Review of Various Indicators for Assessment of Zinc Requirement and Effectiveness

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Zinc (Zn) has been shown to be an essential micronutrient for all organisms including plants, animals and human beings. Deprivation of zinc arrests growth and development and produces multiple system dysfunctions in all these organisms. Because of the involvement of this micronutrient in so many core areas of metabolism, it is common that the features of zinc deficiency in humans are nonspecific with a wide range including growth retardation, alopecia, diarrhea, delayed sexual maturation and impotence, eye and skin lesions and even impaired appetite. Clinical features and laboratory criteria are not always consistent. This inconsistency poses a major difficulty in the search to reliable yet sensitive clinical or functional indicator of zinc status for validation. Further, it has become clear now that the homeostatic mechanisms fall short of perfection and clinically important features of zinc deficiency can occur with only modest degrees of zinc deficiency. In this review article we try to look critically at the available tests and various indicators for assessment of zinc's level for potential requirement and effectiveness and try to conclude about the efficacy of each.

Keywords: Enzymes, Micronutrient, Zinc

BIOCHEMICAL ROLE OF ZINC

Zinc is an essential micronutrient for human health. There is extensive evidence to support diverse, overlapping biological functions of zinc viz. Catalytic, structural, and regulatory. Zinc is defined as a Lewis acid and its being an electron acceptor contributes to its catalytic activity in many of these enzymes. Nearly 100 specific enzymes (*e.g.,* EC 1.1.1.1 alcohol dehydrogenase) depend on zinc for catalytic activity. Examples of zinc metalloenzymes can be found in all six enzyme classes. Well-studied zinc metalloenzymes include the ribonucleic acid (RNA) polymerases, alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase. Still, these metalloenzyme's activity changes by zinc restriction or excess have not been consistent in various experimental studies with humans.

The structural role of zinc involves domains capable of zinc coordination, facilitating protein folding to produce biologically active molecules. The vast majority of such proteins form a "zinc finger-like" structure created by chelation centers, including cysteine and histidine residues.[1] These structural motifs are found throughout biology area including the zinc-containing nucleocapsid proteins of viruses such as the human immunodeficiency virus[2] and in human as deoxyribonucleic acid binding transcription factors, including nonspecific factors such as Sp-1 and specific factors such as retinoic acid receptors and vitamin-D receptors. Zinc also provides a structural function for some enzymes, in which copper-zinc superoxide dismutase is the most notable example. In this instance, copper provides catalytic activity, whereas zinc's role is structural. Zinc is also required for intracellular binding of tyrosine kinase to T-cell receptors, CD-4 and CD-8, which are required for T-lymphocyte development and activation.

The role of zinc as a regulator of gene expression has received less attention earlier than its other functions but currently this area is most promising field of research. Metallothionein expression is regulated by a mechanism that involves zinc's binding to the transcription factor, metal response element transcription factor (MTF1), which in turn activates gene transcription.[3] The number of genes that are activated by this type of mechanism is not known, but some critical genes must be regulated by MTF1 as was proved by null mutation for the same being lethal during fetal development of mice. Among the other metal response element-regulated family of genes zinc transporter proteins of the cell, its influence on both apoptosis and protein kinase-C activity and zinc in normal synaptic signaling processes etc.

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PHYSIOLOGY OF ABSORPTION, METABOLISM AND EXCRETION

Zinc is widely distributed in foods but since virtually none of it is present as a free ion so the bioavailability is a function of the extent of digestion. The vast majority of zinc is absorbed by the small intestine through a transcellular process with the jejunum being the site with the greatest transport rate.[4] Absorption kinetics appears to be saturable, depending on transit time with increase in transport velocity with zinc depletion. Paracellular transport may occur at high zinc intakes. Transfer from the intestine is via the portal system with zinc bound to albumin. But measurement of true zinc absorption needs to take into account zinc from endogenous sources, derived from both pancreatic and intestinal cell secretions. Measurement of true absorption shows regulation of absorption may provide a "coarse control" of body zinc, whereas endogenous zinc release provides "fine control" to maintain balance.[5] Over 85 percent of the total body zinc is found in skeletal muscle and bone.[5] Plasma zinc is only 0.1 percent of total and its concentration is tightly regulated and maintained without notable change when zinc intake is restricted or increased unless these changes in intake are severe and prolonged.[5] Stress, acute trauma, and infection cause changes in hormones (e.g.- cortisol) and cytokines (e.g.-interleukin-6) that lower plasma zinc concentration.

Zinc loss from the body occurs via feces and urine are also attributed to epithelial cell desquamation, sweat, semen, hair, and the menstrual cycle. Major route is secretion into the intestine. It is derived partially from pancreatic secretions, biliary secretion and intestinal cell secretions.[6] These losses may range from less than 1 mg/day with a zinc-poor diet to greater than 5 mg/day with a zinc-rich diet, a difference that reflects the regulatory role that the intestinal tract serves in zinc homeostasis. Urinary zinc losses are only a fraction (less than 10 percent) of normal fecal losses. Albumin is the principal zinc-binding protein in plasma from which most metabolic zinc flux occurs others include plasma amino acids. The increase in plasma amino acids, which constitute a potentially filterable zinc pool, is at least partially responsible for increases in urinary losses concomitant with increases in muscle protein catabolism due to starvation or trauma. A major challenge is posed by the remarkable yet apparently imperfect, homeostatic mechanisms that maintain a narrow range of zinc concentrations within the body in spite of widely diverse dietary intake and bioavailability of this nutrient. Since zinc has a huge impact on the wellbeing of humans the search for a reliable, sensitive and specific index of zinc status has been the subject of considerable research, which has resulted in the identification of a number of potentially useful biomarkers.

AVAILABLE INDICATORS FOR ESTIMATING THE ZINC LEVEL AND REQUIREMENT

Plasma and Serum Zinc Concentration

While both plasma and serum zinc concentrations are used as indicators of zinc status, plasma zinc concentration is preferable because of the lack of contamination of zinc from the erythrocyte. Homeostatic mechanisms are effective in maintaining plasma zinc concentrations for many weeks of even severe dietary zinc restriction.[7]

But to use the serum or plasma levels as a marker of dietary intake is fraught with inconsistencies in different studies. Insufficient and inconsistent data exist for plasma or serum zinc concentrations in apparently normal subjects. Payette et al. observed a significant correlation between dietary zinc intake and serum zinc concentration but the correlation was positive for men and negative for women, furthermore a number of studies have reported no association.[8,9] Cut-off concentrations for lower limits have been established but it depends on the time of day at which collections are made because of the substantial and cumulative effects of meals in lowering concentrations. The cut-off concentration for pre breakfast samples is 70 µg/dL. Thus plasma and serum zinc concentrations do not seem to be sufficiently sensitive to serving as a subsidiary indicator but practically most convenient.

Zinc Concentration in Erythrocytes

The value of erythrocyte zinc concentrations as an indicator of zinc nutritional status is not well defined and the sensitivity is inadequate to provide more than a secondary, supportive indicator of dietary zinc requirements. Erythrocyte zinc concentration is depressed at moderately severe levels of dietary zinc restriction but results from various experimental depletion studies have been mixed.[10,11]

Zinc Concentration in Hair

Associations between low zinc concentration in hair and poor growth have been documented with reports linking low meat consumption or high phytate concentration having relatively low zinc concentrations in hair. However, there is a lack of uniformity in data so no lower cut-off values have been defined. Thus the use of zinc in hair as a supportive indicator for establishing zinc requirements needs further research.[12,13]

Activity of Zinc-Dependent Enzymes

No single zinc-dependent enzyme has found broad acceptance as an indicator of zinc status or requirement. The factors include a lack of sensitivity, the inaccessibility of optimal tissues to assay, inconsistent responses to dietary zinc[14,15] or simply, inadequate research. The activities of

alkaline phosphatase, copper-zinc superoxide dismutase, and lymphocyte 5'-nucleotidase merits specific recognition as a potential marker of zinc status.[16]

Metallothionein and Zinc-Regulated Gene Markers

Erythrocyte metallothionein concentrations have been reported to be responsive to both increased and restricted dietary zinc, Monocyte metallothionein messenger RNA responds rapidly to in vivo zinc supplementation. The sensitivity and precision of this index have not been thoroughly evaluated and merits additional research.[17]

6. Physical Growth Response to Zinc Supplementation

Studies of the effects of zinc supplementation on physical growth velocity in children are useful in evaluating dietary zinc requirements. Confirmation of the effect of zinc supplements on growth velocity (linear growth and weight) in children has been shown in a number of studies from many countries.[18,19] Large numbers of these studies have been undertaken internationally so therefore growth response can be applicable as a functional/clinical indicator of zinc requirement.

Size and Turnover Rates of Zinc Pools

Strong positive correlations have been observed between dietary zinc content, especially the amount of absorbed zinc and estimates of the size of the combined pools of zinc that exchange with zinc in plasma.[20] Pool size and turnover measurements may be of value in future refinements of Estimated average Requirements. Even simpler models involving the measurement of plasma zinc clearance may be useful in assessing zinc deficiency, but dietary data derived by such a method are not available at this time.

Indexes of Immune Status

Zinc is essential for the integrity of the immune system. Though the immune system is sensitive to even mild zinc deficiency, the effects on functional indexes of zinc status are not specific. At this time, therefore, changes in indexes of immune status with manipulation of dietary zinc can serve only as a limited indicator for dietary zinc requirements.[21]

Hormones

The biology of zinc is extensively linked to hormone metabolism, notably since the zinc finger motifs of regulatory proteins is required for gene transcription. Zinc has also been reported to have roles in the synthesis, transport, and peripheral action of hormones. Low dietary zinc status has been associated with low circulating concentrations of several hormones including testosterone, free T4 and IGF-1. However, no studies have directly related hormone concentrations to decreases or increases in zinc intake.[22]

Circulating Hepatic Proteins

Reductions in retinol binding protein, albumin, and pre-albumin concentrations have been reported with moderate dietary zinc restriction. Changes in circulating concentrations of these proteins with changes in dietary zinc may serve as minor supportive indicators on theoretically ground basis only.

KEY MESSAGE

Considering the essential nature of this micronutrient, nowadays there is thrust on zinc supplementation but on the other hand there are isolated case reports of toxicity also. Of all the biomarkers evaluated, both hair zinc and 24-h urinary zinc excretion appear to respond to zinc supplementation in a manner but the effect of zinc depletion is inconclusive due to insufficient data. The erythrocytes or mononuclear cells zinc does not appear to be useful biomarkers of zinc status. In children, physical growth response (linear growth and weight) to zinc supplementation has been tested and established indicator but this cannot be applicable for adult age group. Currently, plasma zinc concentration is the only biomarker of status that can be used to measure zinc status but with arguable limitations and constraint. Latest available literature further advocates that the prevalence of low serum zinc with inadequate zinc intakes may be used to evaluate their impact on the target population's zinc status.[23]

VIEWPOINT

It is clear that there is an urgent need to develop new biomarkers of zinc status and further research is needed to evaluate potentially useful biomarkers, including enzymes and other zinc-binding protein. Kinetic parameters measured using stable isotope techniques, including the exchangeable zinc pool, fractional zinc absorption, and endogenous zinc excretion; also have potential value in the search for ideal biomarker. The level of conformity between the dietary, biochemical and functional indicators of Zn status in national surveys should also be evaluated to conclude about the current trigger levels set for identifying populations at high risk of Zn deficiency.

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