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The impact of zinc supplementation on growth and body composition: A randomized, controlled trial among rural Zimbabwean schoolchildren

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Objectives: To assess the effect of zinc supplementation on growth and body composition among schoolchildren.
Design: Randomized, double-blind, placebo-controlled trial.
Setting and Subjects: 313 rural Zimbabwean schoolchildren (144 boys and 169 girls), 11–17 y).
Interventions: Supplementation with zinc (30 or 50 mg) or placebo on schooldays for 12 months. Due to drought, a food programme was in operation during the last eight months of the study.
Variables: Weight, height, upper arm circumference, triceps skinfold thickness, and weight-for-age, height-for-age, weight-for-height, arm muscle-area-for-age and arm fat-area-for-age Z-scores.
Results: Significant effects on weight gain (0.51 *vs* 0.14 kg, $P = 0.01$), weight-for-age Z (-0.08 *vs* -0.14, $P = 0.01$) and arm muscle area-for-age Z-score (0.10 *vs* 0.01, $P = 0.03$) were seen over the first three months, whereas no effects were seen over the full 12 months.
Conclusions: Zinc deficiency impairing lean body mass and weight gain was documented. However, the effect of zinc seen over the first three months vanished during the last nine months when the food programme was in operation. Zinc deficiency may have persisted, but another nutrient may have become growth limiting during the last nine months.
Sponsorship: Danish International Development Assistance.
Descriptors: Randomized, controlled trial; zinc deficiency; zinc supplementation; growth; body composition; phytic acid; schoolchildren; Zimbabwe

Introduction

The syndrome of dwarfism and hypogonadism among Iranian males, described by Prasad *et al* (1961) represents the first thorough account on human endemic zinc deficiency. A study from Egypt later suggested that this syndrome was associated with zinc deficiency (Prasad *et al*, 1963), probably due to very low bioavailability of zinc in the staple diet (Ronaghy *et al*, 1974). However, subsequent controlled zinc supplementation studies among Iranian and Egyptian boys gave conflicting results (Ronaghy *et al*, 1994; Ronaghy *et al*, 1974; Carter *et al*, 1969), thus questioning the public health importance of zinc deficiency. Nevertheless, several controlled zinc supplementation studies have demonstrated mild, growth-limiting zinc deficiency among infants, toddlers and pre-school children from various affluent countries (Walravens *et al*, 1992; Walravens *et al*, 1976; Walravens *et al*, 1989; Hambidge *et al*, 1985; Walravens *et al*, 1983; Gibson *et al*, 1989), whereas no effect of zinc and multimicronutrients, compared to multimicronutrients alone, on growth Z-scores were seen among Guatemalan children (Cavan *et al*, 1993).

Rural African children are likely to have suboptimal zinc status, since usually the incidence of diarrhoea is high, the nutritional status low and the cereal-based diets high in phytic acid and low in animal proteins (Solomons *et al*, 1990; Ferguson *et al*, 1993). However, knowledge about zinc nutriture in Africa is scarce (Ferguson *et al*, 1993). The recent study of 110 young Gambian children (Bates *et al*, 1993) apparently represents the only previous randomized controlled zinc supplementation trial conducted in Africa. However, zinc given twice weekly had no significant effects on the overall change in either weight, length or mid-upper arm circumference.

As part of a 12-month randomized, placebo-controlled, double-blind zinc supplementation trial to study the role of zinc in susceptibility to *S. mansoni* reinfection (Friis, 1996) the effects of zinc on growth and body composition were assessed. During the first three months of the study, the nutritional situation in the area deteriorated and a school-based food supplementation programme was commenced. The children were examined anthropometrically after three- as well as 12-month, and the results presented here thus reflects the role of zinc in two different dietary situations.

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Subjects and methods

Study area and population

A subsistence farming community, with low nutritional status among schoolchildren and a diet high in unrefined cereals and low in animal protein, was identified in Chiredzi District, in the lowveld region of south-eastern Zimbabwe. The climate in the area was hot and dry most of the year with an annual rainfall below 400 mm, and the vegetation dry savanna woodland and scrub. The staple crops were maize and millet, and occasionally cabbage and other green leaves were grown near the river. Cattle and goats were kept, but mainly used as cash products.

Children attending grades three to six at Mareya or Muteo Primary Schools were eligible for inclusion in the study. From Muteo Primary School 198 (84.3%) of 235 children, and from Mareya 115 (92.0%) of 125 children were included in the study.

Study design and interventions

The study was a randomized, placebo-controlled, double-blind, zinc supplementation trial. Children found infected with *S. mansoni* or *S. haematobium* were treated with a single dose of 40 mg praziquantel per kg body weight after completion of baseline examinations. Concomitantly, supplementation with entero-coated (Vernix entero-solubile) zinc sulphate tablets or identical-looking placebo tablets (The Hospital Pharmacy, Copenhagen County, Denmark) on all schooldays was commenced. Children were allocated to either zinc or placebo according to the result of simple randomization. Children allocated to zinc supplementation and weighing below 29.5 kg were given tablets containing 30 mg of elementary zinc, while those at or above 29.5 kg were given tablets containing 50 mg. At the beginning of the study, one container for each child, with tablets in known excess, was taken to the schools. After four months, and again after eight months, the containers were replaced. Tablets were given out every morning on school days by the school teachers. The administration of tablets was supervised by the local Environmental Health Technician once a week, and by a Research Officer every fortnight. Of the 370 d long study period, school leaves, weekends and public holidays comprised 185 d. The maximum number of tablets that could be taken by a child was thus 185, equivalent to a tablet every other day. After completion of the study, the number of tablets taken by the child was calculated by subtracting the number of remaining tablets from the number doled out. Compliance was expressed as the number of tablets taken in percentage of the maximum of 185. The code was not broken before the data entry, cleaning and analysis were completed.

Food situation during the study

The food situation in the study area was alarming at the time the study was commenced in February 1992, due to the severe drought afflicting southern Africa. The situation further deteriorated in the following months, with the failure of the harvest expected in April–May. A school-based food supplementation programme was therefore introduced by non-governmental organisations in the middle of June 1992, after completion of the three-month follow-up examination, and was still in operation at the time of cessation of the zinc/placebo supplementation. The food supplementation programme provided the children with imported maize, dried fish, sugar beans and oil. The food situation was therefore markedly different during the first three months of the study, compared to the last nine months. The dietary intake of the children in the first three months of the study, although unknown, was presumably very low and based on unrefined cereals. For the latter nine months of the study, the average intake in the schools could be estimated. Information about the amount of food distributed was obtained, and samples of individual food items were collected at the 12-month follow-up examination and kept frozen at -20°C for later analysis of phytic acid and zinc content.

Anthropometry

Heights were measured to the nearest 0.1 cm using a portable height-measuring instrument of own design. Weights were measured to the nearest 0.1 kg using a Health o meter scale (Health o meter Inc., Illinois, USA). The children wore only light clothing and no shoes for the measurements. The measurements were related to references as standard deviation scores (Z-scores). Height-for-age (HA), weight-for-age (WA) and weight-for-height (WH) Z-scores were computed using the nutritional anthropometry module of Epi Info version 5 software (Center for Disease Control, Atlanta)(Dean *et al*, 1990), based on the NCHS-WHO reference curves. HA and WA Z-scores could be computed for boys and girls up to the age of 18 y, while the reference data only allowed WH Z-scores to be computed for boys below 11.5 y of age and less than 145 cm high, and for girls below 10 y and less than 137 cm.

Mid upper arm circumference (AC) was measured to the nearest 0.1 cm and triceps skinfold (TSF) thickness to the nearest 0.1 mm on both arms. The measurements were taken by the same investigator (HF) throughout the study to eliminate inter-observer variation. With the children in the standing position and the elbow flexed to 90°, the midpoint between the lateral projection of the acromial process and the inferior border of the olecranon process of the ulna was identified and a mark was drawn. The TSF was then measured using a Harpenden caliper, in the midline of the posterior aspect of the arm at the marked level, with the arm hanging loosely. At the same level, the AC was measured without compressing the tissue, using Babytape (Raven Equipment Ltd.) (Cameron, 1978). The mean of AC and TSF of both arms were used. From the right arm measurements of AC and TSF, the arm muscle area (AMA) and arm fat area (AFA) were calculated (Frisancho, 1990), using the formulae: arm muscle area, $AMA = [AC - (TSF \cdot \pi)]^2/(4 \cdot \pi)$, and arm fat area, $AFA = [AC^2/(4 \cdot \pi)] - AMA$. Bone area was not adjusted for. From the right AMA and AFA, arm muscle-area-for-age (AMAA) and arm fat-area-for-age (AFAA) Z-scores were computed based on means and standard deviations of anthropometric data from the National Health and Nutrition Examination Surveys (Frisancho, 1990).

S. mansoni and S. haematobium

At three different days during the baseline examination, urine and stool samples were collected from each child between 9 am and noon. Filtration of 10 ml of stirred urine from each sample was done using Nytrel filters (Peters *et al*, 1976). The filters were examined for eggs of *S. haematobium*, and egg output expressed as mean eggs per 10 ml of urine (ep 10 ml). A single 50 mg cellophane faecal thick smear (Peters *et al*, 1980) was prepared from each stool sample and examined for eggs of *S. mansoni*. The egg output was expressed as mean eggs per gram faeces (epg).

From each child a thin and a thick blood slide were examined for malaria parasitaemia.

Blood sampling
Blood samples were taken from the cubital vein of all children between 8 am and noon as part of the baseline examination and 12-months follow-up examinations. The children were not asked to fast for the examination, but due to the severe drought only few children had anything to eat before or during school hours. Serum was collected and kept frozen at -80°C for later analyses of zinc, retinol and ferritin. From 64 children randomly selected for examinations of cell-mediated immunity (data not shown), the blood was used for separation and collection of peripheral mononuclear blood cells, wherefore plasma instead of serum was collected and used for the biochemical analyses. From these children plasma was also collected at the three-month follow-up examination.

Biochemistry
Zinc was determined in the serum and plasma collected at the baseline and 12-months follow-up examination, and in plasma collected at the three-months follow-up examination, while retinol and ferritin were measured in the plasma collected at the baseline and three- and 12-month follow-up examinations. The retinol analysis was undertaken in duplicate by high performance liquid chromatography (Waters Associate, Milford, MA, USA). Values below 0.35 μmol/L were considered to indicate vitamin A deficiency, whereas values between 0.35–0.70 μmol/L were considered to indicate marginal vitamin A status (Suharno *et al*, 1993). The ferritin analysis was done in duplicate by an enzyme-linked immunosorbent assay using the Nova-Path TM Ferritin kit (BIO-RAD, Anaheim, CA, USA). The within-run and between-run coefficients of variation were 4 and 12%, respectively. Values below 12 μg/L were considered to reflect depleted iron stores (Cook *et al*, 1992). For zinc the analysis was done after dilution 1:4 with deionized distilled water by atomic absorption spectrophotometry (Perkin Elmer Model 360, Perkin Elmer, Norwalk, CT, USA). The cut-off value used to define normal zinc concentration was 10.7 μmol/L (Chen *et al*, 1988). No difference was seen between serum and plasma concentrations of zinc (Friis, unpublished observations), and both serum and plasma concentrations were used in the data analysis and referred to below as serum concentrations. Samples of dried fish were analysed for zinc, and samples of sugar beans and maize were analysed for zinc and phytic acid, according to the method described by Davies and Reid (Davies *et al.*, 1979)

Permissions
Permission to conduct the study was obtained from the Medical Research Council of Zimbabwe. The study protocol was also approved by the Danish Central Medical Ethics Committee. The headmasters and teachers of the two schools, and the local Environmental Health Technician were informed about the project and agreed to participate. The village headmen and parents were informed about the study and gave their consent.

Statistical analysis
The Wilk-Shapiro/rankit plot procedure in Statistix Version 4.0 (Analytical Software, Tallahassee, USA) was used to assess whether the variables conformed to normality. The two-sample *t*-test and Wilcoxon two-sample test were used, where appropriate, to test for differences in means or medians between supplementation groups, and Chi-square test to test for differences in proportions. Multiple regression analysis, using a backward elimination strategy, was used to assess possible interactions between zinc supplementation and age, as well as zinc supplementation and sex. Anthropometric measurements and indices were used as dependent variables and sex, zinc supplementation, age, as well as the interaction terms between zinc supplementation and age, and between zinc supplementation and sex were used as independent variables. Age was dichotomised using 12 y as cut-off, in order to separate pre-pubertal from pubertal children.

Results

Of the 313 children between 6–17 y of age included in the study, 169 (54.0%) were girls and 144 (46.0%) boys. Two hundred and ninety one (93.0%) were found infected with either *S. haematobium* (70.3%) or *S. mansoni* (82.1%), while none had malaria parasitaemia.

Baseline comparison
Baseline comparisons of sex ratio, anthropometric measurements and indices, micronutrient status and schistosome infection between the children randomly allocated to zinc or placebo are presented in Table 1. Although baseline equivalence was considered acceptable, comparison of nutritional status between the groups should be based on anthropometric indices, where age and sex differences are adjusted for. As seen, the mean HA and WA Z-scores were similar in the two groups. The mean WH Z-score was lower for the zinc group than for the placebo group, but this comparison was only based on the 122 children for whom this index could be computed.

Growth and body composition
At the three-month follow-up examination, 274 (87.5%) of the 313 children in the cohort were reexamined. The changes in basic anthropometric measurements and indices, stratified for sex, are shown in Table 2. The mean weight gain was 0.51 kg in the zinc supplemented group, compared to 0.14 kg in the placebo group ($P=0.01$). The WA Z-scores declined significantly in both supplementation groups over the first three months, as seen from the confidence intervals, but significantly less in the zinc compared to the placebo group (-0.08 *vs* -0.14, $P=0.01$) (Table 2). In contrast, the placebo group had a mean increase in height of 1.8 cm, compared to 1.6 cm in the zinc group. Although the difference was small, it was marginally significant ($P=0.05$). However, when sex and age differences between the groups were controlled for by using HA Z-scores, no difference was seen (0.01 *vs* -0.01, $P=0.22$). The decline in mean WH Z-scores was similar in the two supplementation groups ($P=$ 0.57). The change in AMAA Z-scores was significantly larger among zinc supplemented children compared to children receiving placebo (0.10 *vs* 0.01, $P=0.03$). The change in AFAA Z-score over the first three months was negligible, and similar in both supplementation groups ($P=0.71$).

At the 12-month follow-up examination, 276 (88.2%) of the 313 children were examined. The effect of zinc supplementation seen over the first three months of the study had vanished over the latter nine months. Neither the change in growth nor body composition Z-scores over the full twelve

Table 1 Baseline characteristics of children allocated to supplementation with placebo and zinc

	Placebo group (n = 157)	*Zinc group (n = 156)*
Sex ratio (female/male)	1.13 (83/74)	1.22 (86/70)
Anthropometric measurements[a]		
Age (y)	11.5 (6.9–17.0)	10.8 (6.3–17.7)
Height (cm)	137.0 (115–173)	136.6 (113–172)
Weight (kg)	29.3 (17.3–55.2)	28.3 (17.7–59.8)
Triceps skinfold thickness (mm)	5.4 (3.4–15.1)	4.6 (3.2–15.5)
Arm circumference (cm)	18.3 (14.1–27.3)	18.1 (14.6–24.7)
Anthropometric Z-scores[b]		
Height-for-age	− 1.18 (1.00)	− 1.18 (0.91)
Weight-for-age	− 1.24 (0.73)	− 1.30 (0.68)
Weight-for-height	− 0.57 (0.72)	− 0.72 (0.76)
Muscle area-for-age	− 0.85 (0.58)	− 0.92 (0.59)
Fat area-for-age	− 1.02 (0.22)	− 1.04 (0.23)
Indicators of micronutrient status[c]		
Zinc (μmol/L)	12.0 (2.5)	11.8 (2.4)
Retinol (μmol/L)	1.09 (0.28)	1.05 (0.26)
Ferritin (μg/L)	22.0 (16.9–31.5)	21.0 (16.3–30.0)
Schistosome infection[d]		
S. mansoni		
Prevalence (%)	80.8 (74.5–87.0)	84.5 (78.8–90.3)
Intensity (epg)	56 (27–140)	50 (24–90)
S. haematobium		
Prevalence (%)	72.6 (65.6–79.7)	68.0 (60.5–75.4)
Intensity (ep 10 ml)	10 (4–30)	9 (3–26)

[a] Median (range).
[b] Mean (standard deviation). WH Z-score only computable for 122 children.
[c] Mean (SD) serum zinc ($n = 287$) and retinol (269), and median (IQR) serum ferritin ($n = 249$).
[d] Prevalence (95% confidence interval) and median (interquartile range) intensity of infection among infected, expressed as eggs per gram of faeces (*S. mansoni*) and eggs per 10 ml of urine (*S. haematobium*).

Table 2 Changes in anthropometric measurements and Z-scores between the baseline and three-month follow-up examinations for placebo and zinc supplemented children

	Placebo group (n = 142)	*Zinc group (n = 132)*	*P*
Anthropometric measurements			
Height (cm)[a]	1.8 (1.7 to 2.0)	1.6 (1.4 to 1.8)	0.05
girls	2.0 (1.8 to 2.2)	1.7 (1.5 to 2.0)	0.93
boys	1.6 (1.4 to 1.9)	1.4 (1.1 to 1.7)	0.17
Weight (kg)[a]	0.14 (−0.07 to 0.35)	0.51 (0.30 to 0.72)	0.01
girls	−0.01 (−0.29 to 0.28)	0.49 (0.25 to 0.73)	0.01
boys	0.29 (−0.01 to 0.60)	0.54 (0.17 to 0.91)	0.30
Triceps skinfold thickness (mm)[b]	0.05 (−0.2 to 0.2)	0.05 (−0.1 to 0.2)	0.79
girls	0.05 (−0.4 to 0.3)	−0.10 (−0.4 to 0.1)	0.99
boys	0.00 (−0.1 to 0.3)	0.25 (0.0 to 0.4)	0.54
Arm circumference (cm)[a]	0.24 (0.12 to 0.37)	0.33 (0.23 to 0.45)	0.28
girls	0.30 (0.12 to 0.48)	0.35 (0.19 to 0.51)	0.69
boys	0.19 (0.01 to 0.36)	0.32 (0.16 to 0.48)	0.26
Anthropometric Z-scores[a]			
Height-for-age	0.01 (−0.01 to 0.04)	−0.01 (−0.03 to 0.02)	0.22
girls	0.03 (0.00 to 0.06)	−0.01 (−0.04 to 0.03)	0.14
boys	0.00 (−0.04 to 0.04)	−0.01 (−0.05 to 0.03)	0.74
Weight-for-age	−0.14 (−0.18 to −0.11)	−0.08 (−0.11 to −0.04)	0.01
girls	−0.17 (−0.22 to −0.13)	−0.10 (−0.14 to −0.06)	0.02
boys	−0.11 (−0.17 to −0.05)	−0.05 (−0.11 to 0.00)	0.17
Weight-for-height[a]	−0.23 (−0.39 to −0.06)	−0.17 (−0.28 to −0.06)	0.57
girls	−0.41 (−0.59 to −0.22)	−0.28 (−0.43 to −0.13)	0.27
boys	−0.10 (−0.35 to 0.15)	−0.07 (−0.24 to 0.10)	0.84
Muscle area-for-age	0.01 (−0.05 to 0.07)	0.10 (0.04 to 0.15)	0.03
girls	0.05 (−0.03 to 0.14)	0.14 (0.06 to 0.22)	0.16
boys	−0.03 (−0.12 to 0.05)	0.06 (−0.01 to 0.12)	0.11
Fat area-for-age	0.01 (−0.01 to 0.04)	0.02 (−0.01 to 0.04)	0.71
girls	−0.01 (−0.04 to 0.03)	0.01 (−0.03 to 0.04)	0.67
boys	0.03 (0.00 to 0.06)	0.04 (0.01 to 0.06)	0.83

[a] Mean (95% confidence interval) and corresponding *P*-values.
[b] Median (95% confidence interval) and corresponding *P*-values.
[c] Only computable for 41 in the placebo and 56 in the zinc group.

Table 3 Changes in anthropometric measurements and Z-scores between the baseline and 12-month follow-up examinations for placebo and zinc supplemented children

	Placebo group (n = 135)	*Zinc group (n = 141)*	*P*
Measurements			
Height (cm)[a]	4.9 (4.7 to 5.2)	4.9 (4.6 to 5.2)	0.89
girls	5.1 (4.8 to 5.4)	5.2 (4.9 to 5.5)	0.67
boys	4.7 (4.3 to 5.1)	4.5 (4.1 to 4.9)	0.39
Weight (kg)[a]	3.12 (2.74 to 3.49)	2.76 (2.46 to 3.05)	0.14
girls	2.83 (2.34 to 3.31)	2.75 (2.35 to 3.15)	0.81
boys	3.45 (2.86 to 4.04)	2.76 (2.32 to 3.21)	0.07
Triceps skinfold thickness (mm)[b]	0.15 (0.0 to 0.4)	0.15 (0.1 to 0.3)	0.68
girls	0.05 (− 0.2 to 0.3)	0.10 (− 0.2 to 0.3)	0.86
boys	0.25 (0.1 to 0.5)	0.25 (0.1 to 0.5)	0.44
Arm circumference (cm)[a]	1.03 (0.90 to 1.16)	0.92 (0.81 to 1.03)	0.20
girls	1.02 (0.83 to 1.21)	0.89 (0.75 to 1.04)	0.29
boys	1.04 (0.87 to 1.20)	0.96 (0.80 to 1.12)	0.50
Anthropometric Z-scores[a]			
Height-for-age	− 0.15 (− 0.19 to − 0.11)	− 0.14 (− 0.19 to − 0.10)	0.81
girls	− 0.14 (− 0.20 to − 0.08)	− 0.13 (− 0.20 to − 0.10)	0.81
boys	− 0.16 (− 0.22 to − 0.10)	− 0.16 (− 0.22 to − 0.11)	0.98
Weight-for-age	− 0.12 (− 0.17 to − 0.06)	− 0.15 (− 0.20 to − 0.10)	0.31
girls	− 0.15 (− 0.21 to − 0.07)	− 0.17 (− 0.23 to − 0.10)	0.68
boys	− 0.08 (− 0.16 to 0.00)	− 0.13 (− 0.20 to − 0.07)	0.33
Weight-for-height[a]	− 0.07 (− 0.27 to 0.12)	− 0.23 (− 0.44 to 0.02)	0.28
girls	− 0.18 (− 0.37 to 0.00)	− 0.48 (− 0.97 to 0.01)	0.25
boys	0.01 (− 0.33 to 0.35)	− 0.07 (− 0.22 to 0.08)	0.61
Muscle area-for-age	0.06 (0.00 to 0.12)	0.06 (0.01 to 0.11)	0.90
girls	0.16 (0.07 to 0.24)	0.11 (0.03 to 0.19)	0.38
boys	− 0.03 (− 0.10 to 0.04)	0.00 (− 0.06 to 0.07)	0.49
Fat area-for-age	0.02 (0.00 to 0.05)	0.02 (0.00 to 0.05)	0.78
girls	− 0.03 (− 0.05 to 0.02)	− 0.03 (− 0.05 to 0.02)	0.97
boys	0.08 (0.05 to 0.10)	0.08 (0.05 to 0.11)	0.99

[a] Mean (95% confidence interval) and corresponding *P*-values.
[b] Median (95% confidence interval) and corresponding *P*-values.
[c] Only computable for 27 in the placebo and 31 in the zinc group.

months study period were significantly different between the two supplementation groups (Table 3).

In multiply regression analysis, neither the interaction term between zinc supplementation and age nor between zinc supplementation and sex were significant in any of the models (not shown). Thus, zinc supplementation had no effects over the full 12 months period in neither age nor sex group. Furthermore, the effects of zinc supplementation over the first three months were not significantly different between the two age groups. And the effects were not, as suggested by the stratified analyses (Table 2), significantly different between the sexes.

For example, in the multiple regression model using weight gain over the first three months as the dependent variable the regression coefficient for the interaction term between zinc supplementation (placebo 0, zinc 1) and sex (girls 0, boys 1) was −0.29 (95% CI: −0.88 to 0.30, *P*0.34). The effects of zinc supplementation among boys and girls separately were estimated by introducing separate covariates in the regression model. One covariate (supl_-boy) taking the value of zinc supplementation for boys and zero for girls, and another (supl_girl) taking the value of zinc supplementation for girls and zero for boys. The corresponding regression coefficients were 0.40 (95% CI: 0.03 to 0.77, $P = 0.04$) and 0.36 (95% CI: 0.01 to 0.71, $P = 0.046$). Thus, the estimated effects of zinc were a 400 g weight gain among boys and a 360 g weight gain among girls.

Micronutrient status

Changes in the concentration of zinc, retinol and ferritin over three- and 12-months are presented in Table 4. Changes in the concentration of zinc over the full 12-month study period could be computed for 243 children. While the concentration of zinc declined in both supplementation groups, the decline was significantly less among the zinc supplemented children (−0.09 *vs* −1.11 μmol/L, $P = 0.002$). Of these 243 children, 69 (28.4%) had concentrations of zinc at baseline below the cut-off of 10.7 μmol/L. Among the 174 with normal baseline concentrations of zinc, the mean concentrations declined in both supplementation groups, but significantly less in the zinc group (−0.89 *vs* −2.12 μmol/L, $P = 0.0001$). In contrast, among the 69 children with low concentrations of zinc, the mean concentration increased in both groups. Although the increase was larger among children given zinc compared to placebo, the difference was not statistically significant (1.99 *vs* 1.36 μmol/L, $P = 0.21$). There were no significant differences in the changes in concentration of either retinol or ferritin.

Dietary intake of phytic acid and zinc

A food supplementation programme was in operation during the last eight of the 12 months study period. Assuming a 100% attendance rate and an equal distribution of the foods to all children, the estimated mean daily intake for the first six and a half of the eight months was 94 g of maize, 17 g of dried fish and 6 ml of vegetable oil. Similarly, for the last one and a half months, the estimated mean daily intake was 79 g of maize, 13 g of sugar beans and 5 ml of vegetable oil. These food items were cooked and served as one meal during schooldays. Neither fermentation, nor any other technique that would serve to degrade phytic acid (inositol hexaphosphate), a strong antagonist of zinc absorption, were used. Only scarce amounts of foods, and probably only cereals, were given to the children at home. The concentrations of phytic acid and zinc, and molar ratios of phytic acid-to-zinc, for individual food

Table 4 Changes in serum concentrations of zinc, retinol and ferritin levels over three and twelve months for children allocated to placebo and zinc

	Placebo group	*Zinc group*	*P*
Indicator of micronutrient status			
Zinc (μmol/L)			
3 months [$n=42$]	−0.85 (3.2)	−0.24 (4.3)	0.61
12 months [$n=243$][a]	−1.11 (2.6)	−0.09 (2.4)	0.002
Retinol (μmol/L)			
3 months [$n=48$]	0.10 (0.22)	0.14 (0.23)	0.48
12 months [$n=48$]	−0.13 (0.37)	−0.05 (0.21)	0.36
Ferritin (μg/L)			
3 months [$n=50$]	1.60 (−3.8 to 17.5)	1.80 (−6.4 to 7.8)	0.48
12 months [$n=48$]	4.4 (−0.6 to 9.5)	6.4 (1.0 to 12.4)	0.33

[a] Plasma and serum concentrations of zinc combined.

Table 5 Content of phytic acid and zinc, and molar ratios of phytic acid:zinc of individual food items and average daily food supplements during the food programme in operation the last eight of the 12-month study period

	Phytic acid	*Zinc*	*Phytic acid:zinc*[a]
Food item	μmol/100 g	μmol/100 g	
Sugar beans	2150	45.4	47
Maize	710	21.2	33
Fish	–	149.5	–
Daily ration[b]	μmol	μmol (mg)	
First $6\frac{1}{2}$ months	667	44.7 (2.9)	15
Last $1\frac{1}{2}$ months	840	22.7 (1.5)	37

[a] Molar ratios of phytic acid:zinc.
[b] The food supplements differed during the first six and a half and last one and a half of the eight-month food supplementation period.

items are shown in Table 5. Based on these analyses, and the estimated mean daily intake of individual food items distributed in the schools as part of the food supplementation programme, the average daily intake of phytic acid and zinc and the molar ratio of phytic acid-to-zinc of the average daily supplements could be estimated (Table 5). Accordingly, for the first six and a half of the eight months food supplementation period the average daily dietary intake of zinc was 2.9 mg and the phytic acid:zinc molar ratio 15, and the corresponding figures for the last one and a half months were 1.5 mg and 37.

Loss to follow-up

Of the 39 (12.4%) children lost to the three-months follow-up examination, 15 were in the placebo group and 24 in the zinc group ($P=0.18$), while among the 37 (11.8%) children lost to 12-months follow-up, 22 were in the placebo and 15 in the zinc group ($P=0.28$).

Compliance

The median compliance for the children followed up anthropometrically for the full 12-month study period was 48% (IQR (interquartile range) 31–68), corresponding to a tablet every other schoolday or twice weekly. The median average daily intake of elementary zinc in the zinc group was 8.4 mg (range 1.8–19.5 mg).

There was no difference in compliance between children in the placebo and zinc groups (47% (IQR 31–70) *vs* 48%, (IQR 31–64), $P=0.17$).

Discussion

Baseline equivalence was considered attained through the simple randomization carried out. Nevertheless, the slight baseline differences in sex distribution and age, offer alternative explanations to the differences found in changes in weight and height, wherefore comparison between the two groups should rest on Z-scores of growth and body composition, instead of the basic anthropometric measurements. For example, although the marginally significant higher increase in height in the placebo group could be due to chance, it was probable due to the confounding effect of age, since the median age was slightly higher in the placebo than in the zinc group. Accordingly, when sex and age differences between the groups were controlled for by using HA Z-scores, no significant difference was seen.

A decline in mean WA Z-scores over the first three-month study period was seen for both supplementation groups, but significantly less so among children allocated to daily zinc supplementation. This is in accordance with the results of the studies of severely malnourished Bangladeshi infants and preschool children (Simmer *et al*, 1988; Khanum *et al*, 1988), but these studies were of short duration, and were neither properly randomized nor controlled. However, in a randomized, pair-matched, double-blind zinc supplementation trial of marasmic infants an effect on weight-for-length % of median was reported (Castillo-Duran *et al*, 1987). In a similar trial among North American infants, with a documented decline in WA of more than 20 percentiles resulting in a WA below the tenth percentile, a significant effect of zinc on WA Z-scores was seen (Walravens *et al*, 1989). In contrast, two randomized, controlled zinc supplementation studies of 12-month duration have shown effects of zinc on linear growth: Significant effects of zinc on HA Z-score were found in a study of Canadian boys with low hair-zinc (Gibson *et al*, 1989), as well as in a study of North American preschool children (Walravens *et al*, 1983). In these trials, the study population was identified based on

low height percentiles. Thus, whether zinc supplementation has effects on weight or linear growth may partly depend on the inclusion criteria used.

The effect of zinc on WA Z-score could be explained by an increased synthesis of lean body mass, since a significantly higher increase in AMAA Z-scores was seen in the zinc compared to the placebo group, whereas there was no effect on AFAA Z-scores. This finding is in accordance with the studies of children recovering from severe malnutrition by Golden and Golden (1981), demonstrating that the synthesis of low-energy, high-zinc lean body mass is impaired in zinc deficiency, while any excess intake of energy results in deposition of high-energy, low-zinc adipose tissue.

A number of the previously conducted randomized controlled zinc supplementation trials have found that the effect of zinc supplementation is larger among boys than among girls. While that may be true, stratified analysis is not appropriate to assess for such interactions, as exemplified by our data. Although the stratified analysis revealed a significant difference in weight gain and WA Z-score over the first three months only among girls, the multiple regression analysis showed that not only was the effect of zinc not significantly different between the sexes, but also that the estimated mean weight gain was of similar magnitude.

That the effects of zinc supplementation found after three months had vanished at the 12-month follow-up examination was not surprising, as the food supplementation programme being introduced after the three-month follow-up examination provided the children with zinc-rich dried fish. However, the content of zinc in the rations was low and the content of the phytic acid, a strong antagonist of zinc absorption, high.

Little is known about zinc requirements, since it vary with age and physiological state of the individual, as well as with composition of the diet. Recommendations for dietary zinc intake are currently being prepared by WHO. In these recommendations, basal (needed to replace losses after adaptation) and normative (needed to maintain equilibrium) physiological requirements are given. The normative physiological requirements are estimated as from 1.1–1.5 mg of elementary zinc/day for girls, and from 1.1–2.0 mg for boys (Sandström, personal communication). The ranges given are due to the zinc requiring growth spurts in puberty.

In our study, the food intakes in the homes were, due to the severe drought, believed to be very low, and with low zinc content and bioavailability. Assuming that the dietary intake of zinc in the homes was negligible, then the daily dietary intake of zinc was close to the estimated intake in the schools of 2.9 and 1.5 mg. The phytic acid:zinc ratios were high, as ratios above 15 are considered to be associated with risk of zinc deficiency (Ferguson *et al*, 1989). The bioavailability of the dietary intake of zinc in our study was considered to be lower than 15%. Thus, the average daily intake of bioavailable zinc was 0.4 and 0.2 mg, respectively, which is well below the lower limit of the estimated normative physiological requirements of 1.1 mg/d. That the children in our study were in fact growing in spite of the obviously low zinc and energy intake may be surprising, and suggests a high degree of adaptation to a low dietary intake of zinc.

The zinc supplement was only administered on schooldays. While on average, the zinc supplement was taken every second schoolday, the intake was probably sporadic. Since there is daily requirement for zinc, the biological effect of the sporadic intake of zinc supplements in our study is likely to be less than expected from the median average daily intake of 8.4 mg. Furthermore, the compliance could have been higher during the first three compared to the last nine months. This could explain the negative findings.

As many as 87.5% of the children were followed-up at the three-month examination, and 88.2% at the 12-month examination. The loss to follow-up was small and not associated with supplementation group, wherefore it is not likely to have affected the validity of the study. Since an effect of zinc supplementation was only seen over the first three months, whereas compliance to supplementation was only determined for the full 12-month study period, a possible dose-response effect could not be assessed. Although treatment of schistosome infections took place at the same time as commencement of zinc or placebo supplementation, this would not have confounded the estimated effect of zinc, as prevalence and intensity were similar in the two groups.

While the study did not demonstrate the presence of growth inhibiting zinc deficiency during food supplementation programme, it does not exclude the persistence of zinc deficiency. Zinc supplementation apparently reduced the intensities of *S. mansoni* reinfection occurring while the food programme was in operation (Friis, 1996). Whether this effect was immune-mediated or due to a pharmacological effect of zinc is not established, but the very low dietary intake and bioavailability of zinc makes the persistence of zinc deficiency conceivable. A likely explanation could be that with the minute amounts of bioavailable zinc given as part of the food supplementation programme, not zinc but another nutrient became growth limiting.

In conclusion, the growth limiting role of zinc among rural Zimbabwean schoolchildren on a typical cereal-based diet during drought conditions was clearly demonstrated. Zinc supplementation had favourable effects on lean body mass and weight gain, but no effects on fat and linear growth. However, the addition of even small amounts of zinc-rich food items may precipitate the growth inhibiting effect of another nutrient.

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