

Original Paper

Vitamin A and Zinc Supplementation of Preschool Children

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Key words: vitamin A supplementation, zinc supplementation, double-blind design, preschool children, covariance analysis, growth, weight gain, hemoglobin

Objective: To determine whether supplementation of vitamin A and/or zinc (Zn) improved serum levels of these nutrients and/or height and weight gains in preschool children, 22 to 66 months, living in Belize, Central America.

Methods: Subjects received either Zn, vitamin A, Zn and vitamin A or a placebo, (70 mg Zn and/or 3030 RE vitamin A, once per week) for 6 months in a 2×2 factorial design. Forty-three children, from a population of 104 prescreened, completed the study; they were selected, prior to treatment, for low/marginal serum concentrations of these micronutrients.

Results: Serum Zn levels were greater (16%, $p < 0.001$) for those who received Zn. In contrast, after vitamin A treatment there were no differences in serum vitamin A among groups. Although increases in height (+4.4 cm, $p < 0.001$) and weight (+0.79 kg, $p < 0.001$), compared with baseline values, were numerically greatest for children who received both supplements, only the vitamin A supplementation effect was significant, resulting in increased height (+1.4 cm, $p < 0.002$) and greater weight gain (+0.15 kg, $p < 0.03$) compared to those receiving no vitamin A. Vitamin A supplementation alone significantly increased ($p < 0.001$) hemoglobin concentration.

Conclusion: The results suggest that the preschool children in this study, prescreened for low/marginal serum concentrations from a larger population prior to treatment, were enduring inadequate vitamin A and, to a lesser degree, Zn nutriture. Height and weight gain were significantly increased in the subjects who received a single weekly supplement 3030 RE of vitamin A.

INTRODUCTION

The essential micronutrients thought to be most limiting in diets of children in developing countries are vitamin A, iodine and iron [1]. Recent estimates by the World Health Organization indicate that 231 million children in more than 90 countries are affected clinically or subclinically by vitamin A deficiency [2], which is the major cause of childhood blindness in developing countries and a contributor to increased morbidity and/or mortality.

Although Zn deficiency is not officially recognized as a global problem, data are accumulating to suggest that Zn nutriture is inadequate for many of the world's children whose

diets are largely plant-derived foods. Because there are no definitive indicators of Zn deficiency that can be used in field studies, the prevalence of this condition has not been well delineated. However, Gibson [3] has assessed the Zn status of children in several developing countries and has suggested that, worldwide, Zn deficiency could rival the documented iron deficiency. Recently, Shrimpton [4] has rhetorically questioned whether "Zn deficiency is widespread but under recognized." Indeed, plant-based diets that lead to poor iron status can induce Zn deficiency because of their high content of fiber and phytate. Calloway [5] has noted that "few (if any) studies of iron deficiency have included evidence showing that only iron is deficient." In addition, a 1993 study involving a low-income

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U.S. population reported that iron and Zn intakes were closely correlated and that both were correlated with energy intake [6]. A nutritional survey in 1990 in two rural areas in Belize suggested that more than 50% of native, non-refugee children under the age of 11 were consuming less than two-thirds of the Recommended Dietary Allowance for Zn and vitamin A [7]. A 1996 study of 613 native Belizean children, two to eight years old, indicated that 24% exhibited inadequate vitamin A status based on concentrations of $<0.87 \mu\text{mol/L}$ [8]. Serum Zn concentrations of $<12.2 \mu\text{mol/L}$ ($80 \mu\text{g/dL}$), suggesting only mild inadequacy, were evident in 35% of males and 40% of females. Other reports indicated that Zn and vitamin A supplementation benefited undernourished children in India [9] and Thailand [10].

The objective of this study was to determine whether biochemical and/or functional (anthropometric) status for vitamin A, Zn, or both, could be improved in preschool refugee children (2.2 to 5.5 years) by supplementing their usual daily diets with these nutrients. In addition, data would be used to test the hypothesis that a nutritional interaction can occur between vitamin A and Zn. Hemoglobin concentrations were examined to determine whether vitamin A supplementation would improve iron status, as had been reported previously [11,12].

SUBJECTS AND METHODS

Subjects/Design

The subjects of this study were refugee children from El Salvador of Mestizo heredity, living in two locations, Camps Los Flores and Salvapan, Cayo District of Belize. The protocol was approved by the Human Research Review Committee of Lincoln University, Jefferson City, MO. Informed consent was obtained from each child's parent or guardian with the aid of a native field assistant who spoke the local dialect after a detailed explanation of the purpose and procedures of the study.

Altogether, 104 children were screened with blood collected for serum vitamin A and Zn analysis. The blood was immediately frozen using dry ice at the field station, stored at -20°C . Within one week it was shipped to Beltsville, Maryland, on dry ice where it was stored at -70°C and analyzed for serum vitamin A and Zn within one month. The mean \pm SEM concentrations for the 104 children who were screened were 0.96 ± 0.02 and $12.4 \pm 0.14 \mu\text{mol/L}$ for serum vitamin A and Zn, respectively. Based on the criterion of low/marginal concentrations of *both* serum vitamin A and Zn, 51 children were selected for the supplemental phase of the study. The mean entry ages and ranges of the children according to treatment were (in months): placebo 48.5 (31 to 72), Zn 48.1 (32 to 68), vitamin A 46.3 (32 to 67), vitamin A+Zn 43.6 (28 to 70). Because of relocation of residence and other causes, eight failed to complete the study, resulting in 43 subjects receiving the supplements for six months.

The pretreatment mean \pm SEM serum vitamin A and Zn concentrations of those who completed the study were $0.84 \pm 0.02 \mu\text{mol/L}$ and $11.5 \pm 0.17 \mu\text{mol/L}$, respectively. Prior to entry into the study, children were examined by the physician in charge (AH); those with fever or serious respiratory infection were excluded from the study.

Within one month following the screening, the supplementation phase of the study began. The screening values for serum vitamin A and Zn were used as pretreatment concentrations for those children who were selected to enter the study. The children selected were randomly assigned to receive one of the following supplements once per week: placebo; Zn, 70 mg as Zn gluconate; vitamin A, 3030 RE as retinyl palmitate; or a combination of vitamin A and Zn. Under the observation of the field nurse, supplements were ingested orally in an orange-flavored powder (10 g), Tang® (Kraft General Foods Inc, White Plains, NY 10625) prepared as a beverage dissolved in approximately 120 mL of water. Supplements, including the placebo, were personally administered in each home by a community nurse, who spoke the local dialect.

For both blood samples, the screening and six months after supplementation, approximately five mL of whole blood was drawn using trace-metal-free Monovet® all-plastic syringes (Sarstedt, Princeton, NJ 08540), with no anticoagulant, in combination with a butterfly infusion set with a 21- or 23-gauge stainless steel needle (Minicath®, Deseret Medical Co., Sandy, UT 84070). The clotted blood was centrifuged ($1500 \times g$) for 15 minutes and separated to yield serum. The serum samples were transferred to trace-metal-free plastic 3.6 mL Cryotubes® (Thomas Scientific, Swedesboro, NJ 08085). They were frozen at -20°C , in Belize, for no more than one week and then at -70°C at Beltsville, until analyzed. The time between collection was approximately one month for the screening samples and two months for the posttreatment samples.

Serum vitamin A concentrations were determined by high-performance liquid chromatography, according to the method of Bieri *et al.* [13]. A certified standard reference material (SRM 968) from the National Institutes of Standards and Technology (NIST, Gaithersburg, MD 20899) was determined along with each series of samples. The concentration obtained by our laboratory for SRM 968 was $1.092 \pm 0.03 \mu\text{mol/L}$ (mean \pm SD), identical to the certified value.

Serum Zn concentrations were measured by atomic absorption spectroscopy using a slight modification of the method of Smith *et al.* [14]. Zinc reference standards were prepared in deionized water instead of in 5% v/v glycerol because our atomic absorption spectrophotometer does not require that the viscosity of standards and diluted sera be similar. A certified SRM (#1598, NIST) for serum Zn was used to verify accuracy. The mean concentration of SRM 1598 in our analyses was $13.8 \pm 0.01 \mu\text{mol/L}$ (mean \pm SD), within the certified concentration range.

Statistical Methods

Analysis of pretreatment data indicated that children who subsequently received Zn supplementation were heavier (1.1 kg) than were non-Zn-treated subjects. The effects of these weight differences were significant variations in body mass index (BMI) and weight-for-age Z score (WAZ). No other differences were found in pretreatment data.

Initial analyses to examine the six month time effects indicated that there were significant changes from pretreatment to posttreatment for all dependent variables. Except for serum retinol and Zn, these significant changes included both treated and non-treated children. Since variation among pretreatment values is a reflection of subject variation, these values should be used to improve the sensitivity of the analysis with respect to posttreatment data.

Given the above considerations, along with the variation in initial age (28 to 66 months) and initial BMI (13.2 to 21.0), analysis of covariance techniques were used to analyze the data at six months posttreatment [15]. The full model for the dependent variables at six months posttreatment included the factorial treatment effects of vitamin A, Zn, and the vitamin A×Zn interaction, plus covariates and interactions between the covariates and treatment sources of variation. Examination of the residuals for each dependent variable did not reveal any problems with model assumptions, but one outlier was identified. This subject, in the vitamin A and Zn combination treatment had a positive residual that was 4.2 standard deviations above the model prediction for WAZ. The statistics reported for WAZ do not include this subject. Had this observation been included, the effect would have been greater significance for all sources of variation and a greater mean for the combination treatment than are being reported for WAZ.

For each dependent variable there were four covariates. The first covered was the pretreatment value of the dependent variable, at baseline. The others were gender, initial age and initial BMI. The full model was then reduced by deleting nonsignificant covariate sources of variation, by the procedure of Hendrix [16], except that the initial value of the dependent variable was never deleted from the model. Figures of significant covariate by treatment interactions, if any, are presented for the final model. All treatment means are reported at the average value of any covariate retained in the model.

Pretreatment values for each dependent variable were included as a covariate to account for variation, among subjects, that existed before treatment. Except for serum vitamin A and Zn, initial values accounted for a significant (76% to 99% reduction, $p < 0.001$, in residual variance) portion of the variation in the dependent variable at six months. Sources of variation involving gender or BMI were not significant and therefore did not appear in the final model for any dependent variable. In only one case (hemoglobin response to Zn treatment) was age significant as described below. Height-for-age Z score (HAZ) and WAZ were calculated from U.S. National

Center for Health Statistics data using the ANTHRO (Centers for Disease, Atlanta, GA 30341) software program [17]. Treatment means, \pm the standard error of the means, are reported unless otherwise indicated, as in the tables. The treatment design was a 2×2 factorial. Thus, “vitamin A supplemented” or “Zn supplemented” refers to the tests of main effects in the analysis of covariance. Significant differences from the 2×2 factorial covariance analysis between the “vitamin A supplemented” or “Zn supplemented” group means are shown in the footnotes of Tables 1 and 2.

Individual group-mean comparisons are also made as indicated by “placebo, vitamin A alone, Zn alone or vitamin A and Zn in combination.” Significant differences among individual group means, if any, are indicated by letter superscripts in Tables 1 and 2, using the least significant difference (LSD) technique.

RESULTS

Serum Vitamin A and Zinc

No significant effects of vitamin A or Zn supplementation were observed in serum vitamin A concentrations after six months of supplementation (Table 1). In contrast, mean serum Zn concentration for those given Zn was significantly greater ($+1.9 \pm 0.48 \mu\text{mol/L}$, $p < 0.001$) compared to the mean of the two groups of children who did not receive Zn supplements. Serum vitamin A and Zn concentrations did not change significantly ($p < 0.05$) from baseline to sixth month posttreatment for the placebo group.

Hemoglobin

Comparison of mean hemoglobin concentrations for the vitamin A supplemented children with the mean of those not given vitamin A yielded a significance of $p < 0.001$ (Table 1). Likewise, when hemoglobin concentrations of individually supplemented groups were compared with the placebo group, the difference was significant for each: vitamin A ($p < 0.001$), Zn ($p < 0.05$), combination vitamin A and Zn ($p < 0.01$) (Table 1). An interaction of vitamin A and Zn tended to affect hemoglobin, yet did not reach statistical significance ($p < 0.07$).

Zinc×Age Interaction Affects Hemoglobin

Subject age significantly ($p < 0.01$) affected the hemoglobin response to Zn treatment (Fig. 1). For non-Zn-supplemented children, hemoglobin concentrations declined significantly at an average rate of $-0.022 \pm 0.008 \text{ g/L/mo}$ from the value at the age of entry into the study; Zn-supplemented children maintained an average hemoglobin concentration of $+0.011 \pm 0.0085 \text{ g/L/mo}$.

Height and HAZ Score

The analysis of height indicated a $1.4 \pm 0.42 \text{ cm}$ greater increase for the vitamin A supplemented children. Specifically,

Table 1. Effect of Vitamin A and/or Zinc Supplementation on Biochemical Indices of Iron (Hemoglobin), Serum Vitamin A and Zinc Concentrations

Biochemical Indices	Post Treatment Means ¹				
	Placebo	Zn	Vitamin A	Zn+Vitamin A	SED ²
Hemoglobin (g/L) ³	109 (9) ^{c 4,5}	113 (11) ^b	117 (10) ^a	116 (7) ^{ab}	1.70
Vitamin A (μmol/L)	0.90 (10) ^a	1.01 (12) ^a	1.03 (10) ^a	0.97 (8) ^a	0.11
Zinc (μmol/L)	11.7 (10) ^b	13.5 (12) ^a	12.1 (10) ^b	14.0 (8) ^a	0.68

¹ In accordance with covariance analysis, treatment means were adjusted to pretreatment means which were hemoglobin, 105 g/L; serum vitamin A, 0.85 μmol/L; serum zinc, 11.5 μmol/L. These values are the means of each of the indices for all of the children, prior to supplementation.

² SED=Standard error of the difference.

³ Likewise for covariance analysis, treatment means of hemoglobin were adjusted to a mean initial age of 48.4 months, which was the mean age of all children with hemoglobin values prior to supplementation.

⁴ Number of subjects in parentheses.

⁵ Superscripts compare individual group means. Means with any identical superscripts are not significantly different by least significant difference (LSD), $p < 0.05$. For 2×2 factorial comparisons, serum Zn concentration differed significantly for Zn-supplemented subjects ($p < 0.001$), and hemoglobin concentration differed significantly for vitamin A-supplemented subjects ($p < 0.001$).

the mean height of those supplemented with vitamin A was 96.3 cm compared with 94.9 cm for those not receiving vitamin A, $p < 0.002$ (Fig. 2, A). Children who received vitamin A supplements also had significantly less negative ($p < 0.001$) mean HAZ scores (-2.11) than those who did not receive vitamin A (-2.44) (Fig. 2, B). In contrast, HAZ scores became significantly more negative, from -2.37 to -2.57, for the placebo group (Table 2). Moreover, the groups receiving vitamin A alone or in combination with Zn exhibited an improvement in HAZ scores ($p < 0.05$) compared to the adjusted pre-treatment mean (comparison not shown). When HAZ scores for groups receiving either one or both supplements were compared individually with the placebo group, the differences were statistically significant (at least $p < 0.05$) in all cases as indicated by the superscripts in Table 2.

Weight and WAZ Score

The two groups of children who received the vitamin A supplements gained an average of 0.15 ± 0.07 kg more weight than non-vitamin-A-supplemented children ($p < 0.03$) (Fig. 3, A). Zinc supplementation resulted in an increase in weight gain

of 0.13 ± 0.07 kg compared to non-Zn supplemented groups, but it was not significant ($p < 0.07$).

The mean WAZ score was significantly greater ($p < 0.05$) for vitamin-A-supplemented children (-1.35) than it was for those not receiving vitamin A (-1.44), (Fig. 3, B). The mean WAZ score for Zn-treated compared with non-Zn-treated subjects was -1.35 vs. -1.44 ($p < 0.09$), which, although not statistically significant, suggests that improved weight gain may be a potential benefit of Zn treatment. The absence of a vitamin A×Zn interaction indicates that the effects of vitamin A and Zn supplementation are additive, resulting in a greater weight gain (0.28 ± 0.096 kg, $p < 0.01$) and WAZ score (0.16 ± 0.064) compared with the same factors for children in the placebo group. Although all groups gained weight during the study, the posttreatment mean WAZ score of the four groups (-1.40) declined as compared with the pretreatment mean of -1.28. These data indicate that the children were falling further behind the norm (of zero) for WAZ score. This decline was significant ($p < 0.05$) for all groups other than the combined vitamin A and Zn supplemented group, which had a final WAZ score of -1.31. These data suggest a beneficial

Table 2. Effect of Vitamin A and/or Zinc Supplementation on Anthropometric (Anthro) Indices

Anthro Indices	Post Treatment Means ¹				
	Placebo	Zn	Vitamin A	Zn+Vitamin A	SED ²
Height (cm)	94.4 ^{b 3}	95.5 ^{ab}	96.2 ^a	96.4 ^a	0.59
HAZ score	-2.57 ^b	-2.30 ^a	-2.09 ^a	-2.13 ^a	0.13
Weight (kg)	14.4 ^b	14.5 ^{ab}	14.5 ^{ab}	14.7 ^a	0.09
WAZ score	-1.48 ^b	-1.40 ^{ab}	-1.39 ^{ab}	-1.31 ^a	0.60

¹ In accordance with covariance analysis, post-treatment means were adjusted to pre-treatment means of height, 92.0 cm; HAZ score -2.37; weight 13.9 kg; WAZ score 1.28. The numbers of subjects for each of the anthropometric indices were placebo 10, vitamin A 12, zinc 11, and zinc+vitamin A 10, except $n=9$ for WAZ (Zn+Vitamin A), where one subject was deleted; see statistical methods for explanation.

² SED=Standard error of difference.

³ Superscripts indicate that, in addition to 2×2 comparisons, individual group means were compared. Means with any identical superscripts are not significantly different by least significant difference (LSD), $p < 0.05$. For 2×2 factorial comparisons, height, HAZ score, weight, and WAZ score differed significantly for A-supplemented subjects ($p < 0.002, 0.001, 0.03, \text{ and } 0.05$, respectively).

effect of the combined micronutrient supplement (Zn+Vitamin A) on WAZ.

DISCUSSION

Possible Multiple Deficiencies

In reviewing nutritional influences on linear growth, Allen [18] stated that there is no “consistent evidence” in the literature that supplementation with a single nutrient benefits linear growth. She included among the reasons for the conflicting results the “strong probability that growth is limited by multiple, simultaneous deficiencies in many populations.” Unfortunately, the preponderance of studies involves supplementation with only a single nutrient instead of two or more, which could in combination result in limiting growth. Therefore, a response would be expected only if the single supplement were the most limiting.

Suggestions for Improved Experimental Designs

A more definitive, but admittedly more difficult and expensive experimental design would be to provide a complete supplement of all nutrients suspected of being limiting, including protein and energy, to a group of “positive control” subjects. Then, one would compare the results from that group with those from a second group receiving the same supplement, but with a single nutrient missing. Such designs, in combination with purified diets, have been used in animal experiments for decades [19]. Indeed, a human study with a positive control group concluded that Zn supplementation stimulated growth of malnourished schoolboys in Iran in 1974 [20]. More recently Penland *et al.* [21], using a double blind trial, have reported that Zn supplements alone had the least effect on growth of six to

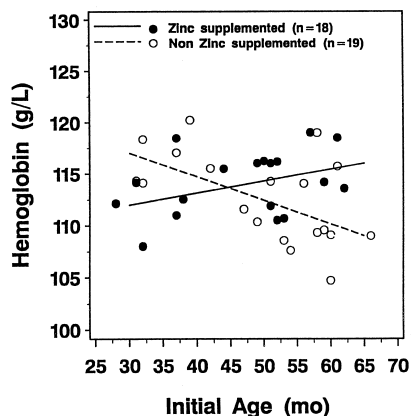


Fig. 1. An age×zinc interaction affecting hemoglobin concentrations. As initial age of entry to the study increased, zinc-treated children maintained hemoglobin levels. In contrast, the children who did not receive zinc exhibited a significant decline (8% predicted decrease) from 28 to 66 months, $p < 0.01$.

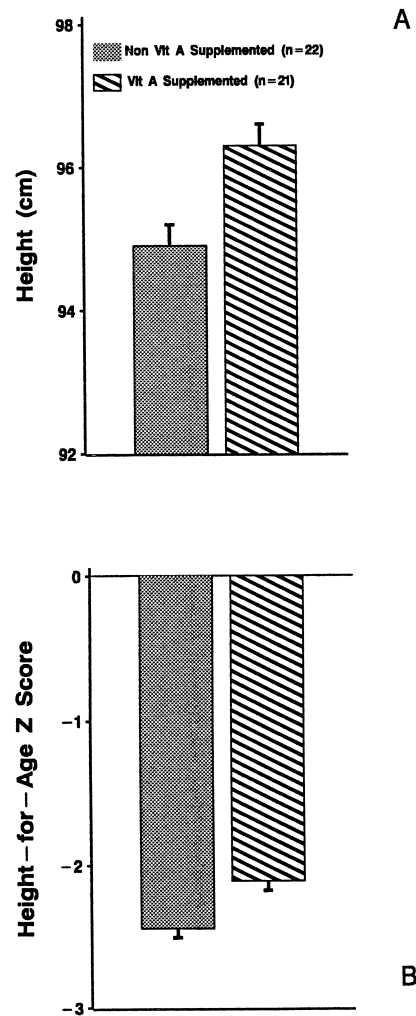


Fig. 2. (A) Comparison of mean height of the children who received vitamin A supplementation for six months (96.3 cm) with the height of those who were not supplemented with the vitamin (94.9 cm). The height increase was 1.4 ± 0.42 cm greater for the vitamin A supplemented children, $p < 0.002$. The pre-treatment adjusted mean was 92.0 cm. The bars represent the mean height increases for the non-vitamin A supplemented compared to the vitamin A supplemented children, e.g. 2.9 cm versus 4.3 cm, respectively. (B) Comparison of mean height-for-age Z score of the children who received vitamin A supplementation with the mean height-for-age Z score of those who were not supplemented with the vitamin, -2.44 vs. -2.11 , $p < 0.001$. The pre-treatment adjusted mean was -2.37 .

nine years old Chinese children compared to Zn combined with mixture of several micronutrients; micronutrients alone had an intermediate effect. These studies confirm that multi-subclinical deficiencies are probable in children whose nutritional status is compromised. Likewise, in a recent review, Solomon and Ruz [22] have suggested a change, beginning with experimental design, from the concept of single nutritional deficiencies, to recognize the reality of nutrient-nutrient interactions.

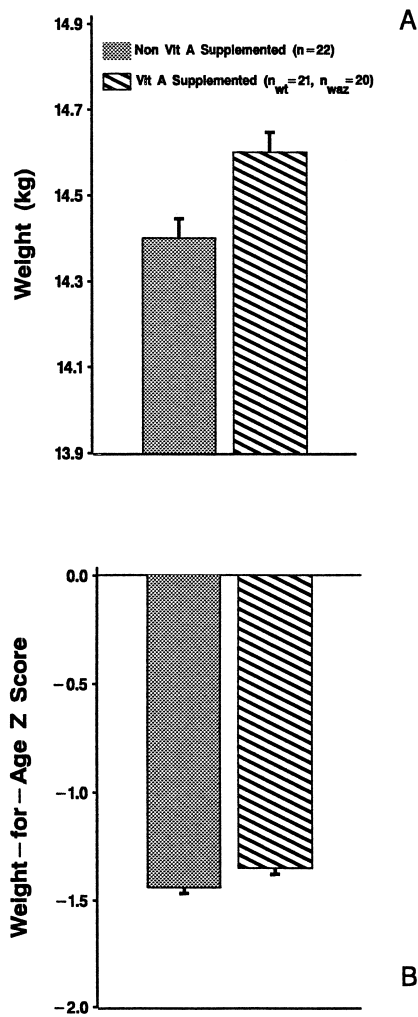


Fig. 3. (A) Comparison of mean weight of the children who received vitamin A supplementation for six months (14.6 kg) with the weight of those who were not supplemented with the vitamin (14.4 kg). The weight increase was 0.15 ± 0.07 kg greater for the vitamin A supplemented children, $p < 0.05$. The pre-treatment adjusted mean was 13.9 kg. The bars represent the mean weight increases for the non-vitamin A supplemented compared to the vitamin A supplemented children, e.g. 0.5 kg versus 0.7 kg, respectively. (B) Comparison of mean weight-for-age Z score of the children who received vitamin A supplementation (-1.35) with the mean weight-for-age Z score of those who were not supplemented with the vitamin (-1.44), $p < 0.05$. Pre-treatment adjusted mean was -1.28.

Effect of Zinc on Growth

The question of whether mild Zn deficiency contributes to poor growth has been addressed in the literature [18,23] and in a recent editorial [24]. Some intervention trials, mostly involving children living in developing countries, have shown an increase in growth attendant upon Zn supplementation [3,24]. Golden and Golden [25] reported increased synthesis of lean tissue and weight gain after Zn supplementation in children

recovering from malnutrition. They stated that Zn supplementation “resulted in a greater net absorption of nitrogen and a higher rate of protein turnover.” Zinc supplementation in our study tended to increase weight gain (0.13 ± 0.07 kg, $p < 0.07$), but we have no body composition data regarding whether the percentage of lean tissue was altered. Recently, repletion studies from several countries, of apparently malnourished children, have reported an effect of Zn supplementation on immunity; there was a positive response on diarrhea and other infections [26–30]. Thus in these cases, Zn repletion may contribute to preventing or minimizing growth deficits.

Response of Anthropometric Indices

The relatively greater response of anthropometric indices to vitamin A than to Zn may suggest that a) subjects entering our study had more adequate Zn than vitamin A status, b) since Zn is poorly stored in body pools, a single weekly dose of 70 mg of Zn may not have been enough to improve Zn status sufficiently to support significantly increased height and/or weight gain. These data also confirm that serum Zn is not a very sensitive clinical index whereas functional indices are more reflective. Compared with placebo treatment, vitamin A supplementation resulted in a significantly greater response for hemoglobin (Table 1), height gain and HAZ score (Table 2). Zinc supplementation alone significantly increased hemoglobin, serum Zn and HAZ score. Moreover, an age \times Zn interaction ($p < 0.01$) indicated that, as age increases, Zn-treated children maintain hemoglobin concentrations, whereas they declined significantly (8% decrease predicted from 28 to 66 mo, $p < 0.01$) in non-Zn-treated subjects (Fig. 1).

The children who received both Zn and vitamin A show the greatest numerical improvement in height and weight gain, suggesting an additive effect. However, these values were not significantly different from those for children who received vitamin A or Zn alone (Table 2).

Zinc Supplementation and Linear Growth

Previous double-blind, placebo-controlled studies involving Zn supplementation for preschool children are summarized in Table 3 [31–36]. In four out of seven studies, growth was greater for the children in the Zn-supplemented group than in the placebo group (controls). Of seven other studies of Zn supplementation involving older, school-aged subjects cited by Gibson [3], but not listed in the table, only two report positive effects on growth. One study was the well-known work in Iran with growth-retarded boys [20]; the other was with growth-retarded boys in Canada [37]. In the study from Canada, the positive effect on height was limited to subjects with low Zn status as indicated by a hair Zn concentration of $< 1.68 \mu\text{mol/g}$.

Perhaps of greater importance in terms of numbers of children who are affected throughout the world is whether marginal Zn inadequacy (not overt, frank deficiency) results in growth retardation. The pretreatment baseline mean \pm SEM serum Zn

Table 3. Effect of Zinc Supplementation on Linear Growth of Preschool Children¹

Country	Number of Subjects	Age (years)	Treatment	Duration (months)	Growth Effect	Reference
USA (Colorado)	40 (20 placebo)	2.0–6	10 mg/day	12	Yes (especially boys)	Walravens <i>et al.</i> , 1983
Gambia	109 (54 placebo)	0.6–2.3	70 mg/2×/week	15	No	Bates <i>et al.</i> , 1993
Chile	98 (49? placebo)	2.0–3	10 mg/day	14	Yes (boys only)	Ruz <i>et al.</i> , 1995
Turkey	25 (9 placebo)	2.5–5.7	10 mg/day	12	Yes	Ince <i>et al.</i> , 1995
Vietnam	146 (73 placebo)	0.3–3	10 mg/day	5	Yes	Ninh <i>et al.</i> , 1996
Mexico	144 (47 placebo)	1.5–3	20 mg/day	12	No	Rosado <i>et al.</i> , 1997
Belize	44 (22 placebo)	2.2–5.5	70 mg/week	6	No	Present

¹ All studies were double-blind placebo-controlled.

concentration of the 43 refugee children completing the study was $11.5 \pm 0.17 \mu\text{mol/L}$. Only six children (14%) had concentrations of $<10.7 \mu\text{mol/L}$ ($<70 \mu\text{g/dL}$), a concentration generally accepted as suggesting inadequate Zn status [38]. Thus, the majority of the children in our study had serum concentrations within normal range. Again, we recognize the limitations of serum Zn as an index to assess status.

In contrast, the mean \pm SEM vitamin A concentration at pretreatment baseline was considered marginal, e.g., $0.85 \pm 0.02 \mu\text{mol/L}$. This concentration has been considered to indicate vitamin A status in which “some individuals (three to eleven years) may improve with increased consumption of vitamin A” [39]. In addition, the response of the anthropometric indices strongly suggests that, at the beginning of the study, the subjects’ vitamin A status was impaired more than was their Zn nutriture.

Vitamin A and Iron Interaction

Evidence has been mounting to suggest a relationship between vitamin A and iron metabolism [40]. The potential importance of such an interaction was recognized nearly two decades ago when iron deficiency anemia was reported to develop in vitamin A deficient adults [41]. A significant correlation between serum vitamin A and hemoglobin ($r = 0.31$,

$p=0.01$) has been reported from a 1993 study of 242 children, five to twelve years of age, living in Bangladesh [12]. The present data demonstrate a highly significant association between vitamin A treatment and an increase in hemoglobin concentration, $p<0.001$.

Vitamin A Status and Growth

The issue of whether vitamin A supplementation will improve height and weight gain in apparently malnourished children has been the focus of several investigations. Results of five studies plus the present one are summarized in Table 4 [42–48]. As indicated in the table, two of the previously reported investigations indicate a significant effect of vitamin A supplementation on linear growth [43,47]; the ages of the children were one and two years [43] and newborns given a single large dose at birth with linear growth measurements at three years [47]. However, for those with a normal fontanelle in the latter study [47], the vitamin A supplemented children grew only 0.68 cm more than placebo controls over the first three years of life. Although statistically significant, the clinical/biologic importance may be minimal.

A major difference between the subjects of the other studies and the present one is that the Belize children were refugees who were screened to have lower serum vitamin A (and Zn)

Table 4. Effect of Vitamin A Supplementation on Linear Growth of Preschool Children

Country	Number of Subjects	Age (years)	Treatment	Duration (months)	Growth Effect	Reference
Indonesia	2,012	1–5	60,000 RE/month	12	No	West <i>et al.</i> , 1988
Indonesia	866	1–5	210 RE/day ¹	12	1 and 2 years only	Muhilal <i>et al.</i> , 1988
India ²	15,419	0.5–5	2500 RE/week	12	No	Rahmathulla <i>et al.</i> , 1991
India ²	592	0.5–3	30,000–60,000 RE/4 months ³	12	No	Ramakrishnan <i>et al.</i> , 1995
Indonesia ²	432	3.0	15,000 RE (only at birth)	see footnote ⁴	Yes	Humphrey <i>et al.</i> , 1998
Nepal ⁵	3377	0.5–5	60,000 RE/4 months	16	No	West <i>et al.</i> , 1997
Belize ^{2,6}	44	2.3–5.5	3030 RE/week	6	Yes	Present

¹ Estimated; fortified monosodium glutamate served as vehicle.

² Double-blind, placebo-controlled.

³ Depending on age, 30 mg for 6–11 months; 60 mg, 12–36 months.

⁴ A single “neonatal” dose of 52 μmol (approximately 15,000 RE) of vitamin A was given soon after birth and growth measured within two weeks of subjects’ third birthdays.

⁵ Randomized placebo controlled, nonxerophthalmic children. Growth of a separate group of non-wasted xerophthalmic children ($n=86$) treated with $\geq 120,000$ RE of vitamin A at baseline was 0.7 cm greater than nonxerophthalmic children. Among a group of initially wasted children treated with vitamin A ($n=34$), growth was approximately 1 cm greater than that of nonxerophthalmic children.

⁶ All children in two refugee villages were screened; subjects were chosen based on having low serum vitamin A (and zinc) concentrations.

concentrations as a prerequisite for entry to the trial. As a result, fewer than one-half of those screened were selected for our study. Evidently for those children selected, vitamin A was at least one or perhaps the most limiting essential nutrient.

Comparison of Supplementation in Thailand with Present Study

A previous trial in Thailand involving 133 children, with an experimental design and duration similar to the present study, determined the effect of supplementing Zn, vitamin A or Zn+vitamin A combined on growth [10]. The status of vitamin A and Zn was considered only marginally inadequate, based on mean serum concentrations of <1.05 and $12.2 \mu\text{mol/L}$, respectively. Unlike their Thai counterparts, the refugee children in Belize who received only vitamin A, responded with statistically significant increases in both height and weight (Table 2). The reasons for these differences, although unknown, may include the following: a) the Belizian children were younger (2.2 to 5.5 years, compared with 6 to 13 years for the Thais) and thus would be expected to have a faster growth rate; b) the refugee children of Belize had poorer vitamin A and Zn status (based on lower initial plasma concentrations), more severe growth retardation (stunting) and/or less nutritionally adequate daily diet. Ethnic differences in stature and weight, especially in regard to universal height and weight standards [17], may also be contributing factors.

Another difference is that, in the Thai study, supplements were given five times per week, whereas in Belize they were administered only once each week because of logistical and financial limitations. However, the more frequent supplementation schedule would favor the Thai study.

Reversibility of Early-Childhood Stunting

Conflicting data have been published regarding the provocative hypothesis that linear growth impairment (stunting) that occurs early in childhood (1 to 3 years) is essentially irreversible [48]. If this is true, it is monumentally important; the stunted child becomes a short-statured adult who could be expected to suffer physical and social consequences. In our study, HAZ scores revealed a high prevalence of stunting. Specifically, applying the suggested HAZ score of ≤ 2.0 as cut-off, 23 of the 43 (53%) of the children were stunted at the beginning of the study. The median HAZ score was -2.37 , with a range of -0.29 to -5.65 , which indicates a severe growth impairment (stunting) compared with the reference population. After six months of supplementation, the median HAZ improved (became significantly less negative) in the three individually supplemented groups; it became significantly more negative for children in the placebo group (Table 2). These data suggest that children who received no supplemental Zn or vitamin A continued to falter in growth as a result of more severe depletion, even during the short study period.

In contrast, supplementation of the two essential nutrients,

either alone or in combination, at a weekly amount equal to approximately seven times the daily RDA, prevented further decrease in relative growth rate and a marked improvement of the HAZ score (Table 2). The improved HAZ score of the children supplemented with the two nutrients, either alone or in combination, does not support the “once stunted always stunted” hypothesis [48]. However, it is recognized that the present study was limited in number of subjects and in duration. Nevertheless, the rapid increase in growth could be considered an attempt to catch up. Golden [49] concludes, “Many children do not catch up because we do not know what component of the diet is critical.”

Effect of Age×Zinc Interaction on Hemoglobin Levels

As indicated, an age×Zn interaction affected hemoglobin concentrations (Figure 1). That is, Zn-treated children maintained serum hemoglobin concentrations, whereas hemoglobin levels declined significantly (8% predicted decline from 28 to 66 months, $p<0.01$) with increasing age in non-Zn-treated subjects. This decline may be due to the unique compromised nutritional status of these refugee children since children progressing in age through the preschool years normally show a slight increase in hemoglobin concentration [50].

To our knowledge, this is the first report of Zn supplementations protecting preschool children, albeit refugee children of marginal vitamin A/Zn status, against a slight but significant decrease in hemoglobin concentration. The clinical significance and the nutritional/biochemical basis for this effect are not readily apparent. However, hematological changes similar to those of iron-deficiency anemia, including reduced hemoglobin, have been reported in Zn-deficient monkeys [51]. In addition, Jameson [52] has speculated that anemia may be a complication of pregnancy in women with inadequate Zn status. Hypothetically, the rate of hemoglobin synthesis could be reduced during Zn deficiency because a required step has been reported to be mediated by a Zn-dependent enzyme, aminolevulinic acid dehydrase [53]. Obviously, the results require confirmation since anemia has not been a salient feature of Zn deficiency.

CONCLUSION

Increases in height and weight were numerically greatest for children supplemented with a combination of vitamin A and Zn. However, the effects were additive, and statistically significant improvement was the result of vitamin A supplementation.

There was a mean significant height increase (+1.4 cm) for children who received vitamin A supplements compared with those who did not ($p<0.002$). Comparing individual groups, there were no significant differences in height increase for

those who ingested the Zn supplement alone compared with those given placebo treatment; the group supplemented with vitamin A+Zn did not show a significantly greater increase in height gain than did the group given vitamin A alone. Mean weight gain of children who had supplements of vitamin A (alone and in combination with Zn) was also significantly greater than it was for children who received no supplement of vitamin A (placebo and Zn supplemented).

Thus, the results could be interpreted as indicating that the children selected to enter the study based on the criterion of low/marginal serum concentrations were enduring inadequate vitamin A and, to a lesser degree, Zn nutriture. Weekly vitamin A supplementation of 3030 RE, approximately sevenfold the daily Recommended Dietary Allowances [54], significantly improved height and weight gain in preschool children. The findings also confirm an effect of vitamin A status on hemoglobin concentration.

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