ABSTRACT  Recent clinical and experimental findings have reinforced the link among zinc deficiency, malnutrition and diarrheal disease. Because there is a strong association between protein and zinc content in virtually all types of foods, insufficient protein intake may often be the cause of zinc deficiency. Compensatory mechanisms operating in monogastric species during malnutrition are less effective for the absorption of transition divalent elements such as zinc, which remain bound to ligands of dietary or endogenous origin. Both protein and zinc deficiencies are strong negative determinants for normal cellular immunity. In zinc deficiency, the organism is more susceptible to toxin-producing bacteria or enteroviral pathogens that activate guanylate and adenylate cyclases, stimulating chloride secretion, producing diarrhea and diminishing absorption of nutrients, thus exacerbating an already compromised mineral status. In addition, zinc deficiency may impair the absorption of water and electrolytes, delaying the termination of normally self-limiting gastrointestinal disease episodes. The gastrointestinal tract may be one of the first target areas where zinc insufficiency may be manifested. A prolonged low zinc intake deprives the organism of the local potential beneficial effects of zinc, including interactions with oxidative free radicals and nitric oxide metabolism. Nitric oxide is a second messenger that plays an important part in the triggering of diarrheal disease. The possible interrelationship among infection, inflammation, free radical damage and its quenching by potential scavengers, such as zinc, in the intestinal lumen or within the enterocyte should be more extensively studied. J. Nutr. 130: 1388S—1392S, 2000.

KEY WORDS:  • zinc deficiency  • malnutrition  • diarrhea  • nitric oxide  • antioxidants

Overview: Interrelationship among malnutrition, diarrhea and zinc status: malnutrition and diarrhea set the stage for zinc deficiency

The close relationship between diarrheal disease and malnutrition has not escaped the attention of the medical community (Gracey 1996). The morbidity and mortality rates due to diarrheal disease are particularly severe in young children. In 1993 there were ~12 million deaths of infants and children < 5 y old, of which one fourth were related to diarrhea. The vast majority occurred in developing countries. Fifty-eight percent, or 1.74 million of the ~3 million premature deaths due to diarrheal diseases have been associated with malnutrition (Fuchs 1998). These statistics suffice to convey the seriousness of the world public health problem. In contrast, since 1968 the United States has experienced a 75% decline in diarrheal disease deaths in children < 5 y old, which now number ~300/y (Kilgore et al. 1995). The interaction of factors linking diarrheal episodes, malnutrition and zinc depletion is diagrammatically presented in Figure 1. Here we do not address immunologic factors associated with zinc deficiency, which is an area that has been extensively reviewed (Shankar and Prasad 1998).

Effects of malnutrition on zinc status. The discovery of zinc deficiency in humans occurred in populations characterized by diets based on staples capable of reducing the bioavailability of metallic elements due to a high concentration of phytate. Another important and often concurrent feature of these diets has been their low protein content. This has been a consistent finding, supported by numerous studies carried out in Asia, Africa and Latin America (Prasad 1998). A typical instance of the link between a low protein intake and zinc deficiency has been documented in Vietnamese children, of whom 50% experience protein energy malnutrition during infancy (Ninh et al. 1996). Protein-energy malnutrition entails a decrease in the immunologic defense mechanisms that leads to greater susceptibility to infections, especially diarrheal disease. Nutritional rehabilitation with a high protein diet is a difficult task that if inappropriately handled can result in catastrophic outcome, because the alterations present in the gastrointestinal tract during severe food restriction can lead to malabsorption, diarrhea, electrolyte depletion and even death.
high zinc concentrations (Rodriguez et al. 1996). In humans, the recovery from protein-energy malnutrition with a high protein diet alone may not be as effective as the same treatment supplemented with zinc (Shrivastava et al. 1993). Important caveats have recently been raised about the amount of supplemental zinc to be provided. Among children admitted to a nutritional rehabilitation unit in Bangladesh, those who received 6 mg/kg of zinc for either 15 or 30 d failed to grow at a greater rate than those who received 1.5 mg/kg for 15 d and had a higher mortality rate than the latter group (Doherty et al. 1998). The cause of this unexpected outcome is yet unclear, although the reasons why high zinc administration may have deleterious effects have been previously examined (Shankar and Prasad 1998). At a minimum, the data suggest that health maintenance and recovery from malnutrition may not be approached with similar dietary prescriptions.

Growth retardation has been one of the most characteristics features of zinc deficiency in children, as it is in protein-energy malnutrition (Prasad 1998). However, zinc supplementation by itself does not necessarily produce the linear growth and weight recuperation needed for catch up (Rosado et al. 1997, Walravens et al. 1989). It is difficult to discriminate between the respective contributions of general nutritional rehabilitation and zinc supplementation during recovery from malnutrition and concomitant zinc deficiency. For example, in a study of children recovering from shigellosis, the effects attributable to a high protein diet could not be separated from the inherent increase in zinc intake. The diet providing additional protein also had more zinc, iron, calcium and vitamin A. While accelerating growth during a follow-up period, it had a marginal effect on the frequency of diarrheal episodes as well as respiratory and febrile illnesses (Kabir et al. 1998). The more rapid recuperation of this type of patients has also been attributed to an improved condition of the intestinal mucosa, which allowed for more effective absorption of nutrients (Kabir et al., 1994).

Many zinc supplementation trials have been carried out since the 1980s. Results have been evaluated in regard to diarrhea duration and, if observations were carried out for longer periods, rate of growth. Data were further assessed by meta-analysis and combined analysis (Black 1998, Brown et al. 1998). The most significant feature is that zinc supplementation has a positive effect on the duration and severity of diarrheal episodes and a lesser effect on height and weight. Children who were short at the beginning of the study benefited from zinc supplementation, whereas those of normal height did not. Similarly, there was an inverse relationship between initial plasma zinc and the benefit accrued by zinc administration.

Severe zinc deficiency is associated with anorexia, which in turn may impede nutritional rehabilitation. The mechanism by which this occurs is unclear. Recent work indicates that the organism may attempt to stimulate food intake by increasing the release of an orexigenic neuropeptide, neuropeptide Y (NPY). It appears as if the number of NPY receptors is not reduced by zinc deficiency. Preservation of hypothalamic NPY receptors has been demonstrated in zinc-depleted rats (Lee et al. 1998). Therefore, other signals may be operating in the observed appetite reduction in humans with zinc deficiency.

Effect of diarrhea on zinc status: contribution of macronutrients. Diarrheal disease in infants <1 y lasting ≥10 d results in abnormally low concentrations of serum zinc, which
inversely correlate with duration of the disease (Naveh et al. 1982). Persistent diarrhea, i.e., a condition lasting >14 d (Black and Sazawal 1998), or repeated episodes of acute gastroenteritis trigger a cascade of deleterious effects discussed later. It appears that a vicious cycle operates between diarrhea and zinc deficiency. Diarrhea generally entails poor absorption of nutrients due to rapid transit in the gut, deterioration of the absorptive mucosa and loss of specific transporters. Diarrhea may produce a secretory state in the small intestine, preventing or reducing net absorption. In addition to the pathophysiologic conditions, nutrient wastage is produced during diarrheal disease of bacterial origin because cellular debris, intestinal flora proliferation and undigested solids may adsorb minerals, including zinc, and reduce their bioavailability. Zinc required in the regeneration of the absorptive mucosa may thus be sequestered or be insufficient, perpetuating the pathology. Conversely, in children with low plasma zinc (8.4 μmol/L: 33rd percentile), an indicator of severe zinc deficiency, there was a greater incidence of severe diarrhea with fever than in those who had higher plasma zinc concentrations (Bahl et al. 1998). In this population, plasma zinc did not correlate with an index of weight for height, suggesting that the incidence of gastrointestinal disease and the degree of zinc depletion were not necessarily linked to a predisposing poor nutritional status.

It has long been known that prolonged diarrheal disease affects the absorption of macronutrients and micronutrients, possibly through damage to the absorptive capacity of the gut (Stern et al. 1980). In laboratory animals, a model of chronic osmotic-secretory diarrhea served to demonstrate a reduction in the absorptive capacity for amino acids (Wapnir et al. 1988) and zinc (Lee and Wapnir 1993). In these studies, the addition of certain long-chain fatty acids in the diets reversed zinc malabsorption. However, an amelioration of zinc status by a higher intake of oils and fats is not realistic for humans in the context of endemic infectious gastroenteritis in populations of tropical countries, where diets are low in fat. Carbohydrates probably are the most accessible macronutrients for most populations. Monosaccharides and easily digestible glucose polymers enhance zinc intestinal absorption. Additional milk intake entails a greater lactose ingestion may produce age-dependent effects on zinc absorption. In animal studies, lactose ingestion appears beneficial early in life but detrimental in mature individuals (Ghishan et al. 1982, Wapnir et al. 1989).

These findings provide a cautionary note for supporting the parsimonious introduction of milk feedings during recovery from diarrhea and treatment of zinc deficiency.

**Mechanisms involved in the triggering of diarrhea episodes: Possible role of zinc deficiency**

Although rotavirus infections prevail in the etiology of diarrheal episodes in most industrialized countries and may account for one third of the cases in reported studies, they are generally self-limiting (Cook et al. 1990). In addition to parasites, such as *Giardia*, *Cryptosporidium* and *Cyclospora*, bacterial infections, particularly those produced by *V. cholerae* and *E. coli*, represent the most threatening public health hazard in the developing countries. *E. coli* may be responsible for ~50% of all worldwide diarrheal episodes. The mechanisms by which cholera toxin (CT) triggers diarrhea include not only the stimulation of adenylate and guanylate cyclases, which evokes chloride secretion, but also another secretagogue, 5-hydroxytryptamine (serotonin) (Bearcroft et al. 1996). Enterotoxigenic *E. coli* strains produce heat-stable and heat-labile enterotoxins, which have substantial homology with CT (Gianella 1995). Activation of the toxin receptors is mediated through phosphorylation by protein kinase C, followed by a cascade of reactions leading to the synthesis of cyclic nucleotides (Crane and Shanks 1996).

Other more specific underlying mechanisms may be involved in the cycle of events regarding zinc and diarrheal disease. Cytokines responsible for induction of acute-phase response, such as interleukin-1 (IL-1), regulate metallothionein mRNA expression (Cousins and Leinart 1988). The injection of IL-1α produced diarrhea in 66% of zinc-deficient rats but in none of the well-fed animals (Cui et al. 1997). This may explain the susceptibility to infectious diarrhea of individuals with a compromised zinc status. Another important contribution has been the finding of an up-regulation of uroguanylin, or guanylate cyclase–activating peptide II, during experimental zinc depletion. Uroguanylin and the structurally similar peptide guanylin are activators of guanylate cyclase C, which leads to the synthesis of cGMP. This cyclic nucleotide in turn regulates the cystic fibrosis transmembrane regulator, as mechanism involved in membrane sodium and chloride balance. Guanylate cyclase C is also one of the targets of one of *E. coli* enterotoxins (Blanchard and Cousins 1997). This subject is discussed more extensively elsewhere in the symposium. Therefore, this potential concatenation of events among zinc depletion, up-regulation of neuropeptides and ultimately secretory conditions in the intestine may explain why zinc deficiency can easily lead to diarrheal episodes.

**Interactions of zinc with free radicals and with nitric oxide as related to intestinal function**

Zinc is an intrinsic constituent of superoxide dismutase, a major scavenger of free radicals, present in the cytoplasm of many types of cells and in the extracellular space. Superoxide dismutase converts the super oxygen free radical $\text{O}_2^-$ to hydrogen peroxide, which is further decomposed by catalase into water and oxygen (Leung 1998). The role of zinc as an antioxidant has been predicated on its ability to be an intramolecular stabilizer, preventing the formation of disulphide bonds and either displacing or competing with cupric or ferric ions, which trigger the formation of free radicals (Bray and Bettrger 1990). However, because the importance of nitric oxide (NO) as a second messenger became increasingly understood in the past several years, new connections among gastrointestinal disease, inflammation, free radicals and tissue damage have entered into consideration regarding the possibility of zinc playing a role in these processes. NO modulates inflammation and can act both as a proinflammatory and anti-inflammatory agent. Proinflammatory cytokines up-regulate inducible NO synthase. This mechanism may be activated, in sepsis and in localized infections. Conversely, a reduction in NO synthesis in endothelia may lead to contraction of endothelial cells, increased vascular protein leakage and inflammation. In the intestine, there also is evidence of the delicate balance between the modulation of NO synthesis and the integrity of the mucosal barrier. Inhibition of NO synthesis may produce a “dysfunctional” epithelial barrier with partial loss of protection against the transcellular passage of potentially deleterious macromolecules, such as antigens, and even bacterial translocation (Alican and Kubes 1996). The role of NO in experimental colitis was also shown to be mediated via proinflammatory cytokines (Southery et al. 1997).

The biphasic outcome of NO synthesis in the gut resulting in proabsorptive or antiabsorptive effects has been shown in vivo by varying the concentration of the NO precursor L-arginine. In the perfused rat jejenum, low concentrations of
l-arginine (1–2 mmol/L) have proabsorptive effects, increasing net water and sodium absorption. At a higher l-arginine concentration (20 mmol/L), a small secretory effect could be measured (Wapnir et al. 1997). These concentration-dependent proabsorptive and antiabsorptive actions of NO have been subsequently confirmed (Schirgi-Degen and Beubler 1998).

It is of greater interest that NO activates the formation of cGMP (Fig. 2). In turn, cGMP activates a cGMP-dependent protein kinase C. On phosphorylation of this enzyme and dephosphorylation of myosin light chain, cell contraction and relaxation of the interepithelial junctions occur with an increase in permeability of the barrier. In addition, protein kinase C acts on transmembrane transporters, resulting in leakage of chloride and, hence, intestinal secretion and diarrhea. A similar phenomenon is produced via the formation of cAMP resulting in secretory conditions (Clancy and Abramson 1995).

Zinc has long been considered to have anti-inflammatory properties. Recently, experiments in zinc-deficient or -sufficient rats injected with lipopolysaccharide to induce inflammation showed that endogenous zinc inhibited lipopolysaccharide- or IL-1β-induced NO formation, as well as reduced the activity of smooth muscle cell NO synthase. This was interpreted as an explanation of the anti-inflammatory activity of zinc (Abou-Mohamed et al. 1998).

In an attempt to investigate whether zinc acts as an NO scavenger, a chemical NO-generating system based on the reduction of nitrite with iodide was exposed to increasing concentrations of zinc chelates with L-histidine and citrate, using the conversion of methemoglobin from oxyhemoglobin as an indicator of NO formation. Concentrations of zinc in the 1–2 mmol/L range reduced ≥50% methemoglobin synthesis, suggesting effective scavenging of NO by soluble zinc complexes (Wapnir et al. unpublished data). It remains to be determined whether these experimental findings are applicable in vivo if zinc were to be used as a pharmacologic agent for scavenging of deleterious free radicals. Zinc as a bishistidine complex has been found to improve postschismic reperfusion injury in rats (Powell et al. 1994) and shown to be effective as a myocardial preservative when added to a cardioplegic solution (Powell et al. 1997). The potential role of soluble organometallic zinc complexes as antioxidants and free radical scavengers has been foretold by Vallee (1995) in his statement, “Nature apparently has availed itself of the redox chemistry of the ligand, not the metal, as a means to control the dynamics of zinc binding.”

**Strategies for redressing the risks of zinc deficiency regarding gastrointestinal disease**

It has become increasingly apparent that several closely linked factors are involved in the often concurrent clinical findings in zinc deficiency, malnutrition and prolonged diarrheal disease. The efforts of many public health workers in developing countries, supported by international agencies such as the World Health Organization, have been directed at decreasing morbidity and mortality rates, especially among infants and children. Numerous field trials directed at obtaining more effective oral rehydration solutions have been steadily providing the clinical basis to reach that goal (Bhan et al. 1995, Santosham et al. 1996). Oral rehydration therapy is probably one of the most cost-effective treatments available for preventing one of the main consequences of protracted diarrhea, i.e., dehydration. How zinc deficiency and the general nutritional status of the populations at greatest risk play into the picture are more complex matters. Questions to be resolved in the future, in this regard, could be viewed in the following sequence of complexity.

1. Is it reasonable to provide zinc supplementation to children at high risk of persistent diarrheal disease? It appears to be very likely that a subpopulation in many developing countries are marginally or definitely zinc deficient and thus at a greater risk of its consequences. Zinc supplementation is attractive as a simple, inexpensive and possibly effective way to cut down on one of the consequences of persistent and repeated bouts of diarrhea, i.e., zinc depletion. Would the inclusion of zinc in oral rehydration solutions be a satisfactory approach? Is it reasonable to argue for an enrichment of zinc with staple foods, possibly together with iron, vitamin A or other critical micronutrients?

2. If the nutritional status of the population at large, especially its younger segments, is improved, then the need for a specific enrichment becomes less pressing. Therefore, is it ethical to make an effort to only supply "cake," in the form of a zinc supplement, when there is insufficient "bread" for the needy? The importance of sufficient high quality protein intake was pointed out in earlier sections. How to achieve it is certainly not an easy task.

3. Ideally, the social and physical environments of the populations at greatest risk determine the conditions and the extent to which malnutrition and disease prevail. Industrialized nations have had 150 y of public health progress, which entailed a decline in endemic and epidemic diseases that are often largely preventable with good sanitation and enlightened social policies. If those responsible for advancement in this front in other parts of the world have the determination and if those who can afford to help contribute to the task, the role of the scientists will be simplified. If this ideal situation develops, the application of physiologic and biochemical new findings will not be clouded by the impossibility of putting them into practice.

**LITERATURE CITED**


