Metabolic adaptations to low zinc intakes in premenarcheal girls 1–4

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ABSTRACT
Background: Zinc deficiency is increasingly recognized as an important cause of mortality and morbidity. Children in developing countries are at especially high risk because of relatively low zinc intakes and poor bioavailability.

Objective: We assessed the effect of 2-wk adaptation to low zinc intake (4 mg/d) on fractional zinc absorption, endogenous fecal zinc excretion, and urinary zinc excretion.

Design: Sixteen healthy 9–14-y-old girls were studied twice in random order after 2-wk adaptation to diets providing either 12 mg/d (high) or 4 mg/d (low) zinc. Fractional zinc absorption and endogenous fecal zinc excretion were measured with use of established stable isotope techniques.

Results: Plasma zinc was not significantly lower during the low dietary intake period (1.06 ± 0.18 mg/L) than during the high dietary intake period (1.14 ± 0.23 mg/L, P = 0.30). Endogenous fecal zinc excretion was significantly lower during the low intake period (1.08 ± 0.62 mg/d) than during the high intake period (1.82 ± 0.95 mg/d, P < 0.026), but there was no significant change in fractional zinc absorption (30.6% ± 12.4% compared with 26.6% ± 9.0%, P = 0.32) or urinary zinc excretion (0.68 ± 0.35 mg/d compared with 0.59 ± 0.24 mg/d, P = 0.30). Approximate zinc balance was significantly lower during the low-intake period than during the high-intake period (P = 0.007) and significantly (P < 0.0001) less than zero.

Conclusion: Short-term zinc restriction in premenarcheal girls leads to a significant decrease in endogenous fecal zinc excretion, which was inadequate to restore normal zinc balance.


KEY WORDS Stable isotopes, mineral metabolism, zinc absorption, zinc balance, zinc excretion

INTRODUCTION
Zinc is a vital mineral for human health and is a component of more than 250 enzymes (1). Human zinc deficiency was first diagnosed in 1960 in men in Iran and Egypt who presented with a syndrome of dwarfism and hypogonadism (2). Subsequently, failure of zinc absorption was found to underlie the autosomal recessive disease acrodermatitis enteropathica, which has the presenting symptoms of diarrhea, dermatitis, alopecia, and failure to thrive (3). Although such extreme zinc deficiency is very rare, it has become increasingly clear that more subtle forms of zinc deficiency are relatively common. Zinc deficiency is especially common in developing countries, where marginal zinc intakes and poor bioavailability combine to make suboptimum zinc status an important cause of morbidity. Several studies have shown that zinc supplementation of at-risk populations can lead to significant reduction in morbidity and mortality from lower respiratory tract infections and diarrhea, 2 of the most important causes of death in children in developing countries (4).

Despite the emerging understanding of the importance of zinc nutrition in children, little is known about the metabolic adaptations that children undergo to try to compensate for low dietary zinc intakes. In adults, zinc homeostasis appears to be maintained by changes in fractional zinc absorption and endogenous fecal zinc excretion and possibly by changes in urinary zinc excretion (1, 5). Whether this is true in children is unclear.

The objective of this study was to assess the effect of adaptation to low intakes of zinc on fractional zinc absorption, endogenous fecal zinc excretion, and urinary zinc excretion in healthy premenarcheal girls.

SUBJECTS AND METHODS
Study population
Healthy girls aged between 9 and 14 y were recruited from the Greater Houston Metropolitan area. Subjects were considered eligible if they were in good health and had no chronic medical conditions. They were considered ineligible if they were taking any medications or vitamin or mineral supplements.

The study protocol was explained to the subjects and their parent(s), and informed written consent was obtained. The protocol was approved by the Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals.

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Study protocol

Once informed written consent was obtained, a registered dietitian conducted a structured interview to assess the subject’s usual zinc intake. Subjects, and their parents, were instructed how to increase or decrease their zinc intake by adding or removing servings of zinc-fortified breakfast cereals, meat, and chicken to the diet (described further in Dietary intervention). Compliance with the diet was assessed with a 24-h dietary recall by telephone during the first week and with a weighed dietary record, to include all food consumed on 2 weekdays and 1 weekend day, in the week before admission (described further in Dietary intervention).

Subjects were studied twice, in random order, after 2-wk adaption to diets that provided ≈4 mg/d zinc and 12 mg/d zinc. At the end of each period the subjects were admitted to the Metabolic Research Unit of the Children’s Nutrition Research Center for 9 d on the prescribed diet, including a 6-d stable isotope-based kinetic study. Subjects were admitted for 3 d before the start of the stable isotope and metabolic studies. All food and drink consumed during the in-patient stay were recorded. Nutrient intake was calculated with use of the Minnesota Nutrition Data System (University of Minnesota, version 2.91, Food database 12A, Nutrition database 27) and averaged to provide an estimate of the zinc intake of each subject. The time period between the 2 studies was variable, reflecting the need for studies to be carried out during school holidays so children could participate without missing school.

The higher zinc intake, 12 mg/d, was selected because it was the higher of the 2 recommended dietary allowances for this age range (children aged 7–10 y = 10 mg/d; girls aged 11–14 y = 12 mg/d), based on the recommendations available at the start of the study (6). The lower zinc intake, 4 mg/d, was selected as the lowest amount that could be reasonably achieved by subjects consuming normal foods without excessive dietary manipulations. Two weeks was felt to be adequate to see changes in zinc metabolism because studies in young men have suggested that apparent zinc absorption increases within 13 d on starting a low-zinc diet (7).

Dietary intervention

After informed written consent was obtained, a registered dietitian conducted a complete 24-h dietary recall to assess the subject’s usual zinc intake. Once subjects were randomly assigned to the high (12 mg/d) or low (4 mg/d) zinc diet, they were instructed on how to decrease or increase their zinc intake. Subjects and their parents were instructed on how to read a food label to determine the zinc content of a food. Next, a list of the zinc contents of commonly eaten foods and their portion sizes was given to each subject. A list of foods that contain relatively high amounts of zinc, such as beef, dairy, and zinc-fortified cereals, was also given. This list was used to advise the subjects which foods (and how many servings) should be added to their diet during the high period to increase their zinc intake to the desired amount. These lists were also used to inform the subjects, and their parents, which foods should be avoided during the low intake period. For example, the subjects were given a list of 3 different cereals with high zinc content that could be used to increase zinc content during the high intake period. They were told to avoid these cereals during the low intake period and given a list of alternative breakfast cereals with low zinc content (Table 1). Planned menus were reviewed with the dietitian to ensure that they met the goals for zinc intake. Finally, the subjects and their parents were instructed on how to conduct a weighed home dietary record.

The adaptation period for both the low zinc and high zinc diets was 2 wk. During the first week of adaptation, a 24-h dietary recall was conducted once by phone to assure compliance to the diet. At that time, any dietary issues were addressed. In the week before admission, the subjects weighed all food and drink consumed on 2 weekdays and 1 weekend day, to again assess compliance. Along with the 3 d of weighed home dietary records, the subjects were admitted to the Metabolic Research Unit of the Children’s Nutrition Research Center for 3 d before the 6-d stable isotope-based kinetic study. The test meal given during the high and low intake periods was designed with use of a structured exchange system (Table 1). For example, the test breakfast during the high intake period contained a choice of 1 of 3 high-zinc-content breakfasts (Table 1), a choice of orange or apple juice, and several other options. Subjects choose an item from each selection of exchanges to build their individual test breakfast. A different selection of options was used to construct each individual’s test breakfast during the low dietary intake period. The menus used reflected the food choices during the home adaptation period. The use of a series of exchanges, rather than a set test breakfast during each period, ensured better compliance with the diet than a single fixed meal that would not be tolerated by children this age. The system of exchanges ensured better compliance with the adaptation meals at home and with the test meals given during the inpatient stay.

All food and drink consumed during the inpatient stay were recorded. Once again, menus were customized for each individual to ensure that the desired zinc intake was achieved, by offering a choice of high (during the high intake period) or low zinc content foods (during the low intake period).

Isotope preparation

Zinc-67 (90% enrichment by mass) and zinc-70 (74% enrichment by mass), produced in Russia, were obtained from Trace Sciences Inc (Toronto) as the oxide. Aqueous solutions of zinc chloride were prepared by the Investigational Pharmacy at Texas Children’s Hospital and tested for sterility and pyrogenicity.
before their use as previously described (8). The isotopic distribution of the tracers was verified by thermal ionization magnetic sector mass spectrometry (Finnigan MAT 261; Thermo Finnigan, Bremen, Germany).

Metabolic study

Subjects were admitted to the Metabolic Research Unit of the Children’s Nutrition Research Center for 9 d. All food and drink consumed during the study period was measured. Diets were individualized with the study subject by a dietitian and were designed to provide the planned zinc intake (either 12 mg/d or 4 mg/d). On the morning of the fourth day, after an overnight fast and after emptying their bladders, subjects received a breakfast meal that provided approximately one-third of their daily zinc intake and included a glass of orange juice that was extrinsically labeled 18–24 h earlier with 1.1 mg oral tracer highly enriched in zinc-67. Subjects received 0.5 mg intravenous tracer highly enriched in zinc-70 by slow intravenous infusion of 1–2 min. Once tracers were administered, a complete 6-d urine and fecal collection was started.

Sample preparation and analysis

Urine was collected in 8-h aliquots, and each fecal sample was collected individually. Samples were stored at −80°C until analyzed. Fecal samples were digested with use of a microwave digestion system (MARS-5; CEM Corporation, Matthews, NC). Aliquots of 1–2 g fecal digest or 5–10 mL urine were digested on a hot plate overnight and resuspended in 1 mL of 6 mol/L hydrochloric acid. An anion exchange resin (2 mL; AG 1-X8 resin; Bio-Rad Laboratories, Hercules, CA) was loaded into columns and washed with 5 mL of 6 mol/L hydrochloric acid. The sample was loaded into the column and washed with 5 mL of 6 mol/L hydrochloric acid, with 5 mL of 3 mol/L hydrochloric acid, with 5 mL of 1 mol/L hydrochloric acid, and then with 5 mL of 0.5 mol/L hydrochloric acid. The samples were eluted with 5 mL deionized water into a polytetrafluoroethylene vial. Ten microliters of 0.23 mol/L phosphoric acid was added to urine samples or 50 μL 3% nitric acid to fecal samples. The samples were dried overnight on a hot plate and resuspended in 10 μL deionized water. Then resulting solution, 2 μL of 0.23 mol/L phosphoric acid, and 6 μL silica suspension were loaded on rhenium filaments for isotope ratio analysis (8).

Zinc isotope ratios were measured by thermal ionization magnetic sector mass spectrometry (Finnigan MAT 261; Thermo Finnigan). Replicate blocks of 10 scans each were carried out until the desired degree of precision was obtained (relative SD < 0.2%). Ratios were expressed with respect to zinc-66 and were corrected for temperature-specific differences in fractionation with use of the ratio of the 2 nonadministered isotopes zinc-64 and zinc-66. Aliquots of the intravenous and oral tracers used in the study had their isotopic ratios assessed in a similar manner.

Ratios of zinc-67 to zinc-66 and of zinc-70 to zinc-66 for the sample, for the intravenous and oral tracer, and for natural abundance were mathematically converted to oral and intravenous tracer:tracee as described elsewhere (9, 10). Zinc content of the urine and fecal aliquots were measured by flame atomic absorption spectroscopy. Zinc-free reagents and disposables were used throughout.

Measurement of zinc absorption

Zinc absorption was measured from the relative fractional excretion of the oral and intravenous tracers in the 48–56-h urine pool and 72–80-h pool (11, 12) with use of the following equation:

\[
\text{Zinc absorption (\%) = } \frac{\text{oral tracer:tracee ratio/dose of oral tracer}}{\text{intravenous tracer:tracee ratio/dose}} \times \frac{\text{intravenous tracer (mg)}}{\text{of intravenous tracer (mg) over 6 d}}
\]

Measurement of endogenous fecal zinc excretion

Endogenous fecal zinc excretion was calculated from the cumulative urinary and fecal excretion of the intravenous tracer over the entire 6-d metabolic study, with use of the following equation (12, 13):

\[
\text{Endogenous fecal zinc excretion (mg/d) = } \frac{[\text{total fecal excretion of intravenous tracer over 6 d (mg)} \times \text{urinary zinc excretion (mg/d)}]}{\text{total urinary excretion of intravenous tracer (mg) over 6 d}}
\]

Zinc balance

Approximate zinc balance was estimated by multiplying fractional zinc absorption (in the 48–56-h urine pool) by the calculated zinc intake (over the 6-d study) and subtracting endogenous fecal zinc excretion (over the 6-d study) and urinary zinc excretion (over the 6-d study). No correction was made for other losses of zinc such as through sweat or hair.

Statistical analysis

Statistical analysis was carried out with use of StatView v5.1 for Macintosh (SAS institute, Cary, NC). Comparisons between 2 dietary periods were made with use of a paired t test. Data are given as mean ± SD, unless otherwise stated, and statistical significance was assumed at \( P < 0.05 \). Zinc balances were compared with the zinc requirement for normal growth estimated by the Institute of Medicine (14) with use of a one-sample \( t \) test.

On the basis of previous studies, we expected zinc absorptions of 20–30% with a SD of 10%. Assuming that the smallest clinically significant difference between the high and low dietary periods was 7.5%, a sample size of 16 was required to have an 80% power (\( \beta = 0.20 \)) of detecting such a difference at \( P = 0.05 (\alpha = 0.05) \).

RESULTS

Subject characteristics

Sixteen healthy girls aged 11.2 ± 0.93 y (range: 9.7–12.9) were studied. All were premenarcheal and in Tanner breast stage 2 at the start of the study. Eight (50%) were white, and 8 were African-American. Their average weight was 43.9 ± 13.9 kg, and their average height was 151 ± 11 cm. Admission for metabolic studies was timed to coincide with school vacations, and the 2 studies were carried out 112 ± 60 d apart.
Dietary intake

The average dietary zinc intake during the high intake period was 12.3 ± 1.2 mg/d and, as designed, was significantly greater than during the low dietary intake period (4.4 ± 0.6 mg/d, \( P < 0.0001 \)). Total fecal zinc differed significantly between the high (12.4 ± 3.4 mg/d) and low (4.3 ± 2.2 mg/d, \( P < 0.0001 \)) dietary intake periods.

Zinc intake from the test breakfast (excluding 67-zinc tracer added) was significantly lower during the low dietary intake period (0.97 ± 0.64 mg compared with 4.1 ± 1.9 mg, \( P < 0.0001 \)), as were the calcium (106 ± 38 mg compared with 322 ± 165 mg, \( P < 0.0001 \)) and total protein (8.3 ± 2.7 mg compared with 18.4 ± 5.9 mg, \( P < 0.0001 \)) intakes. There was no difference in total dietary fiber (low, 3.2 ± 2.6 g; high, 2.1 ± 1.2 g; \( P = 0.14 \)) or phytate (low, 55 ± 65 mg; high, 95 ± 72 mg; \( P = 0.08 \)) intake.

Plasma zinc concentration

Fasting plasma zinc concentration was 1.14 ± 0.23 mg/L during the high dietary intake period. This finding was not significantly different from the low dietary intake period (1.06 ± 0.18 mg/L; \( P = 0.30 \)). In only one subject was plasma zinc <0.8 mg/L, and this occurrence was during her low-intake period.

Zinc absorption

Fractional zinc absorption measured from the 48–56-h urine pool was 26.6 ± 9.0% during the high-intake period, which was not significantly different from the low-intake period (30.6 ± 12.4%, \( P = 0.32 \)). Zinc absorption during the 2 dietary periods was also similar in the urine pool collected between 72 and 80 h after isotope administration (high, 27.3 ± 9.7%; low, 27.7 ± 10.9%; \( P = 0.92 \)).

Urinary zinc excretion and endogenous fecal zinc excretion

No significant difference was found in urinary zinc excretion between the high (0.59 ± 0.24 mg/d) and low (0.68 ± 0.35 mg/d, \( P = 0.30 \)) dietary intake periods. However, endogenous fecal zinc excretion fell by almost one-half during the low-intake period (1.08 ± 0.62 mg/d) compared with the high-intake period (1.82 ± 0.95 mg/d, \( P = 0.026 \)).

Zinc balance

Zinc balance during the high dietary intake period was 0.87 ± 1.54 mg/d, which was significantly greater than during the low dietary intake period (−0.84 ± 0.63 mg/d, \( P = 0.007 \)). Zinc balance during the high-intake period was significantly more than zero (\( P = 0.040 \)) and significantly less than zero during the low-intake period (\( P < 0.0001 \)).

Effect of plasma zinc concentration

A significant negative correlation was found between zinc absorption and plasma zinc concentration \( (y = 0.566–0.254x, r^2 = 0.229, P = 0.006; \text{Figure 1}) \). When the 2 dietary periods were considered separately, this relation was seen only for the low dietary intake period \( (y = 0.744–0.413x, r^2 = 0.339, P = 0.018) \); and not for the high-intake period \( (y = 0.431–0.144x, r^2 = 0.134, P = 0.16; \text{Figure 1}) \).

There was no relation between endogenous fecal zinc excretion and plasma zinc for the 2 time periods combined \( (y = 0.477 + 0.885x, r^2 = 0.043, P = 0.25; \text{Figure 2}) \). However, when the 2 dietary periods were considered separately, a significant positive correlation was seen during the high-intake period \( (y = 2.066x–0.534, r^2 = 0.249, P = 0.049; \text{Figure 1}) \) and a significant negative correlation during the low-intake period \( (y = 3.273–2.072x, r^2 = 0.346, P = 0.017; \text{Figure 2}) \).

If the single individual with a plasma zinc concentration <0.8 mg/L during the low period was excluded, the relation between endogenous fecal zinc excretion and plasma zinc still tended to be significant \( (P = 0.08) \), but the relation between zinc absorption and plasma zinc excretion was no longer significant \( (P = 0.56) \).

There was no relation between urinary zinc excretion and plasma zinc concentration for the 2 time periods combined \( (r^2 = 0.004, P = 0.74) \) or for the high \( (r^2 = 0.083, P = 0.28) \) and low periods \( (r^2 = 0.004, P = 0.82) \) individually.

DISCUSSION

We measured zinc absorption, endogenous fecal zinc excretion, and urinary zinc excretion after 2-wk adaptation to high dietary zinc intakes (12 mg/d) and low dietary zinc intakes (4 mg/d). Children adapted to the lower zinc intake with a significant reduction in endogenous fecal zinc excretion, but there were no significant changes in fractional zinc absorption or urinary zinc excretion. The decrease in endogenous fecal zinc excretion was insufficient to compensate for the decreased absorbed zinc during the low-intake period; therefore, net zinc balance was significantly lower during this period than during the high-intake period.
period. The high intake used in the study was the recommended dietary allowance for zinc in girls aged 11–14 y (12 mg/d) at the time the study was carried out (6). Since then, the recommended dietary allowance for zinc was revised downward to 8 mg/d for girls aged 9–12 y and 9 mg/d for girls aged 14–18 y (14). Zinc balance in children consuming diets containing 12 mg/d was 0.87 ± 1.54 mg/d and was not statistically different from the 0.2 mg/d that the Institute of Medicine estimates is required for growth at this age (14) (hypothesized \( \bar{x} = 0.2 \) mg/d, one-sample \( t \) test). Conversely, a zinc intake of 4 mg/d led to a negative zinc balance and was inadequate to achieve a zinc balance of 0.2 mg/d (hypothesized \( \bar{x} = 0.2 \) mg/d, one-sample \( t \) test), the requirements for normal growth estimated by the Institute of Medicine (14). If we correlate the data for zinc balance and zinc intake from the current study, a zinc intake of \( \approx 10 \) mg/d would be required for zinc balance to average 0.2 mg/d. However, this estimate should be treated with caution, because it assumes a linear relation between zinc intake and zinc balance over this range of zinc intakes, and the data are somewhat skewed by a single subject with a low fasting zinc concentration and high fractional zinc absorption (Figure 1).

A small number of studies in adults have examined the effect of zinc intakes similar to those we studied. Wada et al (7) studied 6 adult men with zinc intakes of either 16.5 mg/d or 5.5 mg/d. Apparent zinc absorption increased significantly during the lower-intake period. However, zinc absorption was calculated from the difference in the dose of isotope given orally and that recovered over the next 12–15 d in the feces. Such a method is incapable of distinguishing isotope that has passed through the gut unabsorbed and that which was absorbed and resecreted into the gastrointestinal tract. Therefore, it is impossible to say whether the change in apparent absorption represented a change in true fractional absorption, endogenous fecal zinc excretion, or a combination of the two.

In a somewhat different study design, Sian et al (15) measured fraction zinc absorption and endogenous fecal zinc excretion in 2 populations of Chinese women with an average zinc intake of either 5.2 mg/d or 8.3 mg/d. There was no difference in fractional zinc absorption between the groups, but the group with the lower zinc intake had significantly lower endogenous fecal zinc excretion (15). There was no significant change in urinary zinc excretion (15). This study confirms the findings of another study showing that urinary zinc excretion does not fall after 5 wk of reduced zinc intake to \( \approx 4 \) mg/d (16). Lee et al (17) studied 8 adult men who were placed on a zinc depletion diet (4 mg/d) for 6 mo. Within 2 mo they could demonstrate significantly increased fractional zinc absorption and reduced endogenous fecal zinc excretion (17). There were no changes in plasma zinc concentration even after 6 mo of the depletion diet (17).

In our study the low intake was one-third of the high intake and was felt to be the lowest zinc intake acceptable to children, with a high likelihood of compliance. This intake was also similar to the studies described earlier (15–17). In adults, a zinc intake of 4 mg/d for 15 d leads to changes in markers of zinc status, although plasma zinc concentration does not decline (18). Indeed, plasma zinc concentrations can remain normal for as long as 6 mo with such an intake (17). In our study, too, plasma zinc concentration did not change significantly after 2 wk of zinc restriction, and only one subject had a zinc concentration <0.8 mg/dL. In our study adaptation to the reduction in dietary zinc excretion was by a decrease in endogenous fecal zinc excretion. This finding is in agreement with animal models of zinc deficiency in which dietary zinc restriction leads to reductions in zinc losses in biliary and pancreatic secretions (19, 20).

Although previous studies have disagreed on whether zinc restriction to the range of 4–5 mg/d increases fractional zinc absorption, Lee et al (17) suggested this increase might happen. There was no significant difference in fractional zinc absorption between the high- and low-intake periods in our study. The failure to identify an up-regulation in zinc absorption during the low period is unlikely to be a result of differences in other components of the diet because calcium content was lower during the low period, and there were no significant differences in total fiber or phytate intake. However, it is possible that an increase in fractional zinc absorption could be identified if the dietary intervention was more severe or prolonged.

Endogenous fecal zinc excretion was significantly lower during the low-intake period than during the high-intake period, although no significant changes in zinc absorption or urinary excretion were observed between the 2 dietary periods. The major metabolic adaptation to zinc restriction appears, therefore, to be a reduction in endogenous fecal zinc excretion. However, during the low-intake period, there was an unexpected negative correlation between endogenous fecal zinc excretion and plasma zinc concentrations. Endogenous fecal zinc excretion during the low-intake period was significantly greater in subjects with
lower plasma zinc concentrations (and therefore presumed worse zinc status). This finding could suggest that there is variability in an individual’s ability to reduce endogenous fecal zinc excretion during zinc restriction. Those individuals least able to reduce endogenous fecal zinc excretion could be at increased risk of poorer zinc status than their peers.

We did not detect any change in urinary zinc excretion in response to dietary restriction, nor have other researchers who used similar zinc intakes (15–17). Several other studies suggested that very low zinc intakes (typically <1 mg/d) do lead to reductions in urinary zinc excretion (21–23), but these diets are synthetic experimental diets, and such low zinc intakes are unlikely to occur in self-selected diets.

In summary, a dietary zinc intake of ~4 mg/d leads to a decrease in endogenous fecal zinc excretion, without changes in fractional zinc absorption or urinary zinc excretion. This adaptation was inadequate to prevent subjects on 4 mg/d zinc being in negative zinc balance.

IGJ was involved in study design and implementation, data collection, and analysis. PDH was responsible for dietary interventions and analysis. LKL was responsible for iso Tup ratio analysis. SAA supervised the sample analysis, was involved in study design and implementation, and assisted with data analysis. All authors contributed to writing the final manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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