

Zinc absorption as a function of the dose of zinc sulfate in aqueous solution¹⁻³

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ABSTRACT

Background: Zinc supplements are used extensively in medicine and research and for public health purposes in the prevention and treatment of zinc deficiency. However, little is known about the efficiency of zinc utilization after different doses.

Objective: The objective was to determine the relation between dose of aqueous zinc and absorbed zinc (AZ) in healthy adults.

Design: Eight healthy adults (3 men and 5 women) aged 33.8 ± 9.8 y ($\bar{x} \pm$ SD) received 3 pairs of zinc doses (2 and 5, 10 and 15, and 20 and 30 mg) in random order in 3 phases (1 pair per phase). There was a 3-wk washout between phases. Aqueous zinc sulfate labeled with ^{70}Zn or ^{68}Zn was orally administered in the postabsorptive state on days 1 and 6, respectively; intravenous ^{67}Zn was administered 1 h after the first oral zinc dose. Two urine samples were collected daily from days 3 to 15; zinc isotopic ratios were determined by inductively coupled plasma mass spectrometry. Fractional absorption of zinc (FAZ) was determined by dual-isotope-tracer ratio; AZ was calculated by multiplying FAZ by dose.

Results: Mean (\pm SD) AZ values at doses of 2.2, 5.2, 10.4, 15.2, 20.3, and 30.1 mg ingested Zn were 1.6 ± 0.4 , 3.5 ± 1.3 , 7.4 ± 1.0 , 9.5 ± 2.2 , 11.0 ± 4.4 , and 11.2 ± 2.1 mg, respectively. A saturable dose-response model, the Hill equation, was selected to model the relation of AZ to ingested zinc. Parameter estimation by nonlinear regression predicted a maximum zinc absorption of 13 mg for larger doses.

Conclusions: Increases in aqueous zinc doses >20 mg result in relatively small and progressively diminishing increases in AZ post-absorptively in healthy adults. *Am J Clin Nutr* 2004;80:1570-3.

KEY WORDS Zinc absorption, zinc sulfate, aqueous solution

INTRODUCTION

Zinc supplementation is being used increasingly in important fields of research, such as in the prevention of diarrhea and pneumonia (1) and in achieving optimal growth (2). Zinc supplementation is associated not only with decreased morbidity but also with decreased mortality (3, 4). It has been estimated that adequate zinc supplementation globally would prevent several hundred thousand early childhood deaths per year (5). Questions remain, however, regarding the optimal dose of supplemental zinc. Doses used in randomized trials have varied widely (1), and in some cases high doses of supplemental zinc in young children have been associated with adverse outcomes (6).

The principal pathway of mammalian zinc absorption is a saturable transport mechanism (7). Dose-response data for the absorption of zinc from a range of oral doses could help establish

dosage guidelines for short-term relatively high-dose zinc supplementation. No such data are available currently for young children, and even adult studies are remarkably limited (8, 9). The objective of this study was to determine the quantity of zinc absorbed from a range of oral doses of an aqueous solution of a soluble inorganic zinc salt administered to healthy young adults in the postabsorptive state.

SUBJECTS AND METHODS

Subjects

Calculations from our previous studies in our laboratory showed that the mean (\pm SD) difference in the fractional absorption of zinc (FAZ) between control and treated groups was 0.3 ± 0.1 . The sample size needed for this study, estimated by Sigma-Stat Statistical Software (version 2.03; SPSS Inc, Chicago)—calculated for analysis of variance with a statistical significance set at a *P* value of 0.05, a power of 0.8, and a minimum detectable difference between means of 0.2 with 6 groups—was 8. Eight apparently healthy adults ($\bar{x} \pm$ SD: 33.8 y ± 9.8) of both sexes (3 men and 5 women) in the metropolitan Denver area were recruited for the study. Subjects were excluded if they had taken zinc supplements within 2 mo before the study. All subjects were omnivorous and consumed a typical North American diet. Precise zinc intakes were not estimated. The study was approved by the Colorado Multiple Institution Review Board at the University of Colorado Health Science Centre and participants provided written informed consent.

Study design

Zinc stable isotope tracers were mixed with solutions of zinc sulfate containing 6 different quantities of zinc ranging from ≈ 2 to 30 mg. Each of these solutions was administered to apparently healthy adult volunteers over a 15-wk period, and FAZ was measured by using a modified version of our standard

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dual-isotope-tracer ratio technique (10). The quantity of zinc absorbed was plotted versus the dose administered and analyzed by regression analysis.

Isotope preparation and administration

Enriched zinc stable isotopes (^{67}Zn , ^{68}Zn , and ^{70}Zn ; Trace Sciences International, Richmond Hill, Canada) were dissolved in 2 mL of 0.5 mol $\text{H}_2\text{SO}_4/\text{L}$. Orally administered isotopes (^{70}Zn and ^{68}Zn) were further diluted in milli-Q water, which is triply filtered and is free of trace minerals, and titrated to pH 3.0 with metal-free NH_4OH or NaOH . The solutions were filtered through a 0.22- μm filter to remove pyrogens. Intravenously (iv) administered isotope (^{67}Zn) was diluted in sterile 0.45% NaCl , adjusted to pH 6.0, filtered through a 0.2- μmol filter to remove pyrogens, and stored in sterile vials. The iv dose was drawn up in a syringe from the sterile vial under a laminar flow hood before administration. The zinc concentration in each isotope preparation was determined in triplicate by atomic absorption spectrophotometry and then adjusted for the different atomic weights of the isotope preparations to calculate the final zinc concentration in each isotope preparation (10). The oral zinc stable isotope preparations added to solutions of natural abundance zinc sulfate were $1.0 \pm 0.01 \text{ mg } ^{70}\text{Zn}$ on day 1 and $2.0 \pm 0.01 \text{ mg } ^{68}\text{Zn}$ (day 6) for each phase.

Six different doses of zinc were administered to each subject (including isotopic zinc): 2.25 ± 0.05 , 5.17 ± 0.05 , 10.44 ± 0.26 , 15.18 ± 0.10 , 20.28 ± 0.12 , and $30.07 \text{ mg} \pm 0.10$ ($\bar{x} \pm \text{SEM}$). A preliminary study indicated that when doses of 20 mg Zn were administered on 2 consecutive mornings, FAZ was considerably lower on the second morning. However, there was no carry over effect when administration was separated by 6 d. Two of the 6 doses, therefore, were administered at 6-d intervals, with a 3-wk washout period before the administration of the next pair and again for the final pair. The pairs were the 2 lowest, the 2 middle, and the 2 highest zinc doses. The order of administration of these 3 pairs was randomized. The zinc solutions were administered between 0800 and 0900 after the subjects fasted overnight, and breakfast was withheld for an additional 2 h.

A dose of ^{67}Zn ($\bar{x} \pm \text{SD}$: $1.01 \pm 0.06 \text{ mg Zn}$; in 12 mL) was administered intravenously 1 h after the first oral zinc dose of each pair. This sterile solution was administered over a 5-min interval via a scalp vein needle in a superficial forearm vein with a 3-way closed stopcock system followed by a rinsing of the delivery syringe with sterile 0.45% NaCl contained in a second sterile syringe.

Sample collection

For each of the 3 phases, 2 spot urine samples were collected daily from days 3 to 15 after administration of ^{67}Zn iv. Before the commencement of the second and third phases, the subjects were required to collect 5 spot, casual (nonfasting) baseline urine samples over 3 d for the measurement of residual isotope enrichment from the previous phase.

Digestion, extraction, and measurement of zinc stable isotopes

Urine samples (5 mL) were wet digested with the use of an MDS-2000 microwave digester (CEM Corporation, Matthews, NC) based on a modified method of Huffer et al (11). The samples were then dried in a beaker on a hotplate and dissolved in 1 mol/L ammonium acetate buffer, pH 5.6. Zinc was then

separated by an ether extraction chelation method from the ammonium acetate suspension (11). Zinc isotope ratios were determined by inductively coupled plasma mass spectrometry (VG Plasma Quad 3; VG Elemental, Cheshire, United Kingdom) run in the hot plasma and peak jump modes (12). Sample isotope enrichments were calculated from the ratio measurements as described previously (12).

Fractional absorption of zinc and absorbed zinc

FAZ was determined with the use of a dual-isotope-tracer ratio method (13) and was calculated by using the following formula (shown for ^{70}Zn): $\text{FAZ of } ^{70}\text{Zn} = (^{70}\text{Zn enrichment}/\text{mg } ^{70}\text{Zn dose}) / (^{67}\text{Zn enrichment}/\text{mg } ^{67}\text{Zn dose})$. FAZ was determined for each urine sample collected ≥ 3 d after zinc administration, and the mean FAZ was calculated. For the second FAZ determination of each pair, urine enrichment with the oral tracer (^{68}Zn) was ratioed to the urine enrichment measurements from precisely 6 d earlier for the intravenously administered ^{67}Zn . It was assumed that zinc metabolism had remained unchanged over this short time interval in healthy subjects consuming their habitual diets.

Before the FAZ calculation for data from phases 2 and 3 of the study, the background residual enrichment from the previous study phases was subtracted from the enrichment data. A long-term simulation with the use of a compartmental model of zinc metabolism (14) showed that the rate of decay of enrichment from the previous tracer administration was low enough during the subsequent measurement period that the background could be approximated by a constant enrichment value without significantly affecting accuracy. The background enrichment value was thus calculated from the mean of the baseline measurements obtained at the start of the study phase and adjusted for the predicted decay (based on the model simulation) in enrichment from the time of baseline measurements to the midpoint of the FAZ measurement period. The amount of absorbed zinc (AZ) was calculated by multiplying FAZ by the aqueous zinc dose.

Statistical analysis

All data are shown as means \pm SDs unless otherwise stated. Several appropriate mathematical models were fitted to the data for AZ versus aqueous zinc dose with the use of least-squares nonlinear regression, and their relative merits were compared. One model, the Hill equation (15), was chosen as the best option on the basis of the quantitative and qualitative criteria. Nonlinear regression was performed by using GRAPHPAD PRISM (GraphPad Software Inc, San Diego).

Incremental FAZ was derived from differentiating the Hill equation model curve. Individual data points for incremental FAZ were determined by dividing the increment in AZ between 2 adjacent doses and the difference (in mg) between these 2 doses of ingested zinc (IZ).

RESULTS

The doses of zinc administered, including the tracer, and the FAZ for each of these doses are given in **Table 1**. The quantities of AZ for each dose of IZ are also shown in Table 1.

A saturable dose-response model sometimes referred to as the Hill equation was chosen as best reflecting the data. The equation for this model is as follows:

$$\text{AZ} = \frac{A_{\text{max}} \times \text{IZ}^H}{\text{IZ}^H + \text{IA}_{50}^H} \quad (1)$$

TABLE 1

Mean ingested zinc (IZ), fractional absorption of zinc (FAZ), and absorbed zinc (AZ) determined in 8 healthy adults in the postabsorptive state¹

	Zinc dose					
	2 mg	5 mg	10 mg	15 mg	20 mg	30 mg
IZ (mg)	2.3 ± 0.1	5.2 ± 0.1	10.4 ± 0.7	15.2 ± 0.3	20.3 ± 0.3	30.1 ± 0.3
FAZ	0.73 ± 0.18	0.68 ± 0.25	0.71 ± 0.11	0.62 ± 0.14	0.54 ± 0.2	0.37 ± 0.07
AZ (mg)	1.6 ± 0.4	3.5 ± 1.3	7.4 ± 1.0	9.5 ± 2.2	11.0 ± 4.4	11.2 ± 2.1

¹ All values are $\bar{x} \pm SD$.

The parameters A_{max} , IA_{50} , and H are the maximum AZ, the IZ that results in AZ of 50% of A_{max} , and the Hill (or sigmoidicity) coefficient, respectively. The estimated values (and 95% CIs) for these parameters from the regression analysis are 13 (9.2, 17), 8.6 (4.5, 13), and 1.7 (0.75, 2.7), respectively. The data and the model curve after regression analysis are shown in **Figure 1**. The curve is graphed beyond the measurement range to show the model's prediction of absorption at higher IZ levels. The incremental FAZ derived by differentiating the model curve shown in Figure 1 is shown in **Figure 2**. Individual increments in FAZ are also included in this figure.

DISCUSSION

The model we used is a general form of the mathematical model widely used in various pharmacokinetic-pharmacodynamic contexts. This model was selected from 6 that were investigated. Three were rejected outright because of unreasonable behavior, ie, a negative slope at higher IZ levels. The 3 remaining models were the one-site binding model, exponential association, and the Hill equation. A comparative evaluation of these 3 models, quantitative measures of goodness of fit and model selection (eg, coefficient of determination and F test where appropriate), and Akaike's Information Criterion gave inconsistent results and indicated that the differences between the models was quantitatively unremarkable. The similarities between the models was due, in part, to the variability in the data. When the models were fitted to the means of the data for each IZ level, the differences were more pronounced. We modeled the individual data to give

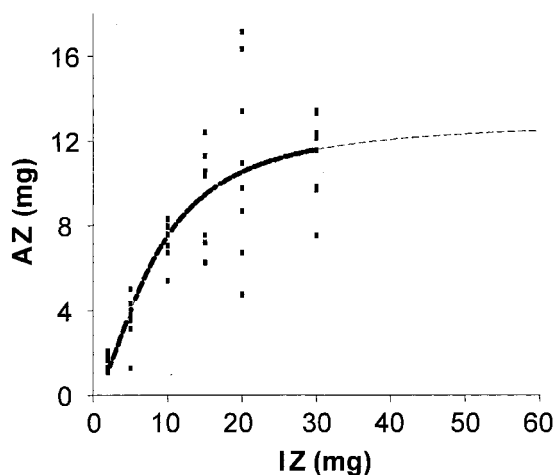


FIGURE 1. Individual measurements of absorbed zinc (AZ) versus ingested zinc (IZ) and the fitted Hill equation model (thick solid line; see Results). $R^2 = 0.73$.

each point equal weight. Taking into account the quantitative information, a final selection was made with the consideration of qualitative criteria, ie, appropriateness of the mathematical expression, morphologic agreement of model and data, and comparison of parameter estimate statistics.

The model selected is consistent with our understanding of the physiology of zinc absorption in the small intestine, ie, it is an active transport process (7) for which several zinc transporter proteins have been identified (16, 17). It is expected that, in addition to the saturable active transport mechanism, there is a passive diffusion component of the absorption process that is linearly related to IZ (18). We investigated this possibility in our modeling of the data and found no evidence of a passive absorption component. We assume, nonetheless, that there is passive absorption, but that it is not evident because of the limited range and variability of the data. Because a passive (linear) component is not likely to become clearly evident until the saturable mechanism is approaching its maximum, it may be necessary to obtain data at higher IZ levels to detect and measure passive absorption.

The regression analysis provided meaningful parameter estimates; all estimates were significantly different from zero ($P \leq 0.001$). The A_{max} of 13 mg is an estimate of the maximum amount of zinc that will be absorbed regardless of the amount ingested. The IA_{50} was not of interest to us at the time. In some circumstances a Hill coefficient greater than one may have a biochemical interpretation, eg, indicates multiple receptor binding sites. We have no basis for such an interpretation at this time and use the parameter only to improve the fit to the data.

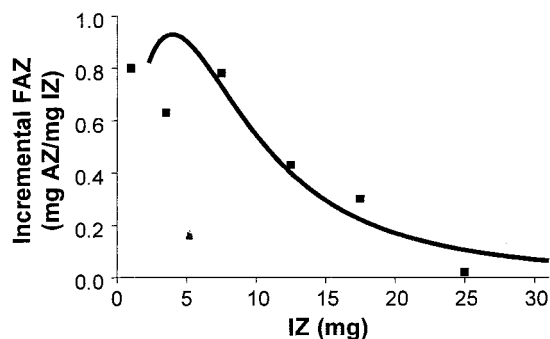



FIGURE 2. The first derivative of the fitted Hill equation model (solid line; see Results). The curve shows the effective fractional absorption of incremental increases in ingested zinc (FAZ) as predicted by the model. Also shown are incremental FAZ data derived directly from the absorption data by calculating the additional amount of absorbed zinc (AZ) with each increase in the amount of ingested zinc (IZ). For example, when we increased the dose of IZ from 10.4 to 15.2 mg, AZ increased by 2.1 mg (see Table 1). The incremental FAZ was then calculated as $2.1/4.8 = 0.44$. This can be viewed as an average value for the range of 10–15 mg IZ and is plotted at the midpoint of that range.

Increments in AZ are progressively smaller at the same increase in dose as the total size of dose is increased. This is illustrated in both Figures 1 and 2. The abrupt decline in the efficiency of utilization of an oral aqueous dose of zinc when the quantity is increased beyond 10 mg Zn is shown in Figure 2. Of particular practical relevance, the regression model predicts that any further increases in dose >20 mg Zn will result in extremely small increments in AZ. The actual mean measured increment in absorption resulting from increasing the dose from 20.3 to 30.1 mg Zn was only 0.2 mg Zn, with a corresponding incremental FAZ of only 0.02. Even though zinc is considered to be relatively safe, the current results provide little justification for exceeding 20 mg Zn in a single aqueous dose for healthy adults. Corresponding data for young children would be especially useful in view of the increasing use of short-term zinc supplements to prevent and treat zinc deficiency in this age group.

Few previous studies have examined absorption from single aqueous zinc doses administered in the postabsorptive state. Istfan et al (19) suggested a hyperbolic relation between IZ and FAZ, with an asymptote for zinc absorption of 56% of the ingested dose based on limited test doses. In contrast, Sandstrom (20) reviewed several small data sets for which the doses used were relatively low. These included data from Valberg et al (8), who studied aqueous doses of 0.5, 4.0, 6.0, and 6.2 mg using radioisotope techniques and reported ^{65}Zn absorption between 69 and 56%; these data were consistent with our previous experience (21). Similarly, Sandstrom et al (9, 22) reported 74% and 46% absorption from 2.6 or 13 mg aqueous Zn doses, respectively, administered orally after an overnight fast.

Our preliminary studies showed that the ingestion of 2 doses of 20 mg Zn on consecutive days in the postabsorptive state resulted in a 40% reduction in FAZ from 0.56 on day 1 to 0.34 on day 2. In addition, data from studies of a single subject showed that the ingestion of 20 mg Zn orally for 6 consecutive d in the postabsorptive state resulted in a 50% reduction in FAZ from 0.49 (day 1) to 0.24 (day 6). However, FAZ (0.53) was unchanged when an oral dose of 20 mg Zn was administered with a 6-d interval (CD Tran, unpublished observations, 2003). These results indicate that FAZ and AZ from single doses are much lower when a 20-mg dose was administered on the previous day or several previous days, a circumstance that is likely to occur with the administration of zinc supplements.

Zinc absorption from an aqueous solution is substantially higher than that from composite meals (23), and increments in AZ with increasing quantities of zinc in or with meals are much lower than those observed in this study in the postabsorptive state (9, 20, 21).

In conclusion, this study examined the relation between the aqueous doses of IZ given in the postabsorptive state and the amount of zinc absorbed in healthy adults. Incremental FAZ decreases progressively with increasing doses of zinc, and there is only minimal additional absorption of zinc with increases in doses beyond 20 mg Zn. These data have practical implications for the administration of oral zinc supplements as a short-term preventive or treatment measure for zinc deficiency. 

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CDT conducted the experimental work, data collection and entry, and analysis of results and had a major role in writing the manuscript. LVM was responsible for the statistical analysis and zinc absorption modeling. SL was

responsible for the analysis of zinc stable isotope enrichment in the urine using inductively coupled plasma mass spectrometry. NFK and KMH oversaw the experimental work and the preparation of this project, performed all of the iv infusions, and had significant input in editing the manuscript. All of the authors contributed to the study design, and none of the authors had a conflict of interest related to this work.

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