

Evaluation of zinc metabolism with use of stable-isotope techniques: implications for the assessment of zinc status¹⁻³

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ABSTRACT Zinc stable isotopes can be applied to the identification of populations at risk for zinc deficiency and to monitoring the effects of zinc intervention studies designed to improve zinc nutriture. Techniques using these isotopes can provide information on how effectively the intestine is absorbing exogenous dietary zinc and conserving endogenous zinc. They can also yield estimates of the quantity of readily exchangeable zinc in the body. Data derived from stable-isotope studies can provide extensive information on zinc status and the bioavailability of dietary zinc, allowing researchers to relate zinc intake to physiologic and pathologic conditions. Application of these techniques in longitudinal studies can provide quantitative data on the effectiveness of prevention programs such as simple community measures aimed at reducing dietary phytate and zinc fortification and supplementation programs. Further, judicious application of zinc stable-isotope techniques could make an important contribution to progress toward the eradication of zinc deficiency in infants and young children in the developing world. *Am J Clin Nutr* 1998(suppl);68:410S-3S.

KEY WORDS Zinc, metabolism, zinc status, stable isotopes, bioavailability, zinc pools, zinc homeostasis

INTRODUCTION

The results of many studies, most of which have been conducted within the past few years, attest to the widespread importance of zinc deficiency as a public health problem in the developing world. Recent progress, which is reviewed in this supplement, has been achieved despite the lack of pathognomonic clinical features of zinc deficiency and sensitive, specific laboratory indexes of zinc status (1). Progress has, however, undoubtedly been hampered by the lack of reliable laboratory assays. More importantly, future progress toward prevention of zinc deficiency in children in the developing world will be facilitated by improved means of identifying population groups at risk of zinc deficiency. In planning future studies, it will also be useful to have information on the magnitude of the deficit of bioavailable zinc from the diet as well as on the quantitative effects of intervention programs, which may range from local measures aimed at reducing the phytate content of the diet to the widespread administration of zinc supplements.

Other pertinent considerations include the importance of knowing whether the observed benefits of zinc treatment pro-

grams, for example those targeted at young children with acute diarrhea, are attributable to a correction of zinc deficiency or to a pharmacologic effect of this cation. Although the former appears more likely, solid documentation is desirable before embarking on any large-scale prevention protocols. This information can be derived in large measure from detailed studies of zinc homeostasis in the small intestine combined with careful measurements of dietary zinc, undertaken in small representative subsamples of the target populations. Ideally, these studies will be supplemented with data on those pools of zinc that exchange rapidly with zinc in plasma, thereby augmenting our ability to interpret information on zinc homeostasis in the intestine.

Fortunately, stable-isotope techniques now offer the capability to undertake such studies (2), not only in adults but even in young children (3). This review will be focused specifically on the potential value of these techniques.

APPLICATION OF ZINC STABLE-ISOTOPE TECHNIQUES

There are 5 naturally occurring stable isotopes of zinc, 3 of which (⁶⁷Zn, ⁶⁸Zn, and ⁷⁰Zn) are present in concentrations low enough to allow enriched preparations of these isotopes to be used in tracer studies of human zinc metabolism. These stable isotopes are being used increasingly for studies of zinc metabolism without any of the safety concerns that limit or preclude the use of zinc radioisotopes (2).

The utility of zinc stable-isotope techniques has certain limitations. The techniques cannot, for example, be used for whole-body or regional measurements, and current techniques generally lack sufficient sensitivity to monitor the metabolism of stable-isotope tracers over a period of more than a few weeks. Hence, there are limitations on the information that can be obtained on the slowly exchanging pools of zinc. In contrast with

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radioisotopes, finite quantities of zinc stable-isotope tracers are required for human studies. Caution is required, therefore, to ensure that these quantities are not sufficient to influence the processes being evaluated (4). The significant cost of stable isotopes also provides a strong incentive for restricting the quantity of tracer used to the minimum necessary. Depending on the sensitivity of analytic techniques, there may be a tradeoff between the quantity of information that can be obtained, especially for the more slowly exchanging pools, and the compelling reasons for restricting the quantity of tracer to a minimum. Although not unique to stable isotopes, decisions on using these techniques also need to be made in recognition of the cost of analytic equipment and the high level of laboratory expertise necessary to obtain reliable measurements of stable-isotope ratios. Finally, the use of zinc stable-isotope techniques does not eliminate the need for meticulous metabolic balance techniques, which are critically important components of most applications.

The application of stable-isotope techniques also has potential advantages over zinc radioisotope techniques, in addition to the safety issue. The most important of these is the availability of multiple tracers that can be administered on the same day to any one study subject with subsequent independent monitoring of the kinetics of these tracers. These isotopes can be used to track the metabolic fate of zinc, including absorption, retention, excretion of endogenous mineral, and turnover rates (5). Combined with model-based compartmental analysis they can be used to determine pool sizes and rate constants and fluxes between pools. They can provide information that allows us to relate zinc metabolism to diet and to a wide range of physiologic and pathologic conditions. They can also provide information of great value in the understanding of zinc status of populations (6).

ZINC METABOLISM: THE SMALL INTESTINE

The small intestine plays a central role in zinc metabolism and the maintenance of whole-body zinc homeostasis. Current theories hold that modulation of absorption of exogenous dietary zinc and intestinal conservation of endogenous zinc are the 2 major mechanisms regulating whole-body zinc homeostasis and maintaining and restoring zinc balance. Not only are each of these processes considered to be responsive to changes in zinc status (7), but both can be affected by dietary factors that may inhibit the absorption of exogenous dietary zinc and the reabsorption of endogenous zinc secreted into the intestinal lumen postprandially (8). These processes are also sensitive to the effect of disease, including malabsorption syndromes (9) and diarrhea (10). Each of these processes can be examined individually and quantitatively with stable-isotope techniques (2), an important goal that cannot be achieved with traditional metabolic balance techniques alone, nor, except with the use of a sensitive whole-body counter, with zinc radioisotope techniques.

ABSORPTION OF EXOGENOUS DIETARY ZINC

Fractional absorption can be determined by several techniques using stable isotopes, including model-based compartmental analysis and deconvolution. In young children, however, practical choices are limited currently to either measurement of the cumulative fecal excretion of unabsorbed tracer administered orally or determination of the ratio of the urine enrichment of an oral tracer to that of a second isotope tracer administered intra-

venously (2). The former technique requires reliable metabolic collections, whereas the latter requires only random urine collections; but administration of an isotopic label intravenously may not always be practical. To derive data of practical value, it is typically advisable to administer the oral tracer with all meals for a minimum of 1 d in quantities that are proportional to the total zinc in those meals. This allows calculation of total daily absorption of dietary zinc. Although these data alone are insufficient to evaluate zinc status, they can contribute to this goal. In one study, for example, without any additional information, the combination of high fractional absorption and relatively high dietary zinc intake observed in normal infants fed a semielemental formula provided strong reassurance about the adequate zinc status of infants fed this formula (11). In contrast, the combination of low dietary zinc and low fractional absorption observed in infants with cystic fibrosis who were breast-fed (2) alerted us to the likelihood of zinc deficiency.

Even in these extreme cases, however, combining absorption measurements with concurrent determinations of losses of endogenous zinc gives information on zinc homeostasis and insights into zinc status that are vastly superior to those from either of these measurements alone. Whereas determination of total urinary zinc excretion provides subsidiary information that is useful in calculating zinc balance and retention, the major route of excretion of endogenous zinc and the site that has a major role in zinc homeostasis is the intestine. Thus, it is fecal measurements of endogenous zinc that are so valuable.

INTESTINAL EXCRETION OF ENDOGENOUS ZINC

Large amounts of zinc are secreted postprandially (12) and most of it must be reabsorbed if zinc homeostasis is to be maintained. It is not yet known to what extent the anatomical sites of reabsorption of endogenous zinc in the small intestine may differ, at least quantitatively, from the sites of absorption of exogenous dietary zinc. Nor is it known whether reabsorption depends on the source of the endogenous zinc. The net difference between secretion and reabsorption can be determined by measuring endogenous zinc excreted in the feces. This measurement requires complete fecal collections and the intravenous or, with careful attention to study design, oral administration of an isotope tracer (2). The information it provides, however, is of exceptional value; this measurement should be included routinely when investigating zinc homeostasis and when isotope techniques are being used to evaluate zinc status.

Intestinal conservation of endogenous zinc appears to be the major mechanism by which subjects restore or maintain zinc balance when zinc intake is reduced or low. Temporary increases in fractional absorption when dietary zinc is reduced may not be maintained (13), whereas populations with habitually low dietary zinc intakes have correspondingly low intestinal excretion of endogenous zinc (6). Fecal excretion of endogenous zinc correlates positively with absorption of exogenous dietary zinc. When the latter is low, endogenous losses in the feces are low (2). Moreover, the mass of zinc in readily exchangeable body pools is also low (6), suggesting that the intestine only conserves endogenous zinc when readily exchangeable zinc pools are already depleted. This relation, which appears to be of cardinal importance to an understanding of zinc homeostasis, was first appreciated many years ago and led to the conclusion that measurement of intestinal losses of endogenous zinc provided the best index of zinc status in animal husbandry (14).

From another perspective, low excretion of endogenous zinc in the feces when dietary zinc is low provides reassurance that the individual or population can conserve endogenous zinc (6). Under certain pathologic conditions this relation does not occur, and inappropriate losses of endogenous zinc by the intestine may be a principal cause of zinc depletion. This has been demonstrated in infants with cystic fibrosis (2) and may be a widespread problem in young children with diarrhea. Of even greater concern, as discussed below, is the potential for dietary factors to impair the ability of the intestine to reabsorb endogenous zinc that is secreted into the intestinal lumen postprandially.

ZINC BIOAVAILABILITY


Whereas factors affecting zinc metabolism, eg, infections, can affect the bioavailability of zinc already retained by the body, the major factors affecting the bioavailability of zinc are typically those influencing fractional absorption of exogenous dietary zinc and reabsorption of endogenous zinc that has been secreted into the intestinal lumen postprandially. Host factors, such as diarrhea, can be important, but the factor of greatest concern is the phytic acid content of the diet. High dietary phytate is regarded as the principal reason for widespread zinc deficiency in the developing world. Although the greatest concern has been for those populations in which the molar ratio of dietary phytate to zinc exceeds 15:1, there is evidence that there is no threshold for the effect of phytate (15). Data for young children are especially limited, although stable-isotope techniques are now being applied to investigate this issue in developing countries (16). These techniques can be used for identifying populations of young children at risk from zinc deficiency by providing quantitative data on zinc bioavailability at different molar ratios of phytate to zinc and in the context of a range of overall dietary circumstances. The latter is important because other dietary factors, such as protein, calcium, and fiber, can also affect the bioavailability of zinc. Furthermore, these techniques can also provide information on the efficacy of intervention strategies designed to reduce the amount of phytate in the diet.

ZINC METABOLISM: READILY EXCHANGEABLE ZINC POOLS

Approximately 10% of body zinc in adults is located in pools that exchange with zinc in plasma within 2 d. This zinc is thought to have a vital role in a wide range of zinc-dependent metabolic processes (17). Readily exchangeable zinc appears to be dependent on recent dietary zinc intake (17). It is likely, therefore, that enhanced knowledge of readily exchangeable zinc and the factors that affect both its quantity and metabolism will provide important new insights into zinc homeostasis, the limitations of homeostasis, and the changes that occur when access to bioavailable zinc is limited. Initial knowledge of the complexity of the rapidly exchanging system was derived from zinc radioisotope studies combined with model-based compartmental analysis (18). Minimal models required to fit data derived recently from the application of stable-isotope techniques are of similar complexity to those required to fit radioisotope data (19). The information necessary for these models requires extensive blood sampling, which is not practical for infants and young children. Studies in adults, however, can be expected to provide valuable information on zinc metabolism, homeostasis, and sta-

tus in different populations within a range of habitual bioavailable zinc intakes. Research over the next few years is also expected to advance interpretation of currently used indexes of zinc status, notably plasma zinc, and also to assist in the identification and validation of new indexes of zinc status (20).

A relatively simple technique for the estimation of the overall size of the combined pools of zinc that exchange with plasma within 48 h was partially evaluated (17). This method requires determination of the coefficient of the simple exponential decay function fitting urine enrichment data between 3 and 9 d after administering a zinc stable-isotope tracer intravenously. Although this method overestimates the mass of this exchangeable zinc pool by $\approx 25\%$, we are encouraged to continue to evaluate this approach because of its applicability to field conditions and because initial results indicate that these estimations do have biological meaning in adults (6, 17) and children (2).

In conclusion, zinc stable isotopes can be used to identify populations at risk from zinc deficiency. These techniques can provide information on how effectively the intestine is absorbing exogenous dietary zinc and conserving endogenous zinc. They can also yield estimates of the quantity of readily exchangeable zinc in the body. Data derived from stable-isotope studies can provide extensive information on zinc status and the bioavailability of dietary zinc, allowing researchers to relate zinc intake to physiologic and pathologic conditions. Application of these techniques in longitudinal studies provides quantitative data on the effectiveness of prevention programs. Further, judicious application of zinc stable-isotope techniques could make a major contribution toward the eradication of zinc deficiency in infants and young children in the developing world. 

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