

Absorbed Zinc and Exchangeable Zinc Pool Size Are Greater in Pakistani Infants Receiving Traditional Complementary Foods with Zinc-Fortified Micronutrient Powder^{1,2}

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Abstract

Adequacy of zinc intake from breast milk alone becomes marginal in relation to infant requirements by around 6 mo of age. Simple and cost-effective strategies are needed at the population level to ensure adequate intakes of zinc in infants and toddlers in populations at risk of zinc deficiency. We determined the amount of absorbed zinc (AZ) from a micronutrient powder (MNP) without and with 10 mg of zinc (MNP+Zn) added to local complementary foods used in Pakistan and the impact on the exchangeable zinc pool (EZP) size. As a nested study within a large, prospective, cluster randomized trial, 6-mo-old infants were randomly assigned to receive MNP or MNP+Zn. Stable isotope methodology was applied after ~3 and 9 mo of use to measure AZ from MNP-fortified test meals of rice-lentils (khitchri) and EZP. Nineteen infants per group completed the first metabolic studies and 14 and 17 infants in the MNP and MNP+Zn groups, respectively, completed the follow-up studies. AZs were (mean ± SD) 0.1 ± 0.1 and 1.2 ± 0.5 mg at the first point for the MNP and MNP+Zn groups, respectively ($P < 0.001$); results were nearly identical at the follow-up measurement. EZP did not differ between groups at the first measurement but was less in the MNP group (3.7 ± 0.6 mg/kg) than in the MNP+Zn group (4.5 ± 1.0 mg/kg) at the second measurement ($P = 0.01$). These data confirm that the MNP+Zn in khitchri were well absorbed and after 1 y of home fortification, zinc status assessed by EZP was significantly better for the MNP+Zn group. Additional field studies may be necessary to ascertain the adequacy of this dose for infants at high risk of deficiency. This trial was registered at ClinicalTrials.gov as NCT00705445. J. Nutr. 144: 20–26, 2014.

Background

Studies in Pakistan and elsewhere indicate that breast milk concentrations of zinc decline during the early months postpartum regardless of maternal zinc intake (1,2). Adequacy of zinc intake from breast milk alone becomes marginal in relation to requirements by around 6 mo of age. Hence, breast-fed infants are prone to develop zinc deficiency if their diets are not complemented with high-zinc foods such as meat or fortified foods. In Pakistan, as in many low-income countries, many of the complementary foods offered to children at 4–6 mo are high in phytate and low in zinc and iron content, contributing to high risk of moderate to severe iron and zinc deficiencies and their consequences.

Large-scale zinc supplementation studies in older infants and toddlers have demonstrated significant positive effects on growth, morbidity, and mortality in infants (3,4). Although zinc supplementation has been promoted on a wide scale as a preventive and therapeutic agent for diarrhea and other illnesses in children, the ecological and biological implications of long-term, routine supplementation at a population level have not yet been fully evaluated. Additionally, given the current thinking on modes of administration, zinc supplementation programs will likely consist of co-administration of zinc with other micronutrients either as supplements or in fortified food products. Home fortification with micronutrient powders (MNPs)⁵ has generally resulted in reduced anemia and iron deficiency, but effects on serum zinc concentration have been modest (5) and no evidence of benefit on growth has been shown (6). The absence of such

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⁵ Abbreviations used: AZ, absorbed zinc; CHW, community health worker; CRP, C-reactive protein; EZP, exchangeable zinc pool; FAZ, fractional absorption of zinc; MNP, micronutrient powder; MNP+Zn, micronutrient powder with zinc; UCD, University of Colorado Denver.

zinc effects has raised questions about the dose and bioavailability of the zinc.

This study was conducted to determine the amount of absorbed zinc (AZ) from a multiple-MNP when added to the complementary diet such as khitchri, a rice-lentil mixture commonly used as a complementary food in Pakistan. Additionally, the effect of zinc home fortification on the size of the exchangeable zinc pools (EZPs), which has been found to correlate with dietary zinc intake (7,8), was determined. Secondary outcomes also included nutritional biomarkers in both groups. Infants participating in the isotope studies were a subset of a larger home fortification trial, designed as an efficacy trial to evaluate the impact of MNP with and without zinc on diarrheal disease burden, respiratory illnesses, intestinal mucosal integrity, intestinal flora, and growth among cohorts of children in Pakistan (9).

Methods

Study design. This longitudinal study applied stable isotope methodology to measure zinc absorption from a test meal containing MNP without zinc or from an identical MNP preparation with zinc (MNP+Zn) and to determine the size of EZZ in a cohort of infants who were a part of a large community-based, cluster randomized trial as described above (9). Infants consumed a standard test meal of rice and lentils (khitchri) to which the MNP sachet was added. Stable isotopes of zinc were orally and i.v. administered to measure fractional absorption of zinc (FAZ) by the dual isotope tracer ratio method, AZ from the test meal, and the size of the EZZ (10,11). Blood samples were also obtained for measurement of plasma zinc and acute-phase reactants at the time of the isotope studies. To allow sufficient time for complementary feeding to stabilize, infants were studied after ~3 mo of receiving the assigned supplement and again after ~9 mo of the intervention, ~6 mo after first isotope measurements (Fig. 1).

Study site. The study was conducted at Bilal Colony, Karachi, Pakistan, where the main randomized controlled trial was being conducted. The research site was selected due to availability of basic demographic surveillance data and established field centers of the Aga Khan University coupled with well-established community and health system liaison. The local population consists of mixed ethnic groups belonging to the 4 provinces of Pakistan. It is an urban squatter settlement in the industrial area of Karachi with a population of >80,000 and 11,000 children <5 y old.

Study participants. Infants were enrolled in the main zinc intervention trial between the ages of 3 and 6 mo and received MNP until 18 mo of age. The MNP sachets were consumed daily by infants mixed with their complementary diet and were replenished fortnightly by the community health workers (CHWs) during scheduled household visits. For the isotope studies, 20 infants from each of the 2 intervention arms (MNP and MNP+Zn) were enrolled for cross-sectional and longitudinal comparisons. The allocation and administration of the stable isotope dosage was done by an independent investigator not involved in the main trial (S.A.). All field researchers were unaware of the contents of the MNP sachets and the group randomization. Any samples that would have suggested group assignment (e.g., duplicate diets) were not analyzed until the main study concluded and the data were decoded. Inclusion criteria for the isotope studies were compliance of daily MNP sachet and consumption of local weaning diet for at least 3 mo. The study protocol was approved by the Ethics Review Committee at Aga Khan University and informed consent was obtained from the mothers or fathers of the infants prior to study participation.

MNP supplement. The MNP sachets were prepared to have identical packing but different colors. The MNP without zinc contained micro-encapsulated iron (12.5 mg), vitamin C (50 mg), vitamin A as retinol (300 µg), cholecalciferol (vitamin D₃) (5 µg), and folic acid (150 µg), and the MNP+Zn was identical except for containing 10 mg elemental zinc as zinc gluconate.

Dietary intake. Approximate daily zinc intakes were estimated from 24-h dietary recall to derive an estimate of dietary zinc intake. Feeding practices related to consumption of high zinc- and iron-containing foods were reported from semiquantitative dietary assessment (12).

Metabolic study. Each infant, following recruitment and consent, was brought to the Bilal Colony Research Centre for the zinc stable isotope metabolic measurements. On day 1 of the metabolic period, infants were offered a test meal (khitchri) and oral zinc isotope was administered by the investigator during the meal. Prior to the isotope administration, a baseline urine sample (30 mL) was collected using aseptic technique. In addition, the research physician (S.A.) recorded participant weight and obtained nutritional history with a 24-h dietary recall.

Preparation of the test meal and isotope administration. Khitchri, a mixture of rice and lentils, is a traditional, commonly used complementary food offered to infants in Pakistan. Khitchri is high in caloric content and contains zinc and iron but is also high in phytic acid. Fresh batches of 250 g of khitchri were prepared for every new recruit at the Bilal research site. Measures were taken to ensure the uniform composition and nutritive value of the test meal throughout the study. For this purpose, raw rice and lentils were purchased in bulk, a single recipe was followed, and test meals were cooked by an assigned CHW throughout the measurement period. Mothers were instructed not to feed infants for at least 4 h prior to the test meal and oral isotope administration. Aliquots of 50 g of the prepared khitchri were removed before adding the MNP sachet. The sample was transferred to a Nalgene bottle and stored at the Nutrition Research Laboratory at Aga Khan University at -80°C until shipment on dry ice to the University of Colorado Denver Pediatric Nutrition Laboratory for phytate, zinc, and iron analyses.

Two MNP sachets (i.e., 20 mg of zinc) were thoroughly mixed into the remaining 200 g of khitchri such that each 100 g of khitchri contained 10 mg of zinc. A 50-g aliquot of the fortified khitchri was then removed for the assessment of phytate, zinc, and iron. The remaining 150 g was fed ad libitum to infants as the test meal, with a goal intake of 100 g. The exact weight of the food provided and consumed by the infant was documented by pre- and post-weights.

Premeasured doses of ⁷⁰Zn oral isotope were administered (see below). The dose was quantitatively transferred to a 3-cc syringe for administration. Small tubing was attached to the syringe to facilitate oral administration to the participant. A few drops at a time were dispensed into the mouth of participants beginning halfway through the meal and continuing throughout the test meal; 3 rinses of the tubing and syringes were also administered during the meal. Any losses of dose vomited or spit out were collected on ashless filter papers that were stored and transported to the University of Colorado Denver (UCD) for isotope analyses.

On the morning of day 2 of the metabolic measurements period, infants were brought to the Clinical Trial Unit of the Aga Khan University, Karachi for i.v. administration of a second zinc isotope tracer (~320 µg ⁶⁷Zn for initial studies and ~450 µg ⁶⁸Zn at 6-mo follow-up). Immediately prior to the infusion, a 3-mL blood sample was collected via venipuncture with a butterfly needle (Jms Singapore Pte) for assessment of biochemical markers [plasma zinc, C-reactive protein (CRP), and hemoglobin]. Two milliliters of blood was collected in zinc-free heparinized tubes, inverted, and centrifuged and plasma removed within 30 min to obtain plasma for zinc analysis; the remainder was used for hemoglobin and CRP concentrations.

Subsequent to the blood draw, a 3-way stopcock was attached to the end of the butterfly tubing and the zinc isotope solution was i.v. infused during a period of 2–3 min. This was followed by rinsing the syringe and tubing twice with equal volumes of normal saline to ensure the entire isotope dose was infused. All isotope losses were collected on ashless filter paper and analyzed as described below. The infusion was carried out by the independent investigator (S.A.) who had earlier received training in tracer isotope studies at the UCD. All precautions were taken to avoid zinc contamination during the procedures.

During days 3–4, the study team (CHW and study physician) visited participants' households to seek general information on health, ensure infants were consuming complementary foods, and monitor compliance of MNP sachets. During days 5–8, urine samples were collected at the

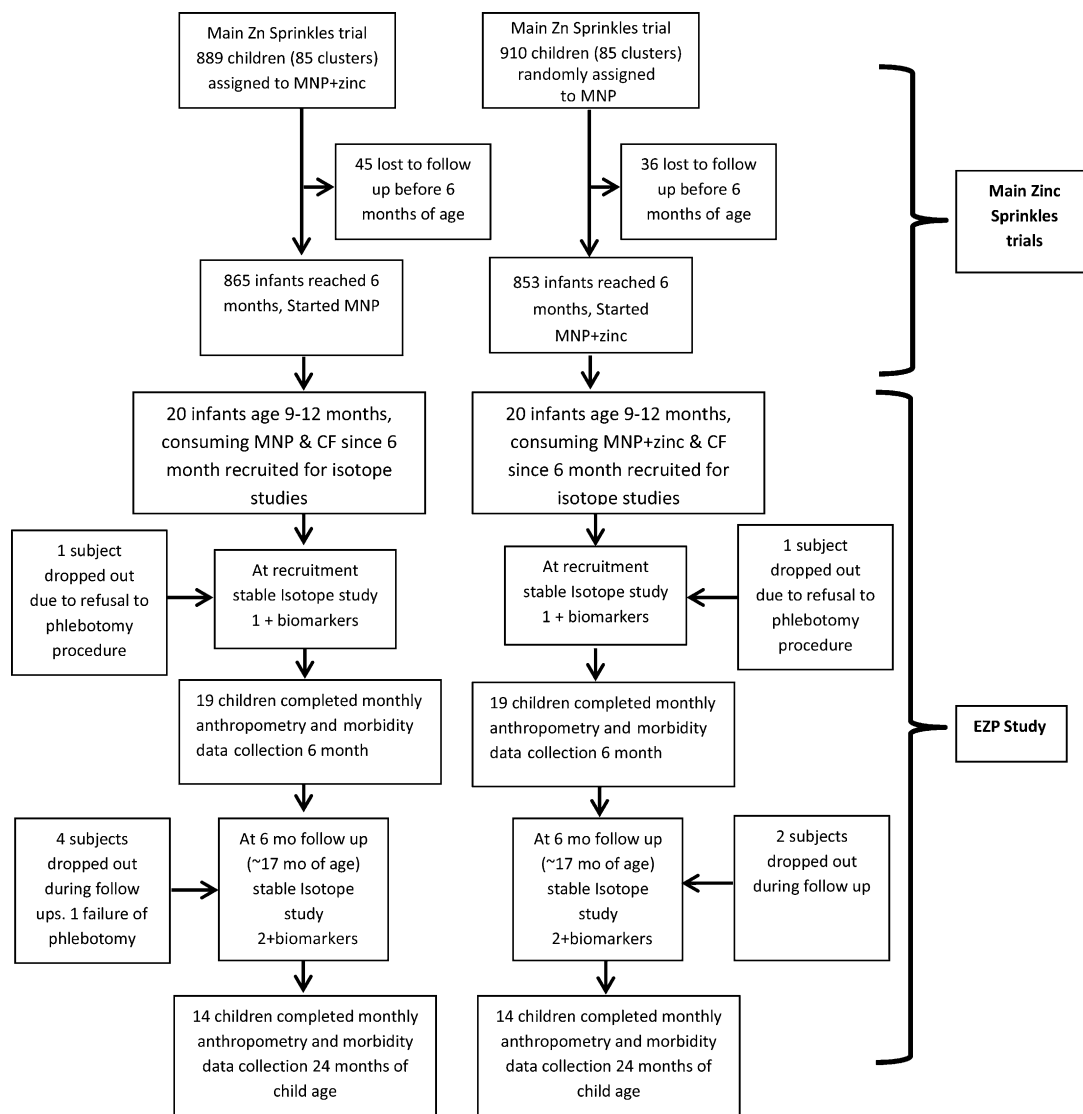


FIGURE 1 Flow diagram showing progress in the 2 assigned MNP and MNP + Zn groups from enrollment in the Main Zn Sprinkles Trial to recruitment in the EZP stable isotope study. The 2 groups underwent EZP isotope measurements and anthropometry and were fed CF. CF, complementary food; EZP, exchangeable zinc pool; MNP, micronutrient powder; MNP+Zn, micronutrient powder with zinc.

homes of the participants by the mothers with assistance from the CHW. Both morning and evening samples were collected for 4 consecutive days for a total of 8 urine samples. Urine was collected in zinc-free standard adhesive pediatric urine collection bags (Briggs Health Care) and then transferred to zinc-free Nalgene bottles and stored at -20°C at the Pediatric Research Nutrition Laboratory of the Aga Khan University. Urine and khitchri samples were later shipped to the UCD for analysis.

Isotope dose preparation. Isotope preparations were performed at the UCD as previously described (8). Enriched stable isotopes of zinc were obtained from Trace Science International. Accurately weighed quantities of each isotopically enriched preparation were dissolved in 0.5 mol/L H_2SO_4 and then diluted with deionized water to prepare stock solutions.

Oral doses were prepared by taking the stock solution of ^{70}Zn (90% enrichment) diluted with purified water and titrating to pH 6.0 with metal-free ammonium hydroxide. The resulting solution was filtered through a 22- μm filter to ensure sterility. Zinc concentrations of solutions were determined by triplicate measure of the preparation using atomic absorption spectrophotometry (model AAnalyst 400, Perkin-Elmer) with adjustment of concentration measurements for different atomic weights of the preparations. Isotope-enriched doses were individually prepared for each infant, with color coding for the MNP and MNP+Zn groups to distinguish dose amounts ($\sim 150\ \mu\text{g}$ or $750\ \mu\text{g}$, respectively).

The filter papers with any lost oral dose were ashed and the amount of isotope was measured according to the procedures described below. The amount of isotope lost during administration was subtracted from the calculated dose to give a final administered isotope dose.

The i.v. doses ($\sim 320\ \mu\text{g}\ ^{67}\text{Zn}$ and $450\ \mu\text{g}\ ^{68}\text{Zn}$) were prepared from the stock ^{67}Zn (94% enrichment) and ^{68}Zn (99.4% enrichment) solutions. The stock solutions were diluted with 0.45% saline and adjusted to pH 6.0. It was then filtered through a 22- μm filter to ensure sterility. The pharmaceutical quality of the sterile solution (i.e., sterility and pyrogenicity) was certified by the University of Colorado Hospital pharmacy and the core laboratory of the General Clinical Research Center, respectively.

Sample size estimations. Sample size determinations were based on data from another study in infants (7) in which a difference in mean EZP sizes of 3.6 mg and a pooled SD of 3.67 mg were observed. To detect a difference of 4.0 mg/kg and assuming a type 1 error rate of $P = 0.05$, 20 infants in each group would give >90% power based on a 2-sample *t* test.

Sample processing and analysis. The test meal samples from the metabolic measurement periods were individually dried in an electric drying oven and then digested at 450°C in a muffle furnace for 24 h. A few drops of concentrated NO were added to each sample and then the

samples were dried on a hot plate and ashed again at 450°C for 24 additional hours. Ashed samples were reconstituted with 25 mL 6 mol/L HCl and the total zinc and iron concentrations were measured by using atomic absorption spectrophotometry fitted with a deuterium arc background lamp (model AAnalyst 400; Perkin-Elmer). A replicate sample of the cooked khitchri was freeze-dried and the phosphorus content was measured using a modification of a previously published colorimetric method (13). The phytate concentration was subsequently estimated from the phosphorus results. Urine samples were digested by using a MARS microwave sample-preparation system (CEM). A 5-mL urine sample was placed into the HP500 vessel system with 3 mL concentrated HNO₃. The microwave gradually ramped up the temperature and pressure in the vessel to ~200°C and 120 psi and digested the sample in 60 min. The digested sample was transferred from the vessel to a 50-mL beaker and evaporated to dryness on a hot plate. The dried sample was reconstituted in 2 mL ammonia acetate buffer (pH 5.6) and zinc was purified by first chelating it with trifluoroacetylacetone and then extracting the chelate with hexane. Isotope enrichment was determined from isotope ratios of ⁶⁷Zn:⁶⁶Zn, ⁶⁸Zn:⁶⁶Zn, and ⁷⁰Zn:⁶⁶Zn by using inductively coupled plasma MS (VG PlasmaQuad 3, VG Elemental, ThermoElectron) (8).

Data processing. The FAZ from the test meal was determined by using a dual isotope tracer ratio method that measured the isotopic ratios of the i.v. and oral isotopes administered, according to the following equation:

$$\text{FAZ} = \text{urine enrichment (oral/intravenous)} \times \text{dose (intravenous/oral)}$$

The mean FAZ from the test meal was calculated for each infant from the spot urine samples collected during the metabolic period.

The AZ was calculated from the zinc in the test meal (mg) × FAZ from the test meal.

The EZP was calculated by dividing the dose of the i.v. isotope (⁶⁷Zn or ⁶⁸Zn) infused by the enrichment value at the y-intercept of the linear regression of a semi-log plot of urine enrichment data from d 5–8 after isotope administration in each measurement period (8,11).

Statistical analysis. Prior to data entry, all forms were checked for completeness and consistency. In case of inconsistency or missing responses, study personnel were consulted for possible explanations. For data entry, databases and entry screens were developed using Microsoft Visual Fox Pro 7.0. A subsample of the data was manually checked to examine data entry errors and monitor error rates of data entry operators. We used SPSS, version 15, for data analysis and a *P* value of <0.05 was considered as a significant difference. Descriptive statistics (mean ± SD, counts and proportions) were reported for all continuous and categorical variables, respectively. An independent sample *t* test was used to compare outcomes between groups at both time points and paired comparison *t* test was used to compare longitudinal change in EZP over time in the participants who completed both metabolic studies.

Results

Forty infants were recruited for the isotope measurements; 20 in each group, 17 males, and 23 females. Thirty-one infants completed the measurements. One participant dropped out from each group at the beginning of the study due to refusal for phlebotomy. Another 6 dropped out between 12 and 18 mo; 4 moved out of the study area and 2 withdrew (Fig. 1). There was one failure of isotope infusion. No adverse events were reported during the period of isotope measurements.

Demographics of the 2 groups were comparable at the start of the study as was supplement compliance during the 6-mo interval between the isotope studies (Table 1). There was no significant difference in the anthropometric measurements between the 2 groups at recruitment and 6-mo follow-up. At recruitment, the weight-for-age *Z*-scores were -1.15 ± 1.06 in the MNP group and -1.52 ± 1.35 in the MNP+Zn group. Similarly, length-for-age *Z*-scores were -1.26 ± 1.19 and -1.75 ± 1.59 in the MNP and MNP +Zn groups, respectively. At the follow-up at ~16 mo of age, weight-for-age *Z*-scores and length-for-age

TABLE 1 Anthropometric data and Zn intakes of infants enrolled in the 2 groups (MNP and MNP+Zn)¹

	MNP	MNP+Zn	<i>P</i> value
Gender, <i>n</i> (%)			
Male	10 (50)	7 (35)	
Female	10 (50)	13 (65)	
Age, <i>mo</i>			
At recruitment ²	10.4 ± 1.2 (19)	9.9 ± 1.5 (19)	0.26
At 6 mo follow-up	17.0 ± 0.9 (14)	16.7 ± 1.3 (17)	0.47
Weight, <i>kg</i>			
At recruitment	7.8 ± 1.2 (19)	7.3 ± 1.0 (19)	0.17
At 6 mo follow-up	9.7 ± 1.5 (14)	8.7 ± 1.1 (17)	0.04
MNP supplement compliance at 6 mo follow-up			
Intake, <i>wk</i>	20.2 ± 5.1 (14)	19.9 ± 6.1 (17)	0.13
Compliance, %	79 ± 18 (14)	70 ± 21 (17)	0.47
Energy, ³ <i>kcal/d</i>			
At recruitment	883 ± 1030	1210 ± 1060	0.60
At 6 mo follow-up	1030 ± 1030	1090 ± 1120	0.59
Dietary Zn intake, ³ <i>mg/d</i>			
At recruitment	5.3 ± 6.2 (19)	7.6 ± 7.9 (19)	0.31
At 6 mo follow-up	6.3 ± 7.6 (14)	6.3 ± 7.3 (17)	0.99
Dietary Zn intake from ASF, ³ <i>mg/d</i>			
At recruitment	0.4 ± 0.3 (19)	0.4 ± 0.4 (19)	0.91
At 6 mo follow-up	2.6 ± 2.1 (11)	1.3 ± 0.3 (11)	0.30

¹ Values are means ± SDs (*n*) unless otherwise noted. ASF, animal source food; MNP, micronutrient powder; MNP+Zn, micronutrient powder with zinc.

² Recruitment refers to recruitment into the isotope substudy.

³ Estimated from 24-h dietary recall data.

TABLE 2 Zinc, iron, and phytate concentrations in the test meal (khitchri) administered to infants in the MNP and MNP+Zn groups¹

	Unlabeled khitchri	Khitchri + MNP ²	Khitchri + MNP+Zn ²
Zinc, mg/100 g	0.3 ± 0.1 (52)	0.3 ± 0.1 (24)	11.1 ± 0.7 (27)
Iron, mg/100 g	0.5 ± 0.1 (50)	13.7 ± 2.1 (22)	14.8 ± 1.6 (27)
Phytate, ² mg/100 g	79 ± 39 (4)		
Phytate:Zn molar ratio	26	26 ³	<1 ³

¹ Values are means ± SDs (*n*). MNP, micronutrient powder; MNP+Zn, micronutrient powder with zinc.

² Estimated from phosphorus content.

³ Estimated from phytate content of unlabeled khitchri.

Z-scores between the 2 groups were comparable (-0.55 ± 1.18 vs. -1.35 ± 1.19) and -1.71 ± 0.96 vs. -2.09 ± 1.01 in the MNP and MNP+ Zn groups, respectively.

Dietary intake data showed approximately similar caloric consumption. The mean zinc content in the daily diet estimated from 24-h recall (excluding zinc from MNP) in both groups and at both time points exceeded the Estimated Average Requirement of 2.5 mg/d (14), with intakes ranging from 5.3 to 7.6 mg/d. Less than 8% of the zinc was from animal source foods at the time of the first isotope measurements but had increased to 20–40% by the time of the second isotope measurements (Table 1).

The analysis of unfortified (without MNP or isotope) cooked khitchri revealed modest inherent zinc or iron contents. Addition of the MNP+Zn sachet significantly increased the iron content of the test meal in both groups and the zinc content in the MNP+Zn group (Table 2). The mean phytate amounts and the phytate:zinc molar ratio in unfortified khitchri were very high, whereas the addition of the MNP+Zn supplement greatly reduced the estimated phytate:zinc molar ratio (Table 2).

All data points were included in a cross-sectional analysis of the variables of zinc homeostasis between groups at each time point. The FAZ was higher in the MNP group compared with the MNP+Zn group at both time points ($P < 0.001$) and zinc intake from the test meal was higher in the MNP+Zn group ($P < 0.001$), leading to a higher AZ from the test meal in the MNP+Zn group at both the first isotope measurement and follow-up ($P < 0.001$) (Table 3).

The mean EZP (expressed as mg or mg/kg) did not differ between the 2 groups at the time of the first measurements (Table 3). However, at the follow-up measurements ~6 mo later, the mean EZP (mg/kg) was higher in the MNP+Zn group compared with the MNP group ($P = 0.01$) (Table 3). Using paired comparisons, a significant longitudinal decline in the mean EZP (mg/kg) was documented within each group between the 2 measurement periods (Fig. 2). The decline in the MNP group was 30% ($P < 0.001$) and in the MNP+Zn group it was 22% ($P < 0.004$); the

degree of the decline was not significantly different between groups ($P = 0.6$).

The mean plasma zinc concentrations in the first measurement, after exclusion of results for infants with high CRP values, was lower in the MNP group [$n = 18$, $53.6 \pm 13.3 \mu\text{g/dL}$ ($8.2 \pm 2.03 \mu\text{mol/L}$)] than in the MNP+Zn group [$n = 19$, $64.6 \pm 15.1 \mu\text{g/dL}$ ($9.8 \pm 2.31 \mu\text{mol/L}$)] ($P = 0.03$). However, at the second measurement, both groups were similar [$n = 13$, $56.3 \pm 7.0 \mu\text{g/dL}$ ($8.6 \pm 1.0 \mu\text{mol/L}$) and $n = 17$, $59.7 \pm 11.7 \mu\text{g/dL}$ ($9.1 \pm 1.7 \mu\text{mol/L}$) in the MNP and MNP+Zn groups, respectively] ($P = 0.4$).

Mean hemoglobin was higher in the MNP group at the first measurement [$10.4 \pm 1.1 \text{ g/dL}$ ($104 \pm 11 \text{ g/L}$) and $9.8 \pm 0.12 \text{ g/dL}$ ($98 \pm 1.2 \text{ g/L}$) in MNP and MNP+Zn groups, respectively; $P = 0.03$] but not at the second measurement [$10.2 \pm 1.2 \text{ g/dL}$ ($102 \pm 12 \text{ g/L}$) in MNP and $9.5 \pm 1.5 \text{ g/dL}$ ($95 \pm 15 \text{ g/L}$) in MNP+Zn] ($P = 0.13$).

Discussion

The results of this study demonstrate that the zinc in the MNP +Zn formulation added to a local complementary food was well absorbed despite the moderately high phytate content of the khitchri test meal. The AZ from the test meal with the MNP+Zn was nearly 10-fold that of the MNP group and exceeded by ~30% the estimated physiologic requirements for both time points (0.84 and 0.74 mg/d, respectively) (14). Although the fortificant would be expected to be the predominant source of zinc in the diet, it is reasonable to assume that zinc absorption from the entire day would be even greater. In contrast, the AZ from the test meals for the MNP group was only a fraction of estimated requirements at both measurements. Although the AZ of the MNP+Zn exceeded the estimated physiologic requirement, the adequacy of this AZ cannot be presumed, because the physiologic requirement was developed from data available for healthy infants in high resource settings. Our participants belonged to a much more marginalized population and hence we cannot rule out the possibility of relatively higher zinc requirements, e.g., owing to recurrent episodes of diarrhea with increased zinc losses and/or chronic intestinal inflammation.

Published zinc absorption data from similar populations receiving MNP are very limited. Fractional absorption and AZ from a meal of maize porridge with added MNP containing 5 mg zinc were lower in Kenyan 9-mo-olds (15). The AZ from the khitchri test meal alone for the MNP+Zn group in these Pakistani infants modestly exceeded the total daily AZ for healthy 9- to 10-mo-old breast-fed infants in the United States who were routinely consuming meat or a zinc-fortified infant cereal (8). Notably, however, the total dietary intake of the U.S.

TABLE 3 Isotope measurements by study groups at different time points¹

Time point	FAZ	Zn intake	AZ	EZP	EZP
		mg/test meal	mg/test meal	mg	mg/kg
First measurement					
MNP (<i>n</i> = 19)	0.29 ± 0.11	0.3 ± 0.2	0.1 ± 0.1	48 ± 12	6.2 ± 1.8
MNP+Zn (<i>n</i> = 19)	0.16 ± 0.08*	8.2 ± 3.8*	1.2 ± 0.5*	44 ± 11	6.1 ± 1.8
Second measurement					
MNP (<i>n</i> = 14)	0.31 ± 0.11	0.2 ± 0.1	0.1 ± 0.0 [§]	36 ± 6	3.7 ± 0.6
MNP+Zn (<i>n</i> = 17)	0.12 ± 0.08*	8.8 ± 4.1*	0.9 ± 0.5*	39 ± 9	4.5 ± 1.0*

¹ Values are means ± SDs. *Different from control, $P < 0.05$. AZ, absorbed zinc; EZP, exchangeable zinc pool; FAZ, fractional absorption of zinc; MNP, micronutrient powder; MNP+Zn, micronutrient powder with zinc.

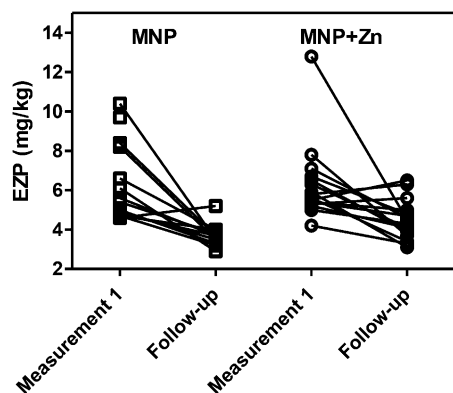


FIGURE 2 Changes between the 2 measurement points in individuals' EZP sizes. The decline was significant for both groups ($P < 0.001$ for MNP and $P = 0.004$ for MNP+Zn); the magnitude of the decline did not differ between groups ($P = 0.6$). EZP, exchangeable zinc pool; MNP, micronutrient powder; MNP+Zn, micronutrient powder with zinc.

infants was only ~ 3 mg/d, or less than one-third the amount provided by the MNP+Zn in the test meal. These observations may represent a combination of the saturation of enterocyte zinc transporters after months of consuming the higher dose of fortificant zinc along with at least some inhibitory effects of the high phytate content of the test meal (16,17). The lack of change in fractional absorption and amount of AZ from the test meals between the 2 measurement periods is also consistent with the current understanding of zinc homeostasis, which suggests that current intake of zinc in a test meal is a more potent determinant of FAZ than chronic intake and zinc status (18,19).

The size of the EZP for both groups was surprisingly high at the time of the first isotope measurement but significantly declined, especially in the MNP group, during the 6 mo between the isotope studies. Although comparable data are very limited, the means for both groups at the first measurement were 30–40% higher than means for U.S. infants of similar age (8). The explanation for the higher than expected initial mean EZP sizes is unclear but may include the relatively generous estimated zinc intakes from diet alone in the form of fortified foods such as cereals and milks for both groups. No significant differences were detected between groups in diarrheal episodes either at recruitment or at the time of the follow-up measurement.

The estimated dietary intakes were well above the Estimated Average Requirement of 2.5 mg/d for this age (14) and were higher than intakes for many older infants in other both low and high resource settings (8,20,21). The lower EZP for the MNP group at the follow-up measurement may reflect a progressively divergent chronic zinc intake provided by the fortificant, even with the only fair compliance by the second measurement period. Norms for EZP size in infants and young children have not been established. The mean EZP size of the MNP+Zn group at the follow-up time point was virtually identical to the mean of 9- to 10-mo-old, breast-fed infants in the United States who had regularly consumed meat or zinc-fortified infant cereal (8). Likewise, the mean EZP of the MNP group at the second measurement, at ~ 17 mo of age, was also very similar to the mean EZP of the 9- to 10-mo-old, breast-fed infants in the United States who received no fortified foods and modest amounts of meat and whose dietary zinc intake was only $\sim 50\%$ of the estimated requirement (8). The limitations of the 24-h recall data for a relatively small sample size may account for the apparent discrepancy in estimated generous dietary zinc intake of the MNP group and their low mean EZP size.

Mean plasma zinc tended to be higher for the MNP+Zn group [64.6 ± 15.1 $\mu\text{g/dL}$ (9.8 ± 2.31 $\mu\text{mol/L}$) vs. 53.6 ± 13.3 $\mu\text{g/dL}$ (8.2 ± 2.03 $\mu\text{mol/L}$); $P = 0.03$] at the first isotope measurement, initially supporting improved Zn "status," but this effect was not sustained through the end of the study. For relatively small sample sizes, as in this study, which was a subsample of the larger trial, plasma zinc has not been found to correlate with dietary or AZ in contrast to the significant correlations observed with EZP (19).

Limitations of the study included the design of conducting the isotope studies after infants had been receiving the MNP for at least 3 mo, which did not allow a true "baseline." We were thus unable to characterize initial dietary zinc intake, AZ, and EZP for the 2 groups and to determine whether it was higher than expected for the MNP group from the outset. A higher dietary intake in the MNP group at enrollment would potentially have explained the lack of difference in EZP between groups at 10 mo of age. Labeling only a test meal with the MNP instead of all foods in an entire day, ideally including breast milk, precluded measurement of total daily AZ, which is correlated with EZP (8). Such demanding metabolic studies were beyond the scope of the resources available in this setting. We also hypothesized an intake of close to 100 g of khitchri in the test meal, whereas in reality the amount consumed by infants varied moderately. This led to varying amounts of zinc intake per test meal; isotope doses were based on the assumption that an entire MNP dose would be consumed. When khitchri intake was low, the isotope dose became a higher percentage of the zinc in the test meal. This variability in intake of the test meal was similar for both groups and did not obscure the striking difference in AZ between the unfortified and fortified test meals.

In summary, the results of this study demonstrate that the zinc in the MNP+Zn was sufficiently well absorbed among infants aged 6–18 mo to exceed the estimated physiologic requirement for this age. Furthermore, they confirm that MNP+Zn added to traditional complementary foods containing high phytate results in a substantial increase in AZ, and administration of such MNPs for 12 mo did not significantly diminish the zinc absorption over time. Whether this amount of absorption is adequate for functional benefits in a population with high risk of recurrent gastrointestinal infections and concurrent losses cannot be determined from this study alone. In fact, the observed excess of diarrhea and modest effect on growth among children consuming MNP alone and MNP+Zn suggest that even this level of dietary intake may be insufficient in the face of excess enteric losses of zinc or systemic infections (9). The first measurement of EZP was surprisingly high for both groups and may have reflected a higher than expected dietary zinc intake for the group receiving micronutrients without zinc. The significantly higher EZP for the MNP+Zn group than the MNP group at the time of the second measurements suggests that, even with imperfect compliance, the zinc fortificant continued to have a positive impact on metabolically available zinc. After 1 y of home fortification, zinc status assessed by EZP was significantly better in the group receiving zinc. The parent study showed only a small impact of MNP+Zn supplementation on growth between 6 and 18 mo of age and hence it is uncertain if this amount of AZ was associated with notable functional benefits (9). MNPs may provide a viable method of home fortification with zinc in low resource settings, but additional studies are necessary to ascertain the ideal zinc dose for high risk conditions.

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Literature Cited

1. Krebs NF. Zinc supplementation during lactation. *Am J Clin Nutr.* 1998;68:S509–12.
2. Brown KH, Engle-Stone R, Krebs NF, Peerson JM. Dietary intervention strategies to enhance zinc nutrition: promotion and support of breastfeeding for infants and young children. *Food Nutr Bull.* 2009;30:S144–71.
3. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2002;75:1062–71.
4. Imdad A, Bhutta ZA. Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. *BMC Public Health.* 2011;11 Suppl 3:S22.
5. Jack SJ, Ou K, Chea M, Chhin L, Devenish R, Dunbar M, Eang C, Hou K, Ly S, Khin M, et al. Effect of micronutrient sprinkles on reducing anemia: a cluster-randomized effectiveness trial. *Arch Pediatr Adolesc Med.* 2012;166:842–50.
6. De-Regil LM, Suchdev PS, Vist GE, Walleser S, Pena-Rosas JP. Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst Rev.* 2011;CD008959.
7. Jalla S, Westcott J, Steirn M, Miller LV, Bell M, Krebs NF. Zinc absorption and exchangeable zinc pool sizes in breast-fed infants fed meat or cereal as first complementary food. *J Pediatr Gastroenterol Nutr.* 2002;34:35–41.
8. Krebs NF, Westcott JE, Culbertson DL, Sian L, Miller LV, Hambidge KM. Comparison of complementary feeding strategies to meet zinc requirements of older breastfed infants. *Am J Clin Nutr.* 2012;96:30–5.
9. Soofi S, Cousens S, Iqbal SP, Khan J, Ahmed I, Zaidi AKM, Bhutta ZA. Effect of provision of daily zinc and iron with several micronutrients on growth and morbidity among young children in Pakistan: a cluster-randomised trial. *Lancet.* 2013;382:29–40.
10. Friel JK, Naake VL Jr, Miller LV, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. *Am J Clin Nutr.* 1992;55:473–7.
11. Miller LV, Hambidge KM, Naake VL, Hong Z, Westcott JL, Fennessey PV. Size of the zinc pools that exchange rapidly with plasma zinc in humans: alternative techniques for measuring and relation to dietary zinc intake. *J Nutr.* 1994;124:268–76.
12. Schröder H, Covas MI, Marrugat J, Vila J, Pena A, Alcantara M, Masia R. Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. *Clin Nutr.* 2001;20:429–37.
13. Vaintraub IA, Lapteva NA. Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Anal Biochem.* 1988;175:227–30.
14. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press; 2001.
15. Krebs NF, Hambidge KM, Justus E Ikemeri JE, Westcott JL, Kemp JF, Sian L, Miller LV, Liechty EA, Esamai F. Zinc (Zn) absorption from Sprinkles™ is not affected by iron (Fe) in Kenyan infants in malaria endemic area. *FASEB J.* 2013 27:107.3
16. Hambidge KM, Miller LV, Westcott JE, Sheng X, Krebs NF. Zinc bioavailability and homeostasis. *Am J Clin Nutr.* 2010;91:S1478–83.
17. Miller LV, Krebs NF, Hambidge KM. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. *J Nutr.* 2007;137:135–41.
18. Krebs NE, Hambidge KM. Zinc metabolism and homeostasis: The application of tracer techniques to human zinc physiology. *Biometals.* 2001;2001:397–412.
19. Chung CS, Stookey J, Dare D, Welch R, Nguyen TQ, Roehl R, Peerson JM, King JC, Brown KH. Current dietary zinc intake has a greater effect on fractional zinc absorption than does longer term zinc consumption in healthy adult men. *Am J Clin Nutr.* 2008;87:1224–9.
20. Sheng XY, Hambidge KM, Zhu XX, Ni JX, Bailey KB, Gibson RS, Krebs NF. Major variables of zinc homeostasis in Chinese toddlers. *Am J Clin Nutr.* 2006;84:389–94.
21. Gibson RS, Abebe Y, Hambidge KM, Arbide I, Teshome A, Stoecker BJ. Inadequate feeding practices and impaired growth among children from subsistence farming households in Sidama, Southern Ethiopia. *Matern Child Nutr.* 2009;5:260–75.