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Zinc Supplementation in Malnourished Children With Persistent Diarrhea in Pakistan

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ABSTRACT. *Objective.* To evaluate the potential benefit of dietary supplementation of a rice-lentil (Khitchri) and yogurt diet with 3 mg/kg/d of elemental zinc (as zinc sulfate) in hospitalized malnourished children (age 6–36 months) with persistent diarrhea for 14 days.

Methodology. Randomized, double-blind placebo-controlled trial.

Setting. Nutrition Research Ward at the National Institute of Child Health, Karachi, Pakistan, where children were admitted for 14 days of inpatient supervised rehabilitation.

Main Outcome Measures. Primary outcome: overall weight gain by day 14. Secondary outcomes: overall energy intake, stool output, time to diarrheal recovery and weight gain (≥ 3 days), plasma zinc, copper, prealbumin, and insulin-like growth factor-1.

Results. Of 87 children randomized for supplementation with either zinc or placebo, the two groups were comparable at admission in terms of severity and duration of diarrhea, as well as nutritional and anthropometric parameters. The overall weight gain, stool volume, stool frequency, as well as the time taken for diarrheal recovery or steady weight gain, were comparable for both supplemented children and controls. Supplemented children had a significant improvement in plasma zinc levels and serum alkaline phosphatase by day 14 of therapy in comparison with controls. Plasma copper levels were low in both groups at admission and although an increase was seen in control children, levels decreased further after zinc supplementation. There was no significant difference between the two groups for hemoglobin, serum albumin, prealbumin, and plasma insulin-like growth factor-1 increments during the course of therapy. Evaluation of primary and secondary outcome criteria among the subset of children with plasma zinc levels $< 60 \mu\text{g/d}$ at admission did not reveal any significant differences.

Conclusions. Although there was satisfactory recovery in malnourished children with persistent diarrhea receiving the Khitchri-yogurt diet, there was no evidence of improved weight gain or acceleration of recovery from diarrhea with zinc supplementation. In contrast, the reduction in plasma copper levels in zinc-supplemented malnourished children suggests that caution should be exercised in supplementing severely malnourished children with zinc alone. *Pediatrics* 1999;103(4). URL: <http://www.pediatrics.org/cgi/content/full/103/4/e42>; persistent diarrhea, Khitchri, yogurt, nutritional rehabilitation, zinc, copper.

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ABBREVIATIONS. PD, persistent diarrhea; NICH, National Institute of Child Health; IV, intravenous; K-Y, rice-lentil (Khitchri) and yogurt diet; IGF-I, insulin-like growth factor-1; L/R, lactulose/rhamnose ratio.

Zinc supplementation during acute diarrhea has been shown to enhance recovery, and continuous supplementation studies have shown a significant reduction in the incidence of persistent diarrhea (PD). There is conflicting evidence of the benefit of zinc supplementation in malnourished children in improving the rate and composition of weight gain. We hypothesized that zinc supplementation in malnourished children with PD might lead to improved nutritional outcome and diarrheal recovery.

There is increasing recognition of the importance of zinc in childhood growth and development¹ and subclinical zinc deficiency has been widely recognized as a significant limiting factor for growth among children in both developing and developed countries.² Given the close association of zinc deficiency with stunting in apparently normal³ as well as malnourished children,⁴ the disorder has been labeled by many as a public health problem in developing countries.⁵ Although the optimal method of assessment of body zinc status is uncertain, plasma levels currently offer the best means of evaluation⁶ and it has also been suggested that the best indication of a deficient state may be the clinical response to supplementation.⁷ The clinical response to zinc supplementation in pathologic states is, however, variable. Although some zinc supplementation studies of malnourished children have suggested improved growth and morbidity,^{8,9} others have failed to identify any improvement in linear growth, despite impressive reduction in morbidity rates.^{10–12}

The major effect during community-based supplementation trials with zinc seems to be on diarrhea-related morbidity.^{9,13,14} Although the mechanisms underlying this beneficial effect remain uncertain, there are several reasons to expect a therapeutic benefit of zinc administration during diarrhea. There is considerable evidence of increased intestinal losses of zinc and other micronutrients in diarrheal states.^{15,16} Zinc deficiency has been shown to induce intestinal mucosal structural changes,¹⁷ and to alter disaccharidase activity¹⁸ and intestinal water/electrolyte transport.¹⁹ Zinc deficiency is especially likely to be associated with longer episodes of diarrhea, such as PD (ie,

episodes >2 weeks in duration).²⁰ Although there is compelling evidence of a beneficial effect of zinc supplementation in acute diarrhea,^{21–23} little information exists on the benefit of such an approach in PD. In a preliminary study of zinc supplementation of malnourished children with PD in India, Sachdev et al²⁴ were only able to demonstrate a benefit in a subset with low rectal tissue zinc levels. A similar study among children with PD in Bangladesh revealed a significant improvement in intestinal permeability among those receiving zinc supplements.²⁵

To further evaluate the role of zinc supplementation in malnourished children with PD, we undertook a prospective randomized, double-blind, placebo-controlled trial of zinc supplementation in Karachi, Pakistan.

MATERIALS AND METHODS

The protocol of the study was approved by the respective institutional ethics committees at the Aga Khan University, National Institute of Child Health (NICH), both in Karachi, Pakistan, and the Harvard School of Public Health in Boston, MA.

Patient Selection, Stabilization, and Randomization

Children (age 6–36 months) with PD, defined as four or more unformed stools per day continuously for at least 14 days and with evidence of malnutrition (weight-for-age *z* score ≤ -2.0), were recruited for the study from the ambulatory care services at the NICH between July, 1993 to September, 1995. After verbal explanation of the study protocol and written consent, the children were admitted to the nutrition research ward, with a doctor and nurse in constant attendance. Children with overt evidence of kwashiorkor, and ocular or skin lesions suggestive of vitamin A or zinc deficiency were excluded. The children were clinically evaluated at admission for dehydration and signs of intercurrent illnesses.

All the children were resuscitated during a fixed stabilization period of 24 hours, in which intravenous (IV) and oral rehydration fluids were administered as necessary and antibiotic therapy for concomitant nonenteric infections was initiated. During this period the stool output was quantified and any coexisting dehydration or electrolyte imbalance was corrected. At the end of the stabilization period, those still needing IV fluids or unable to tolerate oral feeds because of concomitant illnesses, were excluded from the study. In others, dietary therapy with a rice-lentil (Khitchri) and yogurt diet (K-Y), previously validated for the treatment of PD,²⁶ was initiated and continued under supervision for 14 days. The children were allocated to receive either zinc supplement (group A) or placebo (group B) by means of a block randomization process. After 14 days, the children were discharged and advised to continue the supplement or placebo for a further 14 days with home available diets. Appropriate amounts of zinc/placebo were provided at discharge and compliance of therapy was assessed by estimation of remaining supplement volume at return appointment. The randomization code, maintained by the Pharmacy Department at the Aga Khan University Hospital, was not available to the investigators until the end of the study.

Diet Preparation and Zinc Supplementation Protocol

The rice-lentil diet (Khitchri) of standard composition (rice, 60 g; lentils, 30 g; cottonseed oil, 10 g dry weight; and salt, 1 g)²⁶ was prepared on-site daily and fresh live yogurt was procured from a single source (Fine Dairies Ltd, Karachi, Pakistan). Breast-feeding was continued as required and the amount fed estimated by immediate test weighing. Water was offered as necessary and the K-Y diet administered ad libitum in gradually increasing amounts, to provide a minimum of 100 kcal/kg/d by day 4 of therapy. The natural zinc content of the diet was estimated to be <2.5 mg of zinc per 100 g. Both groups received 1.5 times the daily recommended doses of multivitamins in a mixture containing vitamin A (4500 units, 1.35 mg), vitamin D (600 units, 15 μ g), vitamin B₁ (2.2 mg), vitamin B₂ (1.8 mg), vitamin B₆ (1.5 mg),

vitamin B₁₂ (4.5 μ g), nicotinamide (15 mg), and vitamin C (75 mg). The zinc and placebo preparations were prepared at the Pharmacy Department of the Aga Khan University Medical Centre and administered blindly according to a block randomization table maintained in the pharmacy. The zinc-supplemented children received 3 mg/kg/d of elemental zinc sulfate in a single daily dose, whereas the placebo consisted of the vehicle only.

Daily amounts of food consumed were estimated by weighing left-over food. Accurate records of stool, vomitus, and urinary output were maintained by quantifying stool output separately from urine by means of adhesive bags. In the case of female infants, given the high rates of urine-stool admixture, only stool frequency and character were recorded after 72 hours of therapy.

Laboratory Measurements

In all cases baseline stool samples for microscopy, pH, and reducing substances were obtained. If there were >10 white cells/high power field on microscopy, a stool specimen was also obtained for culture. In suspected cholera cases (with watery stool output >100 g/kg/d), a sample was obtained for culture in special transport medium of alkaline peptone water (Oxoid, Basingstoke, UK). In addition to serum electrolytes, all children had baseline preprandial samples drawn for a complete blood count, differential, quantitative C-reactive protein, albumin, prealbumin, alkaline phosphatase, and insulin-like-growth factor 1 (IGF-I) estimation. A 1.5-mL sample of blood was also obtained preprandially for plasma zinc and copper estimation in a trace element-free bottle (Vacutainer Systems, Becton Dickinson, Rutherford, NJ), spun within 30 minutes to separate plasma and stored at -20°C until analysis. Intestinal permeability was evaluated at admission by the dual sugar absorption test using lactulose and rhamnose; the children were administered a 20-mL solution of 1% chloroform water containing xylose (0.5 g), lactulose (3.5 g), and rhamnose (0.5 g) before the first morning feed. All urine was collected during the next 6 hours, preserved with chlorhexidine and frozen at -30°C until quantification of sugars by thin layer chromatography.²⁷ These investigations were repeated at days 7 and 14 of therapy. To evaluate any difference between the groups for lactose absorption, in addition to stool-reducing substances, expired air samples were obtained at baseline, day 7, and day 14 after feeding the K-Y diet and analyzed for hydrogen concentration on a breath hydrogen analyzer (Microlyzer 12-i; Quintron Instrument Co, Milwaukee, WI).

Plasma samples for zinc and copper estimation were analyzed by atomic absorption spectrophotometry (Perkin-Elmer 101, Norwalk, CT) using bovine liver extract as standard (Reference Material 1577b; National Institute of Standards & Technology, Gaithersburg, MD). The mean coefficients of variance for zinc and copper estimations were 3% and 5%, respectively. Samples of serum IGF-I estimation were transported in dry-ice to the Endocrinology Research Laboratory, Karolinska Institute, acid ethanol extracted, and analyzed by an IGF-I specific radioimmunoassay.²⁸

Clinical and Anthropometric Monitoring

The children were weighed unclothed preprandially at admission and daily on a double-beam balance (Detecto, Brooklyn, NY), sensitive up to 10 g. Length was obtained on an infant stadiometer, and the occipito-frontal, mid-arm, and mid-thigh circumferences were obtained in a standardized manner using a paper tape. The left mid-triceps skinfold thickness was obtained in duplicate by application of a skinfold caliper (Holtain Ltd, Crymch, UK). These anthropometric measurements were repeated at days 7, 14, and 28.

The degree of dehydration, body temperature, vital signs, and clinical status was recorded twice daily or more frequently, as clinically indicated. A low threshold of screening for infections was maintained, and in cases of suspected septicemia a blood culture was obtained before initiation of broad-spectrum antibiotics (usually IV ampicillin and gentamicin, or IV ceftriaxone in suspected typhoidal salmonellosis). Suspected bacterial lower respiratory infections were evaluated by a chest radiograph and treated in accordance with standard World Health Organization guidelines.

Outcome Definitions

Although the zinc supplementation was also continued for 2 weeks after discharge, we did not attempt to standardize the

dietary intake at home and therefore the primary outcome was defined as the overall weight gain by day 14 of inpatient therapy. In addition several secondary outcomes were also defined, including overall energy intake (kcal/kg/d), stool frequency (number/d), stool volume (g/kg/d) for males, and changes in laboratory parameters including serum albumin, prealbumin, alkaline phosphatase, IGF-I, plasma copper, and zinc during the same 14-day period of therapy in-hospital. To assess the rapidity of recovery, two main endpoints for estimation of therapeutic response were defined. The "time-to-weight gain" was defined as the time taken to achieve weight gain for 3 or more days consecutively after achieving a caloric intake of 100 kcal/kg/d, whereas the "time-to-diarrheal recovery" was defined as the time taken to achieve a reduction in stool volume to <30 g/kg/d in males, stool frequency <4/d in both, and achievement of a semisoft stool consistency. Given the interest in the impact of zinc supplementation on stool volume, the time taken to achieve a 30% and 50% reduction in stool output (frequency in females and both volume and frequency in males) more than admission values was also estimated.

Sample Size Calculation and Analysis

The sample size for the study was estimated according to the formula suggested by Diggle et al²⁹ for analysis of longitudinal continuous data and was based on the known pattern and rate of weight gain (5 ± 3 g/kg/d) in comparably malnourished children with PD receiving the same K-Y diet.^{26,30} It was thus estimated that to achieve at least a 30% difference in weight gain after 14 days of therapy, with 80% power and a type I error of 0.05, 40 cases would be required in each treatment group. The randomization code was maintained independently by the Pharmacy Department at the Aga Khan University Hospital, who remained unaware of the identity of enrolled patients. A mid-term analysis of morbidity and mortality among the study cohort was conducted independently by consultants from the Applied Diarrhoeal Disease Research Program, and the study was allowed to proceed to conclusion.

Data on all randomized children was analyzed on an intent-to-treat basis, irrespective of length of stay in the study. Differences between the two groups at baseline were evaluated by comparison of categorical data by χ^2 analysis or Fisher's exact test as appropriate. Differences for continuous data were compared by the two-tailed Student's *t* test. Sequential data for primary and secondary outcomes at baseline, day 7, and day 14 were evaluated by analysis of variance for repeated measures, evaluating the interaction of time-trend and treatment effect. In addition, the time taken to different endpoints for the two treatment groups was compared by survival analysis using the log rank test. The aforementioned analysis was repeated for the subgroup of children considered zinc deficient based on plasma zinc levels <60 $\mu\text{g/dL}$ (9.18 $\mu\text{mol/L}$).³⁰ All data were analyzed using SPSS (Windows Version 6.1, 1994; SPSS Inc, Chicago, IL) and significance was set at 5%.

RESULTS

A total of 114 children presented to the ambulatory care services at NICH with a history of PD, who consented to be admitted to the study ward for rehabilitation and randomization. Of these, 14 did not have any significant diarrhea during the stabilization period (formed stools with a volume <20 g/kg/d) and were discharged. A further 13 children could not be stabilized during the stabilization period and at the end of 24 hours were either still in need of IV rehydration or unable to take full oral feeds because of concomitant infections. They were thus excluded from the study, and transferred to the pediatric ward for further intensive care as indicated. Of the 87 children thus randomized for allocation to the two treatment groups, 4 (2 in zinc and 2 in placebo groups, respectively) could not stay for the stipulated 14 days in the ward and were discharged prematurely; a further 6 children were removed from the study at different stages because of development

or exacerbation of concomitant infection precluding full enteral feeds (2 cases in the zinc group and 3 cases in the placebo group) or development of recurrent dehydration (one child on zinc supplementation). In all therefore, 77 children completed the study protocol in-hospital for 14 days, followed by a further 14 days of either zinc or placebo supplementation at home.

Table 1 details the admission characteristics of the two groups. Most children had significant malnutrition, but were closely comparable for all admission clinical, nutritional, and laboratory parameters. The two groups were also comparable for the duration and severity of diarrhea, as assessed by history as well as actual quantification of purging rates during the period of stabilization (Table 2). An equal number of children in both groups revealed stool pathogens on cultures (enteropathogenic *Escherichia coli* and *Campylobacter jejuni* in 2 each, *Salmonella paratyphi* and *Aeromonas hydrophilia* in 2 children in group A, and *Vibrio cholera* ogawa in 1 child from group B). The degree of dehydration at admission was similar in both groups of children and the amounts of IV fluids (48.9 ± 83.6 mL/kg/d vs 27.5 ± 55.3 mL/kg/d) and oral rehydration solution consumed (161.3 ± 131.6 mL/kg/d vs 196.1 ± 179 mL/kg/d) during the initial period of stabilization, were comparable.

Baseline evaluation of plasma zinc revealed that overall 25 (29%) children had plasma levels <60 $\mu\text{g/dL}$ (9.18 $\mu\text{mol/L}$). Although baseline plasma zinc levels were comparable, supplemented children showed a sustained increment in plasma zinc and serum alkaline phosphatase and had significantly higher values at day 7 and day 14 in comparison to controls (Fig 1A and C). Although 30% of children in group A had plasma zinc levels <60 $\mu\text{g/dL}$ at admission, levels remained low in only 3 (8%) by day 7 of supplementation. A significant trend in reduction of serum copper was seen in zinc-supplemented children, whereas values significantly increased in the placebo group by the end of the second week of therapy (72.7 ± 18.3 $\mu\text{g/dL}$ vs 56.2 ± 17.8 $\mu\text{g/dL}$; $P = .02$; Fig 1B).

Table 3 shows the energy intake and correspond-

TABLE 1. Comparison of Admission Characteristics*

	Zinc-supplemented Group	Placebo Group
Number	43	44
Gender (M:F)	27:16	26:18
Age (months)	11.6 \pm 5.6	13.1 \pm 6.2
Weight-for-age z score	-3.47 \pm 0.97	-3.27 \pm 1.33
Height-for-age z score	-1.68 \pm 1.14	-1.44 \pm 1.34
Weight-for-height z score	-3.02 \pm 0.90	-3.13 \pm 1.19
Mid-arm circumference (cm)	11.1 \pm 1.5	11.6 \pm 1.9
Total protein (g/L)	55.0 \pm 9.2	56.8 \pm 8.9
Serum albumin (g/L)	33.7 \pm 7.8	33.5 \pm 6.5
Serum prealbumin (mg/L)	93.8 \pm 40.2	77.4 \pm 35.0
Haemoglobin (g/L)	92.3 \pm 18.2	91.6 \pm 19.0
Hematocrit (%)	29.9 \pm 4.3	29.8 \pm 4.9
C-reactive protein (mg/L)	32.9 \pm 42.5	41.4 \pm 67.6
Plasma zinc ($\mu\text{g/dL}$)	78.0 \pm 32.2	70.3 \pm 19.0
Plasma copper ($\mu\text{g/dL}$)	67.4 \pm 34.2	64.1 \pm 19.2

* All differences are nonsignificant.

TABLE 2. Comparison of Diarrhea Characteristics at Admission*

	Zinc-supplemented Group	Placebo Group
Number	43	44
Duration of diarrhea		
14–30 days	33 (77%)	32 (73%)
>30 days	10 (23%)	12 (27%)
Type of stools at admission		
Watery	32 (74%)	28 (64%)
Bloody	3 (7%)	2 (5%)
Mucoid	3 (7%)	6 (14%)
Mixed	5 (12%)	8 (18%)
Stool volume (g/kg/day)†		
<40	13 (30%)	9 (20%)
40–70	10 (23%)	17 (39%)
>70	20 (47%)	18 (41%)
Stool frequency (n/day)†		
1–5	10 (23%)	8 (18%)
6–10	14 (33%)	15 (34%)
>10	19 (44%)	21 (48%)
Degree of dehydration at admission		
None	23 (53%)	29 (66%)
Mild	16 (37%)	11 (25%)
Moderate	2 (5%)	2 (5%)
Severe	2 (5%)	2 (5%)

* All differences are nonsignificant.

† Observed during initial period of stabilization.

ing outcome of therapy for the two treatment groups. No significant difference was found between the groups for either energy intake, stool output or weight gain for the duration of the study. Although the overall time taken for diarrheal recovery and weight gain were comparable (Fig 2), zinc-supplemented children exhibited a faster initial reduction in stool output (log rank test for time to 30% reduction in stool output, 5.1; $P < .03$). However, survival analysis did not reveal any significant difference between the two groups for the time taken for 50% reduction in stool output more than baseline values (log rank statistic, 1.4; $P = .24$). The serial trends for increment in serum albumin, prealbumin, and IGF-I for the two treatment groups were comparable indicating no significant differences.

A large number (ie, 52 [68%]) of the randomized children had evidence of nonenteric infections and fever during the initial period of hospitalization and several needed systemic antibiotics. Although the rate of weight gain was slower in children with evidence of systemic infection requiring antibiotics, the distribution of these patients between the two treatment groups was comparable (20 [47%] in group A versus 22 [50%] in group B, $P = NS$).

Although our study was not designed for subgroup analysis, evaluation of the aforementioned outcomes for the subgroup with low admission plasma zinc levels ($<60 \mu\text{g/dL}$), also did not reveal any significant differences or trends (Table 4). A similar analysis of outcome among stunted children (height for age z score, <-2.0) also did not reveal any significant differences (data not shown). However, our study had insufficient power to detect significant differences in these subgroups.

Figure 3 shows the lactulose/rhamnose (L/R) ratio for the two treatment groups throughout the course of hospitalization. The L/R ratio was significantly

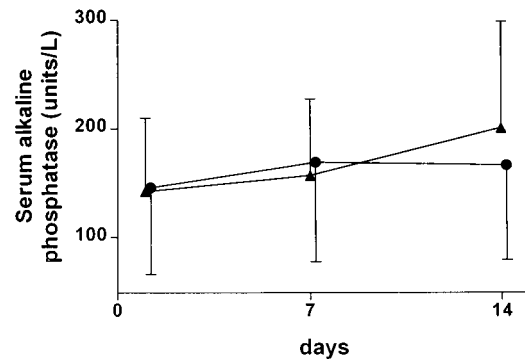
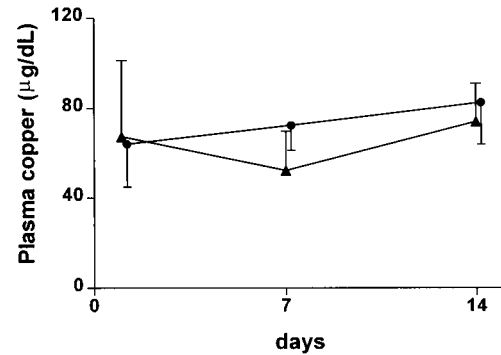
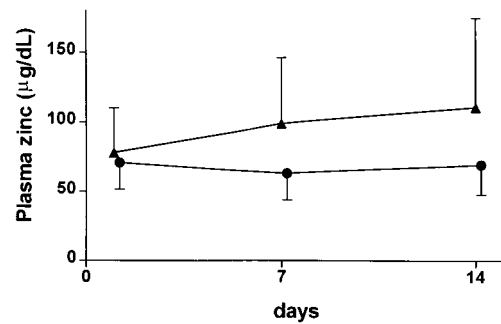


Fig 1. Serial values (mean \pm SD) for plasma zinc, copper, and serum alkaline phosphatase for both treatment groups (▲, zinc supplemented; ●, placebo) throughout 14 days of inpatient therapy. Zinc supplementation results in significantly higher plasma zinc (repeated measures analysis of variance F test for time trend 3.7, $P = .03$; therapy effect F test 3.7, $P = .03$) and alkaline phosphatase (time trend F test 6.4, $P < .01$; therapy effect F test 3.2, $P < .05$). Plasma copper concentrations show opposite and significantly different trends in both treatment groups (time trend F test 4.8, $P = .01$, therapy effect F test 4.0, $P < .05$).

higher in the zinc-supplemented group at admission in comparison with placebo group, but no significant difference was found thereafter between the two groups. No correlation was seen between the L/R ratio and other parameters of nutritional or diarrheal recovery. Although sequential breath hydrogen excretion values did not show any frank evidence of lactose intolerance on the K-Y diet, there was a trend toward higher values among zinc-supplemented children by the end of the second week of therapy (Fig 4).

No child had a relapse of diarrhea and the mor-

TABLE 3. Comparison of Outcome of Therapy During Period of Hospitalization*

	Zinc Group (n = 43)	Placebo (n = 44)	Repeated Measures ANOVA (P Value)
Caloric intake (kcal/kg/d)			
Day 1	83.1 ± 37.5	80.2 ± 28.6	
Day 7	129.6 ± 39.6	123.8 ± 36.9	
Day 14	130.7 ± 46.6	121.1 ± 49.7	.79
Overall increment in caloric intake (kcal/kg/day)	39.9 ± 46.5	40.0 ± 51.3	
Stool frequency (n/d)			
Day 1	10.2 ± 6.4	11.8 ± 7.8	
Day 7	5.9 ± 5.6	5.2 ± 3.7	
Day 14	2.9 ± 1.6	3.0 ± 2.2	.52
Decrease in stool frequency (n/d)	7.4 ± 7.4	8.1 ± 8.8	
Stool volume (g/kg/d) [males]			
Day 1	116.8 ± 103.7	141.9 ± 171.6	
Day 7	66.7 ± 86.1	43.9 ± 40.1	
Day 14	24.9 ± 16.2	27.8 ± 31.4	.42
Decrease in stool volume (n/d)	91.1 ± 103.6	98.0 ± 187.9	
Weight (kg)			
Day 1	6.08 ± 1.32	6.33 ± 1.56	
Day 7	6.27 ± 1.29	6.84 ± 1.41	
Day 14	6.67 ± 1.43	7.13 ± 1.42	.27
Overall weight increment (g/kg/day)	10.3 ± 5.7	8.7 ± 6.5	
Mid-arm circumference (cm)			
Day 1	11.4 ± 1.5	11.5 ± 1.9	
Day 7	11.7 ± 1.4	12.0 ± 1.8	
Day 14	12.0 ± 1.4	12.4 ± 1.8	.66
Overall increment in mid-arm circumference (cm)	0.3 ± 0.3	0.4 ± 0.3	

Abbreviation: ANOVA, analysis of variance.

* Note: The repeated measures ANOVA evaluated the interaction of time trend and therapy effect for both groups during 14 days of therapy. All differences are nonsignificant.

bidity patterns were comparable during the 14-day period of home supplementation and follow-up. The overall weight gain (9.2 ± 46 vs 7.6 ± 5.7 g/kg/d) in zinc-supplemented versus placebo-treated children ($P = \text{NS}$) and increment in mid-arm circumference (0.13 ± 0.28 cm vs 0.19 ± 0.40 cm) during the 14 days of ambulatory home-based supplementation, were also comparable.

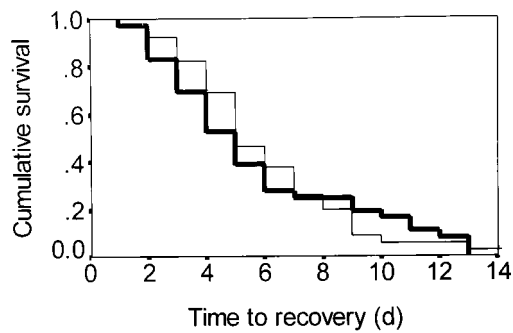
DISCUSSION

Our data indicate that supplementing malnourished children with PD in Pakistan with 3 mg/kg/d of elemental zinc during 14 days neither improved weight gain nor diarrheal recovery, despite significant increments in plasma zinc and alkaline phosphatase. Almost one-third of all children with PD had baseline plasma levels suggestive of zinc deficiency, and the majority also had low values for plasma copper, which decreased further within 7 days of institution of zinc therapy. Although an initial trend toward a relatively faster reduction in stool volume was seen with zinc supplementation, the effect was not sustained. It must be recognized however, that our sample size calculation was primarily based on estimating a clinically significant difference in weight gain. Although the overall weight gain in both groups exceeded our initial estimates, the standard deviations were wide and it is therefore possible that our study had insufficient power to elucidate smaller but potentially significant differences in stool output or weight gain. It can be estimated that the final power of our study to detect a 25% difference rates of weight gain at the aforementioned was <60%.

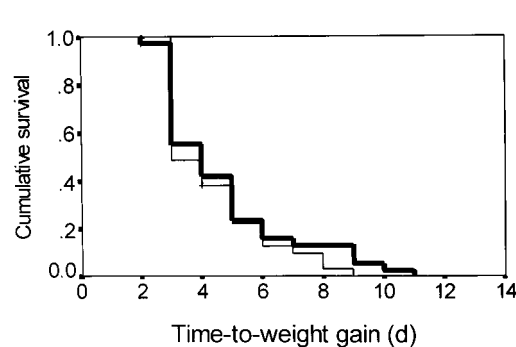
The differential absorption test using lactulose and

mannitol has been used as an indirect measure of intestinal permeability and integrity in gastrointestinal disorders.³¹ We were unable to duplicate the findings of a significant improvement in intestinal permeability after zinc supplementation in children with PD²⁵ and shigellosis,³² as the urinary L/R ratio did not change significantly during the course of therapy. We do not have a satisfactory explanation for the comparatively higher L/R ratio among zinc-supplemented children at admission, as the two groups were otherwise entirely comparable for duration of illness, severity of diarrhea, and degree of malnutrition.

Our data are considerably different from the previous two studies^{24,25} of zinc supplementation in PD and suggest the need for caution before launching into single nutrient supplementation in such malnourished children. However, several important differences from previous studies must be highlighted. Although the duration of zinc supplementation was comparable, our study group was not strictly comparable to the two previous reports on PD treatment from the region,^{24,25} which consisted mainly of stable, uninfected children with lesser degrees of malnutrition. These latter two studies, although randomized and blinded, did not provide data on stool volume and in one study all children also received oral nalidixic acid.²⁴ In contrast, the children in our study were significantly more malnourished and the majority also had associated systemic infections. It is thus likely that the children in our study represented a more severe spectrum of PD. More than half of the children in our study had an initial stool output exceeding 70 g/kg/d. A close correlation between mineral loss and stool output has been reported in



A)



B)

Fig 2. A) Kaplan-Meier plot of time-to-diarrheal recovery (stool volume <30 g/kg/d in males, frequency <4 /d in both and semi-soft stool consistency) after initiation of dietary therapy in zinc-supplemented (■) versus placebo (-) groups. Log-rank statistic 0.14, $P = .713$. B) Kaplan-Meier plot of time-to-weight gain (weight gain for at least consecutive ≥ 3 days after achievement of caloric intake of 100 kcal/kg/d) after initiation of dietary therapy in zinc-supplemented (■) versus placebo (-) groups. Log-rank statistic 0.72, $P = .397$.

children with diarrhea^{15,16,33} and it is conceivable that correspondingly higher levels of micronutrient intake may be necessary in children with more severe diarrhea. Our data however, suggest that despite high purging rates, most children were able to adequately absorb oral zinc sulfate with an increase in plasma levels.

Another consideration is the dose of zinc sulfate used for supplementation. Although the recommended daily zinc intake for infants and young children ranges from 1 to 2 mg/kg/d,³⁴ dietary components such as phytate³⁵ may interfere with zinc absorption, and therefore a comparatively higher intake may be required when feeding such diets.³⁶ It is also likely that children with diarrhea have higher enteric losses of zinc and correspondingly higher intakes may be required to make an allowance for enteric losses. Other studies of zinc supplementation in malnourished children or those with diarrhea have used varying levels of intake, sometimes in pharmacologic doses exceeding 5 to 10 mg/kg/d of elemental zinc.^{21,23–25,32} Some trials have opted to use a fixed amount of zinc supplement daily for the obvious ease of administration.^{22,23} Our decision to administer 3 mg/kg/d of elemental zinc for supplementation, stemmed in the main from an attempt to evaluate a level of zinc intake that provided almost twice the recommended daily allowance, yet was also possible to emulate subsequently from dietary

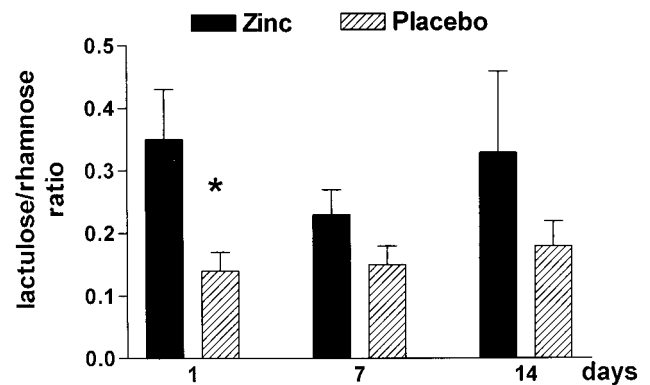


Fig 3. Sequential urinary lactulose/rhamnose excretion in both treatment groups after oral dose of lactulose (3.5 g) and rhamnose (0.5 g). Data are mean \pm SEM. *, $P < .02$ zinc versus placebo group at admission. All other differences are nonsignificant. Repeated measures analysis of variance time trend F test 0.75, $P = .476$; therapy effect F test 0.63, $P = .534$.

sources. We believe that this intake of zinc was sufficient for replenishment. Despite PD and continuing stool losses in excess of 30 g/kg/d, supplemented children demonstrated significant increases in plasma zinc levels during the study period and only 8% had levels <60 $\mu\text{g/dL}$ by the end of the 14 days of supplementation. It is interesting to note that although the pretreatment plasma zinc levels in our patients were comparable to those observed by Sachdev et al²⁴ from North India, values at day 7 were considerably higher than those reported by them after therapy with 5 to 6 mg/kg/d of elemental zinc. Serum alkaline phosphatase measurement has been suggested as an important surrogate marker of zinc status in humans.^{37,38} The serial rise of serum alkaline phosphatase values in supplemented children suggests that sufficient absorption and replenishment of body zinc occurred with the dose of zinc used.

Although both groups received the same multivitamin supplement, an important factor is the possibility of multiple micronutrient deficiencies. It is quite likely that malnourished children with PD may be deficient in more than one micronutrient and care must be exerted when supplementing with single nutrients, as some may interfere with the absorption of others. Significant interaction of zinc absorption with copper and iron has been described.^{39,40} Although a steady increase in serum copper was seen in the control children receiving the K-Y diet, an opposite trend was seen initially in those receiving zinc. The serum copper levels in our patients were somewhat lower than values reported from malnourished children in India⁴ and Morocco,⁴¹ but comparable to those seen among some malnourished children in Chile,⁴² and within the low normal range for humans.⁴³ Although copper supplementation during the early stages of recovery from diarrhea is not recommended,⁴⁴ given the evidence that copper deficiency may impair growth during recovery from malnutrition,⁴² malnourished children with PD should be considered at risk for copper deficiency. The latter could be unmasked during enteral zinc supplementation. We also did not provide any addi-

TABLE 4. Comparison and Outcome in Children With Plasma Zinc <60 µg/dL (9.18 µmol/L)*

	Zinc-supplemented Group	Placebo Group
Number	13	12
Age (months)	12.4 ± 6.3	9.8 ± 4.9
Duration of diarrhea (d)	33.5 ± 26.1	30.2 ± 17.3
Weight-for-age z score	-3.40 ± 0.88	-3.44 ± 0.89
Height-for-age z score	-1.70 ± 1.33	-1.53 ± 1.03
Weight-for-height z score	-2.94 ± 0.85	-3.09 ± 0.61
Serum protein (g/L)	53.1 ± 11.0	54.2 ± 9.1
Serum albumin (g/L)	30.3 ± 9.4	30.7 ± 6.3
Serum prealbumin (mg/L)	95.2 ± 49.4	65.5 ± 30.4
Admission stool volume (g/kg/d)	133.0 ± 107.9	127.2 ± 87.0
Admission stool frequency (n/d)	12.2 ± 7.1	12.7 ± 7.0
Overall caloric intake (kcal/kg/d)	120.9 ± 35.5	136.7 ± 20.8
Overall stool volume (g/kg/d) [males only]	80.1 ± 68.3	89.5 ± 67.7
Overall stool frequency (n/d)	5.3 ± 3.0	7.2 ± 3.7
Overall weight gain (g/kg/d)	7.9 ± 5.1	7.5 ± 3.9
Time to 50% reduction in stool output (d)	5.5 ± 2.4	6.1 ± 4.2
Time to 30% reduction in stool output (d)	3.5 ± 1.1	4.8 ± 3.4
Time to recovery (d)	5.4 ± 3.5	7.1 ± 4.0

* Data as mean ± SD. All differences are nonsignificant.

Breath hydrogen (ppm)

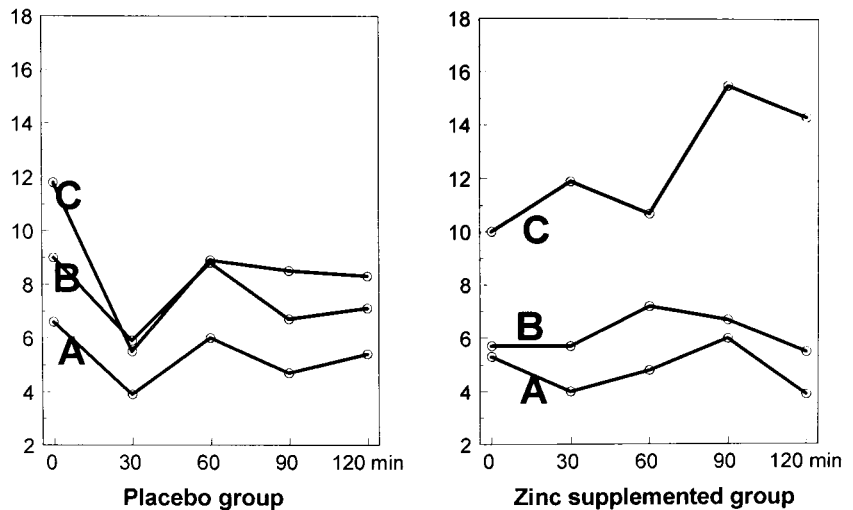


Fig 4. Breath hydrogen excretion on K-Y diet (data as mean values for each time point). A = day 0 values, B = day 7 values, C = day 14 values. All differences are non-significant.

tional oral iron during the course of hospitalization, and the drop in hemoglobin levels in both groups by day 14 suggests a dietary limitation of this important micronutrient. Although we did not observe any significant neutropenia, it is possible that concomitant copper deficiency could also have contributed to this anemia. It is thus likely that instead of isolated zinc supplements, an optimal mixture of micronutrients may be needed in malnourished children with PD to ensure a balanced repletion of body stores. The micronutrient premix recently recommended by the World Health Organization for malnourished children with PD¹ needs to be validated for its effect on plasma zinc and copper levels.

No difference in morbidity patterns was discernible during the 4 weeks of therapy, although admittedly the duration of supplementation is short. As indicated, many of the children in our study had significant systemic infections necessitating concomitant antibiotic therapy. Such coincidental infections are recognized as a major determinant of recovery from PD⁴⁵ and may be an additional reason for the failure of zinc therapy to affect growth during the

rehabilitation of severely malnourished children.⁴⁶ Despite the stated benefits of zinc therapy on immunocompetence, some studies do caution against potentially adverse effects of large dose zinc supplements, especially in malnourished children.^{47,48} We do not have a satisfactory explanation for the relatively higher values of breath hydrogen excretion among zinc-supplemented children by day 14, although the pattern of excretion is suggestive of small bowel bacterial overgrowth.⁴⁹ We did not evaluate small bowel microflora in our study and are unable to comment on a possible effect of enteral zinc supplements on small bowel microflora and bacterial overgrowth. The potential effect of enteral zinc therapy on small bowel microflora needs evaluation in future prospective studies.

In conclusion, our randomized double-blind, placebo-controlled trial of zinc supplementation in severely malnourished children with PD did not show any benefit on either diarrheal recovery or weight gain at either 14 or 28 days of therapy. In contrast, serum copper levels showed a significant drop within 7 days of initiation of zinc supplementation.

Our data suggest the need for caution in the use of zinc supplements alone in severely malnourished children with PD.

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