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Biomarkers of Nutrition for Development (BOND)—Zinc Review[1–5]

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Abstract

Zinc is required for multiple metabolic processes as a structural, regulatory, or catalytic ion. Cellular, tissue, and whole-body zinc homeostasis is tightly controlled to sustain metabolic functions over a wide range of zinc intakes, making it difficult to assess zinc insufficiency or excess. The BOND (Biomarkers of Nutrition for Development) Zinc Expert Panel recommends 3 measurements for estimating zinc status: dietary zinc intake, plasma zinc concentration (PZC), and height-for-age of growing infants and children. The amount of dietary zinc potentially available for absorption, which requires an estimate of dietary zinc and phytate, can be used to identify individuals and populations at risk of zinc deficiency. PZCs respond to severe dietary zinc restriction and to zinc supplementation; they also change with shifts in whole-body zinc balance and clinical signs of zinc deficiency. PZC cutoffs are available to identify individuals and populations at risk of zinc deficiency. However, there are limitations in using the PZC to assess zinc status. PZCs respond less to additional zinc provided in food than to a supplement administered between meals, there is considerable interindividual variability in PZCs with changes in dietary zinc, and PZCs are influenced by recent meal consumption, the time of day, inflammation, and certain drugs and hormones. Insufficient data are available on hair, urinary, nail, and blood cell zinc responses to changes in dietary zinc to recommend these biomarkers for assessing zinc status. Of the potential functional indicators of zinc, growth is the only one that is recommended. Because pharmacologic zinc doses are unlikely to enhance growth, a growth response to supplemental zinc is interpreted as indicating pre-existing zinc deficiency. Other functional indicators reviewed but not recommended for assessing zinc nutrition in clinical or field settings because of insufficient information are the activity or amounts of zinc-dependent enzymes and proteins and biomarkers of oxidative stress, inflammation, or DNA damage. *J Nutr* 2016;146(Suppl):858S–85S.

Keywords: zinc, zinc status, diet zinc, plasma zinc, zinc function

Introduction

The Biomarkers of Nutrition for Development (BOND)[13] project is designed to provide evidence-based advice to anyone with an interest in the role of nutrition in health. Specifically, the BOND project provides state-of-the-art information and service with regard to the selection, use, and interpretation of biomarkers of nutrient exposure, status, function, and effect. To accomplish this objective, expert panels were recruited to evaluate the literature and draft comprehensive reports with regard to specific nutrient biology and available biomarkers for assessing nutrients at clinical and population levels.

Phase I of the BOND project includes the evaluation of biomarkers for 6 nutrients: iodine, iron, zinc, folate, vitamin A, and vitamin B-12. This review represents the third in the series of reviews, and it covers all relevant aspects of zinc biology and biomarkers. The review is organized to provide the reader with a full appreciation of zinc's background as a public health issue, its biology, an overview of available biomarkers and specific considerations for their use, and interpretation of zinc biomarkers across a range of clinical and population-based applications. This article also includes a list of priority research needs for moving this important field forward.

Background: Overview of Zinc Biology and Function

Zinc, a dietary essential trace element, is primarily an intracellular metal involved in numerous metabolic processes, i.e., as a catalyst, structural element, or regulatory ion (1). The seminal events in the discovery of the zinc-related biological functions and assessments of zinc metabolism are highlighted in **Text Box 1**.

With zinc deficiency, multiple nonspecific general shifts in metabolism and function occur, including reductions in growth, increased infections, and the appearance of skin lesions. The generalized, nonspecific response of metabolic and clinical changes

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to zinc deficiency distinguishes it as a type II nutrient that has no characteristic signs or symptoms and results in generalized poor growth, stunting, and wasting (20). This is in contrast to type I nutrients that are required principally for specific metabolic functions in the body rather than for metabolism in general and have deficiencies characterized by specific signs and metabolic or clinical changes (e.g., iron deficiency → reduced hemoglobin, iodine deficiency → goiter, or vitamin A deficiency → Bitot spots) (20).

The inability to link the physiologic effects of zinc depletion to zinc status is due in part to an incomplete understanding of the biochemical and physiologic functions of zinc. **Text Box 2** lists currently known homeostatic responses to an inadequate zinc intake. Although zinc was discovered as being essential for plants and animals nearly 100 y ago, many aspects of its multiple biological functions and processes remain active research areas today.

Zinc functions

More than 300 metalloenzymes require zinc as a catalyst, and ~2500 transcription factors, or 8% of the human genome, require zinc for their structural integrity. Zinc also regulates thousands of genes through the metal response element (MRE)–binding transcription factor-1 (MTF-1), and zinc controls numerous cell-signaling pathways by modulating kinase and phosphorylase activities. The coordination of these zinc-dependent cellular functions appears to be related to 1 or several of the 24 zinc transporters that exhibit differing specificity of expression in different cell types (25, 26). **Text Box 3** contains an outline of general aspects of zinc's 3 primary functions: enzyme catalyst, structural component, and regulation of gene expression. The discovery of the 24 zinc transporters expanded our understanding of zinc metabolism. The current understanding of the roles of the zinc transporters and how they are influenced by cellular zinc is summarized in **Text Box 4**.

Role of zinc in health and disease

Because zinc is essential for regulating numerous aspects of cellular function and metabolism, multiple body functions are affected by zinc deficiency, including physical growth, immune competence, reproductive function, and neurobehavioral development. In low-income countries where zinc intakes are inadequate, these functional disturbances are often associated with impaired growth, increased risk of child morbidity and mortality, and preterm births.

Zinc deficiency. Because there is no functional reserve or body store of available zinc, except possibly in infants (31), a regular, adequate dietary supply is required. Dietary inadequacies may arise from low zinc intakes per se or poor absorption of dietary zinc (32) and may be exacerbated by a physiologic state with higher zinc requirements (i.e., growth). Hence, in low-income countries where diets are predominantly plant-based [plant-based foods, especially cereals, are high in phytate, which inhibits the absorption of zinc and other minerals (discussed in detail in the section entitled "Absorption and excretion")], young children and women during their reproductive years and pregnancy are at greatest risk of zinc deficiency. Breastfed infants may also be at elevated risk after 6 mo of age when zinc intake solely from human milk is inadequate to meet their growth requirements (33). Several pathologic conditions, listed in **Text Box 5**, resulting in poor zinc absorption, excessive losses, or impaired utilization, increase the risk of zinc deficiency.

The prevalence of inadequate zinc intakes by country is shown in **Supplemental Figure 1**. If the prevalence of insufficient zinc intakes is >25%, the population of that country is considered to be at an elevated risk of zinc deficiency (32). The estimates were based on the predicted absorbable zinc content of the national food supply, as described by the IZiNCG, and UN demographic data (43). The potential magnitude of zinc deficiency is the highest in sub-Saharan Africa and South and South-East Asia where the diets are predominantly plant-based and animal-source food intakes are low (44).

Approximately 17% of the global population have inadequate zinc intakes, and approximately one-fourth of the children <5 y of age are stunted (height-for-age z score < -2). This may be an underestimate of the true extent of the problem of zinc deficiency, because a limited number of national surveys have found that the prevalence of low plasma zinc concentrations (PZCs) is ~2-fold greater than the estimated prevalence of inadequate zinc intake (45).

The WHO (46–48) has issued several statements with regard to the potential efficacy of zinc interventions, but it has not produced specific guidelines for preventive zinc supplementation. The WHO and UNICEF issued a joint statement on the

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[5] Supplemental Figure 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[13] Abbreviations used: AAS, atomic absorption spectrometry; AE, acrodermatitis enteropathica; ALP, alkaline phosphatase; AR, Average Requirement; BOND, Biomarkers of Nutrition for Development; CRM, certified reference material; EAR, Estimated Average Requirement; ERK1/2, extracellular signal–regulated kinase 1/2; EZP, exchangeable zinc pool; IAEA, International Atomic Energy Agency; ICP, inductively coupled plasma; IP, inositol phosphate; IZiNCG, International Zinc Nutrition Consultative Group; LIBS, laser-induced breakdown spectroscopy; MGRS, Multicenter Growth Reference Study; MRE, metal response element; MT, metallothionein; MTF-1, metal response element–binding transcription factor-1; MZF-1, myeloid zinc finger 1; ppm, parts per million; PRI, Population Reference Intake; PZC, plasma zinc concentration; RCT, randomized controlled trial; SLC, solute carrier; SRM, standard reference material; SZC, serum zinc concentration; UL, Tolerable Upper Intake Level; XRF, X-ray fluorescence; ZIP, zinc influx transporter; ZnT, zinc efflux transporter; ZTF, Zinc Task Force.

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Text Box 1 Seminal events in zinc history

Highlights in zinc biology

- 1869: Raulin demonstrated the essentiality of zinc in microbial systems (2).
- 1926: The essentiality of zinc in higher plants was shown (3).
- 1934: Zinc essentiality was reported in rats (4).
- 1950–1960: Various reports showed the essentiality of zinc to animals including chickens and pigs (5–7).
- 1963: Prasad et al. (8) described zinc deficiency in patients with hypogonadism/dwarfism.
- 1974: Moynahan (9) described severe zinc deficiency associated with acrodermatitis enteropathica (AE), an autosomal recessive defect associated with a defect in zinc metabolism.
- 1970s–1980s: Hambidge and colleagues (10, 11) conducted several dietary zinc supplementation studies in the United States that confirmed the occurrence of growth-limiting zinc deficiency in otherwise healthy infants and young children.
- 1974: The Institute of Medicine included zinc for the first time in the RDAs (12).

Highlights in zinc assessment

- 1926: Lutz (13) estimated the total zinc content of a 70-kg man to be 2.2 g by using the dithizone technique.
- 1942: McCance and Widdowson (14) performed the first assessment of zinc absorption and excretion in humans that showed that most orally administered zinc was excreted in the feces with a relatively small amount in the urine.
- 1948: Vallee and Gibson (15) reported on improved sensitivity of methods to allow for measurement of zinc at low concentrations in biological samples.
- 1950: Vikbladh (16) reported on serum zinc concentrations (SZCs).
- 1960: O'Dell and Savage (17) reported the impact of phytate on zinc bioavailability.
- 2004: The International Zinc Nutrition Consultative Group (IZiNCG) provided a comprehensive review of all aspects of zinc nutrition and assessment (18, 19).

clinical management of acute diarrhea that includes specific recommendations for administering therapeutic zinc supplementation, along with oral rehydration therapy, in the treatment of diarrhea: 20 mg zinc supplementation/d for 10–14 d (10 mg/d for infants <6 mo old) (49, 50). Given the prevalence of inadequate zinc nutrition in lower-income countries, several international agencies, projects, and task groups are committed to improving zinc nutrition (**Text Box 6**).

Zinc and child growth and development. Zinc is essential for normal growth because of its critical roles in multiple metabolic pathways, including DNA transcription and gene expression, signal transduction pathways, and endocrine function. Mild zinc deficiency consistently reduces weight gain in growing rat pups (23). The growth responses to increased dietary zinc in deficient animals involve both weight gain and linear growth (i.e., length or height). In humans, the probability of a growth response to zinc supplementation is greater if there is evidence of a previous zinc deficiency (59). Although growth faltering occurs in a number of different nutritional settings, the data available consistently suggest that zinc deficiency is a contributing factor for impaired growth among populations in the developing world. Clinical observations in higher-income settings also show that zinc-responsive growth faltering occurs in infants and toddlers with low dietary zinc intakes (60).

Zinc and immune function. Nutrition affects the immune system in a number of diverse ways (61, 62). Of all potential nutritional impacts on immune function, protein-energy malnutrition has the strongest effect, but it is followed closely by vitamin A and zinc deficiencies. Zinc deficiency impairs both innate immune function (via compromised epithelial barrier, macrophage, and neutrophil function) and acquired immunity (e.g., via reduction in the number of CD4 T cells, NF-κB, and IL-2 gene expression) (62). Zinc deficiency causes thymic atrophy, lymphopenia, and compromised cell- and antibody-mediated responses that increase the rates and duration of infections (63). As the deficiency advances, the immune system is reprogrammed, beginning with chronic production of glucocorticoids that accelerate pre–β and pre–T cell apoptosis. This reduces lymphopoiesis and causes thymus atrophy. In contrast, the formation of blood cells in the bone marrow (granulocytes, neutrophils, and monocytes) is preserved with low zinc, thereby preserving the first line of immune defense or innate immunity.

Text Box 2 Metabolic/physiologic responses to an inadequate zinc intake

- In both experimental animals and humans, inadequate zinc intake causes a reduction of excretory losses (21).
  - o Markedly decreased endogenous fecal losses occur within ~2 d.
  - o Urinary zinc losses only decline with severe zinc depletion (<1 mg dietary zinc/d) (22).
- Additional metabolic adjustments may occur if the declines in fecal and urinary losses fail to re-establish zinc balance; tissue catabolism may mobilize zinc for the body's needs from a small, vulnerable pool.
- Zinc-deprived experimental animals fed only 3 parts per million (ppm) zinc develop a fluctuating cycle of anorexia and food ingestion that lasts 4–5 d (23).
  - o Muscle is catabolized and zinc, protein, potassium, and other muscle components are released during the anorexia period.
  - o This cyclic fluctuation between anorexia and food ingestion is thought to permit longer survival.
  - o Prevention of cyclic food intake by tube-feeding a zinc-deficient diet initially allowed growing rats to thrive for 6–7 d, but they then became seriously ill; rats fed the same diet ad libitum underwent cyclical food intakes and stopped growing but remained relatively healthy for 10 d (24).

Text Box 3 Basic zinc functions[1]

- Enzyme catalyst
  - o Zinc is a catalyst for >300 different enzymes (27, 28).
  - o Under conditions of zinc deficiency, zinc metalloenzymes have decreased activity but their protein structure does not change; adding back zinc restores enzyme activity.
  - o Direct links between zinc deficiency symptoms and the function of an individual enzyme or enzymes have yet to be identified in complex organisms.
    - ■ Such direct links are unlikely; they would only occur if the zinc-dependent enzyme were acting at a rate-limiting step in a critical biochemical pathway.
- Structural component
  - o Zinc fingers
    - ■ Motifs were discovered in frogs in 1985; their discovery established a structural role for zinc (27).
    - ■ Contain 4 cysteines that allow zinc to be bound in a tetrahedral complex. Some zinc fingers have histidine substituted for cysteine.
    - ■ Occur in proteins involved in signal transduction, cellular differentiation or proliferation, cellular adhesion, and transcription.
  - o Because of the abundance of zinc fingers, there is tight homeostatic control of zinc metabolism.
  - o Zinc is also at the active site of CuZn superoxide dismutase, where zinc maintains enzymatic structure.
- Regulation of gene expression (29)
  - o An MTF and MRE in the promoter of the regulated gene are the basic components of zinc's role.
  - o The MRE stimulates transcription after interacting with the MTF, which has acquired zinc in the cytosol or nucleus.
  - o Depending on the cellular zinc status, it is thought that MTF-1 negatively or positively regulates numerous genes.
  - o MTF-1 facilitates translocation to the nucleus for MRE binding and stimulating transcription by interacting with dietary zinc that is transported into the cells.
  - o The effects of zinc deficiency on lipid peroxidation, immune function, apoptosis, and neuronal function might be through this mechanism of gene expression regulation.

[1]Data from reference 30.

Changes in gene expression for cytokines, DNA-repair enzymes, zinc transporters, and signaling molecules suggest that cells of the immune system attempt to adapt to the stress of suboptimal zinc. A detailed review of zinc and immune function is available (62).

Zinc and risk of morbidity/mortality. Because of zinc's critical role in sustaining normal immune function, an increased susceptibility to infection is an early sign of zinc deficiency. Current estimates of zinc-associated (or zinc-attributable) mortality are variable, ranging from 97,330 in the Global Burden of Disease Study 2010 (64), to 116,000 in the *Lancet* 2013 Maternal and Child Nutrition series (65), to 453,207 in an earlier review (66). In all cases, these estimates represent an unacceptably high number of preventable childhood deaths attributable to zinc deficiency. Poor zinc nutrition is implicated in >50% of diarrhea deaths (62), 7% of pneumonia deaths, and 10% of malaria deaths (66). Zinc treatment in children has been shown to reduce diarrhea mortality by 13% and pneumonia mortality by 15%, but it does not reduce deaths due to malaria (67). Therapeutic trials of supplemental zinc also reduced the duration of acute and persistent diarrhea (68). On the basis of these studies, in 2004 the WHO recommended treating diarrhea by providing supplemental zinc for 10–14 d with oral rehydration therapy (50) (**Text Box 7**).

Zinc supplementation has also been used to prevent diarrhea. In those trials, a wide range of compounds and doses (1–20 mg zinc/d) with a median of 10 mg/d has been used (67, 70, 73). In addition, the dosing regimens have ranged from daily to weekly and the duration from a few weeks to >1 y. On the basis of these diverse studies, preventive zinc supplementation is estimated to reduce the incidence of diarrhea by 13–20% (67, 70, 73). The specific way in which zinc prevents diarrheal disease is unknown, but it may improve gut function, alter the composition of the enteric microbiome, or improve immune function. Although preventive zinc supplementation has been effective in reducing childhood mortality and morbidity due to diarrheal disease,

Text Box 4 Zinc transporters[1]

There are 2 known classes of zinc transporters (29):

- Zinc influx transporters (ZIPs) are a solute carrier (SLC)[2] (SLC39) family of transporters that move zinc into the cytoplasm from cellular organelles or extracellular space. There are 14 known ZIP transporters.
- Zinc efflux transporters (ZnTs) are an SLC30 family of transporters that move zinc and other metal ions, such as iron, from the cytoplasm to the outside of the cell or into the lumen of the intracellular organelles. There are 10 known ZnT transporters; 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.

The ZIP transporters work in opposition to, but in coordination with, the ZnT transporters to regulate cellular zinc homeostasis.

[1]Data from reference 30.

[2]Solute carrier group of membrane transport proteins that are typically located in the cell membrane.

Text Box 5 Some conditions associated with an increased zinc deficiency risk

- Gastrointestinal and metabolic disorders:
  - o inflammatory bowel disease, ulcerative colitis, Crohn disease, malabsorption syndrome (34)
  - o chronic liver or renal disease, diabetes, malignancies, and other chronic illnesses (35)
  - o chronic diarrhea/tropical enteropathy leading to excessive zinc loss (36)
- Vegetarian diet (37, 38)
- Pregnancy and lactation [the RDA[1] for zinc is higher for pregnant and lactating women than for other women (39)]
- Exclusively breastfed infants (>6 mo) (40)
- Hemoglobinopathies (sickle cell disease/thalassemia) (41, 42)

[1]The RDA is the daily dietary intake level of a nutrient considered sufficient by the Food and Nutrition Board of the Institute of Medicine to meet the requirements of 97.5% of healthy individuals in each life-stage and sex group.

large-scale programs to deliver preventive zinc supplementation do not currently exist in any country.

Data linking zinc deficiency with the incidence, prevalence, and severity of respiratory infections, including pneumonia, are less consistent than the relation with diarrhea. In a systematic review of 6 randomized controlled trials (RCTs) in children from 2 to 59 mo of age, supplemental zinc reduced the incidence of pneumonia by 13% and the prevalence of pneumonia by 41% (74). Other reviews have also shown that zinc supplementation reduced the incidence of lower respiratory tract infections by 15–19% (67, 73). Two studies that examined the impact of zinc as an adjunct to antibiotic therapy for treating pneumonia (75) or probable bacterial sepsis (76) reported lower case fatality rates and treatment failures in young children and infants. Doses varied with age, with those <12 mo receiving 10 mg/d (75, 76) and those >12 mo receiving 20 mg/d (75). Given the high doses used, it is possible that there was a pharmacologic effect on the organisms rather than an enhancement of immune function.

Text Box 6 International agencies, projects, and task groups addressing zinc deficiency

Food Fortification Initiative (51): a public, private, and civic partnership whose mission is to advocate for and support fortifying industrially milled cereal grains. The Food Fortification Initiative sponsored the most recent workshop to review fortification guidelines, including zinc fortification of flour.

Global Alliance for Improved Nutrition (52): an independent nonprofit foundation that was launched to reduce malnutrition by building alliances between governments, businesses, and civil society to develop and deliver solutions to control malnutrition, with a focus on children, girls, and women. Global Alliance for Improved Nutrition programs include large-scale food fortification efforts and developing food products for improved maternal, infant, and young child nutrition, many of which include additional zinc.

HarvestPlus (53): a project that supports efforts to increase the zinc content of selected cereal staples (wheat and rice) through biofortification. HarvestPlus is part of the Consultative Group for International Agricultural Research Program on Agriculture for Nutrition and Health, which helps realize the potential of agricultural development to deliver sex-equitable health and nutritional benefits to the poor. HarvestPlus is coordinated by 2 Consultative Group for International Agricultural Research centers, the International Center for Tropical Agriculture and the International Food Policy Research Institute.

International Zinc Association (54): a nonprofit organization dedicated to the interests and uses of zinc as a metal. It promotes such key end uses as corrosion protection for steel and the essentiality of zinc in human health and crop nutrition. The role of zinc in the environment, health, and sustainability is one of the International Zinc Association's main program areas.

International Zinc Nutrition Consultative Group (IZiNCG) (55): an international volunteer group composed of scientists and public health specialists, whose primary objectives are to promote and assist efforts to reduce global zinc deficiency, with particular emphasis on the most vulnerable populations of low-income countries. The IZiNCG has produced several documents and technical briefs on assessing population zinc status and interventions to control zinc deficiency, which are available on the IZiNCG website.

Micronutrient Forum (56): a consultative group that brings together people from a wide array of sectors who share an interest in reducing micronutrient malnutrition, including researchers, policy makers, program implementers, and the private sector. The Micronutrient Forum facilitates dialogue, fosters collaboration, and disseminates up-to-date research results to improve the design and implementation of scalable programs.

Teck's Zinc and Health Program (57): Teck's Zinc and Health Program has partnered with international organizations to raise awareness and scale up both short- and long-term solutions to zinc deficiency, including therapeutic zinc for the treatment of childhood diarrhea, zinc supplementation, food fortification, and crop nutrition.

Zinc Task Force (ZTF) (58): A collaborative group working toward the accelerated delivery of zinc and oral rehydration salts for the management of diarrhea in low-income countries. The ZTF serves as a technical working group to facilitate joint problem solving for global and national bottlenecks with regard to zinc procurement, delivery, and policy adaptation. The members maintain a repository of evidence, provide programmatic guidance, and work to enhance improved delivery and scale-up around the world. Members of the ZTF include UN agencies; government donor agencies; international nongovernmental organizations, foundations, and associations; and individual researchers from academic institutions.

Text Box 7 Current guidelines for treating diarrheal disease with zinc

- WHO recommendation: provide zinc supplements (20 mg/d × 10–14 d) to infants >6 mo of age to treat acute diarrhea (50); 10 mg/d for infants aged <6 mo.
- Evidence:
  - o Large systematic review (69) that evaluated 24 trials in >9000 children supported previous conclusions in earlier reviews (70): zinc supplementation shortens the duration of acute diarrhea in infants over the age of 6 mo and in young children (under 5 y) by ~6 h.
  - o The effect of zinc supplementation is greater in moderately malnourished children, with an estimated reduction in duration of 27 to 33 h (71).
  - o Zinc supplementation is likely to reduce the number of children with diarrheal symptoms for >1 wk (RR: 0.73; 95% CI: 0.61, 0.88) (69).
  - o For persistent diarrhea, zinc supplementation reduced the duration by ~16 h (69, 72).

Zinc and neurobehavioral function. A number of studies have examined the relation between zinc status and indexes of cognitive function, mood, and depression. The EURRECA (European Micronutrient Recommendations Aligned) network (77) undertook a systematic review and meta-analysis of the relation between zinc intake, status, and cognitive function. Some studies reported a beneficial effect of dietary or supplemental zinc on indexes of cognitive function in children (78–82), whereas others did not show any beneficial effect (83–89). In addition, the meta-analysis of a subset of RCTs did not reveal a significant effect of zinc on cognitive function or motor skills (77). However, only 6 studies were included in the meta-analysis due to the heterogeneity of the methods used to measure aspects of neurobehavioral function (77). There is also no evidence that supplementing mothers during pregnancy or the infant has any impact on neurobehavioral functions several years later (83, 85, 88, 89). A recent RCT (90) investigated the effects of preventing zinc deficiency on cognitive and sensorimotor development in Peruvian infants in a region where micronutrient deficiencies were prevalent. From the age of 6 to 18 mo, infants were supplemented with either iron (10 mg/d) and copper (0.5 mg/d) or an identical supplement with the addition of zinc (10 mg/d). Assessments conducted throughout the supplementation period revealed that the group additionally supplemented with zinc had normal developmental trajectories for attention measures and they performed better than the iron/copper-supplemented group up to the age of 12 mo. Thus, supplemental zinc may support normative neurodevelopment in infants who consume low-zinc diets.

The relation between zinc status and mental health in adults has been studied in both observational and intervention studies (91). In observational studies, an inverse relation existed between zinc status (i.e., PZCs) or dietary zinc and indexes of mood or depression. Low zinc status correlated with symptoms of depression, particularly in women (92), postmenopausal women (93), and the elderly (94). However, it is not clear whether this association was due to a reduced food intake in depressed individuals, a consequence of concurrent inflammation that lowers plasma zinc, or an effect of zinc on neurobiological function (95, 96). Nevertheless, supplemental zinc improves the outcome of patients suffering from depression, particularly when it is used in combination with antidepressant drug therapy (97, 98).

Consequences of zinc excess. Both acute and chronic forms of zinc toxicity exist. Acute effects include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches (39). Although doses as low as 50 mg in adults can have an emetic effect, much higher doses (i.e., several grams) are linked to acute toxicity. One case report cited severe nausea and vomiting within 30 min of ingesting 4 g zinc gluconate (570 mg elemental zinc) (99). An accidental infusion of 7 g zinc over a 60-h period caused death (100). Several cases of severe zinc toxicity have been reported in patients with metal pica involving post-1981 pennies, which are high in zinc (101).

Chronic zinc toxicity has been observed with zinc intakes ranging from 150 to 450 mg/d in adults. Symptoms of chronic toxicity include gastric problems, a reduction in immune function (i.e., reduced lymphocyte stimulation by phytohemagglutinin), decreased HDL cholesterol, and low serum copper concentrations (1). Patients with Wilson disease who were treated with 150 mg zinc/d developed hypocupremia, possibly due to a reduction in copper absorption (102). In addition, several cases of neuropathy occurred in individuals using excessive amounts of denture cream containing up to 17–34 mg zinc/g cream (103). However, zinc is a relatively nontoxic nutrient with supplemental intakes <50 mg/d.

The Food and Nutrition Board established a Tolerable Upper Intake Level (UL) for dietary zinc intakes (**Table 1**). Long-term intakes above the UL increase the risk of adverse health effects (39). The ULs do not apply to individuals who receive zinc for medical treatment, but such individuals should be under the care of a physician who monitors them for adverse health effects. The UL is based on data showing that zinc intakes >50 mg/d in adults reduced the activity of the copper-dependent enzyme erythrocyte Cu,Zn superoxide dismutase. After adjustment for an uncertainty factor, the UL was set for 40 mg/d for adults. The UL for children ranges from 5 mg/d in infants between 6 and 11 mo old to 34 mg/d in adolescents between 14 and 18 y of age. It has been argued that the UL for children is too low. For example, supplementation with 5, 10, or 15 mg/d did not alter the activity of Cu,Zn superoxide dismutase in 6- to 8-y-old boys (104).

Relevant zinc-drug interactions. Given the widespread involvement of zinc in metabolic functions, it is not surprising that a number of therapeutic drugs interact with zinc and alter its function. The interactions with antibiotics, penicillamine, and diuretics are summarized in **Text Box 8**.

Zinc Biology: Factors Affecting Zinc Absorption, Metabolism, and Utilization

Absorption and excretion. During digestion, pancreatic enzymes in the presence of gastric acid break down food components into smaller zinc-containing peptides. Conditions such as pancreatic insufficiency and inflammatory bowel diseases that cause poor hydrolysis of luminal constituents negatively affect intestinal zinc absorption. When zinc is released as a free solute

TABLE 1 Zinc DRI values by life-stage group[1]

	EAR, mg/d		RDA, mg/d			
Life-stage group	Males	Females	Males	Females	AI, mg/d	UL, mg/d
0–6 mo	—	—	—	—	2	4
7–12 mo	2.5	2.5	3	3	—	5
1–3 y	2.5	2.5	3	3	—	7
4–8 y	4.0	4.0	5	5	—	12
9–13 y	7.0	7.0	8	8	—	23
14–18 y	8.5	7.3	11	9	—	34
19–50 y	9.4	6.8	11	8	—	40
≥51 y	9.4	6.8	11	8	—	40
Pregnancy						
14–18 y	—	10.5	—	12	—	34
19–50 y	—	9.5	—	11	—	40
Lactation						
14–18 y	—	10.9	—	13	—	34
19–50 y	—	10.4	—	12	—	40

[1] AI, Adequate Intake; EAR, Estimated Average Requirement; UL, Tolerable Upper Intake Level (unless otherwise specified, the UL represents the total intake from food, water, and supplements). Data are from reference 39.

(Zn^{2+}) during digestion, it may bind with other negatively charged macromolecules (e.g., phytate) and become less soluble and available for absorption.

Zinc is absorbed along the entire small intestinal tract, but the highest rate of absorption occurs in the jejunum (1). However, the largest amount of zinc is absorbed in the duodenum because the highest zinc concentrations after a meal occur there. Endogenous zinc secretions into the gut contribute to the amount of zinc in the upper small intestine that is available for absorption after a meal. Zinc uptake by the small intestine occurs by 2 mechanisms: a saturable, carrier-mediated process and a nonmediated, or passive, process. The sum of the zinc transporters on the enterocyte membrane accounts for the saturable component. A mutation of the gene coding for one of those transporters, Zip4 (SLC39A4), is responsible for the zinc malabsorption disorder AE. After zinc is absorbed into the enterocyte, 2 proteins—metallothionein (MT) and the transporter ZnT7—influence the movement of zinc within the cell. But there is no evidence that these 2 proteins are essential for zinc absorption. The ZnT1 transporter facilitates zinc transfer from the enterocyte into circulation.

At high intestinal luminal zinc concentrations, substantial amounts of zinc may be absorbed by a nonsaturable, or passive, mechanism. Thus, intestinal zinc uptake is not tightly regulated. With high zinc intakes, total absorbed zinc increases and zinc balance is achieved by increasing endogenous zinc losses (30, 110). Dietary intakes of protein, calcium, iron, and organic acids have only a slight or nonexistent effect on zinc absorption (19, 111). Although a negative interaction between iron and zinc absorption occurs when the 2 elements are delivered simultaneously in an aqueous solution or simple food matrix, this inhibitory effect is not seen in complex food matrices, except possibly at extreme conditions with iron-to-zinc ratios >25:1 (112).

Phytate is the major dietary factor that influences zinc absorption (113). Phytate can bind zinc in the intestinal lumen and form an insoluble complex that cannot be digested or absorbed because humans lack the intestinal phytase enzyme (114). This inhibitory effect on zinc absorption in adults can be substantial because there is no evidence of an adaptive response to habitual high-phytate intakes (115, 116). In addition, there are no data supporting an effect of phytate on endogenous fecal zinc losses (117). Because of the impact of phytate on zinc absorption, the European Food Safety Authority in 2014 estimated zinc Average Requirements (ARs) and Population Reference Intakes (PRIs) for 4 different amounts of dietary phytate (**Table 2**) (118). Increasing dietary phytate from 300 to 1200 mg/d essentially doubles the dietary zinc requirement.

Several host-related factors (e.g., enteric infection) may influence zinc absorption and/or utilization over and above that of the dietary factors. The magnitude of these effects has not been quantified and it may depend on the life stage of the individual (119). Hence, at this time, host-related factors are not considered when estimating absorbable zinc from the diet.

Whole-body zinc homeostasis. Cellular, tissue, and whole-body zinc homeostases are tightly controlled (22) to sustain cellular and tissue zinc concentrations over a wide range of zinc intakes. Controlling zinc excretion is the primary way the human body maintains zinc homeostasis. The primary route for zinc excretion is through the gastrointestinal tract. At very low zinc intakes, zinc secretion into the intestinal lumen declines and endogenous fecal zinc losses decline. With an increase in zinc intakes, endogenous losses increase to maintain zinc balance or homeostasis. With extremely low zinc intakes (e.g., <2 mg/d for adults), decreases in endogenous zinc losses are unable to establish zinc balance and small amounts of zinc may be mobilized from more dispensable pools in kinetically active tissues (e.g., liver, pancreas, kidney, and spleen). All cells appear to have a small zinc reserve "stored" in lysosomes (120). However, some tissues, possibly bone, may have labile zinc pools that can be redistributed to maintain zinc-dependent functions in other tissues (121).

Unlike other nutrients, such as iron, zinc absorption does not change in response to changes in whole-body zinc homeostasis or status. Zinc absorption increases, however, in late pregnancy and lactation, especially if dietary intake is low (122). It may also decrease with aging (123). As mentioned earlier, zinc deficiency still occurs despite the robust zinc homeostasis mechanisms to maintain a stable whole-body zinc content over a wide range of intakes (22). Populations with increased needs (i.e., growing infants and children or pregnant women) who consume diets with a low zinc content and/or bioavailability are at greatest risk (43). Conditions that cause increased zinc needs (e.g.,

Text Box 8 Zinc-drug interactions

- Antibiotics: both quinolone antibiotics (such as ciprofloxacin) and tetracycline antibiotics have been reported to interact with zinc in the gastrointestinal tract, causing a reciprocal and negative impact on the absorption of both zinc and the antibiotics (105–107).
- Penicillamine: the absorption of penicillamine, a drug used for treatment of Wilson disease (genetically mediated copper excess) and rheumatoid arthritis, was reduced by zinc supplements (108).
- Diuretics: urinary zinc excretion is increased and plasma zinc concentrations decrease with the use of diuretics (109).

TABLE 2 Estimations of Average Requirements and Population Reference Intakes for zinc according to phytate intake and body weight[1]

Phytate intake	Body weight, kg	Average Requirement, mg/d	Population Reference Intake,[2] mg/d
300 mg/d			
Women ≥18 y	58.5[3]	6.2	7.5
Men ≥18 y	68.1[4]	7.5	9.4
600 mg/d			
Women ≥18 y	58.5[3]	7.6	9.3
Men ≥18 y	68.1[4]	9.3	11.7
900 mg/d			
Women ≥18 y	58.5[3]	8.9	11.0
Men ≥18 y	68.1[4]	11.0	14.0
1200 mg/d			
Women ≥18 y	58.5[3]	10.2	12.7
Men ≥18 y	68.1[4]	12.7	16.3
Pregnancy			Additional 1.6
Lactation			Additional 2.9

[1] Adapted from reference 118 with permission.
[2] Dietary zinc intake of subjects with a body weight at the 97.5th percentile of reference body weights (i.e., 79.4 kg for men and 68.1 kg for women).
[3] The median body weight of 18- to 79-y-old women is based on measured body heights of 19,969 women in 13 European Union Member States and assuming a BMI (in kg/m^2) of 22 (118). At this body weight, the physiologic zinc requirement is 2.9 mg/d.
[4] The median body weight of 18- to 79-y-old men is based on measured body heights of 16,500 men in 13 European Union Member States and assuming a BMI of 22 (118).

infections) or increased losses (e.g., diarrhea) also increase the susceptibility to zinc deficiency.

Dietary considerations. Animal-source foods are the richest source of absorbable zinc, most notably organs and flesh of mammals, fowl, fish, and crustaceans. Of the plant-based foods, fruit and vegetables, starchy roots, and tubers are low in zinc. Cereals, nuts, and legumes have lower and less efficiently absorbed zinc concentrations than animal-source foods (44, 116). Increasingly, ready-to-eat breakfast cereals are fortified with zinc, making them a primary zinc source in some countries (e.g., United States) (124, 125). The zinc content of plant-based foods, most notably maize, rice, and beans, is influenced by soil zinc concentrations (126). Thus, local food-composition tables for plant-based staples should be used to assess zinc intakes.

Unrefined cereals, legumes, nuts, and oil seeds contain very high amounts of phytate, whereas roots and tubers and most leafy vegetables and fruit have low amounts; animal foods do not contain phytate. Local food-processing, preparation, and cooking practices, such as milling/pounding, soaking, germination/malting, nixtamalization, or fermentation, can reduce the phytate content of unrefined cereals, legumes, and nuts (127), making it necessary to adjust the phytate values according to processing, preparation, and cooking practices. During fermentation and germination, phytate is hydrolyzed by phytase enzymes to lower inositol phosphates (IPs; i.e., IP1 to IP4), which do not inhibit zinc absorption (128, 129). Environmental conditions (i.e., climate, soil, and irrigation), fertilizer applications, and stage of maturation also influence the phytate content of seeds and grains; the highest contents are reached at seed maturity (127).

The negative effect of phytate on zinc absorption follows a dose-dependent response. The phytate-to-zinc molar ratio of individual foods or whole diets can be used to estimate the proportion of dietary zinc absorbed (130). Diets with phytate-to-zinc molar ratios >15 generally have poor zinc bioavailability, those with ratios between 5 and 15 are said to have medium bioavailability, and those with ratios <5 have good bioavailability (49).

The World Food System International Mini-list (131) provides the most comprehensive source of zinc and phytate values for low-income countries. It is available from the International Network of Food Data Systems website (132). Zinc and phytate values from the USDA database (133) are available for download from the Nutrition Coordinating Center, University of Minnesota (134). Other useful resources for the phytate values of foods include a phytate publication edited by Reddy and Sathe (135) and phytate values compiled by Wessells and Brown (43).

Zinc-nutrient interactions. An important consideration in the biology of any essential nutrient is the potential for interactions with other nutrients. Zinc shares many aspects of absorption and transport with other essential nutrients. **Table 3** provides a summary of the most common interactions: zinc-copper, zinc-iron, and zinc-calcium. Evidence is also accumulating that selenium interacts with certain zinc finger proteins involved in DNA repair (146, 147), and calcitriol (1,25-dihydroxycholecalicferol) interacts with myeloid zinc finger 1 (MZF-1), which is known to play a critical role in hematopoiesis and myeloid cell differentiation (148). RCTs are needed to confirm the effect of these interactions in human zinc nutrition.

Dietary Zinc Recommendations

National, regional, and international groups have developed dietary zinc recommendations. These recommendations are used to assess, monitor, and evaluate the adequacy of zinc intakes for individuals, populations, and population subgroups. The United States and Canada established a harmonized set of recommendations —the DRIs — for zinc (Table 1) (39). The DRIs include an Estimated Average Requirement (EAR) for zinc that is based on a fixed adjustment for zinc absorption from habitual diets for children and adults. The European Food Safety Authority recently established micronutrient recommendations for Europe (118). Dietary zinc reference values for adults take into account the inhibitory effect of dietary phytate on zinc absorption. Zinc ARs and PRIs were established for 4 phytate intake levels for adults (≥18 y) that cover the average phytate intakes in European populations (Table 2) (118). The European zinc ARs and PRIs for infants and children are summarized in **Table 4**. Those estimates are not adjusted for dietary phytate.

Currently Available Biomarkers

Overview

Table 5 summarizes the 3 recommended zinc biomarkers for research, clinical, and program use: dietary intakes, PZCs or SZCs, and stunting. The biomarkers are graded for the strength of their use for assessing zinc exposure, zinc status, zinc function, and zinc effects.

Biomarker-specific issues

The recommended zinc biomarkers by the BOND Zinc Expert Panel are based on a specific set of issues developed by the BOND Secretariat. This approach, which was used by each of the nutrient-specific BOND expert panels, is summarized on the BOND website (149). The aim of the approach was to provide a common format for all BOND nutrient expert panels and to address core issues of potential importance to various user

TABLE 3 Summary of zinc-nutrient interactions when consumed with food[1]

Nutrient interaction	Possible mechanism	Key features of interactions	Effect of interaction on zinc biomarkers
Copper	Copper may compete for intestinal zinc transport and influence zinc bioavailability; copper may also compete for binding to metallothionein.	Zinc interferes with copper absorption when intakes are very high (≥50 mg/d). High copper intakes have no reported adverse effect on zinc absorption (136).	Usual intakes of copper in normal individuals appear to have no effect on zinc biomarkers, such as PZC (21).
Iron	The mechanism of interaction between iron and zinc is not fully understood. Iron and zinc may compete for a shared absorptive pathway through DMT-1 (137) and/or another common pathway located in the apical membrane of the intestinal cell (138).	Supplemental iron beyond normal amounts of dietary intake may decrease zinc absorption (139, 140). This may be of concern when high-dose prenatal iron supplements (≥60 mg elemental iron/d) are taken routinely. Additional zinc may be warranted in conjunction with high-dose prenatal iron-supplementation programs (141).	PZCs may be reduced as a result of high amounts of supplemental iron (i.e., ≥60 mg elemental iron/d) (141). The effect is diminished when lower amounts (≤10 mg) of iron supplements are given during early childhood (142) or when both minerals are provided in a food matrix (112).
Calcium	Calcium per se has no detrimental effect on zinc absorption. In the presence of phytate, calcium may form insoluble calcium-zinc-phytate complexes in the intestinal tract that cannot be absorbed (143).	Calcium does not impair zinc absorption from diets adequate in zinc irrespective of whether diets have a low (440 mg/d) or high (1800 mg/d) phytate content (144). Whether calcium has an adverse effect in phytate-containing diets low in zinc is uncertain.	There is no evidence of reduced PZCs as a result of prolonged supplementation with high calcium intakes (1000 mg) in women (145).

[1] DMT-1, divalent metal transporter 1; PZC, plasma zinc concentration.

groups. The BOND Zinc Expert Panel selected the PZC or SZC as a biomarker of dietary zinc exposure or zinc status and stunting or height-for-age as a functional indicator of zinc inadequacy (Table 5). The relative strengths and weakness of each of these biomarkers are listed in **Table 6**. An in-depth review of each of the 3 recommended biomarkers follows.

Dietary zinc assessment

An assessment of dietary zinc intakes is the best method for estimating zinc exposure in individuals and populations. Reliable methods have been developed to evaluate dietary zinc intakes and to assess the risk of inadequacy for individuals and population groups (150). As a result, vulnerable individuals can be identified for dietary counseling and at-risk subgroups in the population targeted for intervention programs.

For individuals. The usual zinc intake of individuals can be estimated from a diet history or an FFQ. Usually, intakes are evaluated over a period of ≤1 mo; method details are available elsewhere (151). A diet history or an FFQ provides descriptive, retrospective information about the usual food consumption patterns. An evaluation of the intake of zinc-rich foods (e.g., meat, poultry, and fish), zinc-fortified foods, and high-phytate foods (e.g., unrefined cereals, nuts, and legumes) can be used to predict the intake of absorbable zinc. An Internet-based tool consisting of a meal-based computer-administered semiquantitative FFQ (152, 153) that is designed to assess the usual intakes of total and absorbable zinc for individuals is available. However, its validity and reproducibility need to be confirmed.

Other approaches for estimating the zinc intake of individuals include a 24-h recall, 1-d weighed or estimated food intake records, and duplicate diet composites. To account for individual day-to-day variation in zinc intakes, these methods should be repeated on >1 d. The details of these methods are described elsewhere (151).

TABLE 4 Summary of EFSA Average Requirements and Population Reference Intakes for zinc for infants and children[1]

Age	Average Requirement, mg/d	Population Reference Intake, mg/d
7–11 mo	2.4	2.9
1–3 y	3.6	4.3
4–6 y	4.6	5.5
7–10 y	6.2	7.4
11–14 y	8.9	10.7
15–17 y (males)	11.8	14.2
15–17 y (females)	9.9	11.9

[1] EFSA, European Food Safety Authority. Adapted from reference 118 with permission.

For populations or population subgroups. The zinc intake of populations can be estimated from food balance-sheet data provided by the FAO. These balance sheets provide an estimate of the total amount of zinc available to populations within a country or region; details are given in Brown et al. (130) and Wessells and colleagues (43, 154). This approach, however, does not provide information on the zinc distribution at the household or individual level.

To estimate the proportion of a population "at risk" of inadequate zinc intakes, the distribution of usual zinc intakes of individuals in a group and their estimated zinc requirements are needed. Details of the steps required to determine the proportion of a population at risk of inadequate zinc intakes are available in a 2007 IZiNCG Technical Brief (19) and outlined in **Text Box 9**.

Analysis of dietary intake information. Specialized software programs are available to adjust the distribution of observed intakes to usual zinc intakes (i.e., step 4 in Text Box 9), provided that some repeats are collected on at least a subsample (155). Two software programs available for making those adjustments are the IMAPP (Intake, Modeling, Assessment, and Planning Program) developed by Iowa State University (121, 156) and the National Cancer Institute method (157). The purpose of these specialized software programs is to remove the day-to-day within-person variation in zinc intakes. If this is not done, the variance in intake distributions will be wider, resulting in a higher estimate of the proportion of individuals with intakes below the

TABLE 5 Recommended zinc biomarkers to assess zinc exposure, status, function, and effect[1]

	Usefulness assessment											
	Exposure			Status			Function			Effect		
	Research	Clinical	Program	Research	Clinical	Program	Research	Clinical	Program	Research	Clinical	Program
Dietary assessment	+	0	+	+	0	+	0	0	0	0	0	+
Plasma or serum zinc concentration	+	0	+	+	0	+	+	0	+	+	0	+
Stunting	+	0	0	+	0	0	+	0	+	+	0	+

[1] 0, not useful for the specific purpose; +, useful to some extent and in certain population groups but either not commonly used or important disadvantages (e.g., no reference values); ++, useful in certain population groups, often used with some limitations (e.g., lack of specificity or sensitivity); +++, useful, often used in relevant population groups, with no or only minor limitations. None of the 3 recommended zinc biomarkers met the criteria for ++ or for +++.

EAR. If only 1 d of intake data are collected, then the within-person variation cannot be assessed. However, the IMAPP allows the user to use external estimates of within-person variation to adjust the distribution of observed zinc intakes to usual intakes.

Estimating absorbable zinc for individuals or populations. Because dietary phytate is a key determinant of zinc absorption, estimated intakes of both zinc and phytate permit calculating potential zinc absorption from the phytate-to-zinc molar ratios (19). In nonpregnant, nonlactating adults a prediction equation is available for estimating absorbable zinc from total zinc and phytate intakes (111, 113). If quantitative data on zinc and phytate intakes are not available, the WHO provides guidance for classifying the bioavailability of zinc from various dietary patterns that take into account 3 dietary variables as predictors of zinc bioavailability: protein from meat, fish, or poultry; calcium content; and the dietary phytate-to-zinc molar ratio (49).

PZCs as a biomarker of dietary zinc exposure. Studies of the validity of PZCs as an indicator of zinc intakes are limited. At the population level, conformity exists between the prevalence of low PZCs and the prevalence of inadequate dietary zinc intakes (158). However, at the individual level, the association between PZCs and zinc intakes is poor due to the following factors:

1. Difficulty in estimating an individual's usual dietary zinc intake (and the large number of observation days required to achieve high precision in the estimation).
2. Bioavailability: the need to estimate the proportion of dietary zinc intake that is absorbed, which depends on the amount of zinc, phytate, and possibly other food components in the meal (113) for which information may be missing from food-composition tables.
3. Physiologic state: various physiologic states (e.g., pregnancy) influence the association between PZCs and dietary zinc intake.
4. The possibility that absorbed zinc may be metabolized differently when consumed in food or as a supplement. Several studies have compared the effects on PZC of identical amounts of additional zinc provided as either a supplement delivered between meals or as a fortified food, such as cereal porridge or bread (159–161). In all cases, there was a significant increase in PZCs when additional zinc was delivered as a supplement but not when the same amount of zinc was provided in a zinc-fortified food. These differences in outcome with zinc supplements and zinc-fortified foods may be due to differences in zinc absorption, the postabsorptive metabolism of absorbed zinc, or both.

At the population level, a low mean PZC or a high prevalence of individuals with PZCs that are less than reference norms indicates that the population may be at risk of zinc deficiency. PZC cutoffs based on a statistical definition (e.g., <2 SDs below the mean of the reference population) have been established (**Table 7**). If all of the individuals in the reference population have adequate zinc status, this cutoff will overestimate zinc deficiency because the PZC could be even lower than the established cutoff before true deficiency occurs.

Zinc status assessment: PZCs or SZCs

Although only 1% of the total body zinc is present in circulating blood, several expert committees have endorsed PZCs or SZCs as a useful biomarker of zinc status, especially for assessing the risk of zinc deficiency in populations (32, 130, 163). Before reviewing the validity of plasma or serum zinc as a biomarker of zinc status, it is necessary to consider whether PZCs and SZCs provide the same information (148).

Early studies found that values reported for SZCs were generally slightly higher than those reported for PZCs (164). English and Hambidge (165) hypothesized that this may have been due to the fact that blood samples are often set aside longer before separating serum than is typical for plasma, so as to allow time for the serum samples to clot. This practice could allow more time for zinc to leach from blood cells into serum. When both types of samples (plasma and serum) were retained for identical periods of time before separating the cells, the zinc concentration results for plasma and serum no longer differed. Thus, PZCs and SZCs are both considered valid estimates of zinc status. Throughout this article, PZC is used to indicate this biomarker of zinc status, regardless of the method used to process the blood sample in a particular study. Specific issues concerning the collection and processing of blood samples are discussed in the section entitled "Assay-Specific Queries."

The utility of PZC as a biomarker of zinc nutrition can be assessed in several ways:

1. Measure the PZC response after controlled manipulations of zinc intake, including both zinc-depletion/repletion studies and zinc supplementation trials.
2. Assess the relation between usual dietary zinc intake and PZC.
3. Compare PZCs between individuals by using clinical signs that are generally recognized as functional outcomes of severe zinc deficiency.
4. Compare initial PZCs between individuals who do or do not show a functional response to changes in their zinc intakes.

A review of available evidence with regard to these 4 sets of relations and a discussion of possible approaches for establishing

TABLE 6 Relative strengths and weaknesses of zinc biomarkers[1]

Biomarker	Usefulness for the purpose	Advantages	Disadvantages	Analytical considerations
Dietary assessment	For dietary assessment of zinc intake, the 3 major instruments are FFQs, 24-h food-intake recalls, food diaries or weighed records, or weighed duplicate portions.	The dietary assessment provides an assessment of zinc sources. Dietary assessment methods do not accurately quantify ``usual´´ zinc intake, but dietary data can be used to identify the most important food sources of zinc. If dietary phytate is estimated, the bioavailability of zinc can also be determined. This information is useful to determine risk of zinc deficiency in a population or to design zinc intervention strategies.	It is time consuming to collect data, because it is difficult to identify all zinc sources.	All FFQs and 24-h recalls need to assess specific brands or types of cereal or bread products to determine if the product is zinc-fortified. Questions with regard to the specific cut of meat need to be assessed because zinc content varies with the type of muscle.
	FFQs assess the frequency and portion sizes of zinc-containing foods and/or food groups consumed over a predefined time frame, usually 1 y or several months. The FFQ method captures zinc-rich sources that are irregularly consumed and accounts, to some extent, for day-to-day variation in the overall consumption patterns.	National food balance sheets can be used to estimate risk of zinc inadequacy in a population.	Food-composition databases may not contain information on the zinc content of all foods. The amount of zinc added as a fortificant may vary among similar foods (i.e., breakfast cereals). Dietary phytate, which influences zinc absorption, should also be assessed, but it is rarely included in food-composition tables.	Portion sizes need to be carefully quantified to estimate zinc intakes from meat, fish, poultry, cereals, grains, and seeds.
	Twenty-four-hour recalls assess intakes over the past 24 h. To capture day-to-day variation in dietary zinc intake, 24-h recalls must be repeated preferably on nonconsecutive days, the number of repeats depending on the within-person variation in zinc intakes of the study group.			
Plasma or serum zinc concentration	Plasma zinc may be used to predict a functional response to an intervention, i.e., growth in children or an immune response.	The decline in plasma zinc with severe zinc depletion reflects a change in total body zinc.	A meta-analysis of high-quality studies of the relation between zinc intake and plasma zinc showed a high degree of heterogeneity in all population groups.	Analysis of plasma zinc concentrations requires special care to avoid contamination. Use stainless steel needles and trace element–free tubes and syringes and avoid hemolysis of blood.
	Plasma zinc responds consistently to zinc supplementation. It also decreases with very low zinc intakes (<2 mg/d).	Plasma zinc responds quickly (within 5–10 d) to zinc supplementation in all population groups. This response occurs irrespective of initial plasma zinc concentrations.	Plasma zinc does not respond to short-term exposure for fortified zinc foods; some response may occur when children are given fortified food sources for longer periods.	An AAS, an ICP-OES, or an ICP-MS are required for the analysis.
	Plasma zinc may be used to predict a functional response to an intervention, i.e., growth in children or an immune response.	Plasma zinc reference limits have been established for children, men and women, and pregnant women.	It is not known if functional changes occur without changes in plasma zinc.	
		Different cutoffs are available for fasting, morning, or afternoon blood draws to adjust for diurnal variation.	Biological factors, other than zinc intake, influence plasma zinc concentrations. Examples include infection, food intake, time of day, sex, age, pregnancy, oral contraceptive use, severe stress, position of subject during blood drawing, and length of time subject's arm is occluded with a tourniquet.	

(Continued)

TABLE 6 *Continued*

Biomarker	Usefulness for the purpose	Advantages	Disadvantages	Analytical considerations
Stunting	A growth response to a zinc supplement reflects a pre-existing zinc deficiency. However, it does not rule out other factors that may be limiting growth. There is no pharmacologic effect of zinc on growth. Thus, an increase in growth with supplemental zinc reflects zinc deficiency.	Standardized measurements of length or height are noninvasive and require simple equipment (i.e., a wooden or acrylic length board or stadiometer, preferably fitted with a digital counter). Ideally, the clothing should be minimal for length or height measurements so posture can be seen clearly. Shoes and socks should not be worn. WHO growth standards are available for evaluating the rates of growth in children up to 5 y of age. A WHO growth reference is also available for school-aged children and adolescents, which is closely aligned with the WHO Child Growth Standards at 5 y. The WHO provides computerized programs for calculating the degree of deviation from the age-specific reference median for a male or female child of the same age, i.e., the *z* score.	There are no definitive cutoffs for predicting an elevated risk of zinc deficiency within a population. A prevalence of 20% of low length- or height-for-age (defined as < -2 *z* scores) for children <5 y has been used as a reasonable cutoff. Low height cannot be used to evaluate the prevalence of zinc insufficiency among individuals who are no longer growing. Accurate measurements of length or standing height require calibrated equipment and strict adherence to standard procedures for making the measurements. In longitudinal studies involving sequential measurements on the same individual, 1 person should conduct all of the measurements to eliminate between-examiner errors.	Several well-trained anthropometrists are often rotated in large cross-sectional surveys to reduce measurement bias. To accurately measure growth velocity in infants, the measurements should be made every 2 wk in infants between 2 and 6 wk of age, monthly for ages 2–12 mo, and bimonthly in the second year. Time of measurement should be recorded because the spine gradually compresses during the day.

[1] AAS, atomic absorption spectrometer; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometer.

PZC cutoffs indicative of an elevated risk of zinc deficiency follows.

Effect of dietary zinc restriction and repletion on PZCs. The effect of dietary zinc restriction on PZCs in healthy adult volunteers was reviewed in detail for an interagency meeting on zinc status biomarkers convened by the WHO, UNICEF, the International Atomic Energy Agency (IAEA), and IZiNCG (166). Severe dietary zinc restriction in previously healthy adults, resulting from total zinc intakes <1 mg/d, produces rapid and marked declines in PZCs, which return to baseline levels within a few weeks of resuming the recommended zinc intakes. Moderate dietary zinc restriction, from 3 to 5 mg zinc/d, reduces the PZC only if the restricted diet is continued for several months or is accompanied by high phytate intakes.

Effect of zinc supplementation on PZCs. Several systematic reviews and meta-analyses have provided a sizeable body of information on the effects of zinc supplementation on PZCs among children (33, 40, 59, 73, 167) and adults (163, 168). Most studies and meta-analyses showed that zinc supplementation increases PZCs. In addition to these reviews, several reports are available that examined the dose-response or time course of the PZC response to supplemental zinc. These studies show that PZC responds consistently and fairly rapidly to zinc supplementation, both in children and adults, in relation to the dose or additional amount of zinc provided. After the withdrawal of zinc supplementation, the PZC returns to baseline levels within 1–2 wk.

Information from a nationally representative sample of presumably adequately nourished residents of the United States has been used to develop statistically defined PZC cutoffs (169). After eliminating data for individuals with underlying diseases that might affect zinc status or PZC, cutoffs were established for different age and sex groups and blood sampling conditions, as shown in Table 7. The levels were derived from the 2.5th percentile of the NHANES-II data (162). At the population level, when >20% of individuals in a particular population have a PZC below the age- and sex-specific cutoffs, the population is considered to have an elevated risk of zinc deficiency of public health importance. The basis for this admittedly arbitrary prevalence value is simply that the same prevalence cutoff has been applied for other nutritional conditions, such as childhood stunting and vitamin A deficiency, which, like zinc deficiency, are known to be associated with increased mortality risk. Individual countries may choose to modify this prevalence cutoff depending on available resources to control the condition.

For assessing the relation between PZC and clinical signs of zinc deficiency, data from experimental zinc-depletion/repletion studies and published case reports in individuals diagnosed with AE were examined (170). There was a clear association between the presence of clinical signs and a low PZC among patients with AE, as was the case with the experimental zinc-depletion/repletion studies. Specifically, on days when clinical signs of AE were present, the mean ± SD PZC was 38 ± 21 μg/dL, whereas on days when clinical signs were no longer present after treatment, the mean ± SD PZC was 102 ± 35 μg/dL ($P < 0.001$). The means ± SDs PZCs were significantly lower among individuals subjected to dietary zinc depletion who developed clinical signs associated with zinc deficiency than in those who remained asymptomatic (36.1 ± 16.8 compared with 67.9 ± 13.3 mg/dL; $P < 0.034$). When a cutoff of 50 μg/dL was applied, the sensitivity and specificity of PZC for detecting clinical signs of deficiency were 82% and 92%, respectively.

These data show that, with progressively lower PZCs, there is an increased likelihood of developing clinical signs associated with zinc deficiency, both among previously well-nourished individuals exposed to severe dietary zinc restriction and among patients with AE before and after treatment. These results confirm that a low PZC is related to clinical signs of zinc deficiency and can be used as a biomarker of zinc status.

Text Box 9 Steps in assessing dietary zinc at population levels

1. Select a representative sample of the population.
2. Measure food intake by using either a 1-d weighed or estimated food record or a validated 24-h recall, preferably on at least 2 nonconsecutive days for each individual or for at least a subsample of individuals in the population (30–40/life-stage group).
3. Calculate zinc and phytate intakes and dietary phytate-to-zinc molar ratios for each individual by using an appropriate food-composition database for the country.
4. Adjust the distribution of observed zinc intakes to represent usual zinc intakes by removing the variability introduced by day-to-day variation in an individual's zinc intake.
5. Apply the EAR cutoff method to estimate the prevalence of usual zinc intakes below the EAR. Alternatively, in countries where local food-composition values for zinc and phytate are not available, 24-h duplicate diet composites can be collected from each individual for analysis of zinc and phytate, again with some repeats on a subsample of the population, as described above.

As expected, the statistically derived cutoffs in Table 7 are slightly higher than those just described above based on the presence of clinical signs. In other words, the statistically defined cutoffs based on reference data from healthy individuals provide a greater margin of safety in identifying individuals at risk of zinc deficiency, before the appearance of overt clinical signs. As such, and given the nonspecific nature of the clinical signs of zinc deficiency, it seems that the current statistically defined cutoffs could continue to be used to indicate an increased risk of zinc deficiency, especially at the population level.

Functional outcomes and PZCs. Functional responses to correction of zinc deficiency include increased growth (including linear growth, weight gain, and fat-free mass accrual) and decreased morbidity from diarrhea and respiratory infections, possibly due to changes in immune function and/or mucosal integrity. Because of the lack of a true reference standard for zinc deficiency, functional responses to zinc supplementation have been used to determine whether a particular population is zinc-deficient. Specifically, if there is a greater functional response to zinc supplementation than to placebo in the context of an RCT, this implies that the population from which the 2 study groups were recruited had pre-existing zinc deficiency. By contrast, the absence of a functional response to supplementation does not necessarily indicate that the population has adequate zinc nutrition, because it is possible that other nutrient deficiencies prevented a response to zinc or that the zinc supplements were not adequately absorbed or utilized. In any case, when functional responses are observed after zinc supplementation, it is possible to determine whether any initial characteristics of the population, such as mean PZC, predicts which populations or population subgroups respond to the intervention. As a functional bioindicator, impaired linear growth suggests inadequate intakes or exposure to zinc. Because a lack of growth with supplemental zinc does not rule out zinc deficiency because other factors may be limiting growth, there is a need to complement measures of growth with biomarkers of zinc and other nutrients to make a differential diagnosis of the role of zinc in growth problems.

TABLE 7 Suggested lower cutoffs of plasma zinc concentrations for assessing the risk of zinc deficiency, by age group, sex, and fasting status[1]

Fasting status and time of day	Plasma zinc concentration, μg/dL		
	Children <10 y	Females ≥10 y	Males ≥10 y
Morning, fasting	—	70	74
Morning, nonfasting	65	66	70
Afternoon	57	59	61

[1] Adapted from references 130 and 162 with permission.

In a meta-analysis of the effect of preventive zinc supplementation on children's linear growth and weight gain, which included studies in children hospitalized for treatment of severe acute malnutrition and whose initial mean PZC was as low as 42 μg/dL, the initial mean PZC was associated with the linear growth response to zinc supplementation (171). In other words, zinc-supplemented children with a lower mean initial PZC had a greater gain in length than those children with a higher mean initial PZC. However, in subsequent meta-analyses that only included nonhospitalized children with a mean initial PZC of ≥65 μg/dL, the mean initial PZC was not related to the supplemental growth response (33, 59). Thus, the PZC may only predict a growth response to zinc supplementation when the initial mean PZC is low enough to indicate moderately severe zinc deficiency. Studies of the relation between PZC and the reduction in morbidity after zinc supplementation did not find an association, possibly because these community-based trials did not include a large enough number of children with very low PZCs.

In summary, PZC is a useful biomarker of exposure to severe and moderate dietary zinc restriction and to zinc supplementation. Moreover, clinical signs of zinc deficiency are clearly associated with a low PZC. Thus, PZC is a biomarker of both zinc exposure and the risk of clinical zinc deficiency. Cutoffs have been established to identify individuals and populations with an elevated risk of zinc deficiency by using both clinical data and statistical criteria. However, there are several caveats that must be considered in applying PZC to assess individual and population zinc status. First, PZC seems to respond less to additional zinc provided in food than to additional zinc provided as a supplement administered between meals. This may explain why the associations between measured dietary zinc intake and PZC are weak. Second, the PZC seems to predict functional responses to supplementation only when the initial PZC is very low. Third, there is considerable interindividual variability in PZCs and in responses to changes in dietary zinc intake, so there is a fairly broad range between the proposed PZC cutoff that indicates clinical deficiency and the proposed cutoffs that suggest an increased risk of zinc deficiency.

Measures of functional effect(s) of zinc

The paucity of sensitive and specific zinc biomarkers has resulted in a reliance on measures of relevant functions monitored in

response to zinc supplementation. As opposed to the term "biomarker," which requires a high degree of sensitivity and specificity, these measures of function are referred to as "bioindicators," reflecting changes within specific biological systems. Because bioindicators lack the sensitivity and specificity of biomarkers, they should be used in conjunction with nutrient biomarkers to further expand our understanding of the role of nutrients within these systems (172).

With specific regard to zinc, the gold-standard determinate of deficiency is a positive functional response in the context of a randomized, double-blind controlled trial of zinc supplementation. From trials involving numerous locations, populations, and study designs, general consensus holds that preventive zinc supplementation reduces the incidence of diarrhea and lower respiratory tract infections and growth impairment. However, these functional outcomes are indirect and nonspecific.

A fundamental (and reasonable) assumption is that there is no pharmacologic effect of zinc on growth in zinc-replete individuals. Thus, linear growth is recommended as a zinc functional bioindicator for the following reasons: *1*) low height- or length-for-age is frequently responsive to supplemental zinc and height- or length-for-age are used in routine health- and nutrition-monitoring activities and *2*) standardized methods and growth reference data are available for their measurement and interpretation and (173) linear growth is likely to be the primary response to an increased intake of absorbable zinc, whereas gain in weight is likely to arise as a result of increased linear growth (32). The percentage of children <5 y of age with height- or length-for-age less than − 2.0 SDs below the age-specific median of the reference population is recommended for assessing the zinc status of populations. A prevalence of low height- or length-for-age of ≥20% is indicative of an elevated risk of zinc deficiency (32). Low height is not useful for evaluating current zinc status for populations or individuals who are no longer growing.

The response to supplemental zinc for treating diarrhea or pneumonia has also been proposed as a functional impact of zinc nutrition. However, the morbidity response to supplemental zinc may not be indicative of a zinc deficiency, particularly with the use of pharmacologic doses that are severalfold greater than typical dietary intakes. An example of such a pharmacologic response is the postulated suppression of viral replication as the mechanism by which zinc lozenges shorten the duration of acute viral pharyngitis or upper respiratory infection (173).

The putative effects of zinc on intestinal function in animals with adequate zinc status suggest a therapeutic benefit that is at least partially independent of zinc status (174). Notably, virtually all of the zinc supplementation trials that showed a beneficial effect on diarrhea were conducted in settings where the risk of zinc deficiency is high. Lacking, however, has been definitive evidence that the effect was due to correction of deficiency. A recent trial in Switzerland in a well-nourished population suggested a beneficial effect of zinc supplementation on diarrhea frequency and severity (175). These results support a potential pharmacologic effect of a relatively high dose of zinc on the course of acute diarrhea, independent of zinc status.

Assessment of zinc status in clinical populations

Zinc nutritional status in an individual is difficult to measure with the use of laboratory tests due zinc's distribution throughout the body as a component of various proteins and nucleic acids. PZCs are the most commonly used indexes for evaluating zinc deficiency, but the PZC does not necessarily reflect cellular zinc status due to tight homeostatic control mechanisms. Clinical effects of zinc deficiency, such as growth retardation, loss of appetite, and impaired immune function, can be present in the absence of abnormal laboratory indexes.

When assessing zinc status in a clinical setting, the clinician should first consider risk factors for zinc deficiency, such as inadequate dietary intake, liver disease, alcoholism, vegetarianism, pregnancy, lactation, exclusive breastfeeding beyond 6 mo, and having sickle cell anemia or malabsorptive or maldigestive diseases. The clinician should then assess whether there are clinical manifestations associated with zinc deficiency. In addition to growth retardation, loss of appetite and impaired immune function may occur; more severe zinc deficiency causes characteristic skin lesions (i.e., hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye lesions). The skin lesions are erosive, erythematous, desquamated, and crusted and occur classically in the perioral and groin regions and with persistence on acral surfaces. Weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur. However, most of these symptoms are nonspecific and are often associated with other health conditions, so differential diagnoses will need to be done to rule out other causes of these conditions.

The use of PZC as a laboratory biomarker of zinc status in individuals has the same drawbacks as discussed above for populations. In patients with chronic malnutrition and/or acute illness in whom serum albumin is low, this should be considered as another confounding factor in interpretation of PZCs, because albumin is the primary carrier protein for circulating zinc. Other potential biomarkers of zinc status presented elsewhere in this article are not at a stage where they can be used in clinical settings. Further research is needed to evaluate potentially useful biomarkers. At the present time, PZC is the only biomarker of status that can be used to measure zinc status in individuals with either a low or a high supply of dietary zinc, but with many limitations and constraints. In critically ill patients, especially those with sepsis, PZCs have been reported to be profoundly low but also inversely correlated with cytokine concentrations and markers of oxidative stress, which is postulated to contribute to damage of key proteins associated with the response to sepsis (176). Zinc supplementation has been observed to attenuate experimental liver disease through multiple processes, including stabilization of gut barrier function, decreasing endotoxemia and inflammatory cytokine production, and reducing oxidative stress (177). A limited number of controlled trials, however, have not supported a clear benefit on clinical outcomes (178).

Other Biomarkers

In addition to measuring serum or plasma zinc and growth, a number of other possible biomarkers of zinc status have been tested. The BOND Zinc Expert Panel reviewed the data with regard to these biomarkers and divided them into 3 groups: potentially useful but needing more evaluation to determine cutoffs indicating deficiency, emerging biomarkers (i.e., recently proposed but not fully evolved), and those that are not useful (**Table 8**). A brief review of the current status of these biomarkers follows.

Potential zinc biomarkers

Hair zinc concentrations. The use of hair zinc concentration as a biomarker of zinc exposure is not widely accepted. Uncertainty stems in part from diverse results of hair mineral analyses by commercial laboratories that fail to use standardized methods for sampling and washing and that do not report the accuracy

and precision of the analytical methods, despite the availability of human hair reference material [e.g., Community Bureau of Reference, certified reference material (CRM) no. 397; Institute for Reference Materials and Measurements]. In addition, reference ranges for interpreting hair zinc concentrations have not been established (179–183).

Hair incorporates zinc into its matrix when the developing hair is exposed to the blood supply surrounding the hair follicle. However, as the growing hair approaches the skin surface, it undergoes keratinization, when the zinc accumulated during its formation becomes sealed into the protein structure of the hair. Hence, the zinc content of the hair shaft reflects the quantity of zinc available to the hair follicles at the time of growth, not at the time of sampling. Assuming a normal rate of hair growth (i.e., ~1 cm hair growth/mo), the zinc concentration in the proximal 1–2 cm of hair (i.e., closest to the scalp) reflects the zinc uptake by the follicles 4–8 wk before sample collection (184). Hair zinc concentrations do not change with exposure to the environment (181), and they are not modified by exogenous contaminants such as atmospheric pollutants, water, sweat, or hair beauty treatments. Consequently, the zinc concentration of the hair shaft reflects circulating PZCs at the time of hair synthesis rather than environmental exposure (181).

There are several advantages to using hair zinc as a biomarker of zinc exposure. Hair zinc concentrations are higher than blood and urine concentrations, making their measurement easier; hair samples can be collected, transported, and stored at room temperature without deterioration or need for special preservatives; and hair zinc concentrations are not subject to the rapid fluctuations seen in serum zinc produced by a recent meal, diurnal and circadian variation, or inflammation (166). Nevertheless, hair zinc concentrations are affected by biological factors such as age (185–189), possibly sex (84, 188, 190–195), rate of hair growth (in severe protein-energy malnutrition or AE) (196, 197), and season of the year (198–200). However, hair zinc concentrations are unaffected by hair color (201, 202) or cosmetic treatments (201) if appropriate washing procedures are adopted (181).

A systematic review and meta-analysis of 3 studies in adults (203–205) by the EURRECA network (163, 206) concluded that hair zinc concentrations responded positively and significantly to supplemental zinc intake (weighted mean difference: 13.2 μg/g; 95% CI: 11.9, 14.6 μg/g; I^2 = 0%). However, data were insufficient for assessing the response of hair zinc concentrations to zinc depletion or for identifying subgroups in whom hair zinc may be an effective biomarker. In children, hair zinc responses to supplemental zinc intake have been inconsistent (80, 84, 187, 207–210), although associations were reported in some but not all children between low hair zinc concentrations and high dietary phytate-to-zinc molar ratios (84, 188, 211), impaired taste acuity (84, 86, 187, 212), poor appetite (213), impaired linear growth (187, 194, 213–215), and recurrent respiratory tract infection (216). In sum, more data are needed to establish reference values for hair zinc concentrations in adults and children (217, 218).

Urinary zinc. Typically, ~0.3–0.6 mg zinc is excreted daily in the urine. A systematic review and meta-analysis showed that supplements containing >15 mg zinc/d significantly increased urinary zinc excretion (163). These changes in urinary zinc with zinc supplementation, seen in adults, the elderly, and in both men and women suggest that urinary zinc is a useful marker of increases in zinc exposure in adults with adequate zinc status at baseline (163). However, urinary zinc response to dietary zinc depletion is not evident unless an acute zinc depletion (<1 mg dietary zinc/d) is induced (22). In addition, the use of urinary zinc as a biomarker of zinc exposure is confounded by metabolic or physiologic conditions that increase protein catabolism and therefore increase urinary zinc (22). Starvation, strenuous physical exercise, diabetes, and trauma may all increase urinary zinc excretion. Metabolites that bind zinc tenaciously, such as picolinate, histidine, and cysteine, may also increase urinary zinc excretion (1, 219). Thus, it is important to evaluate the overall diet and health of the individual if urinary zinc is being used to assess zinc nutrition.

Urinary zinc excretion also changes during pregnancy and lactation. An increase is seen during pregnancy, with a return to prepregnant concentrations after delivery (122). The effect of lactation on urinary zinc excretion is uncertain; some researchers reported that lactation causes a decline in urinary zinc excretion (220); however, urinary zinc excretion did not differ significantly among lactating women who consumed a low-zinc diet compared with that in nonlactating women (221, 222). Supplementation with 15 mg zinc/d also did not increase urinary zinc losses in lactating women (223).

In sum, urinary zinc may be a good marker of compliance with a zinc supplementation program provided that at least 15 mg zinc/d is given (163). However, the lack of established cutoffs for evaluating zinc status and the need to collect a 24-h urine sample obviate the usefulness of urinary zinc as a biomarker. Experimental zinc-depletion studies showed that urinary zinc concentrations do not decline unless dietary zinc intake is very low (<3 mg/d) (224). Therefore, it is not a sensitive biomarker of low zinc intakes among free-living populations. The effect of dietary zinc on urinary zinc concentrations has not been studied in infants and children.

Emerging biomarkers

Given the lack of a specific, sensitive zinc biomarker that reflects zinc nutrition across various populations and situations, research is needed to identify or validate new biomarkers. Biomarkers

TABLE 8 Potential, emerging, and not useful zinc biomarkers

	Potential	Emerging	Not useful
Definitions	Biomarkers that show promise, but data are insufficient to establish specific cutoffs indicating zinc inadequacy in populations	Biomarkers for which there is some theoretical basis for a relation to zinc intake or status, but testing is insufficient to confirm the relation	Biomarkers that do not relate consistently to zinc intake or status
Biomarkers	Hair zinc	Nail zinc	Zinc-dependent enzymes
	Urinary zinc	Zinc-dependent proteins	Erythrocyte and leukocyte zinc
	Neurobehavioral function	Oxidative stress and DNA integrity	
		Zinc kinetics	
		Taste acuity	

currently under study are reviewed in this section. All of the biomarkers listed below have a theoretical association with zinc intake or status, but their sensitivity and specificity to changes in zinc nutrition need further study.

Nail zinc concentrations. Nails grow more slowly than hair. The variability in nail growth among individuals ranges from 0.025 mm/d for toenails to 0.1 mm/d for fingernails. Nail zinc concentrations are similar to those in hair (225), with concentrations ranging from 80 to 200 μg/g. The average toenail concentrations in German children (aged 3–7 y) averaged ~130 μg/g, with no obvious effect of season, unlike hair zinc concentrations in children (200). Very high toenail concentrations (~2000 μg/g) have been reported in galvanizers with a high exposure to zinc in the atmosphere.

The use of finger or toenail zinc concentrations as a zinc biomarker has been limited due to the lack of sensitive measurement techniques. However, this is a current field of research. If new sensitive measurement techniques are developed, research is needed to establish the sensitivity and specificity of nail zinc to changes in zinc nutrition.

Zinc-dependent proteins. Zinc is a cofactor for ~3000 different proteins. It is required to sustain protein structure and to regulate metabolic pathways. Recent research suggests that zinc-dependent proteins secreted or leaked from tissue cells into the circulation or existing within circulating cells may reflect zinc status. A focus on zinc-dependent proteins as biomarkers of zinc status assumes that the zinc content/stoichiometry of a protein shifts with limited zinc availability. This has not been proven; however, we know that protein synthesis depends on zinc-dependent transcription factors and that protein degradation is zinc-dependent. However, zinc is not the only factor involved in regulating cellular protein expression or concentrations; many factors are involved. A brief discussion of several proteins thought to reflect cellular zinc status follows.

It has been proposed that MT may be a biomarker of cellular zinc concentrations because its gene expression is highly regulated by MTF-1, a transcription factor sensitive to cellular zinc concentrations (226). As cellular zinc increases, MT synthesis increases and zinc is bound to 1 of 7 potential sites (227). The high cysteine thiol content of MT makes it a target for oxidation, with the consequent release of zinc making the zinc binding to MT dependent on the cellular redox state (228). Controlled human studies indicate that MT expression declines with zinc inadequacy and increases with zinc supplementation (229, 230). However, the utility of MT expression as a marker of zinc status in free-living studies has yet to be confirmed. In fact, a negative correlation between PZC and blood mononuclear cell MT expression was observed in free-living volunteers (231), which may indicate that the stress or inflammation that induces MT, but reduces PZCs, complicates the use of cellular MT expression as a biomarker of zinc status. The lack of sensitive and specific antibodies for the human MT protein also has limited studies of the response of cellular MT concentrations to changes in dietary zinc or zinc status.

With a change in cellular zinc, shifts occur in the expression of 2 sets of zinc transporters. In general, it is thought that the expression of the cellular zinc efflux transporters (e.g., ZnT1 or ZnT2; cellular exporters) is reduced with a decline in cellular zinc, whereas ZIPs (cellular importers; e.g., ZIP1) are increased. In a study in men who were experiencing acute zinc depletion (<2 mg dietary zinc/d), the expression of ZnT1, a cellular zinc exporter, declined within 10 d in leukocytes and whole blood (232). Because the expression of ZnT1 is ubiquitous in all tissues, it could be a biomarker of zinc status or exposure. However, additional studies of its sensitivity to less severe reductions in dietary zinc among individuals are needed to assess the utility of ZnT1 as a marker of exposure under conditions of a more typical range of zinc intake.

Dematin, a cytoskeletal protein involved in the maintenance of cellular morphology, motility, and membrane structural integrity, declined in men who consumed diets providing <2 mg zinc/d for 10 d (233). This observation has not been replicated in other studies.

Several other proteins have been proposed as biomarkers of zinc status, even though they have no direct interaction with zinc. Instead, a zinc-dependent process might regulate their concentrations. An example is the interaction between zinc and vitamin A that was recognized >30 y ago (234). Both plasma retinol and retinol binding protein have been positively correlated with dietary zinc intake (235–238). Possibly, hepatic cellular zinc affects the synthesis of retinol binding protein and therefore plasma retinol. Further studies are needed to determine the sensitivity of retinol binding protein synthesis to changes in cellular zinc.

Zinc also functions as an important cell-signaling agent (239). For example, cellular zinc availability influences signaling pathways, such as extracellular signal–regulated kinase 1/2 (ERK1/2) phosphorylation and insulin receptor signaling (240). The effect of zinc on cellular events may provide targets for monitoring cellular zinc status in the future. Cellular functions may be linked to the concentrations of free zinc within the cell. Shifts in cellular free zinc have not been measured in human zinc-depletion or -supplementation studies.

Oxidative stress and DNA integrity. Zinc occurs as the zinc (II) ion in body fluids and in cells and is redox-inert. Yet, it is widely accepted that it has antioxidant properties (241, 242). Because zinc's antioxidant properties may function as an indirect antioxidant, the term "pro-antioxidant" is more appropriate (243). However, zinc only facilitates pro-antioxidant functions over a limited range of zinc concentrations. Outside this range, zinc is a pro-oxidant. Thus, both zinc deficiency and zinc overload cause oxidative stress and an overproduction of reactive oxygen species (244, 245).

The molecular mechanisms responsible for the oxidative stress due to zinc deficiency stem from the inability to sustain the pro-antioxidant zinc functions. In zinc deficiency, cellular sulfhydryls that usually bind zinc and participate in zinc buffering are no longer protected, and they react with copper and iron to generate reactive oxygen species. In addition, in zinc deficiency, the induction of MT and enzymes involved in antioxidant defense is compromised. At high zinc concentrations, zinc inhibits antioxidant enzymes, such as thioredoxin and glutathione reductase, and components of the mitochondrial respiratory chain (i.e., complexes II and III) with concomitant increases in reactive oxygen species. In addition, high, longer-term zinc supplementation may cause a secondary copper deficiency, which is a pro-oxidant condition.

The pro-antioxidant nature of zinc has been shown in several human studies. Supplementation with 45 mg zinc/d for 8 wk reduced biomarkers of oxidative stress in healthy adult volunteers (246). Biomarkers of inflammation also were reduced when middle-aged or elderly volunteers received 45 mg zinc/d as a supplement for 12 mo (242). Further research is needed to determine the sensitivity of moderate increases and decreases in zinc intake on markers of oxidative stress or inflammation.

Zinc has also been linked to cancer through the effect of zinc deficiency on DNA mutations. Many DNA repair mechanisms involve zinc. For example, the tumor suppressor protein p53 is dysfunctional with low intracellular zinc and DNA repair is compromised (247). In the absence of zinc, an environment with increased oxidative stress and DNA damage occurs. This condition is exacerbated by an inability to adequately signal DNA repair mechanisms, which provides an environment for increased DNA damage. Studies in experimental animals and humans showed that marginal zinc depletion impairs DNA repair and increases the number of DNA strand breaks (248). Although increased DNA strand breaks seem to occur with small changes in zinc intake, these breaks are not a specific marker for zinc depletion. Insufficient intakes of choline, folate, and niacin also cause an increase in DNA strand breaks (249, 250).

In sum, it is clear that zinc and the redox state of the individual are linked, and that this link may be a major factor in disease etiology and pathogenesis. Markers of oxidative stress should be considered as surrogates for zinc status. Unfortunately, because many other conditions alter the redox state, these biomarkers are not specific for zinc nutrition.

Zinc kinetics. Stable isotopes of zinc (^{67}Zn, ^{68}Zn, and ^{70}Zn) can be used as tracers to measure zinc absorption, endogenous fecal zinc excretion, the size of various zinc pools, and shifts in whole-body zinc distribution in response to changes in dietary zinc (251–254). Data are limited, but it appears that shifts in the turnover or size of body zinc pools reflect changes in zinc nutrition.

The observed increase in plasma zinc turnover with zinc depletion has led investigators to explore plasma zinc kinetics, or the rate of movement of zinc into and out of the plasma, as a biomarker of zinc status (238, 254, 255). Plasma zinc kinetics correlate highly with changes in PZCs during acute zinc depletion (237, 254). Normally, total plasma zinc turns over ~150 times/d. With acute zinc depletion, the turnover rate increased to ~200 times/d (22). In another study in 33 premenopausal women, a low intake of beef, a good source of zinc, caused a decrease in the turnover of the plasma zinc pool, and this was associated with a reduction in taste acuity (254). This is the only study to our knowledge that showed a relation between plasma zinc kinetics and the intake of a zinc-rich food or a zinc-related function (taste acuity).

Isotopic tracer studies have also been used to measure the mass (or amount) of a relatively small whole-body exchangeable zinc pool (EZP). The size of the EZP is estimated from tracer-tracee disappearance curves with the use of kinetic modeling software. In adults, the EZP contains 150–200 mg zinc and has a turnover rate of ~12.5 d (251, 252). This pool is made up of the most metabolically active (forms of) zinc in the plasma, extracellular fluid, liver, pancreas, kidney, and intestine (256).

The total EZP mass varies with dietary zinc intakes, zinc absorption, age, and sex, with men having larger masses than women. An acute zinc deficiency in adults, induced by very low dietary zinc intake (<1 mg/d), caused a significant reduction in the size of the EZP (251). Studies in adults and infants showed that the EZP size correlated with the amount of dietary zinc and with the amount of total absorbed zinc (257–261). Furthermore, zinc absorption studies after surgery showed a progressive reduction in total zinc absorption and EZP size (262).

The size of the EZP per kilogram of body weight varies substantially between adults and infants: 2.5 mg zinc/kg in adults compared with 4.5 mg zinc/kg in infants (251, 259). The higher mass in infants may reflect an increased exchange between the zinc tracer and endogenous tissue zinc due to the higher metabolic rates in infants than in adults.

The EZP response to changes in zinc intakes suggests that it may be a good biomarker of zinc nutrition. However, the influence of age and sex on EZP mass indicates that different cutoffs for insufficiency may need to be established for various age and sex groups. More research is also needed to assess the sensitivity of the EZP to small changes in dietary zinc and to determine if the size of the EZP is linked to changes in zinc function.

Taste acuity tests. Diminished taste acuity (hypogeusia) is a nonspecific feature of marginal zinc deficiency and has been investigated as a functional bioindicator of zinc status (84, 86, 187, 212, 263). For example, boys (aged 5–7 y) with low height percentiles and low hair zinc concentrations had impaired taste acuity (86). In another study of acute zinc deficiency in young men, the ability to discriminate differences in bitterness declined during zinc depletion compared with baseline levels (264); however, the changes in perceived bitterness intensity were not related to salivary zinc concentrations or PZCs.

There have been several studies that investigated the efficacy of zinc supplementation in restoring taste acuity in the elderly, but the results have been inconclusive (265). However, zinc supplementation was beneficial in restoring taste loss during irradiation chemotherapy (266, 267).

Detection and recognition thresholds for each taste quality (e.g., sweet, salt, sour, and bitter) can be used to evaluate taste acuity. The detection threshold is defined as the lowest concentration at which a taste can just be detected, whereas the recognition threshold is the lowest concentration at which the quality of the taste stimulus can be recognized. For young children who are easily distracted and have short attention spans, recognition thresholds for only 1 taste quality (salt) can be assessed (268). Alternatively, an electrogustometer, which measures taste thresholds by applying a weak electric current, can be used (153, 269). A threshold value is determined by alternately lowering and raising the current to determine the smallest stimulus that can be correctly discriminated. All tests of taste acuity should preferably be performed midmorning, at least 2 h after a meal, and by the same person on each occasion.

Biomarkers not recommended

Blood cellular zinc concentrations and various zinc-dependent enzymes have been used as biomarkers of zinc status in a number of studies (163). Those studies showed that enzymatic and blood cellular zinc biomarkers do not relate consistently to changes in zinc intakes or PZCs. Thus, the BOND Zinc Expert Panel classified these biomarkers as "not useful."

Zinc-dependent enzymes. Zinc's role as a component of proteins falls into 3 general categories: structural, regulatory, and catalytic. The structural and regulatory roles of zinc are considered emerging biomarkers (see the section on zinc-dependent proteins). Zinc also serves in a catalytic role for >300 zinc metalloenzymes. An enzyme is considered a zinc metalloenzyme if the removal of zinc causes a loss of activity. Examples are nucleotide polymerases, carbonic anhydrases, extracellular superoxide dismutase, aminolevulinic acid dehydratase, angiotensin-converting enzyme, plasma 5′ nucleotidase, and alkaline phosphatase (ALP) (270). The process by which zinc is donated to apometalloenzymes is not well established. It may require post-translational protein modification or a zinc transporter. For example, the ZnT5/ZnT7 complex provides Zn^{2+} to activate tissue-nonspecific ALP (1).

Although zinc is required for the normal function of the zinc-containing enzymes, a consistent, direct link between zinc-dependent enzyme concentrations and signs of zinc deficiency or toxicity has not been seen in experimental animals or in humans. This is probably because signs of zinc deficiency or toxicity would only be evident if the zinc-requiring enzyme were a rate-limiting enzyme in the biochemical pathway. Instead, it is likely that the physiologic symptoms of zinc deficiency or toxicity reflect a series of biochemical changes.

In a systematic review of biomarkers of zinc status (163), the relation between zinc supplementation or depletion and enzyme activity was reviewed for 7 different enzymes. ALP is used most frequently as a zinc biomarker. Six studies of the response of plasma ALP activity and zinc supplementation (3 RCTs) or depletion (3 studies) were available. No consistent effect of zinc intake on overall plasma ALP activity was evident. ALP exists as 3 different isozymes (intestinal, placental, and liver/kidney/bone) and several isoforms (post-translational modification of the isozymes) in humans, and ALP in the circulation is a mixture of these isoforms. Examining the individual responses of these to changes in zinc intake may yield more sensitive and consistent data. On the basis of the current data available, the activity of zinc-dependent enzymes is not a useful biomarker of zinc intake or status.

Erythrocyte and leukocyte zinc concentrations. The zinc concentration of whole blood is 4–8 μg/mL. The zinc content of packed erythrocytes normally is 8–14 μg/g wet weight or 10–11 $\mu g/10^{10}$ cells (271). Leukocytes contain up to 25 times more zinc than erythrocytes and have a much shorter life span than the 120-d life span of erythrocytes (151). Hence, they are thought to be more sensitive to changes in zinc nutrition than erythrocytes. Leukocytic zinc concentrations vary with the type of cell. Monocytes and lymphocytes have the highest concentrations followed by neutrophils. Generally, mixed white blood cells contain 75 μg zinc/10^{10} cells (271).

Studies of the usefulness of blood cellular zinc concentrations as biomarkers of zinc nutrition have yielded mixed results. Experimental human zinc-depletion/repletion studies or prolonged high zinc supplementation (e.g., 50 mg/d) have not shown consistent changes in erythrocyte zinc, erythrocyte membrane zinc, leukocytic zinc, or the zinc content of leukocyte subpopulations (272). Indeed, no response by erythrocyte or leukocyte zinc concentrations was noted in some zinc-depletion studies, even though impaired taste acuity and immune function, 2 potential bioindicators of functional zinc depletion, were evident. In addition, the lack of established reference values for these cellular zinc concentrations makes interpreting the results difficult. Additional factors limiting their use are the relatively large volumes of blood required for the analysis and the difficulties of separating specific leukocytic components from other white blood cell types.

Assay-Specific Queries

Dietary assessment

None of the methods used to measure and evaluate zinc intakes at the individual or population level identify with certainty that the individual has an inadequate zinc intake, because the actual zinc requirement of the individual is unknown. Normal day-to-day variation in food selection and errors in estimating the total quantity of food consumed permit only an approximation of an individual's usual zinc intake. Thus, dietary zinc intake data only provide an estimate of zinc exposure and/or inadequacy.

The DRIs provided by the Institute of Medicine (39) supply guidelines for the qualitative interpretation of the adequacy of the zinc intake of an individual that can be used for clinical evaluations. Alternatively, a quantitative statistical approach can be used to estimate with a certain level of confidence that the usual zinc intake of an individual meets his or her requirement (39). This approach requires an estimate of both the variability of the zinc requirement and the variability of the zinc intake (i.e., within-person variation), which requires multiple 24-h recalls or daily food records. Examining the difference between the reported intake and the EAR enables one to make inferences about the adequacy of an individual's zinc intake. Dividing the estimate by its SD, which reflects uncertainty in both the usual intake and the estimated requirement, standardizes the difference. The result is a z score from which a probability value (P), reflecting the degree of confidence that the individual's usual intake meets his or her requirement, can be determined (39, 273). The choice of the EAR to evaluate the adequacy of the usual zinc intake of the individual by using the quantitative statistical approach depends on the study setting and the likely absorption of zinc in his or her usual diet.

In most industrialized countries, a single zinc EAR is based on a fixed adjustment for zinc absorption from the habitual national diet. For lower-income countries, however, where diet composition is often dependent on geographic location (i.e., urban or rural), socioeconomic status, or religion, the EAR used should reflect the likely absorption of zinc in the habitual diet of the population group under study, which can be predicted by the dietary phytate-to-zinc molar ratio. EARs for zinc from both unrefined, cereal-based diets with phytate-to-zinc ratios >18 and from mixed, refined vegetarian diets with phytate-to-zinc molar ratios of <18 have been set by the IZiNCG (19).

Blood sampling and preparation for analysis

Blood sampling. Blood samples for serum or plasma zinc should be taken under carefully controlled, standardized conditions. Contamination from various sources, such as preservatives, evacuated tubes, lubricants, anticoagulants, water, and rubber stoppers, must be avoided (see the IZiNCG website for practical tips on collecting blood for assessment of plasma zinc concentration). For venipuncture blood samples, trace element–free evacuated tubes with siliconized rather than rubber stoppers must be used. Stainless steel or siliconized needles and Teflon or polypropylene catheters can be used. For capillary blood samples, the use of polyethylene serum separators with polyethylene stoppers and olefin-oligomer is recommended. Fasting status, time of day of blood collection, and time elapsed since the previous meal should always be recorded during large-scale surveys in which serum or plasma zinc will be assayed so that zinc values can be adjusted (274) and the appropriate reference limits chosen (157).

Interpretation of PZC data. The utility of PZC as a biomarker of either short- or long-term zinc status is compromised by the interactions with a number of factors that affect its interpretation. Factors that must be considered during specimen collection and interpretation of results include the following: time of day of specimen collection; time of previous meal consumption; time elapsed until centrifugation; the presence of systemic inflammation or other selected diseases, such as hemolytic conditions; and the administration of certain drugs, hormones, and nutritional supplements. Moreover, blood specimens are susceptible to contamination with ambient zinc during collection and/or processing,

so meticulous handling procedures must be used. The following section provides a brief review of these issues.

Several studies have shown that PZCs fluctuate by as much as 20% throughout the day, mainly in response to meal consumption. In particular, the PZC increases during overnight fasting, and the highest concentrations are generally observed in the morning before breakfast (275). The PZC then decreases progressively for several hours after each meal before rising prior to the next meal. It is not clear whether all of the changes in PZC that occur throughout the day are caused by meal responses, because some population-based studies have found that both factors (time of specimen collection and recent meals) independently predict PZC (274). Thus, the meal status and time of day should be carefully controlled so as not to confound the interpretation of results. Alternatively, the respective information could be recorded so the PZC can be adjusted for these factors (276).

PZCs also decrease in response to several physiologic and pathologic conditions that are not necessarily indicative of low zinc status. For example, PZCs decline progressively during pregnancy, but this is probably due to hemodilution because the zinc-to-albumin ratio remains constant (277). PZCs also decline during acute and chronic infections and other conditions that cause systemic inflammation, such as obesity, surgery, and intensive physical exertion (278). This may reflect the transfer of zinc from the blood to the liver as part of the inflammatory response and cytokine-induced hepatic MT synthesis (279). To adjust for the presence of systemic inflammation, it is recommended that biomarkers of the inflammatory response, such as C-reactive protein and/or α1-acid glycoprotein, be measured along with the PZC. Although a statistical procedure for adjusting the PZC for inflammation has been proposed (62), further research is needed to validate the sensitivity and specificity of the method.

The zinc concentrations of blood cellular components are ~10-fold greater than PZCs. Thus, cellular hemolysis, either in vivo or in vitro, leads to a falsely elevated PZC. Moreover, as described above, allowing whole blood to remain at room temperature before separating plasma or serum causes zinc to transfer from the cells to plasma. To avoid the latter situation, plasma or serum should be separated rapidly from whole blood (ideally within 20–30 min) or the blood can be held at −10°C for up to 24 h to prevent movement of cellular zinc into the plasma (280). Additional information on optimal procedures for collecting and processing blood specimens and methods for analyzing PZC are listed in **Text Box 10**.

Hair and nail zinc. Standardized procedures for sampling, washing, and analyzing hair and nail samples are essential. Hair samples should be collected from close to the occipital portion of the scalp with stainless steel scissors, and only the proximal 1.0–1.5 cm of the hair strands retained for analysis. If necessary, any nits and lice must be removed under a microscope before washing the hair samples with the use of a standardized method. Washing with nonionic detergents [e.g., Triton X-100 (Thermo Fisher Scientific)] with or without acetone is preferred because they are less likely to leach out bound zinc from the hair while effectively removing superficial adsorbed zinc (281). Washing with chelating agents such as EDTA should be avoided because they can remove some of the tightly bound trace elements in the hair samples (282). Nails should be cleaned before analyses to remove exogenous zinc sources by first scraping, then washing in an aqueous nonionic detergent, after which nails should be rinsed and then dried (283).

Laboratory methodologies. AAS is the most frequently used method for analyzing serum or plasma zinc. First, the sample is diluted with 4 or 9 parts of deionized water, an aqueous acid solution (e.g., 0.1 M HCl), organic acids (e.g., n-butanol or n-propanol), or with a signal enhancing mixture. Dilution of the serum reduces the viscosity of the sample, which minimizes both the matrix effect on the rate of aspiration into the AAS and the tendency for the burner head to become blocked. The latter may be especially a problem with plasma because of precipitates that form in these samples. To ensure that the viscosity of the samples and standards are similar, a 5% aqueous glycerol solution is sometimes used as the solvent for the standards (284) or, alternatively, a 6% aqueous solution of butanol as a sample diluent in a 5- to 10-fold dilution. A CV <5% for duplicate zinc samples is the standard when analyzed by AAS. The use of a trichloroacetic acid deproteinization technique is not recommended.

When low zinc concentrations are anticipated, the serum or plasma samples can be acid digested (276) or ashed at low temperatures (285) before analysis. For very small samples, a flameless AAS can be used. Other analytical techniques include ICP-MS, ICP atomic emission spectrometry, ICP optical emission spectrometry, X-ray spectrometry, proton-induced X-ray emission, instrumental neutron activation analysis, and anodic stripping voltammetry.

CRMs, suitable for use when analyzing serum or plasma zinc, include bovine serum (SRM 1598) from the National Institute of Standards and Technology, Seronorm Trace Elements Serum L-1 and L-1 (Accurate Chemical and Scientific Corporation), or animal blood (IAEA A-13) from the IAEA. Because these are expensive, in-house or bench reference materials, such as a pooled serum sample analyzed against an SRM to establish its zinc content, could be prepared and used with every set of analyses to monitor precision.

Several techniques can be used to analyze hair zinc concentrations (183). In the past, flame AAS was most commonly used, although, increasingly, multielement ICP-MS is recommended. Microwave digestion is now the preferred sample preparation method for the washed hair samples (286), although conventional wet or dry ashing can be used. When instrumental neutron activation analysis is used, no ashing is required (182, 183). CRMs for human hair are available [CRM 397 (Community Bureau of Reference, Institute for Reference Materials and Measurements) and IAEA-085 and IAEA-086 (IAEA)]. In-house controls composed of digested hair and finely cut homogenized hair, analyzed in conjunction with a CRM, should also be used to monitor assay variations in the instrument and digestion procedures.

Height or length measurements. For infants and children ≤2 y of age, recumbent length is recommended, preferably with the use of an infantometer with a range of 30–110 cm equipped with a digital counter reader. Recumbent length should be recorded to the nearest millimeter, or even more precisely (i.e., 0.1 mm) when possible. Wooden or acrylic length-measuring boards can be used, but they are rarely fitted with digital counters and therefore are less reliable. It is important to know that the average recumbent length for a child of ~2 y is ~5 mm greater than the standing height for the same child (287).

Children >85 cm and adults should be measured in the standing position, preferably by using a free-standing stadiometer (range: 65–206 cm), again equipped with a digital counter reader capable of measuring stature to 0.1 mm. Platform scales with movable measuring rods should not be used, because they are not accurate. Clothing should be minimal for height

Text Box 10 Practices to minimize contamination during the collection and analysis of zinc in blood[1]

- Prescreen trace element–free polyethylene evacuated tubes, stoppers, and serum separators for zinc contamination before use.
- Prescreen polyethylene storage vials and transfer pipettes for zinc contamination before use.
- Arrange for the subject to be seated. Clean the subject's skin with alcohol at the site of the antecubital vein.
- Limit tourniquet occlusion to no more than 1 min. Prolonged use of a tourniquet may increase SZCs or PZCs due to increased intravascular pressure caused by venous occlusion, which may cause fluid movement into the interstitial space and thereby increasing the zinc concentration.
- Draw blood with the use of stainless steel needles.
- For processing serum, collect the blood into trace element–free evacuated collection tubes without anticoagulant.
- For plasma samples, zinc-free heparin is the preferred anticoagulant. If any other anticoagulant is used, it should be prescreened for adventitious zinc.
- Wear disposable polyethylene gloves, free of talc or other coatings, when handling blood samples.
- All equipment used, with the exception of the prescreened disposable items, should be decontaminated by washing procedures (soaked for 24 h in ultrapure 10%–20% HCl or HNO_3 solution and rinsed 3–4 times in distilled, deionized water).
- All materials and equipment should be covered or sealed during storage and processing to avoid dust contamination.
- Place blood samples in a refrigerator or on ice and allow clotting for 30–40 min. Separation can be delayed up to 1 h, but longer intervals are associated with progressively increasing serum zinc concentrations, due to zinc being released from platelets (165). Refrigeration or putting the samples on ice immediately after collection reduces increases in serum zinc.
- Centrifuge blood samples at 2000–3000 *g* at room temperature for 10–15 min.
- Discard obviously hemolyzed samples.
- Store samples at −20°C unless they are to be analyzed immediately. If necessary, serum or plasma samples can be refrigerated (4°C) for 2–3 wk before analysis.
- Process samples in laminar flow class 100 clean rooms, in a desktop laminar flow hood, or otherwise in a clean dust- and smoke-free laboratory.
- Dilute samples by 5–10-fold in solvents such as 6% aqueous butanol or 10% aqueous propanol.
- Read sample zinc concentrations by using atomic absorption spectrometry (AAS) or with inductively coupled plasma (ICP) emission spectrometry with appropriate standard dilutions, along with in-house quality controls and standard reference materials (SRMs) such as bovine serum (SRM 1598) from the National Institute of Standards and Technology.

[1]Data from references 19 and 130.

measurements so posture can be clearly seen. Shoes and socks should not be worn. The timing of the measurement should be recorded because the spine gradually compresses during the day, causing diurnal variations in height (288, 289). Consequently, in population studies, standing height should always be measured at the same time of day, preferably in the afternoon.

When measuring recumbent length or standing height, attempts should be made to minimize measurement errors. In longitudinal studies involving sequential measurements on the same individuals, 1 person should conduct all of the measurements throughout the study to eliminate between-examiner errors, especially when growth velocity is to be calculated (i.e., cm/y). In the WHO Multicenter Growth Reference Study (MGRS), the minimal interval recommended for reliable data on length increments was every 2 wk for infants from 2–6 wk of age, monthly for ages 2–12 mo, and bimonthly in the second year (290). During adolescence, increments measured over 6 mo are the minimum interval recommended (291). For shorter intervals, the combined errors may be too large in relation to the expected mean increment.

In large cross-sectional surveys, several well-trained anthropometrists are often needed to rotate among the participants to reduce the effect of measurement bias. Regular standardization sessions to assess both intra- and interexaminer reliability should be conducted throughout the data collection period to maintain the quality of the measurements and to identify and correct systematic errors in the measurements; details of the procedures used in the WHO MGRS are provided in de Onis et al. (290).

The WHO MGRS recommends that the maximum allowable difference in length for acceptable precision between measurements by 2 anthropometrists is 7.0 mm (290). Details of the measurement techniques and standardization protocols for both recumbent length and stature are also available in an anthropometric training video prepared for the WHO MGRS and available on request from de Onis et al. (292). Statistical methods exist for removing anthropometric measurement error from cross-sectional anthropometric data; details are given in Ulijaszek and Lourie (293).

Quantifying zinc isotopic ratios by ICP-MS. Zinc stable isotopes can be used as tracers to measure zinc absorption, endogenous fecal zinc excretion, the size of various zinc pools, and shifts in whole-body zinc distribution in response to changes in dietary zinc. Because no radioactivity is associated with these isotopes, their application to vulnerable populations, including infants and pregnant or lactating women, has greatly contributed to the current concepts of zinc bioavailability, metabolism, and homeostasis. The natural abundance of 3 stable zinc isotopes is sufficiently low to allow their utilization as tracers in human studies: ^{70}Zn (0.6%), ^{67}Zn (4.1%), and ^{68}Zn (18.8%). Isotope ratios of ^{67}Zn:^{66}Zn, ^{68}Zn:^{66}Zn, and ^{70}Zn:^{66}Zn are commonly measured by the use of ICP-MS. The majority of studies in human zinc nutrition use extrinsically labeled meals or supplements along with an accurately measured dose that is administered orally or intravenously. Isotopic enrichment can be determined in several biological tissues, including plasma, erythrocytes, urine, and feces (22, 294).

New Directions and Technologies

Bone as a zinc reserve. Bone contains ~30% of total body zinc, that is, ~700 mg total or 66 μg/g body weight, with some differences due to sex (295). Unlike muscle zinc, which accounts for ~60% of body zinc (296) and which appears to be impervious to changes in dietary zinc, there are suggestions that bone zinc is responsive to changes in dietary zinc intake. Studies showed that bone zinc, as well as liver zinc, is mobilized when animals are fed a zinc-deficient diet (297). Because the total content of zinc in bone is 3-fold higher than that of all soft tissues combined, a decrease in bone zinc concentration would indicate a major release of endogenous zinc compared with that from other tissues. Thus, bone zinc may provide a "back-up" source of zinc for other tissues with a vital zinc requirement when the dietary supply is inadequate (298). Nevertheless, there are important biological roles for zinc in bone. In addition to zinc's active role in collagen formation in the epitheses, zinc ions are promoters of bone remodeling by osteoblast proliferation (299), and they contribute to extracellular matrix calcification through the synthesis of matrix proteins in osteoblasts (300). However, a biomarker of changes in bone zinc is not available at this time. Research is needed to better understand the relation between bone zinc, diet zinc, and overall zinc homeostasis.

Cellular biomarkers of zinc status and function. Approximately 99% of whole-body zinc is intracellular (295). Because the total amount of zinc in major tissues is much larger than that in plasma, relatively small variations in the zinc content of tissues, such as liver, can have a dramatic effect on PZCs. For example, treatment with glucocorticoids, which induce hepatic MT, causes a marked decline in plasma zinc as zinc moves into the liver (301). This may explain why systemic markers of zinc status, including whole blood concentrations and PZCs, do not reflect moderate zinc deficiency. Recent studies suggest that the regulation of cellular zinc is complex and may involve both fast and slow mechanisms for regulating cellular zinc turnover (302). Although leukocyte or erythrocyte zinc concentrations are not responsive to changes in dietary zinc, research with regard to subcellular zinc concentrations and various cellular functions may lead to novel biomarkers of zinc status.

Laser technology for measuring zinc concentrations. Zinc concentrations in hair, fingernails, buccal cells, and blood cells are all in the range of 60–200 ppm (dry sample). Thus, a 1-mg sample has 60–200 ng zinc, which is ample material for accurate quantitation by many methods. Perhaps the most useful of these are the new "field-portable" hand-held instruments capable of these assays.

One of these is called laser-induced breakdown spectroscopy (LIBS), which has been in use for approximately 1 decade. LIBS is basically an atomic emission spectrometer, in which a small laser produces a brief nanosecond plasma on the sample and a solid-state photodetector captures the atomic emissions, which in wavelength and intensity correspond to the identity and abundance of the plasma elements. Battery-powered, briefcase-sized LIBS instruments are now commercially available, and they have the capability to accurately measure zinc concentrations in the mid-nanogram mass range. This means that one can clip a bit of fingernail or scrape a bit of buccal tissue, dry the sample, and obtain an LIBS determination of the zinc content in the field within minutes. According to preliminary analysis, even a typical 30-μL drop of whole blood from a finger stick contains enough RBCs, white blood cells, and serum or plasma with which a field-portable LIBS instrument could provide an accurate zinc analysis for each of those blood components. "Lab-on-a-chip" systems are available for separating the 3 blood components from a single drop of whole blood.

Beyond LIBS, another method potentially adaptable to in situ zinc analysis is X-ray fluorescence (XRF). Bone lead has been measured in situ by XRF at Harvard (303) and Mt. Sinai Hospital in New York (304). XRF has also been proposed for measuring (and imaging) zinc within the prostate gland (305). At 200–300 ppm (dry), zinc is ~10-fold more abundant in human bones than lead. Whether the same methods used to measure bone lead could be used for the lighter, but more abundant, element zinc remains unknown.

Research Gaps and Needs

Need for a specific, sensitive, and field-friendly zinc biomarker. The ubiquitous nature of zinc in human biological systems explains the widespread consequences and the complexity of the responses to insufficient zinc intakes. Although marginal zinc deficiency is linked to reduced physical growth in children worldwide, comprehensive studies of the metabolic response to marginal zinc intakes have only been conducted in adults; comparable information is not available for infants or children who are more vulnerable to zinc deficiency. Furthermore, the strong homeostatic mechanisms to sustain tissue zinc concentrations and function with low intakes severely impair our ability to detect zinc deficiency. Currently, the zinc status of populations can be estimated by using the following 3 indicators: prevalence of zinc intakes below the EAR, percentage with low PZCs, and percentage of children aged <5 y who are stunted. A biomarker of zinc status in individuals that is more sensitive than PZCs is needed to improve zinc nutrition in clinical settings. Several potential or emerging zinc biomarkers have been identified (e.g., hair, nail, and urinary zinc concentrations; concentrations of zinc-dependent proteins; zinc kinetic markers; and DNA-repair functions). However, considerable research is required before those biomarkers can be used to evaluate the zinc status of individuals or populations.

Need for evidence to support scale-up of preventive zinc interventions. There are, at present, no preventive zinc programmatic activities at the national or international levels. The WHO has not established guidelines for large-scale zinc interventions that are designed to prevent inadequate zinc nutrition. Furthermore, without a sensitive, specific zinc biomarker, program planners struggle to assess the need for preventive zinc interventions and how to measure their impact. In addition, there is a need to determine the optimal dose, dosing frequency (i.e., weekly, intermittent, or daily), form (i.e., supplement with zinc alone or together with other micronutrients compared with food-bound zinc), delivery channels (e.g., clinics, health centers, market-based access points, community centers, or social protection programs), and distribution platforms (e.g., Child Health Days, Expanded Program on Immunization, growth monitoring, or distribution of multiple micronutrient powders) that are effective and feasible. Until some of these questions are answered, it is very difficult to establish large-scale preventive zinc programs for populations at risk.

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