

Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children¹⁻³

Harold H Sandstead, James G Penland, Nancy W Alcock, Hari H Dayal, Xue C Chen, Jui S Li, Faji Zhao, and Jia J Yang

ABSTRACT The knowledge that zinc is essential for growth and neuropsychologic performance and a report of zinc-responsive stunting in Chinese children prompted this project. This article summarizes findings from a 10-wk, double-blind, controlled trial of zinc repletion in 740 urban, 6-9-y-old first graders from low-income families in Chongqing, Qingdao, and Shanghai, People's Republic of China. Treatments were 20 mg Zn alone (Z), 20 mg Zn with micronutrients (ZM), and micronutrients alone (M). The M mixture was based on National Research Council guidelines. Nutrients that might interfere with zinc retention were excluded or given in lower amounts. Main outcomes were changes in neuropsychologic performance and knee height. Hemoglobin, serum ferritin, plasma and hair zinc, and whole blood and hair lead were also measured. Anemia was not common, and serum ferritin concentrations were usually within the range of normal. Mean baseline plasma zinc concentrations were marginal in children from Chongqing and Qingdao and normal in children from Shanghai. After treatment with ZM or M plasma zinc increased. Hair zinc tended to decrease after all treatments. Mean baseline whole blood lead concentrations were slightly below the limit considered excessive for children by the US Centers for Disease Control and Prevention. Neuropsychologic performance and growth were most improved after treatment with ZM. These findings were consistent with the presence of zinc and other micronutrient deficiencies. *Am J Clin Nutr* 1998;68(suppl):470S-5S.

KEY WORDS Zinc, micronutrients, children, growth, knee height, brain, neuropsychologic performance, cognition, neuro-motor function, computerized testing of brain function, China

INTRODUCTION

The essentiality of zinc for growth of higher animals has been known for >60 y (1). Human zinc deficiency was first suspected in 1940 in malnourished Chinese adults (2, 3). Three decades later, zinc deficiency was shown in adolescent farmers from Iran (4-7) and Egypt (8-10). Zinc deficiency was subsequently described in children and adults of many countries (11) and, based on these findings, appears to be an important public health problem (12).

Pertinent to this report, Chen et al (13) found that zinc repletion of 59 children with short stature was followed by an increased growth rate that exceeded that of 235 untreated chil-

dren of normal stature. These findings, and observations in rats (14), monkeys (15-17), and humans (18-20), provided the basis for this study. This report includes data that were presented at the 1996 "Trace Elements in Man and Animals-9" (TEMA-9) meeting (21), the 1996 annual meeting of the American College of Nutrition (22), and a 1996 workshop on the Chemical Safety of Zinc sponsored by the Government of South Australia (23).

The main cause of human zinc deficiency is consumption of diets that are low in highly bioavailable zinc. Flesh foods, especially red meat, are the best dietary sources of zinc (24). Foods rich in phytate (25, 26) and dietary fiber (27) inhibit zinc retention. Zinc deficiency is also caused by illnesses that impair food intake, cause catabolism or malabsorption, or increase zinc excretion (28).

Zinc mediates growth by affecting activity of insulin-like growth factor 1 (IGF 1) (29). Zinc is necessary for the synthesis of nucleic acids (30, 31) and proteins (32). Zinc is essential for brain maturation and function. In rats, zinc deficiency impaired replication of external granular cells of the cerebellum (33) and retarded arborization of neuronal dendrites (34). Pre- and postnatal zinc deficiency caused behavioral deficits that persisted into adulthood (17, 35). Zinc deprivation of adult rats caused abnormal electrophysiologic function of the hippocampus (36). Neurons of the limbic and cerebrocortical regions are particularly rich in zinc (37). Metallothionein-III is one of the zinc-binding proteins in neurons (38). Zinc from metallothionein is bound by ATP. Zn-ATP is a substrate for pyridoxal kinase (39) and flavokinase (40), which mediate synthesis of pyridoxal-5'-phosphate (PLP) and flavin adenine dinucleotide (FAD), the respective coenzymes for synthesis and degradation of biogenic amines

¹From The University of Texas Medical Branch, Galveston; the US Department of Agriculture Agricultural Research Service Human Nutrition Research Center, Grand Forks, ND; and the Chinese Academy of Preventive Medicine Institute of Nutrition and Food Hygiene, Beijing; the Qingdao University Medical School, Qingdao; the Second Military Medical University, Shanghai; and the Third Military Medical University Chongqing, Peoples Republic of China.

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³Address reprint requests to HH Sandstead, Division of Human Nutrition, Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX 77555-1109. E-mail: hsandste@UTMB.edu.

(41, 42). During neurotransmission, vesicles in presynaptic boutons release zinc into the interneuronal space (37). Zinc inhibits γ -aminobutyric acid-stimulated chloride influx into neurons of the hippocampus, cerebral cortex, and cerebellum (43), affects binding of opioid agonists to μ receptors and decreases the stimulatory effects of magnesium and manganese on μ and δ receptors of cerebral cortex (44). Zinc deficiency reduces the number of glutamate-stimulated N-methyl-D aspartate receptors/calcium channels (45, 46).

Zinc deficiency causes subtle impairments in human neuropsychologic performance. Penland (19) described this phenomenon in 11 men who participated in a double-blind depletion-repletion study. The subjects consumed 1, 2, 3, 4, or 10 mg Zn/10.5 MJ (2500 kcal) daily for periods of 35 d each in random order (47). Intakes of 1–4 mg Zn impaired neuropsychologic performance compared with intakes of 10 mg ($P < 0.05$). Findings from a pilot double-blind zinc repletion trial in women (20) were consistent with Penland's results.

SUBJECTS AND METHODS

This project was approved by Human Study Committees of The University of Texas Medical Branch and the US Department of Agriculture Agricultural Research Service (ARS). During the spring and fall of 1994, effects of zinc repletion on neuropsychologic performance and growth were measured in 740 urban 6–9-y-old first graders from low-income families from the 3 Chinese cities of Chongqing, Qingdao, and Shanghai. Treatments of 20 mg Zn (Z), 20 mg Zn with selected micronutrients (ZM), and micronutrients alone (M) were administered double-blind by teachers 6 d/wk for 10 wk. Subjects were divided equally between treatments. Placebo was not included because the parents were informed their child would be given potentially beneficial nutrients. The decision to not include placebo was justified by findings in Egyptian (48) and Iranian schoolchildren (6) that showed zinc alone had little effect on growth unless latent deficiencies were repleted.

A micronutrient supplement was given to replete latent deficiencies that might inhibit responses induced by zinc (6) (Table 1). The content of the micronutrient supplement was based on recommended dietary allowances (RDAs) of the National Research Council (49). Because we intended to only replete latent deficiencies and wanted to avoid possible pharmacologic effects of micronutrients, the supplement provided 50% of either the RDA or the mean estimated safe and adequate dietary intake for nearly all the nutrients listed in the publication. Because of the possibility that they might interfere with the intestinal absorption of zinc, iron, calcium, magnesium, and phosphorus were excluded, and folate was given at 25% of the RDA (50–52).

Outcomes were measured at baseline and after 10 wk. Parents were asked to have the children fast overnight the evening before blood was drawn. Blood was collected into trace element-free evacuated tubes. Samples were placed in a cold box until delivered to the laboratory where plasma and serum were separated and stored at -20°C or below until analyzed. Hemoglobin and plasma zinc concentrations were measured by using flame atomic absorption spectroscopy (AAS) at each of the 3 cooperating medical schools. Whole-blood lead concentrations were measured by using an AAS method developed by NW Alcock at the Clinical Nutrition Laboratory of The University of Texas Medical Branch (UTMB), Galveston. Analysis of comparison sam-

TABLE 1
Micronutrient supplement¹

Ingredient	Amount	Source
Copper	1 mg	Copper gluconate
Selenium	20 μg	Selenium-rich yeast
Iodine	90 μg	Potassium iodide
Fluoride	1 mg	Sodium fluoride
Manganese	1.5 mg	Manganese citrate
Molybdenum	30 μg	Molybdenum amino acid chelate
Chromium	30 μg	GTF chromium (product of <i>Saccharomyces cerevisiae</i>)
Retinol	2500 IU	Retinol acetate
Cholecalciferol	400 IU	Cholecalciferol
Phytonadione	20 μg	Phytonadione
α -Tocopherol	7 mg	DL- α -tocopherol
Thiamine	0.9 mg	Thiamine mononitrate
Pyridoxine	1.1 mg	Pyridoxine hydrochloride
Riboflavin	1.1 mg	Riboflavin
Niacin	12 mg	Niacin
Folic acid	35 μg	Folic acid
Cyancobalamin	1 μg	Cyancobalamin

¹Prepared by the General Nutrition Products Company, Greenville, SC.

ples from the US Centers for Disease Control and Prevention (CDC), Atlanta, have been consistently within the acceptable range since 1988.

Hair samples were collected from the lower occipital scalp and placed in tarred 15 mL tubes. The instructions specified that the samples should be from next to the scalp and $< 1\text{cm}$ in length. The samples were transported to the Clinical Nutrition Laboratory at UTMB for analysis of zinc and lead by using AAS (53). Serum ferritin concentrations were determined by immunoassay (54) by the Clinical Chemistry Laboratory at UTMB.

Neuropsychologic performance was tested with use of tasks from the Cognition-Psychomotor Assessment System-Revised (CPAS-R) developed by JG Penland. Examiners were instructed to provide encouragement and ensure that the subjects understood each task and operation of the computer mouse. Practice sessions minimized learning effects. Testing required ≈ 50 min. After collection, the data were transmitted by telephone modem to JG Penland at the US Department of Agriculture-ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND. The CPAS software automated the scoring of the neuropsychologic tasks. The tasks were of established reliability and validity, were relatively free of cultural biases, and did not require reading. Fine and gross motor skills and eye-hand coordination were tested by finger tapping, adapted from the Halstead-Reitan neuropsychologic battery (55), and visual-motor tracking adapted from Ammons (56). Sustained attention and dyscontrol were measured by an adaptation of the continuous performance task of Rosvold et al (57). Design matching (visual perception) and delayed design matching (short-term visual memory) were measured by an adaptation of the visual form discrimination subtest of Benton's revised visual retention test (58), with properties of the spatial orientation memory test by Wepman and Turaidis (59). An oddity task adapted from the oddity learning task of Pollitt et al (60) measured concept formation and abstract reasoning by the ability to identify physical and abstract similarities.

Growth was assessed by the change in length of the lower leg with use of an instrument described by Cronk et al (61). Subjects were seated on a low chair in a consistent geometry relative to the

TABLE 2
Changes in plasma zinc concentration from baseline after 10 wk of treatment in spring, 1994¹

City	Baseline	Z	ZM	M
		$\mu\text{mol/l}$		
Chongqing ($n = 118$) ²	12.04 ± 0.38	2.04 ± 0.56 ^{a,3}	11.56 ± 0.52 ^b	7.79 ± 0.62 ^c
Qingdao ($n = 115$) ⁴	12.02 ± 0.38	2.35 ± 0.57 ^a	10.95 ± 0.57 ^b	8.40 ± 0.62 ^c
Shanghai ($n = 112$) ⁵	15.66 ± 0.26	-0.44 ± 0.50 ^a	2.31 ± 0.50 ^b	2.24 ± 0.62 ^b

¹ $\bar{x} \pm \text{SE}$. ANOVA was used to ascertain whether there were significant differences in the changes ($T_2 - T_1$) in plasma zinc concentrations among the groups subsequent to treatment, $P = 0.0001$. Z, 20 mg Zn/d 6 times/wk; ZM, 20 mg Zn and the micronutrients listed in Table 1 6 times/wk; M, micronutrients from Table 1 6 times/wk.

^{2,4,5}The number (n) is smaller at follow-up because blood was not obtained from all subjects who had blood drawn at baseline. ² n at follow-up = 107; ⁴ n at follow-up = 109; ⁵ n at follow-up = 111.

³When the ANOVA was significant, two-tailed t test with Bonferroni adjustment for multiple comparisons was used to determine whether differences between each pair of treatments were significant. Values in the same row with different superscript letters are significantly different from each other after Bonferroni adjustment ($P < 0.05$).

instrument. The lower leg was positioned vertically with a V-shaped steel holder just below the anterior tuberosity of the upper tibia, and the heel was pressed into a V-shaped metal holder, with the foot pressed flat on a floor plate. A pressure of 103 kPa (15 psi) was applied to the top of the knee by the horizontal measuring plate, and the measurement was recorded electronically. Measurements were repeated 4 times, with the leg removed from the device between measurements. For comparison with background growth, knee heights of ≈ 20 untreated children per location were measured in the fall during the 10-wk treatment period.

Outcomes were assessed by using analysis of variance (ANOVA) to learn if there was a significant difference among groups subsequent to treatment. When ANOVA was significant, the significance of differences between each pair of treatments was determined by the two-tailed Student's t -test with Bonferroni adjustments for multiple comparisons.

RESULTS

Anemia was infrequent and serum ferritin concentrations were usually within the reference range. Baseline whole blood and hair lead concentrations ($\bar{x} \pm \text{SD}$) were 9.7 ± 3.9 mg/L and 2.4 ± 2.7 $\mu\text{g/g}$, respectively. After treatment with M, the combined data from the 3 locations showed an increase of whole blood lead of $\approx 30\%$. However, most of the increase occurred in the samples from Shanghai. Little change occurred after the other 2 treatments.

Hair lead concentrations more than doubled after all treatments.

Plasma zinc concentrations from the spring are shown in **Table 2**. Mean baseline concentrations were similar in Chongqing and Qingdao, and near the lower limit of the reference range used by the second National Health and Nutrition Examination Survey (62). Substantial increases occurred after treatment with ZM and M, but not Z. Plasma concentrations from Shanghai were within the reference range at baseline.

Hair zinc concentrations from the spring are shown in **Table 3**. There was a tendency for concentrations to decrease after all treatments. In Chongqing and Qingdao there was no effect of treatment, whereas in Shanghai the hair zinc concentrations in treatment M were different from those in treatments Z and ZM.

The changes in neuropsychologic performance that occurred subsequent to treatment are shown in **Figure 1**. Treatment with ZM was associated with more improvement than with M or Z in 3 of the 4 tasks reported here. The change in 3 of the 4 tasks after M and Z treatments was similar. Complete data will be reported elsewhere (63).

The changes in knee height that occurred in the children from Chongqing and Qingdao are shown in **Figure 2**. The greatest increases occurred after treatment with ZM. Greater increases also occurred after M compared with Z, consistent with repletion of latent deficiencies of other micronutrients needed for growth. In the fall the change in knee height over 10 wk was measured in 19 untreated children from Chongqing. Change in knee height of the Z-treated children during the same interval

TABLE 3
Change in hair zinc concentration from baseline after 10 wk of treatment in spring, 1994

City	Baseline	Z	ZM	M	P^2
		$\mu\text{g/g}$			
Chongqing ($n = 122$) ³	127 ± 2.85	-33 ± 5.18	-32 ± 5.28	-22 ± 5.34	0.3
Qingdao ($n = 120$) ⁴	109 ± 3.14	-26 ± 4.23	-37 ± 4.23	-26 ± 4.37	0.2
Shanghai ($n = 120$) ⁵	152 ± 2.26	-50 ± 4.96 ^{a,6}	-51 ± 4.96 ^a	-32 ± 5.02 ^b	0.02

¹ $\bar{x} \pm \text{SE}$. Z, 20 mg Zn/d 6 times/wk; ZM, 20 mg Zn and the micronutrients listed in Table 1 6 times/wk; M, micronutrients from Table 1 6 times/wk.

²ANOVA was used to ascertain whether there were significant differences in the changes ($T_2 - T_1$) in hair zinc concentrations among the groups subsequent to treatment.

³⁻⁵The number (n) is smaller at follow-up because hair was not obtained from all subjects or the sample was too small for analysis. ³ n at follow-up = 107; ⁴ n at follow-up = 119; ⁵ n at follow-up = 118.

⁶When the ANOVA was significant, two-tailed t test with Bonferroni adjustment for multiple comparisons was used to determine whether differences between each pair of treatments were significant. Values in the same row with different superscript letters are significantly different from each other after Bonferroni adjustment ($P < 0.05$).

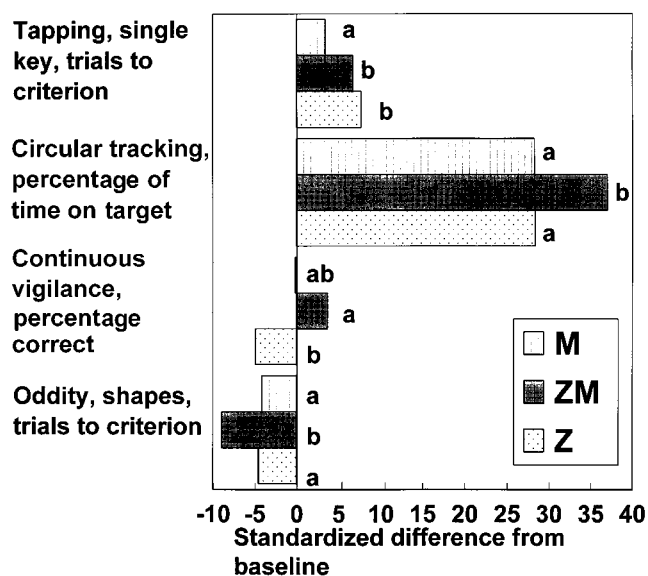


FIGURE 1. Change from baseline in neuropsychologic performance of urban 6–9-y-old Chinese children from Qingdao, Chongqing, and Shanghai after a 10-wk, double-blind, randomized controlled trial of treatment 6 times/wk with micronutrients alone (M), 20 mg Zn with micronutrients (ZM), or 20 mg Zn (Z). Change was determined by the standardized difference score [(week 10 – baseline) / (week 10 + baseline) × 100]. An increase in score indicated a positive response for all but the number of trials needed for correct recognition of odd shapes, for which a decrease was a positive response. Treatment effects and the treatment × sex interaction were evaluated by ANOVA; NS. ANOVA was significant for the following: number of taps of a single key, $P = 0.0009$; percentage of time on the target during circular tracking, $P = 0.0048$; percentage of correct choices during a continuous vigilance task, $P = 0.0092$; and number of trials needed for correct recognition of odd shapes, $P < 0.0067$. The significance of differences in responses among treatments was determined by two-tailed t test with Bonferroni adjustment for multiple comparisons. Means with different letters differed significantly after adjustment ($P < 0.05$).

was similar to that of the untreated children. Technical problems appeared to have interfered with the collection of the knee-height data from Shanghai.

DISCUSSION

Iron deficiency was infrequent; because the diets were very low in foods that are good sources of bioavailable iron, we speculate that iron cooking utensils added iron to diets. Nearly half of the children had baseline whole blood lead concentrations that exceeded CDC guidelines. In Shanghai in particular, the concentration of blood lead increased significantly after treatment with M, but little change occurred after treatment with Z or ZM. In contrast, hair lead increased after all treatments. It is unknown whether these changes were of biological significance. A preliminary evaluation of relations between blood lead concentrations and neuropsychologic performance found no consistent associations.

The finding of marginal baseline plasma zinc concentrations in children from Chongqing and Qingdao but not from Shanghai suggests zinc nutriture might have differed among locations. Plasma zinc concentrations increased after ZM and M, but

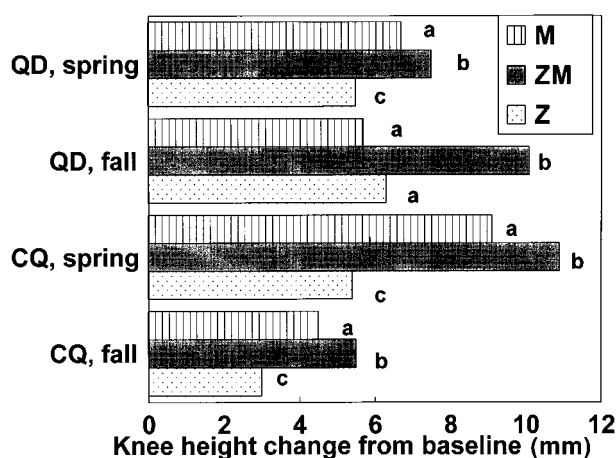


FIGURE 2. Change from baseline in knee height of urban 6–9-y-old Chinese children from Qingdao (QD) and Chongqing (CQ) after a 10-wk, double-blind, randomized, controlled trial of treatment 6 times/wk with micronutrients alone (M), 20 mg Zn with micronutrients (ZM), or 20 mg Zn (Z) alone. Treatments were administered during the spring and fall of 1994. There were 123 and 116 subjects from QD in the spring and fall, and 120 and 124 from CQ, respectively. The subjects were divided equally among treatments. Changes were determined as the difference between the measurement after 10 wk of treatment minus the measurement at baseline ($T_2 - T_1$). Treatment effects and probabilities from the treatment × sex interaction were evaluated by ANOVA; NS. Overall changes were significant ($P < 0.002$) at both locations and in both seasons. When ANOVA was significant, two-tailed t test with Bonferroni adjustment for multiple comparisons was used to determine whether differences between each pair of treatments were significant. Means with different letters differed significantly after adjustment ($P < 0.01$).


showed little change after Z. We suspect the increase was related to increased bone turnover associated with growth.

Baseline hair zinc concentrations were lower than those reported in normal Italian children (64). In 98 boys and 130 girls 6–11 y of age, hair zinc concentrations ($\bar{x} \pm SE$) were 188 ± 9.5 and $112 \pm 9.7 \mu\text{g/g}$, respectively. We found a tendency for hair zinc to decrease after all treatments. In Shanghai only, the decrease after treatment with Z and MZ was greater than the decrease after M. Differences in the response of hair zinc concentrations among the locations might have been caused by differences in the hair sample collection technique. Short samples from next to the scalp reflect recent nutrition. Although the method for collection of the hair was specified, it was not possible to ensure conformity with the instruction. Decreased hair zinc concentrations were reported after zinc repletion in a severely zinc-deficient man (65). It was suggested in that report that the decrease was caused by an increase in hair growth with depletion of zinc available in the hair follicle.

The changes in neuropsychologic performance (Figure 1) were consistent with previous observations (19, 20). They suggest that repletion of other limiting micronutrients is necessary for effects of zinc repletion to be fully expressed. It is notable that others did not find improved cognition in zinc-treated children (66, 67). We suspect that other micronutrient deficiencies might have interfered with the response to zinc, that the tasks selected were insufficiently sensitive, or that the number of subjects studied was insufficient. After completion of this study we repeated the protocol in 720 rural Chinese children. A prelimi-

nary analysis of their neuropsychologic performances found similar improvements in the ZM-repleted group.

Knee height (Figure 2) showed significantly greater increases in children from Chongqing and Qingdao after repletion with ZM and M compared with Z. In the fall, average growth of children from Chongqing after Z alone was similar to that of 19 untreated children. These findings were consistent with those of Carter et al (48) and Ronaghy et al (6) that showed that latent deficiencies of other micronutrients can suppress the growth response after zinc repletion.

In conclusion, repletion with ZM was associated with the largest improvement in neuropsychologic performance in 3 of the 4 tasks reported here. Growth improved subsequent to ZM and M repletion, but showed little change after Z. 

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