British Journal of Nutrition (2007), **98**, 358–363
doi: 10.1017/S0007114507708796

Zinc homeostasis in 1–4 year olds consuming diets typical of US children

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(Received 10 November 2006 – Revised 19 January 2007 – Accepted 12 February 2007)

Few data have evaluated Zn balance in young children after the first year of life. The objective of the present study was to study the relationships among Zn intake, absorption, endogenous faecal excretion, and retention in a group of healthy children. Thirty children, aged 15–48 months, were studied on a diet representative of their usual daily mineral intake. Zn absorption was assessed using a dual-tracer stable-isotope technique. Endogenous Zn faecal excretion and Cu absorption were determined in a subset of children. We found that Zn intake from the in-patient weighed dietary record (5·0 (SD 2·1) mg/d) was significantly greater than the current estimated average requirement (EAR; 2·5 mg/d; $P<0·0001$). Neither fractional Zn absorption, urinary Zn excretion, nor endogenous faecal Zn excretion was significantly related to Zn intake ($r^2<0·1$; $P>0·4$, for all). Absolute Zn absorption was significantly related to Zn intake (r^2 0·696; $P<0·0001$), as was Zn retention (r^2 0·506; $P<0·0001$). Cu absorption was relatively high (75·1 (SD 10·8) %) despite the high Zn intake. The EAR for Zn based on this dataset would appear to be between 4·2 and 4·7 mg/d to allow for a net average retention of 120 μg/d consistent with growth needs. We concluded that at relatively high Zn intakes there was little evidence of down regulation of absorption or up regulation of urinary or endogenous faecal Zn excretion across the intake range studied. Zn retention was positively correlated with intake. A Zn intake between 4·2 and 4·7 mg/d should meet the requirement for normal growth for this age group.

Children: Copper absorption: Mineral homeostasis: Nutrient requirements: Zinc absorption

The dietary recommendations for mineral intakes by the Institute of Medicine were revised in the late 1990s. Intake recommendations for Zn in children aged 1–4 years changed dramatically from an RDA of 10 mg/d in 1989[1] to an estimated average requirement (EAR) of 2·5 mg/d, and an RDA of 3 mg/d[2]. These were based on a factorial approach assuming obligatory urinary losses of 7·5 μg/kg per d, integumental losses of 6·5 μg/kg per d, endogenous faecal losses of 34 μg/kg per d and a requirement for growth of 120 μg/d[2]. Most of these assumptions were based on data in older subjects, due to the lack of suitable data from young children[2].

The principal reason for the absence of data in this age group is the impracticality of prolonged dietary regulation and complete urine and faecal collections that are required for traditional balance studies, especially in active children who are often not toilet-trained. Studies in this age group are now more feasible with modern stable-isotope methods in which Zn absorption can be directly assessed with a single timed urine sample[3,4]. Thus, our goal in the present study was to utilize stable isotopes to evaluate the relationships among Zn intake, absorption, endogenous faecal excretion, and retention in healthy small children on diets common in the USA.

Zn intakes in children 1 to 4 years in the USA (median intake 5·81 mg/d) are well above the current EAR (2·5 mg/d). In fact, according to the Continuing Survey of Food Intakes in Individuals, the 75th percentile of Zn intake in the USA in this age range is 7·74 mg/d, a value higher than the tolerable upper limit for Zn[2]. As the potential hazard identified to determine the tolerable upper limit for Zn intake was a possible adverse effect of high Zn intakes on Cu absorption or Cu status, we were also interested in collecting preliminary data on Cu absorption in children on Zn intakes typical of the 1–4-year-old US population.

We hypothesized that fractional Zn absorption would be significantly negatively correlated with Zn intake, and that urinary Zn excretion, endogenous faecal Zn excretion, absolute Zn absorption and Zn balance would be significantly positively correlated with Zn intake. We also wished to use the relationship between Zn retention and Zn intake to estimate the Zn intake needed to meet the estimated requirement for normal growth (120 μg/d) for 1–4-year-old children, as this would be a suitable basis for determining the EAR.

Subjects and methods

Healthy children aged 1–4 years 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. The Institutional Review Board of Baylor College of Medicine and Affiliated

Abbreviations: ARS, Agricultural Research Service; EAR, estimated average requirement; USDA, US Department of Agriculture.

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Hospitals approved the protocol, and informed written consent was obtained from the subjects' parents for all studies.

Screening visit

Children were eligible for enrolment if they were healthy, not taking any medications (except multivitamins), were born at term (≥37 weeks gestation) and had a birth weight ≥2500 g. Children were excluded from participating if they had chronic health problems, 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 before participating in the mineral absorption study. Families were offered the option of participating in a 2 d in-patient Zn absorption study or a 5 d in-patient study in which faecal samples would also be collected and endogenous excretion would be measured.

The research dietitian met with the parent and obtained a complete dietary history to evaluate usual daily micronutrient and energy intake. Dietary intake data were collected using Nutrition Data System for Research software (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA).

Home dietary adaptation

After screening, a dietary plan was developed for each child that would be consumed at home for the 7 d before the in-patient mineral absorption study. This was to ensure that children did not alter their eating habits immediately before the mineral absorption study, and was designed to be reflective of the child's usual dietary intake. All foods and beverages to be consumed during these 7 d were provided by the research centre and were pre-weighed before delivery to the family. Parents were instructed to return all the uneaten food and beverage items for the first 3 d of the pack-out so items could be post-weighed.

Isotope preparation and mineral absorption study

^{67}Zn (88 % enrichment) and ^{70}Zn (96 % enrichment) were purchased from Trace Sciences Incorporated (Toronto, ON, Canada) and prepared for human use as the chloride salt by the Investigational Pharmacy Service of Texas Children's Hospital, Houston, Texas. All isotopes were tested for sterility and pyrogenicity before use.

At the end of the home adaptation period, patients were admitted to the General Clinical Research Center at Texas Children's Hospital for the mineral absorption study. On the morning of the in-patient study, subjects had a heparin-lock intravenous catheter placed, using topical 4 % lidocaine cream (L-M-X-4; Ferndale Laboratories, Ferndale MI, USA) as an analgesic. Subsequently, 0·5 mg ^{67}Zn was given intravenously over 1 min. The subgroup of subjects in which endogenous faecal Zn was being measured had a higher intravenous dose of 1 mg ^{67}Zn given intravenously. Subjects were then given a breakfast that included 30 ml apple juice to which 0·12 mg ^{70}Zn and 30 μg ^{65}Cu had been added. After the subject consumed the isotope-containing juice, the subject consumed another 30 ml 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.

Each meal provided approximately one-third of the daily mineral intake of the subject. Menus for the in-patient study visit were based on the subject's usual mineral intake that they had received at home for the previous 7 d. All foods and beverages during the in-patient 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 in-patient unit for 48 h and their urine was collected in 24 h 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 out-patient unit 96 h after isotope administration for collection of a spot urine sample. The subset of subjects in whom endogenous faecal excretion was measured remained in the in-patient unit for 120 h during which time their urine and stools were collected in 24 h pools.

Isotope ratio measurement

Urine samples were prepared for MS analysis as previously described[5]. Faecal samples were homogenized with an approximately equal volume of water, the final volume recorded and a sample frozen. A 2 ml portion of the sample was digested in a microwave (MARS 4; CEM Inc., Matthews, NC, USA) until the sample was clear and all organic matter digested. The sample was evaporated on a hotplate at sub-boiling temperature and samples used for Zn and Cu concentration measurement and Zn and Cu isotope ratio measurement.

Samples were analysed for Zn isotopic enrichment with a Finnigan MAT 261 (Thermo Finnigan MAT GmbH, Bremen, Germany) magnetic sector thermal ionization mass spectrometer. Each sample was analysed for the ^{67}Zn:^{66}Zn and ^{70}Zn:^{66}Zn ratios with correction for fractionation to the reference ^{64}Zn:^{66}Zn ratio. Accuracy and precision of this technique for natural abundance samples compared with standard data are 0·15 % or better, depending on the ratio being measured. Cu isotope ratios were measured by a high-resolution inductively coupled plasma MS (Element 2; Thermo Electron, Bremen, Germany) equipped with a 100 μl/min Micromist concentric nebulizer and a Scott type spray chamber. Diluted digested solution containing about 25 μg Cu/l was analysed at a mass resolution of about 4000 for 2 min. Analytical precision for the ^{65}Cu:^{63}Cu ratio was $<0{\cdot}3$ %.

Zn concentrations were measured by flame atomic absorption spectroscopy, and Cu concentrations by inductively coupled plasma MS.

Measurement of zinc absorption and zinc excretion

Zn absorption was measured from the ratio of the urinary excretion of the orally administered stable isotope to the intravenous isotope in the 96 h urine sample as described elsewhere[4,5].

Urinary Zn excretion was calculated from the urine collection (either 48 or 120 h) and expressed as mg/d.

Endogenous faecal Zn excretion was measured from the urinary and faecal excretion of the intravenously administered

tracer and the urinary excretion of tracee Zn in the 5 d urine and faecal collection using the equation[4]:

$$\text{Endogenous faecal Zn excretion} = \text{urinary tracee Zn excretion} \times \frac{\text{faecal excretion of intravenous Zn isotope}}{\text{Urinary excretion of intravenous Zn isotope}}$$

Zn balance was estimated from the equation:

$$\text{Zn balance} = (\text{Zn intake} \times \text{fractional Zn absorption}) - \text{urinary Zn excretion} - \text{endogenous faecal Zn excretion} - \text{integumental Zn losses.}$$

Endogenous Zn excretion was measured in nine subjects. These data were used to extrapolate Zn losses in those subjects (n 21) in whom it was not directly measured (see later). Integumental losses were estimated to be 6·5 μg/kg[2].

Measurement of copper absorption

Cu absorption was estimated from the difference between the amount of oral tracer given, and the cumulative excretion of the oral Cu isotope during the 5 d faecal collection.

Statistical analysis and sample size determination

Assuming that the smallest clinically significant correlation between mineral intake and absorption is when changes in intake explain 25 % of the variability in mineral absorption (r^2 0·25; r 0·5) a sample size of thirty would have a power of greater than 80 % to detect such a difference at $P<0·05$. This degree of correlation is similar to previous studies that have shown a correlation of r 0·5 between intake and net Ca balance in this age range[6], and is therefore biologically plausible.

Relationships between Zn intake and different parameters of Zn homeostasis (Zn absorption etc) were assessed by simple regression analysis. χ^2 Tests, unpaired t tests and one-sample t tests were used as appropriate. Statistical analysis was carried out using StatsView 5·0·1 for Macintosh (SAS Institute Inc., Cary, NC, USA). Data are presented as mean values and standard deviations or as n and percentage, as appropriate. Statistical significance was assumed at $P<0·05$.

Results

Subject demographics

Zn absorption was successfully measured in thirty subjects (sixteen girls and fourteen boys). The ethnic distribution of the study population was 47 % Caucasian, 27 % Hispanic, 17 % African-American, and 10 % multi-ethnic (Table 1). Ten subjects were taking multivitamins; all discontinued them at least 2 weeks before the start of the study. Results are little changed if these subjects are omitted from the analysis.

Table 1. Demographics of the study subjects (numbers and percentages)

Characteristic	*n*	%
Age (months)		
Mean	29·1	
SD	10·6	
Weight (kg)		
Mean	12·7	
SD	2·1	
Height (cm)		
Mean	89·0	
SD	8·1	
Sex		
Male	14	47
Female	16	53
Ethnicity*		
Caucasian	14	47
African-American	5	17
Hispanic	8	27
Multi-ethnic and others	3	10

* Percentages do not equal 100 % due to rounding.

Twenty-one subjects opted for the 48 h admission and only had measurements of Zn absorption and urinary Zn excretion made, nine were admitted for 120 h and also had endogenous faecal Zn excretion and Cu absorption measured. There were no significant differences between the subjects who were admitted for 48 h compared with those admitted for 120 h in terms of sex (χ^2 $P=0·52$), ethnicity (χ^2 $P=0·15$), weight ($P=0·93$), height ($P=0·57$), age ($P=0·18$) or Zn intake ($P=0·51$).

Nutrient intakes

Mean Zn intake was 5·04 (SD 2·08, range 1·09–10·2) mg/d and was significantly greater than the current EAR of 2·5 mg/d ($P<0·0001$), with four subjects (13 %) exceeding the current upper limit for Zn. Mean Cu intake was 408 (SD 116) μg/d, significantly above the EAR of 260 μg/d ($P<0·0001$), but no subjects exceeded the upper limit for Cu (1000 μg/d). Mean Fe intake (6·77 (SD 2·42) mg/d) was also significantly above the EAR ($P<0·0001$).

Zinc homeostasis

Neither fractional Zn absorption (31·1 (SD 7·8) %; $y = 0·324 - 0·0003x$; r^2 0·005; $P=0·71$; Fig. 1) nor urinary Zn excretion was related to Zn intake (0·116 (SD 0·067) mg/d; 9·7 (SD 6·1) μg/kg per d; $y = 0·14 - 0·005x$; r^2 0·022; $P=0·44$).

Endogenous faecal Zn excretion (0·995 (SD 0·401) mg/d; 82·8 (SD 69·0) μg/kg per d; n 9) was unaffected by Zn intake ($y = 0·385 + 0·131x$; r^2 0·060; $P=0·53$), nor was it correlated with the subjects' age (r^2 0·049; $P=0·57$), weight (r^2 0·035; $P=0·63$) height (r^2 0·028; $P=0·67$) or the total amount of Zn absorbed (the product of fractional Zn absorption and Zn intake) (r^2 0·076; $P=0·47$). In the subsequent analysis, therefore, endogenous faecal Zn excretion was estimated to be 0·955 mg/d in those subjects in whom it was

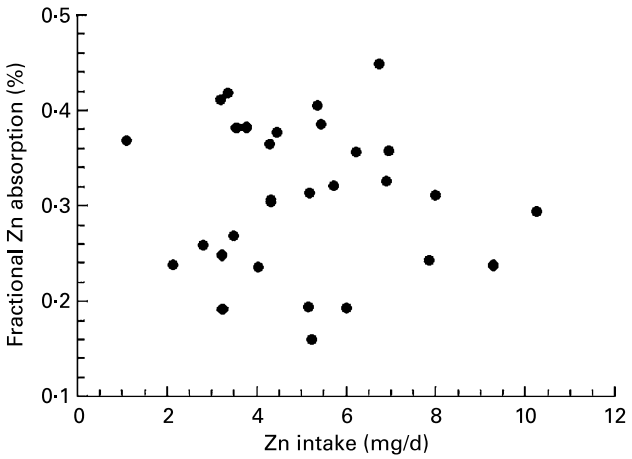

Fig. 1. Fractional Zn absorption in 1–4-year-old children is not related to Zn intake ($y = 0{\cdot}324 - 0{\cdot}0003x$; r^2 0·005; P=0·71).

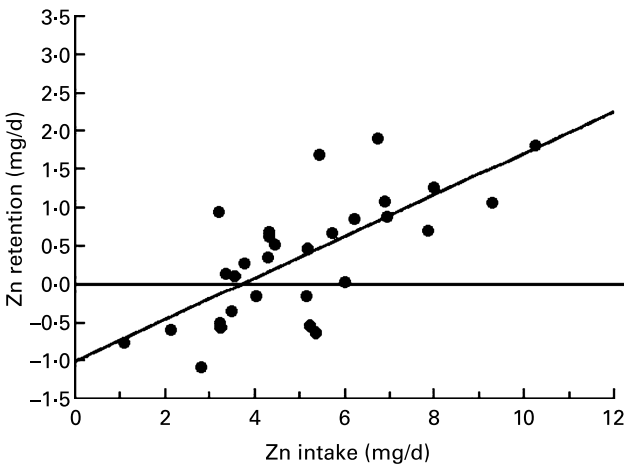

Fig. 3. Zn retention in 1–4-year-old children is significantly positively related to Zn intake ($y = -1{\cdot}008 + 0{\cdot}272x$; r^2 0·506; $P<0{\cdot}0001$).

not measured (equal to the mean value for the nine subjects in which it was measured).

Absolute Zn absorption (Fig. 2) was significantly positively correlated with Zn intake (1·56 (SD 0·71) mg/d; $y = 0{\cdot}111 + 0{\cdot}286x$; r^2 0·696; $P<0{\cdot}0001$).

Zn balance, or Zn retention, was estimated by extrapolating the data on endogenous faecal Zn excretion, and by assuming integumental losses of 6·5 μg/d.[2] For the entire population it was 0·361 (SD 0·794) mg/d which was not significantly different from the 0·120 mg/d estimated to be required for normal growth in 1–4-year-old children[2] (P=0·11). Zn retention was significantly correlated with Zn intake (Fig. 3; $y = -1{\cdot}008 + 0{\cdot}272x$; r^2 0·506; $P<0{\cdot}0001$).

Zinc intake required for normal growth in 1–4 year-olds

Two different methods were used to estimate the dietary Zn intake needed for normal growth.

About 0·12 mg/d is required for normal growth of 1–4-year-old infants[2]. Based on the relationship shown between Zn intake and Zn retention (Fig. 3) this would be achieved at a Zn intake of 4·15 mg/d.

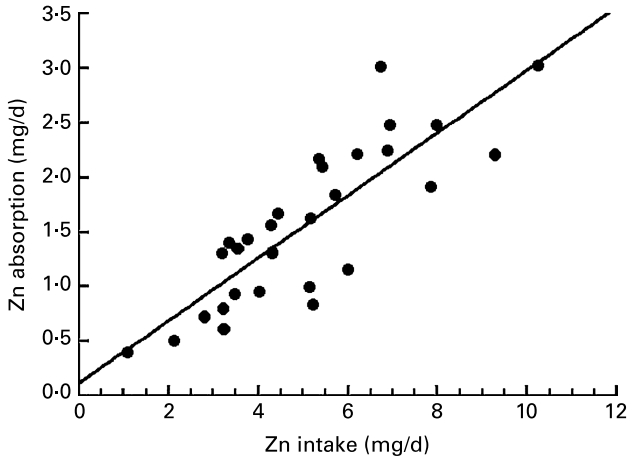

Fig. 2. Absolute Zn absorption in 1–4-year-old children is significantly positively correlated with Zn intake ($y = 0{\cdot}111 + 0{\cdot}286x$; r^2 0·696; $P<0{\cdot}0001$).

The Institute of Medicine[2] calculated the EAR using a factorial approach. They estimated that the requirement for absorbed Zn was 744 μg/d (Table 2)[2]. If we substitute our measurements of endogenous faecal Zn losses (83 μg/kg) and urinary Zn excretion (9·7 μg/d) for the estimates used by the Institute of Medicine, the requirement for absorbed Zn becomes 1410 μg/d (Table 2). If we assume fractional Zn absorption to be 30 % (consistent with the data used by the Institute of Medicine, and our own data from the present study) this equates to a dietary requirement of 4·7 mg/d.

Copper homeostasis

Cu absorption averaged 75·1 (SD 10·8, range 52·5–88·5) %, and was unrelated to Cu intake (n 9; $y = 0{\cdot}866 - 0{\cdot}0003x$; r^2 0·051; P=0·56).

Discussion

We studied 1–4-year-old children on Zn intakes typical of the US population and found no significant relationship between Zn intake and fractional Zn absorption. This was contrary to our hypothesis and to the general expectation that such a relationship should exist[7]. For example, in a study of stunted 3–4-year-old Peruvian children, de Romana *et al.*[8] showed a significant fall in fractional Zn absorption in children receiving different Zn intakes, from 34·2 % in those consuming 2·14 mg Zn/d, to 23·7 % in those on 4·72 mg/d to 13·3 % for those on 10·04 mg/d.[8] Our subjects consumed a similar range of intakes, but fractional Zn absorption of our population was very close to the Zn absorption of the lowest Zn intake group of de Romana's[8] with no fall in fractional absorption as Zn intake increased. There are a variety of possible explanations for these differing findings. The diets used in the present study differed very greatly from those of de Romana[8], and the greater bioavailability from our diets may have obscured the effect of Zn intake on fractional Zn absorption. Alternatively, the length of dietary adaptation may be important. We have previously shown a significant inverse relationship between fractional Zn absorption and Zn intake from a single meal with different amounts of haem Fe and

Table 2. Estimates for total requirement for absorbed zinc for 1–4-year-old children using estimates made by the Institute of Medicine[2] and made by modifying those estimates from data from the present study

	Institute of Medicine[2]		Present study	
	Losses (μg/kg per d × 13 kg)	Losses (μg/d)	Losses (μg/kg per d × 13 kg)	Losses (μg/d)
Obligatory losses				
Intestinal losses	34	442	83	1079
Urinary losses	7·5	98	9·7	126
Integumental losses	6·5	84	6·5	84
Total losses		624		1289
Requirement for growth (μg/d)	120		120	
Required absorbed Zn (μg/d)	742		1409	

inorganic Zn, given without any preceding adaptation period, in 4–8-year-old children[9]. However, in a study of 9–14-year-old girls adapted to intakes of 4 and 12 mg/d for 3 weeks, no relationship between fractional Zn absorption and Zn intake was seen[10]. In the present study we did not modify subjects' usual dietary intake, so they had a prolonged period to adapt to their usual Zn intake. de Romana studied children for 2–3 d and 51–52 d after a dietary modification was made[8] and differences in Zn absorption in relation to intake were seen at both times. However, when adults are placed on marginal Zn intakes, an initial intake in fractional Zn absorption is seen, but this is not maintained and decreases to baseline by 6 months[11].

A final explanation relates to how the dietary changes were made. In those studies where a significant relationship between Zn intake and Zn absorption were seen, either ours[9] or de Romana's[8], the dietary change was affected by the addition of a Zn salt to the test meal[9] or to fortified wheat flour provided to the families[8]. In those studies where no relationship between Zn absorption and Zn intake were seen, the differences in Zn intake were due to differences in foods consumed in the diet – either self-selected by the subject's parents (the present study) or under the guidance of a dietitian to effect the desired changes in Zn intake[10]. The Zn present in whole foods is probably more heterogeneous in its solubility and bioavailability than single salts added to the diet. Furthermore, changes in whole foods (rather than changes in single nutrients) may also lead to changes in the intake of inhibitors and enhancers of mineral absorption. This suggests that modifications in single nutrients in metabolic studies are not likely to produce results generalizable to the 'real world' where subjects consume complex foods rather than single nutrients in isolation. The present results are also consistent with data comparing two populations of young Chinese women whose self-selected diets contained either low or marginal intakes of Zn[12] and where no significant relationship between Zn absorption and Zn intake could be demonstrated. Conversely in a study of 7–9-year-old females where Zn intakes between 5·61 and 14·61 mg/d were produced by adding aqueous zinc sulfate to meals, a significant inverse relationship between Zn intake and fractional Zn absorption was seen[13].

Endogenous faecal Zn excretion may be an important site of Zn homeostasis[11,14] and was measured in a subset of subjects. Ideally, we would have measured it in all subjects but the burden that would have placed on subjects and their families was felt to be too great, and would have greatly increased the difficulty in recruiting subjects. In the small number of subjects we studied we could not identify the expected inverse relationship with Zn intake[11,12,14] or the expected positive relationship with total absorbed Zn[2,12], although our statistical power was very limited and the study was not designed to detect such a relationship. Weight-specific endogenous faecal Zn excretion (89 (SD 69) μg/kg) did, however, tend to exceed the value of 34 μg/kg ($P=0·067$) that the Institute of Medicine assumed for this age group[2]. Although we cannot say whether this higher than expected level of endogenous faecal Zn excretion was due to the relatively high Zn intake or to developmental differences in young children compared with older age groups (from whom the 34 μg/kg per d value was extrapolated from) it clearly has implications for accurate assessment of Zn requirements using a factorial approach.

We could detect no relationship between Zn intake and urinary Zn excretion, and this would be expected to be a late finding in Zn deficiency, or a response to very low Zn intakes[14]. The weight-specific urinary Zn excretion (9·2 μg/kg) was similar to that expected by the Institute of Medicine[2], and somewhat lower than we have previously described in adolescents (averages between 13·4 and 15·5 μg/kg)[10].

Zn retention was linearly related to Zn intake. A Zn intake of 4·15 mg/d would be required to ensure the absorption of the 0·12 mg Zn/d required for normal growth in 1–4-year-old children[2]. An alternative approach to calculating the EAR for this age group, substituting our estimates of endogenous faecal Zn excretion and urinary Zn excretion into the Institute of Medicine's factorial calculation, leads to a slightly higher estimate of 4·7 mg/d. In either case it would appear that the current EAR (2·5 mg/d) is somewhat low and a level nearer 4·5–5·0 mg/d is more appropriate.

The Institute of Medicine also defines a tolerable upper limit of 7 mg/d, based on the theoretical risk of Zn interfering with Cu absorption and hence Cu status[2]. This level has been controversial as it is less than the previous RDA for this age group[1], and is exceeded by many 1–4 year olds based on dietary surveys[2]. The present study design did not allow us to examine the effect of Zn intake on Cu absorption (or vice versa) as the intakes of the two minerals were strongly correlated. However, the demonstration that diets of young children that are high in Zn are also higher in Cu would appear to negate the potential effect of Zn on Cu status. However, in the subgroup of subjects where Cu absorption was measured it was relatively high, making a substantial effect of such Zn intakes on Cu absorption unlikely.

In summary, 1–4 year olds typically consume diets that exceed the current EAR and RDA for Zn; Zn retention increases as Zn intake increases; and a Zn intake of 4·2–4·7 mg/d appears to meet the needs for absorbed Zn in this age group.

Acknowledgements

The present study is a publication of the US Department of Agriculture (USDA)/Agricultural Research Service (ARS) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. This project has been funded in part with federal funds from the USDA/ARS under Cooperative Agreement number 58-6250-6-001 and the National Institutes of Health, National Center for Research Resources (NCRR) General Clinical Research for Children Grant number RR00188. Contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

The authors acknowledge the assistance of the nursing staff of the General Clinical Research Center of Texas Children's Hospital for caring for the study subjects and the investigational pharmacy of Texas Children's Hospital for preparation of the isotopes. We to thank Leslie Cruz and Dana McDonald for their help with patient enrolment and study visits, and Penni Hicks for her support and advice.

None of the authors has any conflict of interest to disclose.

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