Zinc: an essential but elusive nutrient[1–3]

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ABSTRACT

Zinc is essential for multiple aspects of metabolism. Physiologic signs of zinc depletion are linked with diverse biochemical functions rather than with a specific function, which makes it difficult to identify biomarkers of zinc nutrition. Nutrients, such as zinc, that are required for general metabolism are called *type 2 nutrients*. Protein and magnesium are examples of other type 2 nutrients. Type 1 nutrients are required for one or more specific functions: examples include iron, vitamin A, iodine, folate, and copper. When dietary zinc is insufficient, a marked reduction in endogenous zinc loss occurs immediately to conserve the nutrient. If zinc balance is not reestablished, other metabolic adjustments occur to mobilize zinc from small body pools. The location of those pools is not known, but all cells probably have a small zinc reserve that includes zinc bound to metallothionein or zinc stored in the Golgi or in other organelles. Plasma zinc is also part of this small zinc pool that is vulnerable to insufficient intakes. Plasma zinc concentrations decline rapidly with severe deficiencies and more moderately with marginal depletion. Unfortunately, plasma zinc concentrations also decrease with a number of conditions (eg, infection, trauma, stress, steroid use, after a meal) due to a metabolic redistribution of zinc from the plasma to the tissues. This redistribution confounds the interpretation of low plasma zinc concentrations. Biomarkers of metabolic zinc redistribution are needed to determine whether this redistribution is the cause of a low plasma zinc rather than poor nutrition. Measures of metallothionein or cellular zinc transporters may fulfill that role. *Am J Clin Nutr* 2011;94(suppl):679S–84S.

INTRODUCTION

Zinc was shown to be an essential nutrient for rats and mice in the 1930s, for pigs in 1955, and for humans in 1963 (1). In the past 40 y, much has been learned about zinc's essential biochemical and physiologic functions. Although it is a dietary trace element, it is one of the most abundant elements within cells. Approximately 95% of the body zinc is within the cells. A complex, extensive system for cellular zinc homeostasis maintains cellular zinc concentrations within a narrow range.

Even though considerable progress has been made in understanding the regulation of cellular zinc metabolism, there still is much we do not know. The lack of a good indicator of zinc status is a primary limitation. The signs of zinc depletion are diverse and cannot be attributed to a defect in a specific function. Until now, efforts to identify a marker of zinc status have focused predominantly on the response to changes in dietary zinc intake. However, studies of cellular zinc metabolism show that shifts in zinc-dependent functions are frequently linked to intracellular zinc fluctuations. This suggests that cellular zinc metabolism needs to be assessed along with whole-body zinc homeostasis to fully understand zinc status.

This article is divided into 3 parts: an overview of zinc's essential functions, a discussion of *why* assessing zinc status is so elusive, and a summary of cellular and whole-body potential biomarkers of zinc nutrition. Excessively high zinc intakes undoubtedly also alter these biomarkers, but given the limited information on the zinc metabolic response to excessive intakes, that aspect is not addressed in this article.

ZINC ESSENTIALITY

The physiologic effects of zinc deficiency are associated with a number of diverse biochemical changes rather than with a specific function as is the case for other trace elements such as iron and selenium. In general, zinc is essential for normal metabolism. Given its ubiquitous nature and high intracellular concentrations, it is not surprising that zinc has 3 very basic functions: catalytic, structural, and regulatory (2).

Zinc is a catalyst for >50 different enzymes when the same enzyme from different sources (plant, animal, microbial) is counted only once (2). An enzyme is classified as a zinc metalloenzyme if removal of zinc causes a decrease in activity without affecting the protein and if adding back zinc restores enzyme activity. A direct link between zinc deficiency symptoms and the function of an individual enzyme or enzymes has not been identified in complex organisms. Such a direct link is unlikely because it would occur only if the zinc-dependent enzyme were acting at a rate-limiting step in a critical biochemical pathway. For example, alkaline phosphatase is a zinc-dependent enzyme whose activities decline with low zinc intakes. But, it has not been possible to relate the signs and symptoms of zinc depletion with a change in alkaline phosphatase activity.

The discovery of the zinc finger motif in frogs in 1985 established a structural role for zinc (2). Zinc fingers have 4 cysteines within the general structure of the protein that allows zinc to be bound in a tetrahedral complex. Some zinc fingers have histidine substituted for cysteine. Zinc fingers occur in a variety

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of proteins, including those involved in cellular differentiation or proliferation, signal transduction, cellular adhesion, or transcription. Zinc is also involved in maintaining the structure of enzymes such as CuZn superoxide dismutase where copper is at the active site and zinc maintains enzymatic structure. The extent to which dietary zinc intake influences the function of zinc finger proteins is not well known. However, given the abundance of zinc fingers, these structures contribute to the overall zinc requirement and provide a rationale for the tight homeostatic control of zinc metabolism.

The third basic zinc function is regulation of gene expression (2). The basic components include a metal-binding transcription factor (MTF) and a metal response element (MRE) in the promoter of the regulated gene. The MTF acquires zinc in the cytosol or nucleus and then interacts with the MRE to stimulate transcription. MTF-1 is thought to regulate numerous genes either negatively or positively depending on the cellular zinc status (2). It is thought that dietary zinc can be transported into the cells and interact with MTF-1, facilitating translocation to the nucleus for MRE binding and stimulating transcription. This may be the mechanism whereby adaptive changes in lipid peroxidation, apoptosis, immunity, and neuronal function occur with zinc depletion.

These general zinc biochemical functions have not been related to the physiologic effects of zinc depletion. This is because these basic biochemical zinc functions do not provide a complete picture of cellular zinc metabolism and function. Zinc transporters also play a key role in moving zinc to various organelles, thereby regulating cellular function. For example, the secretion of neurotransmitters, such as glutamate from neuronal synaptic vesicles, requires zinc and explains the relation between zinc and cognitive function (3). Also, zinc is required for packaging insulin into pancreatic granules and its subsequent secretion. Thus, zinc transporters also contribute to metabolic regulation by moving zinc into and out of organelles, thereby influencing gene transcription or the secretion of essential hormones or neurotransmitters.

Two classes of zinc transporters have been identified (2, 3). The ZIP (SLC 39) family of transporters move zinc into the cytoplasm from extracellular space or cellular organelles. A total of 14 ZIP transporters have been identified. The ZnT (SLC 30) family of transporters move zinc and other metal ions, such as iron, from the cytoplasm into the lumen of the intracellular organelles or to the outside of the cell. Thus, the ZnT proteins work in opposition to the ZIP transporters. A total of 10 ZnT transporters have been identified (2, 3). Examples include ZnT3, which is found in the synaptic vesicles of neurons, and ZnT8, which is found in the secretory granules of pancreatic β cells. Zinc transporters play a major role in cellular zinc trafficking, thereby regulating zinc function. Although the discovery of zinc transporters has expanded our understanding of zinc metabolism, much remains to be learned. For example, what do all of the 24 zinc transporters do and how is their expression altered to help maintain cellular zinc homeostasis?

WHY IS ZINC STATUS ELUSIVE?

Most nutrients are required for specific metabolic functions rather than for metabolism in general as is the case for zinc (4). When a deficiency of nutrients with specific functions occurs, tissue nutrient concentrations decline and a defect in one or more specific metabolic pathways develop, causing the onset of specific clinical or biochemical signs. The diagnosis of a deficiency of nutrients with specific functions is relatively straightforward. All one has to do is measure the nutrient concentration in an appropriate, convenient tissue; test the metabolic pathways where the defect lies; or simply recognize the specific clinical sign. Nutrients with specific functions are referred to as "type 1 nutrients." Examples of specific indicators of type 1 nutrient deficiencies include microcytic anemia for iron, beriberi for thiamine, pellagra for niacin, scurvy for vitamin C, and macrocytic anemia for folic acid.

Type 2 nutrients are required for multiple general metabolic functions and therefore respond to insufficient intakes quite differently (4). Examples include nitrogen, essential amino acids, magnesium, potassium, and zinc. The first response to an insufficient intake of type 2 nutrients is a marked reduction in endogenous losses and possibly a metabolic adaptation to reduce the need for functions that have a high demand, such as growth or immunity. For example, if a type 2 nutrient deficiency occurs in a growing animal, a marked decline in growth occurs within days, which is soon followed by nonspecific signs of metabolic or clinical dysfunction. General dysfunction—ie, malaise or apathy—is rapid because there is no body store or reserve of type 2 nutrients that lasts for more than a couple of days in growing animals.

Zinc is a classic example of a type 2 nutrient. An inadequate zinc intake in experimental animals or humans causes an avid reduction of excretory losses to conserve zinc (5). Endogenous fecal losses decrease markedly within a couple days. With severe zinc depletion ($<$1 mg dietary zinc/d), urinary losses also decline (6). If the decline in fecal and urinary losses fails to reestablish zinc balance, additional metabolic adjustments occur to mobilize zinc from a small, vulnerable pool for zinc function. Tissue catabolism may also occur to release zinc. Experimental animals fed diets providing $<$3 parts per million (ppm) zinc develop a cyclical food intake pattern in which anorexia and food ingestion fluctuate over a 4–5-d period (7). During the anorexic period, muscle is catabolized, and zinc along with protein, potassium, and other muscle components are released. Flanagan (8) showed that cyclic food intake permits longer survival in rats fed a zinc-deficient diet. Rats tube-fed a zinc-deficient diet thrived for 6–7 d and then became seriously ill, whereas rats fed the deficient diet ad libitum and that then underwent cyclical food intakes stopped growing after 3 d, but they remained relatively healthy for $\approx$10 d.

Because zinc losses are markedly reduced with severe depletion, the total amount of zinc lost is small. For example, men subsisting on a virtually zinc-free diet for 4–9 wk lost a total of $\approx$95 mg ($\approx$1460 μmol) zinc, which represents $\approx$5% of total body zinc (5). In a 5-wk severe-zinc-depletion study, zinc losses totaled $\approx$40 mg ($\approx$615 μmol), or $\approx$2% of body zinc (9). Clinical symptoms of zinc depletion—ie, skin lesions, diarrhea, and sore throats—were evident in both studies. This shows that the vulnerable zinc pool is very small, and it is sufficient only to support body needs for several weeks when the diet lacks zinc. The corollary of this small zinc reserve is that the response to zinc repletion is extremely rapid. Within a matter of days, the clinical picture changes: enzymes are resynthesized (7), a growth response is observed in children (10), and skin lesions are reversed (11). This rapid reversal of clinical symptoms with small

increases in dietary zinc occurs because there is no significant loss of tissue zinc with depletion. There is no large depleted pool that must be replaced before clinical symptoms improve. Instead, only enough is needed to replete a very small, vulnerable pool (4).

It is important to remember that all nutrients excreted as a consequence of muscle catabolism due to zinc deficiency must be replaced during the repletion period. If, for example, an anorexic child with zinc deficiency is supplemented with zinc, he will regain his appetite and, if quality foods are available, his intake of protein, potassium, and intrinsic dietary zinc will increase. However, if the diet staple is a low-quality cereal food, he may not consume sufficient amounts of the other type 2 nutrients lost and the growth response may be limited. The growth response of stunted children given supplemental zinc varies widely in studies done around the world (12). This may reflect an insufficient intake of other type 2 nutrients, such as protein and potassium, during the zinc supplementation trial.

POTENTIAL DIAGNOSTIC BIOMARKERS OF ZINC NUTRITION

Unique features of type 2 nutrients

Aggett (13) defined nutritional status as the ratio of the tissue nutrient need to tissue nutrient availability. If need is greater than availability and the ratio is less than one, nutritional status is inadequate. This definition assumes that a decline in nutritional status is associated with a reduction in tissue nutrient concentration. This is true for type 1 nutrients where a low tissue nutrient concentration causes a decline in one or more specific functions (4). Type 2 nutrients respond differently to dietary insufficiency because their tissue pools are fixed and vary little with reduced intakes. Instead, a small, vulnerable body pool exists that must be sustained by a continual dietary supply. When intakes are insufficient for maintaining balance, this small pool is depleted quickly, often within days in growing animals or weeks in adult humans, giving rise to nonspecific symptoms of nutrient depletion, such as reduced growth, skin lesions, or infection. Because zinc is a type 2 nutrient, the response to zinc depletion and repletion is extremely rapid (4).

Homeostatic response to acute zinc depletion

Studies in animals and humans show that symptoms of severe zinc depletion occur rapidly and recover quickly with repletion (5, 7, 9, 14). For example, growth ceased after 4 d of a zinc-deficient diet (≈1 ppm) in weanling rats (7). In children, a rapid growth response was observed during zinc repletion (15). Also, the onset of clinical signs of zinc depletion occurred rapidly in adult men who consumed zinc-deficient diets (16, 17). A description of the clinical lesions observed in a study of 8 men who consumed a diet virtually free of zinc, the time of onset, and plasma zinc concentration at that time are shown in **Table 1** (17). Only one subject (12) had no health problems, and subject 5 developed only a sore throat without any skin lesions. The plasma zinc concentrations of these 2 men at the end of depletion (day 35 or 36) tended to be higher than that of the other men, ≈49 μg/dL. Three subjects (subjects 2, 3, and 5) complained of sore throats on day 26 of depletion. Toward the end of depletion (day 33 or 40), 6 subjects (subjects 1–4, 10, 11) had developed canker sores of the mouth and nostrils and a rash on their faces. Subjects 1 and 11 also had rashes in the scrotal area. Plasma zinc concentrations had dropped below 35 μg/dL when the skin rashes occurred. After only 6 d of repletion with 12.2 mg zinc/d, all symptoms had vanished. When the first sign of skin lesions occurred (day 28), whole-body zinc losses totaled 42 mg (≈645 μmol or ≈2–3% of whole-body zinc); at the end of depletion, after ≈40 d, total losses averaged 45 mg, showing the marked effect of the decline in excretory losses on total body zinc loss during extended time on the poor diet (16). It appears, therefore, that the vulnerable pool mobilized with zinc deficiency before clinical signs of zinc depletion are evident is <2% of body zinc. Given this limited reserve of only ≈40 mg zinc to maintain function when the diet is insufficient, it is not surprising that zinc endogenous losses rapidly approach zero in an attempt to maintain the pool and tissue zinc homeostasis with a marked reduction in intake.

TABLE 1
Time of appearance of clinical signs of zinc depletion and plasma zinc concentrations

Subject no.	Day of depletion	Plasma zinc	Clinical signs
		μg/dL	
1	32	8.0	Rash on face, canker sores in mouth, rash in scrotal region, fungal infection on feet
2	26	22.2	Sore throat
	32	17.2	Mouth canker sores, facial rash
3	25	<59.7[1]	Sore throat
	34	19.1	Mouth and nasal canker sores
4	37	24.0	Mouth canker sores
	40	15.8	Facial rash with purulent drainage
5	26	<49.0[1]	Sore throat
	35	48.7	Sore throat
10	34	35.5	Mouth canker sores
11	36	27.6	Nasal canker sores
			Rash in scrotal region, lesion on penis
12	36	48.6	No problems
	40	39.7	No problems

[1] Plasma zinc was not measured on this day. The value given is the previous measurement.

Zinc pools sensitive to zinc deficiency

Cellular organelles

The location or locations of the small, vulnerable zinc pool sensitive to zinc depletion has not been established. Components of the small, vulnerable zinc pools are probably found in all tissues (3, 15). In yeast, which does not have zinc efflux systems, excessive zinc is transported into intracellular organelles for storage and release in times of need. This storage organelle is called a zincosome (3). In mammalian cells, it appears that a small reserve of zinc is stored in the Golgi and in the endoplasmic reticulum. To maintain cellular zinc homeostasis, the ZnT (SLC30) transporters efflux zinc into those organelles or across the plasma membrane (3).

Cellular metallothionein

Cellular metallothionein is another means whereby cells can "park" zinc temporarily for future needs (4). Metallothionein is a cysteine-rich, intracellular, metal-binding protein (18). Although metallothionein has a higher affinity for copper and cadmium than for zinc, it normally exists in vivo in the zinc form. Up to 7 zinc molecules can bind to one metallothionein molecule. Four major metallothionein isoforms exist: MT-1 and MT-2 are located throughout the body, MT-3 is found primarily in the brain, and MT-4 is most abundant in stratified tissues (19). MT-2a is the most predominant form in human tissues. The highest concentrations of metallothionein are found in the liver, kidney, intestine, and pancreas (18). Gut and pancreatic metallothionein concentrations respond readily to changes in dietary zinc, suggesting that metallothionein helps maintain zinc homeostasis in these tissues. For example, high concentrations of intestinal zinc induce metallothionein synthesis and limit zinc absorption by sequestering it in the intestinal wall. The role of metallothionein in enhancing zinc absorption with low zinc intakes is uncertain. However, a decline in pancreatic and renal metallothionein occurs with low zinc intakes and facilitates the decline in fecal and urinary zinc losses (18).

Liver metallothionein concentrations respond promptly to tissue injury, infection, inflammation or neoplastic disease (18, 20, 21). In an inflammatory state, hepatic metallothionein content can increase as much as 100-fold within 2–4 h. This sharp rise in hepatic metallothionein causes hepatic uptake of plasma zinc to facilitate the acute stress response associated with inflammation.

Although the largest concentrations of metallothionein are in the liver, kidney, intestine, and pancreas, smaller quantities are located in all tissue cells. Because metallothionein gene expression is regulated by zinc and because it is one of the strongest cellular zinc binding proteins, it is likely that metallothionein also provides a way for cells to maintain a small zinc reserve (2, 18, 20, 21). Furthermore, the release of zinc from metallothionein when needed causes metallothionein to be degraded, freeing the zinc for metabolic functions.

Plasma zinc

Plasma zinc is a pool of zinc readily available for uptake by tissues when needed, and it is a component of the small, vulnerable zinc reserve. Studies in men show that plasma zinc mass declines rapidly with severe deficiency, decliningfrom 3.4 mg to 1.2 mg in 5 wk (9). The important role of plasma zinc, which serves as a source of zinc for essential tissue functions with low intakes, is shown by its high fractional turnover rate. Normally, the total amount of zinc in plasma is replaced $\approx$150 times/d; this increases to $\approx$200 times/d with severe zinc deficiency (9). The decline in plasma zinc concentrations during severe depletion is highly correlated with zinc balance; the higher the loss of body zinc, the greater the rate of plasma zinc decline (22). With moderate dietary zinc restriction (3–5 mg/d), the plasma zinc response is subtle, and shows no change to a slight decrease in zinc because the amount and rate of whole-body zinc loss is lower (23). A comprehensive evaluation of the relation between dietary zinc and plasma zinc concentrations shows that plasma concentrations decrease sharply when intakes are $<$2–3 mg/d but rise slightly and continuously with higher intakes, reaching a peak at $\approx$25–30 mg/d (23). It is interesting that zinc absorption also seems to plateau at intakes $>$20 mg/d. Supplementation with 10 or 20 mg zinc in healthy adult men increased plasma zinc concentrations within 5 d, but concentrations also declined to baseline levels within 5 d of discontinuing zinc supplementation (24).

Although plasma zinc concentrations are sensitive to zinc nutrition, they is not a specific measure of zinc nutrition. This is because plasma zinc is mobilized as a result of metabolic redistribution as well as poor zinc nutrition. Plasma zinc readily decreases with metabolic redistribution induced by endotoxemia, infection, carcinoma, steroid use, and after a meal (4, 25). This decline represents a redistribution of zinc from the small, vulnerable pool to the tissues, not a change in zinc nutrition.

Plasma zinc is primarily bound to albumin (70%) with the remainder bound tightly to α_2-macroglobulin (18%) and other proteins or amino acids, especially histidine and cysteine (23). Hypoalbuminemia is frequently associated with hypozincemia (4), and it is argued that this is due to reduced concentrations of plasma zinc–binding sites that could confound the interpretation of the decline in plasma zinc concentrations. However, even when plasma albumin is low, only $\approx$1 of every 50 albumin molecules is associated with a zinc atom, making it difficult to see how a reduction in albumin could lead to a significant reduction in zinc (4). On the other hand, a marginal zinc status could lead to a reduction in albumin synthesis.

Food intake, plasma volume expansion (pregnancy, overhydration), and steroid or oral contraceptive use all cause a decline in plasma zinc concentrations. A careful assessment of the individual's health status at the time of the blood draw can help determine whether the plasma zinc concentration is low due to one of these conditions. Ruling out the influence of a subclinical infection as a cause of low plasma zinc concentrations is more difficult. Measurements of proteins that are elevated in response to tissue injury or infection—eg, C-reactive protein or α_1-acid glycoprotein—are frequently performed to determine whether infection is present (26). However, these measures of infection are not directly related to plasma zinc concentrations because the stage and severity of infection both influence the change in plasma zinc. Also, the nutritional status of the host will affect the influence of infection on plasma zinc concentrations. The cytokine response to infection is reduced in malnourished animals and humans, suggesting that infection may have a smaller effect on plasma zinc concentrations in malnourished individuals (26).

Theoretically, metallothionein may be the key to diagnosing metabolic zinc redistribution due to infection, trauma, or stress (4). Metallothionein can be detected in plasma and erythrocytes by a radioimmunoassay (27, 28). Hepatic metallothionein concentrations rise with inflammation or stress, which also causes a rise in plasma metallothionein but not erythrocyte metallothionein (20, 21). Thus, in a metabolic state causing tissue zinc redistribution, plasma zinc concentrations will be low and metallothionein concentrations will be high, but erythrocyte metallothionein will be normal (16). If the plasma zinc pool is reduced due to poor zinc nutrition in the absence of infection, both plasma and erythrocyte metallothionein should be low.

This potential procedure for determining if low plasma zinc is due to poor nutrition or metabolic distribution has not been validated experimentally. But, preliminary studies are promising. Human studies have shown that both plasma and erythrocyte metallothionein concentrations are sensitive to dietary zinc (21, 27). However, the effects of infection or stress on plasma and erythrocyte metallothionein concentrations in the presence of low or adequate zinc nutrition need to be evaluated in humans.

Exchangeable zinc pool

Using zinc isotopes and a model-based compartmental analysis, Miller et al (29) and Jackson et al (30) have defined an exchangeable zinc pool (EZP) that exchanges rapidly with plasma zinc. Some, but not all, studies have shown a relation between dietary zinc and the size of the EZP (31, 32). During severe zinc depletion in adult men, EZP size declined nearly as much as the plasma zinc (60% compared with 65%), suggesting that it is part of the small, vulnerable pool susceptible to zinc depletion but not more sensitive than plasma zinc concentrations. Measurements of the effect of infection or stress on the size of the EZP have not been done. But it is likely that EZP size will decline with metabolic zinc redistribution due to stress because plasma zinc is a component of the EZP measure. Presently, EZP size is not used widely as a biomarker of zinc nutrition because it is not more responsive to changes in dietary zinc than is plasma zinc and because its measurement requires the use of expensive stable isotopes and access to a mass spectrometer for analysis.

CONCLUSIONS

Although much has been learned about cellular and whole-body zinc homeostasis and metabolism, a sensitive, specific biomarker of zinc nutrition has not been identified. Given that zinc is required for multiple general metabolic functions, it is unlikely that a specific biomarker of zinc nutrition exists. Nevertheless, the sensitivity of a small, vulnerable zinc pool to dietary zinc provides a means of identifying zinc under- and possibly overnutrition. Plasma zinc is a component of the small, vulnerable zinc pool, and it is a sensitive marker of zinc nutrition. Unfortunately, plasma zinc concentration is subject to metabolic redistribution as well as insufficient dietary zinc. Research is needed to identify biomarkers of zinc metabolic redistribution. Indicators of shifts in the metabolism of cellular zinc pools (zinc transporters or metallothionein) appear to be promising.

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