IMPACT OF ZINC SUPPLEMENTATION ON MENTAL AND PSYCHOMOTOR SCORES OF CHILDREN AGED 12 TO 18 MONTHS: A RANDOMIZED, DOUBLE-BLIND TRIAL

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Objective To evaluate the effect of zinc supplementation on mental and psychomotor scores in children aged 12 to 18 months.

Study design In this double-blind, randomized, placebo-controlled trial, children aged 6 to 30 months received daily elemental zinc (10 mg for infants and 20 mg for others) or placebo for 4 months. Bayley Scales of Infant Development II were used for development assessment in the 12- to 18-month subgroup at enrollment and the end of the study.

Results At the end of the study, the adjusted mean mental ($P = .36$) and psychomotor ($P = .28$) index scores were similar in the intervention and control groups. In a multivariate model, the baseline mental development index score was positively associated with the mother's schooling, the child's height for age, packed cell volumes, hospital birth, and attendance at a day care center, and was negatively associated with the child's age. Breastfeeding, the child's weight for height, and packed cell volumes were positively associated with the baseline psychomotor index score.

Conclusion Zinc supplementation did not affect the mental or psychomotor development index scores in a setting in which zinc deficiency is common. *(J Pediatr 2005;146:506-11)*

Zinc deficiency is common in developing countries because of diets rich in phytate and fiber, a low intake of animal foods, and intestinal zinc losses during diarrheal illnesses.[1,2] Zinc supplementation reduces the incidence of pneumonia and diarrhea, and improves length gain in preschool years.[2-4] The relationship between zinc deficiency and cognition is less clear. Zinc deficiency during rapid brain growth or during the juvenile and adolescent period is reported to affect cognitive development.[5,6] The role of zinc in cognition in infants is biologically plausible. The high concentrations of zinc in the synaptic vesicles of the special "zinc containing" neurons in the forebrain, with its function in biochemical processes like myelination and the release of neurotransmitters like gamma-amino butyric acid and glutamate, suggest that it may be a modulator of neuronal excitability.[7] In a setting in which malnutrition and zinc deficiency are common, we conducted a randomized, placebo-controlled trial in children aged 6 months to 30 months in a low-income community to determine the impact of daily zinc supplementation on diarrhea and pneumonia and, in the subgroup of trial subjects aged 12 to 18 months at enrollment, on mental and psychomotor development. We chose the 12- to 18-month age group because zinc deficiency is very common at this age and the tools for development assessment are more robust than in earlier months of life. The findings related to diarrhea, pneumonia, and plasma zinc levels have been previously reported.[8,9] We report here the findings related to mental and psychomotor development.

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METHODS

Study Setting

The setting and details of the methods have been previously published.[8,9] The trial was conducted in the urban community of Dakshinpuri in New Delhi, which has 15,000 dwellings and 75,000 inhabitants. Childhood malnutrition, zinc deficiency, diarrhea, and lower respiratory tract infections are common in this setting.[10-12]

Randomization and Blinding

Children were included when parental informed written consent was available. Eligible children for the main study were individually randomized by using a simple randomization scheme in blocks of 8. The randomization scheme was generated by a statistician at the Statens Serum Institute, who was not otherwise involved with this study, using SAS software (version 8.1; SAS Institute, Cary, NC). Zinc or placebo syrups, similar in appearance and taste, were prepared and packaged in unbreakable bottles by GK Pharma Aps, Koge, Denmark; they also labeled bottles with unique child identification numbers according to the randomization scheme. Six bottles, 1 for each of the 4 study months and 2 extra, per child were produced and labeled before enrollment commenced.

Enrollment and Intervention Delivery

Children aged 6 to 30 months were identified through a door-to-door community survey. We excluded children for non-consent, when they were planning to move within the next 4 months, when they required hospitalization on the enrollment day, or when they had received a massive dose of vitamin A within 2 months. Information was obtained on the socioeconomic characteristics of the family, the child's feeding practices, recent morbidity experience, birth weight, access to television, and consumption of alcohol by the father. Diarrhea and lower respiratory tract morbidity were monitored after randomization in all enrolled children, as described previously.[8,9] Development assessments at enrollment and study end were limited to children aged 12 to 18 months at enrollment.

Zinc was supplemented as zinc gluconate, with daily doses of 10 mg (6 mL) of elemental zinc for infants and 20 mg (12 mL) for older children for 4 months by a study attendant. One bottle containing 250 mL was kept in the child's home and replaced monthly. Immunizations and treatment for acute illnesses were provided at the study clinic as per the World Health Organization guidelines.[13] The study was approved by the All India Institute of Medical Sciences ethics committee. Informed verbal consent from community leaders and written consent from parents was obtained, and a copy of the form was left with the family.

Sample Size Calculations

Sample sizes were estimated with data from development assessment practice sessions conducted in the same area. Using value of α as 0.05 (95% confidence) and that of β as 0.1 (90% power) to detect a 5% improvement in the mean mental development score and in the mean psychomotor development score, 105 and 204 children, respectively, were required to be enrolled per group. To allow for about 20% attrition, we assessed mental and psychomotor development in 250 children per cell.

Development Assessments

Mental and psychomotor development scores were assessed at enrollment and 4 months after supplementation with the Bayley Scales of Infant Development II, strictly according to the instruction manual.[14] Children were tested in the presence of their mothers at the clinic. When the child was uncooperative, crying, or sleepy during the assessment, the test was abandoned and the child was re-assessed the next day. Three attempts on different days were made before the child was labeled as uncooperative. Children who were sick at the time of assessment were treated and tested after recovery.

Assessments were done in a well-lit, ventilated room, free of distractions. Rapport was established with the child, and the assessment was initiated only when the child was comfortable. The chronological age was first estimated by subtracting the date of birth from the date of testing. For premature births, the months and days by which the child was premature were subtracted from the chronological age to compute the corrected age. Children were given credits for administered items only when they were able to complete them.[14]

The Bayley Scales II were administered by the author and a clinical psychologist, who were both trained. After several practice sessions, standardization exercises were conducted in 100 children. Ten children were assessed on a day, independently by the 2 assessors with a 1-hour interval between the 2 assessments; these exercises were completed within 10 days.

Interobserver agreement during the study was ascertained by testing in duplicate, 10% of the baseline and end study assessments and correlating the mental developmental index and psychomotor developmental index score obtained by the first and the second assessor with the Kappa statistic (mental development index Kappa = 0.88; psychomotor development index Kappa = 0.86).[15]

Plasma Zinc

Blood samples were collected in all children at baseline and for 30% of randomly selected children at study end for the analysis of plasma zinc and copper levels and packed cell volume. The methods and results for these samples have previously been published.[8,9]

Data Management and Analysis

Double data entry followed by validation was completed within 48 hours of filling out the form in the field. A child's raw scores on the mental and psychomotor scales were computed by adding the total number of items for which the child received credit on each of the scales. The raw mental and psychomotor scores were converted into age-adjusted index scores with US norms.[14] Mean mental and psychomotor development scores were compared between groups with the Student *t* test.

A regression analysis was performed to adjust the effect of zinc on mental and psychomotor scores for selected baseline characteristics. For this, the effect of each baseline factor was determined on the regression coefficients of zinc/placebo treatment on the end study mental and psychomotor scores in multiple linear regression models with 2 independent variables (ie, treatment assignment and the characteristic). The factors that changed the crude coefficient by $\geq$10 percent were included in the final model to provide estimates of zinc

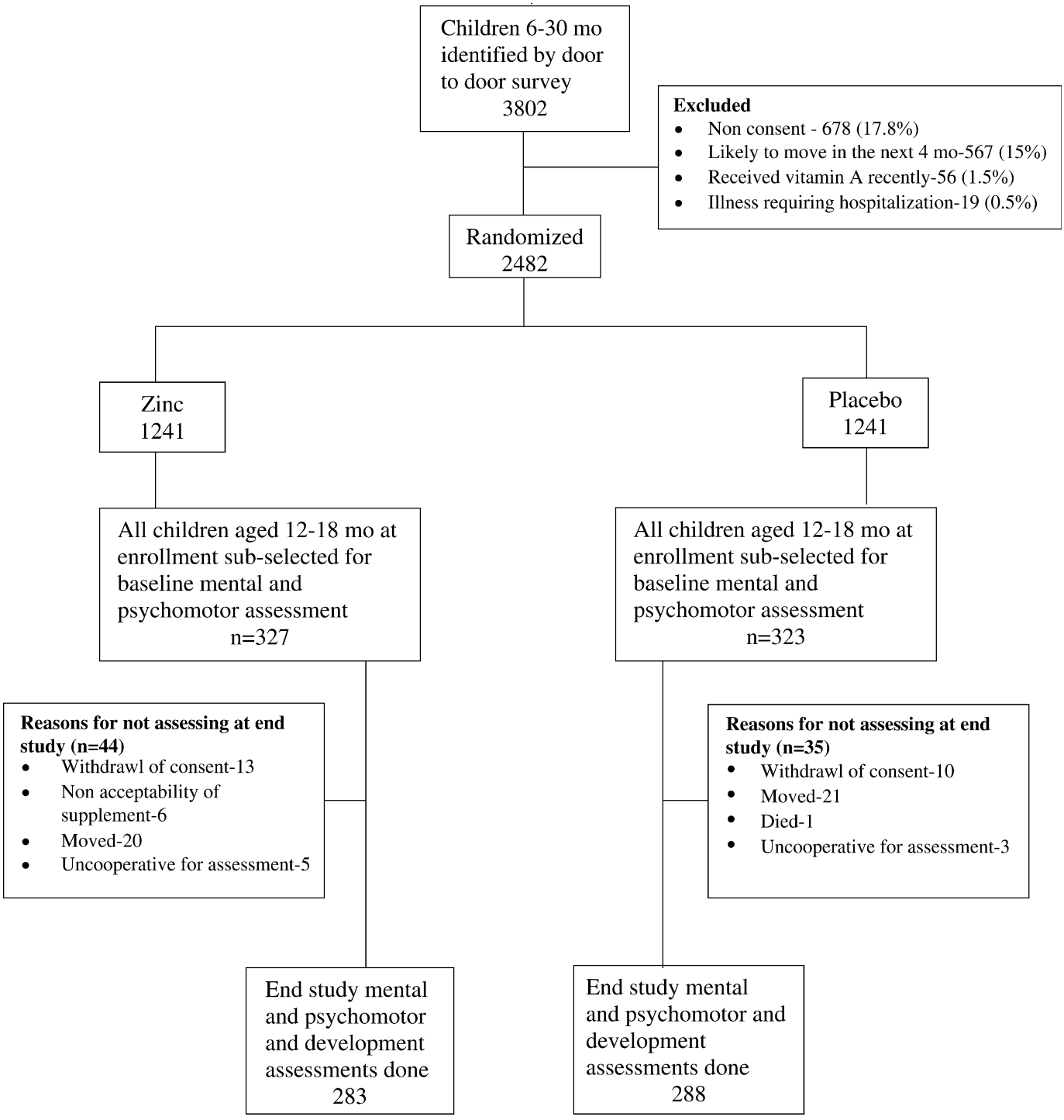

Figure. Trial profile of the main study with outcomes of diarrhea and pneumonia and the substudy on development assessments.

treatment adjusted for potential confounders, and the adjusted and unadjusted results are presented. For mental development scores, the baseline characteristics in the model included baseline mental index score, packed cell volume, age in months, father's alcohol consumption, and years of schooling of parents. In the case of psychomotor development scores, the explanatory variables in the model included baseline psychomotor index score, height for age, packed cell volume, and years of schooling of parents.

Additionally, a secondary analysis was performed to identify covariates associated with baseline mental and psychomotor scores with multiple regression. All factors that were significantly (P <.05) associated with the outcome in the univariate analysis were included in the model in addition to

age as an explanatory variable. Analyses were conducted with Stata software, version 6 (Stata Corp, Union Station, Tex).

RESULTS

A total of 3802 children aged 6 to 30 months were available through the door-to-door survey. Of these, 2482 were randomized (Figure). Those children who were12 to 18 months old at enrollment were subselected for baseline and end-study mental and psychomotor assessment, 327 in the zinc group and 323 in the placebo group. Of these, 283 (86.5%) in the zinc group and 288 (89.2%) in the placebo group were available for end-study assessments (Figure).

Baseline Characteristics

The children in the 2 groups were similar for most variables, but there were a significantly higher proportion of children in the zinc group whose mothers (P = .07) and fathers (P = .004) were literate (Table I). At baseline, there was a significant difference between the mental development index scores of the zinc and placebo groups (P = .0009), but the psychomotor development scores were similar (P = .12).

As reported previously, 45% of the study subjects had low plasma zinc levels at enrollment, and the end-study mean serum zinc level was significantly higher in the zinc group than in the placebo group.[8,9]

Table I. Baseline characteristics of children aged 12 to 18 months whose baseline and end-study mental and psychomotor assessments were done with the Bayley Scales of Infant Development

Characteristic	Zinc n = 283	Placebo n = 288
Age (months) at enrollment	14.8 ± 1.8	15.0 ± 2.0
Males, n (%)	155 (54.8)	152 (52.8)
Breastfed, n (%)	205 (72.8)	216 (75.0)
Number of family members	5.9 ± 2.5	5.9 ± 2.3
Working mothers, n (%)	29 (10.3)	17 (5.9)
Years of schooling of mothers	6.2 ± 4.6	5.2 ± 4.4
Years of schooling of fathers	9.0 ± 4.0	8.2 ± 4.0
Annual family income in Indian rupees (median, interquartile range)	36000 (24000–60000)	36000 (24000–60000)
Weight (kg)	8.3 ± 1.0	8.2 ± 1.1
Length (cm)	73.6 ± 3.4	73.5 ± 3.7
Proportion; n (%) of children with		
Height for age z-score <-2	98 (34.6)	112 (38.9)
Weight for height z-score <-2	51 (18.0)	60 (20.8)
Weight for age z-score <-2	151 (53.3)	172 (59.7)
Packed cell volume (%)	32.8 ± 3.1	32.2 ± 3.4
Birth weight	2.7 ± 0.6	2.6 ± 0.7
Index score Mental	101.2 ± 11.4	98.2 ± 11.0
Index score Psychomotor	99.8 ± 14.3	98.0 ± 14.4
Had television at home, n (%)	251 (88.7)	265 (92.0)
Children attending Anganwadi center, n (%)	9 (3.2)	11 (3.8)
Father consumed alcohol, n (%)	138 (49.1)	146 (50.9)

All values are mean ± SD unless specified.

End Study Mental and Psychomotor Development Index Scores

After 4 months of supplementation, the mean mental development index was similar in the intervention and placebo groups (P = .09; Table II). The mean psychomotor development index (P = .12) was similar in the 2 study groups (Table II). There was no interaction between baseline zinc status and the effect of zinc supplementation on the end-study mean mental development index or psychomotor development index[16] (P = .5 for psychomotor and P <.5 >.2 for mental index scores). Zinc supplementation, therefore, did not have a differential effect among subjects who were zinc deficient at baseline.

Factors Associated with Baseline Mental and Psychomotor Index Scores

The child's age was negatively associated with the mental development scores, and the mother's years of schooling, height for age, packed cell volume, birth at hospital, and attendance at the village Anganwadi (a daycare facility for children aged <6 years, where a daily dietary supplement is also given) were positively associated with the mental development scores. For the psychomotor development index scores, a positive association was found with breastfeeding, weight for height, height for age, and packed cell volume, and a negative association was found with the age of the child. Zinc had no significant effect on the mental (P = .35) or psychomotor development index score (P = .28) after adjustment for several potential confounding factors (Table III).

DISCUSSION

Optimizing intakes of zinc did not lead to improved mental or motor development scores in this study. Some limitations of the study need consideration. Assessments were done only in the 12- to 18-month age subgroup of children. This could potentially have resulted in baseline differences in the intervention and control groups, and although the intervention effects were adjusted for potential confounding factors, there may be others that were not measured. Although the Bayley Scales II were developed and standardized on the basis of a sample representative of a US population, we believe that for the purpose of comparing the impact of zinc versus placebo on cognition, this instrument is valid.

The findings on mental development are consistent with earlier randomized trials in which zinc was supplemented during early infancy.[17,18] In another trial, in which term infants were supplemented with zinc for 5 months, the mental development score was lower in the zinc group than in the

Table II. Adjusted and unadjusted end-study mental and psychomotor scores of children aged 12 to 18 months

Characteristics	Zinc n = 283	Placebo n = 288	Difference in means or difference in proportions (95% CI)*	*P*-value
Mental Development				
Mean (SD) index score	92.8 (10.9)	91.3 (10.8)	1.5 (-0.3 to 3.3)	.358
			-0.7 (-2.1 to 0.8)†	
Psychomotor Development				
Mean (SD) index score	93.9 (11.8)	92.2 (11.4)	1.6 (-0.4 to 3.6)	
			0.8 (-0.7 to 2.3)‡	.284

*All estimates are unadjusted, except those marked with † and ‡, which are values after adjusting for potential confounders.
†Adjusted effects in multiple linear regression models adjusted for baseline mental index score, packed cell volume, age in months, father consuming alcohol, and years of schooling of parents. Model R square = 40%.
‡Adjusted effects in multiple linear regression models adjusted for baseline psychomotor index scores, age in months, height for age, packed cell volume, and years of schooling of parents. Model R square = 39%.

Table III. Factors associated with baseline Mental and Psychomotor Index Scores in multiple regression models

Predictor variable	Coefficient	95% CI	*P*-value
*Mental Development Index Score**			
Age (months)	-2.21	-2.6–-1.80	0
Years of schooling of mother	0.48	0.29–0.67	0
Height-for-age Z Score	1.82	1.01–2.63	0
Baseline packed cell volume (%)	0.38	0.13–0.63	.003
Children born in hospital	2.21	0.50–3.92	.011
Children attending Anganwadi	4.51	0.09–8.94	.046
Psychomotor Development Index Score†			
Age in months	-0.94	-1.51–-0.37	.001
Children breast fed	3.43	0.87–5.98	.009
Weight-for-height Z-score	1.55	0.15–2.94	.030
Height-for-age Z-score	4.09	3.02–5.14	.000
Baseline packed cell volume (%)	0.57	0.23–0.92	.001

*Model R square = 27%.
†Model R square = 16%.

placebo group, although the effect was small.[19] One of the 2 zinc supplementation trials in low birth-weight infants showed significant improvement in motor development with the Griffiths scale, but the other did not find such an effect.[17,18] Two trials in India and Guatemala found significant improvement in the physical activity of infants aged 6 to 9 months who received zinc supplementation; in these trials, activity was assessed with the time sampling observation method.[20,21] In a recent zinc supplementation trial conducted among infants in India who were small for their gestational age, no direct effects of zinc supplementation were seen on the infants' development or behavior.[22]

The conclusion from these studies is that zinc supplementation in infants and young children in developing countries failed to improve mental development. Although a few studies found an impact on physical activity,[20,21] the implications of these benefits are unclear.

Our results confirm previous findings that low maternal education level, stunting, and iron deficiency anemia are associated with impaired mental and psychomotor index scores. Children born in hospitals had better scores, which is probably an indirect indicator of socioeconomic status. Attendance at the day care facility was associated with higher development index scores. This may be related to the stimulation provided by the caregivers or peers or the supplement provided to these children at the facility. The decline in mental development index scores with an infant's age may be because existing care practices and other environmental factors are inadequate, and these may worsen during this period because of the cessation of breastfeeding by most mothers and the increasing demands on the child for interaction at this age. The importance of care practices is also reflected in the positive association between a mother's education level, child's height, and packed cell volume and the psychomotor development scores. Weight for height was directly related to the psychomotor scores, but not to the mental development scores. Malnourished children, particularly those who are wasted, are frequently described as lethargic, possibly because they reduce their activity as a protective strategy to conserve energy.

The lack of benefit of zinc on mental and psychomotor index scores may have several explanations. There may truly be no effect of zinc deficiency on cognition; 4 months may be insufficient time for treatment effects to unfold; or zinc deficiency may affect specific behaviors or processes that are not detected with the Bayley Scales II.[23] It is also possible that the effect of zinc on mental and psychomotor development may have been limited by its interaction with other trace elements, such as iron, copper, folate, and possibly others.[24] Zinc deficiency exists as a part of overall malnutrition and in combination with other micronutrient deficiencies. Concurrent supplementation with other limiting micronutrients may be necessary for the effect of zinc repletion to manifest. Improving zinc intakes in developing countries, such

as India, is, however, a priority because of its effects on diarrhea and respiratory infections.

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