

# Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet<sup>1-3</sup>

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## ABSTRACT

**Background:** Zinc deficiency in children is an important public health concern in the developing world, and the consumption of predominantly cereal-based diets with a high phytate content may contribute to the risk. The gastrointestinal tract plays a central role in absorbing and conserving zinc, yet it has not been carefully studied in such children.

**Objective:** This study investigated zinc homeostasis in healthy, free-living Malawian children with habitually high-phytate diets to better understand the role of the gastrointestinal tract.

**Design:** We evaluated zinc homeostasis in 10 children aged 2–5 y who were consuming a maize-based diet (phytate:zinc molar ratio of 23:1). Zinc stable isotopes were administered orally and intravenously. The tracer and tracee were measured in urine and feces.

**Results:** Endogenous fecal zinc was high in comparison with results for this measure in previous studies. Typical correlations seen in subjects consuming a low-phytate diet between total absorbed zinc, the size of the exchangeable zinc pool, and endogenous fecal zinc were not observed. Fractional absorption of zinc was 0.24.

**Conclusions:** Zinc homeostasis was perturbed, particularly by large, endogenous fecal zinc losses, in this vulnerable population. The effects of interventions to improve zinc status, including dietary phytate reduction, on zinc homeostasis merit further study. *Am J Clin Nutr* 2002;75:1057–61.

**KEY WORDS** Zinc, stable isotopes, zinc homeostasis, Malawi, children, phytate

## INTRODUCTION

Zinc deficiency affects tens of millions of children throughout the developing world (1). During childhood, zinc deficiency contributes to stunting and impaired cognitive development and is associated with an increased incidence and prevalence of infectious diarrhea, pneumonia, and malaria (2–4). Achieving adequate zinc nutriture in developing countries is hindered by its poor bioavailability in predominantly plant-based diets (5). Maize is the major staple food in Malawi. The poor bioavailability of zinc in a predominantly maize-based diet is thought to be a consequence of the diet's high phytate content (6). High dietary phytate intake has been associated with poor zinc status among

Malawian children and pregnant women (7, 8). Phytate forms insoluble complexes with zinc in the gastrointestinal tract and is a potent inhibitor of zinc absorption in animals and humans (9). Homeostatic compensation for low dietary intakes of zinc can be achieved by reducing the amount of endogenous fecal zinc (EFZ), the endogenously secreted zinc lost via the intestine (10). Techniques using zinc stable isotopes provide a safe, accurate, and powerful method of quantifying zinc absorption and endogenous zinc losses in humans (11). This study used zinc stable isotopes to investigate zinc homeostasis in healthy, free-living Malawian children consuming a traditional maize-based diet to determine whether and how adequate zinc nutriture is achieved.

## SUBJECTS AND METHODS

### Subjects and diets

Healthy children aged 2–5 y attending well-child immunizations at the Mpemba Health Center in southern Malawi were eligible for the study. Their mothers first completed a food-frequency questionnaire, and the first 10 mothers who indicated that animal products (meat, eggs, fish, or chicken) were consumed by the family once a month or less were asked to participate. All 10 mothers gave their informed consent to participate in the study. All of the children's families were subsistence farmers, living in mud and thatch homes, without access to electricity or transportation, similar to most rural Malawians. This study received ethical approval from the Washington University

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The food-frequency questionnaire identified the 6 most commonly consumed foods, which accounted for the majority of food intake: unrefined maize flour, red kidney beans, peanut flour, tomatoes, bananas, and Chinese cabbage. Each mother was provided with these 6 foods every 3 d throughout the study. The quantity of food provided was sufficient to prepare daily meals for a large family. Thus, each subject's diet was standardized before the zinc metabolic study, yet it resembled the typical diet consumed at home. Dietary intakes were determined for a 3-d period. Trained field assistants weighed all foods consumed by each child in a central village site in the morning and afternoon. Foods consumed at other times were determined by an interactive 24-h dietary recall method (12). Nutrient intakes from the study diet were calculated with the use of the WORLD FOOD DIETARY ASSESSMENT SYSTEM (version 2.0; University of California, Berkeley).

After receiving the provided foods for 6 d, each child began the zinc metabolic study. On the first day of the zinc metabolic study, the subjects came to the Mpemba Health Center, where they were fed 3 meals and 2 snacks prepared from the same 6 foods identified by the food-frequency questionnaire. All ingredients used in cooking were weighed, and samples of each food were saved and analyzed for zinc content according to standardized procedures (13). Weighed food records were kept for each child. The subsequent days of the zinc metabolic study were completed at home.

### Zinc metabolic study

The stable isotopes  $^{67}\text{Zn}$  and  $^{70}\text{Zn}$  (Martin Marietta Energy Systems, Oak Ridge, TN) were prepared for human administration with standard sterile techniques (14). On day 1 of the zinc metabolic study, the children received a precisely measured intravenous dose of  $\approx 750 \mu\text{g } ^{70}\text{Zn}$ . During the 5 feedings on day 1, an oral dose of  $^{67}\text{Zn}$  was taken with food. The total dose of  $^{67}\text{Zn}$  was 20% of the total zinc intake estimated from the dietary records of the previous 48 h, 1.5–2 mg  $^{67}\text{Zn}$ .

A brilliant blue fecal marker was given 72 h after administration of the  $^{70}\text{Zn}$  and again precisely 4 d later. All stools between and including the markers were collected. Children defecated into zinc-free plastic bags. Twice daily on days 3 through 8, a clean-void midstream urine sample was collected into a zinc-free plastic container. Specimens were frozen and transported unprocessed to the laboratories at the Center for Human Nutrition in Denver.

A blood sample obtained from each child just before the administration of intravenous  $^{70}\text{Zn}$  was used to measure plasma zinc and C-reactive protein. Length and weight were measured with the use of calibrated equipment and standardized methods on day 1 of the zinc metabolic study, and weight was measured again 30 d after completion of the zinc metabolic study to determine the rate of growth.

### Sample analyses

Total urine, fecal, and dietary zinc were measured by flame atomic absorption spectrophotometry, with the use of a deuterium arc background correction lamp (Perkin-Elmer, Norwalk, CT). Fecal samples were analyzed individually or were pooled in 24-h periods. Feces were collected for a 4-d metabolic period, including both stool markers. The number of fecal samples for this period ranged from 8 to 11. Samples were pooled for each day, and daily fecal excretions were analyzed individually. Prepara-

tion of the urine and fecal samples for zinc isotopic enrichment included wet and dry digestion, reconstitution in 6 mol HCl/L, and separation of zinc from other minerals by ion-exchange chromatography (15). Isotope tracer enrichment of samples was determined from measurements of the ratios of  $^{67}\text{Zn}$  to  $^{66}\text{Zn}$  and  $^{70}\text{Zn}$  to  $^{66}\text{Zn}$  by inductively coupled plasma mass spectrometry (16). The isotope ratio analyses were performed with a VG Elemental Plasma Quad 3 mass spectrometer (Winsford, Cheshire, United Kingdom). Prepared samples were taken up in ultrapure nitric acid to a concentration of 50 ng/g, and 8 mL was introduced into a standard pneumatic nebulizer at a rate of 1 mL/min with a peristaltic pump. Data were acquired in peak-jump mode, and mass bias was corrected on the basis of frequent measurements of a natural zinc isotope ratio standard.

Originally, this method used an oral isotope administered with a single meal (17). The validity of applying this method to protocols that divide the tracer between all meals on 1 d was determined both from simulation of this approach with the use of published compartmental models of zinc metabolism (18, 19) and from comparison of data obtained by this method with those obtained by our fecal enrichment method (14). Seven separate data sets yielded virtually identical data for the fractional absorption of zinc as calculated by these 2 methods (KMH, unpublished observations, 2001).

Serum C-reactive protein was measured by rate nephelometry. Plasma zinc was measured by flame atomic absorption spectrophotometry (20). The accuracy of the plasma zinc analysis was determined by using bovine serum albumin standard reference material [SRM-1598; National Institute of Standards and Technology, Gaithersburg, MD; mean ( $\pm$ SD) certified value of  $890 \pm 60 \mu\text{g/L}$  compared with an analyzed value of  $890 \pm 17 \mu\text{g/L}$ ].

### Calculations and statistics

For the determinations of fractional absorption (FAZ), the ratio of the urinary isotopic enrichment of the intravenously administered  $^{70}\text{Zn}$  to the orally administered  $^{67}\text{Zn}$  was used in the following equation (17):

$$\text{FAZ} = \frac{\text{enrichment (oral/intravenous)}}{\text{dose (intravenous/oral)}} \times \quad (1)$$

Ten urine specimens obtained during study days 3–8 were analyzed, and the calculated FAZs for the specimens were averaged to find each child's FAZ.

EFZ excretion was measured by an isotopic dilution technique in which urine enrichment was substituted for enrichment in solid tissue or plasma (14).

$$\text{EFZ (mg/d)} = (F \times f) / (ua \times d) \quad (2)$$

where  $F$  is the total zinc in each sample (mg),  $f$  is the intravenous isotope enrichment in each sample,  $ua$  is the average intravenous isotope enrichment in urine during collection, and  $d$  is the duration of collection (4 d).

Weighed food intakes for each child and the analyzed content of zinc in collected food samples were used in the calculation of total absorbed zinc (TAZ):

$$\text{TAZ (mg/d)} = \text{dietary Zn} \times \text{FAZ} \quad (3)$$

$$\text{Net absorbed Zn (mg/d)} = \text{TAZ} - \text{EFZ} \quad (4)$$

The size of the combined pools of zinc that intermixed with plasma within a 2-d period (the exchangeable zinc pool, or EZP,



**TABLE 1**Demographic and anthropometric characteristics of healthy village children<sup>1</sup>

	Value
Age (mo)	43.6 ± 7.7
Weight (kg)	13.5 ± 1.1
Height (cm)	92.7 ± 8.2
Weight-for-age z score	-1.2 ± 0.5
Weight-for-height z score	-0.2 ± 0.7
Height-for-age z score	-1.9 ± 1.6

<sup>1</sup> $\bar{x} \pm SD$ ;  $n = 5$  F, 5 M.

in mg), a measure of zinc status, was estimated by dividing the mass of intravenously administered <sup>70</sup>Zn by the enrichment value at the y intercept of the linear regression of a semilog plot of urine enrichment data between days 3 and 8 (21).

Results are expressed as means ± SDs. Anthropometric z scores were calculated by using EPI INFO (version 6; World Health Organization–Centers for Disease Control and Prevention, Atlanta). Pearson's correlations and linear regression were used to test for associations between variables with SPSS for WINDOWS (version 10.0; SPSS Inc, Chicago). Statistical differences were considered to be significant at  $P < 0.05$ .

## RESULTS

Ten children were enrolled and studied in April 1999 (Table 1). No child was underweight (weight-for-age z score < -2) or wasted (weight-for-height z score < -2); however, the mean height-for-age z score approached the level of stunting. On average, each child gained 4 g/d. There was no change in height. The mean plasma zinc concentration of 9.8 μmol/L was low (normal: >10.7 μmol/L). The C-reactive protein concentration was not elevated in any subject.

A typical daily diet, made up of the foods provided and determined by the 3-d weighed records and dietary recalls, is shown in Table 2. The study diet provided 4.48 ± 1.37 MJ (1071 ± 328 kcal), 6.1 ± 2.1 mg Zn, 10.5 ± 3.5 mg Fe, 199 ± 80 mg Ca, and 325 ± 95 mg Mg/d. Adequate amounts of magnesium, riboflavin, and thiamine were consumed (22, 23). However, intakes of calcium, iron, vitamin A, and vitamin B-12 were <80% of the recommended amounts (22–24). Energy and protein intakes, when expressed per kilogram body weight, were adequate (25). The phytate content of the unprocessed maize flour was 6.97 ± 0.87 mg inositol hexaphosphate + inositol pentaphosphate/g. The mean phytate:zinc molar ratio of the diet was estimated to be 23:1.

The amount of <sup>70</sup>Zn (intravenous dose) ranged from 0.04% to 0.3% in the fecal samples and from 0.5% to 2% in the urine samples. The amount of <sup>67</sup>Zn (oral dose) ranged from 0.1% to 0.8% in the urine. These values are well above the inductively coupled plasma mass spectrometry detection limits of 0.005% for <sup>70</sup>Zn and 0.012% for <sup>67</sup>Zn.

Zinc intake on the day of isotope administration was 5.4 ± 1.0 mg. FAZ was 0.24 ± 0.04, TAZ was 96 ± 24 μg · kg<sup>-1</sup> · d<sup>-1</sup>, EFZ was 85 ± 24 μg · kg<sup>-1</sup> · d<sup>-1</sup>, and zinc net absorption was 11 ± 26 μg · kg<sup>-1</sup> · d<sup>-1</sup>. The EZP was 3.2 ± 0.9 mg/kg. The absolute values for these variables of zinc homeostasis are given in Table 3. EFZ was inversely correlated with EZP ( $r = -0.66$ ,  $P < 0.05$ ; Figure 1). EZP was not correlated with TAZ but was directly correlated with net zinc absorption ( $r = 0.79$ ,  $P < 0.01$ ).

**TABLE 2**Typical daily diet, made up of foods provided to study participants<sup>1</sup>

Meal	Food	Average intake
		g
Breakfast	Maize flour porridge (14 g unrefined maize flour, 3 g groundnut flour, and 3 g sugar)	250
Morning snack	Banana	100
Lunch	Stiff maize flour porridge (26 g unrefined maize flour)	215
	Green leaf relish, boiled (75 g Chinese cabbage, 14 g tomato, and 5 g groundnut flour)	105
Afternoon snack	Banana	65
Dinner	Stiff maize flour porridge (26 g unrefined maize flour)	190
	Bean relish, boiled (29 g red kidney beans, 14 g tomato, 5 g vegetable oil, and 4 g onion)	135

<sup>1</sup>Derived from 3 d of weighed records and dietary recalls. Ingredients, per 100 g wet wt, in parentheses.

## DISCUSSION

This study was conducted in the home environment of a population of young children expected to be at substantial risk of zinc deficiency. Overall, these Malawian children were zinc deficient. Marked perturbations in EFZ were found that contributed to compromised zinc homeostasis.

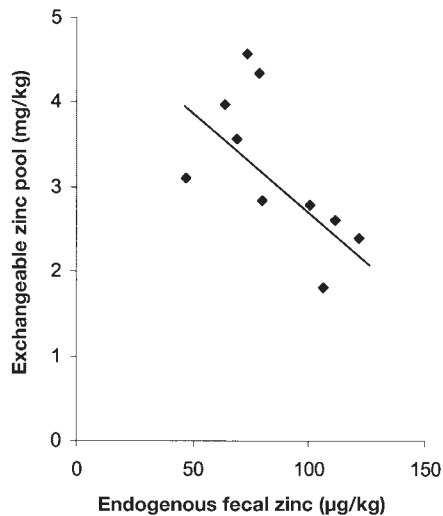
This study is limited in that only healthy Malawian children aged 2–5 y were studied. Therefore, caution should be exercised in extrapolating the findings to other populations, such as ill children and children consuming other types of diets. The methods of determining FAZ and EFZ, although quite sensitive and accurate, require significant cooperation from the subjects and thus could only be done in a small number of children. Because zinc isotope methods are relatively novel, there are no comparison data from children of this age group, which limits the interpretation of these data.

The mean plasma zinc concentration in these children was below the mean for healthy populations, and 4 of the 10 children had distinctly low plasma zinc concentrations, <9 μmol/L. Despite its limited sensitivity (26), plasma zinc remains the most widely accepted biomarker of zinc status. The hypozincemia found in these children, in the absence of clinical evidence of infection or elevated C-reactive protein concentrations, indicates zinc deficiency (27). Theoretically, EZP is also expected to provide a biomarker of zinc status, but cutoffs indicative of zinc deficiency have not been defined. It is plausible that homeostatic

**TABLE 3**Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet<sup>1</sup>

	Absolute value
Fractional absorption of zinc	0.24 ± 0.04
Total absorbed zinc (mg/d)	1.30 ± 0.33
Endogenous fecal zinc (mg/d)	1.15 ± 0.33
Net absorbed zinc (mg/d)	0.15 ± 0.34
Exchangeable zinc pool (mg)	43 ± 11
C-reactive protein (mg/L)	5 ± 7
Plasma zinc (μmol/L)	9.8 ± 3.4

<sup>1</sup> $\bar{x} \pm SD$ ;  $n = 5$  F, 5 M.



**FIGURE 1.** The exchangeable zinc pool is inversely correlated with endogenous fecal zinc in Malawian children aged 2–5 y consuming a high-phytate, maize-based diet ( $r = -0.66$ ,  $P < 0.05$ ). The linear regression line is shown with a scatter plot of the data. The inverse correlation supports the interpretation that endogenous fecal zinc is the primary determinant of zinc status in this population and that greater amounts of endogenous fecal zinc deplete the exchangeable zinc pool.

mechanisms can selectively affect plasma zinc without measurable effects on EZP, and vice versa, and our previous experience (10) did not lead us to expect a positive correlation between plasma zinc and EZP. None was, in fact, observed in this study.

Although the validity of comparisons of EFZ and net absorbed zinc on a body weight basis has not been established, dietary zinc requirements and other variables of zinc homeostasis are related to age or body mass (24); thus we expressed EFZ, TAZ, and net absorbed zinc per unit of body weight. Mean EFZ per kilogram body weight in the children in the present study was more than twice the average amount for adults whose habitual diet is deemed adequate in zinc and low in phytate (24). EFZ in the Malawian children was also higher than the mean amount for breast-fed infants (85 compared with  $50 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) (28). Thus, the EFZ in these children is relatively high. However, EFZ in the present study was similar to what we previously found in 23 hospitalized children, who had EFZ values of  $75 \pm 30 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (29). Typically, for individuals in the developed world consuming low-phytate diets, the quantity of endogenous zinc excreted via the intestine depends on both short-term (30, 31) and long-term (10, 32) zinc absorption. This is thought to be mediated through changes in zinc status. Therefore, because of the poor zinc status of the children in the present study, EFZ might be expected to be relatively low, but in fact the reverse was found. The inverse relation observed between EFZ and EZP is compatible with the conclusion that the excessive losses of endogenous zinc via the intestine have a negative impact on zinc status in proportion to the extent of the losses (10, 32).

Our data also suggest that the excessive EFZ is perturbing the normal correlations between TAZ, EZP, and EFZ. There is usually a positive correlation between TAZ and EZP, such that the more dietary zinc that is absorbed, the larger the body pool size (10, 32, 33). We did not find such a correlation among these Malawian children. In addition, TAZ and EFZ are usually posi-

tively correlated (33, 34), but we did not find this correlation. The only other clinical scenario in which similar perturbations in zinc homeostasis were observed was in infants with cystic fibrosis. In infants with cystic fibrosis, EFZ was exceptionally high and there was no correlation between EFZ and TAZ; however, EFZ was directly correlated with amount of fecal fat (35).

The reason for the comparatively high EFZ observed in the children in the present study is unclear. It has been hypothesized that phytate may complex with endogenously secreted zinc and inhibit its absorption. In our previous, hospital-based study of zinc homeostasis in Malawian children, EFZ was also found to be comparatively high but did not differ between children consuming high-phytate and reduced-phytate diets (29). Another possible explanation is that abnormal permeability of the small intestine, a condition known as tropical enteropathy (36), contributed to the higher obligate EFZ losses noted in this study. Further studies of dietary and physiologic factors are needed to determine why EFZ is increased in these children.


FAZ was relatively low in the Malawian children (24). Apart from bioavailability, the major determinant of FAZ is dietary zinc intake. Dietary zinc intake in the present study was similar to that of children in the United States (37). The relatively low FAZ is most likely a result of the high phytate content of the Malawian diet limiting zinc bioavailability, because substantial increases in FAZ were noted in our previous study of zinc-deficient children consuming a reduced-phytate diet. FAZ was, however, higher than predicted by the World Health Organization (6). The principal basis for the World Health Organization estimates are single-meal or single-day studies in volunteers whose habitual diet is low in phytate. In individuals consuming habitually high-phytate diets, some adaptive mechanisms may allow for a higher FAZ.

The net absorbed zinc with this high-phytate diet was  $11 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . Urinary zinc losses in our previous study of Malawian children were  $9 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (29), and insensible losses have been estimated to be  $7 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (38), which would leave these children in negative zinc balance. However, this estimated net zinc absorption is based on intakes and endogenous losses that occurred on the day of isotope administration and may not reflect the zinc balance that is achieved with the children's habitual diet. If the zinc intake data derived from the 3-d records ( $6.1 \pm 2.1 \text{ mg/d}$ ) are applied, a positive zinc balance results ( $83 \pm \mu\text{g Zn/d}$ ), which would provide approximately enough zinc to cover growth requirements ( $80\text{--}120 \mu\text{g Zn/d}$ ; 6). During the study period, the children gained an average of 4 g/d, somewhat less than the weight gain increment predicted from the World Health Organization growth reference data for children of this age (ie, 5.2 g/d; 39).

It is possible that intakes were lower on the day of isotope administration because the children were constrained in their activity and closely observed during their feeding. On the other hand, it is possible that intakes were higher during the study period because more food was available and parental attention was focused on eating. Therefore, it is uncertain whether these children were consuming adequate amounts of zinc to maintain zinc balance and support normal growth. The low plasma zinc concentrations seen in these children indicate, however, that overall their zinc status was compromised, suggesting that over a longer period, the amount of zinc retained was not adequate. Dietary zinc insufficiency could occur when food is less plentiful or when the children are anorexic during an acute infectious



illness. In addition, pathophysiologic processes that impair zinc absorption, such as diarrhea, could further compromise zinc status. It appears that these children were zinc deficient from a previously accumulated deficit, which they were unable to overcome with their habitual diet.

These results are consistent with substantial and quite complex effects of decreased intestinal absorptive capacity and habitual high dietary phytate intake on zinc homeostasis and status in Malawian children. Although there are no simple interventions to restore intestinal integrity in children living in the tropics, dietary phytate reduction might be achieved through sustainable methods of food preparation. The results of this study highlight the need for carefully designed, long-term studies of the effects of interventions to improve zinc status, including phytate reduction, on zinc homeostasis in populations at risk of zinc deficiency. 

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