Zinc and Health: Current Status and Future Directions

Assessment of Marginal Zinc Status in Humans\textsuperscript{1,2}

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ABSTRACT  The assessment of marginal zinc status is problematic. Currently, there is no universally accepted single measure to assess zinc status in humans. The development of a reliable measure of marginal or moderate zinc status would be useful for a variety of purposes. For example, a simple, yet sensitive and accurate measure of zinc nutritional status is critically needed to further our limited understanding of the possible associations between zinc status and the risk of developing various chronic diseases and in predicting favorable health outcomes in patient populations. A convenient and reliable zinc assessment tool is needed to identify subpopulations who are at a risk of zinc deficiency and as an objective guidepost to determine the need for initiation of zinc supplementation or zinc fortification of the food supply, as well as in the refinement of recommendations of dietary zinc allowances. In frank zinc deficiency, clinical signs and static measures of zinc concentrations in a variety of readily available tissues, such as plasma, various blood cell types and hair, may uniformly confirm the presence of depleted body zinc stores. However, in general, the relative insensitivity or imprecision of these measurements has resulted in general disappointment in their use to assess marginal zinc status. Therefore, the search continues to find a useful and reliable marker of marginal zinc deficiency. In an attempt to speculate on possible future developments in the zinc status assessment field, a number of new and potentially promising approaches to this problem are highlighted. J. Nutr. 130: 1350S—1354S, 2000.

KEY WORDS: • zinc status • metallothionein • zinc-dependent enzymes • differential mRNA display • yeasts genetics

The interested reader is referred to several recent publications that have provided important overviews of zinc metabolism and function (DiSilvestro and Cousins, 1983, King and Keen, 1994, Mills 1989, Prasad 1991, Sandstead 1995, Solomon and Cousins, 1984, Walsh et al. 1994, Williams 1989), as well as the narrower issue of the assessment of zinc status (Aggett 1991, King 1990, Thompson 1991). In brief, zinc is an essential mineral that plays an important functional role in a wide range of zinc-containing proteins, including a large number of zinc-dependent enzymes. Zinc is needed for growth, normal development, DNA synthesis, immunity, neurosensory function and other important cellular processes. Zinc deficiency can be precipitated quite rapidly in young experimental animals by feeding a zinc-deficient diet. In contrast, zinc deficiency is not easily achieved in adults under experimental conditions where very low dietary zinc levels are fed. The characteristics of zinc deficiency in several animal species are well described (Underwood 1977). Moreover, frank zinc deficiency in humans occurs under various circumstances. Acidosis enteropathica is a congenital genetic disorder in humans that causes zinc deficiency (Moynahan 1974). Human nutritional zinc deficiency was originally described in 1961 in young men living in Egypt and coincided with the consumption of diets with very low zinc bioavailability due to high phytic acid content (Prasad et al. 1961). Zinc deficiency in these individuals was characterized by stunted growth and delayed sexual maturation that were reversible with zinc supplementation. Frank zinc deficiency, due to congenital disorders or severe dietary zinc restriction, may be signaled by clinical sequelae such as a prominent skin rash or growth-stunting endocrine dysfunction and can be confirmed by low zinc concentrations in blood and various other tissues. The problem of marginal zinc nutriture and status may be widespread because many studies have reported positive growth response in young children who were administered zinc supplements (Brown et al. 1998, Walravens et al. 1983, 1989). Poor zinc status may also be quite prevalent in clinical populations secondary to various disease states, such as sickle cell
anemia (Prasad 1984). Among more healthy populations, elderly persons are a potentially vulnerable subpopulation (Sandstead 1995) due to low dietary zinc intakes (Mares-Pérlman et al. 1995) and age-associated decreases in intestinal zinc absorption efficiency (Tornlund et al. 1986).

Assessment of marginal zinc status

Despite the potential widespread public health importance of poor zinc nutriture and the possible high prevalence of marginal zinc deficiency (Sandstead 1995), there is still considerable debate and frustration concerning our current methods of assessing zinc status (King 1990). There is no universally accepted single measure suitable to accurately assess the zinc status of an individual. The most often used approach to assessment of zinc status, particularly in large population studies, is the measurement of serum zinc levels. Although relatively convenient, especially for epidemiological investigations, plasma zinc content is generally considered a poor measure of marginal zinc deficiency (King 1990). Thus, it remains an important scientific challenge to develop a reliable and sensitive measure of marginal zinc status. Such a zinc status assessment tool should be of great use in obtaining an accurate picture of the zinc status of both various population groups and the individual patient or subject.

Experimental zinc deficiency in humans. A relatively small number of human zinc depletion-repletion studies have been reported since the initial report of human zinc deficiency in the 1960s. The number of subjects studied in these investigations has been quite limited. Moreover, several zinc depletion-repletion study designs have been used that could differentially influence study outcomes. In general, these studies have revealed that it is difficult to deplete adult humans of a significant amount of body zinc, even when the dietary zinc intake is quite low. This has led many investigators to also feed their subjects various inhibitory substances, such as phytate, which is found in soy-based diets or added directly as sodium phytate, to help minimize zinc absorption. The remarkable homeostatic ability of the body allows it to severely limit obligatory zinc losses from the body. Normally, the quantity of zinc lost in urine is minimal, only ~0.5 mg/d, and in zinc depletion, urinary losses can be even less. The major change in obligatory zinc losses in response to various dietary zinc loads is achieved by altering endogenous fecal zinc losses (King and Keen, 1994). Endogenous fecal zinc is a major regulatory focal point of whole body zinc homeostasis in both animals and humans. These findings from experimental studies are in general in accord with the recent report that endogenous zinc excretion is only ~1–2 mg/d in apparently healthy young Chinese women who typically consume ~5 mg Zn/d (Stam et al. 1996). Thus, assuming there are no other significant zinc losses from the body, obligatory zinc losses apparently can be maintained for long periods of time at ~2 mg/d. This may explain why it has been difficult in human zinc depletion studies to demonstrate significant effects of feeding low dietary zinc intakes on several outcome parameters.

Zinc-dependent enzymes. Because zinc has an important role in many enzymes, human zinc depletion-repletion studies have investigated whether changes in some of these enzyme activities might be a marker of zinc status. For example, Prasad et al. (1978) fed four adult male subjects a soy-based, semipurified, zinc-depletion diet containing 3 mg Zn/d for 6–12 mo and then repleted them for 2–3 mo with 30 mg Zn/d. Measurements of plasma lactic dehydrogenase, plasma ribonuclease and plasma alkaline phosphatase appeared to show promise as possible zinc-dependent biomarkers. In a subsequent study by Baer and King (1984), six young men were fed a semipurified, egg albumen–based diet supplying <0.3 mg Zn/d for 4–6 wk, until their serum zinc concentrations were <70 μg/dL, and then they were repleted for 2–5 wk with zinc. In this study, no effect of zinc was apparent on serum lactate dehydrogenase, alkaline phosphatase, ribonuclease or red blood cell (RBC) δ-aminolevulinic acid dehydrase. Roz et al. (1991) fed 15 young men 0.6 mg Zn/d for 1 wk and then 4 mg Zn/d for 4 wk, followed by a 2-wk zinc-repletion period; the authors reported the lack of an effect of dietary zinc depletion on putative zinc-responsive enzymes such as plasma angiotensin 1–converting enzyme, purine 5'-nucleotidase, α-t-lipoyldehydrogenase and alkaline phosphatase. Thus, for whatever reason, human dietary zinc depletion studies have not identified a reliable zinc-dependent enzyme marker of zinc deficiency.

Metallothionein. Intriguing findings concerning the effects of dietary zinc depletion on metallothionein (MT) have been reported. Grider et al. (1990) studied seven subjects after a short-term, 8-d dietary zinc-depletion (0.46 mg Zn/d) period and found that RBC lysate MT concentrations decreased significantly by ~50% (from 34 to 20 μg/g protein). In a separate group of subjects, RBC MT increased by 10 d of zinc supplementation with 50 mg Zn/d. In a subsequent study by this group, Thomas et al. (1992) showed that RBC MT was responsive to a 12-d zinc-depletion period when 15 young men were fed 0.55 mg Zn/d followed by a 30-d zinc-repletion period with 50 mg Zn/d. Parenthetically, it should be noted that despite the changes found in RBC MT concentration in response to zinc, no changes were observed in plasma zinc levels. This may have been due to tissue breakdown and release of intracellular zinc into plasma. It is interesting that changes in RBC MT were observed in both of the studies (Grider et al. 1990, Thomas et al. 1992) that involved the use of severe, but very short-duration (7–12 d), zinc-depletion periods that presumably would not result in major changes in total body zinc stores. Thus, RBC MT may be responsive to a very labile functional zinc pool and could prove to be a useful marker of zinc deficiency. The rapidity of response of the MT-sensitive intracellular zinc pool is further attested to by the rapid changes reported by this group (Sullivan et al. 1998) in monocyte MT mRNA levels in response to short-term zinc supplementation. Additional study of the potential usefulness of this measure of zinc status under more chronic conditions of zinc depletion appears to be warranted at this time.

Zinc status as a selection criterion in zinc supplementation studies

Zinc nutritionists are fundamentally interested in the influence of zinc status on various bodily functions. In particular, it is very important to define the limits of physiological adaptation to dietary zinc depletion and to investigate the extent of functional accommodation that occurs in the organism when normal physiological adaptations are no longer sufficient to maintain zinc homeostasis. Moreover, identification of the nature and regulation of important functional pools of zinc in the body that are altered in response to zinc intake is a high research priority. One experimental approach to this question is to measure an appropriate biochemical or functional end point that is a reasonable biomarker of zinc status and to explore the effect of altering zinc intake on that putative

Abbreviations: IRE, iron-responsive element; IRP, iron-responsive protein; RBC, red blood cell; MT, metallothionein.
Concerning the effects of zinc supplementation on various subjects who are in fact zinc deficient. This results in a highly considerable from study to study due to a variable number of within the study population with suboptimal zinc status will have an important functional role, only those individuals responsiveness of the measured biomarker. Another important deficiencies, some of which could significantly limit the re-

ment will demonstrably replenish the poor zinc stores of the zinc-deficient subjects. Moreover, a second group of subjects, representative of those with good zinc status, could also be chosen to participate to demonstrate the specificity of the zinc supplement to cause a positive (nutritional) response only in the zinc-deficient group. However, because we do not currently possess an accurate and specific biomarker of zinc status, the ideal approach to demonstrating the functional consequences of zinc supplementation in the population is obviously problematic. In addition, dietary zinc intake information may be of little additional help, even if accurate and available for the population of interest, because of the ability of the body to adapt to a wide range of zinc intakes, including low intakes of zinc, as may be found in the general populace. Indeed, a review of MEDLINE publications on zinc supplementation trials during the past 10 y confirms the lack of the use of any zinc status assessment measure as a selection criterion in many zinc supplement studies.

An alternative approach to the question of zinc-dependent function could be to select a subject population with suboptimal levels of the biomarker (e.g., growth rate or immune status) of interest and then hypothesize that poor zinc status is responsible for a significant portion of the poor biomarker status and thus will likely change in response to zinc supplementation. This approach has been used extensively in trying to define the role of zinc in the immune response of elderly persons and in growth in children. However, there are several potentially important drawbacks to this approach to the definition of zinc-dependent functions in human populations. For example, the depressed immunity or growth function may have a multifactorial pathogenesis that will obscure the role of zinc in the dysfunction of interest. From the strictly nutritional point of view, it is likely that a population with poor zinc nutrition may also have several other unknown nutritional deficiencies, some of which could significantly limit the responsiveness of the measured biomarker. Another important limitation is that it can be expected that even where zinc does have an important functional role, only those individuals within the study population with suboptimal zinc status will respond to treatment. Because the prevalence of poor zinc status is unknown, the overall effect of zinc treatment can vary considerably from study to study due to a variable number of subjects who are in fact zinc deficient. This results in a highly conflicting body of evidence when all of the studies are examined as a whole. This state of affairs seems to be generally true if one looks at all of the studies that have been published concerning the effects of zinc supplementation on various functional outcomes.

Possible new approaches to zinc status assessment

An overall assessment of the current methods available to assess marginal zinc status suggests that new assessment tools are needed. A review of MEDLINE publications concerning zinc status assessment in various populations during the past 10 y indicates that the most common zinc status assessment measure is plasma zinc. In the body, zinc is primarily found intracellularly. However, a small and important portion of body zinc is found in the circulation, mainly bound to plasma protein. Plasma levels of zinc are homeostatically regulated, although the signals and processes involved in this regulation are poorly understood. Plasma zinc levels are also affected by other factors, such as diurnal rhythm, stress, infection, starvation and plasma protein levels. As a consequence of its regulation and other common factors that can influence its distribution, plasma zinc levels are not considered an accurate reflection of dietary zinc intake or status. Given this problem, increased effort has been directed toward evaluating the potential benefit of intracellular zinc concentrations in other tissues and various cell types. However, the added usefulness of these cellular approaches is still under active investigation and can be of limited use in large-scale epidemiological studies. Thus, the search continues for a simple and reliable measure of zinc status.

Ferritin model of iron status assessment. A potentially instructive paradigm concerning the assessment of mineral status that could act as a goal for work in the area of zinc status assessment is the measurement of serum ferritin and serum transferrin receptor concentrations to assess the full range of iron status. Serum ferritin concentration provides a reliable measure of body iron stores in most circumstances, although it can be confounded by inflammatory conditions or during episodes of acute infection (Cook and Finch, 1979). Serum transferrin receptor has been shown to be useful as a biomarker of tissue iron deficiency (Flowers et al. 1989) that is independent of inflammation or the acute phase response (Skikne et al. 1990).

Ferritin is a multichain, ubiquitous cellular iron-storage protein that is translationally regulated according to cellular iron status via the action of cellular iron-responsive proteins (IRP1 and IRP2) that bind to a 5′ iron-responsive element (IRE) on the ferritin mRNA (Theil 1998). Under conditions of cellular iron surffeit, the iron-loaded IRP dissociates from the mRNA IRE, thus allowing translation of the protein subunits to occur. Some of the ferritin protein can be immunologically detected in the serum and has been shown to reflect body iron stores over a considerable range. Very low serum ferritin concentrations have been shown to correspond to the absence of stainable iron in bone marrow and thereby are useful to identify individuals with low iron stores. The measurement of serum ferritin concentration has been confirmed to be a good measure of a wide range of body iron stores, and low serum ferritin can be used as a marker of depletion of body iron reserves, which precedes the development of frank iron deficiency, characterized by reduced hemoglobin synthesis and the development of a microcytic, hypochromic anemia.

Metallothionein as a measure of zinc status. MT is an ubiquitous cellular zinc storage protein that may prove to be a useful measure of zinc status. Grider et al. (1990) first reported in 1990 that RBC concentrations of MT could be shown to decrease in young men fed a zinc-deficient diet for several days and to be rapidly increased after supplementation with 50 mg zinc/d. Thus, RBC MT concentrations appeared to be a promising measure of zinc status. Recently, Sullivan et al. (1998) extended these earlier observations by examining the expres-
sion of MT mRNA in circulating monocytes from humans. Monocyte MT mRNA measured by a competitive reverse transcription-polymerase chain reaction assay responds rapidly to zinc supplementation, resulting in a severalfold induction of mRNA within 2 d of consumption of a 50 mg/d zinc supplement. Thus, this novel measure of zinc-induced transcriptional activity is a sensitive indicator of changes in zinc status caused by zinc supplementation. However, an important question that remains is whether RBC MT or monocyte MT mRNA levels are useful in the detection of marginal zinc deficiency. Because MT is primarily an intracellular zinc storage protein, the measurement of MT mRNA or MT protein is likely to not be an ideal measure of marginal zinc deficiency. On the other hand, it may have significant use as a selection criterion to eliminate from zinc supplementation studies subjects who have robust zinc stores. Similarly, the usefulness of serum ferritin as an index of iron stores becomes limited in the phase of iron depletion immediately preceding the onset of frank deficiency. Under these conditions, the expression of this iron storage protein is quite low, but no obvious iron-responsive functional deficit may be present. Nevertheless, a high prevalence of truly iron-deficient (anemic) subjects will be found among subjects with low serum ferritin. Thus, by analogy, subjects with very low RBC MT concentrations may be at an increased risk of zinc deficiency.

**Serum transferrin receptor and tissue iron deficiency.**

The period of early tissue iron depletion in the absence of anemia can be estimated by the measurement of serum transferrin receptor concentrations (Skikne et al. 1990). The synthesis of ferritin and transferrin receptor reflects cellular iron status but in reciprocal directions. Like ferritin mRNA, the transferrin receptor mRNA also contains an IRE that can bind IRP. However, in the case of the transferrin receptor mRNA, the IRE is 3’. High cellular iron stores result in a disassociation of the IRP from the 3’ IRE in transferrin receptor mRNA, but in this case the dissociation of IRP from the IRE results in increased degradation of the transferrin receptor mRNA, leading in turn to lower levels of transferrin receptor protein. In contrast, when intracellular iron concentrations are low, the levels of transferrin receptor mRNA are increased due to increased stabilization of the mRNA, and more transferrin receptor is synthesized and sent to the cell surface. More transferrin receptor results in an increased ability to take up iron-bound transferrin from the circulation and replenish the depleted cellular iron stores. The increased concentration of transferrin receptor protein during iron deficiency is reflected in increased plasma levels of a truncated form of the cell surface transferrin receptor that can be detected in an antibody-based assay as a measure of tissue iron depletion (Skikne et al. 1990). The search for a functionally parallel protein involved in regulating cellular zinc homeostasis, such as a putative cell surface zinc transporter, may be a fruitful approach to the identification of a potential specific candidate measure of zinc status.

**Novel insights from genetic studies**

**Yeast studies.** As a possible future investigational strategy, it is important to consider that many functional proteins have been well conserved during the evolution of higher multicellular organisms. In this regard, useful insights into mammalian metal homeostasis in simple eukaryotic organisms, such as the yeast Saccharomyces cerevisiae (Wood and Han 1999). Additional research insights into the regulation of metal homeostasis in these more simple organisms will undoubtedly have a significant payoff of a greater understanding of metal regulation in mammals. In the case of zinc, there are an increasing number of genes that are being identified that are involved with zinc metabolism (Eide 1998). In my opinion, two interesting yeast genes, -zap1 and -zrt1, that might have mammalian counterparts deserve particular scrutiny as possible biomarkers of zinc status. -zrt1 is a yeast gene that codes for a high affinity zinc transporter (Zhao and Eide 1996). Presuming a mammalian counterpart exists, does dietary zinc depletion result in an up-regulation of the expression of this gene? If so, could a human zrt homologue be a useful and convenient biomarker of zinc deficiency? The second yeast gene of interest is -zap1 (Zhao et al. 1998, Zhao and Eide 1997), which codes for a transcriptional activator of the zinc transporter gene zrt. An interesting aspect of -zap, at least in yeast, is that it is positively autoregulated, i.e., increasing expression of -zap results in further up-regulation of its own expression. Thus, -zap might be a sensitive cellular zinc sensor, wherein small changes in cellular zinc status may result in significant changes in -zap expression that could be used to monitor cellular zinc status.

**Differential mRNA display.** Increasing knowledge of metal homeostasis in simple organisms will certainly augment our understanding of metal metabolism in higher organisms and hopefully lead to important insights that will help to identify useful markers of zinc status. An additional approach to this problem is the use of differential mRNA display from mammalian tissues. This technique has the potential of identifying mRNAs in various tissues that are preferentially up- or down-regulated by zinc deficiency. Some of these genes may already be known to be regulated by zinc, but some may represent novel zinc-regulated genes. For example, Blanchard and Cousins (1996) recently used this approach in intestinal tissue from zinc-deficient and zinc-replete rats to identify a variety of genes that are differentially regulated by zinc status. Extension of this novel mRNA expression discovery technique to additional tissues may be fruitful in identifying potential protein markers of zinc status. Despite its obvious potential role in the discovery process, gene-based approaches to identifying potential candidate markers of marginal zinc deficiency will likely need to be complemented by more traditional protein-based discovery approaches. Increasing technical advances in both genomic and "proteomic" (Celis et al. 1998, Humphrey-Smith and Blackstock 1997) investigations will hopefully advance our knowledge of cellular zinc homeostasis and result in the refinement of zinc status assessment markers. These novel findings will eventually have to be examined in the context of zinc depletion-repletion studies in humans. The pathway to better zinc assessment tools will likely continue to be tortuous. However, the payoff from this effort can be considerable. The development of better zinc assessment tools will help to define the important role of zinc status in a variety of important health outcomes and lead to a more precise definition of the optimal levels of zinc nutrition for individuals throughout the life cycle.

**LITERATURE CITED**


