Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials[1–3]

Kenneth H Brown, Janet M Peerson, Juan Rivera, and Lindsay H Allen

ABSTRACT

Background: Multiple studies have been carried out to assess the effect of zinc supplementation on children's growth. The results of these studies are inconsistent, and the factors responsible for these varied outcomes are unknown.

Objective: Meta-analyses of randomized controlled intervention trials were therefore completed to assess the effect of zinc supplementation on the physical growth and serum zinc concentrations of prepubertal children.

Design: A total of 33 acceptable studies with appropriate data were identified by MEDLINE (National Library of Medicine, Bethesda, MD) searches and other methods. Weighted mean effect sizes (expressed in SD units) were calculated for changes in height, weight, weight-for-height, and serum zinc concentration by using random-effects models; factors associated with effect sizes were explored by meta-regression techniques.

Results: Zinc supplementation produced highly significant, positive responses in height and weight increments, with effect sizes of 0.350 (95% CI: 0.189, 0.511) and 0.309 (0.178, 0.439), respectively. There was no significant effect of zinc on weight-for-height indexes [weighted mean effect size: –0.018 (–0.132, 0.097)]. Zinc supplementation caused a large increase in the children's serum zinc concentrations, with an effect size of 0.820 (0.499, 1.14). Growth responses were greater in children with low initial weight-for-age *z* scores and in those aged >6 mo with low initial height-for-age *z* scores.

Conclusions: Interventions to improve children's zinc nutriture should be considered in populations at risk of zinc deficiency, especially where there are elevated rates of underweight or stunting. The population mean serum zinc concentration is a useful indicator of the successful delivery and absorption of zinc supplements in children. *Am J Clin Nutr* 2002;75:1062–71.

KEY WORDS Zinc, growth, serum zinc, zinc deficiency, nutritional assessment, indicators, children, meta-analysis

See corresponding editorial on page 957.

INTRODUCTION

Zinc plays a critical role in the cellular growth, cellular differentiation, and metabolism of higher plants and animals. Nevertheless, the importance of zinc for human nutrition and health was not recognized until the second half of the 20th century (1). About 30 y ago, clinicians first noted that human zinc deficiency secondary to acrodermatitis enteropathica, an inborn error of metabolism that causes reduced intestinal absorption of zinc, is associated with impaired growth, increased susceptibility to infections, and other functional abnormalities (2). Since then, a considerable number of intervention trials have been completed in multiple countries to assess the effect of supplemental zinc on children's growth. These studies have yielded inconsistent results, however, possibly because of differences in *1*) the preexisting zinc status of the study subjects, *2*) the content and bioavailability of zinc in the local diets, and *3*) the incidence of common infections that can affect growth independently of an individual's zinc status. Moreover, methodologic aspects of these studies, such as variations in the dose, chemical form, and method of administration of zinc and duration of supplementation, may have influenced their results. Finally, in some cases, the sample sizes may have been inadequate to detect potentially important differences in growth with statistical confidence. For these reasons, a systematic, quantitative review of available studies is needed to determine the overall effect of zinc supplementation on children's growth.

Assessment of the zinc nutriture of individuals is complicated by the fact that no generally accepted, sensitive and specific biomarker of zinc status exists (3). Although it is true that serum (or plasma) zinc concentrations decrease within several weeks of the introduction of a diet containing a severely restricted amount of zinc (4), serum zinc concentrations are generally maintained within the normal range with small or moderate reductions in zinc intake. Moreover, factors unrelated to the level of zinc nutriture, such as recent meals, time of day, infection, tissue catabolism,

[1] From the Program in International Nutrition and the Department of Nutrition, University of California, Davis (KHB, JMP, and LHA), and Centro de Nutrición y Salud, Instituto de Salud Pública, Cuernavaca, Morelos, Mexico (JR).

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[3] Reprints not available. Address correspondence to KH Brown, Department of Nutrition, One Shields Avenue, University of California, Davis, CA 95616. E-mail: khbrown@ucdavis.edu.

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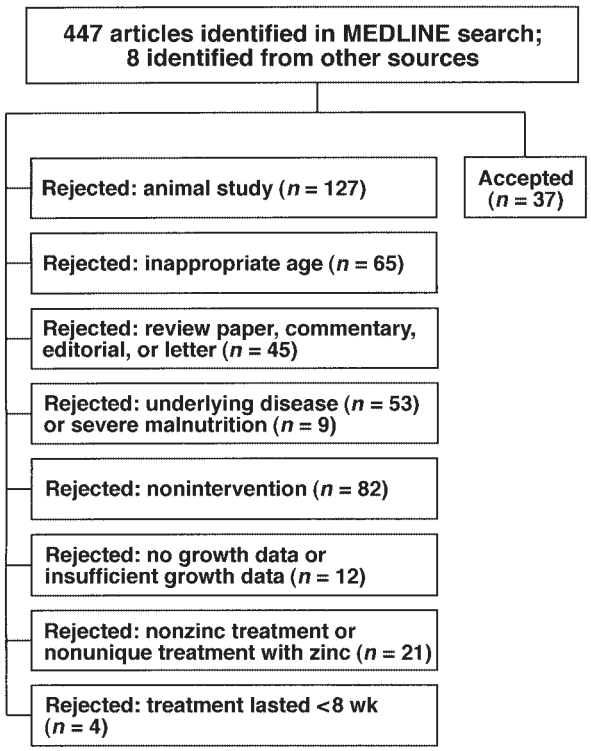

FIGURE 1. Studies excluded and included in the meta-analysis and reasons for exclusions. MEDLINE, National Library of Medicine, Bethesda, MD.

and pregnancy, can also affect serum zinc concentrations (3, 5). Thus, the serum zinc concentration may not always be a reliable indicator of an individual's true zinc status. Although the population mean serum zinc concentration has been proposed as a useful indicator of the zinc status of groups of individuals (6), little information is available on the ability of this indicator to predict the population's functional responses to zinc supplementation. Moreover, information is needed to assess the responsiveness of the mean serum zinc concentration to zinc supplementation to determine whether this measure could be used as an indicator of successful delivery and absorption of supplemental zinc in public health intervention programs.

To address these issues, we completed meta-analyses of intervention trials that were conducted to assess the effect of zinc supplementation on the growth (height, weight, and weight-for-height index) and serum zinc concentrations of prepubertal children. We also explored characteristics of the study populations that could be used to predict these responses to zinc supplementation.

METHODS

This paper presents an updated version of preliminary analyses that were published previously as part of a conference proceedings (7). The current analyses differ from the earlier ones in several important ways: *1*) additional studies were identified by using a newly designed and updated bibliographic search and *2*) inclusion and exclusion criteria for individual studies were modified, with specific exclusion of those studies that lasted <8 wk or enrolled only premature infants or inpatients admitted to a hospital for treatment of severe protein-energy malnutrition (marasmus and kwashiorkor). Furthermore, additional analyses were completed in the present version to determine the characteristics of individual studies that may have influenced the observed responses to supplemental zinc.

Identification of studies

The studies considered for possible inclusion in the current meta-analyses were identified by 2 separate searches of the MEDLINE (National Library of Medicine, Bethesda, MD) computerized bibliographic database spanning the years 1966–2001. The searches were completed on 14 May 2001. For the first search, all articles that included the word *zinc* in the title and the key words *growth* and either *infant* or *child* (or *children*) were selected. For the second search, all articles that contained the word *supplement* or *supplemental* in the title and the key words *zinc* and *growth*, *weight*, *length*, or *height* were selected. The 2 lists of papers were merged, and the resulting 447 articles were then examined for inclusion or exclusion, as described below. The bibliographic citations of each of the articles ultimately selected for inclusion in the analyses were also examined to identify any other acceptable studies that were not captured by the MEDLINE searches. Finally, several unpublished studies that were made available to the authors were also evaluated for their acceptability. These last 2 procedures identified 8 additional sets of results, yielding a total of 455 studies that were assessed for possible inclusion in the meta-analyses.

Inclusion criteria

Studies were considered acceptable for inclusion in the meta-analyses if they met the following criteria: *1*) the study was a randomized, placebo-controlled intervention trial in which the supplemented and control groups were enrolled concurrently; *2*) the subjects were children aged <12 y or specifically stated to be prepubertal throughout the period of intervention; *3*) the subjects were not premature infants; *4*) the subjects were free of chronic diseases, such as sickle cell disease, cystic fibrosis, or severe protein-energy malnutrition (marasmus or kwashiorkor), that might have independently affected their growth; *5*) zinc was the only component of the supplement that differed between treatment groups; *6*) the supplemental zinc was provided for ≥8 wk; and *7*) data for body weight, height, or both were collected during the period of supplementation and were reported in sufficient detail to permit calculation of the change in nutritional status and its SD during that interval. In several cases, supplemental zinc was provided as a component of a food or infant formula. These studies were considered acceptable only if *1*) the zinc was the only difference in the composition of the fortified and unfortified food and *2*) the control (unfortified) diet contained only the amount of zinc intrinsic to that food.

When studies identified during the bibliographic search were excluded from the meta-analyses, the reason for exclusion was categorized according to a single criterion with use of a specific hierarchy as shown in **Figure 1**. This was necessary for the purpose of classifying the basis of exclusion because some studies could have been rejected for several reasons, such as inappropriate age group and presence of an underlying disease in the subjects. In some cases, we were able to retain subgroups of subjects in the appropriate age range after direct communication with the authors, who disaggregated the data accordingly. The number of studies excluded is summarized by category of exclusion in Figure 1; a detailed listing of the excluded studies is available from the corresponding author on request.

Review of studies and extraction of summary data

Each of the coauthors of the present paper independently assessed the suitability of each study for inclusion in the meta-analyses, and the results of these individual assessments were then compared and discussed in working group sessions. In cases in which the original opinions varied, these differences were resolved through consensus by using the preestablished inclusion criteria or further written elaborations of these criteria when necessary.

Once the final set of studies for inclusion in the analyses was established, the coauthors independently prepared written summaries of key descriptive information concerning the study design, baseline characteristics of the study subjects, and outcomes of the intervention. In some instances, the authors of the papers were contacted for additional information not available in the published documents (for example, disaggregated data for the subset of children who were prepubertal or growth data that corresponded directly with the period of supplementation). When there was a lapse in supplementation (as occurred, for example, when supplements were delivered during the school year but not during prolonged, interposed school vacations), only the initial period of supplementation before interruption was included in the analysis. If there was a delay between the termination of supplementation and the collection of outcome information, a maximum interval of 1 mo was considered acceptable for anthropometric variables and of 1 wk for serum zinc concentration. When several amounts of zinc were provided to different study groups, only the 2 groups with the highest and lowest (usually no zinc) doses were included. If the original study used a factorial design in which zinc was given either alone or with some other nutrient or nutrients, only the 2 groups that received zinc alone or a placebo were included.

Because of the stringent criteria applied for inclusion of individual studies, we made no further attempt to score their methodologic quality. However, we did summarize information on the frequency of delivery and the method used to confirm consumption of the supplements; these issues were considered as possible predictors of response to supplementation.

Analysis of data

The primary response variables included in each of the separate analyses were *1*) change in height (length or stature), expressed in cm or height-for-age *z* score; *2*) change in body weight, expressed in kg or weight-for-age *z* score; *3*) change in weight-for-height *z* score; and *4*) change in serum or plasma zinc concentration. For the sake of simplicity, we refer to all results as *serum zinc concentrations* regardless of whether the analyses were conducted in serum or plasma samples. In all cases, the growth and serum zinc outcomes were converted to effect sizes, which were calculated as the difference between the means of the zinc and control groups divided by their pooled SD. The use of effect sizes solves the problem that the units of measurement applied and the durations of observation were not consistent among the studies. In general, effect sizes of ≈0.2 are considered of small magnitude, effect sizes of ≈0.5 are considered moderately large, and those of ≈0.8 are considered large (8), although these must also be evaluated in relation to the effect of other interventions that affect the outcomes of interest.

The overall mean effect size for each outcome variable was estimated from a random-effects model (9). This model assumes that the distribution of the effect size for the studies is the sum of the true study effect size δ_i, which is assumed to come from a normal distribution with mean δ and standard deviation σ, and a normally distributed measurement error with mean 0 and variance $(8 + \delta_i^2)/(2 \times n_i)$, where n_i is the total sample size for study i. Because the total variance for the study effect size is different from one study to the next, the best estimate for the overall mean δ is a weighted mean effect size, in which the weights are equal to the inverse of the total variance. The SAS for WINDOWS (release 8; SAS Institute Inc, Cary, NC) MIXED procedure was used to estimate the weighted mean effect size and its SE.

The heterogeneity of responses was assessed by using the chi-square test, as described by Hedges (10). We explored possible sources of heterogeneity by using 2 methods: *1*) visual inspection of mean effect sizes for subgroups of studies, categorized by selected characteristics of the study design or study subjects that we hypothesized might influence the response to zinc supplementation, and *2*) formal random-effects meta-regression analyses, in which study characteristics were used to explain effect sizes (11, 12). As with any regression, the number of possible explanatory variables was strictly limited by the number of studies available. Explanatory variables were examined separately in a series of bivariate models; then a subset of explanatory variables was entered into a regression model and nonsignificant predictors were removed in a stepwise fashion. Interactions of anthropometric *z* score variables with age (≤ or >6 mo) was assessed because manifestation of stunting and underweight is often delayed for some time postpartum. Nonlinearity was initially assessed with polynomial models, and in one case (relation between initial height-for-age and effect size for height) was followed up with the use of more sophisticated models. Several nonlinear models were examined, and the 2-phase regression model was finally judged to provide the best fit. The SAS for WINDOWS MIXED procedure was used for all of these procedures except the 2-phase regression model, for which the SAS NLMIXED procedure was used.

Formal analyses were completed to detect possible publication bias, which can occur when authors fail to submit papers with nonsignificant results or journals fail to accept these papers for publication. If publication bias is occurring, then studies with both small sample sizes and small effect sizes are less likely to be found, resulting in a negative correlation between absolute effect size and sample size. Therefore, one method of assessing publication bias is to examine the correlation between effect size and sample size. Another method, described by Rosenthal (13), is to calculate how many nonsignificant studies would have to have been carried out, but not published, to overturn a significant result. If this number is small, publication bias might be an issue; but if it is large, the results are not likely to be influenced by unpublished studies.

RESULTS

Description of studies and study subjects

Thirty-seven studies were considered acceptable for inclusion in the analyses (14–49; ME Penny, JM Peerson, RM Marin, et al, unpublished observations, 1998). The general characteristics of these studies and their participating subjects are shown in **Table 1**. Of the potentially acceptable studies, 4 (15, 18, 26, 32) did not contain enough detailed information on the outcome variables to be used in any of the subsequent analyses. These 4 studies did not differ from the other acceptable studies with regard to any aspects

TABLE 1
Selected characteristics of studies and study subjects[1]

Status of study and reference	Country	*n*	Zinc dose	Duration of study	Mean initial age	Mean initial weight	Mean initial Height	Mean initial serum zinc concentration
			mg/d	*mo*	*y*	*kg*	*cm*	*μmol/L*
Included in current analyses								
Bates et al (14), 1993	Gambia	109	20	15	1.5	8.9	76.7	15.2
Castillo-Duran et al (16), 1994	Chile	42	10	12	8.7	—	—	17.5
Castillo-Duran et al (17), 1995[2]	Chile	68	3	6	0.0	2.3	47.2	12.4
Cavan et al (19), 1993	Guatemala	162	7	6	6.8	19.7	112.5	14.2
Dirren et al (20), 1994	Ecuador	96	9	15	2.6	11.2	81.6	11.3
Friis et al (21), 1997[2]	Zimbabwe	191	15	3	10.1	26.6	132.1	12.0
Gibson et al (22), 1989	Canada	60	10	12	6.4	18.8	110.8	16.1
Hambidge et al (23), 1978	USA	75	10	6	4.4	—	—	—
Hambidge et al (24), 1979	USA	96	3	9	4.8	—	—	12.3
Heinig et al (25), 1998[2,3]	USA	85	5	6	0.3	6.9	63.8	10.6
Hong (27), 1982[3]	China	131	—	7	1.6	—	—	10.8
Hong (28), 1987[3]	China	83	7	3	0.0	3.2	49.9	13.2
Hong et al (29), 1992[3]	China	65	8	6	0.0	3.3	50.1	13.2
Kikafunda et al (30), 1998[3]	Uganda	155	10	3	4.7	16.7	103.3	—
Lira et al (31), 1998[2,3]	Brazil	134	1	2	0.0	2.3	46.0	—
Matsuda et al (33), 1984[3]	Japan	39	2	4	0.1	3.2	—	10.8
Meeks-Gardner et al (34), 1998[2,3]	Jamaica	61	5	3	1.2	7.0	68.7	—
Nakamura et al (35), 1993	Japan	21	—	6	5.9	—	—	12.6
Ninh et al (36), 1995	Vietnam	210	10	5	1.5	7.9	71.3	—
Penny et al, 1998[2–4]	Peru	146	9	6	1.6	9.9	76.6	10.9
Rivera et al (37), 1998[2]	Guatemala	89	10	7	0.6	7.2	63.7	—
Rosado et al (38), 1997[2]	Mexico	109	14	12	2.4	11.1	83.1	13.8
Ruz et al (39), 1997	Chile	98	10	14	3.3	15.5	95.6	17.4
Sayeg Porto et al (40), 2000[3]	Brazil	21	—	6	9.9	21.2	121.6	15.4
Sempértegui et al (41), 1996[3]	Ecuador	50	10	2	3.5	—	—	13.3
Shrivastava et al (42), 1993	India	60	6	3	1.3	—	—	13.7
Smith et al (43), 1999[2,3]	Belize	22	10	6	4.0	14.0	92.2	11.5
Udomkesmalee et al (44), 1992[2]	Thailand	68	18	6	9.3	23.0	124.0	13.2
Umeta et al, nonstunted (45), 2000[3]	Ethiopia	94	9	6	0.8	7.5	69.8	—
Umeta et al, stunted (45), 2000[3]	Ethiopia	90	9	6	0.8	6.5	64.5	—
Walravens and Hambidge (46), 1976	USA	68	4	6	0.0	3.2	49.6	—
Walravens et al (47), 1983	USA	40	10	12	4.2	—	—	11.0
Walravens et al (48), 1989	USA	50	6	6	1.5	8.2	74.6	10.7
Walravens et al (49), 1992	France	57	5	3	0.5	8.1	66.5	—
Accepted, but insufficient data[5]								
Castillo-Duran et al (15), 1987	Chile	32	11	3	0.6	5.6	—	15.3
Castillo-Duran et al (18), 2001	Chile	112	5	12	0.0	3.4	50.3	—
Hershkovitz et al (26), 1999	Israel	25	12	3	0.5	—	—	
Mahloudji et al (32), 1975	Iran	50	17	8	9.0	21.8	119.0	—
Included previously, but not currently[6]								
Behrens et al (50), 1990	Bangladesh	64	33	<1	0.9	6.3	68.1	—
Friel et al (51), 1993	Canada	36	2	12	0.1	1.9	41.9	11.6
Gatheru et al (52), 1988	Kenya	82	40	<1	1.7	8.0	—	6.4
Golden and Golden (53), 1992	Jamaica	11	5	2	1.2	5.0	68.0	10.3
Khanum et al (54), 1988	Bangladesh	60	40	<1	2.4	—	—	8.0
Ronaghy et al (55), 1974	Iran	39	27	16	13.0	31.0	133.6	10.9
Schlesinger et al (56), 1992	Chile	39	11	4	0.6	5.1	60.3	21.4
Simmer et al (57), 1988	Bangladesh	23	50	<1	3.2	7.3	78.0	9.8
Smith et al (58), 1985	Australia	204	14	9	10.0	—	—	9.6

[1] For analysis purposes, initial weights, heights, and *z* scores were also estimated from the other anthropometric data available.
[2] Additional data supplied by authors.
[3] Studies included in this paper, but not in previous meta-analyses (7).
[4] ME Penny, JM Peerson, RM Marin, et al, unpublished observations, 1998.
[5] Not used in present meta-analyses because of insufficient information on study variables.
[6] Reasons for exclusion of previously included studies: Behrens et al (50), <8 wk duration; Friel et al (51), premature infants; Gatheru et al (52), <8 wk duration, severely malnourished children; Golden and Golden (53), <8 wk duration, severely malnourished children; Khanum et al (54), <8 wk duration, severely malnourished children; Ronaghy et al (55), some subjects probably postpubertal; Schlesinger et al (56), severely malnourished children; Simmer et al (57), <8 wk duration, severely malnourished children; Smith et al (58), some subjects probably postpubertal.

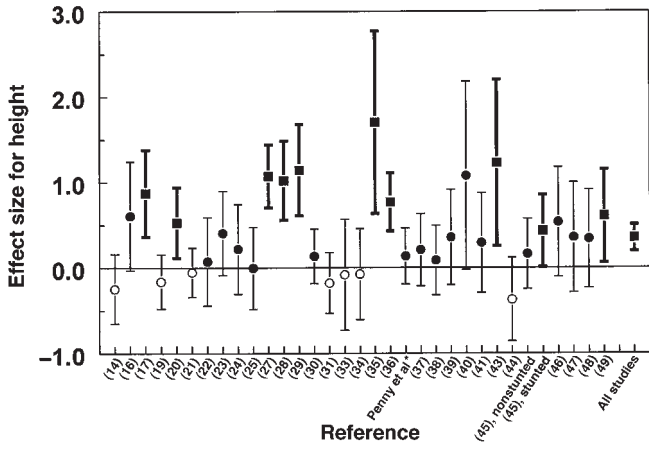

FIGURE 2. Weighted mean effect size and 95% CI (in SD units) for the effect of zinc supplementation on children's linear growth: results of the individual studies and of the meta-analysis. ○, studies with a negative effect size and confidence limits that include zero; ●, studies with a positive effect size and confidence limits that include zero; ■, studies with a positive effect size and confidence limits that do not include zero. *See* Table 1 for the number of subjects in each study. *ME Penny et al, unpublished observations, 1998.

of study design or initial characteristics of study subjects. In 1 of the 33 studies that were ultimately included in the analyses (45), 2 separate strata of subjects, with or without nutritional stunting (height-for-age z score < -2), were enrolled and independently randomly assigned to treatment group, so data from this project were considered as 2 separate studies for the purposes of the meta-analyses, resulting in a total of 34 data sets. Information on the 9 studies (50–58) that were included in the previous version of these meta-analyses but excluded from the present one and the reasons for their exclusion are also provided in Table 1. Currently accepted studies that were newly identified since the previous version of the meta-analyses are also indicated. The 34 data sets that were used for the present analyses provided information for a total of 2945 children. The original 33 studies were published (or otherwise made available) between 1976 and 2001 (median: 1994). Thirteen of the studies were conducted in Latin America or the Caribbean, 8 in Asia, 8 in North America or Europe, and 4 in Africa. The number of subjects in each study ranged from 21 to 210 ($\bar{x}$ ± SD: 87 ± 47 subjects/study), with mean ages ranging from newborn to 10 y ($\bar{x}$ ± SD: 3.1 ± 3.1 y). In most cases, the subjects were fairly evenly balanced between males and females, although one study (22) enrolled only males.

The mean initial height-for-age z scores of the subjects ranged from –2.90 to 0.37 ($\bar{x}$: –1.52 ± 0.97), and their mean initial weight-for-age z scores ranged from –2.78 to 0.76 ($\bar{x}$: –1.18 ± 0.94). All but one (17) of the mean initial weight-for-height z scores fell within the normal limits, with a mean (±SD) of –0.46 ± 0.73. The mean initial serum zinc concentrations ranged from 10.6 to 17.5 μmol/L (69–114 μg/dL) with a mean of 13.0 ± 2.1 μmol/L (85 ± 14 μg/dL).

Most studies (n = 24) provided the zinc supplements in the form of zinc sulfate, although 4 used zinc gluconate (14, 43, 44; ME Penny et al, unpublished observations, 1998), 2 used zinc acetate (17, 33), 2 used amino acid chelates (19, 38), and 1 used zinc oxide (24). The average daily doses of elemental zinc in the supplements (calculated by dividing the total weekly doses by 7) ranged from 1 to 20 mg/d ($\bar{x}$ ± SD: 8.4 ± 4.4 mg/d). The duration of supplementation lasted from 8 wk to 15 mo ($\bar{x}$ ± SD: 6.6 ± 3.8 mo). In 3 cases (24, 33, 46), the supplemental zinc was incorporated in a fortified food. The supplements were offered daily in 21 studies, 5–6 d/wk in 9 studies, and on alternate days, semi-weekly, or weekly in 1 study each. All but one study with relevant information (35) provided the supplements with their identity masked, and 2 studies (27, 33) did not specify whether masking procedures were used. Successful delivery of the supplements was fully monitored in 14 studies, partially monitored in 16, and uncertain in 3.

Change in height

Sufficient information was available from 33 data sets (including a total of 2637 study subjects) to permit calculation of the effect of zinc supplementation on the children's linear growth. The calculated mean effect sizes and their 95% CIs are displayed for the individual studies and for the combined set of studies in **Figure 2**. The effect sizes of the individual studies ranged from –0.37 to 1.70. There was a positive effect size in 25 (75.8%) of the studies, indicating that the children in these studies who received supplemental zinc had greater growth increments than did the children in the respective control groups. In 10 of these studies with a positive effect size, the 95% confidence limits of the effect size excluded zero. Thus, the increased growth that occurred after zinc supplementation in those studies was unlikely to have been due to chance. In 8 (24.2%) of the total group of studies, the effect size was negative or zero. In none of these 8 studies did the confidence limits exclude zero. Overall, the weighted average effect size derived from the random-effects model was 0.350 (95% CI: 0.189, 0.511), which was significantly greater than zero ($P < 0.0001$).

There was significant heterogeneity in the results for change in height from the different studies ($P < 0.001$). The results of the subgroup analyses that were conducted to explore possible sources of heterogeneity are shown in **Figure 3**. Studies that enrolled children with greater degrees of stunting (mean initial height-for-age z score < -2) had a weighted mean effect size of 0.465 (95% CI: 0.179, 0.750), which was nearly 2-fold greater than the effect size of 0.254 (95% CI: 0.057, 0.450) for those studies in which the mean initial

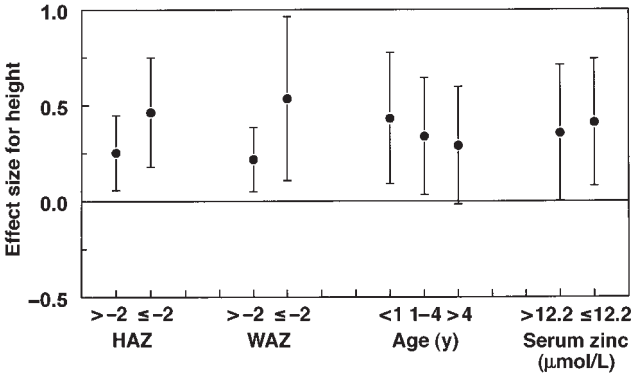

FIGURE 3. Weighted mean effect size and 95% CI (in SD units) for the effect of zinc supplementation on children's linear growth in individual studies, by the subjects' mean initial height-for-age z score (HAZ), weight-for-age z score (WAZ), age, and serum zinc concentration. The n for each subgroup of studies was as follows: 19, 12, 23, 6, 11, 11, 12, 14, and 9, respectively.

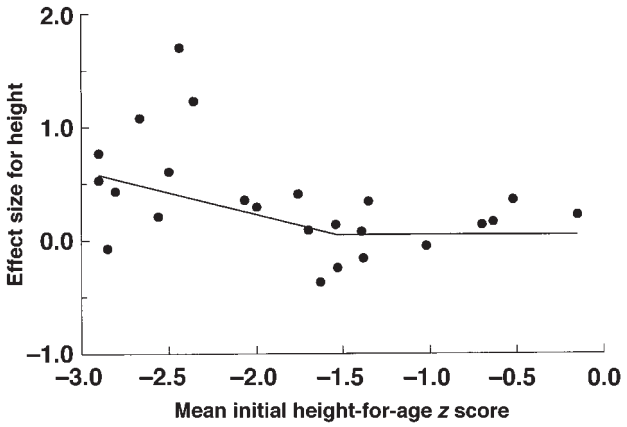

FIGURE 4. Relation between mean initial height-for-age z score and effect size (in SD units) for the effect of zinc supplementation on linear growth among children aged ≥6 mo.

height-for-age z score was >-2, although the results were not significantly different ($P = 0.14$ in the meta-regression analysis). However, there was a significant interaction between age group (< or ≥6 mo) and the presence of stunting ($P = 0.0002$). In other words, in studies in which the children's mean age was ≥6 mo there was a significantly greater effect of zinc supplementation when the mean initial height-for-age z score was <-2.0, but this was not true for studies of younger infants. In a meta-regression analysis of the subset of studies with subjects with a mean initial age ≥6 mo, the mean initial height-for-age z score was a significant predictor of the effect size for height gain; the 2-phase regression model showed a significant negative relation ($P = 0.003$) when the mean initial height-for-age z score was <-1.53 and no significant relation ($P = 0.21$) when the mean initial height-for-age z score was greater than this cutoff (**Figure 4**).

When the results for linear growth were disaggregated according to the presence of underweight in the study subjects, the weighted mean effect size was 0.536 (95% CI: 0.108, 0.963) for those studies with a mean initial weight-for-age z score <-2 compared with an effect size of 0.218 (95% CI: 0.051, 0.386) for those studies in which the initial weight-for-age z score was >-2 ($P = 0.071$ in the meta-regression analysis). Although there was a slight progressive decrease in effect sizes in studies that enrolled older children, these differences were not significant in the meta-regression analysis ($P = 0.77$). No other factors appeared to be related to the variation in effect sizes.

Change in body weight

Thirty-two studies (with a total of 2597 enrolled subjects) provided suitable information on the effect size for change in body weight in relation to zinc supplementation (**Figure 5**). In one study (42), the effect size was >3 times greater than that found in the study with the next largest response to zinc, so the outlying results of the former study were omitted from the combined analysis. The effect sizes of the remaining 31 studies ranged from –0.13 to 1.12. The effect size was positive in 25 studies (80.6% of the total) and significantly greater than zero in 7 of these. The effect size was negative or zero in 6 studies, but in no case did the confidence limits of these 6 studies exclude zero. The overall weighted mean effect size was 0.309 (95% CI: 0.178, 0.439), which was significantly greater than zero ($P < 0.0001$).

Again, there was significant heterogeneity of results ($P < 0.001$). Subgroup analyses are presented in **Figure 6**. The only significant explanatory factor in the meta-regression model was a mean initial weight-for-age z score <-2 ($P = 0.017$). The weighted mean effect size for change in weight was 0.559 (95% CI: 0.92, 1.025) for those studies with mean initial weight-for-age z scores <-2 and 0.202 (95% CI: 0.081, 0.322) for those with mean initial initial weight-for-age z scores >-2.

Change in weight-for-height

Sufficient information on the effect of zinc supplementation on change in weight-for-height z score was available from 15 studies, which provided information on a total of 1191 subjects (**Figure 7**). There was no clear pattern of response, with 8 studies showing a positive effect size and 7 studies showing a negative effect size. In only one case (20) did the confidence limits exclude zero. Overall, the weighted mean effect size was –0.018 (95% CI: –0.132, 0.097), which was not significantly different from zero ($P = 0.77$).

Change in serum zinc concentration

The effect of zinc supplementation on change in serum or plasma zinc concentration could be analyzed for 15 studies, which enrolled a total of 1141 subjects (**Figure 8**). In all but one study, the effect sizes were positive (93.3% of the total). In 12 cases, the confidence limits of the positive effect sizes excluded zero. The weighted average effect size was 0.820 (95% CI: 0.499, 1.14; $P < 0.0001$). There was significant heterogeneity of results. The only factor (negatively) associated with the magnitude of response to supplementation was the mean initial serum zinc concentration ($P = 0.071$ for bivariate correlation), although this was not significant in the random-effects model.

Publication bias

In each meta-analysis the correlation between the number of subjects and the effect size of individual studies was examined to

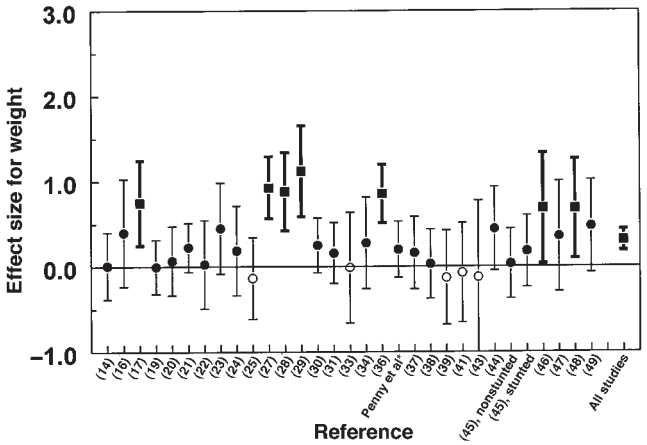

FIGURE 5. Weighted mean effect size and 95% CI (in SD units) for the effect of zinc supplementation on children's weight gain: results of the individual studies and of the meta-analysis. ○, studies with a negative effect size and confidence limits that include zero; ●, studies with a positive effect size and confidence limits that include zero; ■, studies with a positive effect size and confidence limits that do not include zero. *See* Table 1 for the number of subjects in each study. *ME Penny et al, unpublished observations, 1998.

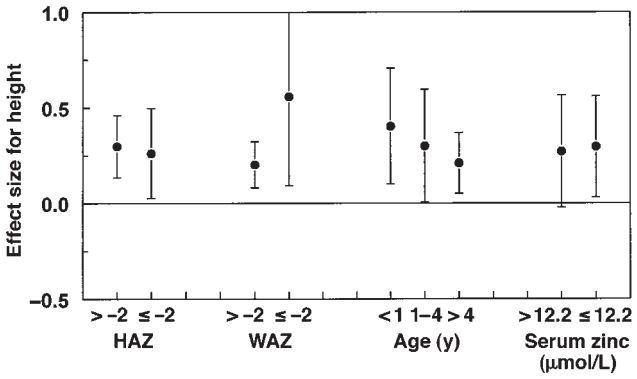

FIGURE 6. Weighted mean effect size and 95% CI (in SD units) for the effect of zinc supplementation on children's weight gain in individual studies, by the subjects' mean initial height-for-age *z* score (HAZ), weight-for-age *z* score (WAZ), age, and serum zinc concentration. The *n* for each subgroup of studies was as follows: 19, 10, 23, 5, 11, 10, 10, 12, and 9, respectively.

explore possible publication bias. The correlation coefficients ranged from –0.28 for the analysis of height increments to 0.20 for weight-for-height increments, suggesting that there was little problem of publication bias. We also examined the strength of the conclusions by estimating how many additional (possibly unpublished) studies with zero effect size would have to be available to negate the significant results of the current meta-analyses examining increments in height, weight, and serum zinc. In each case, >500 studies with zero effect of zinc supplementation would be needed to invalidate the reported findings.

DISCUSSION

Meta-analysis techniques are increasingly being used to consolidate results from multiple studies of the same topic and to develop evidence-based policies for clinical practice and public health programming. The reliability of the conclusions derived from meta-analyses depends on the methodologic quality of the original studies, the appropriateness of the study inclusion criteria, and the thoroughness of the review and synthesis of information. In the current analyses, we included a sizeable number of rigorously designed intervention trials of the effect of zinc supplementation on children's growth and serum zinc concentrations. The results indicate that zinc supplementation results in a highly statistically significant increase in the linear growth and weight gain of prepubertal children, but does not affect the weight-for-height index. Zinc supplementation also produces a large, highly statistically significant increase in children's serum zinc concentrations.

The failure to identify any significant correlations between the sample sizes of individual studies and the magnitude of the effect of supplementation suggests that these conclusions are not likely to have been influenced by publication bias. The strength of the findings is further supported by the fact that all of the studies included in the meta-analyses used a suitable clinical trial design, including a concurrent (usually masked) control group and, in most cases, confirmation that the supplements were successfully delivered to the study subjects. The positive effect of zinc on children's growth is also consistent with results from earlier studies in experimental animals (59).

The criteria for inclusion of studies in the current analyses differed somewhat from those we used previously (7). In particular, for the current analyses we required that the period of supplementation last ≥8 wk because shorter periods may be insufficient for detecting a linear growth response. Furthermore, we eliminated studies of premature infants and of children hospitalized for treatment of severe protein-energy malnutrition because in both cases we felt that the zinc requirements of these children might differ considerably from those of unaffected children. Thus, it did not seem to be appropriate to combine results from the different sets of study subjects in a single meta-analysis. Despite these changes in the inclusion criteria, the results of the current analyses are generally consistent with our previously published findings (7).

Mean initial height-for-age and mean weight-for-age predicted the magnitude of the linear growth responses to zinc supplementation. Studies that enrolled stunted children (mean initial height-for-age <-2 *z* scores) reported nearly 2-fold greater length increments after supplementation than did studies that included nonstunted children (Figure 3), although these differences by initial height status were not significant in the meta-regression analysis. However, there was a significant interaction between mean initial age and presence of stunting, so we reexamined the predictors of linear growth response after excluding all 8 studies (17, 25, 28, 29, 31, 33, 46, 49) that enrolled infants with a mean initial age <6 mo. Among the studies that enrolled older children (mean age > 6 mo), the mean initial height-for-age *z* score was a significant predictor of linear growth (Figure 4). The likelihood that zinc supplementation produces a greater growth response in stunted children is further supported by evidence from the 2 individual studies in which growth responses were examined in subsets of children with and without stunting (37, 45). In both of these studies, there were significantly greater responses to zinc in the subsets of stunted children. The mean initial weight-for-age *z* score was a significant predictor of both linear growth response

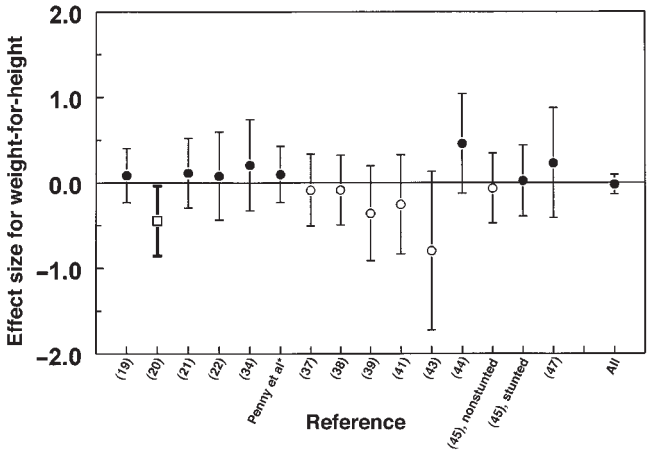

FIGURE 7. Weighted mean effect size and 95% CI (in SD units) for the effect of zinc supplementation on children's weight-for-height index: results of the individual studies and of the meta-analysis. ○, studies with a negative effect size and confidence limits that include zero; ●, studies with a positive effect size and confidence limits that include zero; ■, studies with a positive effect size and confidence limits that do not include zero. *See* Table 1 for the number of subjects in each study. *ME Penny et al, unpublished observations, 1998.

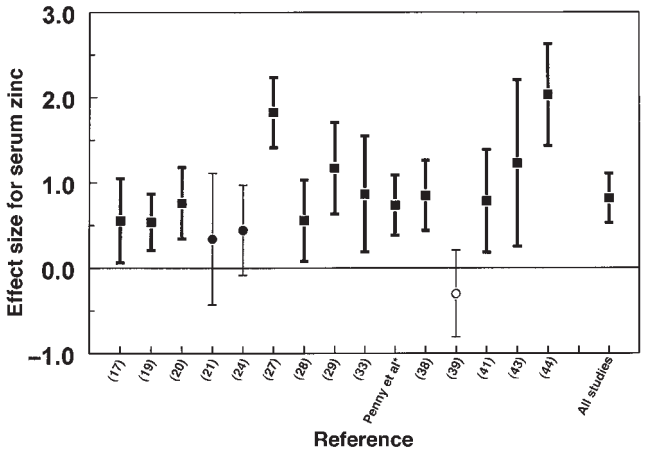

FIGURE 8. Weighted mean effect size and 95% CI (in SD units) for the effect of zinc supplementation on children's serum zinc concentration: results of the individual studies and of the meta-analysis. ○, studies with a negative effect size and confidence limits that include zero; ●, studies with a positive effect size and confidence limits that include zero; ■, studies with a positive effect size and confidence limits that do not include zero. *See* Table 1 for the number of subjects in each study. *ME Penny et al, unpublished observations, 1998.

and weight gain after zinc supplementation. The effect sizes for both height and weight gain were ≈2-fold greater in studies that enrolled underweight children compared with those in which the subjects' mean initial weight-for-age *z* score was > −2.0.

The ability of low height-for-age and low weight-for-age to identify populations that are more likely to respond to zinc supplementation is of tremendous practical importance because routinely collected information on stunting and underweight can be used to assess the likelihood that a population will respond to zinc supplementation. The World Health Organization (60) maintains a database with current information on national rates of stunting and underweight that can be used as readily available, indirect indicators of the national risk of zinc deficiency.

There was no detectable effect of zinc supplementation on children's weight-for-height index, possibly indicating that supplemental zinc is more likely to influence linear growth than accrual of fat mass. This latter notion is consistent with other reports suggesting that zinc promotes increases in fat-free mass rather than fat mass (53, 61).

Information on the mean serum zinc concentration was available from only 15 studies, which limited the statistical power of the analyses to examine the relation between initial serum zinc concentration and growth response to zinc supplementation. In the previous version of these meta-analyses, the serum zinc concentration was a significant predictor of the magnitude of weight gain after zinc supplementation. This was not so in the current analyses, in which the range of values for mean initial serum zinc concentration was reduced by the exclusion of studies of severely malnourished children. Thus, the lack of significance in the current analysis may be due to the limited amount of available information over a broad range of zinc status, and more studies will be needed to resolve this issue. In contrast with the uncertain relation between the baseline mean serum zinc concentration and growth response to zinc supplementation, there was a large and consistent response of serum zinc concentration to zinc supplementation. Thus, the change in serum zinc concentration can be used as a practical indicator to document that zinc supplements have been delivered, consumed, and absorbed successfully by populations exposed to zinc intervention programs.

It is difficult to estimate the absolute growth increments that might be implied by the observed effect sizes because the former are likely to vary according to the age of the child, the duration of supplementation, and possibly other factors. Nevertheless, to gain further insight into the practical significance of the observed overall weighted mean effect size of 0.35 SD units for the effect of zinc supplementation on linear growth, we reanalyzed the subset of 25 studies that presented results in terms of absolute growth increments. The weighted mean effect size for these 25 studies was 0.39 SD, which was not significantly different from the weighted mean effect size for all studies ($P = 0.24$). Among this subset of studies, which had a mean duration of 6.8 mo (range: 2–15 mo) and included children with a mean initial age of 2.8 y (range: 0–10.1 y), the children who received supplemental zinc gained 0.72 ± 0.98 cm more in height during the periods of observation. This absolute difference in the growth increment is within the general range of effect reported previously in studies of food supplementation of young children. For example, in a well-controlled supplementary feeding trial that provided both macronutrients and selected micronutrients to Guatemalan children for almost 3 y (from 3 to 36 mo of age), there was a cumulative effect of 2.5 cm on linear growth (62).

Because of the important functional consequences of zinc deficiency for children's growth and other health outcomes (63, 64), interventions to improve zinc nutriture should be considered in those populations at particularly high risk of zinc deficiency. Additional research will be needed to determine whether the mean serum zinc concentration of a population is a useful predictor of response to zinc supplementation. On the other hand, the population mean serum zinc concentration does increase after supplementation, so this measure can be used to indicate whether public health interventions to promote increased zinc intakes are successful.

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