

## Body Weight-Specific Zinc Compartmental Masses in Girls Significantly Exceed Those Reported in Adults: A Stable Isotope Study Using a Kinetic Model<sup>1</sup>

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**ABSTRACT** Maintaining optimal zinc status is important for normal growth and development in children, but minimal data are available regarding zinc metabolism in this age group. Our objectives were to utilize stable isotope-based compartmental modeling techniques to investigate zinc metabolism in healthy children; to expand a current stable isotope-based model to include red blood cell data; and to compare kinetic parameters in children with those previously reported in adults. Seven healthy girls, age  $9.94 \pm 0.79$  y, received 1.1 mg of a <sup>67</sup>zinc-enriched tracer orally and 0.5 mg of a <sup>70</sup>zinc-enriched tracer intravenously. Blood, urine and fecal samples were collected for 6 d. Stable isotope enrichments were measured by thermal ionization magnetic sector mass spectrometry. A six-compartment model based on a model previously reported in adults was used; the model excluded red blood cell data. Body weight-corrected masses of the body zinc compartments derived using this model were significantly greater in children than those reported in adults. Modification of the model to include a red blood cell compartment increased the total identifiable zinc mass of the nongastrointestinal compartments by ~2.5%. We conclude that compartmental modeling can be used to describe zinc kinetics in children, and that the body weight-corrected zinc pool masses are significantly greater in children than in adults. *J. Nutr.* 130: 2607–2612, 2000.

**KEY WORDS:** • children • compartmental model • stable isotope • zinc metabolism

Despite the importance of zinc in human nutrition, current measures of zinc status have severe limitations (Thompson 1991). Compartmental models have advanced the understanding of mineral metabolism by allowing mathematical estimation of the masses of, and exchanges between kinetically distinct body compartments. Radioisotope-based zinc compartmental models have been used to examine the physiologic responses to high zinc intakes in healthy adults and those with smell or taste dysfunction, as well as the effect of aging on zinc metabolism (Foster et al. 1979, Wastney et al. 1986 and 1992). Radioisotopes cannot be used safely in healthy children, and radioisotope-based models from animal or adult studies cannot be employed in children without extrapolation of the data. Recently, a stable isotope-based model of zinc kinetics was reported in adults (Lowe et al. 1997); however, in contrast to earlier radioisotope models (Foster et al. 1979, Wastney et al. 1986 and 1992), it does not include any compartments representing red blood cell zinc. This model and others (Lowe et al. 1997, Miller et al. 1998) have allowed zinc metabolism in adults to be studied using stable isotopes

rather than radioisotopes. Comparable data have not been available for children due to the technical difficulties involved in performing such studies. The aims of this study were to use stable isotope-based multicompartmental modeling techniques to describe zinc metabolism in children; to extend a current stable isotope model (Lowe et al. 1997) to include red blood cell data; and to compare kinetic parameters in children with those previously reported in adults.

### SUBJECTS AND METHODS

Seven healthy girls were recruited by public advertisement from the Houston, TX area. The protocol was approved by the Institutional Review Board of Baylor College of Medicine and informed written consent was obtained from each subject's parents or legal guardians before the study. The subjects' mean age was 9.94 y (SD 0.79). Four were Hispanic, two were Caucasian and one was African-American. Pubertal stages were assessed by physical examination (Marshall and Tanner 1986).

**Study diet.** After the subjects were recruited, their usual zinc intake was estimated using a 24-h food recall conducted by a registered dietician. Subjects received advice on how to increase their current zinc intake to ~12 mg/d, the current recommended dietary allowance for girls aged 11–14 y (NRC 1989), by increasing their intake of meat, chicken or zinc-fortified breakfast cereals. They consumed this diet at home for 21 d before being admitted to the Metabolic Research Unit of the USDA/ARS Children's Nutrition Research Center in Houston, Texas, for a 6-d zinc metabolic study. Subjects were provided with kitchen scales, and they and their parents were instructed on the proper technique of keeping a weighed

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food record for 3 d before admission. These records, and those of the foods consumed during the 6-d admission period of the metabolic studies, were used to calculate the subjects' zinc intake using the Minnesota Nutrition Data System (University of Minnesota, version 2.91, Food Database 12A, Nutrition Database 27).

**Isotope and sample preparation.**  $^{67}\text{Zinc}$  (74% enrichment by mass) and  $^{70}\text{zinc}$  (90% enrichment by mass) were produced in the former Soviet Union and obtained as the oxide from Tracer Sciences (Toronto, Canada). Aqueous solutions of the tracers, prepared by the Investigational Drug Service of Texas Children's Hospital, Houston, Texas, were tested for pyrogenicity and sterility before use.

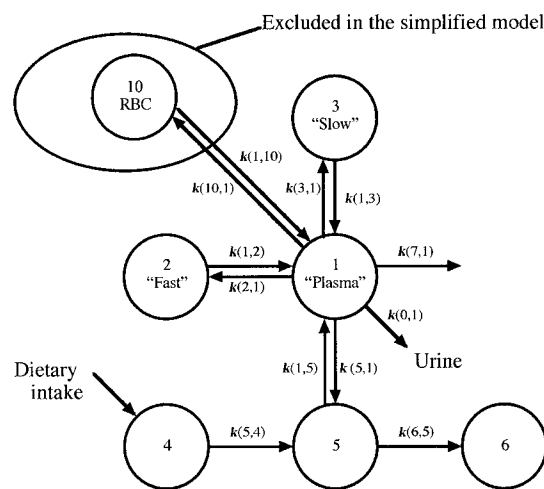
After 21 d of dietary adaptation, followed by an overnight fast, subjects were admitted to the Metabolic Research Unit. An intravenous catheter was inserted in one arm; each subject drank 1.1 mg of the  $^{67}\text{zinc}$ -enriched tracer that had been mixed with milk 18–24 h earlier; 0.5 mg of the  $^{70}\text{zinc}$ -enriched tracer was infused intravenously over 60 s by venipuncture in the other arm. Blood samples were drawn at 5, 10, 15, 20 and 30 min, and at 1, 2, 3, 4, and 8 h from the intravenous catheter; and at 24, 48, 72, 96, 120 and 144 h by venipuncture. A complete 6-d urine and stool collection was started immediately after tracer administration. Urine samples were collected, stored and analyzed in 8-h pools. Stool samples were collected, stored and analyzed individually. Blood samples were collected in zinc-free tubes (Monovette AH, Sarstedt, Newton, NC), and plasma was separated by centrifugation ( $1000 \times g$  for 10 min). An equal volume of 9 g/L sodium chloride was added to the red blood cell pellet and mixed gently. The resulting suspension was centrifuged and the supernatant discarded. This process was repeated three times to remove any residual plasma from the red blood cells. Plasma and red blood cell samples were stored at  $-80^\circ\text{C}$  pending analysis.

Zinc concentrations of plasma, red blood cells, urine and feces were measured by flame atomic absorption spectroscopy. Plasma, red blood cell, urine and fecal samples were purified using an anion exchange method (Abrams et al. 1997). Aliquots of 12 mL of urine, 0.5 mL of plasma, 0.5 mL of red blood cells and 1 g of feces were digested with 10 mL of 15 mol/L nitric acid overnight on a hot plate. The dried sample was dissolved in 1 mL of 6 mol/L hydrochloric acid and loaded onto an anion exchange resin column (AG 1-X8 resin, Bio-Rad Laboratories, Hercules, CA) that had been prewashed with 10 mL of double-distilled water and 5 mL of 6 mol/L hydrochloric acid. The column was washed with serial 5-mL aliquots of 6 mol/L, 3 mol/L, 2 mol/L, 1 mol/L and 0.5 mol/L hydrochloric acid, and the samples eluted with 6 mL of double-distilled water. Ten microliters of 0.23 mol/L phosphoric acid was added and the sample dried on a hot plate overnight before being resuspended in 0.5 mL double-distilled water. The resulting sample solution (10–20  $\mu\text{L}$ ), 2  $\mu\text{L}$  of 0.23 mol/L phosphoric acid and 6  $\mu\text{L}$  of silica suspension were loaded onto rhenium filaments. Trace element-free reagents and disposables were used throughout.

Isotope enrichments were measured by thermal ionization magnetic sector mass spectrometry (Finnigan MAT 261, Bremen, Germany). Isotope ratios were expressed with respect to the nonadministered isotope,  $^{66}\text{zinc}$ , and corrected for temperature- and mass-specific differences in fractionation, using the ratio of  $^{64}\text{zinc}$  to  $^{66}\text{zinc}$ . Ten scans were performed per block, and replicate blocks repeated until the desired degree of precision ( $<0.2\%$ ) was obtained. Isotope ratios were converted to tracer:tracee ratios as described previously (Cobelli et al. 1987, Lowe et al. 1997).

## Kinetic modeling

**Tracee model.** Figure 1 shows the models used; circles represent kinetically distinct compartments, and arrows represent the transfers between compartments. The basic model is based on a stable isotope model described previously in adults (Lowe et al. 1997), which in turn was based on radioisotope models developed by Foster et al. (1979) and Wastney et al. (1986). This model has been shown to have a priori identifiability (Lowe et al. 1997, Saccomani et al. 1994) such that, for any given data set, all of the unknown parameters have one unique solution. The basic model consists of six compartments, i.e., three gastrointestinal compartments, a central "plasma" compartment and two kinetically distinct tissue compartments. Dietary zinc enters the first of a unidirectional chain of three compartments (#4, 5 and 6). Zinc is lost from compartment #6 by excretion in the feces.



**FIGURE 1** Diagrammatic representation of the two tracee models used in the study. Circles represent kinetically distinct compartments and arrows represent the exchange between compartments. The red blood cell (RBC) compartment is included only in the complete model and excluded in the simplified model.

Zinc is absorbed from the second gastrointestinal compartment (#5) to a central "plasma" compartment (#1). Zinc from this compartment undergoes bidirectional transfers with two kinetically distinct compartments (#2 and 3). These are denoted "fast" and "slow" to reflect their different turnover rates. In addition, zinc from the central compartment is lost in urine (urinary excretion) and into the gastrointestinal tract (endogenous fecal zinc excretion). There is a further loss of zinc from the central compartment, which represents transfer of tracer to a large, slowly turning over compartment (Lowe et al. 1997). This compartment turns over too slowly to be resolved in short-term tracer studies (Lowe et al. 1997). Zinc in this compartment has been estimated to have a half-life of  $\sim 50$  d (Lowe et al. 1997), and studies of many months duration are required to identify it (Wastney et al. 1986). In this study, transfer of zinc to this compartment was modeled as an irreversible loss from the system.

On the basis of previous radioisotope studies (Foster et al. 1979, Wastney et al. 1986), the basic model was modified to include a single red blood cell compartment (#10). Previous studies have identified two red blood cell compartments (Foster et al. 1979, Wastney et al. 1986), but the second of these compartments turns over too slowly to be identified in our short-term study. Addition of a second red blood cell compartment did not improve the fit of the red blood cell data.

The total amount of tracee excreted in the urine and feces was calculated from the complete urine and fecal collections, averaged over the duration of the study, and entered as data in the tracee model.

**Tracer model.** Oral tracer was introduced into compartment #4, and intravenous tracer into compartment #1. Plasma data were expressed as tracer:tracee ratio, urine and fecal data as cumulative losses, and red blood cell zinc as total red blood cell tracer (assuming a plasma volume of 70 mL/kg and a hematocrit of 0.38) (Lenter 1984). Each study, therefore, had six sets of tracer data, i.e., the plasma tracer:tracee ratio of the orally administered tracer, the plasma tracer:tracee ratio of the intravenously administered tracer, the cumulative fecal excretion of the orally administered tracer, the cumulative urinary excretion of the orally administered tracer and the cumulative urinary excretion of the intravenously administered tracer. These data sets were used simultaneously to solve the model. The complete seven-compartment model had two additional tracer data sets, namely, the total amount of orally administered tracer in the red blood cells and the total amount of intravenously administered tracer in the red cells.

**Modeling.** Kinetic data were modeled using SAAM II, which uses a weighed, nonlinear least squares iteration algorithm (SAAM Institute, Seattle, WA). Measurement errors were assumed to be normally distributed about a mean of zero, and to have a fractional standard deviation of either 0.1 (for tracer data) or 0.05 (for tracee

TABLE 1

Kinetic parameters and age, weight and fasting plasma zinc concentrations for the 7 study subjects derived from the complete model<sup>1</sup>

Subject	1	2	3	4	5	6	7
Wt, kg	39.1	37.2	43.8	39.4	32.9	43.3	43.8
Age, y	10.88	8.82	9.73	9.73	11.08	9.46	9.90
Plasma zinc, $\mu\text{mol/L}$	15.9	17.1	14.7	13.5	15.3	14.7	14.7
	<i>mg</i>						
$M_1$	2.39 $\pm$ 0.09	2.05 $\pm$ 0.13	3.95 $\pm$ 0.23	1.74 $\pm$ 0.10	1.35 $\pm$ 0.9	2.15 $\pm$ 0.15	3.30 $\pm$ 0.22
$M_2$	20 $\pm$ 1.9	29 $\pm$ 2.5	25 $\pm$ 3.5	7.7 $\pm$ 1.3	9.0 $\pm$ 0.9	12.2 $\pm$ 2.7	7.8 $\pm$ 2.4
$M_3$	64 $\pm$ 3.2	108 $\pm$ 7.8	107 $\pm$ 2.7	62 $\pm$ 2.1	71 $\pm$ 3.1	75 $\pm$ 3.0	63 $\pm$ 2.3
$M_4$	2.30 $\pm$ 0.01	3.60 $\pm$ 0.43	2.87 $\pm$ 0.3	3.27 $\pm$ 0.27	1.49 $\pm$ 0.29	2.08 $\pm$ 0.22	1.75 $\pm$ 0.16
$M_5$	2.57 $\pm$ 0.07	1.08 $\pm$ 0.19	0.85 $\pm$ 0.15	0.54 $\pm$ 0.09	4.84 $\pm$ 0.74	0.42 $\pm$ 0.09	0.31 $\pm$ 0.06
$M_6$	30 $\pm$ 1	60 $\pm$ 4	43 $\pm$ 3	19.2 $\pm$ 2.04	59.9 $\pm$ 5.8	15.7 $\pm$ 1.55	25.8 $\pm$ 2.4
$M_{10}$	7.5 $\pm$ 0.42	8.1 $\pm$ 0.47	6.2 $\pm$ 0.26	1.7 $\pm$ 0.07	8.3 $\pm$ 0.62	6.1 $\pm$ 0.24	1.1 $\pm$ 0.08
	<i>d<sup>-1</sup></i>						
$k(0, 1)$	0.11 $\pm$ 0.01	0.23 $\pm$ 0.02	0.12 $\pm$ 0.01	0.25 $\pm$ 0.02	0.11 $\pm$ 0.01	0.16 $\pm$ 0.01	0.16 $\pm$ 0.01
$k(0, 6)$	0.36 $\pm$ 0.03	0.24 $\pm$ 0.014	0.32 $\pm$ 0.018	0.73 $\pm$ 0.06	0.18 $\pm$ 0.01	0.88 $\pm$ 0.07	0.39 $\pm$ 0.03
$k(1, 10)$	0.38 $\pm$ 0.04	0.47 $\pm$ 0.05	0.93 $\pm$ 0.07	0.36 $\pm$ 0.03	0.25 $\pm$ 0.03	0.67 $\pm$ 0.06	0.28 $\pm$ 0.04
$k(1, 2)$	20.2 $\pm$ 1.5	10.9 $\pm$ 0.7	16.5 $\pm$ 1.7	16.0 $\pm$ 1.9	16.1 $\pm$ 1.2	17.3 $\pm$ 2.3	23.2 $\pm$ 4.8
$k(1, 3)$	1.94 $\pm$ 0.20	0.93 $\pm$ 0.11	1.72 $\pm$ 0.19	2.08 $\pm$ 0.16	1.24 $\pm$ 0.09	2.84 $\pm$ 0.26	4.06 $\pm$ 0.34
$k(1, 5)$	0.43 $\pm$ 0.01	2.09 $\pm$ 0.37	3.59 $\pm$ 0.64	3.64 $\pm$ 0.60	0.53 $\pm$ 0.08	3.38 $\pm$ 0.68	6.33 $\pm$ 1.24
$k(10, 1)$	1.14 $\pm$ 0.07	1.83 $\pm$ 0.18	1.45 $\pm$ 0.12	0.24 $\pm$ 0.02	1.56 $\pm$ 0.11	1.92 $\pm$ 0.17	0.09 $\pm$ 0.01
$k(2, 1)$	170 $\pm$ 7	155 $\pm$ 6	103 $\pm$ 6	71 $\pm$ 6	110 $\pm$ 6	98 $\pm$ 10	55 $\pm$ 7.4
$k(3, 1)$	51 $\pm$ 6	48 $\pm$ 5	47 $\pm$ 6	74 $\pm$ 7	65 $\pm$ 5	99 $\pm$ 12	78 $\pm$ 10
$k(5, 1)$	0.35 $\pm$ 0.02	0.87 $\pm$ 0.07	0.65 $\pm$ 0.05	0.88 $\pm$ 0.07	1.80 $\pm$ 0.14	0.51 $\pm$ 0.04	0.43 $\pm$ 0.04
$k(5, 4)$	4.7 $\pm$ 0.14	4.2 $\pm$ 0.48	5.0 $\pm$ 0.54	4.4 $\pm$ 0.35	7.3 $\pm$ 1.4	6.8 $\pm$ 0.68	6.1 $\pm$ 0.54
$k(6, 5)$	4.1 $\pm$ 0.05	13.4 $\pm$ 2.28	16.4 $\pm$ 2.9	26.1 $\pm$ 4.2	2.1 $\pm$ 0.3	32.5 $\pm$ 6.4	33.0 $\pm$ 6.4
$k(7, 1)$	7.6 $\pm$ 0.39	6.8 $\pm$ 0.86	3.7 $\pm$ 0.34	9.4 $\pm$ 0.60	10.3 $\pm$ 0.74	8.2 $\pm$ 0.60	5.75 $\pm$ 0.40

<sup>1</sup> Data are given as the mean  $\pm$  SD of the parameter estimate, as calculated by the SAAM II software package.

data). SAAM II was used to estimate the mass of each compartment (where  $M_j$  denotes the mass of compartment  $j$ ) and the fractional transfer coefficient between compartments [where  $k(i,j)$  represents the fraction of compartment  $j$  entering compartment  $i$  per unit time]. These can be used to calculate the flux between compartments, where Flux( $i,j$ ) represents the mass of compartment  $j$  entering compartment  $i$  per unit time from the following equation:

$$\text{Flux}(i,j) = M_j \times k(i,j)$$

The fractional transfer coefficient representing loss of zinc to the large slowly exchanging compartment was denoted  $k(7,1)$  to distinguish it from the loss of zinc into the urine,  $k(0,1)$ , and to indicate that it was entering a large unresolved compartment ( $\neq 7$ ). Masses are given as mg, fractional transfer coefficients as  $d^{-1}$  and fluxes as mg/d.

The primary kinetic parameters derived from the model were  $M_1$  and the 13 fractional transfer coefficients. Other compartmental masses and intracompartamental fluxes can be derived from these. In addition, the following could be calculated (Lowe et al. 1997):

$$\text{Endogenous fecal zinc excretion} = \text{Flux}(5,1)$$

$$\text{Urinary zinc excretion} = \text{Flux}(0,1)$$

**Statistical analyses.** Statistical analyses were carried out using StatView version 4.51 (Abacus Concepts, Berkeley, CA) for Macintosh. Normally distributed variables were compared using paired or two-sample  $t$  tests, as appropriate. All values are given as means  $\pm$  SD, unless otherwise stated. Differences were considered significant at  $P < 0.05$ . The precision of kinetic parameters from the compartmental models was assessed using the standard deviation and CV estimated from the model by SAAM II from the covariance matrix at the least squares fit. Differences between compartmental masses and previously published estimates for adults (Lowe et al. 1997) were assessed using two-sample  $t$  tests. Compartmental masses from the six-compartment

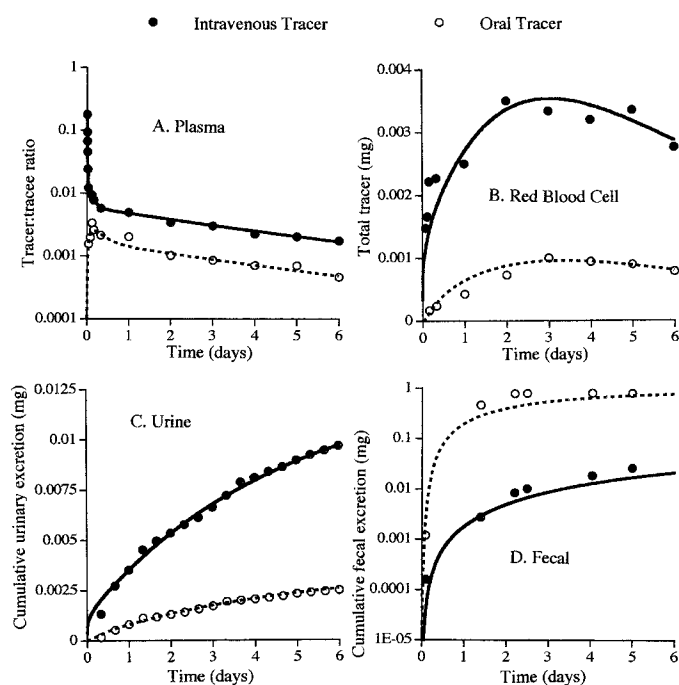
model (excluding the red blood cell compartment) and the seven-compartment model (including the red blood cell compartment) were compared using paired  $t$  test.

## RESULTS

Seven healthy girls were studied. One subject was prepubertal, whereas the others were in the early stages of puberty (Tanner stage 2). The mean dietary zinc intake during the 21-d adaptation period and the 6-d admission period was  $11.46 \pm 1.03$  mg/d. The age, weight and fasting plasma zinc concentration of each subject are given in Table 1.

**Complete model.** The kinetic parameters for each subject determined using the complete model are shown in Table 1. The primary kinetic parameters ( $M_1$  and the 13 fractional transfer coefficients) were all estimated with a CV  $< 20\%$ . The masses of the seven compartments were estimated with precisions (CV) of  $6.9 \pm 1.6\%$  ( $M_1$ ),  $16.2 \pm 8.0\%$  ( $M_2$ ),  $4.8 \pm 1.4\%$  ( $M_3$ ),  $10.9 \pm 4.6\%$  ( $M_4$ ),  $16.1 \pm 5.1\%$  ( $M_5$ ),  $9.5 \pm 1.8\%$  ( $M_6$ ) and  $5.7 \pm 1.4\%$  ( $M_{10}$ ). Figure 2 shows fits for the eight tracer data sets for a representative subject.

**Effect of omitting the red blood cell compartment.** Omission of the red blood cell compartment significantly increased the mass of the central plasma compartment ( $2.4 \pm 0.9$  vs.  $2.5 \pm 0.9$  mg,  $P = 0.05$ ) and the "fast" compartment ( $15.6 \pm 8.9$  vs.  $17.0 \pm 8.8$  mg,  $P = 0.007$ ). The mass of the "slow" compartment also tended to increase ( $78 \pm 20$  vs.  $80 \pm 22$  mg,  $P = 0.12$ ). Overall, however, the omission of red blood cell data decreased the total mass of the identifiable nongastrointestinal compartments by  $2.5 \pm 3.0\%$  ( $96 \pm 28$  vs.  $99 \pm 30$  mg,  $P = 0.02$ ). The masses of the three gastrointestinal com-



**FIGURE 2** Representative plots from one subject of plasma tracer:tracer ratio (Panel A), total red blood cell tracer (Panel B), cumulative urinary tracer excretion (Panel C) and cumulative fecal tracer excretion (Panel D) for the intravenous and oral tracers. Note that for plasma and fecal data, the ordinate axis is logarithmic.

partments did not change significantly when the red blood cell compartment was omitted.

**Comparison with adult data.** The masses of the nongastrointestinal zinc compartments (compartments #1, 2 and 3) derived from the basic model were significantly greater than those determined in adults using the same model (Table 2) (Lowe et al. 1997), when corrected for body weight. The weight-specific masses of two of the gastrointestinal compartments (#4 and 6) also exceeded those reported for adults (Lowe et al. 1997). The fractional transfer coefficients between the plasma and the tissue compartments were similar in adults and children except for  $k(2,1)$ , which was significantly higher in children (Table 2). The fractional transfer coefficient  $k(5,1)$ , representing loss of zinc into the gastrointestinal tract, was significantly lower in children than that previously reported in adults (Table 2). Significant differences in the fractional transfer coefficients between the gastrointestinal compartments were also noted;  $k(5,4)$  and  $k(0,6)$  were significantly lower in children than adults, and  $k(6,5)$  was significantly higher (Table 2).

Urinary zinc excretion was higher in children than in adults, when expressed either as mg/d or mg/(kg·d) (Table 2). Fecal zinc excretion was  $1.66 \pm 0.07$  mg/d in our subjects compared with  $2.79 \pm 1.70$  mg/d in adults ( $P = 0.055$ ). No difference was seen when fecal excretion was corrected for body mass (Table 2).

## DISCUSSION

We report here the first use of a stable isotope-based compartmental model to describe zinc metabolism in a group of healthy children. The data are shown to be well fitted using either a seven-compartment model based on plasma, urine, fecal and red cell data, or a modified six-compartment model that excluded the red blood cell data.

Limited data are available on the total body zinc content of children, but a value of 22.3 mg/kg fat-free mass, or ~17 mg/kg, has been suggested (De Kok et al. 1993). The total zinc mass resolvable by the seven-compartment model was therefore only ~15% of the estimated total body zinc mass. This was not unexpected because previous radioisotope studies found that ~90% of body zinc in adults was located in very slowly turning over body pools, largely in bone and muscle (Wastney et al. 1986). These pools are not resolvable in short-term studies (i.e., <14 d), but can be detected only in studies lasting many months (Wastney et al. 1986). We contend that this should not limit the value of such short-term models in studying the physiologic adaptations to zinc deficiency. The clinical and biochemical effects of established zinc deficiency correct very rapidly after zinc supplementation, suggesting that these features are associated with depletion of rapidly turning over tissue compartments. If the symptoms of zinc deficiency were due to depletion of slowly turning over tissue compartments, then prolonged zinc supplementation would be required to replete these compartments, and resolution of symptoms would be much slower.

In this study, the mass of the central compartment was 0.60 mg/kg. This is higher than we would have expected on the basis of measurements of plasma zinc and estimates of plasma volume. Assuming a plasma zinc concentration of ~15  $\mu\text{mol/L}$  (~1 mg/dL), a blood volume of 70 mL/kg and a hematocrit of 0.38 (Lenter 1984), the plasma zinc mass would

**TABLE 2**

*Kinetic parameters for the 7 study subjects and for adults derived from the basic model and urinary and endogenous fecal zinc excretions*

Parameter	Current study <sup>1</sup>	Adult data <sup>2</sup>	P-value
	<i>mg/kg</i>		
$M_1$	$0.06 \pm 0.02$	$0.04 \pm 0.01$	0.006
$M_2$	$0.43 \pm 0.22$	$0.14 \pm 0.05$	0.010
$M_3$	$2.0 \pm 0.6$	$1.4 \pm 0.2$	0.032
$M_1 + M_2 + M_3$	$2.5 \pm 0.8$	$1.6 \pm 0.1$	0.027
$M_4$	$0.06 \pm 0.02$	$0.01 \pm 0.01$	0.009
$M_5$	$0.04 \pm 0.04$	$0.05 \pm 0.03$	0.63
$M_6$	$0.9 \pm 0.6$	$0.17 \pm 0.06$	0.010
	<i>d<sup>-1</sup></i>		
$k(0, 1)$	$0.152 \pm 0.058$	$0.102 \pm 0.059$	0.37
$k(0, 6)$	$0.411 \pm 0.280$	$0.847 \pm 0.348$	0.045
$k(1, 2)$	$16.7 \pm 3.9$	$22.3 \pm 17.4$	0.42
$k(1, 3)$	$2.17 \pm 1.01$	$1.49 \pm 0.44$	0.16
$k(1, 5)$	$2.86 \pm 1.93$	$1.79 \pm 1.25$	0.27
$k(2, 1)$	$105.4 \pm 38.7$	$65.0 \pm 21.1$	0.044
$k(3, 1)$	$64.3 \pm 16.7$	$57.7 \pm 10.2$	0.58
$k(7, 1)$	$7.2 \pm 1.8$	$7.3 \pm 1.5$	0.91
$k(5, 1)$	$0.72 \pm 0.38$	$1.39 \pm 0.64$	0.039
$k(5, 4)$	$5.48 \pm 1.23$	$24.9 \pm 19.8$	0.025
$k(6, 5)$	$17.9 \pm 12.0$	$4.63 \pm 4.68$	0.028
Urinary zinc excretion			
mg/d	$0.38 \pm 0.14$	$0.21 \pm 0.12$	0.043
mg/(kg · d)	$0.009 \pm 0.003$	$0.004 \pm 0.002$	0.007
Endogenous fecal zinc excretion			
mg/d	$1.66 \pm 0.65$	$2.780 \pm 1.21$	0.055
mg/(kg · d)	$0.04 \pm 0.02$	$0.05 \pm 0.02$	0.393

<sup>1</sup> Mean  $\pm$  SD,  $n = 7$ .

<sup>2</sup> Mean  $\pm$  SD, from Lowe (1997).

be expected to be  $\sim 0.43$  mg/kg. The central "plasma" compartment identified in our study may therefore include other physiologic spaces in addition to the plasma. The difference may be related to zinc in rapidly equilibrating extravascular compartments, or zinc bound to cellular elements in the blood. Equilibration must occur so rapidly that zinc in these compartments is not distinguishable kinetically from that in plasma.

We identified one red blood cell compartment; the inclusion of additional red blood cell compartments did not improve the fit of the model. Previous radioisotope studies in adults have identified two red blood cell compartments (Wastney et al. 1986). The second of these red blood cell compartments, however, turns over too slowly to be resolved in this 6-d study.

Whether these red blood cell zinc pools are useful measures of zinc status is unclear. There is, however, preliminary in vitro evidence that uptake of zinc into red blood cells is affected by zinc status (De Kok et al. 1993) and that zinc uptake into the red blood cells changes during periods of zinc loading (Wastney et al. 1986). Although the inclusion of the red blood cell compartment involves additional effort and cost, it allows a more complete understanding of zinc metabolism. Furthermore, inclusion of the red blood cell pools may be particularly useful when studying subjects with concurrent infective or inflammatory processes such as Crohn's disease or infective diarrhea. In these conditions, plasma zinc may decrease due to sequestration into the liver (Brown 1998) such that the mass of the plasma compartment may fall, whereas the mass of the nongastrointestinal compartments may increase. However, zinc influx into red blood cells appears to be unaffected by inflammation (Naber et al. 1994), and the red blood cell compartment may therefore provide important information concerning zinc status that is not confounded by a concurrent inflammatory process.

A simplified version of the model that excluded the red blood cell compartment also fit the observed data well. The mass of the central plasma pool increased significantly when the red blood cell data were omitted, but the absolute change was small ( $0.003 \pm 0.003$  mg/kg). Similarly, the total mass of the identifiable nongastrointestinal zinc compartments decreased by only 2.5% after omission of the red blood cell data. The masses of the three gastrointestinal compartments were not affected significantly by omission of red blood cell data.

This six-compartment model is comparable to one previously described in adults (Lowe et al. 1997) but the compartmental masses in our subjects, corrected for body weight, significantly exceeded those reported previously (Lowe et al. 1997). The greater size of the nongastrointestinal compartments may be explained by the fact that the adolescent growth spurt begins at about the age at which our subjects were studied (Marshall and Tanner 1986). This period is associated with a significant increase in lean body mass. Approximately 60% of body zinc is in skeletal muscle (Cousins 1996); thus it is possible that increased amounts of kinetically labile zinc are present to support the rapid growth in lean tissue mass. This difference in zinc pool masses at different ages is also compatible with studies of calcium metabolism, which have shown larger compartmental masses in children than in young women (Wastney et al. 1996).

Two of the three gastrointestinal compartments, corrected for body mass, were larger in children than those previously reported in adults. This may reflect a difference in gastrointestinal transit or may be explained by the higher dietary zinc intakes in our study than that of Lowe et al. (1997).

The fractional transfer coefficients between tissue zinc compartments did not differ significantly from those described in adults (Lowe et al. 1997), except for  $k(2,1)$ , which was signifi-

cantly higher in children. The reason for this difference is unclear. The fractional transfer coefficients between the three gastrointestinal compartments were significantly different than those previously described in adults (Lowe et al. 1997);  $k(5,4)$  and  $k(0,6)$  were significantly lower in children and  $k(6,5)$  was significantly higher. These differences may relate to subtle differences in the manner in which the oral tracer was administered. In the previous adult study, the oral tracer was given 15 min after a low zinc breakfast (Lowe et al. 1997). In our study, the oral tracer was given at the same time as a breakfast that included zinc-fortified breakfast cereal. The observed differences in fractional transfer coefficients would be compatible with a difference in the time course of zinc absorption when zinc is given in these different ways, with absorption occurring more rapidly when given 15 min after a low zinc breakfast than with a meal containing higher zinc.

It is unclear whether fecal excretion should be compared in absolute terms (mg/d) or on a body weight-specific basis [mg/(kg·d)]. Because the recommended dietary allowance for zinc is expressed as mg/d and does not change in females between the ages of 11 and 51 y (NRC 1989), the former method may be preferable because it relates more intuitively to net zinc balance. Using this method, endogenous fecal zinc excretion tended to be lower in children than that reported in adults ( $P = 0.02$ ). This is consistent with our finding that the fractional transfer coefficient  $k(5,1)$ , representing loss of zinc into the gastrointestinal tract, was significantly lower in children than that reported previously in adults. Endogenous fecal zinc excretion is a major site of regulation of zinc homeostasis (Cousins 1996, Wastney et al. 1986), and the lower value in children may be a compensatory mechanism to meet the high zinc requirements that are likely during the adolescent growth spurt. No difference in endogenous fecal zinc excretion was seen, however, after correction for differences in body mass. Urinary zinc excretion was significantly higher in children than in adults, although the absolute difference was small.

We have described the use of two related compartmental models to study zinc kinetics in children and demonstrated the suitability of stable isotope-based models to study zinc metabolism in this population. The current study demonstrates significant differences in zinc metabolism between children and adults, with children having greater weight-specific zinc compartmental masses, higher urinary zinc excretion and possibly lower endogenous fecal zinc excretion. We believe that the further use of such compartmental models offers a novel approach to the study of zinc metabolism in health and disease, and will provide insights that could not otherwise be obtained.

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