Zinc and calcium apparent absorption from an infant cereal: a stable isotope study in healthy infants

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Fractional apparent absorption of Zn and Ca from a wheat–milk-based infant cereal was studied in six healthy infants (18–30 weeks old). Mineral absorption was measured by a stable-isotope technique based on faecal excretion of the isotopes. Each test meal (40 g cereal) was extrinsically labelled with ^{70}Zn and ^{42}Ca before intake. All faecal material passed during the 21 d following intake of the labelled test meal was collected on trace-element-free nappies. Individual stool samples were analysed for their content of ^{70}Zn and ^{42}Ca by thermal ionization mass spectrometry. Apparent absorption was calculated as intake minus total faecal excretion of the isotopes over 68–92 h after administration. The fractional apparent absorption values for Zn and Ca were 33·9 (SD 16·4)% (range 19·2–63·9%) and 53·5 (SD 12·6)% (range 36·7–71·7%) respectively. Re-excretion of absorbed ^{70}Zn (> 68–92 h to 21 d after intake of the labelled meal) was 0·44 (SD 0·38)% of administered dose while only one infant re-excreted detectable amounts of ^{42}Ca (1·74% of administered dose). The analysis of individual stool samples confirmed that 72 h is a sufficient time period for complete collections of non-absorbed isotopes in faecal material from infants during the weaning period and that re-excretion of initially absorbed ^{70}Zn and ^{42}Ca (> 68–92 h to 21 d after intake of the labelled meal) is negligible.

Zinc: Calcium: Weaning cereal: Stable isotope

The estimation of mineral and trace element requirements of infants, and the ability to establish dietary recommendations for these nutrients during early life, is limited by the lack of information from studies with infants. Nutrient bioavailability, which includes absorption and retention of the nutrients, is a necessary component in these estimates since only the absorbed and retained fraction of a nutrient can be utilized by the body. The bioavailability of trace elements and minerals varies over a wide range, depending on the amount of the mineral in the diet, the presence of enhancers and inhibitors as well as on the chemical form of the mineral in the diet (Turnlund, 1991). Information on the total content of minerals in the diet is thus only of limited value when evaluating the nutritional impact of different diets.

The stable-isotope technique is an excellent tool for studies of absorption and retention of trace elements and minerals, which does not introduce any risk to the subjects (Janghorbani *et al.* 1985; Turnlund, 1991; Davidsson, 1994). The 'faecal monitoring' method is the most commonly used for studies in infants. Previous studies of Zn and Ca absorption from infant foods by infants, using the stable-isotope technique, have measured absorption by analysing the non-absorbed isotope in faeces (Serfass *et al.* 1989; Ziegler *et al.* 1989). In some studies two isotopes of different elements were added simultaneously to

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the same test meal: Zn and Ca (Ehrenkranz *et al.* 1984, 1985), Zn and Cu (Ehrenkranz *et al.* 1989; Johnson & Canfield, 1989) and Mg and Ca (Liu *et al.* 1989). All these studies used infant formula and/or human milk as the labelled test meal administered to young or premature infants, followed by analysis of the excretion of non-absorbed isotopes in faeces collected for 72 h. For Ca a double-isotope technique also exists which is based on the simultaneous administration of two isotopes, one given orally and the other administered intravenously (Moore *et al.* 1985; Hillman *et al.* 1988; Yergey *et al.* 1990; Abrams *et al.* 1991, 1992, 1994). However, the need for venepuncture has limited the use of this technique for studies in healthy, non-hospitalized infants.

Little information is available on the absorption of minerals from weaning foods such as infant cereals and other semi-solid or solid foods consumed by older infants. In the present study we report on the use of the stable-isotope technique to measure the apparent absorption of Zn and Ca from a weaning cereal consumed by infants aged 18–30 weeks. Servings of the weaning cereal were extrinsically labelled with ^{70}Zn and ^{42}Ca and the faecal excretion of the stable isotopes was quantitated by thermal ionization mass spectrometry (TIMS). Faecal material was collected over 21 d in order to determine the time necessary to recover all the unabsorbed isotopes as well as to quantitate the rate at which the absorbed ^{70}Zn and ^{42}Ca were subsequently re-excreted in faeces after ingestion of extrinsically labelled weaning cereal.

MATERIALS AND METHODS

Infants

Six healthy term infants (two girls, four boys) were recruited at 'well-baby' clinics in Aberdeen at 18–30 weeks of age. All infants were fed with commercial cows' milk formulas. At least one serving per day of the infant cereal was fed as part of the infants' normal diet for approximately 1 week before the administration of the labelled test meal to ensure acceptance of the product.

Stable isotopes

$CaCO_3$ enriched with ^{42}Ca and ZnO enriched with ^{70}Zn were purchased from Medgenix (Ratingen, Germany) and Oak Ridge National Laboratory (Oak Ridge, TN, USA) respectively. The $^{42}CaCO_3$ was dissolved in 10·2 M-HCl to obtain $^{42}CaCl_2$ while ^{70}ZnO was transformed into $^{70}ZnCl_2$ by dissolution in 0·5 M-HCl. The final concentration of HCl was 0·05 M (pH 1·3) in the $^{70}ZnCl_2$ solution. Two batches of the enriched labels were prepared with slightly different concentrations. Total Zn was measured by atomic absorption spectrometry (AAS; model 975, Varian Techtron, Mulgrave, Australia). Allowance was made for the different atomic weights in the $^{70}ZnCl_2$ solution compared with the Zn standards. Total Ca was determined by isotope dilution using TIMS (Thermoquad, Finnigan MAT Model THQ, Bremen, Germany). Total concentrations of Zn and Ca were 508 and 551 μg Zn/ml and 6·16 and 5·51 mg Ca/ml respectively in the separate solutions. The isotopic composition of the stable-isotope solutions was checked by TIMS (Finnigan MAT). The solutions were filled into acid-washed, Teflon bottles and kept refrigerated until used. The ^{42}Ca labels contained 79·35% ^{42}Ca, 19·60% ^{40}Ca, 0·164% ^{43}Ca, 0·862% ^{44}Ca and 0·0343% ^{48}Ca. The stable-isotope solution of ^{70}Zn contained 88·35% ^{70}Zn, 4·68% ^{64}Zn, 2·84% ^{66}Zn, 0·494% ^{67}Zn and 3·64% ^{68}Zn. Abundances are given as atom%.

Test meal

A weaning cereal based on wheat with low (70%) extraction rate (400 g/kg total solids) and skimmed milk powder (290 g/kg total solids), (Cerelac, Nestlé SA, Vevey, Switzerland) was used in the present study. The cereal was fortified with $CaCO_3$. One 40 g serving of the

cereal was made up by the addition of 160 g deionized distilled water (16 MΩ, Millipore (UK) Ltd., Watford, Herts.). The cereal was labelled with 370 μg ^{70}Zn and either 4·9 mg ^{42}Ca (infants 1, 3 and 4) or 4·4 mg ^{42}Ca (infants 2, 5 and 6) immediately before administration. The Zn and Ca contents in the weaning cereal were 12·7 μg Zn/g and 5·13 mg Ca/g resulting in 508 μg native Zn (927 μg total Zn) and 205 mg native plus fortification Ca (211 mg total Ca) in the labelled test meal. Each test meal also included 50 mg carmine red as a faecal marker, except for the test meal given to infant no. 3. The infants were given the labelled test meal at midday, about 4 h after the last intake of infant formula, under close supervision by one of the investigators to make sure that each child consumed the complete serving. No food or fluid was allowed for a period of approximately 2 h after intake of the test meal.

Collection of faecal material

A baseline faecal sample was collected before administration of the stable isotopes. The collection of all faecal material passed during 21 d after administration of the isotopes started immediately after intake of the test meal. Faeces were collected on disposable nappies (Lewis Wolf, Selly Oak, Birmingham, W. Midlands) and nappy liners (Boots, Nottingham, Notts.), previously deionized by soaking in 1 M-EDTA solution (Na_2EDTA, BDH Ltd, Poole, Dorset) for 24 h followed by copious rinsing with deionized distilled water (16 MΩ, Millipore (UK) Ltd). The nappies and liners were air-dried before being packed individually in zip-lock plastic bags until used. During the period of collection of faeces the infants' perineums were kept clean with deionized distilled water and no ointments were applied. Disposable plastic gloves were worn whilst the infants' nappies were changed. Soiled nappies and liners were placed in the zip-lock bags, labelled with name, time and date. Each home was visited daily by one of the investigators to supervise the parents and to collect faecal samples. The samples were kept frozen (−20°) until prepared for analysis.

Sample preparation and analysis

Solid pieces of faecal material were removed from the nappies and liners using an acid-washed plastic spatula. The remaining faeces were removed by soaking nappies and liners in 0·1 M-HCl for 24 h after which they were squeezed and rinsed with deionized distilled water to remove any staining. The washings were then added to the solid faecal material, homogenized using a titanium-bladed blender, frozen and freeze dried. All samples collected during the first 5 d, as well as on day 21, were processed separately. Remaining samples were pooled over several days: days 6–10, 11–15 and 16–20. Pooled faecal samples were prepared by homogenization with 0·1 M-HCl, using the blender equipped with a titanium blade.

Freeze-dried samples of faecal material and of the weaning cereal powder were mineralized in quartz Erlenmeyer flasks in a muffle furnace at 520° for 48 h. The residual ash was dissolved in 4 ml sub-boiled HCl and diluted to 25 ml with ultra-pure water before analysis of total Zn and Ca by AAS. For Ca analysis. La_2O_3 was added to the samples equivalent to a final concentration of 10 g La/kg. Accuracy of the analysis was tested by analysing standard reference materials (Bovine liver SRM 1577a, National Institute of Standards and Technology, Gaithersburg, MD, USA) and a pooled faecal sample as a laboratory standard. CV for Zn and Ca analysis of the pooled faecal sample were 2·6% (n 25) and 4·6% (n 27) respectively. Dry weight was determined after drying at 105° for 24 h.

For mass spectrometric analysis, Zn was separated from matrix elements in faecal samples by ion exchange, using a slightly modified procedure to that described by Gotz & Heumann (1987). Econo columns (Bio-Rad Laboratories, Glattbrugg, Switzerland) with

an inner diameter of 7 mm were filled to a height of 70 mm with anion exchange resin (Type AG1-X8, 200–400 mesh, Cl^- form, Bio-Rad). The resin was washed with 60 ml 2 M-HNO_3 using an Ismatec IPN 4 peristaltic pump (Ismatec SA, Zürich, Switzerland) at a flow rate of 1 ml/min and then regenerated to the chloride form with 100 ml 1 M-HCl. After addition of the sample in 6 ml 2 M-HCl the column was washed with 30 ml 0·5 M-HCl. The first 15 ml of solution eluted from the column after sample loading and rinsing was recovered for Ca separation. The Zn was eluted with 20 ml 1 M-HNO_3, evaporated to dryness and redissolved in 0·1 M-HCl to give a final concentration of 3 g Zn/l. Ca was isolated from the sample by precipitation with ammonium oxalate (Merck, Darmstadt, Germany). A solution containing approximately 1000 μg Ca was made alkaline with 250 g/l NH_3, heated to 70° and 5 ml of saturated ammonium oxalate solution (pH 10) was added. After cooling in an ice bath, samples were centrifuged and the precipitate was washed twice with 1 g/l ammonium oxalate solution. The Ca oxalate was then converted to $CaCO_3$ in a muffle furnace at 600° overnight. 0·3 M-HNO_3 was added to obtain a final concentration of 5 g Ca/l.

Stable-isotope ratios were measured with a computer-controlled quadrupole TIMS (Model THQ, Finnigan MAT) equipped with a Faraday cup, secondary electron multiplier, thirteen-sample turret and a reference pyrometer. Rhenium (Re) filaments of 0·04 × 0·7 mm (99·98% purity, Wagner Analysentechnik, Worpswede, Germany) were cleaned by out-gassing in a bake-out device (Finnigan MAT) under vacuum at 4·5 A for 20 min to remove impurities. For Zn analysis, samples were loaded onto Re single filaments together with a mixture of silica gel and H_3PO_4. The ^{68}Zn:^{70}Zn isotope ratio was determined at 1510° using the secondary electron multiplier operated at an amplification of 25 relative to the Faraday detector. Ca samples were loaded according to the procedure described by Moore & Machlan (1972) and measured by using Re double filaments at 1600°. Three blocks each with twelve scans across the isotope pattern were collected for each element in the peak-jumping mode. Data were analysed for outliers by a Dixon test (Dixon & Massey, 1969). Measurements which had an internal standard deviation greater than the target precision of 0·5% for ^{40}Ca:^{42}Ca and ^{68}Zn:^{70}Zn were discarded and analysis was repeated. Accuracy of the isotope ratio measurements was verified by analysis of $ZnNO_3$ (Merck) and $CaNO_3$ (Merck) as standards for natural isotopic composition. Relative accuracy of the ^{68}Zn:^{70}Zn isotope ratio measured was within 0·4% of the accepted value (International Union of Pure and Applied Chemistry (IUPAC), 1991). Relative external precision was 0·6% (n 13). The natural abundance ^{40}Ca:^{42}Ca was within 0·6% of the accepted value given by IUPAC (1991) with a relative external precision of 0·5% (n 9). The smallest enrichment that could be detected was consequently 1·8% for ^{68}Zn:^{70}Zn and 1·5% for ^{40}Ca:^{42}Ca.

All acids used during analysis of Zn and Ca as well as for preparation of samples for mass spectrometric analysis were purified by sub-boiling in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). Other chemicals were analytical grade purity. Only ultrapure water (18 MΩ, Milli-Q water system, Millipore AG, Zürich, Switzerland) was used. To minimize contamination from vessel material only acid-washed quartz, Teflon and polyethylene containers were used.

Calculations

Fractional apparent absorption values for ^{70}Zn and ^{42}Ca were calculated according to Turnlund *et al.* (1982). The calculations were based on the faecal excretion of isotopes during 68–92 h after intake of the labelled test meal, using the following equations:

$$M^{AA} = M^s + M^n, \quad (1)$$

where M^{AA} is the total mass of Zn or Ca recovered in the faecal sample measured by AAS, M^s is the mass of enriched ^{70}Zn or ^{42}Ca (spike) in the faecal sample and M^n is the mass of naturally occurring mineral, Zn or Ca;

$$M^s/M^n = F(R_j^i) = \omega^s/\omega^n \times A_j^n/A_j^s \times \frac{A_j^n/A_j^n - R_j^i}{R_j^i - A_i^s/A_j^s}, \quad (2)$$

$$M^s = \frac{M^{AA} \times F(R_j^i)}{1 + F(R_j^i)}, \quad (3)$$

where R_j^i is the measured ratio of isotope i to isotope j in the faecal sample, ω^n is the atomic weight of naturally occurring mineral, Zn or Ca, ω^s is the atomic weight of enriched ^{70}Zn or ^{42}Ca (spike), A_i^n is the atomic abundance of isotope i for naturally occurring mineral, A_i^s is the atomic abundance of isotope i for enriched ^{70}Zn or ^{42}Ca (spike), A_j^n is the atomic abundance of isotope j for naturally occurring mineral, A_j^s is the atomic abundance of isotope j for enriched ^{70}Zn or ^{42}Ca (spike), i is the reference isotope and j is the isotope enriched in ^{70}Zn or ^{42}Ca (spike).

In the present study the following equations for $F(R_j^i)$ were obtained:

$$^{42}Ca \text{ faecal excretion: } F(R_{42}^{40}) = 41{\cdot}593/40{\cdot}08 \times 0{\cdot}647/79{\cdot}35 \times \frac{96{\cdot}941/0{\cdot}647 - R_{42}^{40}}{R_{42}^{40} - 19{\cdot}60/79{\cdot}35},$$

$$^{70}Zn \text{ faecal excretion: } F(R_{70}^{68}) = 69{\cdot}447/65{\cdot}362 \times 0{\cdot}589/88{\cdot}35 \times \frac{18{\cdot}16/0{\cdot}589 - R_{70}^{68}}{R_{70}^{68} - 3{\cdot}641/88{\cdot}35}.$$

Fractional apparent absorption was calculated according to the following equation:

$$\text{fractional apparent absorption} = \frac{\text{dose} - M^s}{\text{dose}} \times 100,$$

where dose is the amount of enriched ^{70}Zn or ^{42}Ca administered and M^s is the amount of enriched ^{70}Zn or ^{42}Ca excreted in faeces.

Cumulative faecal excretion was determined from ^{70}Zn and ^{42}Ca in faecal samples collected after completed excretion of non-absorbed isotopes (after > 68–92 h after intake up until 21 d post-administration) according to the following equation:

$$\text{dose re-excreted isotope (\%)} = \frac{\Sigma M^s_{(68\text{–}92\,\text{h until } 21\,\text{d})}}{\text{dose}} \times 100.$$

Ethical considerations

Parents were fully informed about the aims and the procedure of the study and their informed consent was obtained. The protocol was approved by the Joint Ethical Committee of the University of Aberdeen and the Grampian Health Board, Aberdeen.

RESULTS

Infant characteristics are shown in Table 1. Transit time through the gastrointestinal tract, measured as the time lapse between the administration of the faecal marker until it first appeared in the stool, ranged from 6 to 29 h with a mean of 18 h.

Individual values for fractional apparent absorption of ^{70}Zn and ^{42}Ca, calculated as the difference between the dose ingested and the total excretion of the isotope up to 92 h, are given in Tables 2 and 3. Since a second faecal marker was not given after 72 h, we included

Table 1. *Sex, age, body weight, length, gastrointestinal transit time and hours of faecal excretion included in the calculation of fractional absorption of zinc and calcium by infants*

Infant	Sex	Age (weeks)	Body wt (kg)	Length (m)	Transit time (h)	Faecal excretion (h)
1	F	28	8·4	0·67	29	74
2	M	29	9·6	0·71	21	68
3	F	30	8·2	0·68	nd	69
4	M	18	6·2	0·64	6	81
5	M	20	6·8	0·64	23	92
6	M	20	8·2	0·68	9	75

nd, not determined.

Table 2. *Individual values for fractional absorption and cumulative re-excretion of ^{70}Zn by infants**

Infant	Administered dose (%)	
	Absorption of ^{70}Zn	Cumulative re-excretion of absorbed ^{70}Zn
1	19·2	0·85
2	25·4	0·88
3	41·4	0·12
4	27·6	0·06
5	26·1	0·16
6	63·9	0·59
Mean	33·9	0·44
SD	16·4	0·38

* For details of procedures, see pp. 292–295.

Table 3. *Individual values for fractional absorption and cumulative re-excretion of ^{42}Ca in infants**

Infant	Administered dose (%)	
	Absorption of ^{42}Ca	Cumulative excretion of absorbed ^{42}Ca
1	47·0	1·74
2	36·7	< d.l.
3	46·8	< d.l.
4	62·5	< d.l.
5	56·2	< d.l.
6	71·7	< d.l.
Mean	53·5	
SD	12·6	

< d.l., below the detection limit.
* For details of procedures, see pp. 292–295.

the isotopes excreted in faeces during a time period of 68–92 h in the calculation of fractional absorption for the individual infants (Table 1). The re-excretion of absorbed ^{70}Zn and ^{42}Ca in faeces during the period > 68–92 h to 21 d after administration is also given in Tables 2 and 3. Mean fractional apparent absorption values for ^{70}Zn and ^{42}Ca were 33·9 (SD 16·4)% (range 19·2–63·9%) and 53·5 (SD 12·6)% (range 36·7–71·7%) respectively. Re-excretion of absorbed isotopes (> 68–92 h to 21 d after administration) was found to be 0·44 (SD 0·38)% of the administered dose (^{70}Zn) while only one infant (baby 1) re-excreted detectable amounts of absorbed ^{42}Ca; 1·74% of administered dose.

DISCUSSION

During the weaning period the infant is very vulnerable nutritionally (Herveda & Newman, 1992). The increased amounts of semi-solid and solid foods that are being introduced, at the expense of human milk or infant formula, must provide energy and nutrients for the rapid phase of growth and development. Traditionally, weaning cereals are one of the first semi-solid foods to be given to the infant at about 4–6 months of age (American Academy of Pediatrics Committee on Nutrition, 1980; Clark & Laing, 1990) and the nutritional composition and bioavailability of nutrients in this type of product are therefore of great importance.

Relatively high fractional apparent absorption values for both Zn and Ca from the weaning cereal labelled with stable isotopes were found in the present study. The infant cereal was based on white wheat flour with a low extraction rate and skimmed milk powder and can thus be expected to contain only low amounts of phytic acid, a potent inhibitor of mineral absorption. These results demonstrate that weaning cereals containing low amounts of inhibitors can play an important role in providing Zn and Ca to the rapidly growing infant. Very little information on Zn and Ca absorption in term infants is available for comparison with our results. Fractional Zn absorption from cows'-milk-based infant formulas, studied in infants by the stable-isotope technique, has been reported to be dependent on the Zn level in the diet, higher fractional absorption taking place at lower intakes (Ziegler *et al.* 1989). In the study by Ziegler *et al.* (1989) the mean true Zn absorption was found to be 16·8 (SD 5·8)% at the normal level of Zn in infant formulas (6·58 mg Zn/l) and 41·1 (SD 7·8)% from formula containing 1·47 mg Zn/l. The labelled formula (1 l) was administered over approximately 24 h. The present results thus seem to indicate that the apparent absorption of Zn from infant cereal containing about 1 mg Zn in a single meal (when measured under these conditions) is relatively high. More information on mineral bioavailability from weaning foods is clearly needed together with identification of dietary inhibitors and enhancers. For example, the effect of increased phytic acid levels in weaning products with a higher fibre content on mineral bioavailability in infants has not been reported. This lack of information is primarily related to the methodological difficulties involved in this type of study.

The results from the present study demonstrate that 72 h faecal collections are adequate for complete collections of non-absorbed stable isotopes when measuring Zn and Ca apparent absorption in infants during the weaning period. However, since some of the initially absorbed isotope will be re-excreted via the gastrointestinal tract (and in urine) during the 72 h following administration, true absorption will be underestimated. It is thus important to estimate the magnitude of the re-excretion of absorbed isotope in order not to introduce any significant error in the experimental data. The extent of re-excreted absorbed mineral lost via the gastrointestinal tract can be monitored by the appearance of isotopes in faeces following injection (Janghorbani *et al.* 1985). This technique is based on the assumption that an injected isotope is excreted in an identical manner to the native element. However, the need for venepuncture limits the use of this method when studies are

being done in healthy, non-hospitalized infants and an alternative approach is needed. Studies of the cumulative faecal excretion of isotopes given orally can be used for this purpose. The positive slope of the excretion curve after the elimination of unabsorbed mineral is used for estimating the magnitude of re-excretion of absorbed isotope by this technique. This method has been used in a study of premature infants, demonstrating that re-entry of absorbed isotope into the gastrointestinal tract was insignificantly small for ^{70}Zn. However, re-entry of ^{46}Ca was shown to be higher, about 0·5% per 24 h period in two premature infants (Janghorbani *et al.* 1985). Re-excretion of absorbed mineral is not quantitatively the same as endogenous excretion as the latter requires isotopic equilibration in the body pool which is the major reason for the observed negligible re-excretion of ^{70}Zn in contrast to the significant endogenous secretion of this element (Janghorbani *et al.* 1985).

Prolonged collections of faecal material in the present study showed only minor amounts of isotopes being excreted after completing the excretion of non-absorbed isotope (> 68–92 h after administration). In our study we could quantify only small amounts of ^{70}Zn being re-excreted in faeces and found values below the detection limit for ^{42}Ca, except for one infant (baby no. 1). Our results thus support the earlier conclusion that the simple faecal pooling method should provide a sufficiently accurate estimate of apparent absorption of Zn and Ca. The more elaborate method involving analysis of individual stools and extrapolations to zero transit time in order to estimate the magnitude of re-excretion is therefore not needed (Janghorbani *et al.* 1985).

In the present study it was not possible to ensure a separate collection of urine and faeces since no special collection devices were used. The inclusion of urine in some of the faecal samples cannot be ruled out, resulting in an underestimate of the apparent absorption of Zn and Ca. The magnitude of the underestimation is difficult to ascertain since very little information is available about the excretion of newly absorbed Zn and Ca in urine. We have recently measured the excretion of newly absorbed ^{70}Zn and ^{44}Ca after administration of bread fortified with $FeSO_4$ or NaFeEDTA in ten adult women (Davidsson *et al.* 1994). Very small amounts of the administered dose of ^{70}Zn were found in complete 6 d collections of urine during the consumption of $FeSO_4$ and NaFeEDTA-fortified diets; 0·29 (SD 0·21)% and 0·91 (SD 0·34)% while the excretion of absorbed ^{44}Ca was 8·8 (SD 1·9)% and 9·8 (SD 2·2)% during the two dietary regimens respectively.

The total contents of Zn and Ca in the test meal were increased by 82 and 3% respectively in the test meal after addition of the stable-isotope solutions. The increase in Ca content can thus be regarded as negligible while the extrinsic labelling with ^{70}Zn increased the Zn content significantly. The increased amount of total mineral in the test meals is a frequently discussed problem related to the stable-isotope technique since stable isotopes cannot be regarded as 'true' tracers. This problem cannot be completely overcome, due to the nature of stable isotopes, but can be minimized by careful study design and high precision measurements of stable-isotope ratios. In the present study we chose to administer a relatively high dose of ^{70}Zn since one of our aims, related to the methodological part of this study, was to be able to measure accurately the faecal excretion of absorbed isotope over an extended period of time in individual stool samples. The fractional apparent absorption of Zn from the infant cereal would thus be assumed to be underestimated since percentage Zn absorbed depends on the Zn content of the test meal (Ziegler *et al.* 1989).

Another methodological aspect of the stable-isotope technique which needs to be considered is the procedure used when labelling test meals. By far the most commonly used labelling technique, which was used in the present study, is to add stable isotopes as extrinsic labels. An alternative approach would be intrinsic labelling of test meals. However, due to the very elaborate study design needed when preparing intrinsically

labelled foods this approach was not considered in this case, The extrinsic labelling technique with Zn and Ca stable isotopes has been validated in previous studies in infants and adults. Zn absorption from intrinsically and extrinsically labelled cows' milk infant formula was identical for both infants (Serfass *et al.* 1989) and adult women (Egan *et al.* 1991), as was the absorption of Ca and Mg by low-birth-weight infants fed with intrinsically and extrinsically labelled human milk (Liu *et al.* 1989). Similarly the absorption of intrinsically and extrinsically added Ca was not significantly different in adults given whole-wheat flour (Weaver *et al.* 1992) or milk (Martin *et al.* 1989). Thus, there is little reason to believe that intrinsic and extrinsic stable-isotope labels added to infant cereals would be utilized differently by infants.

We hope that the recent development of stable-isotope techniques for studies of mineral absorption in infants will result in increased research activity in this field of infant nutrition. The large inter-individual variation in Zn and Ca absorption observed in the present study needs be studied further to evaluate also the day-to-day and meal-to-meal variation in mineral absorption by infants. Thus, carefully designed studies are needed when evaluating the influence of different dietary factors on mineral absorption during infancy. The possibility of studying several minerals simultaneously and of repeating studies over a period of time to investigate the effect of age on mineral absorption should be considered for future studies.

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