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Review

The use of stable isotope techniques to assess zinc metabolism

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The refinement of techniques that can accurately measure small changes in zinc stable isotope ratios in biological samples provides new opportunities for advancing our understanding of human zinc metabolism. The feasibility of utilizing more than one zinc stable isotope label simultaneously is invaluable for more complex kinetic studies. These techniques are especially valuable for investigations of the regulation of Zn homeostasis in infants and in women during the reproductive cycle in whom problems with zinc nutriture may be relatively frequent and of concern for pre- and postnatal growth and development. Initially, these techniques have been applied to studying the role of the intestine in the maintenance of zinc homeostasis and have served to emphasize the importance of the modulation of fecal excretion of endogenous zinc. Application of stable isotope techniques to explore zinc metabolism beyond the intestinal tract is still limited but has considerable potential for advancing our understanding of zinc metabolism in health and disease. (J. Nutr. Biochem. 6:292–301, 1995.)

Keywords: zinc; stable isotopes

Introduction

Since the biological importance of zinc as an essential trace metal was first recognized over a century ago, numerous and diverse roles in cell biology have been established for this micronutrient.[1] Our previous understanding of the physiologic roles of zinc, however, has been dwarfed by recent recognition that the "zinc finger" is the most common motif for DNA binding sites of transcription proteins.[2] A similar motif has been found to have a crucial role in several ligand-activated nuclear receptors which include steroid and thyroid hormones, retinoic acid, and vitamin D receptors.[3] Concurrently with these recent and impressive advances in our understanding of the biology of zinc, there has been a steady growth in recognition of the practical importance of adequate zinc nutriture for normal human growth and development and disease prevention. Still unclear is the precise manner in which zinc homeostasis is maintained normally and how it is impaired when clinical manifestations of zinc deficiency occur. Little is also known about the accompanying changes in whole body zinc metabolism. Resolution of these unanswered questions should greatly improve our ability to prevent as well as to detect zinc deficiency.

Our understanding of human zinc metabolism has, until quite recently, depended to a great extent either on traditional metabolic balance studies and/or investigations employing zinc radioisotope labels. While carefully performed zinc metabolic balance studies coupled with insightful interpretation of analytical data have contributed to our understanding of zinc homeostasis,[4] progress has been restricted by the inevitable limitations of these experimental data. Studies employing zinc radioisotopes have yielded invaluable information on zinc absorption[5–9] and, all too rarely, on endogenous zinc excretion.[10] When combined with compartmental modeling, techniques employing radioisotopes offer the potential for more comprehensive studies of whole body human zinc metabolism.[11–21] Yet, radioisotope techniques also have their limitations. Although with high quality detection systems it is possible to use doses that are low enough to be safe, there remain restrictions on the use of these radioisotopes. This applies especially to some of the population groups in which studies are most needed,

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e.g., young children or women in the reproductive cycle. These limitations are magnified by the long half-life (243.6 days) of ^{65}Zn, while the utility of ^{69m}Zn is limited by the very short half-life of this isotope (13.5 hr).

There were very few investigations utilizing stable isotopes of minerals or trace elements prior to 1980.[22] During the next decade, advances in mass spectrometry techniques were a major reason for a steady growth in the application of stable isotopes to studies of human mineral/trace element metabolism, especially studies of metal absorption. While these isotopes circumvented the restrictions on use of radioisotopes, they also have several limitations. An important example is the inability to detect stable isotope labels by total body counting or regional scanning. A second potential limitation is the quantity of the zinc stable isotope label that is required, which, in contrast to radioisotope tags, may be sufficient to disturb the physiology of the tracee.[23] Considerable care, therefore, must be taken to optimize the dose administered.

There are, however, also several potential advantages to the use of stable rather than radioisotope zinc labels. Outstanding among these is the availability of multiple zinc stable isotopes, which permits the administration of multiple labels simultaneously in any one human study. The stable isotopes of zinc, together with their abundance in naturally occurring zinc, are given in *Table 1*. Three of these isotopes, ^{67}Zn, ^{68}Zn, and ^{70}Zn, occur naturally in low enough abundance that it is feasible to use enriched preparations of these isotopes to study human zinc metabolism. A second important advantage of these stable isotopes is that they are entirely safe and there is thus no restriction on their use in infants and young children or during the reproductive cycle.

Technical aspects of measuring zinc stable isotopes in biological samples

Mass spectrometry is the analytical technique of choice for measuring stable isotopes.[22,24–27] There are several types of inorganic mass spectrometry that are most likely to be applied to the analysis of stable isotopes in tracer studies of mineral nutrition and metabolism: thermal ionization mass spectrometry (TIMS), inductively coupled plasma mass spectrometry (ICPMS), and fast atom bombardment mass spectrometry (FABMS).

With a long history of development and use in geochemical and nuclear isotope applications, TIMS is the most precise and accurate of these techniques. Relative precision figures of 0.05 to 0.5% have been reported for zinc isotope ratio measurements on 2 to 6 μg samples. This level of precision comes at a high price, however. At 1 to 3 hr/measurement, the analysis time is so long as to be almost impractical for tracer kinetic studies involving frequent multitissue sampling.

Table 1 Zinc stable isotopes and their natural abundance

Isotope	Abundance (%)
^{64}Zn	48.6
^{66}Zn	27.9
^{67}Zn	4.1
^{68}Zn	18.8
^{70}Zn	0.6

Furthermore, rigorous sample cleanup is required and one isotope must be reserved for ratio normalization in sample-by-sample fractionation correction. Thermal ionization mass spectrometers require considerable operating skill to attain the level of performance of which they are capable and, due primarily to their expense and specialized nature, these instruments are relatively rare compared with other types of mass spectrometers discussed here. Nonetheless, TIMS has played a major role in the coming of age of stable isotope studies of mineral nutrition and metabolism and will continue to be the precision and accuracy benchmark for isotope ratio analysis in the foreseeable future.

ICPMS, on the other hand, is of fairly recent origin and has experienced explosive development and growth over the past decade. The reported precision of zinc isotope ratios measurements by ICPMS is 0.3 to >1.0%. These analyses require <20 μg of zinc in 1 to 2 mg/L solution. While this technique's precision is not as impressive as that of TIMS, the use of ICPMS presents some significant advantages: analysis time is only 5 to 10 min (after an instrument startup time of 1 to 2 hr); sample cleanup need not be as extensive as that required by TIMS; and ICP mass spectrometers are in widespread use, their major application being quantitative elemental analysis. Though there is a significant instrumental background (molecular ions) inherent to ICPMS which precludes the analysis of certain isotopes of other metals, the measurement of zinc isotopes is minimally affected. Also, careful use of isotope standards is necessary to assure measurement accuracy since instrumental mass discrimination is evident with the technique. The continuing development and refinement of ICPMS instrumentation and methods have made it the most widely used metal isotope analysis technique.

While not being as actively developed or as popular as ICPMS, FABMS has been shown to be similarly well-suited to metal isotope, including zinc, analysis.[28] FABMS falls under the more general category of secondary ion mass spectrometry (SIMS) and, by the name FABMS, is more widely known as a powerful technique for analyzing large, labile polar molecules. Its application to metal isotope analysis has occurred primarily in the field of nutrition and metabolism research because FAB mass spectrometers designed for molecular analysis became widely available in biomedical research laboratories in the mid-1980s and could, with minor modification, become multipurpose instruments. This remains a unique advantage to the use of FABMS for metal isotope analysis.

With FABMS, using ion counting detection and peak switching, we attain a relative precision of 0.2 to 0.6% (depending on the ratio) on analyses of 1 μg samples of aqueous zinc standard or zinc isolated from biological material (L. Miller et al, unpublished data). A complete analysis of three ratios ($^{67}Zn/^{66}Zn$, $^{68}Zn/^{66}Zn$, $^{70}Zn/^{66}Zn$) as well as a hydride signal takes 20 to 25 min. As with TIMS, samples must be rigorously isolated and purified from their biological matrices. Remaining interferences, with the ex-

ception of the monohydride of zinc, are eliminated using selective ion energy filtering. The effects of isobaric interference from ^{66}ZnH and ^{67}ZnH are mathematically corrected using the measurement of $^{68}ZnH/^{68}Zn$. FABMS exhibits isotope fractionation and, like the other techniques, requires the use of ratio normalization or isotope enrichment standards to ensure measurement accuracy. Like ICPMS, the potential of FABMS as an accurate, precise high sample throughput metal isotope analysis method has yet to be fully realized.

Application of zinc stable isotopes to studies of human zinc metabolism

When utilizing either radio or stable zinc isotope techniques, it is desirable to design studies whenever possible that provide the opportunity to examine experimental data in relation to whole body human zinc metabolism. This approach offers the potential to extract the maximal amount of information from the experimental data. It is also, however, relatively complex and necessitates sampling regimens that are frequently impractical, e.g., multiple blood samples. To date, the majority of published reports of the application of zinc stable isotope techniques have focused specifically on the gastrointestinal tract.

Zinc homeostasis in the gastrointestinal tract

While additional sites involved in the regulation of zinc metabolism have been identified,[17] the gastrointestinal tract is of great importance in maintaining whole body zinc homeostasis. It is the location of two key aspects of regulation: the absorption of exogenous zinc and the secretion/reabsorption of endogenous zinc (*Figure 1*). The latter, in turn, determines the quantity of endogenous zinc excreted in the feces. One major advantage conferred by the use of isotope techniques is that these two processes can be distinguished quantitatively, a feat which cannot be accomplished by traditional balance techniques alone.

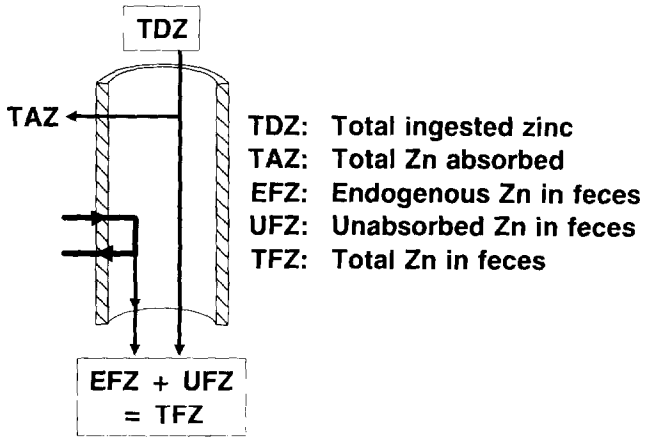

Figure 1 Schematic of small intestine depicting absorption and excretion of zinc.

Zinc absorption

Conceptually, the simplest technique for measuring fractional absorption of exogenous zinc (FAZ) is to give an extrinsic isotope label orally, measure cumulative fecal excretion of this label, and subtract the latter from the oral dose administered:

$$FAZ = 1 - Fraction\ of\ dose\ excreted\ in\ feces$$

Total absorbed exogenous zinc per day (TAZ) can then be calculated as follows:

$$TAZ = FAZ \times TDZ$$

where TDZ = total dietary zinc (mg of zinc/day).

There is one potential source of error in the determination of FAZ and TAZ that is specific to this cumulative fecal excretion method of determining absorbed zinc. This is the difficulty of distinguishing unabsorbed label in the feces from label that has been absorbed, then secreted into and excreted from the gastrointestinal tract. Following the intravenous administration of zinc isotope label, approximately 0.5 to 1.0% of the dose appears in the feces each day; this quantity gradually diminishes with time.[29] It is, therefore, reasonable to assume that a similar percentage of absorbed label from an orally administered isotope will be returned to the lumen of the intestinal tract and excreted in the feces. Early stable isotope analytical techniques lacked sufficient sensitivity to detect this excretion of absorbed/secreted label.[30] With the advent of more sensitive mass spectrometry techniques, as well as with the application of radioisotope techniques, a small quantity of isotopic enrichment can be detected for many days after the elimination of unabsorbed label is apparently complete. The latter is generally within the first five fecal samples after oral administration of label, though in the occasional study fecal excretion of unabsorbed isotope appears to be more protracted. It is thus desirable to collect a minimum of 10 stools. Calculation of FAZ will vary by several percent according to whether this continuing excretion of small quantities of label is disregarded,[22,24,31,32] considered as unabsorbed label,[33,34] or as label that has been absorbed, secreted, and excreted.[35] According to calculations based on a compartmental model of whole body zinc metabolism, unabsorbed label continues to make a substantial contribution to fecal enrichment for many days after administration of label.[20] This model was, however, based on research that included limited experimental data related directly to the intestine.[17]

We have tested the hypothesis that this residual enrichment, after approximately 5 fecal samples, could be attributed entirely to isotope that had been absorbed, secreted back into the lumen of the intestine, and then excreted in the feces.[35] The study design included the simultaneous oral and intravenous administration of different stable isotopes of zinc followed by determination of cumulative fecal excretion of both isotopes. If this hypothesis is correct, the slopes of cumulative excretion of orally and intravenously administered label should be identical, after elimination of the unabsorbed isotope is complete, when expressed as a

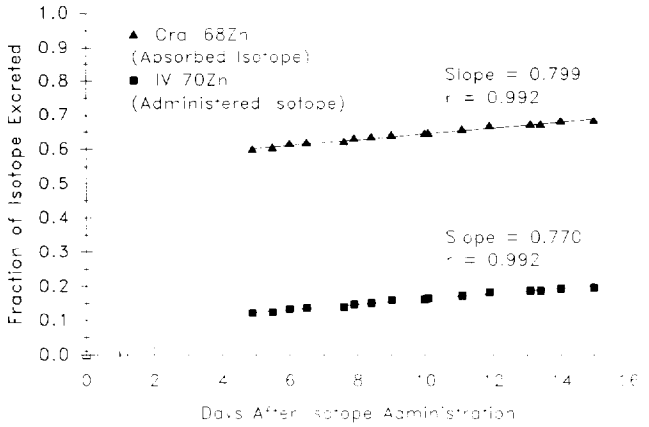

Figure 2 Cumulative fecal excretion of orally (^{68}Zn) and intravenously (^{70}Zn) administered stable isotopes of zinc. Data for ^{68}Zn are expressed as a fraction of the absorbed isotope excreted. Data for ^{70}Zn are expressed as a fraction of the intravenously administered isotope excreted in feces.

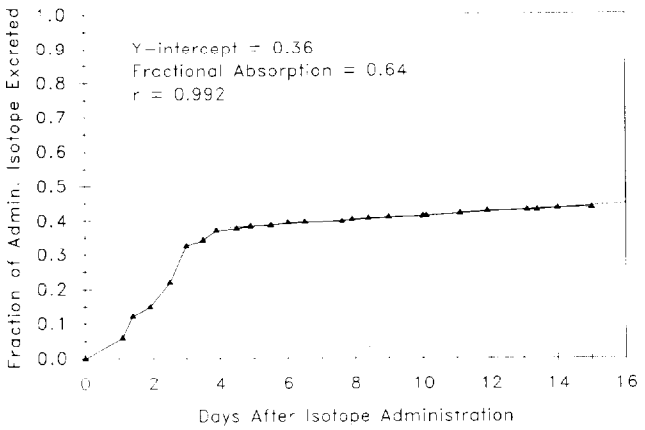

Figure 3 Cumulative fecal excretion of orally administered isotope depicting correction for isotope that has been absorbed, secreted back into the intestinal lumen, and excreted in the feces.

fraction of absorbed and administered dose, respectively (*Figure 2*). While these plots are not truly linear, the approximation is very close. The mean (±SD) r value for linear regression through these points was 0.966 ± 0.065 for studies in 12 normal adults. The slopes obtained from linear regression analysis for the orally and intravenously administered isotopes were, therefore, compared. The mean slopes for the intravenously and orally administered isotopes, respectively, were 0.76 ± 0.08 and 0.86 ± 0.19% of administered isotope per day (P = 0.63). While the potential for a beta error in this study is recognized, these results strongly suggest that the fecal isotope enrichment subsequent to the first five fecal samples is attributable primarily to isotope that has been absorbed and then secreted back into the intestinal lumen and subsequently excreted in the feces. Since absorption is a rapid process and secretion of a small percent of absorbed isotope is likely to start soon thereafter, we adjust routinely for secreted isotope by extrapolating this regression line back to the time of isotope administration (*Figure 3*). It is recognized that there is a risk of overestimating absorbed zinc, but our data indicate that the magnitude of this potential error is small compared with the potential for underestimating absorption if no adjustment is made.

There are other potential pitfalls with determination of TAZ by extrinsic label that are not limited to the cumulative fecal excretion method. One consideration is the quantity of label. When radioisotopes are used, this is extremely small and can be ignored, but the same is not necessarily true for zinc stable isotope labels. Beyond a certain threshold, FAZ decreases progressively with increasing total quantity of zinc ingested.[23,36] When the zinc is ingested with water alone, this threshold for adults appears to be approximately 5 mg of zinc. When the zinc is ingested with a meal, the threshold is lower (≤1 mg) and less well defined.[23] The quantity of zinc stable isotope label used should always be kept to the minimum that it is necessary to achieve an accurate measure of enrichment in the target pool(s).

Another potential concern is the extent to which the behavior of the extrinsic isotope label reflects that of the intrinsic zinc in the diet. Results of one animal study indicated comparable absorption.[37] Studies have been undertaken in humans in which single food items have been intrinsically labeled with a zinc stable isotope.[38–40] These foods have been fed together with a second stable isotope of zinc as an extrinsic label. In one,[39] there was a good correlation between intrinsic and extrinsic but absolute values differed by about 25%. In the others, the ratios of fractional absorption of the extrinsic to that of the intrinsic label were not significantly different from unity, though the risk of a beta error appeared to be substantial.[38,40]

We have undertaken pilot studies using a simpler and less expensive technique. The approach is as follows: The quantity of endogenous zinc excreted in the feces (EFZ) is measured by an isotope dilution technique (see next section) and subtracted from the total fecal zinc (TFZ) to calculate the quantity of unabsorbed exogenous dietary zinc excreted in the feces (UFZ): TFZ − EFZ. Total absorption of exogenous dietary zinc is then determined as follows: TDZ − UFZ. Total absorption of extrinsic label is determined simultaneously by the administration of extrinsic label with each meal over the course of one day during the study period. Initial comparisons in infants fed human milk or infant formula are given in *Table 2*. Apart from the economy of this method, absorption of exogenous dietary zinc can be determined from composite meals which is not feasible when selected food items are intrinsically labeled.

Table 2 Comparison of two methods of calculating total absorbed zinc (mg Zn/day) from human milk or cow milk–based infant formula (subjects are normal infants)

Extrinsic label Technique	Net absorption + endogenous fecal zinc	Δ
0.67	0.68	−0.01
0.55	0.61	−0.06
0.14	0.18	−0.04
0.26	0.26	0.00
0.85	0.80	+0.05

r = 0.99, $P < 0.001$

The cumulative fecal enrichment method for determination of fractional absorption is labor intensive, requiring the separate analysis of at least eight individual samples. Any error in sample collection, preparation, or analysis, especially in the early postlabel samples, will affect the accuracy of results without any obvious means of detecting such an error. Accordingly, an alternative technique for determining fractional absorption has been evaluated which is based on the ratio of enrichments of orally and intravenously administered isotopes (adjusted for doses administered) in urine.[41] Separate estimates of absorption can be derived from each random urine sample. Repeated urine sample collections between 3 and 10 days postlabel administration can thus yield multiple estimates of fractional absorption. In our experience, this is a simpler and generally more reliable approach than the cumulative fecal excretion technique. An example of data derived from one study[41] is depicted in *Figure 4*.

The substitution of urine for plasma enrichment measurements has been emphasized because a large proportion of our application studies has been in infants and children or in other circumstances in which it is difficult to collect multiple blood samples. Where detailed plasma kinetic data are available, deconvolution[15] and/or compartmental modeling techniques[20] can be used to determine absorption. Pilot studies have found close agreement between results derived from compartmental modeling and from the simpler techniques described above (N. F. Krebs et al., unpublished observations).

Endogenous fecal zinc

Determination of fractional absorption of extrinsic zinc label by one of the methods discussed in the previous subsection can be combined with a traditional metabolic balance study to obtain an estimate of endogenous fecal zinc (EFZ):

$$EFZ = TAZ - NAZ$$

where TAZ = total absorbed zinc (mg/day) = total dietary zinc × fractional absorption of zinc. NAZ = net (apparent) absorption of zinc (mg/day) = total dietary zinc − total fecal zinc.

This approach has been used to estimate EFZ in several recent human studies.[32,42] However, it depends on the traditional balance technique and on two equations that require the subtraction of one relatively large number from another number of similar magnitude.

Because of these concerns, we have preferred to use an isotope dilution technique to determine EFZ. This technique is based on that first described and validated in animal models by Weigand and Kirchgessner[43] and first applied in the human by Jackson.[44] It is based on the assumption that the endogenous zinc excreted in the feces is derived from a pool of zinc that exchanges rapidly with zinc in plasma and in certain solid tissues. Weigand and Kirchgessner[43] demonstrated that urine enrichment could be substituted for enrichment in selected solid tissues, while Jackson[44] used plasma enrichment in his initial human study. We have applied the following modification of Jackson's formula:

$$EFZ = \Sigma\ (TFZ \cdot f)/p \cdot d \text{ or } EFZ = \Sigma\ (TFZ \cdot f)/u \cdot d$$

Where TFZ = total zinc in fecal sample, f = isotope label enrichment in corresponding fecal sample, p = average plasma enrichment during metabolic collection, u = average urine enrichment during metabolic collection, and d = days of metabolic study. Our experience[45] has supported the validity of using urine rather than plasma which offers considerable advantages for many human studies.

For the isotope dilution technique as first described, the zinc stable isotope was administered parenterally and, in the case of human studies, intravenously. Theoretically, it is feasible to substitute the oral route of administration provided that the metabolic studies are delayed until after all unabsorbed extrinsic label has been eliminated from the intestinal tract as discussed above. This assumption has received experimental support[45] from comparisons of data of EFZ derived from simultaneous oral and intravenous administration of zinc stable isotopes to the same individuals (*Figure 5*). Use of the oral route has obvious practical advantages, especially in studies of young infants.

Whole body zinc metabolism

Human zinc metabolism is complex and it is beyond the scope of this review to do more than make passing reference

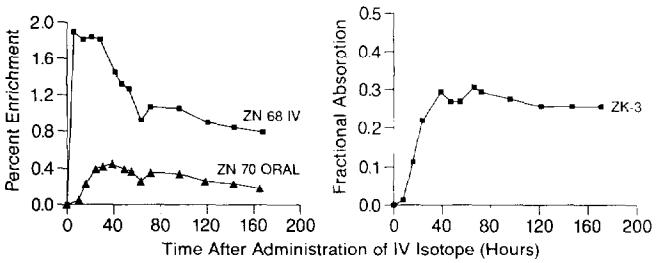

Figure 4 (a) Urine enrichment with ^{68}Zn(intravenous) and ^{70}Zn (oral). (b) Calculated fractional absorption of ^{70}Zn at each data point from 2 to 7 days.

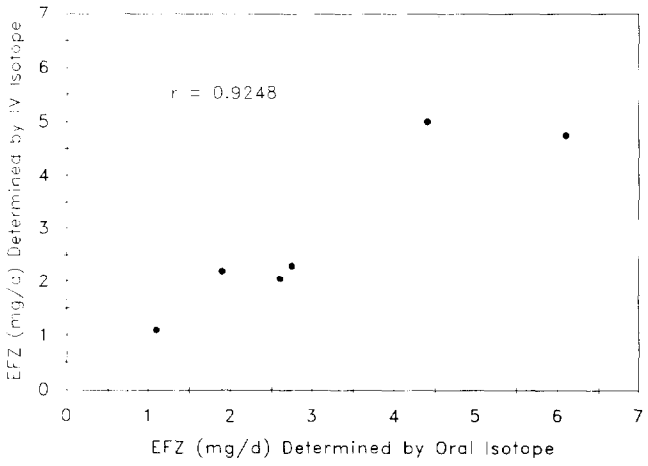

Figure 5 Endogenous zinc excreted in feces (EFZ) determined by isotope dilution technique. Correlation of EFZ determined simultaneously in the same individuals with orally and intravenously administered isotopes.

to the challenges it presents. Whole body metabolism of zinc in the human adult has been investigated most effectively and completely using compartmental analysis based on experimental data derived from radioisotope techniques that included whole body and regional counting as well as plasma and erythrocyte kinetics in a substantial number of normal adults.[17] During the later phases of data collection, these adults received a large quantity of supplemental zinc daily. This allowed identification of five sites of regulation of zinc metabolism: two in the intestine, one each in the kidneys, muscle, and red blood cells. This model has been applied in investigations of zinc metabolism in selected disease states[16] and disorders of taste and smell,[13,14] and to assess the effects of dietary fiber[18] and of aging[21] on zinc metabolism. In each of these studies, ^{65}Zn has been used, but similar data have been obtained by application of stable isotope data to the whole body model[46] (N. F. Krebs, unpublished data).

It is currently not feasible to derive such extensive experimental data in the human with the use of stable isotope techniques. Hence, it is necessary to either develop more limited and simpler models to assist with data analysis or to use the existing model of whole body zinc metabolism derived from more extensive radioisotope data to fit experimental data from stable isotope studies.[46] The latter is, theoretically, an attractive choice provided investigators are cognizant of the potential limitations of this model and are aware how to use it effectively for their own research.

Zinc that exchanges rapidly with zinc in plasma

The pools of zinc that exchange rapidly with zinc in plasma are thought to have a vital role in most of the body's zinc-dependent metabolic processes and are of particular potential interest in studies of zinc nutriture. Several recent reports have focused on this rapidly exchanging zinc.[47–49] We have been especially interested to estimate the total size of the combined pools of zinc that exchange with plasma zinc within 48 hr (exchangeable zinc pool, EZP). Apart from its potential value in the assessment of zinc nutriture, it is possible to achieve an estimate of the size of EZP without frequent or indeed any plasma sampling. The method uses the simple exponential function fitting the plasma enrichment data between 2 and 10 days after intravenous administration of an isotopic label. The y-intercept of this function gives an approximate isotope enrichment resulting from dilution of the isotope in the EZP. Therefore, the EZP size can be estimated by dividing the dose administered by this enrichment value.

We have ascertained that sampling can be further simplified by substituting urine enrichment for plasma enrichment. This has allowed preliminary extension of research on the size and other parameters of the EZP to otherwise inaccessible study populations. These include infants, children, and populations it is desired to evaluate under "field" conditions. The inherent sources of error and imprecision in this approach have been carefully evaluated and recently discussed.[49] While we are continuing to investigate these limitations with the use of compartmental modeling and the development of correction algorithms, our tentative conclusion is that they are not of sufficient magnitude to negate the potential value of this technique in studies of human zinc metabolism.

Theoretically, an additional concern regarding this approach has to be considered when studying the infant and growing child. The growth process implies that steady-state kinetics are not applicable. The impact of growth, however, is very small and will not add appreciably to the inaccuracy of this approach except possibly in exceptional circumstances such as the extremely low birth weight infant during a phase of very rapid postnatal growth.

Examples of application of these techniques

This review will be concluded with an overview of selected examples of the application of these techniques.

Zinc homeostasis in the breastfed infant

It has been widely accepted for some time that net absorption of zinc from human milk must be exceptionally high. This conclusion has been based on the apparently adequate zinc status of most breastfed infants despite the relatively low zinc content of mature human milk. While previous studies of zinc absorption from human milk have been reported in the human adult[50] and in the rat pup model,[51] it has not been possible to undertake quantitative measurements of zinc absorption and fecal excretion of endogenous zinc in the breastfed infant prior to the development of zinc stable isotope techniques. Recent measurements using zinc stable isotopes have demonstrated favorable fractional absorption and only modest excretion of endogenous zinc in the feces.[52] Fractional absorption averaged slightly more than 50% and daily absorption of zinc between 2 and 4 months in fully breastfed infants averaged 0.6 mg/day. With excellent conservation of losses of endogenous zinc via the intestine, net absorption averaged a little over 0.3 mg of zinc/day. Despite favorable absorption and conservation of endogenous zinc, this is considerably less than previous estimates of requirements for net absorption by infants at this age.[53] No data are available on the composition of the weight gain of these infants during the period of the metabolic studies, and it is possible that this weight gain included a high proportion of adipose tissue. Other possibilities are that the data from which the earlier estimates were derived are inaccurate or that zinc retention in these infants was less than optimal. Some fully breastfed infants have been reported to develop zinc deficiency which may be clinically severe[54] and there is some evidence to suggest that milder growth limiting zinc deficiency may be quite common in the breastfed infant.[55] It will require further research, including but not limited to stable isotope techniques, to determine which of these alternatives alone or in combination provides the correct explanation.

Studies of the efficacy of intestinal conservation of endogenous zinc

A notable finding in the studies of normal breastfed and formulafed infants is that differences in zinc intake are narrowed by the absorptive process, but it requires modulation of endogenous zinc excretion by the intestine to "fine tune"

zinc homeostasis. With this fine tuning, net zinc absorption remains quite similar across a wide range of zinc intake as depicted in *Figure 6*.[52] The correlation observed between the quantity of zinc absorbed and the quantity of endogenous zinc excreted (*Figure 7*) is also compatible with a role for endogenous zinc excretion in maintaining zinc homeostasis.

Although traditional balance studies without the addition of any stable isotope techniques will theoretically yield similar data for net absorption, they provide no insight into the differences in fractional and total absorption or/and excretion of endogenous zinc in the feces that result in similar net absorption despite widely differing intakes of zinc.

Further evidence of the importance of regulation of excretion of endogenous zinc by the intestine in achieving zinc homeostasis has been derived from studies of adult women of childbearing age living in northeast China (S. Lei, et al., unpublished data). Two groups were studied: a rural group with an average zinc intake of 5 mg of zinc/day and an urban group with an average intake of 8 mg of zinc/day. Both groups had a similar dietary phytate: zinc molar ratio of approximately 10 to 1. Fractional absorption was virtually the same for both groups (approximately ⅓ dietary zinc) which resulted in total absorption being substantially lower in the low zinc rural group. This was accompanied, however, by a lower excretion of endogenous zinc so that net absorption was the same for both groups, each of which was in balance. The results of this study have highlighted the key role of endogenous zinc excretion via the intestine in long-term zinc homeostasis as well as for short- and medium-term periods of homeostatic adjustments.[56]

Though the studies referred to above all emphasize the ability of the human to maintain zinc homeostasis in a variety of circumstances, preliminary data from studies of young infants with cystic fibrosis illustrate how homeostatic mechanisms can be disrupted in abnormal circumstances. These studies have been undertaken at approximately 6 weeks of age in infants diagnosed with cystic fibrosis as a result of the Colorado Newborn Screening Program. While fractional absorption appears to be somewhat lower than in normal infants, the striking difference between these infants and normal infants has been in the losses of endogenous zinc in the feces. In general, these have been inappropriately high for the quantity of zinc absorbed leading, in some instances, to grossly negative net zinc absorption (*Figure 8*).[57]

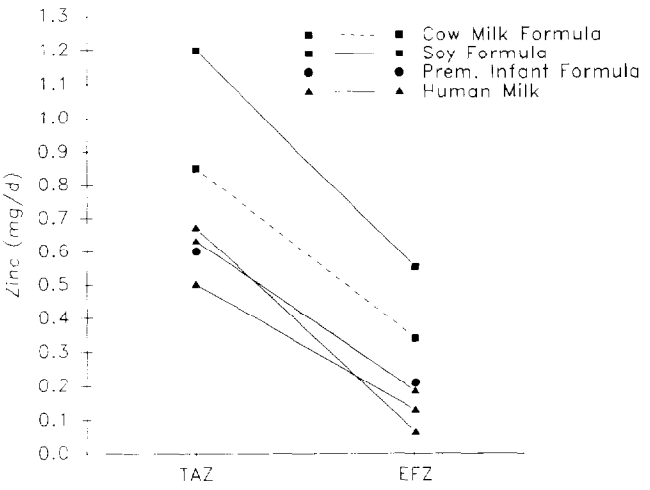

Figure 7 Total absorbed zinc (TAZ) versus endogenous fecal zinc (EFZ) in the same infants whose data are depicted in *Figure 6* ($r = 0.93$, $P < 0.01$).

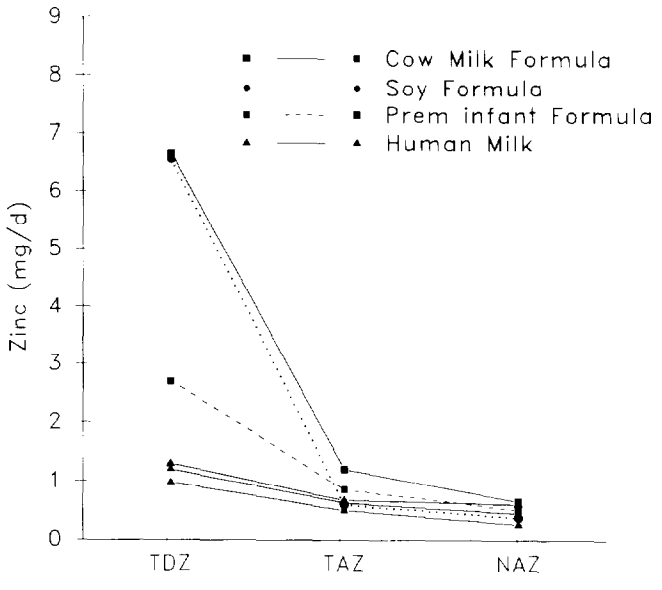

Figure 6 Total dietary zinc (TDZ) versus total absorbed zinc (TAZ) versus net absorbed zinc (NAZ) in normal young infants including one delivered prematurely. NAZ calculated by subtracting endogenous zinc in feces (EFZ) from TAZ. EFZ determined by isotope dilution technique.

Exchangeable zinc pool

The estimated quantity of zinc in the EZP has previously been found to have a linear relationship with dietary zinc

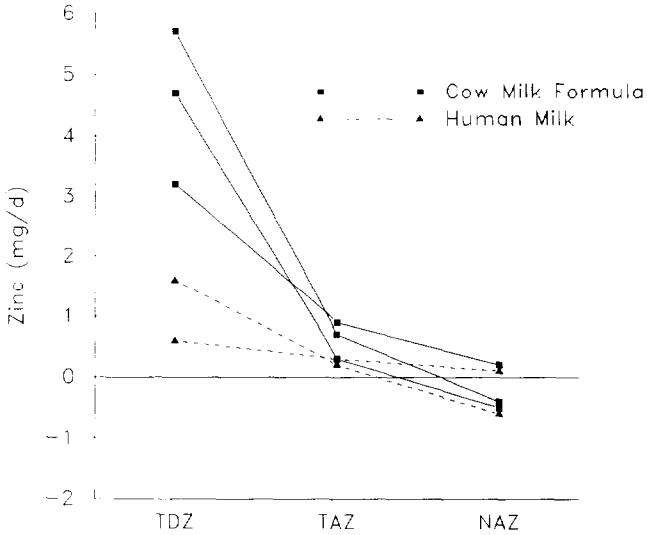

Figure 8 Total dietary zinc (TDZ) versus total absorbed zinc (TAZ) versus net absorbed zinc (NAZ) in young infants with cystic fibrosis. High endogenous zinc losses in feces resulted in severe negative NAZ in three infants.

intake.[49] These data have now been extended to include subjects from northeast China who had generally lower dietary zinc intakes. Analysis of combined Colorado and Chinese data indicated a strong positive correlation between EZP size and dietary zinc. Regression analysis demonstrated an equally good fit for linear ($r = 0.863$) versus exponential ($r = 0.878$) or versus polynomial ($r = 0.881$) regression (*Figure 9*).

For the Chinese subjects alone the correlation was quite weak, probably reflecting the small range of dietary zinc. There was, however, a strong positive correlation between the quantity of zinc absorbed (TAZ) and the quantity of zinc in the EZP ($r = +0.762$, $P < 0.001$) for the Chinese subjects (S. Lei, et al., unpublished data). A similar correlation had previously been observed in our infant studies (*Figure 10*).[58]

These results suggest that the size of the EZP may depend on recent zinc absorption. They also provide a measure of zinc nutritional status by giving information on the mass of a lumped pool of zinc that is considered to be of special importance for zinc-dependent metabolism. Finally, a weaker but persistent correlation has also been observed between the size of EZP and the magnitude of endogenous zinc excretion in the feces[58] (L. Sian, et al., unpublished data). The combination of these observations provokes speculation on the mechanisms of zinc homeostasis. For example, it appears that any adaptive changes in fractional absorption in response to changes in available dietary zinc intake are not adequate to maintain the mass of readily exchangeable zinc at a constant level. This conclusion has received support from studies of zinc depletion/repletion.[49] It also suggests the possibility that changes in the size of the EZP, or of a certain compartment within the EZP, lead to a corresponding change in the quantity of endogenous zinc excreted by the intestine. In addition to providing new insight into whole body human zinc homeostasis, this approach should assist in the design of parallel mechanistic studies at a subcellular level.

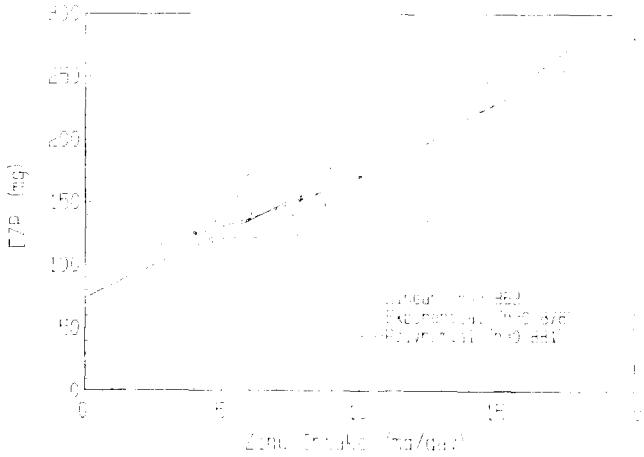

Figure 9 Correlation between dietary zinc intake with estimate of the combined size of the pools of zinc that exchange with zinc in plasma within 2 days (EZP). Data from normal adults in Denver, Colorado, Beijing, and rural northeast China. Data analyzed by linear, exponential, and polynomial regression.

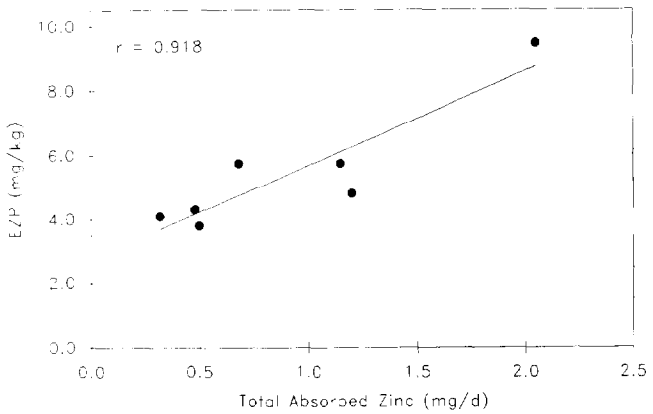

Figure 10 Correlation between total absorbed zinc and estimate of combined size of the pools of zinc that exchange with zinc in plasma within 2 days (EZP). Data from normal infants.

Conclusion

The application of stable isotope techniques, linked with compartmental modeling, to studies of human zinc metabolism is still in an early stage and its full potential has not been realized. This technology can make a major contribution to our understanding of the regulation of human zinc metabolism and of factors that contribute to deficiency of this essential micronutrient.

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