ZINC: WHAT ROLE MIGHT SUPPLEMENTS PLAY?

A conference sponsored by the NIH
Office of Dietary Supplements

November 6, 1998

Uniformed Services University
of the Health Sciences
Building B Auditorium
4301 Jones Bridge Rd
Bethesda, Maryland, USA

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CONFERENCE ON ZINC: WHAT ROLE MIGHT SUPPLEMENTS PLAY?

INTRODUCTION

The 1994 Congressionally mandated Office of Dietary Supplements was formally established at the end of November 1995, within the Office of the Director (OD) at the National Institutes of Health. Among the tasks for this office are:

> "to explore more fully the potential role of dietary supplements as a significant part of the efforts of the United States to improve health care; … and to conduct and coordinate scientific research within the National Institutes of Health relating to dietary supplements …" (Public Law 103-417).

In June, 1996 in keeping with the focus on activity heightened by publication of Physical Activity and Health, A Report of the Surgeon General, the Centennial Olympic Games, and the 1996 Paralympic Games, the ODS held its first major workshop, "Dietary Supplements for Physically Active People." This conference **Zinc: What Role Might Supplements Play?**, is the second in the ODS initiated series which will present a state-of-the-art review of zinc as it relates to health. The last five years have seen a virtual explosion of interest and understanding of the spectrum of functions of zinc at sub-cellular levels. Zinc is a versatile trace element required as a cofactor by more than 300 enzymes in every organ of the body. Zinc supports the work of numerous proteins in the body—among them are the metalloenzymes, which are involved in a variety of metabolic processes. The zinc atom is so ubiquitous in cellular metabolism, that even minor impairment of an adequate supply is likely to have multiple biological and clinical effects. Given the central roles of zinc in cellular growth and differentiation, it is no surprise that the effects of zinc deficiency are pronounced in the rapid turnover of tissues and organs, especially the immune system. "Zinc is a prime candidate for attention at the close of this and the beginning of the next millennium." D. Michael Hambidge, M.D. (personal communication, fall, 1998)

It is likely that identifying and correcting borderline nutritional zinc deficiency will offer widespread health benefits. Pharmacological doses of zinc may also be beneficial in certain circumstances and harmful in others. In the public health arena, the positive results of zinc supplementation trials on childhood morbidity and mortality in developing countries have been remarkable and offer promise as a low cost solution to specific health problems.

The goal of this conference is to provide a broad overview of the biochemical, cellular, and nutritional requirements of zinc in health and disease. Attention will be focused on key areas where zinc supplementation may play a role in the prevention, reduction, or treatment of disease. The following topical areas will be addressed: overview of the essentiality of zinc, dietary zinc requirements, the regulation of zinc metabolism, zinc and development, zinc and

immune function, zinc metabolism in disease, and zinc intake of the US population. In addition, new and emerging roles of zinc in human health will be discussed.

The conference will bring together leading experts in zinc research from several scientific disciplines who will present a timely update and critical needs assessment on zinc and health to researchers, nutritionists, and public health advisors and policy makers. These scientists will present an overview of the current state of scientific knowledge regarding zinc nutriture, requirements, and function that will be applicable to many basic science, clinical and public health programs across the country.

In closing, copies of this program booklet will be available for downloading from the Office of Dietary Supplements web site at http://dietary-supplements.nih.gov as well as a copy of a "Zinc and Health Bibliography" prepared in conjunction with the National Library of Medicine. We would also like to acknowledge the assistance of Donna F. Allen of the ODS project staff for her editing and word processing of the program booklet, and the assistance of Kristine M. Scannell and Adam Glazer for production of the Zinc and Health Bibliography.

Rebecca B. Costello, Ph.D.

Bernadette M. Marriott, Ph.D.

GENERAL INFORMATION

Conference sessions will be held in Auditorium B, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD. The conference will run from 8:00 AM to 4:00 PM on Friday, November 6, 1998. The telephone number for the conference message center is (301)-295-3466.

CAFETERIA

The cafeteria is located on the street level of the conference building, and it is open daily from 7:00 a.m. to 3:00 p.m.

CONTINUING EDUCATION CREDITS

For Physicians

The goal of this conference is to summarize and present the recent findings on zinc research on selected topics related to public health. The concluding presentations will summarize zinc requirements and intakes with emphasis on changes across the life cycle.

The conference will present in open public sessions state-of-the-art scientific information focused on a number of key areas where zinc supplementation may play a role in the prevention, reduction, or treatment of disease.

The National Institutes of Health is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

The National Institutes of Health designates this continuing medical education activity for a maximum of 7.0 credit hours in Category I of the Physician's Recognition Award of the American Medical Association.

For Dietitians

Participation in this conference meets the American Dietetic Association's requirement for continuing education criteria for 7.0 credit hours.

SPONSORS

The primary sponsor for this workshop is the NIH Office of Dietary Supplements. Co-sponsors include the The American Dietetic Association, the American Society for Clinical Nutrition, the Centers for Disease Control and Prevention, Department of Defense, and the Food and Drug Administration/Center for Food Safety and Applied Nutrition. The NIH co-sponsors include the Fogarty International Center, National Institute on Dental and Craniofacial Research, National Institute of Drug Abuse, National Institute of Diabetes and Digestive and Kidney

Diseases, National Institute of General Medical Sciences, and the Office of Research on Women's Health.

AGENDA

Friday, November 6, 1998
Uniformed Services University of the Health Sciences
Auditorium, Building B
Bethesda, MD, USA

Sponsored by the Office of Dietary Supplements, NIH
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National Institute of Diabetes and Digestive and Kidney Diseases
National Institute on Drug Abuse
National Institute of General Medical Sciences
Office of Research on Women's Health

Friday, November 6, 1998 8:00 AM–4:00 PM

8:00-8:30 AM	**Welcoming Remarks and Introduction** **Bernadette M. Marriott, Ph.D.** *Director, Office of Dietary Supplements, Bethesda, M.D.*
8:30-9:10 AM	Overview/Essentiality of Zinc D. Michael Hambidge, M.D. *University of Colorado, Denver, CO*
9:10-9:20 AM	Discussion
9:20-10:00 AM	Impact of Dietary Zinc Status on the Immune System Pamela Fraker, Ph.D. *Michigan State University, East Lansing, MI*
10:00-10:10 AM	Discussion

10:10-10:50 AM	Regulation of Zinc Metabolism Robert J. Cousins, Ph.D. *University of Florida, Gainesville, FL*
10:50-11:00 AM	Discussion
11:00-11:40 AM	Zinc and Development Carl Keen, Ph.D. *University of California – Davis, CA*
11:40-11:50 PM	Discussion
11:50-1:00 PM	LUNCH
1:00-1:40 PM	Zinc Metabolism in Disease Nancy F. Krebs, M.D. *University of Colorado, Denver, CO*
1:40-1:50 PM	Discussion
1:50-2:30 PM	Dietary Zinc Requirements Janet C. King, Ph.D. *USDA, Agricultural Research Service, Western Human Nutrition Research Center, San Francisco, CA*
2:30-2:40 PM	Discussion
2:40-3:20 PM	Zinc Intake of the U. S. Population Ronette Briefel, Dr.P.H., R.D. *National Center for Health Statistics, Hyattsville, MD*
3:20-3:30 PM	Discussion
3:30-4:00 PM	Panel Discussions/Discussant Questions
	Conference Conclusions/Summary **Bernadette M. Marriott, Ph.D.** *Director, Office of Dietary Supplements, Bethesda, MD*

SPEAKERS

Ronette Briefel, Dr.P.H., R.D.
National Center for Health Statistics,
Centers for Disease Control and Prevention,
Department of Health and Human Services
Hyattsville, MD

Robert J. Cousins, Ph.D.
University of Florida
Gainesville, FL

Pamela J. Fraker, Ph.D.
Michigan State University
East Lansing, MI

D. Michael Hambidge, M.D.
University of Colorado
Denver, CO

Carl L. Keen, Ph.D
University of California, Davis
Davis, CA

Janet C. King, Ph.D.
Western Human Nutrition Research
Center/Agricultural Research Service,
US Department of Agriculture
Presidio of San Francisco, CA

Nancy F. Krebs, M.D.
University of Colorado
Denver, CO

PLANNING COMMITTEE

Carolyn K. Clifford, Ph.D.
National Cancer Institute Program
Rockville, MD

Jerry Cott, Ph.D.
National Institute of Mental Health
Rockville, MD

Robert J. Cousins, Ph.D.
University of Florida-Gainesville
Gainesville, FL

Robert W. Eisinger, Ph.D.
Office of AIDS Research
Office of the Director
Bethesda, MD

Robert Eiss, Ph.D.
Fogary International Center
Bethesda, MD

Nancy D. Ernst, Ph.D.
National Heart, Lung & Blood
Bethesda, MD

Pamela J. Fraker, Ph.D.
Michigan State University
East Lansing, MI

Stephen C. Groft, Pharm.D.
Office of the Director
National Institutes of Health
Bethesda, MD 20892-9120

Dr. Joan A. McGowan, Ph.D.
National Institute of Arthritis and
Skin Disease
Bethesda, MD

Kenneth A. Gruber, Ph.D.
National Institute on Deafness & Other
Communication Disorders
Rockville, MD
Carole Hudgings, Ph.D.
National Institute of Nursing
Bethesda, MD

Richard D. Irwin, Ph.D.
National Institute of Environmental Health
Bethesda, MD

Carl L. Keen, Ph.D.
University of California
Davis, CA

Jag H. Khalsa, Ph.D.
National Institute on Drug Abuse
Rockville, MD 20857

Lori Klein
National Library of Medicine
Bethesda, MD

Nancy F. Krebs, M.D.
University of Colorado
Denver, Co

Donna Krasnewich, M.D., Ph.D.
National Human Genome Research Institute
Bethesda, MD

Dr. Dennis Leszczynski
Office of International Science
Center for Scientific Review (CSR)
Rockville, MD

Bo Lonnerdal,Ph.D.
University of California
Davis, CA

Kenneth May, Ph.D.
National Institute of Diabetes & Digestive

& Kidney Disease
Bethesda, MD

Jean Pennington, Ph.D., R.D.
National Institute of Diabetes & Digestive & Kidney Disease
Bethesda, MD 20892-6600

Peter D. Preusch, Ph.D.
National Institute of General Medical
Bethesda, MD

Pamela Starke-Reed, Ph.D.
National Institute of Aging, Biology of Aging
Bethesda, MD

Lois A. Salzman, Ph.D.
National Institute of Dental and Craniofacial Research
Bethesda, MD

Charles R. Sherman, Ph.D.
Office of the Director
National Institutes of Health
Bethesda, MD

Martina Vogel-Taylor, M.T.
Office of the Director
Office of Disease Prevention
Bethesda, MD

ABSTRACTS

The following abstracts of presentations to the conference. **"Zinc: What Role Might Supplements Play?,"** were furnished by presenters in advance of the conferennce. This book is designed for the use of the conference participants and as a pertinent reference document for anyone interested in the conference topic. We are grateful to the authors who have summarized their materials and made them available in a timely fashion.

Bernadette M. Marriott, Ph.D.
Director, Office of Dietary Supplements
Office of the Director
National Institutes of Health
Bethesda, Maryland

OVERVIEW/ESSENTIALITY OF ZINC

Michael Hambidge

Zinc is one of that group of micronutrients that are termed trace elements, each of which contributes less than 0.01% to the body weight of the human. The total body zinc of an adult human is approximately 1.5–2.0 grams, or about 0.003% of total weight. As much as 90% of this zinc is incorporated in muscle and bone where it remains for relatively long periods of time. Though this slowly turning over zinc performs a number of essential biological functions, its metabolic importance is overshadowed by that of the remaining 10% of body zinc which is metabolically very active, exchanges very rapidly and is very sensitive to changes in access to bioavailable zinc in the diet. For example, all of the zinc in the liver, which is an organ of central importance for zinc metabolism, exchanges with zinc in plasma within two days. Application of tracer, especially zinc stable isotope, techniques—and parallel research *in vitro* at a subcellular/molecular level—is now allowing us to achieve a clearer understanding of how zinc metabolism is regulated. Of greater practical importance, these techniques are starting to (and can increasingly) help us understand in what circumstances and how zinc metabolism can be disturbed by disease and other host factors as well as by inadequate or excessive intakes of bioavailable zinc.

Each of the nutritionally essential trace elements (and major minerals) has its own unique combination of atomic characteristics that underlie their special individual roles in human biology. Of special note among the numerous biologically useful properties of the zinc atom is its outstanding ability to form strong but readily exchangeable and highly flexible ligands with the side chains of organic molecules. This special property has assured its versatile role at the catalytic site of a wide range of enzymes; its still poorly understood but potentially very exciting intracellular regulatory role, for example, in cellular growth and differentiation and in the process of apoptosis; and its roles as a structural ion in biomembranes and proteins. Of recent note has been the elucidation of its structural role in the zinc finger motif of transcription proteins and steroid hormone nuclear receptors.

Another notable feature of the zinc atom is its lack of redox properties. Thus, in striking contrast to iron and copper, the body can transport zinc in physiological quantities with relative impunity both in the circulation and intracellularly without concern about oxidant damage. This characteristic has undoubtedly facilitated the incorporation of zinc into so many metabolic pathways where its biological utility depends to a large extent on its ability to freely exchange its ligand binding.

The zinc atom is so ubiquitous in cellular metabolism, that even minor impairment of an adequate supply is likely to have multiple biological and clinical effects. For this reason it is difficult to identify the biochemical correlates of zinc deficiency. Furthermore, the clinical features of zinc deficiency are, almost without exception, non-specific. In combination, these factors dictate that the unraveling of human zinc deficiency is a challenging process. This task is further complicated by incomplete understanding of whole body human zinc metabolism and how zinc homeostasis is—or is not—maintained under a variety of host and environmental circumstances. At a molecular level, while progress with our understanding of zinc transporters is accelerating, clear understanding of the regulation of zinc metabolism still eludes us. To add to the challenge, there is the long-term frustration of the lack of adequate, sensitive, simple biomarkers of zinc nutriture. While plasma zinc is of some value, homeostatic mechanisms tend to maintain circulating zinc levels within a normal range even when available intake of zinc is sufficiently low to have detectable clinical consequences. Despite all of these difficulties, progress has been achieved and is accelerating at this time. Moreover, we have a much clearer idea today of where we need to go and the tools we need to achieve this than was the case a few years ago.

The first evidence for a biological role for zinc—in microorganisms—came little more than a century ago, long after the role of iron was recognized, and it was less than 40 years ago when the occurrence of human zinc deficiency was first postulated. Subsequent progress in our understanding of the complexities of human zinc deficiency has provided an outstanding example of the value of combining nutrition research in this country with parallel research internationally, especially in developing countries. The mutual benefits derived from this “two pronged” approach have, in the case of zinc, taken on a global significance. The first hypothesis

of human zinc deficiency, in 1960, was related to a syndrome of adolescent dwarfism and delayed sexual maturation in the Mid-East. Regretfully, though quite extensive investigations were undertaken in Egypt and Iran, general acceptance of these findings was slow to materialize. This appears to have been attributable in part to tardy appreciation of the potential widespread significance of these observations and also in part to a paucity of well-designed, randomized intervention trials. This latter shortcoming subsequently limited the value of other early investigations of the putative value of zinc supplements—for example, in wound healing and burns—and continues to be a shortcoming in some patient-oriented research, such as in the evaluation of the possible benefits of zinc in ameliorating the symptoms of the common cold. The value of, indeed the necessity for, well-designed, adequately controlled, randomized, and professionally executed intervention studies is of special importance with this particular micronutrient, given the lack of adequate biomarkers and the lack of specific clinical features of zinc deficiency. Equally important is to work with the smallest possible quantities of zinc in order to help differentiate between correction of a deficiency state and a possible pharmacologic effect. This consideration gains importance as we become progressively more sensitive to the disadvantages of too much as well as too little zinc.

A major advance in our appreciation of the extraordinary importance of zinc for human health (and, indeed, survival) resulted from recognition, in the late 1960s, that the phenotype of the autosomal recessively inherited disorder *acrodermatitis enteropathica* was attributable to severe, relatively acute zinc deficiency. This was followed by the recognition of similar acquired severe zinc deficiency states, most frequently the result of failure to add zinc supplements to "total" parenteral nutrition infusates. Another well-documented cause is the rare failure of lactating mothers to secrete a normal quantity of zinc in their milk despite normal zinc nutritional status. Apart from dramatic benefits to the subjects concerned, recognition of the central role of zinc deficiency in acrodermatitis enteropathica syndromes advanced our understanding of the consequences of human zinc deficiency, most notably, perhaps, on a wide range of immune functions. Given the central roles of zinc in cellular growth and differentiation, it is no surprise that the effects of zinc deficiency are especially pronounced in rapidly turning over tissues and organs of which the immune system is an outstanding example.

For the same reason, it is to be expected that the effects of human zinc deficiency will be most pronounced during periods of rapid growth both pre- and postnatally. Despite extensive documentation of the importance of adequate zinc for embryogenesis, organogenesis, and fetal growth in mammalian models, progress with human research during the reproductive cycle has been both slow and at times contradictory. Nevertheless, there is now an accumulating body of evidence that human maternal zinc deficiency does exist, both in this country and overseas, with deleterious effects on fetal growth, development of the immune system, and of the central nervous system—effects which could be long-lasting.

A series of randomized controlled studies of dietary zinc supplementation in infants and young children in Colorado starting in the early 1970s conclusively demonstrated the occurrence of growth-limiting zinc deficiency in otherwise normal subjects. Food intake was also shown to increase significantly in zinc-supplemented children compared with controls. Similar observations were made in Ontario, Canada, and have recently been extended in school-aged children in Texas.

These Colorado studies contributed a cornerstone for a series of outstanding intervention trials in the 1990s. For these, the setting moves principally to the developing world. These studies have provided definitive information on the effects of zinc supplements in enhancing growth velocity in young children from many countries—an effect which provides clear evidence of a growth-limiting zinc deficiency state in these children. Equally strong evidence has accumulated for a beneficial effect of zinc supplements in preventing and/or ameliorating acute diarrhea. While zinc deficiency does not have a monopoly on the etiology of acute diarrhea, the magnitude of the benefits of zinc supplementation appear similar to those derived from a clean water supply and adequate sewage system. There is also evidence, though not yet so extensive, for a beneficial effect of zinc supplements in preventing persistent diarrhea and in decreasing morbidity and mortality from pneumonia to a remarkable extent. Zinc supplementation may also decrease the risk of *plasmodium falciparum* malaria in young children by as much as 40%. Improvements in brain function are being increasingly documented. This constellation of clinical features, taken together, account for a major proportion of morbidity and mortality in young children in the developing world and the implications of the benefits of zinc supplements—some

definitively documented, others requiring further confirmation—are extraordinary. More than anything else, it is this progress which has given a new sense of purpose and urgency to research directly and indirectly related to human zinc nutriture.

Despite these recent advances, indeed in no small part because of them, there is a challenging research agenda ahead if we are to really come to grips with detecting, managing, and, preferably, preventing human zinc deficiency. Many of the tools are now available, e.g.:

a. Molecular techniques that will allow identification of key zinc transporters and other zinc proteins that are central to the regulation of zinc metabolism and to the biological activity of this metal—perhaps with special emphasis on the gastrointestinal tract and its associated organs that have such a central role in zinc homeostasis and on organs that are of most vital importance for the clinical effects of zinc deficiency, including the immune and central nervous systems.

b. A sophisticated range of subcellular techniques that should allow elucidation of the biological effects of zinc at a subcellular level, including this metal's putative roles as an intracellular control ion.

c. A second generation of zinc stable isotope techniques that will allow us to understand the nuances of zinc homeostasis and whole body zinc metabolism in a range of different environmental and host circumstances. These techniques can make a major contribution to our understanding of why zinc deficiency occurs, what are the limits to adaptation / accommodation to low intakes of bioavailable zinc, and at what sites regulation fails. They can assist in identifying improved indices of zinc nutritional status and in the optimal means of preventing and managing zinc deficiency.

d. Well-honed intervention studies, for which there has been a wealth of experience, accumulated during the 1990s. In addition to second- and third-generation investigations on a global basis, there is a very substantial need for high-quality, interdisciplinary, patient-oriented research in General Clinical Research Centers in this country.

In conclusion, this three-day NIH conference on zinc—sponsored by the Office of Dietary Supplements—is most opportune in its timing, especially if this can be translated into specific

actions to enhance support for high-quality research not only by the NIH but also by the USDA and by public and private agencies concerned with improving micronutrient nutrition in the developing world. Zinc is a prime candidate for attention at the close of this and the beginning of the next millennium.

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Impact of Zinc Status on the Immune System

Pamela Fraker

Background: Nutritional deficiencies are probably the single greatest cause of immunodeficiency states in humankind. It is now well established that a variety of types of nutritional deficiencies have a rapid and deleterious effect on the immune system. Probably the best characterized nutritional-immunological paradigm would be the effects of suboptimal zinc deficiency (ZD) on host defense of rodents, rhesus monkeys and humans (1-3). Though a great deal has been learned to date much more remains to be investigated. There are large deficits in our understanding of how this deficiency affects different branches of the immune system. In addition, clinical information regarding the impact of ZD on immune defense systems are greatly in need of further investigation.

Zinc deficiency is thought to be one of the more frequent nutritional deficiencies in the human population (1). In the USA, suboptimal zinc status has been noted in children of lower socioeconomic groups, low birth weight infants, pregnant teenagers and some of the elderly (1). However, it also accompanies a variety of diseases and disease states where it is evident that it can adversely impact immune status (1-3). Thus, zinc deficiency in the USA probably has its greatest prevalence in the diseases listed below. However, more information is needed to determine the effect of these diseases on host defense.

Chronic Diseases and Disease States Where Suboptimal Zinc Status Alters Immune Defense: Areas In Need of Further Investigation

1. GI disorders, Crohn's disease, chronic diarrhea
2. Renal disease
3. Sickle cell anemia
4. Some cancers
5. AIDS
6. Alcoholism

Impact of Zinc Deficiency on Host Defense of Human Subjects: Research conducted on human subjects who had suboptimal zinc status or a genetic defect in assimilation of zinc such as *Acrodermatitis enteropathica* exhibited lymphopenia and thymic atrophy (1-4). The latter became hallmarks of the deficiency in earlier studies. Reduced levels of serum immunoglobulins, lymphopenia, reduced delayed type hypersensitivity responses, reduced natural killer function, etc., were frequently noted in these subjects (1-4). More importantly, these subjects had increased incidences of secondary infections that provided clear evidence that cell and antibody mediated responses were impaired. As a result, increased incidences of sepsis, pneumonia, colds, flu, *Candida*, etc., were noted (4,5). Indeed, in the case of chronic illnesses where ZD and/or protein calorie malnutrition are a component, one still loses patients to infections rather than the primary disease. Thus, the need for better maintenance of immune defense systems through better supplementation and nutritional management is evident.

Contributions of Animal Models: Mice have been used successfully to expand and delineate more clearly the specific effects of ZD on host defense systems. Our lab and others have found that as little as 30 days of suboptimal intake of zinc reduced cell mediated, delayed type hypersensitive, tumor defense and antibody mediated responses by 30% to 80% in the case of young adult mice and rodents (2,3). This represents a rapid and adverse effect on host defense. It was evident that lymphopenia was significantly altered by ZD since a 22% body weight loss translated into a 50% loss in thymus weight and a 50% loss of lymphocytes in the blood (3,6). More recent work from our lab shows that this 30 day period of ZD depletes the B-cell compartment of the marrow by 40% to 90% (7,8). Thus, the underlying cause of the lymphopenia in zinc deficiency is inhibition of the ability of the marrow to carry out lymphopoiesis and replenish the lymphoid population (7,8). This, in turn, reduces antibody and cell mediated defense. We also have evidence that suboptimal zinc induces apoptosis in developing precursor cells and reduces the rate of proliferation - maturation of T and B-cell is in the marrow (3,7). Thus, animal models provide the opportunity to define the mechanisms of change in immunity created by ZD. ZD rodents challenged with subacute levels of human parasites, nematodes, etc., exhibited high levels of proliferation of the pathogen followed by

deaths (9,10). Clearly the rodent model parallels changes noted in ZD humans thereby providing a highly useful model for these studies. Collectively, the human and animal studies make clear that zinc is absolutely essential to the integrity of the immune system!

Important Branches of Immune Defense in Need of Investigation: Most of the focus of past studies has been on the peripheral immune system. The impact of zinc status on the areas of immune function listed below are virtually unknown. Each represents an important area where zinc may have a significant impact on health status.

Areas of Immune Defense Where Little is Known About the Effects of Zinc Deficiency or Zinc Supplementation: Area of Need:

1. Mucosal immunology
    A. Respiratory Function
2. Viral defense mechanisms
3. Cancer defense mechanisms: rate of remission
4. Acute phase responses
5. Immune senescence
6. Fetal - neonatal immune development
7. Incidence of infection: Rate of remission

Potential Value of Zinc Supplementation: An additional and very important need is to develop more information on the ability of zinc supplementation to prevent or reduce the destruction of the immune system as chronic diseases advance. The questions listed below are just a few that require investigation.

Role of Zinc Supplementation in Preventing Or Repairing Losses in Immune Integrity: Areas of Need:

1. Will aggressive zinc supplementation significantly reduce the destruction of the immune system that occurs in chronic diseases and dietary deficiencies where zinc is suboptimal?

2. Can zinc be used in combination with cytokine and/or drug therapy in clinical settings to bring about even better protection of the immune system?
1. Will zinc supplementation bring about full repair of an immune system altered by zinc deficiency during growth and development or by chronic diseases, or disease states where zinc was suboptimal?
2. How soon will zinc supplementation bring about repair and restoration of immune function and will the time required vary among age groups?

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Zinc and Endocrine Function

Robert J. Cousins

The potential influence of zinc on the endocrine system has been the subject of considerable research and debate. Much of this interest developed from the growth limiting effects of zinc deficiency in animals and humans. Zinc restriction has been shown to limit growth in plants and single cell organisms as well. The dramatic observations by Prasad and coworkers that human zinc deficiency presents as reduced stature for age and hypogonadism in males (1) led to the suggestion that growth hormone (somatotropin) and/or testosterone and other androgenic hormones were involved in this nutritionally produced phenotype. Subsequent studies have not produced a definitive biochemical answer for the etiology of the zinc-related decreased growth.

Zinc nutritional status could influence endocrine function by one or multiple modes of action. These include hormone synthesis, activation and/or degradation, zinc-related conformational changes that effect the hormone or its receptor, and zinc-related changes in DNA-binding transcription factors that regulate transcription of genes or gene families. A great deal of information in this area has been derived from in vitro experiments, thus the integrative aspects of these findings are not clear. For example, there is substantial data to suggest that zinc-dependent membrane metallopeptidases are important in the postsecretory processing of biologically active peptides (2). Examples include endothelin-converting enzymes and neutral endopeptidases which have important substrates associated with cell-specific regulatory pathways. Clearly, a reduction in the dietary zinc supply and/or redistribution of cellular/extracellular zinc that could remove Zn (II) from these enzymes could alter hormone processing and activation. To date no clear relationship has been demonstrated, however, examples exist that could relate to this mode of zinc action. Specifically, the upregulation of some propeptides for peptide hormones in zinc deficiency may be a compensation for the lack of active hormone. The upregulation of uroguanylin and cholecystokinin expression in zinc deficiency may be examples of this effect (3,4). Another example is the activation of peptide hormones or factors by direct zinc binding. The recent demonstration that human endostatin has

a zinc binding site adjacent to the precursor cleavage site suggests a zinc dependency for activation of this antiangiogenic factor (5).

Another equally interesting zinc-hormone relationship is that of the hormonal regulation of zinc metabolism. The latter has been investigated for three decades. It is clear that glucocorticoid hormones and specific cytokines (e.g., interleukins 1 and 6) produce major changes in zinc metabolism which is manifested as tissue specific uptake and transient changes in blood (plasma) zinc concentrations (6,7). The functional significance of the reduction in plasma concentrations, if any, is not known. Nonetheless, these concentrations are maintained within a very constant physiologic level unless the dietary supply is deficient or in great excess. During stresses such as acute infection or trauma, a transient hypozincemia occurs concomitant with zinc uptake into specific tissues including liver, thymus, and bone marrow (8–10). This may provide zinc for protein synthesis, cellular defense, and/or may remove zinc from the plasma to prevent its acquisition by pathogenic microorganisms or to directly remove zinc from sites from factors that are activated by zinc removal or from zinc-metalloenzymes associated with activation/processing.

Strong evidence is gathering from studies of the cell and structural biology of zinc which suggest zinc has effects on endocrine action. Integrative evidence is developing to suggest that zinc may be an important mediator or modifier of the action of some hormones or factors involved in cell regulation.

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Primary and Secondary Zinc Deficiency on Factors Contributing to Abnormal Embryonic and Fetal Development

CL Keen, LA Hanna, MA Jankowski, LM Wiley,GP Daston, JM Rogers and Michael S Clegga

Zinc deficiency during embryonic and fetal development can result in numerous gross structural and biochemical abnormalities. Such a deficiency can arise through a variety of mechanisms, including low maternal zinc intake, disease-induced or drug-induced changes in maternal and conceptus zinc metabolism, or a combination of these. Evidence will be presented that cytokine-induced changes in zinc metabolism may be a common mechanism which contributes to the developmental toxicity associated with a large class of diverse compounds. These issues will be discussed in detail along with the use of *in vitro* preimplantation, peri-implantation, and postimplantation embryo culture models to study the mechanisms underlying zinc deficiency-induced teratogenesis. Current data show that cell cycle kinetics, cell differentiation, and gene expression are affected by zinc deficiency during development. Specifically, inappropriate apoptosis occurs in areas found malformed as a result of zinc deficiency. Likewise, embryos grown in a low zinc environment exhibit reduced cell differentiation which may be associated with changes in expression of genes that are regulated by zinc-finger transcription factors. In addition, cell culture studies suggest that excessive oxidative damage and alterations in cell migration may play a role in zinc deficiency-induced anomalies.

Zinc Metabolism in Disease

Nancy F. Krebs

The processes by which zinc metabolism may be impacted by disease conditions may be broadly considered as situations with increased losses, increased requirements, and redistribution or sequestration. Examples of these will be discussed below.

Increased losses occur especially from the gastrointestinal tract and the kidneys. Diarrhea has been shown to be associated with zinc deficiency, especially severe deficiency, and zinc deficiency can be caused by diarrhea. The pathophysiologic mechanisms are not well characterized in either condition, but may well be multifactorial. For example, zinc deficiency is associated with flattening of villous architecture with loss of absorptive surface along with certain digestive enzymes, reduced crypts, inflammatory infiltration in the intestine, and perturbation of the gut associated immune system. Such changes in the small intestine could clearly be associated with diarrhea, through such processes as impaired absorption and resultant osmotic effects and/or increased susceptibility to infectious agents. Recent findings from molecular probes have suggested zinc deficiency may result in increased intestinal secretions by induction of specific protein. One group of investigators has suggested that interleukin -1(IL-1) stimulated expression of inducible nitric oxide synthase, which can cause tissue damage, is enhanced in zinc deficient animals. Any malabsorption associated with diarrhea, especially if prolonged or recurrent, can also cause zinc deficiency by impaired absorption of dietary zinc, but perhaps more importantly by interference with reabsorption of endogenously secreted zinc in the intestine. Thus the stage is set for a vicious cycle of diarrhea causing zinc deficiency, which can, in turn, cause diarrhea.

It has increasingly been appreciated that without efficient reabsorption of endogenous zinc secreted into the intestine, there is likely to be in effect a “leaching” of zinc from the body, quickly resulting in a deficiency state. In addition to infectious causes of diarrhea, malabsorption from other processes has been linked with excessive zinc losses. One such example is that of pancreatic insufficiency, as occurs in cystic fibrosis. We have found a direct significant correlation between fecal losses of endogenous zinc and the amount of malabsorbed fat. We speculate that the zinc is being bound by unabsorbed fatty acids. Excessive endogenous fecal

zinc losses have also been observed in celiac disease, which is associated with mucosal damage and with generalized malabsorption, including fat malabsorption. In the patients with cystic fibrosis, absorption of exogenous zinc is also impaired, but to a lesser extent than the losses of endogenous zinc.

These observations may be relevant to any disease state associated with chronic diarrhea, such as with intestinal manifestations of AIDS, short bowel syndrome, inflammatory bowel disease (especially regional enteritis), and others. The dramatic benefits of zinc supplementation on both acute and persistent diarrhea in infants and young children, without particular selection for etiologic entities supports the critical role of zinc in normal gastrointestinal function. Whether the effect of supplementation has been mediated by treatment of an underlying deficiency or by a local effect within the gastrointestinal tract is an area of considerable debate. Either "mechanism" could have a net effect of interrupting the cycle of diarrhea, deficiency, diarrhea.

The kidney is involved in zinc homeostasis, but the magnitude of zinc excretion in urine is normally very modest compared to that in the gastrointestinal tract. Hyperzincuria and hypozincemia have been observed, however, in a number of conditions, notably chronic liver disease, diabetes, cancer, and chronic inflammatory conditions. In both adults and children, orthotopic liver transplantation has been associated with correction of the hypozincemia. In children, we have described significant hyperzincuria prior to transplant in those patients with hypozincemia, and rapid correction of both after transplant. Data from patients with diabetes, cancer, and rheumatoid arthritis are limited but also suggest a pattern of hyperzincuria and hypozincemia. For example, measurements N-acetyl-beta-D-glucosaminidase (NAG), an indicator of renal tubular dysfunction, have been significantly and positively correlated with urine zinc excretion. These correlations have also been associated with immune system activation, including cytokine release.

The physiologic explanation for the apparent renal zinc "wasting" is not known. Possibilities include concurrent hypoalbuminemia and altered filtration and reabsorption, although reported significant correlations between plasma zinc and albumin have been relatively weak; reduced hepatic synthesis of other zinc binding proteins, including metallothionein (MT); altered circulating amino acid profile (e.g. increased cystine) which may alter zinc binding and

reabsorption in the renal tubules; and primary alteration in renal transport in response to chronic inflammatory illness. Recent characterization of zinc transporters associated with zinc efflux and expression localized to the kidney may also offer new mechanistic insights.

Zinc's critical role in the immune system has raised much interest in the role of zinc deficiency and altered metabolism in the face of infectious diseases. In response to infection or injury, cytokines such as IL-1 and tumor necrosis factor are secreted primarily by monocytes and activated macrophages; the effect of these mediators is a predictable redistribution of zinc. The acute phase response is characterized by leukocytosis, fever, increased synthesis of certain plasma proteins, increased plasma copper concentrations, and hypoferremia and hypozincemia. IL-1 stimulates secretion of interleukin-6 (IL-6) and glucocorticoids, which stimulate synthesis of hepatic metallothionein (MT), an intracellular metal binding protein, and decreases in plasma zinc concentrations. This has been demonstrated repeatedly and convincingly by measurement of absolute quantities of the protein and by measurement of mRNA levels in response to injection of endotoxin and of IL-1. More recently experiments with knockout mice which cannot produce MT have confirmed the role of MT synthesis in the hypozincemia and increased hepatic zinc in response to endotoxin injection. IL-1 stimulates expression of MT –1 and MT-2 not only in the liver but also in bone marrow and thymus. The induction of MT represents one of the most thoroughly investigated examples of micronutrient responses to the acute phase response which has been directly related to changes in transcription. How (or whether) the synthesis of MT with its binding of zinc then functions to regulate transcription is not clear. The MT may be a vehicle to transfer zinc from cytosol into nuclear DNA transcription/translation processes or it may sequester zinc and maintain low nuclear zinc concentrations, which for some proteins is critical to transcription.

Lymphocytes activated by either cytokines or mitogens take up zinc by multiple mechanisms. The increased cellular zinc is critical to activity of numerous enzymes fundamental to replication and transcription, including thymidine kinase, DNA polymerase, DNA-dependent RNA polymerase, among others. Protein kinases are involved in the signalling pathway underlying zinc-induced cell stimulation. Thus cytokine release alters transcription by impacting *intra*cellular zinc availability. Cytokines also suppress availability of *extra*cellular zinc to microbiostatic concentrations; this may be another function of the sequestration of zinc by MT.

Alternatively, the decline in plasma zinc concentrations during the acute phase response also may serve as an important regulator of leukocyte cytokine production and a modulator of the immune response.

In view of the complexity of the putative roles of zinc in modulation of the immune response, it is not surprising that zinc supplementaion has been associated with both positive and adverse outcomes. Diseases for which there have been reported therapeutic benefits include (in addition to diarrhea) malaria (*Plasmodium falciparum*), lower respiratory infections, and the common cold. For all of these entities, however, results from published trials have been mixed. Furthermore, since zinc may have a synergistic effect with endotoxin on cytokine production, zinc supplementation in gram negative infections could be deleterious. In one recent human study, prevention of the decline in plasma zinc concentration associated with "catheter sepsis" resulted in excessive IL-6 concentrations and in an exaggerated febrile response.

The last 5 years have seen a virtual explosion of interest and understanding of the spectrum of functions of zinc at subcellular levels. In the public health arena, the positive results of zinc supplementation trials on childhood morbidity and mortality in developing countries have likewise been remarkable. In clinical medicine, knowledge about potential therapeutic roles for zinc in improving outcomes is much more rudimentary. It is likely that correcting nutritional zinc deficiency will offer benefit. Pharmacologic doses of zinc may also be beneficial in certain circumstances and harmful in others. Distinguishing each of these scenarios, however, is not always obvious and will require well-controlled clinical trials.

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Dietary Zinc Requirements

Janet C. King

The requirements for dietary zinc are determined by two factors – the endogenous or tissue needs to replace losses and maintain function, and the bioavailability of zinc from the foods consumed (1), In general, as tissue needs increase during growth or lactation, the efficiency of zinc absorption increases offsetting the need to increase dietary zinc intakes (2). The efficiency of zinc absorption is up-regulated in individuals consuming low zinc diets. The amount of dietary zinc required to replace tissue losses in individuals fully adapted to a low zinc diet is considered the minimal or basal zinc requirement.

The physiological requirement for zinc is the amount of zinc that must be absorbed from the diet to replace all sources of zinc loss plus provide any additional need for tissue growth or synthesis. In adult males, the physiological requirement is 1.4 mg zinc/d; it is 1.0 mg/d for females (1). The difference between males and females reflects differences in the amount of lean tissue. The physiological requirement is thought to be about 0.57 μg/basal kcal. Metabolic rate is considered an indirect measure of lean body mass and body surface, both of which are closely related to zinc losses. The zinc requirement per basal kcal has been used to estimate the requirements of infants and children.

Zinc requirements for infants under one year of age are influenced by the type of feed. Infants use the zinc in breast milk more efficiently than that in formula (3). Thus, the maintenance requirement of absorbed zinc is 20 μg/kg for breast-fed infants and 40 μg/kg for those fed formula. An allowance for growth must be added to the maintenance requirement. This is about 180 μg/kg during the first three months of life; it drops to about 25-30 μg/kg during the adolescent growth spurt.

The total amount of zinc retained during pregnancy in the fetus and other pregnancy tissue is about 100 mg (4). The physiological requirement for the three trimesters of pregnancy is estimated from this value. Since about 60% of the additional zinc need is new fetal tissue, the additional need occurs primarily in the last trimester when fetal growth is most rapid. This is about 0.7 mg/d.

The zinc concentration in human milk varies from a high of 2.5 μg/ml in the first month to 0.7 μg/ml after four months (5). Total zinc output in breast milk could amount to 1.4 mg/d. That amount plus the maintenance zinc need of the mother add up to 2.4 mg/d of absorbed zinc. The efficiency of zinc absorption increases during lactation to meet the cost of zinc for lactation (6). Furthermore, another 0.5 mg zinc per day may be released from bone during bone resorption and from uterine muscle during involution early in the postpartum period. There is no evidence that milk zinc output is influenced by typical dietary variations in maternal zinc intake.

The chemical and physical properties of the diet determine the amount of zinc that must be consumed to provide the physiological requirement for absorbed zinc. Zinc absorption can range from as little as 5 percent from cereal-based diets high in phytate to more than 70% from chemical zinc salts consumed with water in the fasting state (7,8). An adult consuming 10 mg zinc/d absorbs about 25% of the dietary zinc from highly available sources. As zinc intake increases, the fractional absorption declines but the total amount of zinc absorbed increases and endogenous losses rise to achieve balance.

The phytate-zinc molar ratio can be used to estimate dietary zinc bioavailability. If the ratio is <5, maximal zinc absorption can be as high as 50%; if the ratio is between 5-15, maximal absorption would be about 30%; if the ratio is > 15, maximal absorption would be about 15%. Diets with phytate/zinc ratios >15 are high in unrefined, unfermented and ungerminated cereal grain. At least 50% of the energy is supplied by high phytate foods; animal protein sources are lacking in the diets (1).

Functional endpoints have not been used to estimate zinc requirements. Currently, the Food and Nutrition Board of the Institute of Medicine are revising the Recommended Dietary Allowances and using functional endpoints for estimating requirements to the extent possible (9). Very few functional indicators of nutrient requirements have been established. An ideal functional endpoint should be related to the nutrient's biological action, should demonstrate a dose response relationship, should provide consistent estimates across studies, should be temporally correct, and should be quantitative and specific for the nutrient under study (10).

Data from studies of human zinc requirements demonstrate the importance of using functional endpoints for establishing zinc requirements. The capacity to adjust endogenous losses to the level of dietary zinc provided is great. Therefore, if requirements are estimated from endogenous losses, the lower the intake, the lower the requirement (2). Zinc function may be impaired with low intakes even though balance is established. Future studies need to identify sensitive markers of zinc function that can be used to estimate dietary requirements.

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Zinc Intake of the U.S. Population: NHANES III, 1988-94

Ronette Briefel, Jacqueline Wright, Jocelyn Kennedy-Stephenson, Karil Bialostosky, Bethene Ervin, and Margaret McDowell

The National Center for Health Statistics (NCHS) conducts periodic national surveys to assess the health and nutritional status of the U.S. civilian, noninstitutionalized population. The most recent survey, the third National Health and Nutrition Examination Survey (NHANES III), was conducted in 1988-94 on a cross-sectional representative sample of the U.S. population ages 2 months and older (1). Children under 6 years, persons ages 60 years and older, blacks, and Mexican Americans were over sampled to produce more reliable statistics for these population groups. The NHANES is unique in that information is collected on dietary intake, vitamin and mineral supplement usage, nutritional biochemistries, and health parameters for the same individuals.

Signs and symptoms of dietary zinc deficiency include loss of appetite, growth retardation, skin changes, and immunologic abnormalities (2). Zinc toxicity due to acute or chronic ingestion of high quantities of zinc supplements can also occur and lead to impaired immune response, hypocupremia, microcytosis, neutropenia, and a decline in high density lipoproteins (HDL) (2). The effects of moderately elevated zinc intakes are difficult to assess and require biochemical and other data for proper interpretation. Although dietary intake alone is insufficient to evaluate nutritional status, estimates of dietary intakes in the population can be evaluated with respect to estimates of nutrient requirements (3). Current estimates of dietary and total zinc intake collected in NHANES III are used to assess zinc intake in the U.S. population and to indicate population groups for which zinc status may be a concern.

Methods

In NHANES III, complete and reliable information on dietary intake and vitamin/mineral supplement usage was available for 28,663 persons ages 2 months and older. Intakes from food and beverages are referred to as dietary zinc intakes. Dietary intake estimates for the population were assessed using a single 24-hr dietary recall per person, and adjusted using a second 24-hr recall collected on a subsample of the population based on the method to

remove within-person variability described in the 1986 National Academy of Sciences (NAS) report on *Nutrient Adequacy* (4,5). Food composition data for the dietary zinc intakes were based on the USDA Survey Nutrient Data Base (6).

Information collected on vitamin/mineral supplement usage included brand name, if known, and frequency and amount of use in the past month. NCHS staff compiled a data base for the content of the supplements. The contribution of zinc from supplements was calculated for each person for all products the person reported over the past month. For each product reported, the zinc content in a single dose was multiplied by the dose and frequency per day reported by the person. Intake was then summed over all products reported. Daily intakes from diet (food and beverages) plus supplements are referred to as total zinc intakes. Since the average requirements and coefficients of variation (CV) for most nutrients are unknown, approximations for mean nutrient requirements are based on assumptions that the 1989 Recommended Dietary Allowance (RDA) approximates the mean requirement plus 2 standard deviations with a CV of 15% (2, 7). Mean zinc requirements were then calculated as 77% of the RDA. Total zinc intakes were compared to 77% of the age-sex specific 1989 RDA value for zinc to determine "adequate" intakes for the population and specific population groups.

Age determination was made at the time of the household interview. Pregnancy status was based on self-reported information or a positive urine test (8). There were 346 pregnant females and 99 lactating females with complete 24-hr recall data. Mean intakes for pregnant and lactating females are reported separately due to different zinc requirements.

Since dietary zinc intakes were skewed, the data were log transformed to normality. Appropriate sample weights and statistical techniques (SAS and SUDAAN) were used to produce population estimates of mean dietary and total zinc intakes for the U.S. population (8).

Results

Mean dietary and total zinc intakes are shown by age and sex in Table 1. Mean daily dietary intakes increased from 5.5 mg in infants to 10-11 mg in adolescents and adults 70 years and younger, and then declined to 9.2 mg in the oldest age group over 70 years. Mean total zinc intakes increased across age groups to approximately 13 mg in adults. Table 2 shows the prevalence of "adequate" zinc intake was 56% overall in the population. The proportion with

"adequate" total zinc intake was highest in infants (96%) and lowest in young children ages 1-3 years (19%). The percent of the population with "adequate" total zinc intakes was lower in females compared with males for all age groups.

Other major findings include:

- Mean dietary intakes are similar for males and females 10 years and younger. For those over the age of 10 years, mean dietary zinc intakes averaged 3-4 mg higher for males compared with values for females of the same age group.

- Mean total zinc intakes were approximately 0.7 mg higher in adolescents and 2.5-3.5 mg higher in adults compared with the mean dietary intake, indicating the average contribution of supplements to total zinc intake.

- Females who were pregnant or lactating had mean dietary zinc intakes which were comparable to their non-pregnant, non-lactating counterparts; however, mean total zinc intakes (~22 mg) were approximately 10 mg higher than those for non-pregnant, non-lactating females.

- In general, mean dietary zinc intakes were significantly higher in non-Hispanic whites (12.6 mg $\pm$ 0.15) compared with non-Hispanic blacks (11.0 mg $\pm$ 0.13) and Mexican Americans (11.1 mg $\pm$ 0.10) of the same age group (crude data unadjusted for age; data not shown).

- Persons at or below 130% of poverty (the Food Stamp Program income eligibility cutoff) had a significantly lower percent of persons with adequate zinc intake (49.0%$\pm$1.04) compared with persons above 130% of poverty (58.1%$\pm$0.75) (data not shown).