

Experimental studies in human volunteers confirm an influence of zinc nutriture on the balance between lymphocyte TH1 elements and functions (cell-mediated immunity) and TH2 elements and functions (humoral immunity).

Is zinc deficiency a widespread endemic public health problem or a rare condition that only accompanies severe gastrointestinal, endocrinologic, or neoplastic diseases? This is currently an important question in public health circles in developing nations.^{1,2} It remains partly unresolved because of the age-old problem of lack of suitable diagnostic markers of zinc status in humans.³ It also confronts a shifting paradigm in nutrition from concern only about nutrient deficiency or excess to the promotion of health and prevention of disease.⁴ Because of the experimental model and outcome variables used, a recent study⁵ conducted in collaborative institutions in the United States is relevant to the problems of zinc in its broadest public health domain.

The goals of a study by Beck et al.⁵ were threefold: (1) to manipulate zinc status prospectively using dietary variation in zinc intake and inhibitors of zinc absorption, (2) to assess zinc status using a battery of laboratory indicators, and (3) to determine the functional consequences in terms of cytokine production from peripheral mononuclear cells and from altering the relative proportions of subpopulations of lymphocytes as determined by flow cytometry and specific antibody segregation.

Beck et al.⁵ studied five young adult, healthy, non-smoking male volunteers in an experimentally induced zinc deficiency protocol. This was an outpatient study with all meals consumed at the metabolic unit. A hospital diet containing the recommended dietary allowance (RDA) of zinc (12 mg) was consumed daily for 4 weeks in what was the baseline period. This was followed by a phase involving a 20- to 24-week period of consuming a zinc-restricted diet containing 2–3.5 mg zinc daily along with 1 g phytic acid in a texturized soy protein semisynthetic diet. All other essential nutrients were provided at RDA levels. This is a relevant model because the presence of inhibitors of zinc absorption (e.g., phytic acid, dietary fiber, tannins [polyphenols], oxalate, etc.) in diets of developing nations is considered to be the cause of any marginal zinc status that may be endemic. Lower intakes would hasten the devel-

opment of deficiency in persons who presumably have normal zinc status. Finally, subjects were given either daily supplemental zinc at 25 mg ($n = 3$) for 12 weeks or 50 mg ($n = 2$) for 8 weeks during the repletion phase. An additional, sixth subject had been entered into the study but was dismissed for noncompliance. Even for a metabolic unit study, the total sample size of five offers little statistical power, except for the most robust effects.

In each of the study's phases (baseline, zinc depletion, zinc repletion), blood was collected and analyzed for plasma zinc and for the respective zinc content of separated lymphocytes, granulocytes, and platelets. Plasma zinc fell from a baseline concentration mean of about 110 $\mu\text{g/dL}$ to about 92 $\mu\text{g/dL}$ with dietary restriction and returned to the original levels with repletion. In parallel fashion, the zinc content of lymphocytes fell by 13% during the depletion phase, only to rebound above original levels with repletion, and the interim declines with granulocytes (–11%) and platelets (–29%) were consistent. The interim concentration in these cellular elements fell below one standard deviation (SD) of the established reference levels⁶ in all volunteers. The authors interpreted this to mean that they had indeed produced a mild zinc deficiency state.

During each of the phases a specific panel of immune assays aimed at characterizing the number of lymphocytes of distinctive subclasses related to humoral and cellular immunity was repeated. The lymphocyte subpopulations were identified using flow cytometric analysis following staining with commercially available fluorochrome mouse monoclonal antibodies to specific cell markers.

The capacity of the volunteers' lymphocytes to produce cytokines was determined by the supernatant of cell cultures of peripheral mononuclear cells that had been separated by Histopaque density gradient and stimulated with lipopolysaccharide (for interleukin-1) and phytohemagglutinin (PHA). The cytokines of interest were the interleukins IL-1 β , IL-2, IL-4, IL-6, and IL-10, γ -interferon, and tumor necrosis factor- α , assayed by commercially available ELISA kits. The variables for humoral immunity surround the TH1 group of lymphocytes. These included the cellular production of γ -interferon, TNF- α , and IL-2. The variables for cell-mediated immunity surround the TH2 group of lymphocytes. These included the production of IL-4, IL-6, and IL-10.

The period of zinc restriction was associated with a decreased cellular production of γ -interferon and TNF- α and with borderline (but not significant) decrease in IL-2. Production of IL-1 β was numerically, but not significantly, elevated. The modulation of zinc status of the hosts had

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no effect on the *in vitro*, PHA-stimulated production of IL-4, IL-6, and IL-10. Better and poorer zinc status had a significant effect on the CD4:CD8 ratio. There was also a suggestion of a downward shift in the ratio of CD4 cells that are precursor “memory” T lymphocytes (CD4+CD45RA+) to those with committed antibody memory (CD4+CD45R0+). Finally, zinc restriction decreased the percentage of CF73+ cells that are precursors to cytotoxic T lymphocytes in the CD8+ subset. As such, the results of this study add nothing radical, unexpected, or new to the basic biology of zinc and immunity. The findings confirm the elements of the *a priori* hypothesis based on the literature. The close parallels in the results in humans to those in laboratory animals, however, adds validity to the findings and to the zinc deficiency model.

The authors of the Beck et al. study⁵ include a member of the team (Dr. Ananda Prasad) that first identified zinc deficiency in a nonclinical, community setting. The authors do not hide the fact that these findings are aimed at this paradigm and at the emerging questions of the magnitude of endemic zinc deficiency.^{1,2} The authors state that their “observation in the Middle East indicated that respiratory tract infections, probably viral in etiology, and parasitic infestations were common in zinc-deficient dwarfs.” However, no competent or rigorous comparative epidemiologic approaches were applied 35 years ago to differentiate between acute respiratory infections and intestinal helminthiasis in zinc deficiency cases versus unaffected controls. Such anecdotal evidence at best sets the basis for prospective hypothesis testing. Great care must be taken, however, in trying to relate the implications of these findings to the real world of community nutrition.

The significance of the fact that cellular elements and hormonal signaling of cell-mediated immunity are diminished by mild zinc deficiency in a reversible fashion could be clinically relevant or irrelevant. Its implications for real-world experience of disease remain a matter of speculation, but certainly rich speculation. Will the changes in function demonstrated on an experimental zinc-restricted diet substantively influence the course of naturally acquired infections, as suggested by the authors? Moreover, because one of the findings relates to a preservation of the CD4:CD8 ratio in zinc sufficiency, and because this balance of lymphocytes is crucial in human immunodeficiency virus (HIV) infection for the transition to overt acquired immune deficiency syndrome (AIDS), efforts to improve the zinc status of populations in areas of high rates of HIV infection might extend the symptom-free life of HIV-seropositive individuals where the cost of case finding and antiviral therapy is currently prohibitive.

A dampened immune response is not always disad-

vantageous. Less robust activation of the acute-phase response may allow for preservation of metabolic functions and growth in the face of microbial contamination. It may also allow for survival of an individual who would have been killed by a normally vigorous outpouring of cytokine mediators. Solomons⁷ has speculated that “we must confront the nutritionally heretical hypothesis that sometimes lower intakes or stores of nutrients are adaptively favorable for the species in the process of evolution.” If this speculation is true, zinc deficiency may be mediating the adaptation of human population to life under adverse environmental and dietary conditions.

Perhaps the firmest result from the present study is additional ammunition for the epidemiologic assessment of zinc status. As early as 1973, Sandstead⁸ suggested that, at times, the only way to determine whether a population is zinc deficient is to administer zinc and observe the evolution of a zinc-dependent function(s). Twenty years of frustration with nonavailability of a valid and reliable index of zinc nutriture at both the individual and the population level³ continue. Hence, although flow-cytometry and cell-culture studies require large amounts of blood and are not routine or cheap, this study provides a series of new “functional indexes” in the immune function domain which could be added to the battery of tests currently used in conjunction with zinc supplementation trials.

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