -tocopherol equivalents (α -TE), or 40 IU, vitamin E (as *all-rac*- α -tocopherol), with soybean oil as an excipient. The placebo capsule contained 26.8 mg α -TE (as *all-rac*- α -tocopherol), with soybean oil as an excipient. The vitamin A and placebo capsules looked identical.

The children were randomly assigned by a person not involved in the study who used permuted blocks of random numbers. Sets of 2 bottles and 1 capsule for each child were serially numbered according to the randomization list and corresponding to the study serial numbers.

Sample size

A total of 800 children were randomly assigned; blood samples were collected from the first 411 children enrolled in the study after informed consent was obtained. This sample size was adequate to see an effect of combined zinc and vitamin A on the biochemical indexes of vitamin A status. The larger sample ($n = 800$) was needed to evaluate the effect of zinc and vitamin A supplementation on morbidity and is reported elsewhere (36). Characteristics of the 411 children from whom blood samples were collected were similar to those of the remaining children.

Supplementation procedure

At enrollment, a health assistant fed the child 5 mL (1 tsp) syrup from the numbered bottle that corresponded to the randomization list and showed the child's mother how to administer the medicines at home. The mother was given one bottle containing 50 mL (10 tsp) syrup and was instructed to feed her child one spoonful of syrup each morning after breakfast. Also, the mother was instructed to save the bottle after the syrup was finished and to request a replacement if the bottle was broken or lost. After 7 d, the health assistant visited the child at home and calculated the amount of syrup given by subtracting the amount left over from 50 mL. Each mother was asked whether she had encountered any problems when feeding the syrup (eg, whether the child liked the syrup or vomited) and was then given another 50-mL bottle of syrup. On day 14, the health assistant again visited the child at home and measured the amount of intake and recorded her finding. Mothers were asked about any problems encountered while feeding the syrup. Then the health assistant fed the child a vitamin A or placebo capsule.

Collection of blood samples

Venous blood (3 mL) was obtained at enrollment and again on day 21 and after 3 mo. For blood drawing, the children were brought to the International Centre for Diarrhoeal Disease Research, Bangladesh, hospital. The blood samples were kept for 0.5 h to allow the blood to clot and were then transferred to the nutritional biochemistry laboratory located in the same building. In the laboratory, the serum was separated and stored at -70°C . The serum samples were later analyzed for retinol, RBP, C-reactive protein, and zinc.

Biochemical analysis

Serum retinol was measured by HPLC (Millipore Corp, Bedford, MA) (37). RBP was measured by radioimmunoassay with a commercially available kit from Boehringer Diagnostics (La Jolla, CA) (38). C-reactive protein was measured by the immune turbidimetry method (38). Zinc was measured by atomic absorption spectrophotometry (Shimadzu AA 65015; Shimadzu, Nakagyo, Japan) (39).

Anthropometric measurement

Weight was measured by using a balance with a precision of 10 g (Seca, Hamburg, Germany). Length was measured with a locally constructed length board with a precision of 0.1 cm.



TABLE 1
Baseline characteristics of the study children¹

	Z group (n = 84)	A group (n = 85)	ZA group (n = 86)	Placebo group (n = 84)
Age (mo)	23.0 ± 7.3 ²	24.1 ± 7.2	24.2 ± 7.7	24.2 ± 7.7
12–23 mo [n (%)]	42 (50.0)	35 (41.2)	36 (41.9)	35 (41.7)
24–35 mo [n (%)]	42 (50.0)	50 (58.8)	50 (58.1)	49 (58.3)
Female sex [n (%)]	39 (46.4)	34 (40.0)	37 (43.0)	43 (51.2)
Breast-feeding continued [n (%)]	61 (72.6)	48 (56.5)	62 (72.1)	66 (78.6)
Weight-for-age z scores	−2.38 ± 0.86	−2.37 ± 0.90	−2.53 ± 0.76	−2.31 ± 0.89
<−2.0 [n (%)]	56 (66.7)	58 (68.2)	61 (70.9)	50 (59.5)
≥−2.0 [n (%)]	28 (33.3)	27 (31.8)	25 (29.1)	34 (40.5)
Weight-for-height z scores	−1.27 ± 0.72	−1.29 ± 0.76	−1.31 ± 0.70	−1.23 ± 0.76
<−2.0 [n (%)]	9 (10.7)	14 (16.5)	14 (16.3)	12 (14.3)
≥−2.0 [n (%)]	75 (89.3)	71 (83.5)	72 (83.7)	72 (85.7)
Height-for-age z scores	−2.34 ± 1.19	−2.23 ± 1.78	−2.54 ± 0.97	−2.29 ± 1.23
<−2.0 [n (%)]	48 (57.1)	51 (60.0)	59 (68.6)	52 (61.9)
≥−2.0 [n (%)]	36 (42.9)	34 (40.0)	27 (31.4)	32 (38.1)
Serum zinc <10.0 μmol/L [n (%)]	35 (41.7)	27 (31.8)	34 (39.5)	30 (35.7)
Serum retinol <0.7 μmol/L [n (%)]	32 (38.1)	32 (37.6)	30 (34.9)	34 (40.5)
Family income (\$/mo)	63 (17–244) ³	63 (32–168)	63 (21–231)	63 (11–273)

¹Z group, received 20 mg Zn/d for 14 d; A group, received 60000 retinol equivalents (200000 IU) vitamin A on day 14; ZA group, received both zinc and vitamin A.

² $\bar{x} \pm SD$.

³Median; range in parentheses.

Data analysis

Data were entered into a personal computer with use of SPSS for WINDOWS (version 8.0; SPSS Inc, Chicago). Anthropometric calculations (weight-for-age, weight-for-height, and height-for-age z scores) were made with the National Center for Health Statistics package (version 3.0; Centers for Disease Control and Prevention, Atlanta). Data were cleaned by visual and logical checks and were analyzed by using the SPSS program. Initially, descriptive statistics were analyzed. The proportion of children in each group who were vitamin A deficient after supplementation was compared by using the chi-square test. An analysis of covariance with the day 1 value as the covariate was done on postsupplementation changes in serum zinc, retinol, and RBP concentrations. Bonferroni corrections were done to adjust for multiple comparisons. Statistical significance was accepted at a probability level of 0.05.

RESULTS

Of the 411 children supplemented, 103 were in the Z group, 104 were in the A group, 103 were in the ZA group, and 101 were in the placebo group. The number of children available for the second blood drawing on day 21 was 84, 85, 86, and 84 from the 4 groups, respectively (total $n = 339$). Of the 72 children who dropped out, 40 were excluded from the study because they had received an additional vitamin A capsule through the national Expanded Program on Immunization clinic; parents of the other 32 children who dropped out withdrew their consent for blood drawing. Baseline characteristics of these 72 children did not differ significantly from those of the 339 children who completed the study. The baseline characteristics of the children in the 4 groups were also not significantly different (**Table 1**). Forty-four percent of the children were <2 y of age. All children received breast milk at least for some time, and 70% of them were still receiving breast milk at the time of enrollment. Most of children were underweight (weight for age < −2.0 z scores) or

stunted (height-for-age < −2.0 z scores). Ninety-two percent of the children had received a vitamin A capsule before their enrollment in the study. The median (range) time gap between enrollment and prior vitamin A supplementation was 5 (4–12) mo; in most instances it was between 4 and 6 mo. Ninety percent of the mothers reported that their children took the syrup without any problem, 6% of the mothers forced their children to feed because they usually do not want to take any type of medicine, and 4% of the children vomited once or twice during the 14 d.

Baseline and postsupplementation serum zinc concentrations are shown in **Table 2**. Compared with the baseline values, serum zinc concentrations increased only in the Z and ZA groups, suggesting that the zinc supplementation was effective. The postsupplementation serum zinc concentration in the Z group was significantly higher than that in any other group (data not shown).

The mean postsupplementation changes in serum retinol were not significantly different among the 4 groups (**Table 3**). However, there were trends in the A and ZA groups ($P = 0.05$ and 0.12 , respectively). The mean changes in serum RBP were also not significantly different among the 4 groups (**Table 3**).

The proportion of children with low serum retinol concentrations (serum retinol < 0.7 μmol/L) after supplementation was significantly lower in the ZA and A groups than in the placebo group (**Figure 1**). The proportion of children with low RBP concentrations (<17.0 mg/L) was significantly lower in the ZA group than in the other 3 groups (**Figure 1**), indicating an improvement in RBP status with combined zinc and vitamin A supplementation.

The postsupplementation vitamin A status in a subgroup of children who were initially vitamin A deficient (serum retinol < 0.7 μmol/L) is shown in **Figure 2**. The proportion of vitamin A-deficient children after supplementation was significantly lower only in the ZA group. Although 37.5% of the children remained vitamin A deficient in the A group, only 13% remained vitamin A deficient in the ZA group.



TABLE 2

Baseline (day 1) serum zinc concentrations and changes in concentrations after supplementation (day 21 – day 1)¹

	Z group (n = 84)	A group (n = 85)	ZA group (n = 86)	Placebo group (n = 84)
	$\mu\text{mol/L}$			
Day 1	11.2 (10.5, 11.3)	11.0 (9.5, 10.6)	10.7 (10.1, 11.2)	11.3 (10.8, 11.9)
Change ²	1.2 (0.6, 1.7) ³⁻⁵	-1.0 (-1.6, -0.5)	0.4 (-0.2, 0.9) ^{5,6}	-0.5 (-1.0, 0.06)

¹ \bar{x} ; 95% CI in parentheses. Z group, received 20 mg Zn/d for 14 d; A group, received 60 000 retinol equivalents (200 000 IU) vitamin A on day 14; ZA group, received both zinc and vitamin A.

²Adjusted for multiple comparisons by Bonferroni's correction.

^{3,6}Significantly different from A group (Bonferroni's correction, ANCOVA with day 1 value as the covariate): ³ $P < 0.001$, ⁶ $P < 0.005$.

⁴Significantly different from placebo group, $P < 0.001$ (Bonferroni's correction, ANCOVA with day 1 value as the covariate).

⁵Significantly different from day 1, $P < 0.01$ (Bonferroni's correction).

DISCUSSION

The results of the present study show that only combined zinc and vitamin A supplementation successfully reverted the deficiency in vitamin A-deficient children; supplementation with zinc or vitamin A alone did not. This finding indicates that there is a synergistic effect of zinc and vitamin A on vitamin A status. Although >90% of the children in the present study had received a vitamin A capsule as part of the National Vitamin A Week campaign 4–6 mo before enrollment, 38% of these children were still vitamin A deficient. After receiving another large dose of vitamin A during the study, a significant number of children in all groups except the ZA group remained vitamin A deficient. Thus, vitamin A status improved significantly in children who received both zinc and vitamin A, but not in those who received vitamin A alone.

This failure to improve vitamin A status in a significant number of children in the A group is consistent with our previous findings (31–33). In our earlier study, 61% of infants aged <6 mo remained vitamin A deficient despite receiving 3 doses of 15 mg (15 000 RE, or 50 000 IU) vitamin A at monthly intervals (32). We propose that these failures are related to a concomitant zinc deficiency in these vitamin A-deficient children.

Vitamin A binds to RBP within the cytoplasm of the hepatocyte, forming holo-RBP, which is secreted into the blood. Prealbumin, which is also synthesized in the liver, forms a trimolecular complex of retinol, RBP, and prealbumin circulating in the liver, apparently to prevent filtration and loss of vitamin A in the urine. Thus, zinc affects vitamin A status because it affects synthesis of the transport protein, which, in turn, transports retinol from the liver to the blood and other target tissues (40, 41). Zinc also participates in the synthesis of cRBP, which is essential for the intra-

cellular transport of retinol within liver hepatocytes. Hence, zinc is essential for both intra- and intercellular transport of vitamin A. In the present study, although the mean RBP concentrations on day 21 were not significantly different among groups, the proportion of children with low RBP was significantly lower in the ZA group than in the A group (Figure 1). This observation can likely be explained by the synergy between zinc and vitamin A, which resulted in the improved circulating retinol concentrations.

Other than its essential role in vitamin A transport, zinc aids in the absorption of vitamin A in the intestine (42, 43). In adult male rats with experimentally induced zinc deficiency, retinol absorption is markedly reduced; in contrast, essential fatty acid deficiency exerts only a mild effect on the absorption of retinol (42). Compared with the essential fatty acids, dietary zinc has a more pronounced effect on phospholipids, which are necessary for the absorption of vitamin A (42, 43). Addition of essential fatty acids fails to counteract the adverse effect of zinc deficiency on vitamin A absorption (43). In zinc deficiency there is a defect in the biliary secretion of phospholipids into the gut lumen. It was postulated that, because of the lack of phospholipids, the enterocyte of zinc-deficient rats fails to form chylomicrons, the principal carriers of dietary lipids and lipid-soluble nutrients.

In contrast with our findings, Udomkesmalee et al (27) found no synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutriture in Thai children. The Thai study differed from ours in several ways: those children were much older (6–13 y) than the children in the present study; their total plasma protein and albumin concentrations were within normal ranges, suggesting better nutritional status; and they had normal

TABLE 3

Baseline (day 1) serum retinol and serum retinol binding protein (RBP) concentrations and changes in concentrations after supplementation (day 21 – day 1)¹

	Z group (n = 84)	A group (n = 85)	ZA group (n = 86)	Placebo group (n = 84)
Serum retinol ($\mu\text{mol/L}$)				
Day 1	0.83 (0.76, 0.90)	0.79 (0.73, 0.87)	0.82 (0.74, 0.89)	0.85 (0.75, 0.90)
Change ²	0.07 (0.002, 0.14)	0.19 (0.12, 0.26) ³	0.17 (0.11, 0.24) ³	0.06 (-0.01, 0.13)
Serum RBP (mg/L)				
Day 1	19.5 (18.2, 20.9)	19.8 (18.5, 21.1)	20.1 (18.8, 21.5)	20.1 (18.7, 21.4)
Change ²	2.3 (0.7, 3.8) ³	3.5 (2.0, 5.0) ³	4.2 (2.7, 5.7) ³	2.0 (0.5, 3.5)

¹ \bar{x} ; 95% CI in parentheses. Z group, received 20 mg Zn/d for 14 d; A group, received 60 000 retinol equivalents (200 000 IU) vitamin A on day 14; ZA group, received both zinc and vitamin A.

²Adjusted for multiple comparisons by Bonferroni's correction.

³Significantly different from day 1, $P < 0.01$ (Bonferroni's correction).

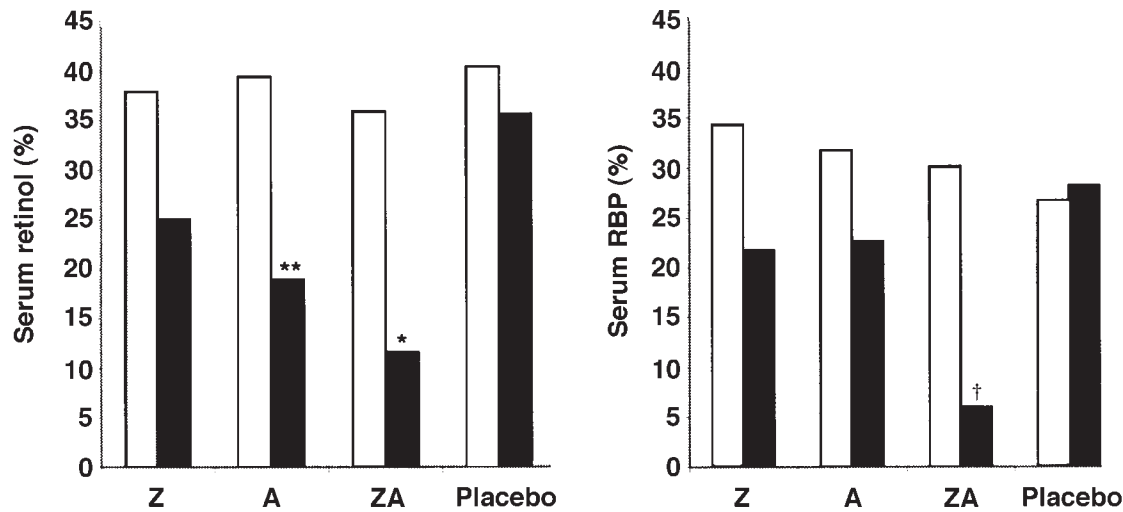


FIGURE 1. Improvements in vitamin A status with supplementation. Shown is the percentage of children with low serum retinol ($<0.7 \mu\text{mol/L}$) and low retinol binding protein (RBP; $<17.0 \text{ mg/L}$) concentrations before (\square) and after (\blacksquare) supplementation. Z group, received 20 mg Zn/d for 14 d; A group, received 60 000 retinol equivalents (200 000 IU) vitamin A on day 14; ZA group, received both zinc and vitamin A. ***Significantly different from placebo group (chi-square test with Bonferroni's correction); * $P < 0.001$, ** $P < 0.05$. †Significantly different from all other groups, $P < 0.01$ (chi-square test with Bonferroni's correction).

results on relative-dose-response tests, indicating normal vitamin A stores. Hence, no improvement of serum retinol with simultaneous zinc and vitamin A supplementation was possible. In our study, serum retinol also did not increase in children with adequate vitamin A status. Only in children who were vitamin A deficient did serum retinol concentrations improve. Although circulating retinol and RBP did not improve in the Thai children, improved dark adaptation time was observed in the zinc group. Also, the proportion of abnormal conjunctival impression cytology results decreased in children who received both zinc and vitamin A (27). In another study (44), children who were supplemented with both zinc and vitamin A tended to show higher proliferative responsiveness of T lymphocytes to purified protein derivatives than did those treated with placebo ($P = 0.08$). All of these observations suggest the existence of an interaction between zinc and vitamin A in children.

In the present study, postsupplementation concentrations of RBP and retinol in the zinc-supplemented group were not significantly different from those in the placebo group. However, the change from baseline was significant in the zinc group, but not in the placebo group. Despite the increment in RBP, the increment in circulating retinol was not significant. The reason for this could be that the zinc status of our study children, although lower than that of the Thai children, was not very severely depleted ($\bar{x} \pm \text{SD}$ serum zinc concentration: $11.2 \pm 2.9 \mu\text{mol/L}$). It is likely that the beneficial effect of zinc supplementation on serum retinol is observed only when zinc is limiting (ie, deficient to the extent that it may interfere with protein and enzyme synthesis). Above a certain threshold of serum zinc, vitamin A transport is not dependent on serum zinc concentrations; below that threshold, however, vitamin A release and transport from the liver are strongly influenced by serum zinc concentrations (45).

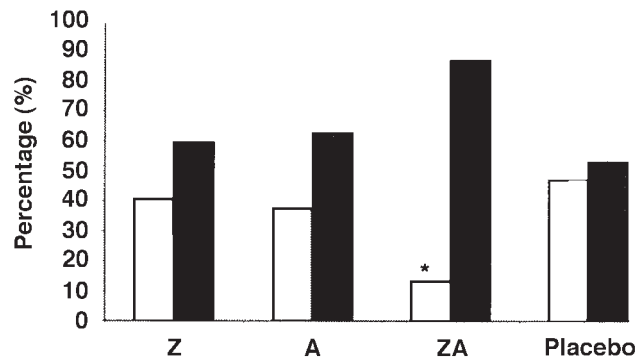



FIGURE 2. Percentage of initially vitamin A-deficient children (\square ; serum retinol concentration $< 0.7 \mu\text{mol/L}$) whose serum retinol concentrations returned to normal (\blacksquare) after supplementation. Z group, received 20 mg Zn/d for 14 d; A group, received 60 000 retinol equivalents (200 000 IU) vitamin A on day 14; ZA group, received both zinc and vitamin A. *Significantly different from placebo group, $P = 0.01$, and significantly different from Z group, $P = 0.04$ (chi-square test with Bonferroni's correction).

An earlier Indian study that reported a significant improvement in RBP and retinol with short-term (5 d) zinc supplementation was conducted in severely malnourished children whose plasma zinc concentrations were very low (26). In a group of American preterm infants, those receiving intravenous zinc supplementation showed significant improvements in serum retinol concentrations (25). These findings suggest that zinc supplementation may be beneficial only in vitamin A-deficient subjects.

In this randomized controlled trial we carefully adjusted for confounding factors, including age, sex, initial nutritional status, and concentrations of C-reactive protein, serum zinc, and retinol. We observed that vitamin A alone failed to correct vitamin A deficiency in a large proportion of the children, whereas combined zinc and vitamin A supplementation effectively corrected the vitamin A deficiency, suggesting the existence of a synergistic effect of zinc and vitamin A. These findings support combined zinc and vitamin A supplementation in undernourished children, in children with suspected zinc deficiency, and, more generally, in children from very poor communities where both undernutrition and low zinc concentrations may be prevalent. 

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