Stable Isotope Methods in Micronutrient Research

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Introduction

We will consider specific examples of three areas in which mineral stable isotopes are used, and then discuss the issues regarding increasing the use of stable isotope-based research.

Evaluation of Food Fortification in Indonesia

We assessed iron and zinc absorption in fortified wheat flour in collaboration with the Nutrition Research and Development Center in Bogor, Indonesia. One of the important questions weighed in this study was whether the bioavailability of zinc sulfate added as a fortificant to wheat flour would be the same as that of zinc oxide [1, 2].

Zinc fortification is increasingly frequent in developed countries, where the most common forms of zinc used are zinc oxide and zinc sulfate. In Indonesia, consideration is being given to co-fortifying iron-fortified flour with zinc. Identifying the optimal form of zinc to add (best bioavailability and lowest cost) is crucial to implementing such a program. Ninety healthy children (45 male and 45 female) were recruited from a rural clinic south of Jakarta,
Indonesia. Healthy subjects, 4–8 years old, were enrolled after informed written consent was obtained.

We prepared $^{67}\text{Zn}$ as both the oxide and the sulfate. Doses were mixed with steamed dough balls of the food product to be fortified. The iron and zinc isotopes were added to water, which in turn was added to the dough. After an overnight fast, subjects received an intravenous infusion of $^{70}\text{Zn}$. Shortly thereafter, the subjects received a meal consisting of the dough balls. Subjects collected urine samples for zinc isotope ratio analysis at 48 and 72 h. Urinary zinc isotope ratios were measured following acid digestion and anion exchange. Isotope enrichments were measured by magnetic sector thermal ionization mass spectrometry. Isotope ratios were expressed with respect to the non-administered isotope, $^{66}\text{Zn}$, and corrected for differences in fractionation using the $^{64}\text{Zn}/^{66}\text{Zn}$ ratio.

We found no difference in zinc absorption between the children who received the zinc oxide (24.1 ± 8.2%) and those who received the zinc sulfate (23.7 ± 11.2%; $p = 0.87$). This result was somewhat unexpected. Because zinc oxide is less soluble than zinc sulfate, it has been generally expected that zinc absorption would be greater from zinc sulfate than from zinc oxide. However, there is little objective evidence to support a bioavailability difference. As zinc absorption was similar from zinc sulfate and zinc oxide, we suggest that zinc oxide might be a preferable choice, especially as it is cheaper than zinc sulfate (a significant consideration) [1]. Whether these findings would hold true for smaller children including those in the first 2 years of life, however, is uncertain and should be further tested before choosing a zinc fortificant for foods intended for this age group.

Children with Chronic Illnesses

The use of stable isotopes may be especially relevant in studies involving children and adolescents with chronic illnesses [3, 4]. Such patients are unlikely to tolerate the dietary regulation and long-term collections associated with metabolic balance studies. One recent study used stable isotopes to assess calcium absorption in children in cystic fibrosis (CF). Earlier radioisotope studies suggested that adults with CF had decreased calcium absorption but data using a dual tracer system and data in children were lacking [5].

Schulze et al. [3] studied children with CF who were pre-, early-, or late-pubertal. Data were compared with results from similarly studied healthy children (table 1) [6, 7]. They found no evidence of decreased calcium absorption in the children with CF. This might be related to the relatively high calcium intakes and normal vitamin D levels in their study subjects. These findings suggest that it may not be simple to enhance calcium status in children with chronic illnesses by increasing their calcium or vitamin D.
intake. This area of research also points out another potential use of stable isotopes. That is, evaluating the effects of medical interventions on mineral homeostasis in adults with CF. Brown et al. [8] administered calcitriol and demonstrated an increase in radioisotope calcium absorption. Ultimately, it is reasonable to assume that this type of therapy or therapy with direct bone-acting agents, such as bisphosphonates, may be evaluated by their effects on calcium absorption and bone kinetics (see below) as determined using stable isotopes. This would obviate the need for radiation exposure and allow the studies to be more readily performed in children.

It is important that future studies also address the secretory losses of minerals in children with conditions that might affect gut losses of nutrients such as inflammatory bowel disease or CF. Although measuring dietary absorption is useful in assessing the bioavailability of fortificants and supplements, ultimately it is the net mineral retention which matters. In the case of healthy subjects, especially for calcium, and magnesium, it is likely that relatively little regulation is via endogenous excretion and oftentimes this can be omitted [9–10]. However, this assumption may not be valid in cases of chronic bowel-related illnesses.

**Kinetic Studies of Mineral Metabolism**

Although the majority of published radioisotope or stable isotope mineral kinetic studies have involved calcium and more recently zinc, we will use magnesium as our example in this report (fig. 1) [9, 11–14]. This is because, until recently, analytical difficulties and dosing issues made the use of magnesium stable isotopes extremely limited and therefore many are not aware of the potential involving magnesium stable isotopes. Even radioisotope studies of magnesium metabolism were severely limited by the limited availability and safety concerns with using $^{28}$Mg [11]. Recently, the use of high-resolution mass spectrometric analytical techniques has led to the opportunity to expand our knowledge on the physiology of this crucial, but under-researched, mineral.

Stable isotope techniques permit a unique approach to the assessment of magnesium metabolism, including absorption, excretion, pool sizes, and turnover [10, 11, 15]. We evaluated the relationship between magnesium
kinetic values and other measures of body composition. We found that fat-free mass represents the single body composition parameter that most closely correlates with magnesium kinetic data [14] (table 2). Of note is that body composition has a stronger relationship with magnesium kinetics than with calcium kinetics. These relationships provide justification for basing dietary magnesium requirements in children on body composition measures such as body weight or, where available, fat-free mass. Further studies to evaluate these relationships are indicated in situations where either magnesium status or body composition is abnormal.

Based on available human data, it is not currently possible to directly relate magnesium deficiency conditions to changes in the exchangeable pool or pool turnover rates. As with calcium and zinc, whole-body deficiency conditions may lead to an increase in both the mass and turnover of magnesium.

### Table 2. Correlation coefficients for body composition/anthropometric values and mineral kinetics (n = 22) [14]

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<th>Mg pool mass</th>
<th>Mg turnover&lt;sup&gt;1&lt;/sup&gt;</th>
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<tr>
<td>Age</td>
<td>0.39</td>
<td>0.50*</td>
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<tr>
<td>Weight</td>
<td>0.76**</td>
<td>0.55**</td>
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<tr>
<td>Height</td>
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<td>0.68**</td>
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<tr>
<td>Body mass index</td>
<td>0.72**</td>
<td>0.45*</td>
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<tr>
<td>Fat-free mass</td>
<td>0.77**</td>
<td>0.67**</td>
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<sup>1</sup>Mg turnover refers to the forward flow of magnesium from the largest identifiable pool to long-term storage, primarily in bone.

* <i>p < 0.05</i>; ** <i>p < 0.01</i>.
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from some of these pools [10, 11]. However, unlike calcium, there is no readily available method (e.g., dual energy X-ray absorptiometry) for determining whole-body magnesium content during growth. Therefore, development and enhancement of stable-isotope based methods of evaluating magnesium kinetics offers a unique opportunity to study this mineral and the effects of nutritional and medical conditions on it.

Methodological Issues

An important aspect of mineral stable isotope studies is that they can be used in any subject population [9]. This includes children of all ages, as well as pregnant and lactating women. However, special considerations are involved when performing pediatric studies. First, it is frequently difficult to obtain the same volume or sampling frequency in children relative to adults. Secondly, isotope doses must be tailored based on the body pool distribution and turnover rates which are affected by growth and puberty [15]. Finally, analgesia during painful procedures, as well as ethical issues, must be carefully considered in performing pediatric studies.

Mineral stable isotopes have been used in thousands of clinical studies with no reported complications related to their use. Complications would only be expected related to inappropriate isotope preparation or administration, especially of intravenous doses. That these have not been reported provides testimony to the exceptional safety profile of the isotopes and the care used in their clinical administration.

Our foremost concern is that the isotopes be obtained from sources which have thoroughly tested and demonstrated their purity, and which provide evidence, in the form of formal certification, documenting the origin of the isotopes, the isotopic content (enrichment), and the lack of excessive trace mineral contamination (table 3). Isotopes prepared for intravenous dosing must always be prepared in a sterile environment and rigorously tested before human dosing, which must also be done in a sterile environment.

Table 3. Recommended guidelines for purchasing mineral stable isotopes [2, 9]

<table>
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<tr>
<td>1. Original manufacturer’s assay (translated)</td>
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<tr>
<td>2. A certificate of origin from the country of isotope production</td>
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<tr>
<td>3. Independent assay of composition</td>
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Accompanying letter from the manufacturer that provides the date of manufacture, the company to which the material was sold, when it was sold, and how it was transported from the source country to the distributor.

It is recommended that purchasers obtain, or be able to readily obtain from any distributors the following documents:

1. Original manufacturer’s assay (translated)
2. A certificate of origin from the country of isotope production
3. Independent assay of composition

Accompanying letter from the manufacturer that provides the date of manufacture, the company to which the material was sold, when it was sold, and how it was transported from the source country to the distributor.
fashion by trained medical personnel who monitor patient status during and after the infusion.

An important consideration is that infusion procedures be made as painless as possible. Children have a limited ability to tolerate uncomfortable procedures, a concern which should be kept in mind when interacting with those who volunteer to participate in research studies. It should be noted that often, pediatric subjects receive no direct benefit from the study. Clearly, on principle, any potential risks and discomforts that may be associated with a child’s participation in an individual study should be very carefully scrutinized and minimized. To minimize discomfort from venipuncture, we utilize an analgesic agent for all phlebotomies. The universal use of numbing creams has made such studies better tolerated by children.

**Ongoing Issues Related to Mineral Stable Isotope Studies**

There is little doubt that the central issue remaining currently for mineral stable isotope studies, especially those involving iron, is identification of the optimal form and method for dosing. This issue extends to a long-standing controversy regarding the benefits, risks and interpretation related to the use of an intravenous iron stable isotope dose. A detailed description of these issues is beyond the scope of this review, but it remains to be determined whether the relatively smaller particle size and greater purity of centrifuge-produced isotopes leads to levels of absorption, especially for iron, that substantially exceed those obtained in food-fortification programs. This issue does not seem to be a concern for calcium where stable isotope absorption values have been shown over many years to be comparable to those obtained from both mass balance studies and radioisotope studies.

An important issue is developing the supply line and enhancing the connection between the various contributors to research regarding stable isotopes (table 4). This line begins with the scientist or physician, frequently in a developing country who has a question he or she wishes to ask. They must then find collaborators, suppliers, funding sources, research subjects and ultimately a ‘market’, such as government agencies, for the research. This is an extremely daunting task whether in the United States or other countries.

It is crucial to organize these efforts both to enhance the quality of the science, by standardizing approaches and methods, and more importantly, to make it feasible for these studies to be conducted. This requires organizing the analytical laboratories and suppliers so that scientists know who and how these may be accessed. Organization would involve providing consistent availability, purity and source information for the isotopes and analytical speed, precision and cost by the analytical laboratory. There are, in reality, many geology and other non-biological science laboratories that have the equipment to perform very high precision analysis of mineral stable isotopes.
Organization of these efforts might induce some of them to more readily consider participating in this research thereby providing more opportunities for nutritional scientists searching for analytical collaborators.

Funding agencies that have supported this type of research include, notably, the International Atomic Energy Agency, other United Nations-based organizations, the National Institutes of Health, the National Research Council (UK), and many other such government and non-governmental organization. Important research has been funded via private corporations, either directly or via foundations such as the Nestlé Foundation [16, 17].

Of note is that our group has found that virtually everyone who initially contacted us about conducting such research has overestimated the costs involved in doing these studies. We believe that many more potential scientists do not even get that far in proposing mineral stable isotope studies because they believe that these studies are impossibly expensive or even that the isotopes cannot be obtained.

This thinking is no doubt a legacy issue generated from the fact that before 1990, virtually all of the mineral stable isotopes were obtained from the US Department of Energy Calutron Program in Oak Ridge, Tenn. This program was based on full-cost recovery and thus prices for isotopes increased substantially over a short period of time, especially between 1980 and 1990. Moreover, since the Oak Ridge calutrons were placed in a shut-down mode at
various times in the 1990s (they are now completely out of service), it was thought that isotopes were in either short supply or very expensive [18]. Technological advances in isotope separation, namely gas centrifugation, have allowed large quantities of isotopes to be isolated at a relatively low cost. While not applicable to all elements the isotopes of zinc, iron and selenium are now available at higher enrichments at lower prices using the gas centrifugation approach. With respect to the isotopes of calcium and magnesium, which cannot be produced by gas centrifugation, there is an abundant supply produced on an ongoing basis from the calutrons located in Russia. There are reputable distributors who validate, assay, and alter the chemical form of the isotope when this is required for the nutrition market (table 3). Russia has been thought of as an ‘unstable source’ of isotopes but in fact they have been the key supplier to the industry for over 10 years, and reliability has not been an issue when dealing with reputable agents.

Advocacy for this type of research is also important. Often both public policy administrators as well as those trained in other nutrition disciplines may not appreciate the value or the way such data may be used. The rapidly expanding publication list of mineral stable isotope studies may alleviate much of this problem, but it remains important to emphasize the inter-relationship of epidemiological and physiological research as represented by mineral stable isotope studies.

References

7 Abrams SA, Griffin IJ, Davila PM, Hawthorne KM: Calcium absorption is similar from milk and two types of fortified orange juice. J Bone Miner Res 2003, in press.
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Discussion

Dr. Guesry: I know the cost is very different for bioavailability studies, but do you think that extrinsic tags give as reliable data as intrinsic labeling?

Dr. Abrams: I think that one cannot assume that this is the case. For every mineral and every circumstance one has to look at the data out there. The good thing is that, for the last 50 or so years, people have been looking into this issue and there is a lot of information about where the extrinsic tag is working and where it doesn’t work, so one is entirely in the dark. In general, for example, cow’s milk extrinsically tagged with calcium is a very good marker, whereas more complex markers, for instance soy milk with calcium, are very bad, it is overestimated. So it is not simple, but it is also not true that it can’t be done. For iron one virtually has to use the same source at least as what you are measuring, but it depends on the circumstances.

Dr. Castillo-Durán: For infants, what is the amount of the fluid, urine, saliva, that you need for this kind of analysis?

Dr. Abrams: For blood analysis of iron we routinely use 0.5 ml of whole blood, so that is not really problematic. Usually between 5 and 10 ml of urine is plenty for calcium and zinc. We had a problem in Nigeria because the children had such low calcium in their urine that in fact we could not find it, so occasionally one runs into special problems. But in general it is extreme volumes for what we are doing.

Dr. Castillo-Durán: And for saliva?

Dr. Abrams: Saliva is widely used by NASA for astronauts. I have to check to see how much they got, but it was not very much, the astronauts routinely did that. The question is just how accurate saliva analyses are? They have been used especially for calcium, but not very much.

Dr. Castillo-Durán: Concerning the selection of stable isotopes or radioisotopes. I understand that radioisotopes are still a lot cheaper, and I wonder whether stable isotopes are good mainly in terms of safety or accuracy?

Dr. Abrams: The shift of stable isotopes is certainly not a cause of accuracy. Stable isotope is more accurate in terms of the measurement of absorption for different things you do. It has primarily been safe, and also the overall cost of isotope studies has come down quite a bit. We can talk about details of cost, but it is a typical subject in terms of iron and zinc study. It might cost between USD 500 and 1,000 for the isotope after analysis for single subjects. If studies involve 20–50 people we might be talking about USD 10–15,000 for a study, which is not very large relative to a USD 1 million
supplementation trial. I think the real issue has been safety and the maintenance of global levels of safety.

**Dr. Hurrell:** I think that radioisotopes are much less expensive. In Kansas City we have done a lot of radioisotope studies and we calculate on maybe USD 3,000/study. With stable isotopes it is somewhat more expensive. With radioisotopes we investigate many more variables; we could look at many more levels of phytate for example. However, one of the main differences between the radioisotope and the stable isotope studies is that with radioisotope studies no additional mineral is added, but with the stable isotope studies there is. So with stable isotope studies we are always investigating a food which has milligrams of iron added to it. You can never investigate meals which are not iron fortified, which you can do with the extrinsic tag radio iron studies.

**Dr. Abrams:** In terms of the cost, I fully agree in terms of the total amount. There are issues of disposal, transport of isotopes and things like that in different areas that may affect the cost, but I would not deny that radioisotope studies are cheaper than stable isotope studies, only that the safety margin prefers the use of stable isotope in my opinion, that is why I said not everybody agrees with me. The issue of adding the mineral too is a very real one. I think for the most part, for most of the clinically relevant issues that we must ask about whether this food is a good source of iron, is this iron supplement useful, can we give doses of the isotopes so that we are not exceeding what would otherwise be given in a food that we are testing as supplement? There are some special issues certainly involved in breast milk that may be problematic, but for most issues I think that we can make use of the stable isotopes for practical questions.

**Dr. Pettifor:** Just one issue related to what Dr. Hurrell said about the use of non-labeled iron or calcium. If we talk about the calcium issue, there is going to be a difference if one adds non-isotope calcium to the meal compared to when you are using a purely tracer amount of calcium. When you are looking at absorption, just a very small amount of absorption, then you may well be looking at the effects of diffusion of calcium across the gut as opposed to the active transport of calcium.

**Dr. Abrams:** We can do studies using the stable isotope $^{46}$Ca, giving a dose of less than 1 mg of calcium total in an adult.

**Dr. Pettifor:** But the issue is which is the most relevant? I think the issue of just using a tracer amount is not particularly helpful in assessing what is happening in real life.

**Dr. Abrams:** I guess it doesn’t mix in again with the query of calcium and that again depends on the circumstances. There are certainly some questions one might ask.

**Dr. Vasquez-Garibay:** Could you explain a little bit about the use of iron incorporation and iron absorption in babies with iron deficiency anemia because that can influence the results and also the rate of erythrocyte incorporation of iron in this particular case.

**Dr. Abrams:** I will pass on the clinical aspects of segmentation rate because that is not my field, but I will say that red blood cell incorporation of iron well reflects the iron absorption of small babies, and this an area of considerable controversy right now. There is some evidence that the 14-day incorporation method does not really represent true incorporation. Comparing the studies, I am not entirely sure that it is a big effect, but there is some effect. The bigger concern is that small babies don’t actually incorporate most of the iron that they absorb. So I think that at least for now we have to recognize that for small babies there are some limitations in the red blood cell method, but it does give you at least some basis for comparison. The only way to correct that would be to do fecal collections at the same time and therefore do simultaneous mass balance, which has extreme limitations, or to give an intravenous dose
which is what we did in premature infants and some others have also done, which also has extreme limitation, so I think there are still some unknown issues here.

Dr. Barclay: Coming back to the question raised earlier, could you comment on whether to use short-term or longer-term bioavailability studies depending on the question being asked?

Dr. Abrams: The iron/calcium interaction is one of my favorites and we took 12-month-old babies and gave them calcium supplement and iron, actually gave milk and iron, and found a marked decrease in iron absorption with calcium. We adapted them for anywhere from 2 to 6 weeks in different studies and we found complete adaptation just as in adults. So one of the big questions in the studies is, let’s suppose you have a weaning food and you want to know how much iron is absorbed from it. You have two basic choices: tomorrow you can bring in 30 children from the community, give them the food, label with the isotope and measure the absorption, or you can put them on it for 2, 4 or 6 weeks, 1 year, and then measure the absorption. You are going to get different numbers, so you have to decide what the question is that you are asking, and I think they answer very different questions. My personal bias is to go ahead and adapt them for a brief period of time beforehand, but that gives you a different answer than just bringing them in tomorrow.

Dr. Gebre-Medhin: Would you comment on the issue of steady-state. Do the individuals have to be in a steady-state? How do you measure that? Does it have any importance if they are not?

Dr. Abrams: Other than what I just said, upon the change in isotope absorption or any mineral absorption over time as the iron status improved, the biggest problem we have for steady-state is when we look at body pool masses and turnover rates, especially in small children. So they are ways mathematically within the SAMM program that actually counts and models growth into those. So it is not an impossible thing to model within the SAMM program but it is certainly true that there are some limitations there. The reality is when you are looking at absorption, there isn’t any real steady-state. As long as you continue to give somebody a supplement the status is going to change and the absorption may change. So you simply have to identify some points on which you are going to make your measurement.

Dr. Zlotkin: In your institution there are some people who have been using stable isotopes to intrinsically label growing plants. I wonder if you could just talk about that for a moment because I think that the use of plant breeding is important for understanding the bioavailability of minerals from new plant sources. It might be important in the future.

Dr. Abrams: There is no question that the extrinsic tag is not the way to go if you are working with broccoli, it has to be labelled intrinsically. The use of hydropomax allows that to be done. The really big problem is that of cost because a considerable amount of isotope is going to be lost in the growing process. So one of the things that plant physiologists have to do is to recycle the water and keep the losses to a minimum. In fact, depending on the plant and the situation, they have become pretty good at that. As we see all sorts of new types of crops develop a stable isotope, with higher levels of iron or whatever, there is no question that, if scientifically the technique currently exists to intrinsically label them and to test those in people, it could be done today.

Dr. Hurrell: You mentioned pools, and I am a little unclear what you mean by the fast pool and the slow pool. Could you explain what parts of our physiology they are? The second question is about trying to use the size of the pools to measure micronutrient status. Can you explain this?

Dr. Abrams: One of the things noticed about calcium a long time ago is when a patient became deficient in calcium some of the body pools increased. The same thing
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is probably true for zinc as well. When a kinetic study is made it is critical what the model is and how it is divided up, what type of equations are used, and a multi-equation model or a multi-component exponential model are the same model to determine what period of time you are looking at. These are purely theoretical constructs until animal studies are done. This has been done somewhat with zinc to try to figure out where they are and what tissues they are in. For example, for calcium we know that even the fastest turnover pool is mostly serum plasma versus a small amount of bone. With zinc we have all sorts of tissues that are mixed into these, including liver and so on. But the bottom line is that the pool is a theoretical construct, and there is no question that being deficient may make some of your pools increase because when there is a deficiency the turn over increases to protect the initial serum pool.

Dr. Barclay: Could you comment on the doses of stable isotopes or the amounts of calcium administered in calcium absorption studies? If you look at absorption values for different calcium fortification compounds, there are large differences between studies and a lot of this could be due to methodological differences. What are the most important factors to take into consideration in this context?

Dr. Abrams: Calcium absorption intensely depends upon the role of total calcium given and what the meal components of that are. Heaney et al. [1] have spent many years documenting that beautifully. They have a perfect curve relating the dose of total calcium and absorption, and this was published about 12 years ago. So there is no question that when these things are used, they must be standardized. The high dose of the isotope itself isn’t much of an issue, it is only a tiny amount. When giving the meal, typically a glass of milk in a calcium study, it depends on whether the isotope is given at the beginning or at the end of the meal or half an hour after a meal. So any given group certainly has to standardize what it is doing and recognize those supplementations. We have done a lot of studies trying to relate what we get with isotopes to other methods including mass balances, so we have a reasonable idea of what the best way is to do them, but it is very critically time dependent. That is one of the reasons why the numbers vary all over the place and why there is so much controversy about things like whether or not calcium citrate or calcium carbonate is better absorbed.

Dr. Villalpando-Carrión: I was wondering if you are aware of this pool model? Does the iron pool model stimulate, correlate or to some point move from one pool to the zinc model?

Dr. Abrams: Theoretical kinetics, that is working with iron pools, is a method that pretty well died with radioisotope studies about 30 years ago. Very few people have done them in humans since then. We are doing them because until very recently it was hard technically to do iron isotope ratios and anything other than red blood cells.

Dr. Barclay: One calcium isotope that has not been discussed today is $^{41}$Ca.

Dr. Abrams: I removed that slide. For those of you who are not familiar with it, $^{41}$Ca is an extreme along isotope with a half life of millions of years. It is somewhat radioactive and somewhat stable and it has a multi-million year half life with a radiation exposure that is essentially negligible. The nice thing is that it exists naturally in every one’s tissues so you could have a tiny dose in the permanent lifetime turnover rates. The drawbacks are first of all that special isotope analysis techniques are not widely available, and the second is that you have to be looking over a very long period of time. It is the cutting edge of calcium isotope work. The reason I am not too involved in it is that I decided a long time ago that I would not give a radioisotope to a child no matter what, and I stand by that. Other people might have another ethical perspective because the doses are extremely small in practical terms, the problem is really safety. In adults I think it is a tremendous opportunity to look at long-term turnover rates. It is something that will be seen in the future. A Swiss group and some others are actively involved in this work.
Dr. Barclay: Would you also give a rough estimate of the cost per subject of an iron or a calcium bioavailability study?

Dr. Abrams: Just to give you an idea, right now a $^{58}$Fe study runs at more than USD 50 or 60/mg. A study in a typical child might use 1–2 mg $^{58}$Fe. So if you are going to give that, it again depends upon what you are doing, you may be talking about USD 100. If you are going to give a reference dose of 2–5 mg $^{57}$Fe, it is running at about USD 10–15/mg. So we are roughly talking about USD 200 to study a small child. That can be roughly doubled or tripled if you are talking about a pregnant woman. As you go up, it depends on what you are looking at. So from USD 200 to 500 is the current price for the isotope analysis. We would generally charge anywhere between USD 50 and 150/sample, and iron study is one sample so we are not talking that much in general. A zinc study is 1 or 2 samples, a calcium is 1 or 2 samples. So I think we are talking about isotope dosing in the range of USD 300–500, 600/mineral tested.

I only want to comment that in terms of safety it is not causing any harm. There is nothing different to what we do than draw blood. I think every single one of you would not hesitate to do a research study in which you draw blood from the subject. There is not one bit of difference between that and the stable isotope study in terms of what the child must go through. We use numbing cream for every single child, for every single blood sample draw, any single site, we have a very large team of medical college students in the laboratory to provide amusements and distractions, etc. But it does involve a velocity of research, and we have done this in many of the countries that are represented here, virtually all of them are represented here.

Dr. Zlotkin: I think that understanding physiology in the year 2003 really does involve using stable isotopes in children to understand both the normal child and a pathological condition. This really does demonstrate that it can be done. It can be done safely; it can be done within a reasonable price range. We have an obligation to understand the metabolism of micronutrients as we go forward with programs, and I think this exemplifies that very well.

Reference
