Zinc absorption and intestinal losses of endogenous zinc in young Chinese women with marginal zinc intakes

Lei Sian, Xiang Mingyan, Leland V Miller, Li Tong, Nancy F Krebs, and K Michael Hambidge

ABSTRACT  The objective of this study was to determine fractional absorption of exogenous zinc and intestinal excretion of endogenous zinc in women of childbearing age whose habitual dietary zinc intake was marginal. The target population (L group) comprised residents of a remote farming village in northeast China and the control subjects (M group) were residents of Beijing. Mean (± SE) calculated dietary zinc intakes were 5.2 ± 0.2 and 8.1 ± 0.2 mg/d, respectively. The phytate-zinc molar ratio in the diet of both groups was ≈ 10.1. 70Zn was administered intravenously before breakfast and 67Zn orally with three main meals in 1 d. Subsequently, all feces were collected quantitatively until the second visible marker had been excreted and 12-h urine samples were collected on days 3–9. Fractional absorption was determined by measuring cumulative fecal excretion of nonabsorbed 67Zn and endogenous fecal zinc by an isotope-dilution technique (70Zn). Fractional absorption values for L and M groups, respectively, were 0.31 ± 0.03 and 0.34 ± 0.03 (P = 0.45). Corresponding figures for endogenous fecal zinc were 1.30 ± 0.07 and 2.34 ± 0.20 mg Zn/d (P < 0.001). Both the estimated total size of the pools of zinc that exchange with zinc in plasma within 2 d (r = 0.762, P < 0.001) and the excretion of endogenous zinc in the feces (r = 0.706, P < 0.0001) were positively correlated with calculated total daily zinc absorption. We conclude that fractional absorption of zinc does not differ between women consuming marginal and adequate quantities of zinc in their diets, but endogenous zinc is conserved effectively by the intestine in women whose habitual dietary zinc is marginal. Am J Clin Nutr 1996; 63:348–53.

KEY WORDS Zinc, stable isotopes, absorption, endogenous fecal excretion

INTRODUCTION  The emergence of stable-isotope techniques has facilitated studies of human zinc metabolism and especially of the role of the intestine in maintaining zinc homeostasis (1). Current understanding of how the human intestine adapts to low zinc intake has been influenced by studies of experimental dietary zinc restriction in healthy volunteers (2–7). The duration of dietary zinc restriction has ranged from a few days to 6 mo, with results that depend on the duration of this restriction (5). A large percentage of the world’s population is habitually dependent on a relatively low dietary zinc intake that may be, at best, marginally adequate. The objective of this study was to delineate the efficiency of zinc absorption and the extent to which the intestine conserves endogenous zinc when dietary zinc intake is habitually low. The documented importance of maternal zinc nutriture for normal embryogenesis (8) influenced our selection of young women of childbearing age as the subjects for this study.

SUBJECTS AND METHODS

Study design  Absorption of exogenous dietary zinc and excretion of endogenous zinc in the feces were examined in rural Chinese women whose dietary zinc intake was considered marginal and were compared with that of urban Chinese women who had a higher zinc intake. Fractional absorption of an extrinsic zinc isotope label (67Zn) administered with meals was measured by determining cumulative fecal excretion of unabsorbed label (1). Endogenous fecal zinc was determined by an isotope-dilution technique after the administration of a second stable zinc isotope label (70Zn) intravenously (1). In addition, we derived estimates of the size and other parameters of the combined pools of zinc that intermixed with zinc in plasma within a 2-d period (exchangeable zinc pool, EZP).

Subjects  All subjects were nulliparous young Chinese women who had no evidence of either acute or chronic disease. The low-income rural group (L) were residents of the remote farming village of Jiuduhe, which is located ≈ 100 km north-northwest of Beijing. A preliminary study had documented marginal zinc intakes for residents of this and similar villages (9). The comparison group of middle-income urban Chinese women (M) were residents of Beijing, were nurses in a Beijing hospital, and lived in a hospital dormitory. The average income of each family member of the L group was < 120 RMB yuan ($20 US)

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per month. The average income of each family member of the urban group was > 200 RMB yuan ($30 US) per month. The means for age, height, and weight are given in Table 1. Subjects did not smoke or drink alcohol. All subjects gave their written informed consent to participate in this study. Ethical approval for the study was provided by the Academic Committee of Beijing Children’s Hospital.

**Dietary intake**

Before the metabolic studies began each subject provided a 3-d diet record after careful instruction. Food staples in Jiuduhe were rice, wheat flour, and Chinese cabbage. Other foods consumed by several subjects were egg, corn, and a variety of other vegetables and fruit. Although the major food staples were the same in Beijing, these subjects also ate pork, and most ate tofu and a wider variety of other foods. These records were analyzed by using tables of Chinese foods prepared by the Chinese Academy of Preventive Medicine (10). Intake of phytic acid was calculated from data obtained from a previous study (11).

Subjects were instructed to consume a diet identical to the one reported in the diet records for 1 wk before isotope administration and during the study period. During any one season, there is typically little day-to-day variation in the diet of these populations, especially in Jiuduhe (12).

**Site of studies**

Studies were conducted under free-living conditions for the M group with subjects continuing their normal routines. All meals were provided by the hospital cafeteria, which offered a limited menu. All participants in the rural village stayed in the home of one of the senior village women for the entire course of the metabolic study. This carefully selected individual was responsible for supervising the metabolic data collections.

**Isotope preparation and administration**

Enriched stable zinc isotopes (65Zn and 70Zn) were obtained as the oxide from The Institute of Atomic Energy of China (Beijing). Isotopes were dissolved in 0.5 mol H2SO4/L to prepare a stock solution (13). For preparation of orally administered doses, the stock solution was diluted with triply deionized water and then adjusted to pH 5.0 with NH4OH. This solution was filtered through a 0.2-μm filter to remove pyrogens. Sterile techniques were used for preparing doses from the stock solution for intravenous administration. pH was adjusted to 6.0 with NH4OH and the stock solution was diluted with sterile normal saline to a concentration of 1.5 mmol isotopic zinc/L. The solution was filtered as for the oral preparation. Zinc concentrations were determined by atomic absorption spectrophotometry. Concentration measurements were adjusted for the different atomic weights of each preparation. Solutions were tested to ensure that they were pyrogen-free.

An accurately weighed quantity of 70Zn (~1.0 mg) was administered intravenously over a 5-min period via a peripheral forearm vein. Accurately weighed quantities of 65Zn were administered with water at the midpoint of each of the three main meals of the day. The total administered dose was ~2 mg 65Zn, which was divided in proportion to the total zinc content of each meal. The containers for 67Zn were carefully rinsed three times with water and this water was also consumed. Isotope administration was performed and supervised by one of us (LS). Brilliant blue was administered as a visible fecal marker on the 6th day, and precisely 96 h later on the 10th day after isotope administration.

**Collection of samples**

A baseline fecal sample and urine sample were collected before isotope administration. Individual fecal samples were collected separately and quantitatively in plastic bags until 10–13 d after administration of the isotopes, the collection being completed with passage of the second fecal marker. Twelve-hour urine samples were collected each day from 3 to 10 d after isotope administration. Acid-washed plastic containers were used for collection. One small blood sample was collected by peripheral venipuncture after an overnight fast and again 2 h after breakfast for plasma zinc determination.

**Sample preparation and analysis**

Individual fecal samples and 150-mL aliquots of individual urine samples were dried to constant weight in an electric oven. An accurately weighed aliquot of finely ground, well-mixed dried feces and the dried urine samples were then ashed in a muffle furnace at 425 °C for 24 h. A few drops of concentrated nitric acid (Baker Instra-Analyzed for Trace Metal Analysis, Phillipsburg, NJ) were added to the ash, which was then dried before reheating again at 425 °C for 24 h. Ashed fecal samples were reconstituted quantitatively in 10 mL, 6 mol HCl/L, and ashed urine samples in ~40 mL, 6 mol HCl/L (13). The concentration of total zinc in these reconstituted fecal samples was determined by flame atomic absorption spectrophotometry with an atomic absorption spectrophotometer fitted with a deuterium-arc background correction lamp (model 2380; Perkin-Elmer Corporation, Norwalk, CT). Measurements were corrected for variations in average atomic weight of isotope-enriched samples. Zinc in ashed fecal and urine samples was separated from other inorganic constituents by ion-exchange chromatography. Zinc isotopic ratios were determined by fast-atom bombardment-induced secondary ion-mass spectrometry on a double-focusing mass spectrometer (model VG 7070E HF; Fisons-VG Analytical, Manchester, United Kingdom) equipped with an Ion Tech (London) atom gun. The mass spectrometer was operated at low resolution and polyatomic isobaric interferences were eliminated by using secondary ion energy selection. The overall precision for this technique was determined to be 1.5% CV.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Subject and diet profile and fecal zinc excretion*</th>
<th>( M (n = 10) )</th>
<th>( L (n = 10) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21.5 ± 0.9</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.9 ± 2.4</td>
<td>51.7 ± 1.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 ± 2</td>
<td>156 ± 1</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>7500 ± 1430</td>
<td>5075 ± 700</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>49.9 ± 3.4</td>
<td>30.6 ± 1.7</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>8.1 ± 0.2</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>Molar ratio of phytic acid to zinc</td>
<td>9.7 ± 0.3</td>
<td>10.5 ± 0.4</td>
</tr>
<tr>
<td>Fecal zinc (mg/d)</td>
<td>7.9 ± 0.3</td>
<td>4.8 ± 0.4</td>
</tr>
</tbody>
</table>

*\( x \pm SE. \) M, control group (Beijing); L, test group (Jiuduhe).
Data processing

Isotopic enrichments were calculated from measured isotopic ratios by using established standard curves. Adjustments were made to isotopic enrichment data to correct for the presence of the other isotope label. For each particular isotope label used, enrichment is defined as all zinc in the sample from an isotopically enriched source divided by the total amount of zinc in the sample.

For determination of fractional absorption the fraction of orally administered $^{67}$Zn excreted in each individual stool sample was determined. Cumulative fecal excretion of this extrinsic isotope label was plotted against time after the isotope was administered. Excretion was adjusted for label that had been absorbed and then secreted back into the intestinal lumen and excreted by linear regression through the final three to seven (average five) points of the plot (after excretion of unabsorbed isotope was apparently complete), and extrapolation to the y axis (1). This adjusted result for cumulative fecal excretion, expressed as a fraction of the $^{67}$Zn dose administered, was subtracted from 1.0 to determine fractional absorption. The mean adjustment was 0.04 ± 0.02.

Endogenous fecal zinc excretion was measured by an isotope-dilution technique in which urine enrichment (1, 14) was substituted for enrichment in either solid tissues (15) or plasma (16). It was calculated as follows:

$$\text{Endogenous fecal zinc (mg/d)} = \sum (F_i \times f_j)(u_o \times d)$$

where $F_i$ is the quantity of zinc (mg) in each individual fecal sample between the appearance of the visible markers, $f_j$ is the enrichment of intravenously administered isotope ($^{70}$Zn) in each of these fecal samples, $u_o$ is the average $^{70}$Zn enrichment in urine between administration of the two visible markers, and $d$ is the duration (d) of the collection period (ie, 4.0 d).

An estimate of total absorbed zinc was derived from multiplying calculated daily dietary zinc intake (mg) by fractional absorption of the extrinsic label as follows:

$$\text{Total absorbed zinc (mg/d) = total dietary zinc} \times \text{fractional absorption of zinc}$$

The validity of using this extrinsic label was assessed by comparing these data with results of an alternative method of calculating absorption of dietary zinc that was based on metabolic balance calculations and was independent of the extrinsic label, as follows (16):

$$\text{Total absorbed zinc} = (\text{total dietary zinc} - \text{total fecal zinc}) + \text{endogenous fecal zinc}$$

An estimate of net (apparent) absorption of zinc (mg/d) was derived by subtracting endogenous fecal zinc from total absorbed zinc.

The size of the combined pools of zinc that intermixed with plasma within a 2-d period, EZP, was estimated by dividing the mass of $^{70}$Zn administered intravenously by the enrichment value at the y intercept of the linear regression of a semilog plot of the urine enrichment data between days 3 and 9 (17). The rate constant $k$ of the total flow of zinc out of EZP is equal to the negative of the slope of this linear regression. The half-life of $^{70}$Zn label in EZP is the natural log of 2 divided by $k$, the turnover time of EZP is $1/k$, and the flow rate out of EZP was calculated by multiplying $k$ with the EZP size.

Statistical analysis

Results are expressed as means ± SEs. Data were analyzed by using STATISTIX 3.5 (Analytical Software, St Paul). Data were initially plotted and inspected and mean differences were tested by Student's $t$ test. Pearson correlation coefficients were determined and regression analysis was also performed.

RESULTS

The mean calculated dietary intakes of zinc, energy, and protein and the estimated phytate-zinc molar ratio are given in Table 1. This table also includes mean excretion of zinc in the feces of the L and M groups. An individual example of the calculation of fractional absorption of the orally administered extrinsic label is given in Figure 1. Mean (± SE) fractional absorption values for the L and M groups were 0.31 ± 0.03 and 0.34 ± 0.03, respectively ($P = 0.45$). The mean endogenous fecal zinc calculated by the isotope-dilution technique was 1.30 ± 0.07 mg Zn/d for the L group and 2.34 ± 0.20 mg Zn/d for the M group ($P < 0.001$).

When the calculations of dietary zinc derived from the diet records was used, mean total absorption of dietary zinc (TAZ) estimated from fractional absorption of extrinsic label was 1.63 ± 0.23 and 2.75 ± 0.24 mg Zn/d for the L and M groups, respectively ($P = 0.003$). Corresponding means for total absorption calculated from metabolic balance data were 1.74 ± 0.25 and 2.96 ± 0.30 mg Zn/d. The mean calculated net absorption for the L group did not differ from that of the M group (Figure 2). Endogenous fecal zinc was positively correlated with TAZ (Figure 3).

An individual example of the calculation of the EZP is given in Figure 4. The mean calculated sizes of the EZP for the L and M groups were 139 ± 2 and 150 ± 2 mg Zn, respectively ($P = 0.20$). The half-lives of $^{70}$Zn label in EZP was 5.6 ± 0.1 and 6.7 ± 0.1 d, respectively, for the L and M groups. Corresponding turnover times were 9.3 and 9.7 d, respectively. The half-life of $^{70}$Zn label in the EZP of the combined groups was

positively correlated with the calculated quantity of zinc in the EZP (r = 0.762, P < 0.001). The calculated fluxes of zinc from the EZP via excretion and to the more slowly exchanging compartments were 15.2 ± 0.2 and 15.7 ± 0.2 mg Zn/d for the L and M groups, respectively. There was no correlation between the flux and quantity of zinc in the EZP.

The size of EZP was positively correlated with dietary zinc (r = 0.468, P = 0.04), fractional absorption (r = 0.454, P = 0.04), total absorption (r = 0.767, P < 0.001), and endogenous fecal zinc (r = 0.568, P < 0.001) (Figure 5). After stepwise-regression analysis, only the relation between EZP and TAZ remained significant: EZP = 110 + (16 × TAZ) (r = 0.762, P < 0.001).

Mean plasma zinc concentrations before and 2 h after breakfast were 14.3 ± 0.6 and 11.9 ± 0.5 μmol/L, respectively, for the L group. Corresponding figures for the M group were 13.6 ± 0.4 and 11.3 ± 0.4 μmol/L, respectively. Neither the means of plasma zinc concentrations nor the mean differences at the two points was significantly different between the L and M groups. There was no significant correlation between plasma zinc and fractional absorption, total absorbed zinc, endogenous fecal zinc, or EZP.

**DISCUSSION**

There are several technical aspects of this study that merit discussion. First, the fecal zinc data are supportive of the reliability of the calculations of zinc intake derived from dietary data. This encouraged us to extend our investigation of fractional absorption of zinc and fecal excretion of endogenous zinc to include calculations of total and net absorption of zinc. Second, the study design allowed us to determine fractional absorption of zinc from urine ratios of enrichment of orally and intravenously administered enriched zinc stable-isotope preparations (18) and from ⁶⁷Zn enrichment data in feces. There was a positive correlation between these two methods (r = 0.768, P < 0.001) and substitution of fractional absorption of zinc derived from urine data did not significantly affect the results or conclusions. Third, although we cannot be certain that the quantity of ⁶⁷Zn label was not large enough to depress fractional absorption, our experience suggests that any effect would at most have been very small, i.e., ~0.01 fractional absorption of zinc (19). Fourth, absorption of extrinsic labels of stable iron isotopes was reported to differ from the absorption of iron in the diet itself when the iron label was administered in solution (20, 21), as the zinc label was in this study. We administered the label in water during the meal because in practice it is often difficult, if not impossible, to uniformly mix
the zinc label with a meal of solid foods or even with the main dietary staple unless the label is incorporated intrinsically (22).

The validity of our extrinsic labeling technique received support from the very similar mean daily total absorption data derived simultaneously from the same subjects by application of an alternative method not dependent on extrinsic labeling (16). Incidentally, this close agreement also gives support to the accuracy of calculations of dietary zinc intake and endogenous fecal zinc. In addition, it is reassuring that calculated endogenous fecal zinc values derived from a combination of extrinsic labeling and traditional metabolic balance data (1) (L group, 1.48 ± 0.27 compared with 1.30 ± 0.07; M group, 2.30 ± 0.36 compared with 2.34 ± 0.20) were similar to those derived from the independent isotope-dilution technique (r = 0.633, P = 0.003). This is especially reassuring given the uncertainty related to the dietary data and the well-recognized hazards of the traditional balance technique.

The major sites of whole-body zinc homeostasis are in the gastrointestinal tract and involve both zinc absorption and excretion of endogenous zinc in the feces. The results of several animal studies are compatible with the conclusion that excretion of endogenous zinc is of primary importance for zinc homeostasis at low zinc intakes (23, 24). It has also been concluded from animal studies that endogenous fecal zinc excretion is affected by both current diet and body zinc status, i.e., long-term dietary changes, whereas zinc absorption is affected only by current intake (25). Information in humans is still quite limited. In normal young infants with an essentially constant diet and zinc intake, endogenous fecal zinc was positively correlated with total absorbed zinc (26). One recent study of normal adults suggested that although fractional absorption and endogenous fecal zinc are both affected by short-term restriction of dietary zinc, only the changes in excretion of endogenous fecal zinc persist when the zinc-restricted diet is continued for 6 mo (5). The results of the present study extend these observations. Although confirmation in other populations and with other dietary regimens will be important, these data suggest that regulation of endogenous zinc excretion via the intestine is responsible principally, if not exclusively, for long-term zinc homeostasis in humans when dietary zinc intake is relatively low.

The inherent inaccuracies in the calculation of EZP were examined previously (17). Although the mass of zinc in the combined pools of zinc that exchange (intermix) with plasma zinc within 2 d is overestimated by the technique used for EZP calculation in this study, this error may not detract severely from the potential usefulness of the data derived. The estimated size of the EZP for these Chinese subjects was generally lower than that of adults in Colorado (17). This was anticipated because of a lower dietary zinc intake even for the group of urban Chinese. Consistent with observations in infants (27), there was a strong positive correlation between total absorbed zinc and EZP mass. This suggests that the quantity of readily exchangeable zinc in the body is dependent, at least within a certain range, on the quantity of zinc absorbed recently. In turn, a larger EZP may be associated with excretion of more endogenous zinc in feces, as suggested by the positive correlation between EZP and endogenous fecal zinc.

Overall, although the possibility of subtle impairment of normal biology and physiology secondary to marginal impairment of zinc status cannot be ruled out, the results of this study provide evidence of humans’ long-term ability to maintain zinc homeostasis with a dietary zinc intake of 5 mg/d and a moderate phytate intake. This does not provide total reassurance that maternal zinc status will be adequate under these conditions during the critical period of embryogenesis. Although zinc requirements are not substantially increased at this early stage of pregnancy, zinc intake may be reduced and the limits of adaptive responses are unknown. There is also a need for detailed studies of zinc homeostasis in this and similar populations during later stages of pregnancy and during lactation when zinc requirements are substantially increased.

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REFERENCES


