A 14-mo zinc-supplementation trial in apparently healthy Chilean preschool children[1–3]

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ABSTRACT Apparently healthy preschool children (46 boys, 52 girls) aged 27–50 mo from low socioeconomic conditions who attended daycare centers in Santiago participated in a 14-mo long double-blind zinc supplementation trial. Unlike most previous studies, no additional inclusion criteria such as short stature or slow growth rate were considered. Subjects were pair matched according to sex and age and randomly assigned to two experimental groups: the supplemented group, which received 10 mg Zn/d, and the placebo group. Selected anthropometric, clinical, dietary, biochemical, and functional indexes were determined at the beginning of the study and after 6 and 14 mo of intervention. Actual dietary zinc intake was 66% of the recommended dietary allowance. Height gain after 14 mo was on average 0.5 cm higher in the supplemented group ($P = 0.10$). The response, however, was different between sexes. Boys from the supplemented group gained 0.9 cm more than those in the placebo group ($P = 0.045$). No effect was seen in girls. Although no significant differences were observed in the rest of the variables studied, trends ($0.05 < P < 0.10$) in the supplemented group compared with the placebo group for increased midarm muscle area in boys, improved response to tuberculin, and reduced rates of parasite reinfestation were noted. We conclude that in preschool children of low socioeconomic status, zinc is a limiting factor in the expression of growth potential. *Am J Clin Nutr* 1997;66:1406–13.

KEY WORDS Zinc, growth, preschool children, zinc supplementation, low socioeconomic status, Chile

INTRODUCTION

Several attempts have been made to identify reliable laboratory indexes of zinc status (1). Nevertheless, despite promising results obtained with the determination of erythrocyte metallothionein concentrations (2), thymulin activity (3), or the activity of selected enzymes such as erythrocyte membrane alkaline phosphatase (4), and lymphocyte 5′-nucleotidase (5), no definitive test is available. The observation of the response of static and functional (laboratory and clinical) tests to a clinically controlled, double-blind zinc supplementation trial has provided useful information regarding the zinc status of selected groups (6).

Studies conducted in infants and children recovering from protein-energy malnutrition have consistently shown beneficial effects of additional zinc (7–9). Also, zinc supplementation trials have provided evidence of the occurrence of zinc deficiency in free-living populations. On the basis of this methodology, mild degrees of zinc deficiency have been confirmed in preschool children of short-stature from Denver (10), growth-retarded schoolchildren in Chile (11), and short boys from southern Ontario (12), among others.

In the present study we evaluated the response of 98 preschool children attending daycare centers in the city of Santiago to a 14-mo double-blind zinc supplementation intervention. The study was conducted in periurban populations considered to be of middle-to-low- or low socioeconomic status. The main difference, in relation to previous zinc supplementation trials in preschool children, is the absence of any inclusion criteria related specifically to suspected zinc deficiency, such as short stature, low zinc intakes, or slow growth velocity. The only possible predisposing factor was their low socioeconomic status.

SUBJECTS AND METHODS

Subjects

Preschool children aged 27–50 mo of both sexes were recruited at two daycare centers run by the governmental agency Junta Nacional de Jardines Infantiles in periurban Santiago. This agency sponsors daycare centers intended for middle-to-low- to low-income families. After a detailed explanation of the nature and purposes of the study and the procedures to be implemented, parents voluntarily agreed to the participation of their children. A written consent form was signed by the parents or guardians. This protocol was approved by the Ethics Committee of the Department of Nutrition of the Faculty of Medicine, University of Chile.

All children were examined by a pediatrician, and any subject having a clinical condition predisposing to growth failure was dismissed. Ninety-eight children were included (46 boys

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[2] Supported by FONDECYT, research project no. 91–1017. Laboratorios Rhodia-Merieux, Santiago, Chile, donated one-third of the multitest kits used to evaluate the cell-mediated immune response.

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and 52 girls). The general characteristics of the individuals entering the trial are shown in **Table 1**. Anthropometric values are well within the range observed in large-scale studies conducted in this socioeconomic stratum of the Chilean population (13).

Experimental groups

Subjects were pair matched exclusively according to sex and age (± 3 mo), and then randomly assigned to one of two experimental treatments. The study was conducted in a doubly-blinded fashion, and the code was broken only after the project had been finished. The random allocation of each member of the pair to the experimental groups (identified as group A or B, to avoid bias) was done by a member of our staff not involved with the study. The randomization procedure was followed strictly. It yielded comparable groups for most of the variables of interest. The only exception was initial height-for-age z score. This situation was primarily the consequence of the chance allocation of the five subjects with the lowest height-for-age (< 2 z score) to the placebo group. Results including or excluding these subjects were similar.

Supplement

The zinc supplement solution given to the supplemented group consisted of 0.2% $ZnSO_4$, prepared as a glucose-based syrup. A 5-mL dose, supplying 10 mg elemental Zn, was administered daily. The placebo was a glucose-based syrup without added zinc. The zinc and placebo solutions were indistinguishable in appearance and taste. Solutions were bottled in individual containers labeled with the name of the child. The code was only known to the pharmacist in charge.

Compliance was assured by the administration of the supplements at the daycare center. Daily, teacher administered the corresponding solution and simultaneously kept attendance and morbidity records on specially designed forms. Because children attended the centers only 5 d/wk, an additional supplement solution was given to the parents to cover those days on which the children did not attend. Periodical reinforcement was given to the parents to maintain the administration of the supplements. These take-home bottles were replaced at the end of every month and if consumption appeared to not agree with what was expected, we personally spoke with the parents to stimulate adherence to the protocol.

TABLE 1
General characteristics of the subjects at the beginning of the study[1]

	Zinc-supplemented group (n = 49)	Placebo group (n = 49)
Age (mo)	39.8 ± 6.2	39.8 ± 6.2
Weight (kg)	15.8 ± 2.5	15.3 ± 1.9
Height (cm)	96.5 ± 5.3	94.7 ± 4.8
Triceps skinfold thickness (mm)	12.3 ± 2.7	12.0 ± 2.2
Midarm circumference (cm)	16.2 ± 1.4	16.0 ± 1.1
Bone age (mo)	28.9 ± 8.6	28.2 ± 19.9
Height-for-age (z score)	−0.27 ± 0.84	−0.77 ± 0.95[2]
Weight-for-age (z score)	0.26 ± 1.04	−0.01 ± 0.92
Weight-for-height (z score)	0.64 ± 0.96	0.67 ± 0.87

[1] $\bar{x}$ ± SD.
[2] Significantly different from zinc-supplemented group, P = 0.01.

The total duration of the study was 14 calendar months. After the first 6 mo of supervised supplementation, there was a 3-mo interruption because of summer school holidays. To maintain the supplementation regimens during the summer months, enough solution was given to the parents to provide for unsupervised continuation. After this break, the supervised supplementation protocol at the daycare centers was resumed.

Sampling and measurements

At three time points—the beginning of the study and after 6 and 14 mo of supplementation—a series of anthropometric, dietary, functional, and clinical variables was measured. Biochemical determinations in blood were conducted on only two occasions during the trial (ie, at baseline and 6 mo) for two reasons: to reduce the invasiveness of the study and because it was expected that any changes induced by the treatments should be evident after this period. After 6 mo of intervention, 19 individuals had dropped out of the study, leaving 79; after 14 mo, the total number of children still participating was 56. Despite the high number of dropouts, the number of individuals remaining at the end of the study was greater than the minimal sample size calculated to detect differences in height gain (24 subjects/group). Initial values of selected variables (height, height-for-age, plasma and membrane alkaline phosphatase, and plasma and hair zinc) were comparable between the group of subjects leaving and those remaining in the study.

Anthropometric assessment

Weight was measured to the nearest 0.05 kg with a portable beam balance (Detecto scales Inc, Brooklyn, NY). Height was measured to the nearest 0.1 cm by using a Harpenden stadiometer (Holtain Ltd, Crosswell, United Kingdom). Midarm circumference (MAC) was determined at the midpoint of the upper left arm. Triceps skinfold thickness (TSF) was measured on the left arm by using a precision Lange calipers (Cambridge Scientific Industries Inc, Cambridge, MA). All measurements were conducted by highly trained personnel according to standardized procedures (14). To eliminate interexaminer error, measurements were taken on each child by the same anthropometrist.

The height-for-age, weight-for-age, and weight-for-height indexes, expressed as z scores, were calculated according to the NCHS growth charts by using the ANTHRO software released by the National Centers for Disease Control and Prevention (Atlanta). Midarm muscle area (MAMA) and midarm fat area (MAFA) were derived from MAC and TSF measurements by using Frisancho's formulas (15).

Bone age was evaluated on admission and at 14 mo by means of a carpal X-ray and use of the atlas of Greulich and Pyle (16) for comparison. This evaluation was conducted both times by the same experienced radiologist, who did not know to which group each child belonged.

Dietary assessment

Mothers or guardians of the children were interviewed by an experienced nutritionist to recall food intake. Also, information regarding foods consumed at the preschool was obtained directly from the teacher. At baseline, 6 mo, and 14 mo, two 24-h recall interviews were conducted on each occasion. One of the interviews was carried out on a weekday and the other on a

weekend. Weekdays and weekends were represented proportionately (5:2) in the final data.

Mean daily intakes of energy, protein, iron, and zinc were computed by using FOOD PROCESSOR 2 software (ESHA Research, Salem, OR). The food-composition data used were of local origin (17), completed with information contained in the software database. The adequacy of energy and protein intakes was calculated according to the FAO/WHO/UNU reference values (18). Adequacy of iron and zinc intakes was evaluated in relation to the recommended dietary allowance (19).

Biochemical and functional measurements

Blood

Blood samples from a peripheral venipuncture were taken between 0800 and 1000, after a fast of $\geq$ 10 h. Five to seven milliliters of blood was collected into plastic syringes. Two milliliters were received in a test tube containing EDTA as anticoagulant. This was used for the determination of hemoglobin, hematocrit, and white blood cell differential count with standardized routine procedures. The remaining blood sample was placed in a plastic tube containing heparin as anticoagulant. From this portion, plasma was separated within 2 h of collection and kept frozen at -20 °C until analyzed. Erythrocytes were washed with isotonic phosphate buffer and erythrocyte membranes were isolated as indicated later.

Determinations in plasma included zinc and copper concentrations by atomic absorption spectrophotometry according to the method suggested by Smith et al (20), plasma alkaline phosphatase (EC 3.1.3.1) activity by using *p*-nitrophenyl phosphate as substrate (Sigma diagnostic procedure 104; Sigma Chemical Co, St Louis), and serum ferritin by radioimmunoassay (Gamma Dab ^{125}I Ferritin Radioimmunoassay Kit, Travenol Laboratories, Cambridge, MA).

Erythrocyte membranes were prepared as indicated by Steck et al (21), and alkaline phosphatase activity was determined by the method of Ruz et al (4). Enzyme activity was expressed as nmol product formed · mg membrane protein^{-1} · min^{-1}. Membrane protein was determined according to the method of Markwell et al (22).

Hair

Scalp hair samples were collected from close to the occipital portion of the scalp by using stainless steel scissors. Only the proximal 1–2 cm was retained for analysis. Samples were washed with nonionic detergent and the zinc content was determined by atomic absorption spectrophotometry (23). All materials used during blood and hair collection and processing were carefully controlled to avoid contamination with zinc.

Immune response

Immunologic function was measured in a subsample of 60 children (30 from each group) by using a delayed-hypersensitivity skin test kit (Merieux Multitest; Institute Merieux, Lyon, France). The kit contained standardized doses of seven antigens (*Streptococcus, Candida, Trichophyton, Proteus*, tuberculin, tetanus, and diphtheria) plus glycerin as control. Antigens and control were applied to the right forearm of each child by a physician member of our team (XL). Induration sites were measured after 48 h. Indurations $\geq$ 2 mm were recorded as positive responses. Both total number of positive responses and the sum of induration sites were computed.

Clinical assessment of morbidity

Information regarding morbid episodes was recorded daily by the teacher on a specially design form. A physician (XL) standardized all steps from identification to recording of morbidity information. In addition, continuous surveillance was maintained throughout the study. The physician collected all the morbidity forms from the teachers every week. The categories used were upper respiratory infection, lower respiratory infection, diarrhea, and dermic infection. A "new episode" was defined as the episode beginning after $\geq$ 7 d of the absence of any sign or symptom of disease.

Parasitologic assessment

Parasitologic tests were conducted on stools to identify enteroparasites (eg, *Ascaris lumbricoides, Trichuris trichura, Hymenolepis nana, Entamoeba histolytica,* and *Giardia lamblia*) by using the modified Telemann procedure (24). In addition, the Graham test was applied to identify the presence of *Enterobius vermicularis* (25).

Statistical analysis

Parametric [*t* tests, two-way analysis of variance (ANOVA), and two-factor repeated-measures ANOVA] and nonparametric (Wilcoxon's, Friedman's, Fisher's exact, and McNemar's) tests were performed to assess the significance of observed changes during the study where appropriate. Multiple comparisons were conducted with the Bonferroni procedure. A probability value < 0.05 was considered to be significant (26).

RESULTS

Anthropometric assessment

In **Figure 1**, the anthropometric changes (ie, measurement at 14 mo minus measurement at baseline) are displayed. The height gain of the supplemented group was, on average, 0.5 cm more than for the placebo group ($P = 0.10$). A difference of 0.9 cm in favor of boys receiving zinc was observed ($P = 0.045$). Girls, in contrast, did not show any differential response. These sex-related differences were also observed when growth velocity data were expressed as *z* scores (boys, $P = 0.024$; girls, $P = 0.70$). Change in height-for-age *z* score was negative. No significant effects attributed to zinc treatment were observed. As a consequence of the absence of a treatment effect on weight gain but a significant effect on height gain in males, the responses of the weight-for-age and weight-for height *z* scores were diverse. Thus, apparently paradoxic effects were observed in girls receiving placebo whose improvement in weight-for-age and weight-for height *z* scores contrast with those of their counterparts receiving zinc.

Changes in (14 mo value minus initial) body-composition measures (data not shown) were not different between the supplemented and placebo groups. After the groups were separated by sex, a trend toward increased changes in MAMA was apparent, again, only in males. The average change in MAMA was 96.1 $\pm$ 118.6 mm^2 in the supplemented group compared with 13.9 $\pm$ 112.3 mm^2 in the placebo group ($P = 0.08$).

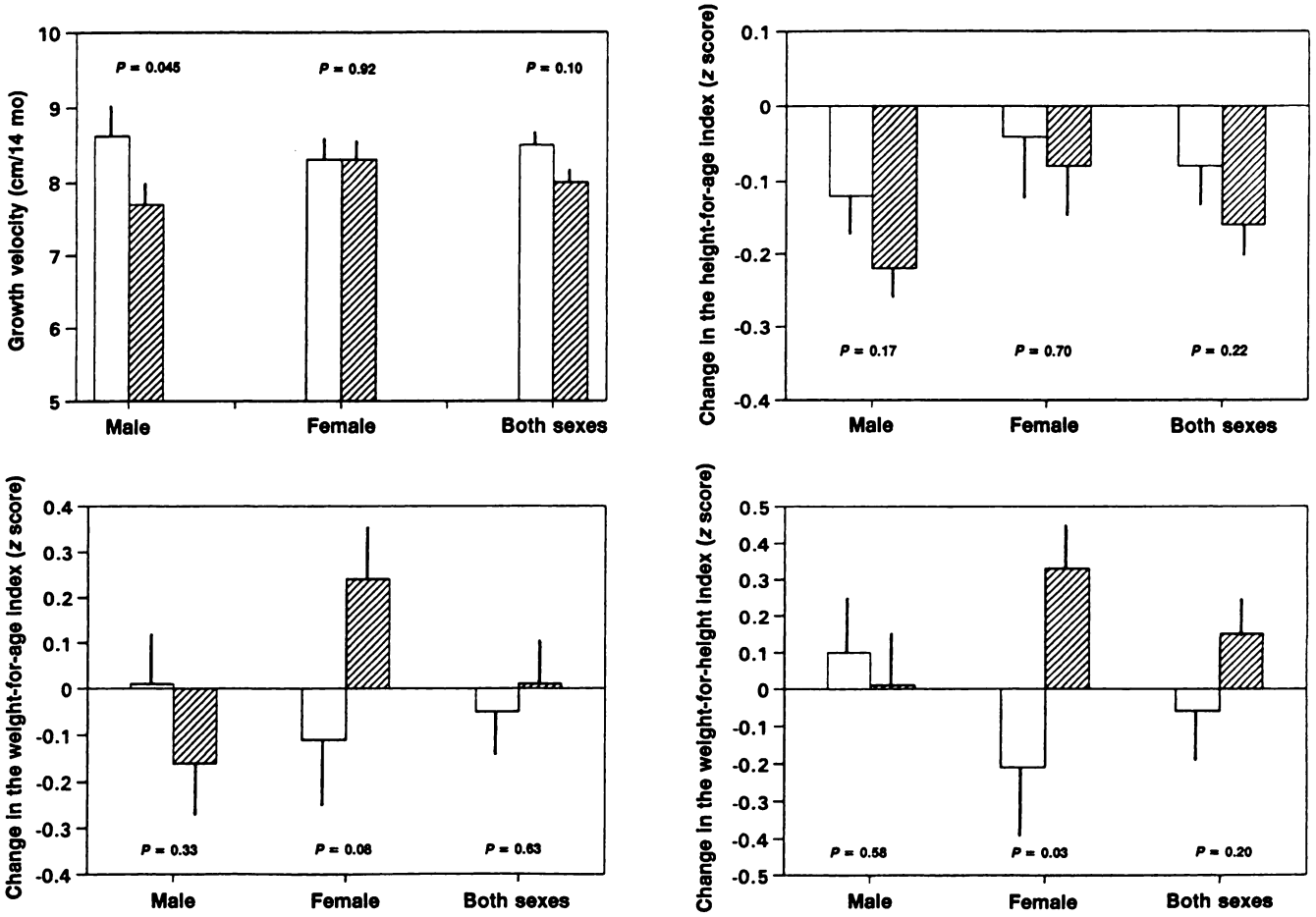

FIGURE 1. Growth velocity and change (final minus initial value) of the anthropometric indexes height-for-age, weight-for-age, and weight-for-height of the zinc-supplemented (□) and placebo (▨) groups, according to sex. $\bar{x} \pm$ SEM.

Bone age showed a mean retardation of 11.8 mo in boys and 8.0 mo in girls at baseline. Change of bone age after the 14-mo intervention was similar in both groups.

Dietary assessment

Daily dietary intakes of energy, protein, iron, and zinc are given in **Table 2**. At the beginning of the study, both groups had similar intakes. As a whole, the adequacy of energy was 99%, of protein was 236%, of iron was 112%, and of zinc was 66%. The main contributors to total zinc intake were cereals and milk products. Meat, poultry, fish, and meat products, highly bioavailable zinc sources, supplied a median of 18% of the total zinc. Fifteen percent of the children interviewed at baseline consumed no flesh foods. Two-factor repeated-measures ANOVA identified a significant effect of time for energy ($P = 0.03$) and zinc ($P = 0.02$) intakes. No significant interactions of time with treatment group were observed.

Biochemical, functional, and clinical assessments

Initial and 6-mo values of selected variables used as indexes of zinc, iron, and copper status measured in blood and hair are given in **Table 3**. All indexes were comparable at baseline. All but one group × time interaction was not significant. Contrary to what was expected, plasma alkaline phosphatase activity increased more in the placebo than the supplemented group. Indexes of iron status (hematocrit, hemoglobin, and serum ferritin) and copper status (plasma copper) were not affected by the experimental manipulations.

Two-way ANOVAs were conducted to determine which variables were most closely associated with the observed height velocity changes (**Table 4**). Baseline results of the main zinc-related variables were arbitrarily dichotomized. The cutoff points were selected to try to combine a representative number of subjects from the lower part of the distribution and a value with some degree of biological meaning. This kind of analysis indicated the interaction between low initial hair and erythrocyte membrane alkaline phosphatase activity and zinc treatment.

None of the comparisons between the supplemented and placebo groups showed any significant difference at the 0.05 limit in terms of indexes of immune response, parasitic infestation, and morbidity (data not shown). Nevertheless, some trends could be identified. For instance, response to tuberculin using the McNemar test showed that in the supplemented group, the number of responders tended to increase from 47% to 80% ($P = 0.07$). The placebo group did not change. For parasitic infestation, the supplemented group tended to have a lower infestation rate than the placebo group (Fisher's exact test, $P = 0.08$).

TABLE 2
Daily energy and nutrient intakes at the beginning of the study and at 6 and 14 mo after intervention

	Zinc-supplemented group	Placebo group
Energy (kJ)		
Initial	6521 ± 1133 [49]	6521 ± 1116 [49]
6 mo	6893 ± 1233 [28]	6609 ± 1049 [24]
14 mo	7047 ± 1049 [24]	6671 ± 1099 [22]
Protein (g)		
Initial	50.3 ± 12.4 [49]	48.8 ± 9.4 [49]
6 mo	51.8 ± 13.3 [28]	49.6 ± 8.1 [24]
14 mo	53.1 ± 10.5 [24]	48.7 ± 8.4 [22]
Iron (mg)		
Initial	10.3 ± 2.9 [49]	10.2 ± 2.4 [49]
6 mo	11.4 ± 3.4 [28]	9.9 ± 3.2 [24]
14 mo	10.4 ± 2.5 [24]	9.8 ± 2.5 [22]
Zinc (mg)		
Initial	6.9 ± 2.1 [49]	6.3 ± 1.3 [49]
6 mo	7.1 ± 2.2 [28]	6.4 ± 1.3 [24]
14 mo	7.5 ± 1.9 [24]	6.9 ± 1.4 [22]

[1] $\bar{x}$ ± SD; *n* in brackets. There were no significant group × time interactions (two-factor, repeated-measures ANOVA).

TABLE 3
Initial and final (6 mo) biochemical indexes of zinc, iron, and copper status for zinc-supplemented and placebo groups[1]

	Zinc-supplemented group	Placebo group
Plasma zinc (μmol/L)		
Initial	17.7 ± 2.4 [44]	17.2 ± 2.2 [41]
6 mo	17.6 ± 2.2 [36]	17.7 ± 1.9 [33]
Hair zinc (nmol/kg)		
Initial	1.74 ± 0.50 [41]	1.75 ± 0.62 [34]
6 mo	2.23 ± 0.61 [23]	2.14 ± 0.72 [17]
Plasma alkaline phosphatase (U/L)		
Initial	70.6 ± 16.8 [45]	70.2 ± 17.4 [46]
6 mo	81.3 ± 21.7 [39]	89.1 ± 21.8 [34][2]
RBCM alkaline phosphatase (U)		
Initial	0.48 ± 0.27 [46]	0.46 ± 0.18 [45]
6 mo	0.51 ± 0.28 [38]	0.45 ± 0.21 [33]
Ferritin (μg/L)		
Initial	23.7 (3–95) [45][3]	24.3 (3–69) [43]
6 mo	22.5 (2–46) [35]	19.9 (5–60) [33]
Hemoglobin (g/L)		
Initial	123 ± 74 [47]	122 ± 65 [46]
6 mo	125 ± 89 [40]	124 ± 85 [34]
Plasma copper (μmol/L)		
Initial	17.8 ± 2.6 [45]	17.7 ± 2.6 [42]
6 mo	17.3 ± 2.1 [37]	16.7 ± 1.9 [32]

[1] $\bar{x}$ ± SD; *n* in brackets. RBCM, red blood cell membrane.
[2] Significant group × time interaction, $P = 0.02$ (two-factor, repeated-measures ANOVA).
[3] Median (range); *n* in brackets.

DISCUSSION

An accepted method for evaluating zinc status is the response of selected indexes during a long-term zinc supplementation trial. In subjects undergoing growth and development the evaluation strategy has relied mostly on the observed changes in linear growth, because this is markedly impaired in zinc deficiency (6).

In the vast majority of the zinc-supplementation studies conducted, the selection criteria included one or more characteristics potentially associated with the existence of zinc deficiency. The studies by Golden and Golden in Jamaica (7) and by Castillo et al in Chile (8) were carried out in severely malnourished children. The study by Walravens et al in Denver (10), included in the trial only those children consuming less than two-thirds of the recommended dietary zinc and with height-for-age less than the 10th percentile. Gibson et al (12) in Canada, only included children with low height-for-age percentiles. Our protocol did not consider any additional inclusion criteria other than socioeconomic status. This was done with the intention of obtaining results potentially applicable to a significant segment of the underprivileged Chilean population of children.

Results of zinc-supplementation trials have provided evidence of suboptimal zinc nutriture in both developed (10, 12, 27) and developing (28, 29) countries. Recent studies on this topic have been published for Chilean children and adolescents with short stature (11), in 12–48-mo-old Ecuadorian children (30), in Mexican preschoolers (31), in 7–9-mo-old Guatemalan infants (32), and in 4–36-mo-old Vietnamese children (33).

Although several zinc-supplementation trials have reported a positive growth response, this has not been universal. Bates et al in Gambia (34), Cavan et al in Guatemala (35), and Udomkesmalee et al in Thailand (36) did not observe improved growth with supplemental zinc. Differences in design, the contents of the supplements (zinc alone or zinc plus other nutrients), the duration of the intervention, and the age group targeted, are among the explanations for the inconsistencies found. Brown and Allen (unpublished observations, 1995) performed a meta-analysis of zinc supplementation and growth studies in infants and children. The main conclusion was that zinc did induce a positive linear growth response provided that participants had low (< -2) initial height-for-age z scores. These authors did not examine whether such a response could be obtained with less severe degrees of height retardation.

In our study, as in most similar trials (10, 11, 27, 30), the response to supplemental zinc was clearly sex dependent. Boys gained significantly more height if they were in the supplemented group. Girls did not show any differential response to treatment group status. In terms of observed height gain compared with reference data (37), boys in the supplemented group gained 100.6% of the median and those in the placebo group gained only 89.7%. In contrast, girls from both groups gained 96.3% of the median. Bone age determinations showed that increased growth was not accompanied by acceleration of bone maturity.

A reasonable explanation of the sexual dimorphism in increased growth velocity is not yet available. Some authors have postulated differences in zinc requirements (12). There are some animal data supporting this hypothesis (38). Others have suggested that the distinct effect of zinc is mediated by changes induced in growth hormone and testosterone concentrations (39, 40). Clearly, more research is needed on this issue.

When discussing the effects of zinc supplementation on height gain, we cannot completely disregard the potentially confounding effects of certain factors, such as the lower initial

TABLE 4
Growth velocity according to selected initial anthropometric and biochemical indexes[1]

	Zinc-supplemented group	Placebo group
	cm/14 mo	
Initial height-for-age (z score)		
< -1.0	8.3 ± 1.1 [6]	7.7 ± 0.9 [16]
≥ -1.0	8.5 ± 1.2 [21]	8.4 ± 0.8 [10]
Initial plasma zinc (μmol/L)		
< 16.8	8.7 ± 1.4 [10]	8.0 ± 1.0 [10]
≥ 16.8	8.3 ± 1.0 [17]	7.9 ± 0.8 [16]
Initial hair zinc (nmol/kg)		
< 1.53	8.9 ± 1.3 [13][a]	8.2 ± 1.0 [13][ab]
≥ 1.53	8.1 ± 0.9 [14][ab]	7.7 ± 0.8 [13][2b]
Initial RBCM alkaline phosphatase (U)		
< 0.35	9.6 ± 0.8 [9][a]	7.8 ± 0.9 [10][b]
≥ 0.35	7.9 ± 0.9 [18][b]	8.1 ± 0.9 [16][3b]
Initial plasma alkaline phosphatase (U/L)		
< 65	8.5 ± 1.4 [14]	7.8 ± 0.9 [8]
≥ 65	8.4 ± 0.9 [13]	8.0 ± 0.9 [18]

[1] $\bar{x}$ ± SD; *n* in brackets. Means with different superscript letters are significantly different, $P < 0.05$. RBCM, red blood cell membrane.
[2,3] Significant interaction of factors (two-way ANOVA): [2] $P < 0.05$, [3] $P < 0.01$.

height-for-age *z* score of the placebo group, and the high dropout rates seen during the trial. Nevertheless, as explained in the Methods section, on one hand the randomization procedure was carefully conducted, and on the other, initial values of selected zinc-related variables were comparable between the group of individuals leaving and those remaining in the study. We are therefore confident of the robustness of the conclusions reached.

Anthropometric assessment should also include body-composition measurements. The importance of this component is illustrated by the study of Cavan et al (35) in Guatemala, who did not observe effects of zinc on growth but did observe a significant increase in TSF *z* score and a smaller deficit in median MAC values in their zinc-treated group. In our study, MAMA tended to increase ($P = 0.08$) in the group of boys receiving zinc.

The measurement of a battery of indexes provides for increased chances of accurately diagnosing zinc status. In our study, we used biochemical, functional, and clinical indexes. None of the biochemical measures showed a significant positive effect of supplemental zinc. Nevertheless, when their results were related to the observed linear growth response, a significant association with arbitrarily low initial hair zinc and low red blood cell membrane alkaline phosphatase activity was seen.

In addition to growth, immune response and morbidity are other functional responses used to evaluate effects of zinc supplementation. In children recovering from severe malnutrition, several studies have confirmed the stimulation of the immune capacity by zinc (7–9). Observations in children under free-living conditions have not shown consistent results (34, 35, 41). Immune response variables are dramatically reduced in moderate and severe but not in mild zinc deficiency. The absence of significant differences in terms of delayed-hypersensitivity skin reaction and leukocyte and lymphocyte counts in our study are therefore not surprising.

Zinc nutrition and parasitism has been a topic covered in few studies. Fenwick et al (42) in an animal model showed the beneficial effect of zinc in the response to infestation with *Trichinella spiralis*. Friis et al (43) reported a significant decrease in the reinfestation rate with *Schistosoma mansoni* in Zimbabwean children supplemented with zinc. On the contrary, Grazioso et al (44) did not observe any effect of supplemental zinc on the reinfestation rate of helminths and protozoa in school-age Guatemalan children. In our investigation, a major impediment to reaching firm conclusions was the marked reduction in parasite tests performed in children at the end of the experimental period, despite continuous reinforcement of parents and guardians. With this important consideration, a trend observed indicated a potential beneficial effect of supplemental zinc on the reinfestation rate of *G. lamblia*.

No significant differences nor trends toward positive effects of zinc treatment were seen for morbidity. Studies conducted lately in Mexico (31), Vietnam (33), and Guatemala (45), have shown a consistent effect of zinc supplementation on reducing diarrheal episodes. In our study, the prevalence of diarrhea was low, possibly too low to detect differences even if they had existed. The effects of additional zinc on other types of morbidity, such as respiratory infections, is not consistent, and whereas the Mexican and Vietnamese studies found a positive effect of zinc, the Guatemalan study did not.

In developing countries, several predisposing conditions for zinc deficiency frequently coexist, such as low intake of flesh foods, high intake of zinc-inhibiting factors, frequent episodes of diarrhea, and high parasite infestation rates (46). The extent to which these predisposing factors determine zinc nutritional status vary widely among Third World countries. In Chile, national-level health statistics indicate low rates of morbidity and mortality compared with most other Latin American countries. Probable causes of the marginal suboptimal zinc status detected in this study likely relate to food zinc. In terms of the amount of zinc consumed, adequacy (66% of the recommended dietary allowance) was similar to the figures observed in previous studies conducted in the country (11, 47). This figure is commonly seen not only in zinc-deficient but also in zinc-sufficient populations. Bioavailability of zinc seems to be the key factor in determining the biological adequacy of the diets. In our study, a high proportion of children reported no consumption of flesh foods at the initial dietary interviews. In addition, the highest proportion of dietary zinc came from cereals and milk products and both also strongly inhibit zinc absorption because of the high amounts of phytate and calcium provided by these foods. Highly available zinc supplied by meat, poultry, and fish represented only 18% of the total zinc intake.

In summary, a carefully controlled, randomized, double-blind zinc-supplementation study was conducted in apparently healthy preschool children from medium-to-low- and low socioeconomic conditions in periurban Santiago. It suggested subnormal zinc status in this population. The magnitude of the observed changes in selected indexes of zinc status indicate that the severity of the zinc deficiency was mild. The effects observed may be relevant to larger groups because the children studied here did not represent a segment particularly selected as

having increased risk of zinc deficiency, as in most of previous studies. On the contrary, the children studied here were regular preschoolers attending daycare centers designed to serve the low-income segments of the population.

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