Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men[1–3]

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ABSTRACT

Background: Zinc homeostasis and normal plasma zinc concentrations are maintained over a wide range of intakes.

Objective: The objective was to identify the homeostatic response to severe zinc depletion by using compartmental analysis.

Design: Stable zinc isotope tracers were administered intravenously to 5 men at baseline (12.2 mg dietary Zn/d) and after 5 wk of acute zinc depletion (0.23 mg/d). Compartmental modeling of zinc metabolism was performed by using tracer and mass data in plasma, urine, and feces collected over 6–14 d.

Results: The plasma zinc concentration fell 65% on average after 5 wk of zinc depletion. The model predicted that fractional zinc absorption increased from 26% to essentially 100%. The rate constants for zinc excretion in the urine and gastrointestinal tract decreased 96% and 74%, respectively. The rate constants describing the distribution kinetics of plasma zinc did not change significantly. When zinc depletion was simulated by using an average mass model of zinc metabolism at baseline, the only change that accounted for the observed fall in plasma zinc concentration was a 60% reduction in the rate constant for zinc release from the most slowly turning over zinc pool. The large changes in zinc intake, excretion, and absorption—even when considered together—only explained modest reductions in plasma zinc mass.

Conclusion: The kinetic analysis with a compartmental model suggests that the profound decrease in plasma zinc concentrations after 5 wk of severe zinc depletion was mainly due to a decrease in the rate of zinc release from the most slowly turning over body zinc pool.   *Am J Clin Nutr* 2001;74:116–24.

KEY WORDS   Zinc depletion, compartmental model, kinetic analysis, rate constants, plasma zinc, zinc homeostasis, men

INTRODUCTION

Studies in experimental animals and humans have shown that the whole-body content of zinc remains relatively constant over a wide range of intakes. Adjustments in gastrointestinal zinc absorption and endogenous excretion appear to be the primary means of maintaining zinc homeostasis (1). In a study in weanling rats, as the efficiency of zinc absorption decreased and endogenous excretion increased, a constant whole-body zinc content was maintained as dietary zinc increased from 10 to 100 μg Zn/g (2). Studies in humans also showed that zinc losses and

absorption are adjusted to match intakes over a 10-fold range (3); these studies were conducted in men fed diets adequate in zinc that supported zinc balance and maintained normal physiologic function. When diets virtually free of zinc were fed, balance was not achieved, plasma zinc concentrations declined, and the clinical symptoms of zinc depletion developed (4). Usually, plasma zinc concentrations decline before the onset of clinical symptoms of zinc depletion. In animals fed diets extremely low in zinc, plasma zinc concentrations fall before tissue zinc concentrations change in those tissues most sensitive to depletion, eg, the skin (5, 6). Possibly, the fall in plasma zinc concentrations with acute depletion reflects adjustments in the release of zinc from tissues to maintain normal tissue function (7). To further understand the relation between plasma and tissue zinc concentrations when there is severe depletion, we used a compartmental model to describe zinc kinetics in men fed a diet virtually free of zinc.

Compartmental models of zinc metabolism in humans have been formulated by using radioactive or stable-isotope tracers of zinc (8–12). Monitoring the oral and intravenous tracer data in plasma, urine, and fecal samples has allowed the development of models describing zinc absorption, fecal and urinary endogenous excretions, and the sizes and turnover rates of extravascular pools that exchange with plasma zinc. Using such a compartmental model of zinc metabolism, Wastney et al (9) identified 5 sites at which zinc homeostasis was regulated when zinc intake increased 11-fold: gastrointestinal zinc absorption, urinary zinc excretion, erythrocyte exchange of zinc, muscle zinc release, and secretion of zinc into the gut. These adjustments, along with a nearly 2-fold increase in the plasma zinc concentration, maintained normal physiologic function when there was an excess of zinc.

TABLE 1
Subject characteristics at baseline

Subject	Age	Height	Weight	Ideal body weight
	y	*m*	*kg*	*%*
2	21	1.79	68	100
3	35	1.70	67	104
4	24	1.80	74	102
10	34	1.75	68	96
11	25	1.80	93	120
$\bar{x} \pm SD$	28 ± 6	1.77 ± 0.1	74 ± 10	105 ± 9

Similar studies of zinc metabolism and homeostasis in humans fed a zinc-deficient diet have not been conducted with the use of a compartmental model. Therefore, the purpose of the present study was to apply a mathematical model of zinc metabolism developed previously (11) to the tracer and tracee data in plasma, urine, and feces obtained from 5 men fed a diet virtually free of zinc to determine the relation between changes in plasma zinc, zinc absorption and excretion, and tissue zinc kinetics.

SUBJECTS AND METHODS

Subjects

Twelve men aged 20–35 y were recruited for the study; 7 subjects completed the study. Of these 7 subjects, 1 did not comply with the study design and 1 was unable to provide critical blood samples. Data for the remaining 5 subjects are reported in **Table 1**: 3 subjects were white, 1 was Hispanic, and 1 was white and Asian. All of the subjects were nonsmokers and judged to be healthy on the basis of a routine blood screening, medical history, physical examination, and psychological profile. Before the study, all of the subjects had an adequate zinc intake on the basis of estimates of zinc intake from a food-frequency questionnaire and a dietary history evaluated by a dietitian. The mean (±SD) estimated zinc intake was 11.5 ± 6.4 mg/d. Fasting plasma zinc concentrations at the time of the prestudy physical were all within the range of 10.7–15.4 μmol/L (0.70–1.00 μg/mL).

The study protocol was reviewed and approved by the Committee for the Protection of Human Subjects (University of California at Berkeley), the Committee on Human Research (University of California at San Francisco), and the Human Studies Review Committee of the US Department of Agriculture, Agricultural Research Service. All subjects gave written, informed consent.

Study design

The subjects were housed in a metabolic ward at the Western Human Nutrition Research Center (San Francisco) for 57 d. The study was divided into 2 metabolic periods: a 16-d baseline period in which 12.2 mg Zn/d was provided and a 41-d depletion period in which 0.23 mg Zn/d was provided. Intravenous stable-isotope tracers of zinc were administered on days 6 or 7 of the baseline period and at the end of the depletion period (day 35) to develop the mathematical model of zinc metabolism.

Body weight, temperature, and blood pressure were measured between 0700 and 0800 daily after the subjects' first urinary void in the morning. To maintain physical activity comparable with prestudy levels, the subjects walked 9.6 km (6 miles)/d at a brisk pace, 4.8 km (3 miles) in the morning and 3 mi (4.8 km) in the afternoon.

Diet

To provide a diet virtually free of zinc, an egg-albumin-based, semipurified formula diet adequate in all nutrients except zinc (4) was fed throughout the study (**Table 2**). The basic formula provided 761 kJ/d, with 10% of the energy from protein, 60% from carbohydrate, and 30% from fat. The extra-energy formula—composed of oil, sugar, and dextromaltose—was added to the basic formula in varying amounts to maintain a constant body weight. The total energy intake ranged from 155 to 192 kJ·kg^{-1}·d^{-1}. Each subject's protein intake was also adjusted for body weight by using egg albumin powder to provide ≥0.8 g protein/kg. The actual protein intake ranged from 0.7 to 1.2 g·kg^{-1}·d^{-1}. All adjustments in energy and protein intakes were made during the first 7 d; no further adjustments were made thereafter. Fiber (methylcellulose) was added to the formula to ensure regular fecal flow. A total of 4 g α-methylcellulose/d (1 g/meal) was given to all but one subject, who required 8 g/d to control constipation. Multivitamin tablets were taken once daily (Long's Daily Vitamin; Long's Drug Stores, Walnut Creek, CA). Trace minerals—except zinc, copper, and iron—were supplied as capsules (prepared by the University of California at San Francisco School of Pharmacy, Drug Product Services Laboratory) and were taken once daily. A solution of iron (FeSO$_4$) and copper (CuSO$_4$) providing a total daily intake of 10 mg Fe and the upper level of the estimated safe and adequate intake of copper, 3 mg/d, was added to the formula at each meal to ensure an adequate intake of those trace elements known to influence zinc metabolism. Likewise, a solution of ZnSO$_4$ providing 12 mg Zn/d was added to the formula during the baseline period and withdrawn during the depletion period.

Meals were served 4 times daily at 0800, 1200, 1630, and 2000. Liquid flavorings (McCormick & Co, Inc, Hunt Valley, MD) were provided for addition to the formula ad libitum. The following food items were served with the formula: a low-protein rusk (Aprotein; Dietary Specialties, Inc, Rochester, NY) with the 0800 meal, a low-protein wafer (Dietary Specialties, Inc, Rochester, NY) with the 1200 and 2000 meals, and raw carrots (20 g) at the 1630 meal. A sugar-free breath freshener was available after each meal (Certs mini-mints; Warner Lambert Co, Morris Plains, NJ). Deionized water was available ad libitum. Initially, each subject selected up to 4 nonnutritive beverages per day to consume throughout the study. All food items, breath fresheners, beverages, and flavorings were analyzed for zinc by atomic absorption spectrometry (AAS) and were found to contain negligible amounts of zinc.

The mean (±SD) daily zinc intake from the basic formula diet and all foods fed during the study (excluding the ZnSO$_4$ solution) was 0.22 ± 0.07 mg/d.

Kinetic studies of zinc metabolism

Kinetic studies were performed in the middle of the baseline period (on day 7 in subjects 2–4 and on day 6 in subjects 10 and 11) and at the end of the depletion period (on day 27 in subject 2 and on day 35 in subjects 3, 4, 10, and 11). Subject 2 developed clinical symptoms of zinc deficiency (ie, erythematous dermatitis) by week 4 of depletion; therefore, the depletion kinetic studies were conducted earlier and zinc depletion was terminated on day 33. At baseline, 1.6 ± 0.06 mg tracer highly enriched in ^{67}Zn was administered intravenously; 0.3 ± 0.02 mg tracer highly

TABLE 2
Composition of the daily diet

Ingredient	Amount
Basic formula (g/d)	
Egg albumin	59.71
Dextrimaltose	119.81
Cornstarch	119.81
Sucrose	39.95
Vegetable oil	59.9
Biotin	0.0002
Cholesterol	0.4
Choline	0.25
Magnesium oxide	0.58
Calcium carbonate	2.00
Potassium phosphate	3.52
Potassium chloride	1.77
Sodium chloride	4.08
Extra-energy formula[1]	Variable
Protein supplement[2]	Variable
Trace element solution[3]	—
Vitamin supplements (tablet/d)[4]	1
Trace element supplement (capsule/d)[5]	1
Fiber (methylcellulose)	Variable

[1] Mixture of dextrimaltose, cornstarch, sucrose, vegetable oil, and deionized water formulated to supply 2 kcal/g (8.368 kJ/g).

[2] Mixture of dried egg albumin and deionized water to provide 0.2 g protein/g.

[3] Solution of iron (10 mg $FeSO_4$), copper (3 mg $CuSO_4$), and zinc (12 mg $ZnSO_4$). No zinc was added during depletion.

[4] Composition: 4750 IU vitamin A (acetate), 250 IU vitamin A (β-carotene), 60 mg vitamin C (ascorbic acid), 1.5 mg thiamine, 1.7 mg riboflavin, 20 mg niacin (niacinamide), 400 IU vitamin D (ergocalciferol), 30 IU vitamin E (all-rac-α-tocopheryl acetate), 2 mg vitamin B-6 (pyridoxine HCl), 0.4 mg folic acid, 6 μg vitamin B-12 (cyanocobalamin), and 10 mg pantothenic acid (d-calcium pantothenate).

[5] 3.5 mg Mn, 0.15 mg Mo, 0.1 mg Cr, 0.07 mg Se, 0.15 mg I, and 2.5 mg F.

enriched in ^{70}Zn was administered at the depletion time point. Before the intravenous tracer was injected, a catheter was placed in the opposite arm and was used for blood sampling (8.0 mL) at the following times after the tracer infusion: 2, 5, 10, 20, 30, 45, and 60 min and 2, 3, 6, 9, and 12 h. The catheter was removed after 12 h and additional blood samples were collected by venipuncture at 24, 48, 96, and 144 h. All samples were kept on ice until the plasma was separated by centrifugation at 1145 × g for 15 min at 4°C (Sorvall Instruments, Dupont Corp, Wilmington, DE) within 1 h of collection.

Preparation of stable-isotope tracers

Stable-isotope tracers of zinc, highly enriched in ^{67}Zn (90.09% abundance) or ^{70}Zn (85.03% abundance), were purchased as zinc oxide from Oak Ridge National Laboratory (Oak Ridge, TN). The tracers were prepared for intravenous administration as described previously (11). Sterilization, pyrogen testing, and packaging into individual, sealed, sterile vials were performed at the pharmacy of the University of California at San Francisco.

Sample collection and analysis

Twenty-four–hour urine and complete fecal samples were collected throughout the study. The zinc tracer concentration was measured in each plasma sample, in each complete 24-h urine collection for 7 d, and in each stool specimen for 14 d after

administration. Precautions against environmental zinc contamination were taken for all diet, blood, and excreta collections and analysis. Before use, all glassware was acid washed in 10% nitric acid and rinsed 3 times with triply deionized water.

The total zinc content of the plasma, fecal, and urinary, samples was determined by AAS (Smith-Hieftje-22; Thermo Jarrell Ash, Franklin, MA). Plasma and urine samples were diluted with 0.125 mol nitric acid/L (trace metal grade; Fisher Scientific, Pittsburgh) before aspiration directly into the atomic absorption spectrophotometer as previously described in detail (11). Individual stool samples were freeze-dried to constant weight and ground to homogeneity. Weighed aliquots (0.2 g) were digested by using microwave digestion (MDS 2000; CEM Corporation, Matthews, NC) and the total zinc content was determined by AAS as previously described (11).

The ratios of zinc isotopes in plasma, urine, and fecal samples at baseline were determined by using inductively coupled plasma mass spectrometry (ICP-MS). Because the plasma zinc concentrations at depletion were low, isotope ratios were determined by magnetic sector thermal ionization mass spectrometry (model MAT 261; Finnigan, Bremen, Germany) in the laboratory of one of the authors (SAA). Detailed methods for the preparation of samples for ICP-MS were published elsewhere (11). In brief, plasma (3–4 mL) and freeze-dried fecal samples (0.3–0.5 g) were digested by using microwave digestion in 5 mL concentrated nitric acid (Fisher trace metal grade). Zinc was purified from the mineral digest by ion-exchange chromatography (type AGIX-8 ion exchange resin; Bio-Rad Laboratories, Mississauga, Canada). Urine samples were centrifuged (230 × g, 4°C, 10 min) and the inorganic salts were removed by using a chelating resin (Chelex 100 resin; Bio-Rad Laboratories); zinc was purified from the eluant by ion-exchange chromatography. Isotope ratios were expressed with respect to the nonenriched isotope, ^{66}Zn, and corrected for temperature- and mass-specific differences in fractionation by using the ratio of ^{64}Zn to ^{66}Zn. Ten scans were performed per block, and replicate blocks were repeated until the desired degree of precision (<0.2%) was obtained.

Isotope ratios of baseline plasma samples and of all urinary and fecal samples were measured by using a Sciex ELAN 500 ICP-MS instrument (Perkin-Elmer, Norwalk, CT) equipped with a U-5000AT ultrasonic nebulizer (Cetac Technologies Inc, Omaha) and a model 212B autosampler (Gilson Medical Electronics Inc, Middleton, WI).

Treatment of stable-isotope tracer data for kinetic analysis

All isotope ratios were converted to tracer-tracee ratios (mg/mg) for kinetic analysis by using ^{66}Zn as the reference isotope as previously described (11). Although the tracer-tracee ratios in plasma were used directly in the kinetic analysis, those for the urine and feces were first converted to tracer amount (mg) and expressed as cumulative tracer in urine and feces. Finally, the zinc concentrations in plasma, urine, and fecal samples measured by AAS were corrected for tracer mass as previously described (11).

Kinetic analysis

The compartmental model used to analyze the zinc tracer and steady state mass data is shown in **Figure 1**. This model is a simplification of that used previously to analyze a double-isotope tracer study in humans (11), which itself was a simplification of a more elaborate compartmental model of zinc metabolism
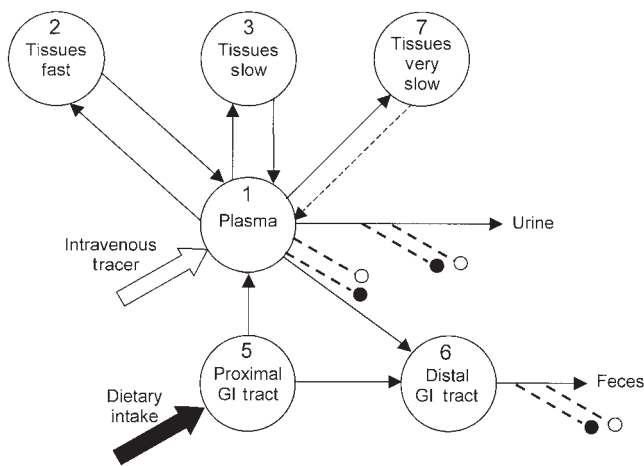
FIGURE 1. Tracer and tracee compartmental model of zinc metabolism includes the gastrointestinal (GI) tract, plasma, plasma equilibrating pools, and irreversible losses from plasma into urine and feces. The intravenous stable-isotope tracer was administered into the plasma compartment by bolus injection (open arrow); open bullets represent the tracer-tracee ratios in plasma, urine, and feces. The solid arrow represents the dietary intake of zinc; solid bullets represent mass measurements in plasma, urine, and feces.

(9, 10). In the current study, only a single intravenous isotope tracer was administered. The circles represent kinetically distinct pools of zinc (mg); the arrows represent the rate constants (per day) of the model, where $k_{i,j}$ is defined as the fraction of tracer or tracee in pool j being transported to pool i per day; the numeral 0 refers to irreversible losses in urine or feces.

Compartment 1 is assumed to be plasma zinc and it exchanges with kinetically distinct zinc tissue pools 2 and 3, referred to here as "tissues fast" and "tissues slow," respectively. A third compartment, equilibrating zinc pool 7, referred to as "tissues very slow" has a fractional turnover rate (dashed arrow from compartment 7 to compartment 1) too slow to be resolved by a tracer experiment over only 6 d. Nevertheless, this rate constant is needed in the model to explain an apparent irreversible loss of tracer from the plasma that is not accounted for by measured tracer losses in the urine and feces and estimated tracer lost from the integument. Because the mass transport of zinc into compartment 7 from plasma, flux$_{7,1}$, is determinable from the zinc tracer and plasma mass data and because input into an equilibrating pool must equal output at any given steady state, a value for $k_{1,7}$ is calculable given an estimate of the zinc mass of compartment 7, M_7. An estimate of M_7 is possible if an assumption is made about the magnitude of total body zinc, defined as the sum of the zinc masses of compartments 1, 2, 3, and 7. This value, assumed to be 30 mg/kg for fat-free body mass, estimated to be 72% of body weight (13), was used in the baseline model to calculate M_7. Thus M_7 is estimated from the difference between total body zinc and $M_1 + M_2 + M_3$ [ie, the exchangeable zinc pool (EZP)], which is determined from the baseline steady state solution of the model at the least-squares fit. The value for M_7 at depletion was calculated as total body zinc at baseline minus the net loss of zinc from the body during the depletion process minus the size of the EZP at depletion. The net loss of zinc was estimated from urinary and fecal zinc losses determined by

balance measurements, plus estimated integumental losses of 0.3 mg/d (14), offset by an estimate of the decrease in zinc content in the gastrointestinal tract between the baseline and depletion studies. The latter was calculated from the steady state solutions of the compartmental model at baseline and depletion. Any error in estimating total body zinc at baseline would lead to comparable errors in estimating M_7 and, therefore, $k_{1,7}$ at baseline and depletion. Nevertheless, the relative change in $k_{1,7}$ between baseline and depletion would not be affected.

The fractional turnover rate of compartment 5, referred to as the proximal gastrointestinal tract and equal to the sum of $k_{1,5}$ and $k_{6,5}$ was not determinable from our data because no oral tracer was administered. Consequently, we assumed a value for the fractional turnover rate of compartment 5 equal to the average value previously reported from our group of 6 female subjects: 6.42/d (11). Fractional zinc absorption (FZA) was then estimated as $k_{1,5}$/6.42. The absolute magnitude of the assumed fractional turnover rate of compartment 5 had no significant effect on any measures of zinc metabolism obtained from our model including FZA. Because no oral tracer was given, we were also unable to separate endogenous zinc secretion into the gastrointestinal tract from endogenous zinc excretion into the feces. Consequently, endogenous zinc secretion was set equal to endogenous zinc excretion in our model even though it is known that the former is greater than the latter. Finally, the infrequency of stool samples in either of the tracer studies at baseline or depletion for a given subject led to poorly determinable values for the mean transit time through the distal gastrointestinal tract, $1/k_{0,6}$. This problem was resolved by assuming values for $k_{0,6}$ to be determinable from the data in a given subject but invariant between the baseline and depletion studies. Any errors in determining $k_{0,6}$ would not have any significant effect on any of the measures of zinc metabolism extracted from the compartmental model.

The baseline and depletion tracer studies were analyzed concurrently subject to 2 constraints that linked the fitting process between the 2 data sets. First, the plasma volume was assumed to be invariant between the 2 tracer studies (body weight remained constant), the implication being that the plasma zinc mass is proportional to the average plasma zinc concentration calculated from all of the plasma zinc concentration measurements obtained during each tracer experiment. The second constraint, as described above, assumes that the fractional turnover rate of the distal gastrointestinal tract ($k_{0,6}$) is estimable from both sets of tracer data but invariant between baseline and depletion.

The plasma zinc masses at baseline and depletion were calculated directly in the fitting process and used as the input information for calculating the steady state solutions. Mass flux information, including dietary intakes and measurements of urinary and fecal zinc excretion, were used in the data array as added constraints on the fit of the model to the tracer data.

Two sets of the model in Figure 1 were fitted concurrently to the baseline and depletion tracer and mass data for each participant subject to the above-mentioned constraints. Data were fit by using SAAM II (version 1.2; SAAM Institute, Seattle), a program that uses a weighted, nonlinear, least-squares parameter estimation algorithm. Measurement errors were assumed to be independent and Gaussian with a mean of 0 and a fractional SD of 0.1. Weights were chosen optimally, ie, equal to the inverse of the variance of the measurement error. The precision of the parameter estimates was determined from the covariance matrix at the least-squares fit.

TABLE 3
Steady state zinc mass and balance data

Measure and state	Subject number					$\bar{x} \pm$ SD	P^1
	2	3	4	10	11		
Plasma zinc (mg/L)[2]							
Baseline	0.79	0.72	0.58	0.72	0.73	0.71 ± 0.08	0.0007
Depletion	0.22	0.17	0.24	0.37	0.26	0.25 ± 0.07	
Urinary excretion (mg/d)[3]							
Baseline	0.303	0.376	0.427	0.348	0.837	0.458 ± 0.216	0.0105
Depletion	0.018	0.018	0.011	0.011	0.009	0.013 ± 0.004	
Fecal excretion (mg/d)[4]							
Baseline	8.86	8.93	11.67	10.07	9.2	9.75 ± 1.18	0.0001
Depletion	0.412	0.369	0.334	0.375	0.435	0.39 ± 0.04	
Loss by balance (mg)[5]	63.0	82.7	42.5	37.1	48.2	54.7 ± 18.4	
Loss from GI tract (mg)[6]	37.4	17.8	12.6	11.6	15.2	18.9 ± 10.6	
Net loss (mg)[7]	25.6	64.9	29.9	25.5	33.0	35.8 ± 16.6	
TBZ estimates (mg)[8]							
Baseline	1469	1447	1598	1469	2009	1598 ± 237	0.0085
Depletion	1443	1382	1569	1443	1976	1563 ± 241	

[1] Probability of null hypothesis.

[2] Mean of 16 measurements over 6 d of study, corrected for tracer mass.

[3] Mean of six 24-h urine collections for each study, corrected for tracer mass.

[4] Total fecal zinc divided by number of days in collection period, corrected for tracer mass.

[5] Difference between zinc input and output over length of study; output includes 0.3 mg/d estimated integumental loss.

[6] Difference in gastrointestinal (GI) tract zinc content between baseline and depletion, calculated from the compartmental model.

[7] Net loss from total body zinc, ie, loss by balance minus loss from GI tract.

[8] Total body zinc estimate based on body weight at baseline and corrected for net loss at depletion.

To gain some insight into the possible mechanism underlying the changes in plasma zinc mass during zinc depletion, an average zinc model was formulated by using the mean values for the rate constants for the 5 subjects at baseline. The steady state solution for this average baseline model was calculated and the various compartmental mass values were assigned to their respective compartments as initial conditions, thereby formulating a mass model of zinc metabolism at baseline. Simulations over 35 d on this mass model were then performed under conditions of low zinc intake and with various changes in rate constants to their depletion values to determine the relative importance of these changes, taken individually and together, in explaining the fall in plasma zinc mass.

Statistics

Differences between the mean values (n = 5) of the rate constants and steady state measures for the baseline and depletion states were evaluated by using paired t tests. Significance was defined as $P \leq 0.05$.

RESULTS

Steady state zinc mass and balance data are shown in **Table 3**. The average plasma zinc concentration fell significantly from a baseline value of 0.71 ± 0.08 to 0.25 ± 0.07 mg/L at the time of the depletion tracer study. During this time, urinary and fecal rates of zinc excretion also fell significantly from 0.458 ± 0.216 and 9.75 ± 1.18 mg/d to 0.013 ± 0.004 and 0.39 ± 0.04 mg/d, respectively. Zinc losses by balance in urine, feces, and integument during depletion averaged 54.7 ± 18.4 mg. When corrected for the decrease in gastrointestinal zinc mass during depletion, averaging 18.9 ± 10.6 mg, the net loss from total body zinc averaged 35.8 ± 16.6 mg.

The model shown in Figure 1 fit all of the data well from each subject. A typical fit to the baseline and depletion plasma zinc tracer data, expressed as a fraction of the tracer dose, is shown in **Figure 2**. A typical fit to the entire data set (6 d) is shown in the top panel; the fit to data over the first 6 h if shown in the bottom panel. The fits from the same subject represented in Figure 2 to the cumulative fecal (top) and urinary (bottom) tracer data, as a fraction of the administered dose, for baseline and depletion studies are shown in **Figure 3**.

The estimated rate constants for each subject at baseline and depletion are shown in **Table 4**. The precision of the rate constants for each subject, expressed as CVs (%), were acceptably good for all parameters. Significant changes in the rate constants between baseline and depletion were found in $k_{0,1}$, the fractional rate of plasma zinc excretion to the urine, falling significantly from 0.143 to 0.005/d; in $k_{6,1}$, the fractional rate of plasma excretion into compartment 6 or the feces, falling significantly from 0.83 to 0.22 per day; and in $k_{1,7}$, the fractional rate of zinc release into plasma from the very slowly turning over zinc pool, falling significantly from 0.015 to 0.006 per day. The only other significant change in rate constants was the FZA, estimated as the ratio of $k_{1,5}$ to the sum of $k_{1,5}$ and $k_{6,5}$, which increased significantly from a baseline mean of 0.26 to a value of essentially unity at depletion. This limiting value of unity for FZA at depletion was the result of our inability to detect any dilution of the plasma tracer-tracee ratio in the feces.

The zinc masses and fluxes obtained from the steady state solution of the model at baseline and depletion states and their estimated uncertainties are shown in **Table 5**. The number of significant changes in these measures between baseline and depletion was greater than that found for the rate constants because of the significant fall in plasma zinc mass (M_1) in all 5 subjects. The average plasma zinc mass fell significantly by
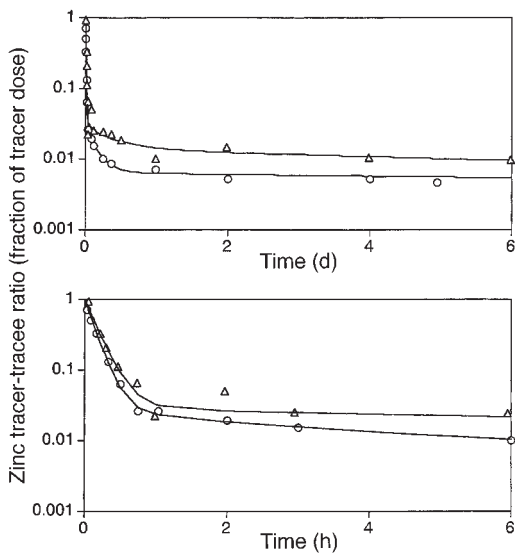
FIGURE 2. Typical fit of the compartmental model to plasma zinc tracer-tracee ratio data at baseline ($\triangle$) and depletion ($\bigcirc$) over the entire 6-d study period (top) and over the first 6 h of the study (bottom).

a 35-d period corresponding to the interval during which zinc intake was reduced from a baseline value of >12 mg/d to a depletion value of 0.22 mg/d. The decrease in zinc intake by itself, ie, no changes in baseline rate constants, resulted in a modest 16% decrease in plasma zinc mass by day 35, from 3.36 to 2.81 mg, whereas the measured plasma zinc mass actually declined by 65% to 1.17 mg. When the increases in FZA and decreases in fractional zinc losses from plasma to urine and feces (corresponding to the depletion values) were added to the simulation model on day 1 of depletion and were maintained throughout the depletion period, the decrease in plasma zinc mass from baseline to day 35 was even more modest (only 4%), from 3.36 to 3.22 mg. When the values for the rate constants of the equilibrating zinc pools 2 and 3 were changed to their depletion values immediately at the beginning of the simulation process and when the changes cited above were taken into account, the plasma zinc mass fell to 2.98 mg at day 35, an 11% decrease. Finally, when $k_{1,7}$ at baseline (0.015/d) was changed to its depletion value (0.006/d) at the beginning of the simulation and was considered together with all of the other changes cited above, the plasma mass decreased significantly by day 35 to 1.22 mg, a decrease of 64% from baseline. This reduction in simulated plasma zinc mass was similar to that actually seen (M_1 fell from 3.36 to 1.17 mg; Table 5).

DISCUSSION

During this severe, 5-wk zinc-depletion study, the average plasma zinc concentration fell by 65% and average total urinary and fecal zinc losses fell by 96%. Simulations with use of a mass model of zinc metabolism formulated from our kinetic analysis of tracer and mass data at baseline and at depletion suggest that the fall in plasma zinc concentrations during acute, severe zinc depletion was caused mainly by a 60% decrease in $k_{1,7}$, the fractional rate of zinc release from the very slowly turning over zinc pool back to plasma. The reduction in fecal zinc losses, although mainly due to the large decrease in dietary zinc intake, also

65% from 3.36 mg at baseline to 1.17 mg at depletion. This decrease in M_1, in association with no significant changes in the rate constants defining plasma equilibration kinetics [($k_{2,1}$, $k_{1,2}$, $k_{3,1}$, $k_{1,3}$, and $k_{7,1}$], resulted in significant decreases in M_3 (the relatively slowly turning over but still resolvable equilibrating zinc pool) from 143 to 101 mg and in the total EZP ($M_1 + M_2 + M_3$) from 166 to 106 mg. Even though the mean zinc mass of the relatively rapidly turning over equilibrating zinc pool, M_2, decreased from 19.60 mg at baseline to 3.62 mg at depletion, this change was not significant—a finding likely related to the small sample size.

The decrease in M_1 was also mirrored by significant decreases in fluxes to and from compartments 3 and 7; $flux_{3,1}$ and $flux_{1,3}$ fell from 242 to 116 mg/d, $flux_{7,1}$ and $flux_{1,7}$ fell from 21.1 to 8.8 mg/d, and the total plasma flux fell from 475 to 231 mg/d. Considerably larger percentage decreases were seen in those fluxes that reflected the decline in M_1 and significant decreases in rate constants between baseline and depletion. Such changes were seen in the rate of endogenous zinc excretion ($flux_{6,1}$), which fell significantly (91%) from 2.72 mg/d at baseline to 0.25 mg/d at depletion; the rate of urinary excretion ($flux_{0,1}$) decreased significantly (99%) from 0.47 to 0.005 mg/d.

The total zinc lost from the body, estimated by balance measures, during the entire 5-wk depletion (Table 3) averaged 54.7 ± 18.4 mg, of which 18.9 ± 10.6 mg could be accounted for by the movement of unabsorbed dietary zinc into the feces during the early phase of the depletion process. The difference of ≈ 36 mg represented the net zinc loss from the body during depletion, an amount enough lower than the 60-mg decrease in mass of the total EZP (Table 5) to at least suggest the possibility of zinc sequestration in the very slowest turning over zinc pool during the depletion period. At the very least, our analysis indicates the absence of any significant decrease in size of this very slowly turning over zinc pool during acute depletion.

To identify those model parameters that account for the bulk of the decline in plasma zinc mass during depletion, we used the average mass model (*see* Methods) to perform simulations over
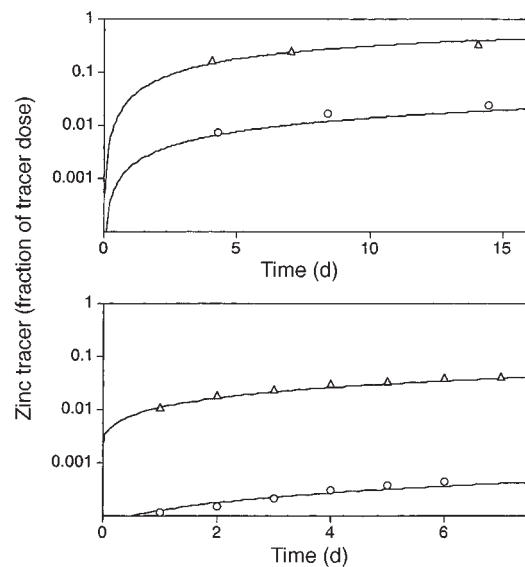

FIGURE 3. Typical fit of the compartmental model to cumulative zinc tracer mass in feces (top) and urine (bottom) for baseline ($\triangle$) and depletion ($\bigcirc$) studies.

TABLE 4
Effect of zinc depletion on rate constants of model for zinc kinetics[1]

Rate constants and state	Subject number					$\bar{x} \pm SD$	P^2
	2	3	4	10	11		
$k_{2,1}$							
Baseline	64.5 [16]	77.9 [18]	28.0 [30]	84.3 [13]	51.0 [17]	61.1 ± 22.5	0.24
Depletion	88.3 [15]	58.6 [12]	38.6 [15]	107.1 [13]	157.7 [16]	90.1 ± 46.1	
$k_{1,2}$							
Baseline	11.9 [22]	5.6 [20]	21.1 [46]	17.1 [20]	19.7 [26]	15.1 ± 6.4	0.07
Depletion	42.6 [14]	10.5 [15]	18.6 [23]	40.4 [14]	38.8 [13]	30.2 ± 14.6	
$k_{3,1}$							
Baseline	46.1 [23]	42.9 [32]	95.1 [11]	89.9 [13]	104.4 [11]	75.1 ± 28.6	0.14
Depletion	89.8 [8]	95.1 [7]	73.6 [8]	98.2 [8]	145.3 [10]	100.4 ± 26.8	
$k_{1,3}$							
Baseline	1.86 [21]	0.93 [36]	2.72 [11]	1.61 [15]	1.73 [12]	1.77 ± 0.64	0.09
Depletion	1.28 [8]	0.72 [16]	1.10 [13]	1.52 [9]	1.23 [10]	1.17 ± 0.30	
$k_{7,1}$							
Baseline	6.39 [9]	3.66 [39]	6.89 [10]	7.95 [12]	7.10 [16]	6.40 ± 1.63	0.56
Depletion	14.30 [9]	4.40 [72]	5.00 [27]	5.70 [23]	8.40 [40]	7.55 ± 4.07	
$k_{1,7}$[3]							
Baseline	0.020	0.011	0.015	0.016	0.012	0.015 ± 0.004	0.007
Depletion[4]	0.012	0.003	0.004	0.006	0.005	0.006 ± 0.004	
$k_{0,1}$							
Baseline	0.086 [7]	0.118 [7]	0.145 [7]	0.116 [8]	0.248 [9]	0.143 ± 0.062	0.009
Depletion[4]	0.005 [9]	0.010 [7]	0.003 [8]	0.004 [8]	0.003 [10]	0.005 ± 0.003	
$k_{6,1}$							
Baseline	0.67 [10]	0.83 [9]	0.75 [9]	1.05 [9]	0.84 [11]	0.83 ± 0.14	0.0007
Depletion[4]	0.20 [8]	0.28 [7]	0.18 [8]	0.20 [8]	0.26 [10]	0.22 ± 0.05	
$k_{0,6}$[5]							
Baseline	0.35 [10]	1.09 [NR]	1.38 [19]	1.59 [17]	1.03 [19]	1.09 ± 0.54	—
Depletion	0.35 [10]	1.09 [NR]	1.38 [19]	1.59 [17]	1.03 [19]	1.09 ± 0.54	
FZA[6]							
Baseline	0.26 [8]	0.28 [8]	0.24 [7]	0.25 [7]	0.28 [8]	0.26 ± 0.02	0.0001
Depletion[4]	1.00 [ND]	1.00 [ND]	1.00 [ND]	1.00 [ND]	1.00 [ND]	1.00	
fTR-PI[7]							
Baseline	118 [7]	125 [5]	131 [7]	180 [7]	164 [8]	144 ± 27	0.12
Depletion	193 [10]	158 [5]	117 [8]	211 [9]	312 [12]	198 ± 73	

[1] SDs expressed as CVs [(SD ÷ value) × 100] in brackets. ND, not determinable (value hit uppper limit); NR, not resovable from data because of too few stool samples; fixed at the average value for other 4 studies.

[2] Probability of null hypothesis.

[3] Not resolvable from data based on an experiment lasting only 6 d; requires assumption of total body zinc (*see* text).

[4] Significantly different from baseline, $P < 0.05$.

[5] Fractional turnover rate of distal gastrointestinal tract assumed invariant between baseline and depletion.

[6] Measure of rate constants $k_{1,5}$ and $k_{6,5}$, where fractional zinc absorption (FZA) = $k_{1,5}/(k_{1,5} + k_{6,5})$; the denominator is assumed to be 6.42/d (*see* text).

[7] Fractional turnover rate of the plasma compartment: sum of all rate constants leaving plasma compartment ($k_{2,1} + k_{3,1} + k_{7,1} + k_{0,1} + k_{6,1}$).

reflected an increase in FZA and a reduction in endogenous fecal zinc excretion. FZA increased from 0.26 to essentially unity. The model suggested that at depletion, all of the dietary zinc entering the small intestine was transferred to the plasma; none entered the lower bowel. In addition to an increase in FZA, the average fractional plasma zinc loss into the feces, estimated from $k_{6,1}$, fell by 74%. This reduction in fractional plasma zinc losses into the feces, along with a fall in the plasma zinc mass, was associated with a 91% decline in endogenous fecal zinc excretion at depletion. Urinary zinc losses fell to nearly zero during depletion. The fractional plasma zinc losses in the urine ($k_{0,1}$) declined by 97%, whereas the rate of urinary zinc excretion dropped by 99%. The average decrease in the total EZP (compartments 1, 2, and 3) between baseline and depletion of 60 mg was larger than could be accounted for by zinc losses measured by balance and corrected for the decrease in gastrointestinal zinc content. This finding suggests that the mass of the very slowly turning over zinc pool was unchanged or perhaps slightly increased in size at the end of the depletion process.

Zinc tracer kinetics have not been studied in humans during severe depletion. However, the effect of dietary zinc loading on zinc tracer kinetics was previously investigated (9, 15). In contrast with our findings, supplementation with 100 mg Zn for 9–10 mo caused marked reductions in FZA and increases in gastrointestinal and urinary zinc excretion and rate of release of zinc from slowly turning over zinc stores. The signals for adjusting zinc utilization at these sites are unknown. The sites in gut and kidney are probably under separate feedback control (1). Studies in experimental animals and humans showed that changes in endogenous fecal zinc excretion occur quickly with a low zinc intake but have a relatively limited capacity to change (5, 16). Adjustments in FZA take longer to occur but can cope with larger fluctuations in zinc intake.

TABLE 5
Effect of zinc depletion on fluxes and compartmental masses of the zinc kinetic model[1]

Measures and state	Subject number					$\bar{x} \pm SD$	P^2
	2	3	4	10	11		
Flux$_{2,1}$ (mg/d)							
Baseline	271 [17]	277 [19]	90 [31]	225 [13]	161 [17]	205 ± 79	0.09
Depletion	103 [13]	49 [12]	51 [14]	146 [11]	181 [12]	106 ± 58	
Flux$_{3,1}$ (mg/d)							
Baseline	193 [22]	153 [31]	305 [10]	232 [11]	329 [8]	242 ± 74	0.008
Depletion[3]	104 [5]	80 [6]	97 [6]	134 [5]	166 [5]	116 ± 34	
Flux$_{7,1}$ (mg/d)							
Baseline	26.8 [6]	13.0 [38]	22.1 [7]	21.2 [10]	22.4 [14]	21.1 ± 5.0	0.0004
Depletion[3]	16.6 [7]	3.7 [75]	6.6 [26]	7.8 [22]	9.6 [39]	8.8 ± 4.8	
Plasma flux (mg/d)[4]							
Baseline	494 [5]	467 [5]	419 [6]	480 [6]	516 [5]	475 ± 36	0.0014
Depletion[3]	224 [7]	133 [4]	155 [5]	288 [6]	357 [7]	231 ± 93	
Plasma M (M_1) (mg)							
Baseline	4.20 [7]	3.56 [6]	3.21 [7]	2.67 [7]	3.16 [9]	3.36 ± 0.57	0.002
Depletion[3]	1.16 [7]	0.84 [6]	1.32 [7]	1.36 [7]	1.15 [9]	1.17 ± 0.21	
Tissues fast M (M_2) (mg)							
Baseline	22.80 [33]	49.70 [36]	4.30 [67]	13.10 [27]	8.20 [37]	19.60 ± 18.20	0.12
Depletion	2.41 [13]	4.69 [23]	2.75 [26]	3.61 [14]	4.66 [14]	3.62 ± 1.06	
Tissues slow M (M_3) (mg)							
Baseline	104 [8]	164 [14]	112 [5]	143 [8]	191 [8]	143 ± 36	0.006
Depletion[3]	82 [7]	112 [15]	88 [10]	88 [7]	135 [10]	101 ± 22	
Total EZP ($M_1 + M_2 + M_3$) (mg)							
Baseline	131 [16]	217 [19]	120 [13]	159 [10]	202 [13]	166 ± 43	0.008
Depletion[3]	86 [9]	118 [13]	92 [11]	93 [9]	141 [11]	106 ± 23	
Tissues very slow M (M_7) (mg)							
Baseline[5]	1338	1230	1478	1310	1807	1433 ± 228	
Depletion[6]	1358	1265	1476	1351	1835	1457 ± 224	
Absorption (flux$_{1,5}$) (mg/d)							
Baseline	3.17 [6]	3.37 [6]	2.88 [5]	3.10 [5]	3.43 [6]	3.19 ± 0.22	0.0001
Depletion[3]	0.23 [5]	0.25 [4]	0.24 [4]	0.27 [4]	0.30 [4]	0.26 ± 0.03	
Endogenous zinc excretion (flux$_{6,1}$) (mg/d)							
Baseline	2.81 [7]	2.95 [7]	2.41 [6]	2.79 [5]	2.65 [7]	2.72 ± 0.20	0.0001
Depletion[3]	0.23 [5]	0.24 [4]	0.23 [4]	0.27 [4]	0.30 [5]	0.25 ± 0.03	
Fecal excretion (flux$_{0,6}$) (mg/d)							
Baseline	11.80 [5]	11.80 [5]	11.70 [5]	11.90 [5]	11.50 [5]	11.80 ± 0.20	0.0001
Depletion[3]	0.23 [4]	0.24 [5]	0.23 [4]	0.27 [4]	0.30 [5]	0.25 ± 0.03	
Urinary excretion (flux$_{0,1}$) (mg/d)							
Baseline	0.36 [3]	0.42 [3]	0.46 [3]	0.31 [4]	0.78 [4]	0.47 ± 0.19	0.005
Depletion[3]	0.006 [6]	0.008 [5]	0.005 [5]	0.005 [4]	0.004 [6]	0.005 ± 0.002	

[1] SDs expressed as CVs [(SD ÷ value) × 100] in brackets. EZP, exchangeable zinc pool; TBZ, total body zinc.
[2] Probability of null hypothesis.
[3] Significantly different from baseline, $P < 0.05$.
[4] Product of M_1 and the fractional turnover rate of the plasma compartment [sum of all rate constants leaving plasma compartment ($k_{2,1} + k_{3,1} + k_{7,1} + k_{0,1} + k_{6,1}$)].
[5] TBZ (baseline) − EZP (baseline).
[6] TBZ (baseline) − net zinc loss during depletion − EZP (depletion).

Adjustments in urinary zinc excretion occur only when zinc intakes are very low, <3 mg/d (3), or very high (15). A shift in the ratio of glucagon to insulin may be one of several mechanisms causing a change in renal tubular zinc transport (16, 17).

To gain some insight into the mechanism by which the plasma zinc mass fell by 65% in 35 d, we formulated a dynamic model of zinc mass movement based on the average values of the rate constants from the tracer model at baseline along with the associated steady state solution. Once formulated, changes in rate constants from baseline to depletion could be tested individually and collectively to determine their effects on plasma zinc mass. Selected rate constants at baseline were changed to their depletion values at the beginning of a 35-d simulation by using compartmental masses at baseline as initial conditions. The model was solved for the next 35 d of severe depletion, subject to the very low zinc intake, and the changes in plasma zinc mass were generated over time. The only simulation that came close to explaining the decrease in plasma zinc mass from 3.36 to 1.17 mg was the change in $k_{1,7}$ from a baseline value of 0.015 to 0.006 per day. All other changes in rate constants, including those describing the equilibrating zinc pools 2 and 3, the increase in FZA, and the decreases in fractional plasma zinc losses into feces and urine produced only modest decreases in plasma zinc mass by day 35. Our analysis suggests that the most slowly turning over zinc pools, depicted in our model as compartment 7, are sensitive to extreme reductions in zinc intake. The changes in rate

constants between compartments 1 and 3 also have an acute, significant effect on reductions in plasma zinc mass, but that effect was dissipated over a 35-d time span because the turnover time of compartment 3 was <1 d, ie, a new equilibrium was reestablished between M_1 and M_3 in a few days. The effect of a change in the rate constants between compartments 1 and 2 on plasma zinc mass was even more evanescent because the turnover time of compartment 2 was <1 h.

The skeletal muscle, which contains ≈60% of the whole-body zinc (7), is likely to be a major component of compartment 7 (18). Studies of growing experimental animals showed that the skeletal muscle conserves zinc even when the animals are fed diets so deficient in zinc that growth ceases and protein synthesis is severely impaired (18–20). Our data and analysis suggest that zinc release in muscle also declines in men consuming diets severely restricted in zinc. The relatively rapid response of this slowly turning over pool to a deficient zinc intake also suggests that the tissue signal is not local cellular zinc deficiency or a reduction in circulating zinc concentrations. Possibly, the signaling pathways of the endocrine receptors are altered early in zinc depletion. McNall et al (21) reported that the impaired growth in zinc-deficient rats is associated with a decreased expression of hepatic insulin-like growth factor I and the growth hormone receptor genes. Further studies of the underlying mechanisms mediating tissue zinc conservation with depletion are needed.

In our acute depletion study, the 65% decrease in the plasma zinc concentration was about twice that of the percentage decrease in the total EZP (from 166 to 106 mg), whereas in more modest, or chronic, zinc-depletion states, plasma zinc concentrations did not decline significantly even though the total EZP was lower (22). Possibly, plasma zinc concentrations stabilize at normal or near-normal concentrations under conditions of chronic low zinc intakes because of changes in gastrointestinal absorption and excretion, which require months rather than weeks for complete equilibration with all extraplasma zinc pools to occur. Furthermore, there also may be a change in the rate constants of the plasma equilibrating pools that results in a decrease in the total EZP, but over a time span measured in months rather than weeks and therefore not reflected in a tracer experiment performed during the first 5 wk of acute zinc depletion. If this process is confirmed, estimates of the total EZP may turn out to be a good reflection of zinc status with a long-term low zinc intake, whereas the plasma zinc concentration may be a better marker of acute, severe depletion. Nevertheless, neither of these markers, in absolute terms, should be considered a totally reliable marker of either chronic or acute zinc deficiency without further study.

In sum, this kinetic study of zinc metabolism showed that acute, severe zinc depletion increased FZA to essentially unity and decreased the excretion of zinc into the feces and urine by 91% and 99%, respectively. No significant changes in the plasma distribution rate constants were detected, suggesting that the kinetics of the zinc pools is not an effective means of determining zinc status during acute depletion. Although the total EZP decreased significantly, the percentage reduction was only about one-half that of the plasma mass, suggesting that the plasma zinc concentration is a better indicator of zinc status than is the size of the total EZP in acute depletion. Within the context of our compartmental model of zinc metabolism, the 65% decrease in plasma mass that occurred over a 5-wk period of severe zinc restriction could only be explained by a marked reduction in the rate of zinc release into the plasma from the large very slowly turning over zinc pool.

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