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Mineral homeostasis in young children consuming typical U.S. diets*

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Abstract: Mineral requirements in young children are poorly understood, and current recommendations rely heavily on data collected in older children or adults. Our objectives were to assess the relationship between mineral (Ca, Mg, Fe, Zn, and Cu) intake in young U.S. children, and mineral absorption, excretion, and retention; and to use these data to re-examine the most recent recommended intakes. Thirty children, 1–4 y old, were studied on their usual diet. After 7 d of home adaptation they were admitted for either a 2-d or 5-d metabolic study where multiple stable isotope or Ca, Mg, Fe, Zn, and Cu were administered and mineral absorption (2-d study), or absorption and excretion (5-d study) were assessed. Fractional (%) absorption of some (Ca, Mg) but not all (Fe, Zn) minerals decreased as intake increased. Absolute (total) absorption and net retention of all the minerals increased as their intake increased. Mineral homeostasis was related to changes in fractional absorption (Ca and Mg), fecal excretion (Zn), or whole body mineral status (Fe). Our results support the current U.S. recommended intakes for 1–4-y-old children for Mg and Fe, but suggest that those for Ca and Zn are too low.

Keywords: calcium; copper; iron; magnesium; mineral absorption; mineral balance; mineral excretion; mineral homeostasis; stable isotope; zinc.

INTRODUCTION

The requirements of young children for many minerals (including Ca, Mg, Fe, Zn, and Cu) are poorly characterized and often rely on extrapolation from data in older children or adults [1,2].

For example, in the 12–48-m age range [1] some direct data exists to guide the Ca requirements in the form of balance data on almost 100 2–8-y-old children [3,4] largely carried out in the 1960s, although how many of these children were aged less than 4 y is unclear. Mg requirements for this age group, however, were extrapolated from the limited data in 10–15-y-olds [1], Cu requirements were extrapolated from adult data [2]. Fe and Zn requirements were estimated using a factorial method—from the estimated mineral requirement to meet obligatory losses and for growth, multiplied by an estimate of fractional absorption. The estimate of fractional mineral absorption is derived from adult studies (Fe) or from studies in children less than 1 y of age (Zn) [5,6]. Only Ca requirements are directly based on experimental data on 1–3-y-olds (Table 1), although this is combined with data from older age groups.

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Table 1 Methods used by the IoM to estimate Ca, Mg, Zn, Cu, and Fe requirements for 1–3-y-old children. [1,2].

Mineral	Method used	Source of experimental data
Calcium	Balance data	Older children: 2–8 y old (balance, n = 99) 5–12 y old (isotope, n = 51)
Magnesium	Extrapolated using metabolic weight	Older children: 10–15 y old
Zinc	Factorial method	Younger children: 9 m old (n = 11) 18–30 wk (n = 6)
Copper	Extrapolated using metabolic weight	Adults
Iron	Factorial method	Adults

In recent years, stable isotope methods to examine mineral metabolism have improved with decreasing costs of isotopes and for sample analysis, as well as reduction in sample sizes needed for analyses. The objective of this study was to assess the relationship between mineral (e.g., Ca, Mg, Fe, Zn, and Cu) intake, and mineral absorption, urinary excretion, endogenous fecal excretion, and net balance (retention) among 1–4-y-old children consuming typical U.S. diets.

SUBJECTS AND METHODS

We carried out an observational cohort study on 1–3-y-old children from the Greater Houston area, consuming diets typical of those consumed in the United States. Detailed methods are discussed elsewhere [7–10].

Healthy children ages 1–4 y in the greater Houston area were recruited through public advertising. Subjects were selected to reflect the approximate racial and ethnic distribution of the greater Houston population. Children were eligible for enrollment if they were healthy, not taking any medications (except multivitamins), were born at term (≥37 wk gestation) and had a birth weight of at least 2500 g. Children were excluded from participating if they were below the 3rd or above the 97th percentile of weight- or height-for-age, or were below the 5th or above the 95th percentile of weight-for-height. Those subjects taking multivitamins were required to discontinue them for 2 weeks prior to participating in the mineral absorption study.

Families were offered the option of participating in a 2-d inpatient Zn absorption study or a 5-d inpatient study in which fecal samples would also be collected and endogenous excretion would be measured. The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals approved the protocol, and informed written consent was obtained from the subjects' parents. A research dietitian met with the parent and obtained a complete dietary history to evaluate usual daily micronutrient and calorie intake. Dietary intake data were collected using Nutrition Data System (NDS) for Research software (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). After screening, a dietary plan was developed for each child that would be consumed at home for the 7 days prior to the inpatient mineral absorption study. This was to insure that children did not alter their eating habits immediately prior to the mineral absorption study. All foods and beverages to be consumed during these 7 days were provided by the research center and were pre-weighed prior to delivery to the family. Parents were instructed to return all the uneaten food and beverage items for the first 3 days of the pack-out so items could be post-weighed. At the end of the home adaptation period, patients were admitted to the General Clinical Research Center at Texas Children's Hospital (TCH) for the mineral absorption study.

On the morning of the inpatient study, subjects had a heparin-lock intravenous catheter placed, using topical 4 % lidocaine cream (L-M-X-4, Ferndale Laboratories, Ferndale, MI) as an analgesic. Subsequently, intravenous isotopes (^{46}Ca, ^{25}Mg, ^{70}Zn) were administered over 1 min each, separated by an infusion of 2–3 ml of normal saline. Subjects were then given a breakfast that included 30 ml of apple juice to which 0.12 mg of ^{70}Zn and 30 mcg of ^{65}Cu had been added. After the subject consumed the isotope-containing juice, the subject consumed another 30 ml of apple juice without isotope from the same cup as a rinse to ensure that none of the isotope was left in the cup. This process was repeated with lunch using the same amount of isotopes as breakfast. In the evening of the first inpatient day, 3 mg of ^{57}Fe mixed with 30 ml of white grape juice and 1 ml of Tri-vi-sol, containing 35 mg of ascorbic acid was given as a "reference dose". This represents Fe absorption under optimal conditions (aqueous solutions given with vitamin C in the fasted state). It is an alternative measure of Fe status, and a method of correcting for between-subject differences in Fe absorptive capacity. No food or drinks were given for 2 h before or 2 h after this dose.

Menus for the inpatient study visit were based on the subject's usual mineral intake that they had received at home for the previous 7 days, which in turn were based on the reported usual intake of the children. All foods and beverages during the inpatient visits were pre- and post-weighed to accurately determine intake. Dietary intakes used in the Results section were based on these intakes.

Subjects remained in our inpatient unit for 48 h, and their urine was collected in hour pools for the duration of their hospitalization. If the subject was not well toilet-trained, urine bags were used for the sample collection. Subjects returned to the outpatient unit 96 h after isotope administration for collection of a spot urine sample. The subset of subjects in whom endogenous fecal excretion was measured remained in the inpatient unit for 120 h during which time their urine and stools were collected in 24-h pools.

Isotope ratios in blood, urine, and feces were measured using induction coupled plasma-mass spectrometry or magnetic sector thermal ionization mass spectrometry. Fractional absorption, urinary excretion, endogenous fecal excretion, and mineral balance (Table 2) were measured as described elsewhere [7–10].

Table 2 Metabolic variables measured during the 48-h and 120-h inpatient visits for each mineral.

Mineral	Ca	Mg	Zn	Cu	Fe
Intake	Yes	Yes	Yes	Yes	Yes
Fractional absorption (%)	Yes	Yes	Yes	Yes	Yes
Absolute absorption (mg)	Yes	Yes	Yes	Yes	Yes
Urinary excretion	Yes	Yes	Yes	Yes	N/A
Endogenous fecal excretion	120 h only	120 h only	120 h only	No	N/A
Balance	120 h only	120 h only	120 h only	No	N/A
Reference dose absorption	No	No	No	No	Yes

RESULTS

A total of 32 subjects were recruited and studied. For each mineral, data is available on 30 subjects [7–10].

Mineral Intake

The reported intake of all minerals was similar to those published from the National Health and Nutrition Examination Survey (NHANES) III dietary survey. Actual intakes measured at home by the parents and in hospital by the research dietician were very similar, but both were less than reported by the parents.

Intakes of Ca and Fe were significantly greater than the median intake reported from the NHANES III dataset (Table 3) [1,2]. Mean Ca intake was similar to the current U.S. Institute of Medicine (IoM) adequate intake (AI), but mean intakes of Mg, Cu, Zn, and Fe were significantly greater than the IoM estimated average requirement (EAR). Four subjects (13 %) consumed Zn intakes above the current IoM upper limit (UL).

Table 3 Mean (SD) and range of intake of Ca, Mg, Cu, Zn, and Fe in the study.

Mineral	Intake (mg)	
	Mean (SD)	Range
Calcium	551 (219)	124–983
Magnesium	106 (25)	44–152
Copper	0.41 (0.12)	0.26–0.76
Zinc	5.0 (2.1)	1.1–10.2
Iron	6.9 (2.4)	3.1–11.7

Fractional absorption (%)

Fractional Ca ($r^2 = 0.26$, $p = 0.006$) and Mg ($r^2 = 0.144$, $p = 0.304$) absorption were negatively correlated with mineral intake, with the relationship being strongest for Ca.

The intake of Fe, Zn, and Cu was unaffected by Fe, Zn, and Cu intake ($p > 0.05$). Fe intake was significantly affected by body Fe status as assessed by either the serum ferritin concentration ($r^2 = 0.32$, $p < 0.0001$) or the reference dose Fe absorption ($r^2 = 0.65$, $p < 0.0001$). This remained true when Fe intake was included in the analyses, and the relationship was stronger for reference dose Fe absorption ($t = -3.5$, $p < 0.0001$) than for serum ferritin concentration ($t = 6.6$, $p < 0.0001$). Fractional Cu absorption was unaffected by Zn intake.

Absolute absorption (mg)

Mineral intake was significantly positively correlated with the absolute (net) absorption of Ca ($r^2 = 0.26$, $p = 0.006$), Mg ($r^2 = 0.566$, $p < 0.0001$), and Zn ($r^2 = 0.696$, $p < 0.0001$). No threshold was apparent across the range of intake studied, such as has been seen in earlier studies of Ca absorption [4].

Endogenous fecal excretion

Intake had no significant effect on the endogenous fecal excretion of Ca or Zn. The relationship between Mg intake and endogenous fecal Mg excretion did not reach statistical significance ($p = 0.12$) despite the relatively large amount of variance explained ($r^2 = 0.31$) due to the small sample size.

Urinary excretion

There was no significant relationship between mineral intake (across the range studied) and urinary excretion of the mineral, for any of the mineral studied.

Overall mineral balance

There was a significant linear relationship between Ca intake and Ca retention ($r^2 = 0.36$, $p < 0.0001$). A similar relationship was noted for Mg ($r^2 = 0.16$, $p = 0.304$) and Zn ($r^2 = 0.51$, $p < 0.0001$).

Re-examination of requirements

Calcium

Contrary to expectations, and to previous data [4], the relationship between Ca retention and Ca intake did not show a threshold; an intake level above which little if any extra Ca was retained. This relationship was highly significant if fitted as a linear function, and even more significant if fitted as an S-shaped curve [9].

The determination of what is an adequate intake therefore depends on what is felt to be an adequate rate of Ca retention. Assuming this value is 140 mg/d (110 mg/d for bone mineralization and 30 mg/d for dermal losses [9], an adequate intake would be about 470 mg/d [9], very similar to the current AI quoted by the IoM of 500 mg/d, and considerably less than the 770 mg/d suggested by some previous data [4].

It should be noted that, even above this intake, further Ca is retained and presumably deposited to the skeleton. Whether this has any short- or long-term benefits is unknown.

Magnesium

The IoM estimates that Mg retention of 8–10 mg/d should meet the needs for growth of 1–3-y-olds [1]. Based on our data [7] an intake of 60–80 mg/d should meet this need. This is very similar to the IoM's EAR of 65 mg/d [1].

Zinc

The EAR for Zn has been calculated using a factorial method [1]. If our data for urinary excretion, endogenous fecal Zn excretion, and fractional absorption are substituted for the estimates used by the IoM, this yields an EAR of 4.7 mg/d. This is considerably in excess of the 2.5 mg/d quoted by the IoM [1], and above the current UL for Zn (4 mg/d). The UL was set at a maximum level that no one should exceed due to concerns that Zn intakes at or above this level might reduce Cu intake. We have relatively limited data on Cu absorption, but could find no evidence that Zn intakes in the range we studied had an adverse effect on Zn absorption.

Fate of absorbed mineral

The metabolic fate of the absorbed minerals was examined by considering the mass of mineral that was subsequently excreted in urine or feces, and the amount retained (Fig. 1). For example, of the 260 mg Ca absorbed on average by the population, 185 mg was retained, only 44 mg was excreted into the gut, and only 28 mg into the urine (Fig. 1b). When expressed as percentages, 72 % of the total Ca absorbed was retained (Fig. 2a), compared to 35 % of Mg (Fig. 2b) and 23 % of Zn (Fig. 2c).

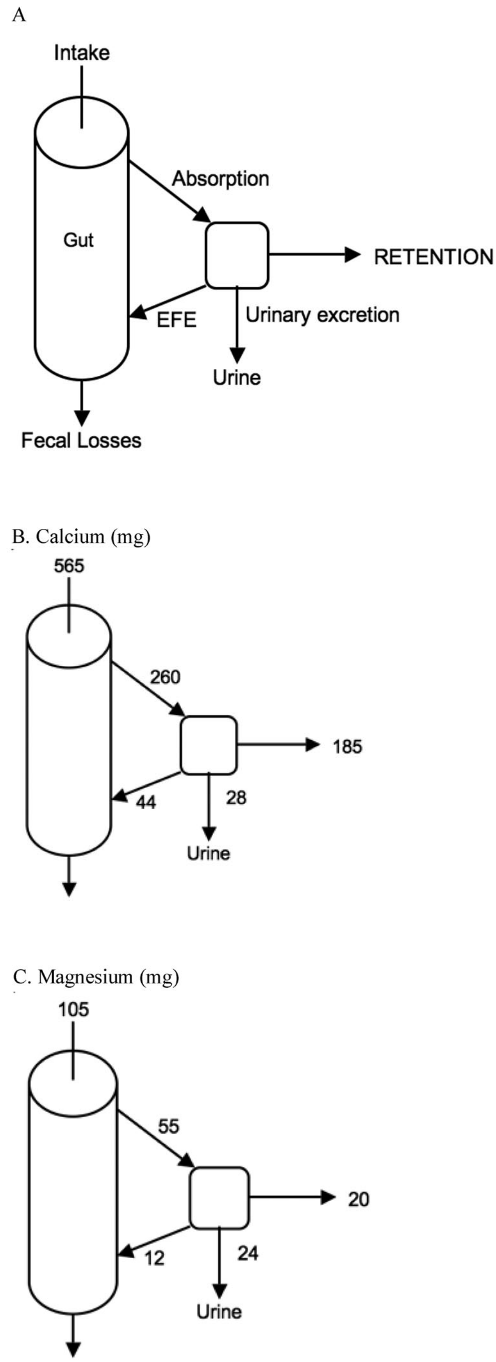

Fig. 1 Overview of the fates of dietary minerals (a) schematic overview, (b) Ca, (c) Mg, (d) Zn.

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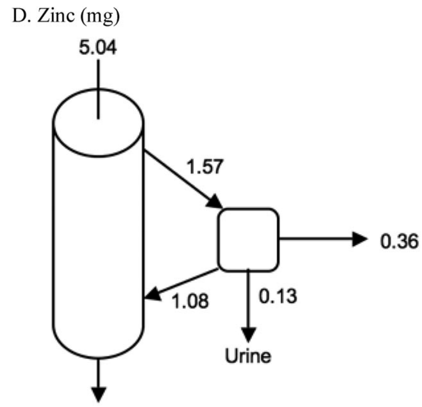

Fig. 1 (*Continued*).

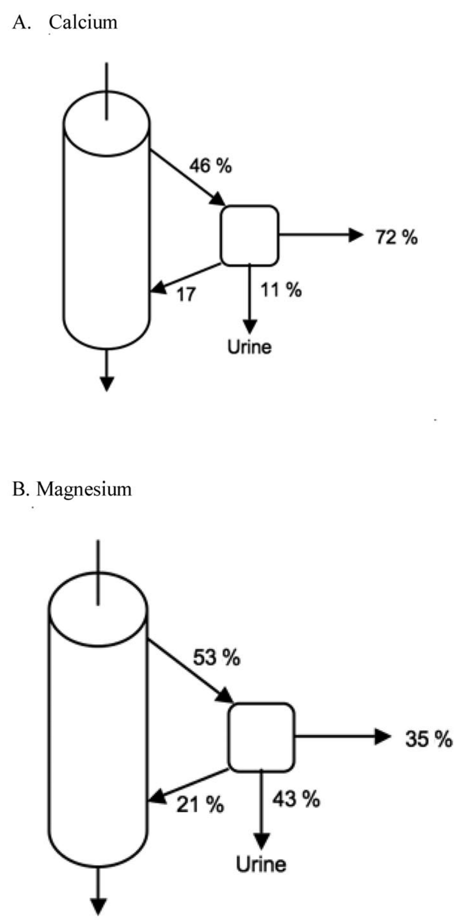

Fig. 2 Fate of absorbed Ca (a), Mg (b), and Zn (c) as a fraction of the total dose absorbed from the diet. Also shown is the percentage of dietary Zn absorbed.

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C. Zinc

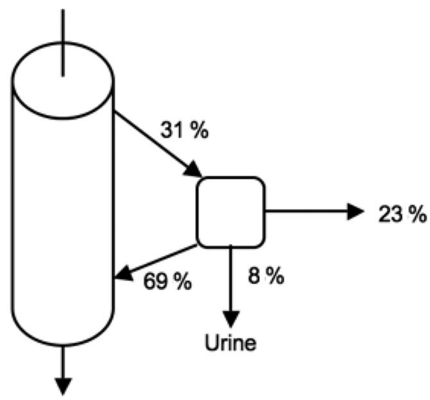

Fig. 2 (*Continued*).

DISCUSSION

The data presented here add considerably to our knowledge of mineral requirements in young (1–3-y-old) children. In some instances, they are the first direct data in this age group. It is reassuring, therefore, that in most instances the AIs estimated from these data are similar to the current AIs or EARs developed by the IoM.

For example, the AI for Ca seems to match the data in this study well, and it is perhaps time that this recommendation graduate from "adequate intake" status to an EAR, reflecting the increased certainty that can be attached to it.

The situation for Zn is somewhat different. Our data suggest that intakes significantly above the current EAR are required to support adequate Zn retention rates. Furthermore, intakes above the current UL may not only be acceptable, they may be desirable to maintain Zn retention. Finally, although some of the subjects in our study exceeded the UL for Zn, we saw no adverse effect on Cu absorption. It is also worth noting that Cu intakes were relatively high in this population as well, and correlated with Zn intake (data not shown). The effect of apparently "excessive" Zn intakes may be mitigated by the fact that diets high in Zn are also likely to be high in Cu and thus limit any potential effect of Zn on Cu absorption. The Zn requirements of children, and the interaction (if any) between Zn intake and Cu absorption is worthy of further study.

Fe absorption was determined by Fe status, not by Fe intake *per se* although there must be a relationship between Fe status and Fe intake, it was not detectable in this population with a relatively high Fe intake, albeit representative of the general U.S. population. Of the two methods of assessing Fe status (serum ferritin and reference dose Fe absorption), the reference dose Fe absorption was superior as it explained a larger amount of the variance in Fe absorption from the diet.

A major limitation of our study was the relatively small sample size. Although 30 subjects were studied for most minerals, as few as 10–12 elected to do the 120 h study. The number of children in whom endogenous fecal excretion data is available is therefore limited. When calculating balance data, the data on endogenous fecal losses was extrapolated from this smaller sunset of infants. Despite this, the data presented here still represents a notable increase in the data on mineral homeostasis in 1–3-y-old children.

The study demonstrates the difference ways mineral homeostasis is maintained across a range of intakes. For Ca and Mg, changes in fractional absorption were the only site of potential homeostasis that changed with mineral intake. For Zn, neither fractional absorption, endogenous fecal Zn excretion, nor urinary excretion changed consistently as intake changed. Zn retention was determined by Zn intake, with little (if any) evidence of metabolic adaptation across these intakes. This does not meant that changes might not occur at lower intakes (either up-regulation of intake, reduction of fecal, or urinary losses, or a combination thereof) or at higher intakes.

Once minerals are absorbed, their metabolic fate and degree of retention varies greatly. Although similar fractions of dietary Ca and dietary Mg are absorbed (46 and 52 %) the fate of the absorbed mineral differs. More than twice as much of the absorbed Ca (72 %) is retained by the body than absorbed Mg (35 %, Fig. 2). Absorbed Ca is excreted in approximately equal amounts in the urine and in the feces (Fig. 2). However, 2/3 of absorbed Mg is excreted in the urine (Fig. 2). In contrast, relatively little of the absorbed Zn is retained, and the majority is exceeded into the gut (endogenous fecal excretion), with a minor amount being excreted in the urine.

REFERENCES

1. U.S. Institute of Medicine, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes: For Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*, National Academy Press, Washington, DC (1997).
2. U.S. Institute of Medicine, Panel on Micronutrients. *DRI: Dietary reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: A Report of the Panel on Micronutrients ... and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine*, National Academy Press, Washington, DC (2001).
3. V. Matkovic. *Am. J. Clin. Nutr.* **54**, 245S (1991).
4. V. Matkovic, R. P. Heaney. *Am. J. Clin. Nutr.* **55**, 992 (1992).
5. L. Davidsson, J. Mackenzie, P. Kastenmayer, P. J. Aggett, R. F. Hurrell. *Br. J. Nutr.* **75**, 291 (1996).
6. S. J. Fairweather-Tait, S. G. Wharf, T. E. Fox. *Am. J. Clin. Nutr.* **62**, 785 (1995).
7. I. J. Griffin, M. F. Lynch, K. M. Hawthorne, Z. Chen, M. Hamzo, S. A. Abrams. *J. Am. Coll. Nutr.* **27**, 349 (2008).
8. I. J. Griffin, M. F. Lynch, K. M. Hawthorne, Z. Chen, M. G. Hamzo, S. A. Abrams. *Br. J. Nutr.* **98**, 358 (2007).
9. M. F. Lynch, I. J. Griffin, K. M. Hawthorne, Z. Chen, M. Hamzo, S. A. Abrams. *Am. J. Clin. Nutr.* **85**, 750 (2007).
10. M. F. Lynch, I. J. Griffin, K. M. Hawthorne, Z. Chen, M. G. Hamzo, S. A. Abrams. *J. Nutr.* **137**, 88 (2007).

