A comparison of the short-term kinetics of zinc metabolism in women during fasting and following a breakfast meal

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The physiological importance and mechanism of the postprandial fall in plasma Zn concentration is not well understood. In order to gain further information on this apparent redistribution of plasma Zn, a stable isotope, ^{70}Zn, was used to study the effect of a breakfast meal on plasma Zn kinetics. Nine women participated in two trials, a fasting trial and a breakfast-meal trial; five of the women participated in a third trial in which the energy content of the breakfast meal was doubled. At each trial, 0·1 mg of ^{70}Zn was infused intravenously, and the plasma disappearance of the isotope was analysed using a two-compartment model of Zn kinetics. Plasma Zn concentration fell significantly following the two trials in which the subjects were given meals, reaching low points that were 13 and 19 %, respectively, below concentrations at comparable times during the fasting trial. Kinetic analysis revealed that after the doubled breakfast meal there was a significant fall ($P < 0·007$) in the size of the most rapidly turning over Zn pool (pool (a)) from 2·90 (SE 0·13) mg in the fasting state to 2·47 (SE 0·14) mg postprandially. The fractional turnover rate of pool (a) to other extravascular Zn pools, i.e. outside the two-compartment system, was also significantly elevated after the doubled breakfast meal ($P < 0·05$). These results suggest that the decline in plasma Zn concentration following a meal is due to a redistribution of Zn from the plasma to other more slowly turning over extravascular pools that may be involved in the assimilation and metabolism of fuels following food intake.

Zinc: Postprandial metabolism: Kinetics

Human plasma Zn concentrations vary throughout the day (Hetland & Brubakk, 1973; McMillan & Rowe, 1982; Markowitz *et al*. 1985). This variation is influenced by the food intake pattern of the individual (Davies *et al*. 1968; Sweeney *et al*. 1986; Hambidge *et al*. 1989; Wallock *et al*. 1993; King *et al*. 1994), with plasma Zn levels falling by as much as 15 % postprandially. If no further meals are consumed over the next 4–5 h, plasma Zn concentrations gradually return to baseline levels. The typical pattern of three or more meals or snacks during a day does not allow sufficient time, however, for plasma Zn concentrations to recover to baseline levels before food is consumed again. Consequently, plasma Zn concentrations may be as much as 25 % lower after the last meal of the day compared with the fasting, morning concentrations (Markowitz *et al*. 1985; Hambidge *et al*. 1989).

The physiological importance and mechanism of this postprandial plasma Zn response are not understood. Zn is an essential component of many enzymes involved in fuel metabolism (Reinstein *et al*. 1987) and protein synthesis (Clegg *et al*. 1989). The liver is the primary site of postprandial, non-lipid nutrient metabolism, and it has been proposed that plasma Zn is redistributed postprandially to the liver to supply Zn for hepatic fuel metabolism (Wallock *et al*. 1993). It has also been suggested that plasma Zn may be redistributed to the pancreas to replenish the Zn that is secreted into the duodenum during digestion (Davies *et al*. 1968). As much as 4·8 mg Zn may be secreted into the duodenum after a meal (Matsheshe *et al*. 1980). The pancreas also requires Zn for the synthesis of Zn-containing enzymes involved in digestion, such as carboxypeptidase.

Isotope tracer studies have been used to develop mathematical models of Zn kinetics (Wastney *et al*. 1986; Lowe & Jackson, 1991; Fairweather-Tait *et al*. 1993; Lowe *et al*. 1993, 1997). Those studies show that isotopic Zn exchanges with only about 6 % of the total body Zn during the first 2 d following isotope administration. The pattern of disappearance of the isotope from the plasma indicates the

Abbreviations: BRKFT, breakfast meal trial; BRKFT × 2, breakfast meal trial in which breakfast energy was doubled; FSTNG, fasting trial; ICP-MS, inductively coupled plasma mass spectrometry; Qa, size of pool (a); Qb, size of pool (b).

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number, size and turnover rates of the metabolic pools of Zn. During the first 2 h after isotope administration, the tracer distributes into two distinct pools (Lowe & Jackson, 1991; Lowe *et al*. 1993). Anatomical measurements of the isotope in experimental animals showed that the first pool is composed primarily of plasma Zn (pool (a)) and that the second pool is located predominately in the liver (pool (b)).

The purpose of the present study was to investigate further the underlying mechanism of the fall in plasma Zn concentration postprandially. The short term (2 h) Zn kinetics following two breakfast meals, one containing twice as much energy as the other, were compared with those in a fasting state. Using a simple two-compartment model, the size and turnover rate of the plasma Zn pool (pool (a)) were measured in the fasting and postprandial states. The postprandial transfer of Zn to a putative liver Zn pool (pool (b)) was also measured.

Subjects and methods

Subjects

Nine premenopausal women aged 29 (SD 8) years participated in the study. Eight of the women were Caucasian; one was Filipino. None of the women was taking oral contraceptives, other medications, or vitamin–mineral supplements at the time of recruitment and for the duration of the study. The height of the women averaged 1·650 (SD 0·073) m, their weight averaged 63·3 (SD 12·3) kg, and their BMI was 23·1 (SD 3·7) kg/m^2. The usual intakes of Zn were assessed before the study using a questionnaire designed specifically to evaluate Zn intake. The estimated Zn intake averaged 6·3 (SD 2·8) mg/d; daily intakes ranged from 1·8 to 10·9 mg. The experimental design of the study was approved by the University of California at Berkeley Committee for the Protection of Human Subjects, and all participants gave written, informed consent.

Methods

Experimental design. All nine women completed two trials, a fasting (FSTNG) trial and a breakfast-meal (BRKFT) trial; five of the nine women participated in a third trial in which the energy content of the breakfast meal was doubled (BRKFT × 2). The foods consumed and nutrient content of the meals are shown in Table 1. The composition of the diet was analysed by a computerized nutrient database (Nutritionist III, N-Squared Computing, Salem, OR, USA) that had been updated by us to include Zn values for every food in the database. The percentages of the energy supplied by fat and carbohydrate were about 6 and 83 respectively, in both breakfast trials.

The FSTNG and BRKFT trials, administered in a random manner, were scheduled at a 4-week interval to allow time for isotope washout and to standardize for phase of the menstrual cycle, assuming an average cycle length of 28 d. To control for the effects of the amount of Zn in the previous meal and the time of that meal on plasma Zn concentration (Hambidge *et al*. 1990), the women were given a standard dinner to eat between 18.30 and 19.30 hours the evening before the trial day. This meal consisted of either Chicken Fettucini (Budget Gourmet, Orange, CA, USA) or Stouffers Vegetarian Lasagna (Nestle Frozen Food Company, Solon, OH, USA), plus two slices of white bread, 227 g non-fat blueberry yogurt (The Dannon Company Incorporated, Jacksonville, FL, USA) and 227 g milk. The Zn content of the meal averaged 5 mg (Nutritionist III). The women were instructed not to eat anything further until the test was conducted the following morning; water was permitted *ad libitum*.

The subjects arrived at the metabolic unit at 07.30 hours in the fasting state. After height and weight were measured, an intravenous catheter was inserted into the antecubital vein and a 10 ml baseline blood sample was drawn into a Zn-free polypropylene syringe (Sarstedt Monovette, NH_4-heparin, Sarstedt Inc., Newton, NC, USA). Samples were stored on ice for no more than 1 h. Plasma was separated by centrifugation, transferred into polyethylene tubes, and stored at –20° until analysis.

During the BRKFT trial, a 1388 kJ breakfast meal was consumed at 08.00 hours (Table 1). The meal provided 83 % of the total energy as carbohydrate, 11 % as protein and 6 % as fat (Wallock *et al*. 1993). The five women participating in the BRKFT × 2 trial consumed a 2678 kJ breakfast meal at 08.00 hours. The energy content of the meal was increased by including three additional slices of toast and 37 g jelly

Table 1. Composition of breakfast meals used in the present study*

Food item	Amount (g)	Energy (kJ)	Carbohydrate (g)	Protein (g)	Fat (g)	Zinc (mg)
BRKFT trial						
Orange juice	210	397	22·6	1·4	0·1	0·11
White bread, toasted	50	561	24·4	4·1	2·0	0·31
Jam	22	267	15·9	tr	tr	–
Non-fat milk	104	153	5·0	3·5	0·2	0·42
Coffee, decaffeinated	104	10	0·6	tr	tr	–
Total		1388	68·5	9·0	2·3	0·84
Items added to the breakfast meal in the BRKFT × 2 trial						
White bread, toasted	75	841	36·6	6·2	3·0	0·47
Jam	37	449	26·7	tr	tr	–
Total added		1290	6·3	6·2	3·0	0·47
Total intake		2678	131·8	15·2	5·3	1·31

BRKFT, 1388 kJ breakfast; BRKFT × 2, 2678 kJ breakfast; tr, trace.
* Values from computer database, Nutritionist III, N-Squared Computing, Salem, OR, USA.

(jam). The time allowed to consume the BRKFT or BRKFT × 2 meals was 20 min.

Sample collection. Two blood samples were drawn via the catheter at 30 and 60 min after initiation of either the FSTNG trial or completion of the meal. At 90 min, 0·1 mg ^{70}Zn in 0·8 ml solution was infused into the antecubital vein of the opposite, non-catheterized arm using a 'butterfly' infusion set (Becton Dickinson and Company, Sandy, UT, USA). The butterfly tubing was flushed with 5 ml sterile saline (9 g NaCl) to ensure that all of the isotope dose had been administered. The isotope infusion was administered over a time period of 1 min and the midpoint of the isotope infusion was taken as the time of dose administration. Blood samples (10 ml) were drawn at 5, 10, 15, 30, 45, 60, 75, 90, 120 and 150 min after infusion of the isotope. Packed cell volume was measured in all samples. All samples were stored on ice for no more than 60 min before centrifugation; plasma was removed and stored at −20° until analysis.

Sample analysis. The total Zn content of the plasma was determined by atomic absorption spectroscopy (Thermo-Jarrell Ash 22, Franklin, MA, USA). Plasma samples (0·2 ml) were diluted 8-fold with 0·125 M-HCl (Trace Metal Grade, Fisher Scientific, Pittsburg, PA, USA) and aspirated directly into the atomic absorption spectrometer. A bovine liver standard (National Bureau of Standards, Gaithersburg, MD, USA) was used as an internal control. The measured Zn concentration fell within the acceptable range of 14·15 (SD 0·92) μmol/l. The CV for the measurement of Zn in bovine liver standard was 1·6 %.

The enrichment of the plasma with the isotope, ^{70}Zn, was determined using a method adapted from that of Turnlund & Keyes (1990). All plasma samples were ashed and purified by ion-exchange chromatography. Only ultrapure acids were used for the purification procedure (Seastar HCl, Seastar Chemicals Inc., Seattle, WA, USA and Optima HNO_3, Fisher Scientific, Pittsburgh, PA, USA). Plasma samples were thawed and weighed into acid-washed Pyrex petri dishes. Concentrated HNO_3 (4 ml) was added to each sample. The petri dishes were then placed on a hotplate under a fume hood and the plasma slowly evaporated to dryness. After the samples had cooled, they were placed in a low-temperature asher (Bronson/IPC, Hayward, CA, USA) overnight to oxidize and eliminate the organic material. The resulting mineral ash was dissolved in 10 ml 2·5 M-HCl.

Zn was purified from the mineral ash solution using ion-exchange chromatography. An anion exchange resin (Type AG1X-8, BioRad Laboratories, Richmond, CA, USA) was prewashed and packed into polypropylene columns to a saturated resin volume of 0·6 ml. The resin was acidified by sequentially adding two column volumes each of 0·005 M-HCl, then 0·5 M-HCl, then 2·5 M-HCl. The dissolved mineral ash was then applied to the column and washed with two column volumes of 2·5 M-HCl followed by two volumes of 0·5 M-HCl. The eluent was discarded. Zn was eluted from the column using 10 ml 0·005 M-HCl. This fraction was collected, evaporated to dryness in a teflon beaker, and then dissolved in 0·16 M-HNO_3 for a final concentration of 0·5 mg Zn/l.

Isotope ratios were determined by inductively coupled plasma mass spectrometry (ICP-MS). A Perkin-Elmer Sciex ELAN 500 mass spectrometer (Perkin-Elmer, Norwalk, CT, USA), equipped with a Cetac U-5000AT ultrasonic nebulizer (Cetac Technologies Inc., Omaha, NE, USA) and a Gilson 212B autosampler (Gilson Medical Electronics Inc., Middleton, WI, USA) was used for isotope ratio determination. The data aquisition parameters, detection limits and precision were as previously described (Roehl *et al*. 1995). In order to determine the precision of the chromatography purification and analysis by ICP-MS for determining isotope ratios of enriched biological samples, it was necessary to prepare enriched biological samples followed by ICP-MS analysis. Non-enriched plasma and urine samples were spiked with ^{67}Zn and ^{70}Zn to enrichment levels of 0·1 %–10 %. A large enough sample size was spiked in order to have six samples per enrichment level. Each sample was separately purified using ion-exchange chromatography, and then analysed by ICP-MS as an individual sample. Plasma enriched to 0·1 % with ^{67}Zn yielded an isotope ratio CV of 0·19 %; plasma samples enriched to 0·1, 1·0 and 10·0 % with ^{70}Zn yielded isotope ratio CV of 0, 0·19 and 0·26 % respectively. Urine enriched to 0·1 % with ^{67}Zn yielded an isotope ratio CV of 0·16 %; urines enriched to 0·1 and 1·0 % with ^{70}Zn yielded isotope ratio CV of 0·21 and 0·74 %. These data show very good precision in both the purification procedures used for the sample preparation and the ICP-MS analysis.

Preparation of ^{70}Zn for intravenous infusion. ^{70}ZnO (85·03 % enriched, Oakridge National Laboratories, TN, USA), was dissolved in concentrated HCl (Seastar) (2·68 μl HCl/mg ZnO). The solution was diluted with triple deionized water to a final total Zn concentration of 0·152 g Zn/l, at pH 2·0. The solution was sterilized by filtration and pyrogen tested by the pharmacy at the University of California, San Francisco. Doses containing 0·13 mg ^{70}Zn/ml were stored in sealed, sterile vials.

Kinetic analysis. A plot of the ^{70}Zn:^{67}Zn ratio in the plasma *v.* time was analysed using a computerized program for regression analysis (Blackwell Scientific Software, Oxford, Oxon, UK). Previous work by us in the rat demonstrated that there was no preferential binding of an intravenously administered radioisotope of Zn between the albumin or non-albumin fractions of the plasma (Lowe & Jackson, 1991). The dose administered represents only 4 % of the total exchangeable plasma Zn pool. The minimum number of exponential terms required to describe each decay curve was determined. The goodness of fit was assessed by a visual comparison of the experimental data and the data predicted using the exponential equation. As in our previous studies (Lowe & Jackson, 1991; Lowe *et al*. 1993), one exponential term was insufficient to describe the decay curve over a period of 2 h. In all the subjects studied, two exponential terms were minimal and sufficient to describe the observed data. Compartmental analysis of the data was undertaken using techniques described by Shipley & Clark (1972) as previously described by us (Lowe & Jackson, 1991; Lowe *et al*. 1993).

Statistical analysis. The statistical significance of the difference in the measurements between the FSTNG and BRKFT trials and between the FSTNG and BRKFT × 2 trials was determined using a two-tailed Student's *t* test (paired); a P value $< 0·05$ was considered to be significant.

Results

Plasma zinc concentration

Plasma Zn concentrations did not deviate significantly from baseline values during the FSTNG trial. Following the meal in the BRKFT trial, plasma Zn concentrations declined significantly, reaching a value that was 13·5 (SE 3·0) % below the baseline value 165 min after completion of the meal (Fig. 1(a)). The concentrations had fallen significantly by 100 min following the meal and they remained significantly lower than the FSTNG values throughout the remaining length of the 240 min trial.

Over the time period studied during the BRKFT × 2 trial, the average plasma Zn concentration reached a lowest value at 210 min after completion of the meal, that was 18·7 (SE 2·9) % below the baseline value (Fig. 1(b)). There was no significant difference between the lowest plasma Zn concentration reached during the BRKFT trial and the lowest value of the BRKFT × 2 trial.

Two individuals did not display the expected fall in plasma Zn concentration postprandially. The data from those two subjects were not included in the kinetic analyses.

There was no change in packed cell volume during any of the trials suggesting that the declines in plasma Zn concentrations following the meals were not due to shifts in plasma volume.

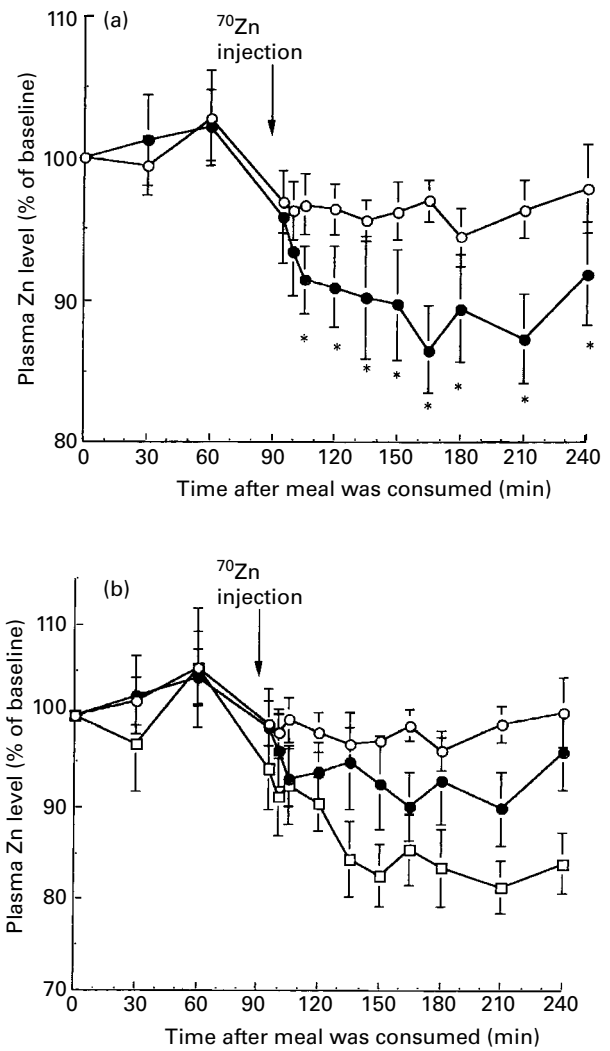

Fig. 1. (a) Plasma Zn concentrations in human subjects during fasting (○) and after a 1388 kJ breakfast meal (●), expressed as a percentage of the baseline value. Values are means for nine subjects, with their standard errors represented by vertical bars. Mean values were significantly different from those during fasting, $*P < 0{\cdot}05$. (b) Plasma Zn concentrations in human subjects during fasting (○), after a 1388 kJ breakfast meal (●) and after a 2678 kJ breakfast meal (□), expressed as a percentage of the baseline value. Values are means for five subjects, with their standard errors represented by vertical bars.

Zinc kinetics

The minimum number of exponential terms required to fit the plasma isotope decay was two. Therefore a two-compartment model was used to describe the Zn kinetics during the study period of 90–240 min following a meal (Fig. 2). The exponential equation and the dose of ^{70}Zn administered were used to calculate the size of pool (a, Qa), the size of pool (b, Qb), the fractional turnover rates of the pools (*k* values) and the transfer rate of Zn between pools (F values), as previously described (Lowe *et al*. 1993). A typical fit of the isotope ratio data to the model-predicted data from one subject in the FSTNG and BRKFT trials is shown in Fig. 3. At the end of the sampling period, the ^{70}Zn tracer

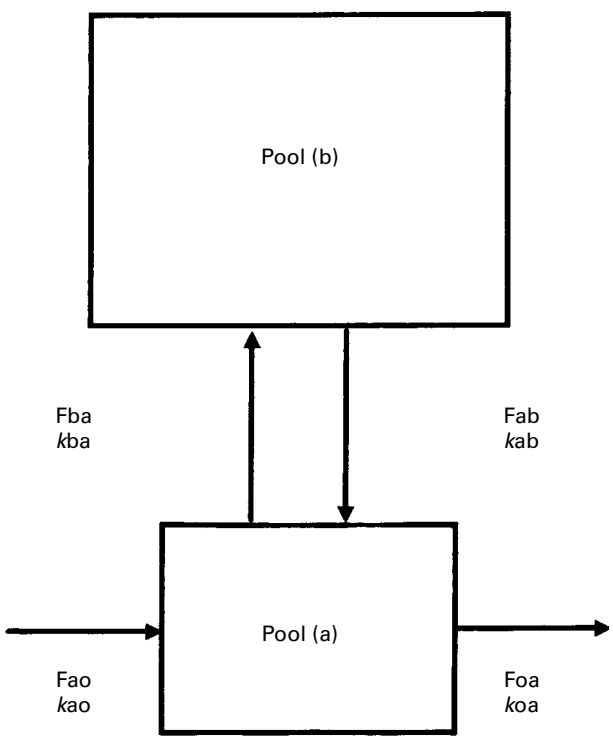

Fig. 2. Two-compartment model of Zn metabolism. Fab, Zn transfer rate to pool (a) from pool (b); Fba, Zn transfer rate to pool (b) from pool (a); Fao, Zn transfer rate to pool (a) from outside; Foa, Zn transfer rate to the outside from pool (a). Zn transfer rate is measured as mg/min. *k*ba, fractional turnover rate of pool (a) into pool (b); *k*ab, fractional turnover rate of pool (b) into pool (a); *k*oa, fractional turnover rate of pool (a) to pools outside the system.

in the plasma was still detectable above background levels (Fig. 3); the natural abundance ratio ^{70}Zn:^{67}Zn was 0·1509 (Oakridge National Laboratories, Oak Ridge, TN, USA).

The results of the kinetic analysis for the FSTNG and BRKFT trial data are shown in Table 2. The sizes of the two pools did not change significantly following ingestion of the BRKFT meal. However, there was a significant correlation ($P < 0·05$) between the change in the plasma Zn concentration postprandially and the change in the size of pool (a) compared with the FSTNG trial, r 0·34 (Fig. 4). (For each subject, the change in plasma Zn concentration between the FSTNG and BRKFT trials was the difference in the average plasma Zn concentration, calculated using all the plasma samples taken after the isotope infusion, for each trial.)

Table 2. Calculated values of zinc pool size, zinc turnover rate and zinc transfer rate in human subjects during fasting (FSTNG) and after a 1388 kJ breakfast meal (BRKFT)*

(Mean values with their standard errors for seven subjects)

	FSTNG		BRKFT	
	Mean	SE	Mean	SE
Qa (mg)	2·58	0·18	2·40	0·20
Qb (mg)	8·27	0·36	7·97	0·50
*k*oa (/min)	0·0038	0·0008	0·0042	0·0007
*k*ba (/min)	0·0719	0·0062	0·0751	0·0034
*k*ab (/min)	0·0227	0·0021	0·0231	0·0025
Fao and Foa (mg/min)	0·010	0·002	0·014	0·005
Fab and Fba (mg/min)	0·188	0·020	0·180	0·017

Fab, Zn transfer rate to pool (a) from pool (b); Fba, Zn transfer rate to pool (b) from pool (a); Fao, Zn transfer rate to pool (a) from outside the system; Foa, Zn transfer rate to the outside from pool (a); *k*ba, fractional turnover rate of pool (a) into pool (b); *k*ab, fractional turnover rate of pool (b) into pool (a); *k*oa, fractional turnover rate of pool (a) to pools outside the system; Qa, size of pool (a); Qb, size of pool (b).

*For details of subjects and procedures, see pp. 364–365.

The transfer rates of Zn between the pools (Fab and Fba) and the fractional turnover rates of Zn in the pools (*k*oa, *k*ba, and *k*ab) also did not differ significantly between the FSTNG and BRKFT trials. Pool (a) turned over about 0·080 times/min (*k*oa + *k*ba), or about 4·8 times/h following the meal, with about 93 % of it going into pool (b) (*k*ba). The

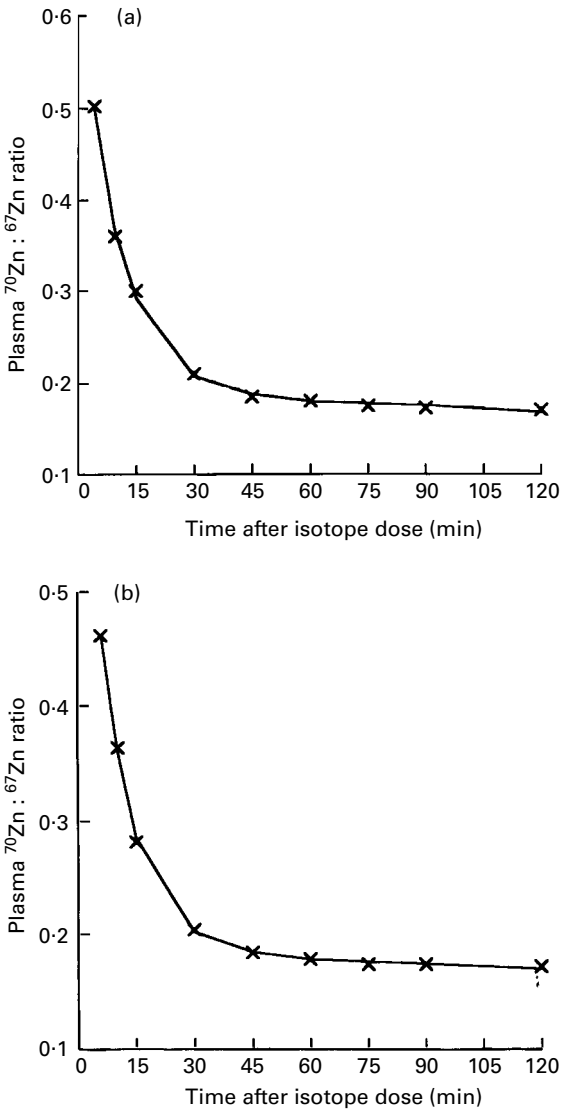

Fig. 3. Typical fit of the observed ^{70}Zn:^{67}Zn ratio in the plasma to that predicted from the model, from individual subjects. (a) Values obtained during fasting. The curve is described by the equation $Y = 0·524e^{-0·109t} + 0·192e^{-0·0012t}$. (b) Values obtained after a 1388 kJ breakfast meal. The curve is described by the equation $Y = 0·540e^{-0·112t} + 0·186e^{-0·0008t}$. (×), Observed data points; (–), model-generated data.

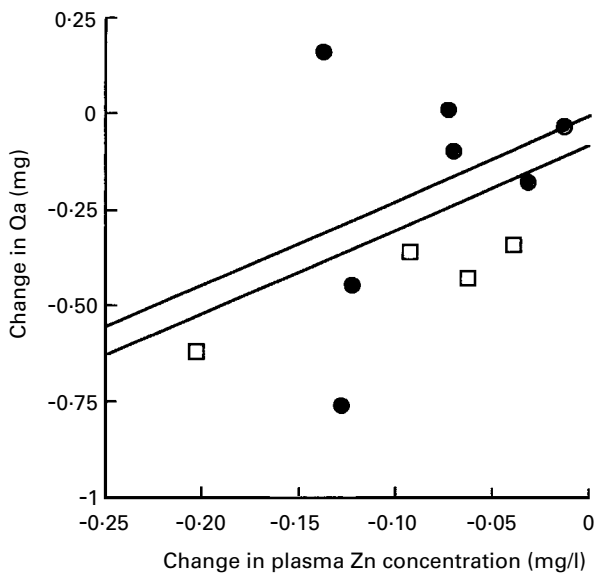

Fig. 4. Change in plasma Zn concentration *v*. change in the size of pool (a) (Qa) in subjects after a 1388 kJ breakfast meal (●) and after a 2678 kJ breakfast meal (□). The upper line represents the linear regression of the 1388 kJ breakfast values (r 0·34, $P < 0·05$); the lower line represents the linear regression of the values from both breakfast studies (r 0·45, $P < 0·04$). For each subject, the change in plasma Zn concentration between the fasting and the breakfast trials is the difference in average plasma Zn concentration, calculated using all the plasma samples taken after the isotope infusion, for each trial.

fractional turnover rate of pool (b) (*k*ab), approximately 0·023 times/min, was significantly ($P < 0·0001$) lower than that of pool (a). About 180 μg Zn/min transferred between the two pools (Fab and Fba). The transfer rate of Zn out of pool (a) to other body pools (Foa) tended to be higher following a meal than during fasting, 14 *v.* 10 μg/min, but this difference was not significant ($P = 0·50$).

The data for the four women participating in the BRKFT × 2 trial are shown in Table 3. Pool (a) was significantly ($P < 0·01$) smaller following the BRKFT × 2 trial than that of the FSTNG trial. The size of pool (b) tended to increase following the BRKFT × 2 trial compared with the FSTNG trial, but the difference did not reach statistical significance ($P = 0·10$). Doubling the size of the meal resulted in a significant increase in the fraction of pool (a) moving to other, more slowly turning over, extravascular pools (*k*oa; $P < 0·05$). No significant changes in Zn transfer rate were seen after the BRKFT × 2 trial.

There was a significant correlation ($P < 0·04$) between the change in the size of pool (a) and the change in the plasma Zn concentration postprandially compared with the FSTNG trial, when the data from the two breakfast trials were combined, *r* 0·45 (Fig. 4).

Discussion

Plasma Zn concentrations decline 10–15 % following a meal (Goode *et al*. 1991; King *et al*. 1994), but the mechanism for this decline is not known. Packed cell volume values measured postprandially did not differ from those measured in the fasting state in the present study. These data, along with previous reports of no change in serum albumin or Ca concentrations postprandially, suggest that haemodilution does not explain the fall in plasma Zn (Goode *et al*. 1991; King *et al*. 1994).

The liver plays an important role in nutrient metabolism postprandially. In a previous study, serum P concentrations were 10 % below baseline values 90 min after a meal (King *et al*. 1994). The authors concluded that plasma Zn may be sequestered by the liver postprandially to participate in phosphorylation reactions. Erythrocyte Zn exchanges rapidly with plasma Zn (Wastney *et al*. 1986), but there is no evidence that erythrocyte uptake of Zn accounts for the postprandial decline in plasma Zn concentrations (Wallock *et al*. 1993).

Radioactive tracer studies of Zn metabolism in human subjects and animals show that the pancreas actively takes up Zn (Siegel *et al*. 1961), and that pancreatic Zn has a rapid turnover rate (Sheline *et al*. 1943). Zn is required for the synthesis of pancreatic enzymes that are released into the duodenum. The most important stimulation of pancreatic enzyme secretion occurs during the intestinal phase of digestion, enzyme secretion beginning at once and continuing steadily throughout the course of gastric emptying. The stimulation of the pancreas to secrete enzymes is mediated primarily by two hormones, secretin and cholecystokinin. Secretin is released from the duodenal mucosal cells when duodenal pH declines to a threshold value of 4·5–5·0; maximal release occurs at a pH range of 0–3. Gastric contents reach a pH of 3·5 approximately 60 min after ingestion of a meal which coincides with the start of the postprandial decline in plasma Zn concentration. Over the range of pH from 0–3, the release of secretin is directly proportional to the amount of acid entering the duodenum. Therefore, the larger the meal, the greater the amount of secretin released. Cholecystokinin release from duodenal cells is stimulated by the presence of the products of protein and fat digestion arriving in the duodenum. This hormone augments the action of secretin.

Previous studies, evaluating the effect of meal composition on the rate and amount of plasma Zn decline, compared isoenergetic meals composed of fat emulsion, glucose, or a self-selected meal (Goode *et al*. 1991). They found that meal composition had no effect on the magnitude of the fall of plasma Zn concentration. However, the rate of decline was greatest following the glucose meal and least following the self-selected meal. Our present study suggests that doubling the energy load, thereby increasing the quantity of food consumed, increased the fall in plasma Zn concentration postprandially (Fig. 1(b)). Possibly the postprandial decline in plasma Zn is due, in part, to Zn uptake by the pancreas to facilitate the synthesis of digestive enzymes. The larger the meal the greater the release of secretin and the greater the amount of enzyme synthesis required. The absence of a postprandial decline in plasma Zn in an individual with pancreatitis supports this hypothesis (Davies *et al*. 1968). Intravenous infusions of glucose or nutrient media also induce a decline in plasma Zn concentrations (Davies *et al*. 1968; Sweeney *et al*. 1986; Goode *et al*. 1991). The release of insulin, as a result of intravenous glucose infusions, also stimulates gastric acid secretion (Davenport, 1980) and a concomitant release of pancreatic juices. Thus, the postprandial fall in plasma Zn may be due to an uptake of Zn by both the liver and pancreas.

Models of Zn metabolism derived from the plasma kinetics of radioactive and stable-isotope tracers provide a valuable tool for studying Zn metabolism. With the increase in the availability and improvement in the sensitivity of

Table 3. Calculated values of zinc pool size, turnover rate and zinc transfer rate in human subjects during fasting (FSTNG) and after a 2678 kJ breakfast meal (BRKFT × 2)†

(Mean values with their standard errors for four subjects)

	FSTNG		BRKFT × 2	
	Mean	SE	Mean	SE
Qa (mg)	2·90	0·13	2·47**	0·14
Qb (mg)	8·64	0·55	9·91	0·05
*k*oa (/min)	0·0038	0·0011	0·0072*	0·0004
*k*ba (/min)	0·0752	0·0090	0·0778	0·0077
*k*ab (/min)	0·0250	0·0021	0·0200	0·0018
Fao and Foa (mg/min)	0·012	0·004	0·018	0·001
Fab and Fba (mg/min)	0·215	0·025	0·198	0·021

Fab, Zn transfer rate to pool (a) from pool (b); Fba, Zn transfer rate to pool (b) from pool (a); Fao, Zn transfer rate to pool (a) from outside the system; Foa, Zn transfer rate to the outside from pool (a); *k*ba, fractional turnover rate of pool (a) into pool (b); *k*ab, fractional turnover rate of pool (b) into pool (a); *k*oa, fractional turnover rate of pool (a) to pools outside the system; Qa, size of pool (a); Qb, size of pool (b).

Mean values were significantly different from those for FSTNG: *$P < 0·05$; **$P < 0·007$.

† For details of subjects and procedures, see pp. 364–365.

ICP-MS that have occurred in recent years, the use of stable isotopes for the study of trace mineral metabolism has become more routine. Stable isotopes have obvious ethical advantages over radioiostopic tracers. However, because of the natural abundance of stable isotopes, the dose required to achieve a sufficent enrichment above baseline for the duration of the sampling period may be so large as to disturb normal mineral kinetics. In the present study, ^{70}Zn was chosen since it has the lowest abundance of all the Zn isotopes at 0·62 %. The stable-isotope dose administered represented only 4 % of the total exchangeable plasma Zn pool and was sufficient to raise the ratio at the final measurement time point above background values (Fig. 3). In a previous human stable-isotope study by Fairweather-Tait *et al*. (1993), two different doses of ^{70}Zn were administered, 2·078 mg and 0·445 mg, representing approximately 32 and 14 % respectively, of the subject's exchangeable plasma Zn pool. The authors claimed that there was no perturbation of the plasma Zn pool, even at the higher isotope dose, which was 20-fold greater that the dose used in the present study. Thus, we assumed that our intravenously administered isotope did not perturb the plasma Zn pool and behaved in a manner indistinguishable from endogenous Zn.

In the present study, a simple two-compartment model of Zn metabolism was used to investigate the effect of food intake on Zn kinetics. A comprehensive model of Zn metabolism (Wastney *et al*. 1986) indicates that only 6 % of the total body Zn, or about 95 mg, exchanges with the plasma over a period of 2 d. This pool of Zn exchanging in 2 d is composed of a number of discrete metabolic pools located within a variety of tissues, including the liver and erythrocytes. In our simple two-compartment model, the isotopic Zn exchanged with about 10 mg Zn, or approximately 0·5 % of the whole-body Zn, over a 2 h period.

The size of pool (a) in our subjects averaged 2·6 (SE 0·18) mg Zn. We believe that pool (a) is composed primarily of Zn in the plasma. If the blood volume is assumed to be 7·3 % of body weight (Foster *et al*. 1979), the total amount of Zn circulating in the plasma can be estimated from the packed cell volume and fasting plasma Zn concentration. Using this approach, the total amount of plasma Zn in our subjects was estimated to be 2·1 (SE 0·18) mg, or about 85 % of the amount of Zn in pool (a). The second pool exchanging with the tracer, referred to as pool (b), is thought to be composed primarily of a portion of the Zn in the liver (Lowe & Jackson, 1991). The fractional turnover rate of pool (a) to pools other than pool (b) (*k*oa) increased after meals in comparison with fasting; it went from 0·0038/min to 0·0042/min following the BRKFT trial and from 0·0038/min to 0·0072/min following the BRKFT × 2 trial. This suggests that the postprandial fall in plasma Zn is due in part to transport of Zn into other pools besides pool (b). Zn exchange with pool (b) represented the majority of the movement of Zn from the circulation, however. Of the total amount of Zn flowing out of pool (a), 93 % went into pool (b).

Although plasma Zn concentrations declined 13·5 % following the BRKFT meal, the 7 % fall in Qa was not significantly different from that seen in the FSTNG trial. However, there was a significant relationship between the fall in plasma Zn concentration and the fall in the size of pool (a) (Fig. 4). Doubling the breakfast meal resulted in a significant fall in Qa of 15 % ($P < 0·007$).

The compartmental modelling technique used to analyse the data from our study assumes that the system is in steady state. Since the postprandial drop in plasma Zn concentration occurred primarily within the first 90 min following the meal in our previous studies (Wallock *et al*. 1993; King *et al*. 1994), the kinetic study was not initiated until 90 min after the meal and samples were collected for the next 150 min. In the present study, however, plasma Zn concentrations continued to drop for the first 15 min in the BRKFT trial and the first 45 min of the BRKFT × 2 trial. Since the drop during the kinetic study represented less than half of the total percentage change in plasma Zn, a steady state was assumed. The fit of the data to the steady-state model was good (Fig. 3).

In sum, these kinetic data show that changes in the concentrations of Zn in the plasma postprandially are associated primarily with changes in the size of a 2·5 mg pool (pool (a)). An increased fractional turnover rate of pool (a) after a 2678 kJ meal suggests that the postprandial decline in plasma Zn concentrations is due to tissue uptake rather than an increase in plasma volume and haemodilution. Uptake of Zn by a second pool (pool (b)), putatively the liver, accounts for most of the Zn transferred from the circulation.

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