Zinc absorption during late pregnancy in rural southern Ethiopia1–3

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ABSTRACT

Background: Little is yet known about zinc absorption in late pregnancy, and no information on absorption from the total diet is available.

Objective: The objective was to measure the fractional absorption of zinc (FAZ) and to estimate the total quantity of absorbed zinc (TAZ) each day during the third trimester of pregnancy in poor rural southern Ethiopian women.

Design: The participants (n = 17) were a convenience sample from a larger study population. The third stage of pregnancy was estimated from fundal height by the Bushulo Health Center prenatal outreach program. FAZ was determined with a dual-isotope tracer ratio technique that uses measurements of urine enrichment with zinc stable isotopes administered intravenously and orally, as an extrinsic label, with all meals in 1 d. Total dietary zinc (TDZ) was calculated from weighed diet records and Ethiopian food-composition tables supported by zinc and phytate analyses of major food items for individual meals. Plasma zinc and exchangeable zinc pool size were also estimated.

Results: Mean (±SD) FAZ was 0.35 ± 0.11, TDZ was 6.0 ± 3.2 mg/d, TAZ was 2.1 ± 1.0 mg/d, phytate intake was 1033 ± 843 mg/d, plasma zinc was 44.1 ± 6.0 μg/dL, and the exchangeable zinc pool size was 142 ± 39 mg. The molar ratio of phytate to zinc was 17:1.

Conclusions: Women from a poor rural population who were dependent on a moderately high-phytate diet had low TDZ and low plasma zinc concentrations in the third trimester of pregnancy. TAZ was modestly higher than that predicted but did not meet physiologic requirements.

INTRODUCTION

Because of substantial residual uncertainties about dietary zinc requirements (1, 2) and the effect of dietary factors and physiologic circumstances on these requirements, measurement of the quantity of zinc absorbed from the habitual diet can provide potentially useful baseline information for determining the need for and magnitude of interventions to increase absorbed zinc. Late pregnancy is an outstanding example of a physiologic circumstance for which there is a lack of data for zinc absorption over an entire day.

This study was part of a more extensive cross-sectional pilot investigation of zinc nutriture during the third trimester of pregnancy in very poor rural communities in the Sidama region of Southern Ethiopia. Exceptionally low zinc intakes (3) and very low plasma zinc concentrations (4) have been documented in this population during the third trimester of pregnancy. The principal purpose of these studies was to provide data for the optimal design of intervention studies to improve zinc absorption and status.

The principal objective of this study was to determine the quantity of TAZ each day. It was hypothesized that the estimated quantity of zinc absorbed is substantially lower than current estimates of physiologic zinc requirements during the third trimester of pregnancy.

SUBJECTS AND METHODS

Study design

A convenience sample of women in the third trimester of pregnancy participated in a cross-sectional study. Subjects were studied under free-living conditions while consuming their habitual diet. Weighed records of food intake for an entire day were collected by trained community research workers (CRWs) in the participants’ homes. The trained CRW also administered a zinc stable-isotope tracer orally with each of the 3 meals consumed during the day. After lunch, a second zinc stable isotope was administered intravenously. Morning spot urine samples were collected daily for 5 d, commencing 3 d after isotope administration, for the measurement of zinc stable-isotope ratios. Fractional absorption of zinc (FAZ) was measured with the dual-isotope tracer ratio (DITR) technique (5, 6).

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Subjects

The population that participated in the study was the Sidama ethnic group, a primarily rural population located in the Rift Valley in Southern Ethiopia. The participants were from the community of Alamura, which is served by the Bushulo Health Center. As is typical of rural populations in this region, the community of Alamura is very poor, especially the women and children. Families depend for their food almost entirely on subsistence farming on progressively diminishing family plots. The food staples are maize and fermented Enset. The tuber of Enset provides little apart from carbohydrate and dietary fiber, but, because it is very drought resistant, this tuber is especially valuable in the dry season when it may be the principal food staple. These studies were performed in July, shortly before the start of the rainy season.

The subjects included in this zinc stable-isotope study were a convenience subsample of a larger study population of 100 subjects whose dietary intakes were reported elsewhere (3, 7). The prenatal outreach program of the Bushulo Health Center confirmed, on the basis of fundal height, that the participants were in the third trimester of pregnancy. The mean (±SD) age of the women was 27.8 ± 4.7 y, and they had a mean (±SD) parity of 3.3 ± 2.6. Mean weights and heights were 51.2 ± 6.7 kg and 153.4 ± 7.1 cm, respectively.

The protocol was approved by the University of Colorado’s Multiple Institution Review Board, Oklahoma State University’s Ethics Committee and by the Ethics Committee of Debub University. The protocol was explained verbally and in writing to community leaders and health workers by one of the investigators (YA) in Amharic and by the community representatives to the potential participants in Sidama. Translation was monitored by senior community research workers who speak both languages. Eligible participants returned from 1 to 3 days later if they wished to participate. Consent was given verbally and was witnessed by a local health worker or community leader who would be the individual who signed the written consent form. This procedure was approved by the ethics review boards for cultural reasons.

Dietary zinc intake

Twenty-four-hour weighed food records were obtained from all the subjects as part of a larger study on the dietary intake of this population (3, 7). These records were obtained in their homes by trained CHWs on the day of isotope administration. Small aliquots of staple foods were collected at each meal from each participant for chemical analysis of zinc and phytate.

Preparation of tracer

Enriched stable isotopes of zinc oxide were obtained from Trace Sciences International, Ontario, Canada. Accurately weighed quantities of each isotopically enriched preparation were dissolved in 0.5 mol H₂SO₄/L and then diluted in triply deionized water to prepare a stock solution. For preparation of orally administered doses, the stock solution of enriched ⁷⁰Zn was diluted and titrated to pH 5.0 with metal-free ammonium hydroxide. This solution was filtered through a 0.22-μm filter to make a sterile solution. Sterile techniques were used to prepare intravenously administered doses of ⁶⁷Zn. The stock solution was diluted and adjusted to pH 6.0 and then filtered through a 0.22-μm filter to make a sterile solution. Concentrations of zinc in the isotope preparations were determined in triplicate by atomic absorption spectrophotometry (AAS) and concentrations were adjusted for the different atomic weights of the preparations.

Isotope administration

The women in this community consumed either 2 or 3 meals per day of approximately equal size. No animal products were consumed, except for the occasional consumption of small amounts of cow or goat milk with coffee. A standard accurately weighed quantity of ⁷⁰Zn was administered orally by the CRW at each meal, which was supervised by one of the investigators (RSG). The total quantity of ⁷⁰Zn administered was ≈300 μg. Administration was by our standard method for extrinsic labeling, starting approximately halfway through the meal and taking in sips of the solution over the remainder of the meal from a plastic tube including 2 rinses from the tube. Between 1300 and 1400 on the day of oral isotope administration, a CRW accompanied the participant to the open-air Alamura community center. A sterile solution of an accurately weighed quantity (≈500 μg) of ⁶⁷Zn was administered intravenously over a 10-min interval by one of the authors (KMH). The solution was administered into a peripheral forearm vein via a 3-way stopcock; the syringe was rinsed twice with sterile 0.5 N saline (8). Simulation with the use of our compartmental model of zinc metabolism (9) indicated that the optimal time for administration of the intravenous zinc tracer for a 1-d total diet study is in the late evening (LV Miller, unpublished observations, 2005). Administration at 1300–1400 requires a downward adjustment of 4% in the calculated FAZ.

Sample collection, storage, and transport

On the dosing day, trained CRW accompanied participants for the entire day under the supervision of one of the authors (RSG). They weighed all foods and beverages consumed throughout the day and collected aliquots of all major staple food items consumed. A midstream urine sample was collected at baseline and subsequently at ≈0800 on days 4–8 into a zinc-free plastic container. The urine samples were collected from the homes by a senior CRW by midmorning each day and transported to the program’s research laboratory facility at Debub University. Samples were stored there at −20 °C until transported to the Pediatric Nutrition research laboratories at the University of Colorado Health Sciences Center. A blood sample was obtained from the same peripheral venous access immediately before the intravenous administration of the zinc tracer. The samples were separated within 2 h in the laboratory of the Bushulo Health Center by using zinc-free techniques. Plasma was then stored at −20 °C until hand-carried frozen to the University of Colorado Health Sciences Center for analyses.

Sample processing and analyses

Urine samples were digested by using an MDS-2000 microwave sample preparation system (CEM Corp, Mathews, NC). Five milliliters of urine was placed into an Advanced Composite Vessel (CEM, Matthews, NC) and combined with 1 mL concentrated HNO₃; the pressure was gradually increased to a maximum of 120 psi. Total digestion time was ≈90 min. Digested samples
were transferred to 50-mL beakers, evaporated to dryness on a hot plate, and reconstituted in 2 mL ammonia acetate buffer (pH: 5.6). Zinc in the sample was purified by chelation with trifluoroacetylacetone and then extraction of the chelate with hexane (10). Isotope enrichment was determined by measuring the isotope ratios $^{67}$Zn/$^{66}$Zn and $^{70}$Zn/$^{66}$Zn with an inductively coupled plasma mass spectrometer (VG Plasma Quad 3; VG Elemental, Cheshire, United Kingdom). Tracer enrichment was defined as all of the zinc in the sample derived from the isotopically enriched tracer preparation divided by the total amount of zinc in the sample.

Ion-pair HPLC with refractive index detection (410 Differential Refractometer; Waters, Milford, MA) was used to measure hexa-inositol (IP-6) and penta-inositol (IP-5) phosphates (11) in dried powdered food samples after their extraction and separation by a Hypersil column (H30DS-250A; HiCHROM, Berkshire, United Kingdom). Myo-inositol phosphates with <5 phosphate groups were not measured because they do not have a negative effect on zinc absorption (12). The accuracy of this HPLC procedure was assessed through interlaboratory comparison of the IP-5 + IP-6 content of white maize flour (65% extraction). Our analyzed value ($\pm$ SD) certified value of 89 $\pm$ 0.5 g/g was 89 $\pm$ 2 mg/100 g compared with our previously reported value of 89 $\pm$ 0.7 mg/100 g. The analysis of dried-powdered aliquots of each staple food sample were dry-ashed at 450 °C, dissolved in 6 mL Ultrapure HCl (Arista, BDH Laboratory Supplies, Cheshire, United Kingdom). Myo-inositol phosphates with <5 phosphate groups were not measured because they do not have a negative effect on zinc absorption (12). The accuracy of this HPLC procedure was assessed through interlaboratory comparison of the IP-5 + IP-6 content of white maize flour (65% extraction). Our analyzed value ($\pm$ SD) ($n$ = 3) for IP5 + IP6 was 292 $\pm$ 21 mg/100 g compared with our previously reported value of 284 $\pm$ 22 mg/100 g. For zinc analysis, dried-powdered aliquots of each staple food sample were dry-ashed at 450 °C, dissolved in 6 mL Ultrapure HCl (Arista, BDH Laboratory Supplies, Dorset, United Kingdom), and analyzed by flame AAS (AA-800; Perkin Elmer Corp, Norwalk, CT). National Institute of Standards and Technology Standard Reference Materials (SRMs) citrus leaves (SRM-1572) and rice flour (SRM-1568a) were analyzed to check on the accuracy and precision of the AAS procedure for zinc. The means ($\pm$ SDs) and CVs (as %) for the zinc content of citrus leaves (SRM-1572) ($n$ = 5) and rice flour (SRM 1568a) ($n$ = 5) were 29.0 $\pm$ 1.3 μg/g (CV: 4.5%) and 19.1 $\pm$ 0.2 μg/g (CV: 1.0%) compared with the certified values of 29.2 $\pm$ 2 and 19.4 $\pm$ 0.5 μg/g, respectively.

Plasma zinc was measured by flame AAS (13). The precision of the plasma zinc analysis was determined by using a reference plasma pool. Accuracy was established by analyzing an SRM [Inorganic Constituents in Bovine Serum, SRM-1598; National Institute of Standards and Technology Gaitersburg, MD; mean ($\pm$ SD) certified value of 89 $\pm$ 6 μg/dL compared with an analyzed value of 89 $\pm$ 1.7 μg/dL].

Data processing and statistical analysis

Intakes of total dietary zinc (TDZ) and phytate for each subject were calculated from the weighed food records by SPSS software (SPSS Inc, Chicago, IL) with the use of a food-composition table based mainly on the analyzed values for the staple foods consumed by the corresponding subject (3, 7).

For the determination of FAZ, the ratio of the urinary isotopic enrichment of the orally administered $^{67}$Zn to the intravenously administered $^{70}$Zn was used in the following equation (5):

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

Five urine specimens obtained during study days 4–8 were analyzed, and the calculated FAZs for the specimens were averaged to determine each participant’s FAZ. Dixon-type tests were used to check for single outliers (14). These FAZ values were multiplied by 0.96 to adjust for the time of administration of the intravenous isotope.

TDZ was calculated from each individual’s weighed diet records. TAZ for each individual was calculated as follows:

$$\text{TAZ (mg/d)} = \frac{\text{TDZ (mg/d)}}{\text{FAZ}} \quad (2)$$

Urine isotopic enrichment data were also used to estimate the size of the exchangeable zinc pool (EZP) (15), defined as the sum of those pools of zinc that interchange with zinc in plasma within 3 d as follows:

$$\text{EZP (mg)} = \text{intravenous dose (mg)} / y \text{intercept} \quad (3)$$

The y intercept was estimated by extrapolation from a linear regression analysis of the natural log of the percentage enrichment of the intravenous isotope in the 5 daily urine samples. To examine the relation between the quantity of TAZ and the quantity of TDZ, both linear regression (16) and nonlinear regression were applied. For the latter we used the saturable response model:

$$\text{TAZ} = \frac{(A_{\text{max}} \times \text{TDZ})}{(I_{\text{A50}} + \text{TDZ})} \quad (4)$$

where $A_{\text{max}}$ is the maximal absorption of zinc, and $I_{\text{A50}}$ is the quantity of zinc intake required for half maximal absorption (15). The saturation model and linear regression were compared with Akaike’s Information Criterion (AIC) by using a second order correction for small sample size (17). Pearson’s correlation coefficients were determined for plasma zinc versus EZP and for each of these variables versus TDZ, FAZ, and TAZ. Data are presented as means ($\pm$ SDs) unless otherwise stated. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed by using GRAPHPAD PRISM (version 4.03; GraphPad Software Inc, San Diego, CA).

RESULTS

Eighteen of the 20 participants had urine samples suitable for analyses. The calculated TDZ for one of these subjects was extremely high (22.6 mg Zn/d), which was attributable entirely to an extraordinarily high zinc concentration in the aliquot of fermented corn bread collected from the home on the isotope administration day. Data from this subject were not included. For the remaining 17 subjects, the mean ($\pm$ SD) FAZ was 0.35 $\pm$ 0.11. The calculated zinc intake (TDZ) on the same day for these subjects was 6.0 $\pm$ 3.2 mg/d. Mean TAZ was 2.1 $\pm$ 1.0 mg Zn/d.

Mean dietary phytate was 1033 $\pm$ 843 mg/d, and the average molar ratio of phytate to zinc was 17:1. Plasma zinc ($n$ = 15) was 44.1 $\pm$ 6.0 μg/dL. The estimated size of the combined pools of zinc that exchange with zinc in plasma within 3 d (EZP) was 142 $\pm$ 37 mg or 2.76 $\pm$ 0.79 mg Zn/kg body wt.

The best fit for TAZ versus TDZ was obtained with nonlinear regression using the saturable response model described in the methods (Figure 1). AIC showed the nonlinear saturation response model to be superior to linear regression for modeling the relation between TAZ and TDZ. The AIC scores for the nonlinear and linear models were $-10.14$ and $-7.79$, respectively. The ratio of probabilities derived from the difference between the scores was 3.24, which indicated that this nonlinear model is $>3$ times as likely to be the correct model than is the linear model. The $r^2$ for the nonlinear regression was 0.64, compared with 0.58.
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FIGURE 1. Daily total absorption of zinc (TAZ) versus daily total dietary zinc (TDZ) for rural Ethiopian women in late pregnancy. Data were fit with a basic saturable response model (solid line) described by the equation TAZ = (A_{max} \times TDZ)/(IA_{50} + TDZ), where A_{max} is the maximal absorption of zinc and IA_{50} is the quantity of zinc intake required for half maximal absorption (15).

for the linear regression. The fitting of the saturable response model to the data with the use of the nonlinear regression produced parameter estimates of 7.0 and 13.0 mg/d for A_{max} and IA_{50}, respectively. No significant correlations were observed for plasma zinc or EZP.

DISCUSSION

As with other data sets for the quantity of zinc absorbed versus that ingested (18, 19), nonlinear saturation response modeling (Figure 1) provided a better fit than did linear regression. This is to be expected because active zinc absorption is a saturable transport-receptor-mediated process (20). Maximal absorption was estimated to be similar to that calculated previously for men (18), but with a wider CI.

Evaluation of data on FAZ and, more importantly, on TAZ, requires examination of data as a function not only of TDZ (18) but also of dietary phytate (2, 9). With a dietary zinc intake of 6.0 mg Zn/d and a molar ratio of phytate to zinc of 17:1, the means for this study, the predicted FAZ and TAZ, were 0.31 and 1.8 mg Zn/d, respectively, for nonpregnant adults. These reference values were determined by 2 independent models of zinc absorption as a function of dietary zinc and phytate:zinc ratios, both of which give the same results for nonpregnant adults of both sexes (2, 21). The mean FAZ and TAZ values for the participants in this study were 0.05 and 0.3 mg Zn/d higher, respectively, than these predicted values for nonpregnant adults. Despite these modestly higher than predicted values, the quantity of zinc absorbed by these Ethiopian women was very low in comparison with estimated physiologic zinc requirements for late pregnancy (1). The low quantity of absorbed zinc was not unexpected in view of the low dietary zinc. However, this additional information provides a specific target for increasing the quantity of absorbed zinc.

On the basis of the results of single-meal studies of zinc absorption, it has been reported that FAZ increases by one-third (22) to one-half (23) between prepregnancy and/or the first trimester and the third trimester of pregnancy. The modestly higher zinc absorption values in this study compared with that predicted by the 2 models are marginally consistent with the conclusion that adaptation during late pregnancy enhances the efficiency of zinc absorption. Confirmation will require longitudinal total diet studies of zinc absorption. However, it is clear from the results of this study that any pregnancy-related adaptive increase in active intestinal absorption of zinc that may have occurred was insufficient to counterbalance the low zinc intake of this population.

It has been suggested that adaptation in late pregnancy is greater in subjects with low zinc intakes (22). Comparison of the results of this study, in which zinc intakes were exceptionally low, with those reported by Donangelo et al (22) and Fung et al (23) did not support this conclusion. However, the study designs were different; specifically this study was not longitudinal but it did measure zinc absorption over a whole day rather than from a single meal. Donangelo et al (22) also observed a negative correlation between FAZ and plasma zinc and suggested that there was up-regulation of zinc absorption with impaired zinc status. The results of the present study, in which the participants’ plasma zinc concentrations were exceptionally low, do not support this conclusion. In fact, these results are consistent with the conclusion (18, 24) that zinc status, in contrast with recent zinc intake (19), does not have a major effect on the efficiency of zinc absorption.

Data on the EZP are “byproducts” of our DITR method for measurement of FAZ. No comparable data during late pregnancy are available. Our ability to interpret the values for these Ethiopian women is, therefore, limited. The mass of the EZP of poor, rural, nonpregnant, nonlactating Chinese women with comparable heights and weights was similar; the mean was 131 mg Zn (25). The mass of the EZP at 2 mo postpartum for lactating Chinese women with adequate zinc intake and absorption (26) was higher; the mean was 184 mg (S Lei et al, unpublished observations, 2002). In contrast with some other data sets (27), no correlation between the mass of the EZP and the quantity of zinc absorbed was observed in this study.

In conclusion, the quantity of zinc absorbed by this population of rural southern Ethiopian women was very low compared with estimates of physiologic requirements for zinc during the third trimester of pregnancy. FAZ and TAZ were modestly higher than predicted by modeling FAZ and TAZ as a function of TDZ and dietary phytate for nonpregnant women. Determination of the quantity of zinc absorbed each day from habitual diets provides more precise data on the deficits that require correction than can be achieved at present from dietary data and algorithms.

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YA, KMH, NFK, RSG, JEW, and BJS participated in the study design and data interpretation. YA, KMH, RSG, IA, AT, and BJS were responsible for the human studies. KBB was responsible for the dietary phytate and zinc measurements. SL conducted the total and isotopic zinc (inductively coupled plasma mass spectrometry) laboratory analyses. KMH, JEW, and LVM analyzed the data. KMH drafted the manuscript. None of the authors had any conflicts of interest related to the findings of this study.

REFERENCES

1. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron,


