Review Article

Using stable isotopes to assess mineral absorption and utilization by children[1–6]

Steven A Abrams

ABSTRACT Adequate mineral intake is a crucial part of a healthy diet for children—it supports appropriate growth and development and provides protection against childhood conditions like anemia and helps to prevent future adult diseases such as osteoporosis. Challenges in performing and interpreting studies in infants and children have hampered the accurate assessment of their mineral utilization. Many of the most powerful techniques used in adults, such as radioisotope testing, are not appropriate for use in children. In recent years, advanced mineral stable-isotope techniques have been developed to fill this gap. Pediatric applications include studies of calcium absorption and kinetics during puberty and evaluation of the calcium-iron interaction in infants and toddlers. The effects of genetics in determining calcium absorption and bone turnover may become an important research area. The goals and methods of ongoing mineral stable-isotope research in infants and children are examined in this report. In the past, the cost and difficulties in obtaining isotopes have limited such research. This situation has improved considerably, although relatively few nutrition research laboratories are prepared to perform sample analyses. *Am J Clin Nutr* 1999;70:955–64.

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INTRODUCTION

Interest is increasing in the role of mineral nutrition in the health care of children. Mineral nutrition not only meets children's growth and developmental needs, but also may limit or prevent disease processes (eg, diarrhea) and protect against future diseases (eg, adult osteoporosis). Children are a particularly challenging group in which to perform nutritional research, not only because of their rapid growth, but also because of the difficulties inherent in dietary regulation and sample collection in children. Use of stable isotopes offers a unique opportunity to meet the need for evidence-based dietary guidelines. Stable-isotope studies may also provide physiologic information regarding nutrient metabolism that is otherwise unobtainable in children.

There are numerous methods for evaluating mineral requirements in children. One important approach to assessing requirements is to determine the amount of minerals absorbed and retained by children consuming diets providing various intakes. This can be done by several methods. These include mass-balance measurements, radioactive mineral administration, and stable-isotope methods.

Mass-balance measurements

In these studies, the net dietary balance, often referred to as retention, of the nutrient is determined from simultaneous measurements of intake and excretion (urinary and fecal) of the nutrient. The effects of different intakes on balance are calculated and an attempt is made to determine an optimal intake on the basis of these data. Mass-balance studies, however, have many limitations, especially when applied to pediatric populations. These include potential errors in identifying accurately both intake and total excretion of minerals and the high cost and substantial difficulty of conducting long-term nutrition balance studies. Children do not readily tolerate long-term dietary regulation or urinary and fecal collections.

Because of these problems, few mass-balance studies have been performed in recent years in children, except in small infants (1, 2). Although such studies have provided important information used in making dietary recommendations, they remain a limited tool for investigating mineral requirements (3). An additional problem is that mass-balance studies do not provide direct information regarding key aspects of mineral

[1] From the US Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center and Section of Neonatology, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston.

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[6] Address reprint requests to SA Abrams, Children's Nutrition Research Center, 1100 Bates Street, Houston, TX 77030-2600. E-mail: sabrams@bcm.tcm.edu.

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metabolism. For example, endogenously secreted (and then excreted) mineral cannot be distinguished from nonabsorbed dietary intake. Furthermore, information regarding the kinetics of mineral transport and utilization is not provided by mass-balance studies. Finally, it is very difficult to evaluate nutrient-nutrient interactions (eg, calcium-iron interactions) when using mass-balance studies.

Radioactive isotope studies

Radioactive isotopes began to be widely used during the 1940s to study mineral absorption and turnover. Iron (^{55}Fe and ^{59}Fe) and calcium (^{45}Ca and ^{47}Ca) radioisotopes continue to be used in studies of both healthy adults and those with mineral-deficiency conditions (4–6). However, concern exists regarding the appropriateness of using radioactive isotopes in research involving children or pregnant women. This concern has been highlighted by ethical questions raised regarding several studies performed in the 1940s and 1950s (7–9). A public investigation of some of these studies was conducted by the Department of Energy (9, *see* 10 for the complete text of the Department of Energy's Advisory Committee on Human Radiation Experiments report).

Concerns about the safety of radioactive isotopes, as well as the difficulties and expenses associated with elimination of radioactive wastes, have led an increasing number of researchers to prefer stable rather than radioactive isotopes for their studies in adults (11). Even recent studies of calcium absorption and kinetics on the Mir Space Station were performed by using stable rather than radioactive calcium isotopes (12). It is likely that the increasing availability of both stable isotopes and resources for their analysis will increase this trend toward investigators using stable rather than radioactive isotopes in mineral studies involving adults as well as children.

GOALS OF MINERAL STABLE-ISOTOPE STUDIES IN CHILDREN

Stable-isotope techniques can facilitate research into the mineral requirements of children in several ways, as follows: *1*) relating mineral metabolism to growth and pubertal development, *2*) evaluating dietary mineral interactions, *3*) evaluating the effects of acute and chronic illnesses on mineral absorption and metabolism, and *4*) evaluating the mineral needs of breast- and formula-fed infants to consider the optimal intake of minerals in formulas or other foods for infants.

Relating mineral metabolism to growth and pubertal development

Stable-isotope studies, because of their safety and relative simplicity, are more readily adaptable to repeated measurements over time than are other balance techniques. This ability to perform longitudinal studies of minerals, especially calcium, provides a unique method for establishing the effects of growth and development on dietary mineral requirements. For example, kinetically determined mineral turnover rates can be related to body composition and biochemical or hormonal changes in children.

Evaluating dietary mineral interactions

Vitamin and mineral supplements are being taken by an increasing number of children (3). The interactions of the minerals within these supplements, or between supplemental and dietary minerals, have not been well studied in adults or children. For example, combining calcium and iron supplements may decrease iron absorption from the supplements (11, 13). Multimineral stable-isotope studies can be used to directly assess the consequences of multimineral supplementation on mineral bioavailability.

Evaluating effects of acute and chronic illnesses on mineral absorption and metabolism

The widespread availability of bone mass measurement techniques has led to the publication of many reports documenting the high rate at which chronic pediatric illnesses lead to a loss of bone mass. These include many common pediatric diseases such as juvenile rheumatoid arthritis, cystic fibrosis, and inflammatory bowel disease (14–16). However, few studies have analyzed the dynamics of mineral losses in the presence of these conditions. This lack of data is the basis of uncertainty regarding the need for, and potential risks and benefits of, mineral supplementation. For example, we showed that high calcium intakes did not prevent a net calcium loss (ie, a negative calcium balance) in adolescent girls with anorexia nervosa (17). Relatively few other pediatric conditions have been similarly evaluated (18, 19).

Other minerals are being increasingly linked to disease states in children. For example, zinc deficiency may be an important contributor to morbidity from diarrheal illnesses. Stable isotopes may be used to evaluate the magnitude of direct secretory zinc losses in diarrhea or chronic malabsorptive conditions as well as the effect of supplementation on these losses and body pools of zinc (20).

Evaluating the mineral needs of breast- and formula-fed infants to consider the optimal intake of minerals in formulas or other infant foods

Evaluation of the mineral needs of infant is a special circumstance in which stable isotopes have been relatively widely used and continue to contribute to the understanding of nutrient requirements. Human milk provides adequate minerals as the sole source of food for full-term infants during the first 4–6 mo of life (21), despite relatively low concentrations of minerals such as calcium, iron, and zinc (20, 22, 23). To understand the infant mineral requirements of infants, it is necessary to understand the bioavailability of minerals in human milk and the effects of both supplemental solid foods and infant formulas on mineral metabolism (22–25). Very-low-birth-weight infants require supplemental minerals beyond those provided by human milk. Numerous stable-isotope studies have specifically addressed these issues in premature infants (26–33).

RESEARCH USING MINERAL STABLE ISOTOPES

I will now focus on the methods and results of specific mineral research studies involving stable isotopes. The 2 primary minerals that will be described are calcium and iron, followed by briefer discussions of magnesium and zinc.

Calcium

Early studies using calcium stable isotopes

A limited number of studies using radioactive isotopes of calcium (^{45}Ca and ^{47}Ca) have been performed in children. Bronner and Harris (34) measured calcium absorption and kinetics in a group of adolescent boys (mean age: 12.6 y) who received ^{45}Ca.

The initial human studies using mineral stable isotopes were reported in the 1960s. McPherson (35) reported on methods for using stable calcium isotopes analyzed by neutron-activation analysis in 1965. During the next 20 y, however, relatively few calcium stable-isotope studies were performed because of the lack of readily available methods for performing sample analysis. A few landmark studies during this period are described below.

In 1971, Heaney and Skillman (36) reported a remarkable study using ^{48}Ca administered intravenously to 15 women aged 15–28 y to measure calcium kinetics in different stages of pregnancy. These studies showed increases in calcium absorption and kinetic values during pregnancy relative to values in nonpregnant women of similar ages. This study remains the standard for understanding many of the changes in calcium metabolism during pregnancy.

Barltrop et al (26) reported in 1977 on calcium absorption and endogenous fecal excretion in a small group of preterm infants. These infants were given ^{46}Ca orally, the least abundant calcium isotope, and fecal samples were subsequently collected.

The studies described above used neutron activation (irradiation) to determine the isotopic content of blood, urine, and fecal samples. However, that technique is relatively cumbersome compared with mass spectrometry determinations. In 1972, Moore and Machlan (37) reported a mass spectrometric technique to measure calcium stable-isotope enrichment in blood and urine samples using a specially constructed thermal ionization mass spectrometer (TIMS). Measurement precision for isotope ratios <0.1% was achieved, which is comparable with the precision obtained currently with commercially available techniques (38).

A turning point in the use of these isotopes came with the possibility of performing analysis with more widely available mass spectrometers. The use of a standard quadrupole TIMS to measure calcium isotope ratios was reported by Yergey et al (39) in 1980. Similarly, in 1985, Smith (40) reported using fast atom bombardment mass spectrometry to perform these analyses. These reports led to an increase in the use of mineral stable isotopes in human nutrition studies.

Isotope preparation

There are 6 naturally occurring stable isotopes of calcium. Typical doses of isotopes used in clinical studies are shown in **Table 1** (41). The most abundant calcium isotope, ^{40}Ca (96.97% natural abundance), is rarely used in nutrition research, although very highly enriched (>99.9%) ^{40}Ca can be administered over time to wash out the lower-abundance isotopes (42). The large quantity of ^{40}Ca needed for this purpose makes this relatively impractical. The existence of a very-low-natural-abundance isotope, ^{46}Ca (0.003% natural abundance), and 4 other low-abundance isotopes makes calcium a favorable mineral for tracer studies. Furthermore, the cost and availability of calcium stable isotopes have improved considerably in recent years, as discussed below.

Calcium stable isotopes are usually purchased as powdered calcium carbonate. Preparations for human use are made under sterile conditions. The powder is dissolved in nitric oxide and then, after drying, is converted to calcium chloride by mixing it with sodium chloride. Isotopes are then filtered and tested for sterility and pyrogenicity before use in humans. Established protocols for isotope preparations and testing are readily available from pharmacies experienced in these procedures, including the Investigational Drug Service of the Texas Children's Hospital, Houston, or the Pharmaceutical Development Section, Pharmacy Department, of the Clinical Center at the National Institutes of Health, Bethesda, MD.

TABLE 1
Mineral stable isotopes frequently used in pediatric nutritional research

Isotope	Natural abundance	Typical dose for mineral absorption studies[1]
	%	
^{42}Ca	0.65	1–2 mg iv
^{44}Ca[2]	2.08	3–16 mg iv (10–15 mg po)
^{46}Ca	0.0032	15–20 μg po
^{57}Fe[3]	2.14	5–15 mg po
^{58}Fe[3]	0.29	1–3 mg po (0.2–0.4 mg iv)
^{67}Zn	4.10	1–3 mg po
^{70}Zn	0.62	0.2–0.5 mg iv
^{25}Mg	10.0	0.3–0.5 mg/kg iv
^{26}Mg	11.0	0.2–0.4 mg/kg iv

[1] po, orally; iv, intravenously. Typical doses are based on dual-tracer studies involving adults and children >1 y of age being analyzed with thermal ionization mass spectrometers (TIMSs). Intravenous isotope doses of calcium, magnesium, and zinc must be increased from these values for complete studies in which endogenous fecal excretion of the mineral is assessed; for these measurements, about double the dose listed for absorption studies is usually given.

[2] For analysis with most magnetic sector TIMSs, it is preferable to not administer both ^{42}Ca and ^{44}Ca to the same subject because this limits the precision of the final measurement.

[3] In studies of preterm and full-term infants <1 y of age, ^{57}Fe doses of 1–4 mg and ^{58}Fe doses of 0.2–0.5 mg are given po (TIMS analysis).

We prefer to have each dose of intravenously administered isotope prepared, labeled, and dispensed by a registered pharmacist using aseptic techniques and standard protocols. Before any subject receives a stable isotope, informed written consent is obtained from the appropriate caregiver based on the guidelines of the Institutional Review Board for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals. We are unaware of any known risk to subjects of any age or health status specifically related to the use of calcium stable isotopes in the doses administered for research purposes. The total amount of calcium infused is always small, and is far below the amount of calcium used therapeutically (43). All isotope infusions are performed by a physician or registered nurse.

Measurement of calcium absorption

In a mass balance study, the net absorption of a nutrient is calculated by measuring the difference between mineral input from the diet and total fecal mineral output (1–3). The fecal output of mineral includes both unabsorbed dietary mineral and mineral that has been secreted into the intestine and not reabsorbed (usually referred to as endogenous fecal excretion). These 2 sources of mineral that appear in the feces cannot be distinguished by a classic balance study (44, 45).

With use of isotopic methods, however, fecal calcium from these 2 sources may be measured and the "true" dietary absorption fraction of the mineral can be measured. Several methods have been used to calculate mineral absorption from isotope studies. With the single-isotope technique, an isotope of the mineral is given either with a meal or separately. A complete fecal collection is made until virtually all of the unabsorbed oral tracer is recovered. The fraction of administered tracer that was absorbed is calculated from the difference between the amount ingested orally and the amount recovered in the feces. The single

oral-tracer technique has the benefit that the calculated absorption represents only the dietary component of the element that is absorbed and does not include endogenous secretory losses (26). A disadvantage of this oral-tracer approach is that extended fecal collections are required.

We prefer to use a dual-tracer technique to measure calcium absorption. With this technique, one low-abundance calcium isotope is given orally and a different isotope is given intravenously. Early in the morning of the study, subjects are instructed to empty their bladders and are then given breakfast. Toward the end of the meal, the subjects are given an isotope of calcium that had been premixed (and allowed to equilibrate in the refrigerator for 12–24 h) with milk or juice. After breakfast, a different calcium isotope is administered intravenously over 2–3 min.

The orally administered isotopic tracer is absorbed into a central body pool, which, for calcium, is believed to represent serum, extracellular fluid, and some metabolically active bone (26, 40, 42). The oral tracer mixes with the intravenous tracer, which serves to normalize variations in calcium distribution pool mass among subjects (41).

After administration of the tracers, a complete 24-h urine collection is carried out. The relative fraction of the oral compared with the intravenous tracer dose in this 24-h urine pool is determined and represents the fraction of the oral tracer dose that was absorbed. Spot determinations of urine or serum isotope concentrations may also be used (46, 47). However, for calcium, this method may not be as accurate as complete 24-h collections (48). A spot urine sample similarly collected 2–3 d after dosing may be useful for measurement of zinc absorption, however (33, 49). Because absorption is calculated from total urinary isotope recovery, it is not necessary to carefully sequence the time of administration of the oral and intravenous isotopes, as would be necessary if a single peak serum value were used.

Measurement of endogenous fecal calcium excretion

The direct assessment of endogenous fecal calcium excretion requires intravenous administration of a calcium isotope and collection of feces for a period of 6–7 d (3–4 d in infants). It is necessary to infuse a larger dose of isotope intravenously to ensure adequate enrichment of the feces with the secreted isotope (44, 50). If each component of calcium balance is to be measured directly, these fecal collections are necessary to assess endogenous fecal calcium excretion. In other cases in which dietary absorption is the primary endpoint, fecal collections may be omitted and estimates used for endogenous fecal excretion to calculate net retention. Although endogenous excretion of calcium is not a primary regulatory point for calcium balance in adults (45), adequate data regarding this issue are as yet unavailable for children. Developmental changes in endogenous fecal calcium excretion are shown in **Table 2**. Few data are available for full-term infants and young children.

Measurement of bone calcium deposition and pool masses

Although isotopic methods are important as a means of simplifying the measurement of mineral absorption, a second important benefit of their use is that they allow for kinetic models to be developed to understand the distribution and turnover of minerals after absorption (17, 28, 53–55). To perform these kinetic studies, the calcium absorption protocol must be expanded to collect sequential serum and urine samples after isotope administration. Serum samples (0.5 mL) are obtained for isotope enrichment determination 6, 12, 20, 30, 45, 60, 120, 180, 240, and 480 min after completion of the intravenous infusion of the calcium isotope. Studies in premature infants have been done with fewer serum samples (27, 28). Additionally, ≥2 spot urine samples are collected daily for a total of 5–6 d after isotope administration (17, 53). Calculation of the kinetic pool masses is described under "Compartmental modeling."

TABLE 2
Typical values for endogenous fecal calcium excretion

Age group	Endogenous excretion[1]	Urinary excretion
	$mg \cdot kg^{-1} \cdot d^{-1}$	
Premature infants	15–25	2–8
Older infants and small children	2–5	1–4
Adolescents and adults	1–2	1–4

[1] Data from references 3, 44, 45, 50–52. Few data are available for full-term infants and young children.

Analytic methods

Calcium can be isolated from most serum and urine samples by precipitation with ammonium oxalate (27, 39). Adequate amounts of calcium can usually be recovered from 3 mL urine or 0.5 mL serum. Fecal samples require acid digestion and sometimes ion exchange chemistry before precipitation and analysis. After precipitation, samples are baked in a muffle furnace and resuspended in dilute nitric acid. Five microliters of suspension are loaded onto a multisample turret and placed in the mass spectrometer for analysis. With TIMS, calcium samples are analyzed indirectly by using a dual-filament technique. For magnetic sector instruments, accuracy of this technique for natural-abundance samples is 0.1–0.2% of accepted values (41). Precision, including sequential measurement of the same sample (on different filaments) over a period of time, is similar.

Compartmental modeling

The compartmental model used for calcium kinetic interpretation (53, 54) is similar to that originally described in adults by Neer et al (55). This model is based on a series of sequential pools before calcium deposition in the "deep" bone calcium pool. Bone calcium deposition (V_o+) is the flow rate of calcium to the final pool. The compartmental modeling of the data is done with the aid of the SAAM program or its SAAM II successor (56).

Results: overview

During the past 10 y, the use of calcium stable isotopes has dramatically increased in pediatric research. Two areas in which they have been used will be highlighted to show the use of the method to evaluate mineral requirements. These are a series of studies of calcium metabolism in premature infants and in early adolescent and adolescent girls.

Results in premature infants. It might seem surprising that some of the first uses of calcium stable isotopes involved premature infants (26, 27). However, the importance of understanding the bioavailability and utilization of minerals by preterm infants has made them a focus of nutrition balance studies. For example, infants <1500 g at birth remain at high risk for bone demineralization and rickets (57), even though the latter has become uncommon among children.

Several groups have measured calcium absorption and excretion in preterm infants using stable isotopes. In studies of small preterm infants, we showed that using special formulas or human milk fortifiers intended for premature infants led to a net calcium retention similar to that achieved in utero. We further showed a significant positive relation between the kinetically determined rate of bone calcium deposition and net calcium absorption (28, 29).

One unique aspect of calcium balance in preterm infants is the high rate of endogenous fecal calcium excretion in these infants relative to that of older children or adults (Table 2). This can represent a larger source of calcium loss than urinary calcium excretion, a situation somewhat different from that seen for adults (3, 28, 29, 44, 45, 51, 52).

Because of the need for rapid growth in preterm infants, formulas designed for their use maximize macronutrient as well as mineral absorption (57). The nature of different fat blends used in these formulas may have a marked effect on calcium absorption. Lucas et al (30) compared calcium absorption from a routinely used formula with that from a formula in which the fat blend was optimized with β-triacylglycerol to more closely resemble human milk. The result was increased calcium absorption, likely related to reduced calcium soap formation. This study showed the use of a relatively straightforward calcium absorption study protocol to answer a specific nutrient bioavailability question concerning infants.

Results in adolescents. The preadolescent and early adolescent period is one of rapid growth and skeletal mineralization associated with pubertal development. Genetic factors including ethnicity, a family history of osteoporosis, and putative genetic markers may have a substantial effect on the timing and maximal rate of calcium absorbed and retained by the body during puberty. We have used stable isotopes to evaluate these genetic factors in girls.

We assessed the effects of ethnicity on calcium absorption and kinetics in groups of white, African American, and Mexican American girls. With diets relatively low in calcium, we reported greater calcium absorption postmenarche in African American than in white girls (58). Smaller ethnic differences were seen in girls premenarche. In contrast, we did not identify any difference in calcium absorption or kinetics in prepubertal 7- and 8-y-old Mexican American girls relative to white girls (59). Further studies are needed, however, to identify the adaptability of different ethnic groups to very low calcium intakes and to assess ethnic differences in the roles of other factors such as exercise in calcium metabolism.

A second approach we have used to evaluate the genetics of calcium metabolism was the examination of calcium kinetics in families with a history of early osteoporosis. We found that families with a history of osteoporosis show evidence of alterations in calcium kinetics relative to families without such a history (60).

Ultimately, the most important tools available to assess genetic factors in the development of peak bone mass and osteoporosis are those provided by recent advances in molecular biology. Genetic markers such as polymorphisms of the vitamin D receptor (VDR) gene have been implicated as capable of identifying groups at high risk of low bone mass (61, 62).

We evaluated the relation between calcium absorption and restriction fragment length polymorphisms of the *VDR* gene in 72 healthy children aged 7–12 y (63). We found that the *Fok* 1 polymorphism at the *VDR* translation initiation site was significantly associated with calcium absorption ($P = 0.04$). Children who were *FF* homozygotes had a mean calcium absorption that was 41.5% greater than that of *ff* homozygotes and 17% greater than that of *Ff* heterozygotes.

Eventually, if these data are confirmed and further genetic links to bone mass, calcium absorption, and kinetics are identified, it may be possible to target groups of children who, based on their genetic background, have a higher (or lower) risk of osteoporosis than other groups. If such identification can be done, it will then be necessary to conduct controlled trials to evaluate the effects of long-term calcium and vitamin D intakes on these risks. However, this level of knowledge remains quite distant, and, at present, the data do not support altering the calcium dietary requirements on the basis of known risk factors. It seems reasonable to assume that the genetic component of osteoporosis is related to multiple genetic sites, and that these are only beginning to be understood. As these evaluations progress, it is likely that calcium stable-isotope studies in children can play an important role in clarifying these genetic factors.

Iron

Overview

Although iron stable-isotope use was reported in the 1960s, as with calcium, there were relatively few studies that used these isotopes until the early 1980s, when they began to be used primarily in studies involving adults (64–66). To date, most pediatric studies using iron stable isotopes have been conducted in preterm or full-term infants (22–25, 67–71). This inclusion of full-term infants in iron stable-isotope studies contrasts with the virtual lack of such studies for calcium. This difference is presumably because of the relatively greater clinical problem of iron deficiency compared with calcium deficiency for otherwise healthy full-term infants and toddlers.

Iron stable isotopes

There are 4 naturally occurring iron stable isotopes. Of these, the lowest-abundance isotopes, ^{58}Fe and ^{57}Fe, are most commonly used in human nutrition research. Iron stable isotopes are usually provided as iron metal and are converted to ferrous sulfate before oral administration. Both the supply and cost of these isotopes have remained relatively constant or have decreased in recent years (Table 1). Because dosing of iron stable isotopes is dependent on enriching the circulating body iron pool (*see* below), the dose administered is usually dependent on the subject's weight, and increases in proportion to weight and hemoglobin concentration (68, 69).

We use [^{58}Fe]citrate as the form of iron to be administered when it is given intravenously. There may be a very small risk of an allergic reaction associated with the use of intravenously administered iron. An adverse reaction is extremely unlikely because of the form and the small doses (always < 0.5 mg total Fe) used (70–72). However, because of this potential risk, we have chosen to administer iron isotopes intravenously only within a hospital setting. We administer the [^{58}Fe]citrate over 30 min with careful monitoring of vital signs.

Measurement of iron absorption

Isotope studies using iron stable isotopes usually take advantage of their localization in red blood cells to directly estimate the fraction of isotope that is incorporated into red blood cells (RBC_{inc}) from an orally administered dose. A sample of blood is obtained 14–28 d after dosing and the enrichment of the administered isotopes is calculated (67).

The ratio of the administered isotope (^{57}Fe or ^{58}Fe) is determined relative to ^{56}Fe in the sample of blood, and the quantity of administered isotope incorporated into erythrocytes (Fe_{inc}) is determined from enriched (enr) and baseline (base) isotope ratios as follows (for ^{57}Fe):

$$^{57}Fe_{inc} = \frac{[^{57}Fe/^{56}Fe_{enr} - {}^{57}Fe/^{56}Fe_{base}] \times Fe_{circ} \times NA_{57}}{^{57}Fe/^{56}Fe_{base}} \quad (1)$$

where Fe_{circ} is the child's total circulating iron (calculated as the product of the child's blood volume, the measured hemoglobin concentration, and the concentration of iron in hemoglobin of 3.47 mg/g) and NA_{57} is the natural fraction (by weight) of ^{57}Fe (0.0214). Identical calculations are done for $^{58}Fe_{inc}$, except that 0.00287 is used as the natural fraction of ^{58}Fe.

In our initial studies, we measured baseline ^{57}Fe-^{56}Fe and ^{58}Fe-^{56}Fe ratios. However, variability in these ratios was extremely small and did not suggest that there is a substantial natural variation in iron isotopic distribution (68, 69). To calculate RBC_{inc}, the total isotope incorporated is divided by the dose. In multitracer studies, a correction of the dose of ^{58}Fe administered is required because of the small amount of ^{58}Fe present in all sources of ^{57}Fe tracer (67).

An important issue in this calculation is the value used for the child's blood volume. It is not practical to measure this directly for each subject. Rather, an estimate is usually used. Although estimates of 65 mL/kg are generally used in adults, blood volume may be greater in small children, especially premature infants. In infants, most calculations use a volume of 80 mL/kg. It should be recognized that the final calculated RBC_{inc} may have some imprecision. However, most iron isotope studies are done by comparing values within individual subjects consuming 2 different diets, so this small uncertainty in the actual RBC_{inc} may not have an important effect on the final study findings (67, 71–74).

We usually prefer to report the value for RBC_{inc} as the endpoint for iron bioavailability (74). If the actual dietary iron absorbed by the body is the endpoint of interest, several methods may be used for its determination. The most common method is to simply assume that 90% of all the iron absorbed from the diet is incorporated into RBCs (67) and to divide the RBC_{inc} by 0.9 to calculate the fractional absorption.

Alternately, it is possible to infuse an iron isotope intravenously and use a dual-tracer method similar to that applied for other minerals to directly measure iron absorption. This method is more difficult than oral isotope administration because of the cost and difficulties associated with infusing the iron isotopes. Furthermore, the calculated absorption may not be accurate if the intravenous isotope is transported differently within the vascular system than is the absorbed oral isotope. With these limitations in mind, Zlotkin et al (71) and McDonald et al (70) reported that much less than 90% of absorbed iron is incorporated into red blood cells by preterm infants. The lower values seen in preterm infants are likely related to the tissue deposition (storage) of absorbed iron that would have occurred in utero had the infant not been delivered prematurely.

Results

One of the key issues in providing iron to children is the determination of the optimal timing of its administration. This is because other dietary components, especially calcium, may decrease iron absorption. We evaluated these issues in a series of studies in preterm infants and young children (**Table 3**) (69, 70, 74, 75).

In formula-fed premature infants and in 1-y-old infants, we found that giving an iron supplement between meals (separate from formula or milk) resulted in a significantly greater RBC_{inc} than when the iron supplement was given with a feeding (69, 70). However, the difference was small in formula-fed premature infants and no effect was seen in fortified breast-fed premature infants (74).

Recently, we studied this effect in children 3–5 y of age and found no significant effect of calcium intake on RBC_{inc} after 5 wk of adaptation to relatively high calcium intakes (≈1100 mg/d) compared with lower intakes (≈500 mg/d) (75). Our results suggest partial or complete adaptation to the effect of calcium over time. This study was small, and more data are needed regarding this relation, although recent studies in adults (76) and in miniature piglets (77) support these findings.

The interaction of iron with other vitamins and minerals is a key nutrition issue requiring continued evaluation. The increasing frequency with which many foods and supplements are fortified with minerals has led to a situation in which the default assumption is that more vitamins and minerals are always better. However, with regard to iron fortification, 2 important issues need to be considered: *1*) the potential for interference with iron absorption when other minerals are also supplemented (eg, calcium or zinc), and *2*) the potential for iron overload by high rates of iron absorption from highly iron-fortified (or ascorbic acid–fortified) food products. These issues should be addressed directly by researchers performing studies in children with multimineral stable-isotope studies.

Magnesium

The measurement of magnesium absorption, endogenous excretion, and kinetics with stable isotopes in children is a signi-

TABLE 3
Effects of calcium-containing foods on iron absorption in children[1]

Subject group and reference no.	Low-calcium food, high-calcium food	Relative RBC_{inc}[2]	*P*
Premature infants (74)	No meal, fortified human milk	1.0	0.67
Premature infants (70)	No meal, formula	1.4	0.04
1-y-olds (69)	Juice, milk	2.9	<0.01
3- to 5-y-olds (75)	Low-calcium meal, high-calcium meal	0.8	0.67

[1] In the case of the preterm or premature infants, studies were performed comparing iron absorption from iron supplements given between feedings (ie, low calcium administration) or mixed with feedings (high calcium administration). Studies in 1-y-old infants compared absorption from an iron supplement given either with juice containing ascorbic acid (low calcium administration) or with milk (high calcium administration). Studies in 3- to 5-y-olds compared absorption from iron supplements given with high- or low-calcium-containing meals after an extended adaptation period of the children to low- or high-calcium diets.

[2] The ratio of red blood cell incorporation of the supplemental iron from the low compared with the high calcium-containing sources.

ficant methodologic challenge (78). The difficulty lies in the fact that, unlike calcium, zinc, and iron, which have ≥4 naturally occurring stable isotopes, there are only 3 stable isotopes of magnesium. None of the 3 isotopes of magnesium are of low abundance (ie, <5%). Therefore, to achieve measurable enrichment of a serum or urine specimen, a relatively large dose of isotope needs to be given. This dose represents a significant fraction of the exchangeable magnesium pool and therefore may not function as a true tracer. Furthermore, although magnesium isotopes are readily available for purchase, the large doses required make these studies somewhat expensive to perform.

Nonetheless, several studies using magnesium stable isotopes have been performed, beginning with the work of Schwartz et al (78) in the 1970s. Limitations in analytic capabilities for these early studies were substantial. Further work in this area has depended on improvements in analytic precision, with subsequent lowering of the necessary doses of isotopes (79–81).

We recently completed a study measuring magnesium absorption and kinetics in boys and girls aged 9–14 y (50, 80, 81). We showed a close correlation between weight and fat-free mass and both the size of the exchangeable pool of magnesium and the rate at which this pool exchanges with the longer-term storage pool (82). These relations are closer for magnesium than for calcium. These relations provide support for basing dietary magnesium requirements in children on body-composition measures such as body weight or, when available, fat-free mass (83). These studies have further suggested that current intakes of magnesium may only be marginally adequate during the rapid growth of early adolescence (50).

Zinc

There has been increased interest in zinc in pediatrics because of recent studies suggesting an association between low zinc status and infections, especially diarrheal illnesses and respiratory infections (84–86). The possibility of using supplemental zinc to decrease the consequences of these infections or to enhance growth in children, especially in underdeveloped nations, is an important area for ongoing research.

There are 5 naturally occurring stable isotopes of zinc. Three of them, ^{67}Zn, ^{68}Zn, and ^{70}Zn, are in sufficiently low natural concentrations to allow enriched preparations of these isotopes to be used in tracer studies of human zinc metabolism. As with calcium, early zinc stable-isotopes studies in children generally used a single oral tracer followed by fecal collections to assess absorption (87, 88). Serfass et al (89) used this approach to show that infant formulas may be extrinsically tagged to assess absorption in infants. More recently, Fairweather-Tait et al (23) used this approach to show that zinc absorption from a vegetable-based weaning food was ≈30% and was not affected by iron fortification of the food. This value is similar to that reported using this technique for infant zinc absorption from a wheat-based infant cereal (24).

Friel et al (33, 49) have described a dual-tracer method similar to that used for calcium to determine zinc absorption. This method was successfully applied to a group of premature infants as well as to older populations. We used this technique to assess zinc absorption from an isotope mixed with breast milk in full-term 5- to 7-mo-old infants (22). We found ≈50% absorption from that dose, similar to that described in single-isotope fecal-monitoring studies (20).

Multicompartmental models of zinc metabolism have been reported for adults and for premature infants (31, 90). Few comparable data are available for older children or adolescents. We recently presented preliminary data indicating that body pool masses and turnover rates in adolescents exceed those of adults on a body-weight basis (91). However, further work relating zinc kinetics to both growth and disease processes is needed. The potential use of such data is shown, for example, in the report by Krebs et al (20) based on stable-isotope studies showing that endogenous fecal zinc excretion was markedly elevated in 2 infants with cystic fibrosis.

ISOTOPE SOURCES AND ANALYTIC AVAILABILITY

For many who have wished to take advantage of mineral stable isotopes in nutritional research, the difficulties inherent in obtaining the isotopes and having biological samples analyzed have been major limitations (92). I will review these issues, especially the problem of purchasing high-quality mineral stable isotopes.

The only source of mineral stable isotopes produced in the United States is Oak Ridge National Laboratories (ORNL; Oak Ridge, TN). Isotopes are prepared at ORNL by electromagnetic separation with the calutron reactors originally designed for production of enriched uranium toward the end of World War II (93). After they were no longer needed for military purposes, a few calutrons remained in operation and were converted to the production of commercially needed isotopes for research (especially biomedical research). Because of the limited use of the nutritionally interesting mineral isotopes throughout the 1970s and the early 1980s, limited supplies of these stable isotopes were available at relatively low cost for nutrition research. Beginning in the mid-1980s, however, the Department of Energy (which by then had taken over operation of the calutrons) was required to more carefully recover the costs of isotope production. This led to a rapid escalation in the costs of the isotopes. This situation reached a peak in the early 1990s when, for example, newly produced calcium stable isotopes became virtually impossible to obtain and their cost reached prohibitive levels (92). Eventually, in 1992, the perception of a limited need for more isotopes and the high costs of maintaining the calutron facility led the Department of Energy to stop mineral stable-isotope production at ORNL and to place the calutrons on standby status.

At about the same time, calutron operators in Russia became increasingly interested in producing stable isotopes that could be sold by distributors in the United States and Europe. These isotopes filled an important cost and availability gap for several years, but the quality and supply of these isotopes were not always optimal.

This seemingly bleak situation has improved considerably over the past several years. First, in 1995, ORNL restarted production of isotopes (which has now again been stopped), but more importantly, it made the large isotope reserves available for purchase at a discount price through a commercial isotope distribution company. Second, isotope production facilities in Russia (**Appendix A**) agreed to produce high-quality mineral isotopes under contract according to user needs. Finally, methods of producing some isotopes (including iron and zinc, but not calcium) using an alternative so-called centrifugation process became available in both Russia and Europe.

The net effect of these changes has been a dramatic recent increase in the availability and quality of mineral isotopes available for nutritional research as well as a marked decrease in their price. This is especially true for high-volume purchases, a consideration that may be important for research-granting agencies.

Although the unstable political and economic situation in Russia limits absolute confidence in its ability to continue calutron operation, the supply of mineral stable isotopes appears to be adequate and the costs reasonable during the foreseeable future. The potentially permanent closure of the calutron facility at ORNL would be of concern, however, in that it would force reliance for calcium isotopes on stockpiled ORNL isotopes and Russian production.

The situation regarding analytic capacity is not as well defined at present. Most TIMS equipment was purchased for geologic rather than nutritional research. New TIMS equipment is too expensive and impractical for most laboratories. Available alternatives include other forms of mass spectrometry such as fast-atom bombardment mass spectrometry and inductively coupled plasma mass spectrometry (ICP-MS). These techniques are reasonable and potentially less expensive alternatives for the analysis of nutritionally important minerals, especially zinc and iron (94). The recent introduction of magnetic sector–based ICP-MS may enhance the use of ICP-MS for mineral stable-isotope analysis.

CONCLUSIONS

Mineral stable-isotope research remains a field still relatively new in its application to assessment of nutrient absorption and utilization. Recently, human nutritional research has shifted away from radioisotope use toward increased use of stable isotopes. Advances in identifying genes related to nutrient bioavailability may open up an important new role for stable isotopes. However, the availability of more facilities to perform sample analysis may remain a limiting factor in the development of this field in the near future.

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APPENDIX A

Internet sources for further information regarding mineral stable isotopes

Urenco Industries (http://www.urenco.com/isotope/home.htm): produces isotopes in Europe using centrifugation techniques; its web site has pictures and a detailed description of its isotope production.

Euriso-Top, France (http://www.eurisotop.fr/gasmet/metal/metaux.htm) and AEA Technologies, United Kingdom (http://www.aeat.co.uk/pes/aeat/aeathome.html): provide mineral stable isotopes throughout Europe.

Kurchatov Institute (http://www.kiae.ru/eng/inf/tex/texnol.htm): produces isotopes both by electromagnetic separation and by centrifugation (see the "New material" section of this page); a fascinating history is provided about this famous Moscow facility.

Trace Sciences International (http://www.isotopetrace.com/): an international distributor of stable isotopes; its page describes the electromagnetic isotope facility in Lesnoy, Russia (http://www.isotopetrace.com/ekp.htm), where calcium stable isotopes are currently being produced.

Oak Ridge National Laboratories (http://www.ornl.gov/isotopes/catalog.htm): provides a list of isotopes and prices as well as a description of the electromagnetic separation (calutron) facility, placed on standby status again on January 30, 1998, pending a decision regarding its permanent closure.

Pennwood Chemicals, Inc, Great Neck, NY (http://www.pennwoodgroup.com/home.htm): distributes stable isotopes in both the United States and Russia.