Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials[1–3]

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ABSTRACT

Background: Zinc deficiency is prevalent in children in developing countries. Supplemental zinc provides therapeutic benefits in diarrhea.

Objective: We sought to measure the effect of supplemental zinc given with oral rehydration therapy during recovery from acute or persistent diarrhea.

Design: We conducted pooled analyses including all available published and unpublished randomized controlled trials of the effects of supplementary oral zinc in children aged <5 y with acute or persistent diarrhea. We used Cox survival regression analysis to evaluate the overall effect of zinc on continuation of diarrhea and possible differential effects in subgroups divided by sex, age, weight-for-height, and initial plasma zinc concentration. Dichotomous outcomes were analyzed by logistic regression. To assess the effects of excluding studies without original data from the pooled analyses, effect-size was estimated for all studies by using random-effects models.

Results: Zinc-supplemented children had a 15% lower probability of continuing diarrhea on a given day (95% CI: 5%, 24%) in the acute-diarrhea trials and a 24% lower probability of continuing diarrhea (95% CI: 9%, 37%) and a 42% lower rate of treatment failure or death (95% CI: 10%, 63%) in the persistent-diarrhea trials. In none of the subgroup analyses were the 2 subgroups of each pair significantly different from each other; however, in persistent diarrhea there tended to be a greater effect in subjects aged <12 mo, who were male, or who had wasting or lower baseline plasma zinc concentrations.

Conclusion: Zinc supplementation reduces the duration and severity of acute and persistent diarrhea. *Am J Clin Nutr* 2000;72:1516–22.

KEY WORDS Diarrhea, diarrheal disease, malnutrition, meta-analysis, randomized controlled trial, zinc, children, infants, developing countries, zinc supplementation, nutrition, zinc deficiency

INTRODUCTION

It is estimated that diarrheal diseases cause >3 million deaths of children in developing countries each year and contribute substantially to malnutrition in surviving children (1). Diarrheal episodes of longer duration, commonly called persistent diarrhea, have the greatest effect on these outcomes (2, 3). Treatment of acute diarrhea with oral rehydration solution has become widespread, resulting in reduced mortality from dehydrating diarrheas but no decrease in the duration of episodes or their consequences, such as malnutrition (4). Furthermore, adherence to recommendations regarding fluid therapy in children with diarrhea is poor because caregivers want to reduce the duration of illness and this often leads them to use antibiotics and other treatments of no proven value (5).

Two well-documented determinants of diarrheal duration are low weight-for-age and decreased cell-mediated immunity (6, 7). A common determinant of both of these factors is zinc deficiency (8, 9), thought to be prevalent in children in developing countries (10). Furthermore, zinc supplementation was shown to reduce the duration and severity of childhood diarrhea in randomized controlled trials (11–20).

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We conducted pooled analyses of available randomized controlled trials that evaluated the effects of supplementary oral zinc given as an adjunct to other therapy in children with acute or persistent diarrhea. We also attempted to identify any differential effects in subgroups of children. Finding these differential effects might allow for targeting of this therapy toward children who would derive the greatest benefit. For the pooled analyses, we formed a group of investigators who had conducted trials of zinc supplementation. To reduce possible publication bias, we attempted to include all investigators of published and unpublished trials. The use of original trial data from individual study children allowed standardization of outcome and subgroup definitions, use of information not included in the publications, and use of proper statistical methods. We present here the pooled analyses of a total of 7 trials of zinc supplementation; there were 3 trials in children with acute diarrhea and 4 trials in children with persistent diarrhea. We also performed a meta-analysis of the effect of zinc on diarrheal duration; we included all known trials to ensure that our results were not biased because we excluded from the pooled analyses 3 trials for which original data were not available.

SUBJECTS AND METHODS

We attempted to locate all published and unpublished randomized controlled trials of oral zinc supplementation in preschool children in developing countries. We systematically searched MEDLINE, SCI-SCIMATE, CURRENT CONTENTS, Cochrane Clinical Trials Register, and references from articles. Potential funding agencies were also contacted to identify trials. International agencies such as the World Health Organization (WHO) and UNICEF were contacted. The principal investigators of known trials and researchers in the micronutrient field were asked to identify trials.

Studies eligible for inclusion were randomized, controlled, and masked trials that assessed the adjunctive therapeutic benefit of zinc supplements containing ≥50% of the US recommended dietary allowance (RDA) per day in children aged <5 y. The children had acute (<14 d pre-enrollment duration) or persistent (≥14 d pre-enrollment duration) diarrhea and resided in a developing country. Subgroups for the analyses were defined a priori as follows: sex, age (<12 mo or ≥12 mo), and weight-for-height z score (<−2 or ≥−2 compared with the National Center for Health Statistics reference) (21). Subgroups for baseline plasma zinc concentration were defined as those subjects below or above the median plasma zinc concentration for that study. The subgroups were selected because some original trial reports suggested differential effects by sex, age, nutritional status, or baseline plasma zinc concentrations.

From the search, 26 zinc supplementation trials were identified and 10 of these were therapeutic trials that met the inclusion criteria (11–20). Of the 5 trials of zinc therapy for acute diarrhea, only 3 were incorporated into the pooled analysis because original, individual case data were no longer available for one trial (11) and the investigator did not provide data for another (16). Of the 5 trials on persistent diarrhea, 1 could not be included because original data were not available (12). The published data from the 3 trials that could not be included in the pooled analysis were incorporated in other analyses of effect size for all known trials. All trials used standard fluid and dietary case management of diarrhea as recommended by the WHO (4).

Principal investigators of the trials included in the pooled analysis agreed to join the Zinc Investigators' Collaborative (ZINC) Group, which also included 2 external advisors (SMB and RM) selected by the WHO Programme on Child Health and Development and 1 advisor selected by the coordinators of the ZINC Group (KHB). An initial meeting with some of the investigators and correspondence with the others resulted in consensus on trial inclusion criteria, subgroup and outcome definitions, and procedures for the pooled analyses. After a preliminary analysis, the ZINC Group met to consider conclusions and implications and to evaluate the need for additional analyses. Final analyses and draft manuscripts were approved by all investigators who had trials included and by the advisors.

For the acute-diarrhea trials, diarrhea was defined as 3 or 4 loose stools (depending on the original trial definition) in a 24-h period. The final day of diarrhea was defined as the last day meeting the above definition followed by 48 h without diarrhea. For the persistent-diarrhea trials, the definitions of diarrhea and recovery used in the original trials were retained, as was the definition of treatment failure (generally an increase in diarrheal severity, occurrence of dehydration, or continued diarrhea for >7 d or >14 d). These trials differed in their withdrawal criteria. In the pooled analysis of recovery after enrollment, data from children who withdrew from the study, who were declared a treatment failure, or who died were included up to the time that they dropped out of the study. Children who had no diarrhea after enrollment in the study or who had an unknown pre-enrollment duration were excluded from the analyses of diarrheal recovery. In the acute-diarrhea analysis of 2446 children, 1 did not have a pre-enrollment duration and 30 had no diarrhea after enrollment. In the persistent-diarrhea analysis of 640 children, 3 did not have a pre-enrollment duration and 84 had no diarrhea after enrollment.

Individual subject data were provided from each trial with outcomes redefined as necessary, along with descriptive information on the trial methods and study populations. For the Indonesian trial that included >1 diarrheal episode per child, only the first episode for each child was used.

A detailed methodologic assessment and scoring system (available on request) for assessment of study quality was used. This system incorporated standard randomized controlled trial quality assessments and items specific to these trials, such as definitions of recovery and degree of co-intervention. Trials were independently evaluated by REB and SS and any disagreements were resolved by further review of the methods and consensus. At a meeting of the ZINC Group, the methodologic scores were reviewed by the principal investigators of the trials and any errors and inconsistencies were corrected.

For the pooled analysis of trials with available individual child data, the therapeutic effect of zinc on the duration of diarrhea was analyzed by using Cox survival regression models stratified by individual trial (22, 23) using the exact method for handling ties (23, 24). In the simplest of these, continuation of the episode after enrollment was modeled as the dependent variable and treatment group and pre-enrollment duration were independent variables. Additional analyses were performed with nutritional status, sex, and age added as covariates. The analyses were performed with SAS (version 8.0; SAS Institute, Cary, NC). These models permitted calculation of the relative hazard (RH) for continuation of the episode and its 95% CI. The control group was coded as 1 and the zinc group as 0, resulting in an RH of <1 for a beneficial effect, consistent with the beneficial effect

TABLE 1
Characteristics of trials that evaluated the therapeutic effects of zinc supplementation in acute or persistent diarrhea

Trial	No. of children in zinc group	No. of children in control group	Enrollment		Zinc supplement	Control supplement	Frequency of supplementation
			Age	Nutritional criteria			
			mo				
Acute diarrhea[1]							
Indonesia (18)	739	659	3–35	None	4–5 mg/kg as acetate	Placebo	Divided into 2 doses daily
India (13)	456	481	6–35	No severe malnutrition	20 mg as gluconate + vitamins A, B, D, and E	Vitamins A, B D, and E	Daily
Bangladesh (14)	57	54	3–24	Weight-for-age below 76th percentile	20 mg as acetate + vitamins A, B, D, and E	Vitamins A, B, D, and E	Divided into 3 doses daily
Persistent diarrhea[2]							
Peru (19)	139	136	6–35	None	20 mg as gluconate	Placebo	Daily
Bangladesh (15)	95	95	3–24	None	20 mg as acetate + vitamins A, B, and D	Vitamins A, B, and D	Divided into 3 doses daily
Bangladesh (20)	44	44	6–24	Weight-for-age below 76th percentile	20 mg as acetate + vitamins A, B, C and D[3]	Vitamins A, B, C, and D[3]	Divided into 2 doses daily
Pakistan (17)	43	44	6–36	Weight-for-age z score ≤ -2	3 mg/kg as sulfate + vitamins A, B, C, and D	Vitamins A, B, C, and D	Daily

[1] Defined as <7 d, except in the Bangladesh study (14), in which the definition was <3 d.
[2] Defined as ≥14 d.
[3] Only half the subjects received vitamin A.

expressed by the odds ratio (OR). The dichotomous dependent outcomes, which were duration ≤7 d compared with >7 d postenrollment in acute-diarrhea trials and treatment failure or death in persistent-diarrhea trials, were analyzed by using logistic regression models. These models were also stratified by trial, with treatment group, subgroup categories, and potential interaction terms as independent variables, to calculate the ORs and 95% CIs (25). To evaluate a possible clustering effect in the survival analysis, we used random-effects extensions of the Cox models (26); the robust correlation matrix and SEs were calculated with use of the PHREG procedure in SAS version 8.0 (27).

To estimate summary effects, including data from the 3 eligible trials for which original data were not available, we performed a meta-analysis of effect size. Means and SDs of diarrheal duration in the zinc and control groups were used to estimate the effect size. By using Bayesian methods, we estimated a joint posterior probability distribution for the variable of interest (28). From these joint distributions, effect size and 95% CI were calculated for each study. By using a random-effects hierarchical model (28), the summary effect size and the 95% CI were estimated. The confidence profile method was also used to estimate the ORs and CIs for effects on episodes lasting >7 d in acute-diarrhea trials and effects on treatment failure or death in the persistent-diarrhea trials; summary estimates were determined by using random-effects hierarchical models (28). Of the 3 trials not included in the pooled analysis, only 1 (16) reported data on these outcomes and so was incorporated into the analysis. Finally, to evaluate heterogeneity across studies, we determined the chi-square for heterogeneity.

RESULTS

The 3 acute-diarrhea trials, conducted in Indonesia (18), India (13), and Bangladesh (14), were similar in terms of the age of the children and the dose of zinc (**Table 1**). The study in Bangladesh enrolled only children who were underweight. In part because of this, the nutritional status of children in the 3 trials differed (**Table 2**). In the trial for which plasma zinc concentrations before and after treatment were available, the mean plasma zinc concentration increased in the zinc group after treatment but did not increase in the control group; this difference between the zinc and control groups was not statistically significant.

In the acute-diarrhea trials (**Table 3**), zinc-supplemented children had a 15% lower probability of continuing diarrhea on a given day (95% CI: 8%, 22%) than did the children in the control group. The random-effects extension of survival analysis yielded similar estimates (Table 3).

In the effect-size analysis, which used data from all 5 acute-diarrhea trials, mean duration of diarrhea was lower in the zinc-supplemented group in all 5 studies, significantly so in 2 studies (**Table 4**). The effect size for reduction in the mean duration of the diarrheal episode in individual trials ranged from 10% to 24%. The summary estimate of the effect size for reduction in duration was 16% (95% CI: 7%, 26%). The results of these 5 trials were not significantly heterogenous (chi square = 2.99, $P = 0.56$). For the analysis of the effect on episodes lasting >7 d, zinc-supplemented children had a 27% lower rate of prolonged episodes than did control children (OR = 0.73; 95% CI: 0.55, 0.98). These results were also not significantly heterogenous (chi square = 1.39, $P = 0.71$).

The 4 persistent-diarrhea trials in Peru (19), Bangladesh (15, 20), and Pakistan (17) were similar to each other and to the acute-diarrhea trials in terms of age group and dose of zinc (Table 1). One of the trials in Bangladesh (20) and the one in Pakistan had nutritional-status criteria for enrollment. The background characteristics of children in these 4 trials differed: the children in the Peru study were substantially better nourished than were children in the other studies in terms of weight, but not height (Table 2). This study also differed in that it enrolled children in the community rather than in a health facility. In 3 of the 4 trials, the plasma zinc concentrations showed a significant

TABLE 2
Background characteristics and plasma zinc concentrations before and after supplementation in therapeutic zinc trials

Trial	Percentage with illiterate mothers	Percentage with height-for-age z score < -2	Percentage with weight-for-height z score < -2	Plasma zinc concentration			
				Zinc group		Control group	
				Before	After	Before	After
	%	%	%	μmol/L			
Acute diarrhea							
Indonesia (18)	32	23	15	NA[1]	NA	NA	NA
India (13)	81	67	21	10.0 ± 0.1[2]	NA	9.9 ± 0.1	NA
Bangladesh (14)	57	58	51	11.2 ± 0.4	12.6 ± 0.8	12.6 ± 0.7	12.3 ± 0.6
Persistent diarrhea							
Peru (19)	5	30	1	11.4 ± 0.3	17.0 ± 0.7[3]	11.0 ± 0.2	11.7 ± 0.4
Bangladesh (15)	40	34	42	13.4 ± 0.5	13.6 ± 0.6	13.4 ± 0.5	11.9 ± 0.5[4]
Bangladesh (20)	51	52	46	14.0 ± 1.0	17.0 ± 1.2[5]	14.4 ± 1.0	13.2 ± 0.8
Pakistan (17)	83	87	35	11.9 ± 0.8	15.8 ± 1.2[3]	10.8 ± 0.5	11.7 ± 0.8

[1] NA, not available.
[2] $\bar{x}$ ± SE.
[3–5] Significantly different from before supplementation (t test): [3] $P < 0.01$, [4] $P = 0.03$, [5] $P = 0.02$.

response to zinc supplementation but did not change significantly in the control group (Table 2).

In a stratified analysis of the persistent-diarrhea trials (Table 3), zinc-supplemented children had a 24% lower probability of continuation of diarrhea on a given day (95% CI: 8%, 38%) than did control children. The random-effects survival analysis yielded similar results (Table 3). Zinc-supplemented children in these trials had a 42% lower rate of treatment failure or death (OR = 0.58; 95% CI: 0.37, 0.40) than did control children. For this outcome, the trials were significantly heterogeneous (chi-square = 8.6, $P = 0.04$), which was due mainly to the results of the study conducted in Pakistan. After exclusion of this study, there was no significant heterogeneity (chi-square = 3.2, $P = 0.20$). Random-effects analysis for this outcome yielded an OR of 0.61 (95% CI: 0.26, 1.46).

In the effect-size analysis, which used data from all 5 persistent-diarrhea trials including 1 trial (12) not included in pooled analysis, the mean duration of diarrhea tended to be lower in the zinc-supplemented group in all 5 studies, although this result was significant in only 1 study. The effect size for reduction in the mean duration of the diarrheal episode in individual trials ranged from 12% to 53%. The summary estimate of the effect size for reduction in duration was 29% (95% CI: 6%, 53%). The results of these 5 trials were not significantly heterogenous (chi-square = 2.26, $P = 0.69$).

In the subgroup analyses in acute-diarrhea trials, the effect of zinc supplementation in each of the subgroups by age, wasting, and sex was significant (**Figure 1**). The subgroups did not differ from each other in terms of the magnitude of this effect. In persistent diarrhea, age <12 mo, wasting, and male sex were

TABLE 3
Pooled analysis of the therapeutic effect of zinc supplementation on acute and persistent diarrhea

Trial	Effect on recovery				Effect on continuation for >7 d, failure, or death[1]		
	Recovered	Censored[2]	Excluded[3]	Relative hazard (95% CI)	Zinc	Control	Odds ratio (95% CI)
	n	n	n		n (%)	n (%)	
Acute diarrhea							
Indonesia (18)	1368	0	30	0.92 (0.83, 1.02)	47 (6.4)	57 (8.6)	0.72 (0.48, 1.07)
India (13)	931	6	0	0.79 (0.69, 0.90)	70 (15.4)	85 (17.7)	0.85 (0.60, 1.19)
Bangladesh (14)	101	9	1	0.85 (0.57, 1.28)	14 (24.6)	16 (29.6)	0.77 (0.33, 1.79)
Pooled				0.85 (0.78, 0.92)			0.79 (0.61, 1.01)
Pooled multifactorial				0.85 (0.78, 0.92)			0.80 (0.62, 1.02)
Pooled random effect				0.85 (0.76, 0.95)			0.78 (0.56, 1.09)
Persistent diarrhea							
Peru (19)	164	24	87	0.82 (0.60, 1.12)	11 (7.9)	13 (9.6)	0.81 (0.35, 1.88)
Bangladesh (15)	138	52	0	0.85 (0.61, 1.19)	7 (7.4)	17 (17.9)	0.37 (0.14, 0.92)
Bangladesh (20)	55	32	1	0.45 (0.26, 0.78)	9 (20.5)	22 (50.0)	0.25 (0.10, 0.64)
Pakistan (17)	55	32	0	0.98 (0.57, 1.67)	16 (37.2)	12 (27.3)	1.58 (0.64, 3.91)
Pooled				0.76 (0.62, 0.92)			0.60 (0.38, 0.93)
Pooled multifactorial				0.75 (0.62, 0.91)			0.58 (0.37, 0.90)
Pooled random effect				0.76 (0.63, 0.91)			0.61 (0.26, 1.46)

[1] Effect on continuation for >7 d was used for acute-diarrhea trials; effect on treatment failure or death was used for persistent-diarrhea trials.
[2] Includes withdrawal, treatment failure, and death.
[3] Exclusions were because the postenrollment duration of diarrhea was zero, except for 4 cases for which pre-enrollment duration was missing.

TABLE 4
Meta-analysis of the therapeutic effects of zinc supplementation on the mean duration of acute and persistent diarrhea

Trial	Zinc group	Control group	Effect size (95% CI)
	d	*d*	
Acute diarrhea			
Indonesia (18)	3.5 ± 2.4[1]	3.8 ± 2.6	0.096 (−0.010, 0.201)
India (13)	4.5 ± 3.6	5.4 ± 3.4	0.238 (0.109, 0.367)[2]
Bangladesh (14)	5.1 ± 2.5	5.5 ± 2.7	0.122 (−0.269, 0.513)
India (11)	3.4 ± 1.8	3.8 ± 1.7	0.199 (−0.357, 0.755)
Bangladesh (16)	6.1 ± 5.1	7.1 ± 5.1	0.178 (0.028, 0.329)[3]
Summary estimate			0.162 (0.068, 0.256)
Persistent diarrhea			
Peru (19)	2.2 ± 1.7	3.0 ± 2.5	0.360 (0.051, 0.670)[3]
Bangladesh (15)	6.5 ± 3.7	7.0 ± 3.8	0.135 (−0.199, 0.470)
Bangladesh (20)	2.9 ± 1.4	3.5 ± 1.4	0.421 (−0.059, 0.901)
Pakistan (17)	5.1 ± 3.3	5.5 ± 2.7	0.122 (−0.408, 0.652)
India (12)	3.7 ± 1.1	4.5 ± 1.9	0.530 (−0.101, 1.160)
Summary estimate			0.293 (0.060, 0.525)

[1] $\bar{x}$ ± SD.
[2] $P < 0.01$.
[3] $P = 0.03$.

associated with significant effects of zinc on continuation of diarrhea, but their corresponding alternatives were not (Figure 1). However, comparisons between the 2 categories for each subgroup analysis in acute and persistent diarrhea did not show significant differences.

In the analysis of subgroups with lower or higher initial plasma zinc concentrations and acute diarrhea, there was a significant pooled effect in both subgroups, and this effect tended to be greater in the subgroup with lower baseline zinc concentrations (**Table 5**). In this analysis in children with persistent diarrhea, there was a significant pooled effect only in the subgroup with lower baseline zinc concentrations. The subgroup with higher baseline zinc concentrations had more variability among the trials and a pooled effect that suggested a benefit of zinc supplementation, although this result was not significant.

The methodologic score of the trials ranged from 76 to 96 of a possible 96. This score, which served as an indicator of study quality, did not correlate with effect size. A possible dose effect for the zinc supplement could not be examined because of the narrow range of zinc doses used in the trials. The effect of zinc alone versus zinc given with selected vitamins (which were also given to the control group) was evaluated by modeling an interaction term of vitamins and zinc. For both the acute- and persistent-diarrhea analyses, the interaction was not significant; however, only one acute- and one persistent-diarrhea trial that did not involve vitamin supplementation was available.

DISCUSSION

These pooled analyses of data from trials of acute and persistent diarrhea in developing countries show that zinc, given in a daily dose of about twice the RDA, significantly reduces the duration of acute or persistent diarrhea. Three trials (11, 12, 16) that met the inclusion criteria could not be used in the pooled analysis but were included in meta-analyses of all eligible trials (Table 4). Note that one of the trials conducted in Bangladesh used a factorial design with zinc and vitamin A; the zinc effect on recovery from diarrhea was significant but there was no effect of vitamin A (16).

It is important to examine the effect of zinc supplementation on other measures of severity, such as diarrheal stool output, occurrence of dehydration, treatment failure, or death. The trials provided some information on these outcomes, but the different types of study data available precluded many pooled analyses. Three acute-diarrhea trials with appropriate outcome measures all found reductions in diarrheal severity in zinc-supplemented children compared with control children. Of the 2 trials conducted in India, one found 18% fewer diarrheal stools/d ($P < 0.1$) (11) and the other found 39% fewer watery stools/d ($P < 0.02$) (13). In the only hospital-based trial of acute diarrhea, zinc-supplemented children had a 28% lower measured diarrheal stool output/d ($P = 0.06$) (14). Of the 4 persistent-diarrhea trials that included severity measures, 2 found no significant difference between the groups (15, 19). The trial conducted in India reported a 21% lower diarrheal stool frequency ($P = 0.08$) (12) and a hospital-based trial in Bangladesh found a 37% lower measured stool output ($P < 0.02$) in zinc-supplemented children (20). Although there were too few

FIGURE 1. Therapeutic effect of zinc supplementation assessed by the relative hazard of continuation of diarrhea in subgroups of children with acute or persistent diarrhea (pooled analysis).

TABLE 5
Effect of zinc supplementation on diarrhea by baseline plasma zinc subgroup

Trial	Baseline plasma zinc below median	Baseline plasma zinc above median
Acute diarrhea		
India (13)	0.74 (0.62, 0.89)[1]	0.83 (0.70, 1.00)
Bangladesh (14)	0.90 (0.52, 1.59)	0.89 (0.57, 1.39)
Pooled	0.75 (0.63, 0.90)	0.83 (0.69, 0.99)
Persistent diarrhea		
Peru (19)	0.79 (0.51, 1.23)	0.83 (0.53, 1.31)
Bangladesh (15)	0.69 (0.43, 1.11)	1.01 (0.63, 1.61)
Bangladesh (20)	0.46 (0.24, 0.91)	0.43 (0.17, 1.11)
Pakistan (17)	0.82 (0.40, 1.70)	1.12 (0.48, 2.70)
Pooled	0.70 (0.53, 0.91)	0.88 (0.67, 1.17)

[1] Relative hazard for continuation of diarrhea (95% CI).

treatment failures or deaths for a pooled analysis of acute-diarrhea trials, these outcomes were reduced by 42% with zinc supplementation in the pooled analysis of persistent-diarrhea trials.

The results of these pooled analyses and additional information from other published randomized trials indicate that zinc, given during acute or persistent diarrhea, can have substantial clinical benefit and suggest that this adjunctive therapy could reduce the risks of dehydration and death from diarrhea. The findings of these trials, which were performed in several different developing countries, indicate that therapeutic use of zinc may have wide applicability. The similar benefits seen in subgroups divided by age, nutritional status, and sex and the relative safety of oral zinc (29) suggest that there is no need to target specific population groups. This further enhances the feasibility of this therapy.

The reduction in the duration and severity of diarrhea as a result of zinc supplementation may be perceived as desirable by the caregivers of children with diarrhea. Perhaps the use of this effective and inexpensive nutrient supplement would be helpful in efforts to reduce the now common treatment of diarrhea with unnecessary antibiotics and other drugs (5). At the same time, it will be important to continue the promotion of appropriate fluid and dietary therapy as the mainstay of efforts to reduce mortality from diarrhea (4).

Pooled analyses have a number of strengths (30). These include *1*) the use of rigorous methodologic assessment, *2*) the ability to use the most meaningful clinical outcomes, *3*) the standardization of subgroup and outcome definitions, *4*) the use of optimal statistical procedures made possible by the availability of original trial data, and *5*) the involvement of both trial investigators and outside advisors to ensure the most valid results and conclusions. The trials included in this pooled analysis fully met the standards for this mode of evaluation. Four of the trials involved a difference in taste between the zinc and control supplements, but this did not appear to compromise masking. Additional geographic representation (eg, children from Africa) would have been desirable.

Children who were not given zinc supplements generally had stable or declining plasma zinc concentrations; net loss of zinc during diarrhea has been found (31, 32). Zinc-supplemented children generally had increases in plasma zinc concentrations, indicating an effect of the supplement. The subgroup analyses for initial plasma zinc concentration were done by classifying children in each trial as above or below the median zinc concentration for that trial. Because the different distributions of plasma zinc concentrations in different settings may have reflected methodologic differences among the trials, we thought this approach was more appropriate than selecting a single plasma zinc concentration for use in all the trials. Furthermore, plasma zinc can be reduced by illness; therefore, some of the variability could reflect the severity of diarrhea or concomitant infections (33). In acute diarrhea, both of the subgroups with lower and higher baseline zinc concentrations showed a significant effect of supplemental zinc, but the effect tended to be larger in the group with lower baseline zinc concentrations. In children with persistent diarrhea, the group with lower baseline zinc concentrations showed a significant benefit of zinc supplementation and the group with higher baseline zinc did not, although the pooled effect estimate did suggest benefit. These effects suggest that zinc should be provided to all children with acute and persistent diarrhea in such settings.

The mechanisms of these effects of zinc on diarrhea are unclear. Zinc deficiency is associated with many immunologic deficits, and zinc supplementation was shown to improve immune function in children in developing countries (9, 34) and to reduce the incidence and prevalence of diarrhea (35). Other possible mechanisms include effects of zinc deficiency on intestinal permeability (36, 37), regulation of intestinal water and electrolyte transport (38), brush border enzymatic function (39, 40), and intestinal epithelial tissue repair (41, 42).

The use of zinc as adjunctive therapy has the potential to improve the management of diarrhea and increase survival in children, if it can be incorporated into diarrheal disease control programs in developing countries. Primary prevention of zinc deficiency would be expected to reduce infectious disease morbidity and improve the growth and development of children (8, 35, 43–45) and might also reduce the severity of diarrhea. Attention should now focus on the best means of providing zinc during diarrhea or on other ways to improve the zinc nutriture of children in developing countries.

Robert Black and Sunil Sazawal organized and conducted the pooled analyses and drafted the manuscript. Zulfiqar Bhutta, Adi Hidayat, Farida Khatun, Mary Penny, Swapan Roy, and Sunil Sazawal were principal investigators of zinc therapeutic trials. Julie Meeks Gardner, Nguyen Ninh, Jorge Rosado, Marie Ruel, and Anu Shankar were principal investigators of zinc preventive trials and participated in meetings of the ZINC Group. Kenneth Brown, Sheila M Bird, and Reynaldo Martorell served as advisors. Members of the ZINC Group are listed in alphabetical order. Other investigators in the therapeutic trials in the pooled analyses were A Achadi, S Soedarmo, and Sunoto in Indonesia; MK Bhan, N Bhandari, RE Black, S Jalla, and A Sinha in India; S Akramuzzaman, R Behrens, G Fuchs, R Haider, D Mahalababis, M Abdul Malek, NR Sarkar, and A Tomkins in Bangladesh; RE Black, KH Brown, A Duran, CF Lanata, B Lönnerdal, RM Marin, and JM Peerson in Peru; and Z Issani, S Niazi, and S Nizami in Pakistan.

REFERENCES

1. Bern C, Martines J, de Zoysa I, Glass RI. The magnitude of the global problem of diarrhoeal disease: a ten-year update. Bull World Health Organ 1992;70:705–14.
2. Victora CG, Huttly SRA, Fuchs SC, et al. International differences in clinical patterns of diarrhoeal deaths: a comparison of children from Brazil, Senegal, Bangladesh, and India. J Diarrhoeal Dis Res 1993;11:25–9.
3. Black RE. Persistent diarrhea in children of developing countries. Pediatr Infect Dis J 1993;12:751–61.
4. Richard L, Claeson M, Pierce NF. Management of acute diarrhea in children: lessons learned. Pediatr Infect Dis J 1993;12:5–9.
5. Harris S, Black RE. How useful are pharmaceuticals in managing diarrhoeal diseases in developing countries? Health Policy Plan 1991;6:141–7.

6. Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrheal duration, but not incidence, among young children in a longitudinal study in rural Bangladesh. Am J Clin Nutr 1984; 39:87–94.
7. Baqui AH, Sack RB, Black RE, Chowdhury HR, Yunus M, Siddique AK. Cell-mediated immune deficiency and malnutrition are independent risk factors for persistent diarrhea in Bangladeshi children. Am J Clin Nutr 1993;58:543–8.
8. Brown KH, Peerson JM, Allen LH. Effect of zinc supplementation on children's growth: a meta-analysis of intervention trials. Bibl Nutr Dieta 1997;54:76–83.
9. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 1998; 68(suppl):447S–63S.
10. Sandstead HH. Is zinc deficiency a public health problem? Nutrition 1995;11:87–92.
11. Sachdev HPS, Mittal NK, Mittal SK, Yadav HS. A controlled trial on utility of oral zinc supplementation in acute dehydrating diarrhea in infants. J Pediatr Gastroenterol Nutr 1988;7:877–81.
12. Sachdev HPS, Mittal NK, Yadav HS. Oral zinc supplementation in persistent diarrhoea in infants. Ann Trop Paediatr 1990;10:63–9.
13. Sazawal S, Black RE, Bhan MK, Ghandari N, Sinha A, Jalla S. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med 1995;333:839–44.
14. Roy SK, Tomkins AM, Akramuzzaman SM, et al. Randomised controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. Arch Dis Child 1997;77:196–200.
15. Roy SK, Tomkins AM, Mahalanabis D, et al. Impact of zinc supplementation on persistent diarrhoea in malnourished Bangladeshi children. Acta Paediatr 1998;87:1235–9.
16. Faruque ASG, Mahalanabis D, Haque SS, Fuchs GJ, Habte D. Double-blind, randomized, controlled trial of zinc or vitamin A supplementation in young children with acute diarrhoea. Acta Paediatr 1999;88:154–60.
17. Bhutta ZA, Nizami SQ, Isani Z. Zinc supplementation in malnourished children with persistent diarrhea in Pakistan. Pediatrics 1999; 103:1–9.
18. Hidayat A, Achadi A, Sunoto, Soedarmo SP. The effect of zinc supplementation in children under three years of age with acute diarrhea in Indonesia. Med J Indonesia 1998;7:237–41.
19. Penny ME, Peerson JM, Marin RM, et al. Randomized, community-based trial of the effect of zinc supplementation, with and without other micronutrients, on the duration of persistent childhood diarrhea in Lima, Peru. J Pediatr 1999;135:208–17.
20. Khatun UHF. Impact of zinc and vitamin A supplementation in malnourished hospitalized children suffering from persistent diarrhoea. PhD thesis. The University of Dhaka, Dhaka, Bangladesh, 1998.
21. National Center for Health Statistics. Growth curves for children birth–18 years. United States. Vital and health statistics. Washington, DC: US Government Printing Office, 1977. [Series 11. DHEW publication (PHS) 78 1650.]
22. Cox DR, Oakes D. Analysis of survival data. London: Chapman & Hall, 1984.
23. Allison PD. Survival analysis using the SAS system. A practical guide. Cary, NC: SAS Institute Inc, 1995:233–49.
24. Delong DM, Guirguis GH, So YC. Efficient calculations for subset selection probabilities with application to Cox regression. Biometrika 1994;81:607–11.
25. Matthews DE, Farewell VT. Using and understanding medical statistics. 2nd ed. Basel, Switzerland: Karger, 1988:164–6.
26. Cantor A. Extending SAS survival analysis techniques for medical research. Cary, NC: SAS Institute Inc, 1997:103–33.
27. SAS Institute Inc. SAS/STAT changes and enhancements, release 6.10. Cary, NC: SAS Institute Inc, 1994:93–102.
28. Eddy DM, Hasselblad V, Shachter R. Meta-analysis by confidence profile method. The statistical synthesis of evidence. New York: Academic Press Inc, 1992:109–69.
29. Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc: health effects and research priorities for the 1990s. Environ Health Perspect 1994;102:5–46.
30. Blettner M, Sauerbrei W, Schlehofer B, Scheuchenpflug, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. Int J Epidemiol 1999;28:1–9.
31. Castillo-Duran C, Vial P, Uauy R. Trace mineral balance during acute diarrhea in infants. J Pediatr 1988;113:452–7.
32. Ruz M, Solomons NW. Fecal excretion of endogenous zinc during oral rehydration therapy for acute diarrhea. J Trace Elem Exp Med 1995;7:89–100.
33. Brown KH, Lanata CF, Yuen ML, Peerson JM, Butron B, Lönnerdal B. Potential magnitude of the misclassification of a population's trace element status due to infection: example from a survey of young Peruvian children. Am J Clin Nutr 1993;58:549–54.
34. Sazawal S, Black RE, Jalla S, Mazumdar S, Sinha A, Bhan MK. Effect of zinc supplementation on cell-mediated immunity and lymphocyte subsets in preschool children. Indian Pediatr 1997;34:589–97.
35. Zinc Investigators' Collaborative Group. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. J Pediatr 1999;135:689–97.
36. Moran JR, Lewis JC. The effects of zinc deficiency on intestinal permeability: an ultrastructural study. Pediatr Res 1985;19:968–73.
37. Roy SK, Behrens RH, Haider R, et al. Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome. J Pediatr Gastroenterol Nutr 1992;15:289–96.
38. Blanchard RK, Cousins RJ. Upregulation of rat intestinal uroguanylin mRNA by dietary zinc restriction. Am J Physiol 1997;272:G972–8.
39. Gebhard RI, Karouani R, Prigge WF, McClain CJ. Effect of severe zinc deficiency on activity of intestinal disaccharidases and 3-hydroxy-3-methylglutaryl coenzyme A reductase in the rat. J Nutr 1983;113:855–9.
40. Jones PE, Peters TJ. Oral zinc supplements in non-responsive coeliac syndrome: effect on jejunal morphology, enterocyte production, and brush border disaccharidase activities. Gut 1981;22:194–8.
41. Bettger WJ, O'Dell BL. A critical physiological role of zinc in the structure and function of biomembranes. Life Sci 1981;28:1425–38.
42. Arcasoy A, Akar N, Ors U, Delibasi L, Karayalcin S. Ultrastructural changes in the mucosa of the small intestine in patients with geophagia (Prasad's syndrome). J Pediatr Gastroenterol Nutr 1990; 11:279–82.
43. Sazawal S, Bentley M, Black RE, Dhingra P, George S, Bhan MK. Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. Pediatrics 1996;98:1132–7.
44. Bentley ME, Caulfield LE, Ram M, et al. Zinc supplementation affects the activity patterns of rural Guatemalan infants. J Nutr 1997;127:1333–8.
45. Sandstead HH, Penland JG, Alcock NW, et al. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. Am J Clin Nutr 1998; 68(suppl):470S–5S.