

Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries^{1,2}

Kenneth H Brown

ABSTRACT The development of intervention programs to control zinc deficiency is hampered by the lack of sensitive, specific, low-cost indicators of zinc status. The mean plasma zinc concentration of groups of individuals has been suggested as a possible indicator of a population's zinc status because the plasma zinc concentration seems to predict whether growth will increase in response to zinc supplementation. However, experimental studies in both animal models and adult human volunteers as well as clinical studies of infected and noninfected adults indicate that systemic infections that produce an acute phase response also cause the plasma zinc concentration to fall. Therefore, concerns have been raised about the usefulness of plasma zinc concentration as an indicator of zinc status in populations with high prevalences of infections. By contrast with the aforementioned studies in adults, cross-sectional, community-based surveys of children with and without common infections have not found any association between the presence of infection and plasma zinc concentration, possibly because the severity of those infections observed in children in field settings was less than that of the infections studied in adults. Thus, it appears that the mean plasma zinc concentration may be a useful indicator of population zinc status for children in low-income countries despite the high prevalence of common childhood infections encountered in these settings. *Am J Clin Nutr* 1998;68(suppl):425S–9S.

KEY WORDS Zinc, plasma zinc, infection, nutritional status, nutritional assessment, children, adults, low-income countries

INTRODUCTION

Despite evidence suggestive of possible widespread zinc deficiency in low-income countries, attempts to implement large-scale intervention programs to improve their zinc status have been few (1). A major limiting factor in establishing relevant programs has been the difficulty of reliably identifying individuals and populations with a high likelihood of benefiting from increased intake or absorption of zinc. For assessment techniques to be useful in this context they should be sensitive, specific, non-invasive (from both clinical and sociocultural perspectives), and inexpensive. Moreover, they should not require sophisticated laboratory instruments or highly trained personnel if they are to be applied widely. Although assessment techniques should ideally characterize correctly the zinc status of both individuals and populations, techniques that classify accurately the status of popula-

tions are probably adequate for programmatic purposes even if they misclassify some individuals within the populations.

The full range of techniques available to assess zinc status or the risk of low zinc intake was reviewed previously (2). Of them, the ones that seem most promising for field application are dietary assessment and measurement of plasma zinc concentration. Dietary assessment requires both quantitative estimation of zinc intake and appraisal of the likely absorption of zinc from local mixed diets (3, 4). Because of current uncertainties in the estimates of zinc absorption from different diets, however, further work is needed to validate the use of dietary data to predict zinc status. Thus, despite its limitations, some of which will be described below in more detail, analysis of plasma zinc concentration remains the most practical method currently available to assess the zinc status of populations.

This article will review the use of plasma zinc concentrations to assess zinc status and will discuss some of the factors that may confound the interpretation of this indicator. Background information on the transport of zinc in plasma and general metabolic responses to infection will be reviewed briefly, as will selected technical issues about the collection and analysis of blood specimens for plasma zinc determination. Special attention will be directed to the influence of infection on plasma zinc concentration because of both the high prevalence of infections in many of those populations likely to be zinc deficient and the large body of experimental evidence indicating that infections reduce plasma zinc concentrations. Whether the magnitude of the effect of infection on plasma zinc concentration is sufficient to undermine the use of this tool for population assessment will also be considered. For the sake of simplicity, the words *plasma zinc* will generally be used preferentially in the review unless individual studies specifically indicate that serum specimens were obtained. As indicated below, plasma zinc is generally preferred because of the greater ease of rapid separation from blood cells.

TRANSPORT OF ZINC IN PLASMA

The mean concentration of zinc in human plasma is ≈ 15 $\mu\text{mol/L}$ (≈ 100 $\mu\text{g/dL}$), which is considerably less than in many

¹From the Department of Nutrition and Program in International Nutrition, University of California, Davis.

²Address reprint requests to KH Brown, Department of Nutrition, University of California, Davis, CA 95616. E-mail: khbrown@ucdavis.edu.

other tissues (5, 6). The total amount of zinc circulating in plasma is <0.2% of the total body zinc content. Because the concentration of zinc in tissues such as muscle and liver is \approx 50 times greater than in plasma, small differences in uptake or release of zinc from these peripheral sites can have a profound effect on the plasma zinc concentration. For these reasons, it is not surprising that plasma zinc concentrations do not indicate total-body zinc stores reliably under all circumstances. For example, release of zinc from muscle tissue that is catabolized during starvation can result in transient, seemingly paradoxical, elevations in plasma zinc (7). On the other hand, consumption of either standard meals or glucose alone induces a postprandial reduction in plasma zinc concentration, even though dietary zinc intake and tissue reserves are presumably adequate (8). Nevertheless, when zinc intake of volunteers is severely restricted, the plasma zinc concentrations diminish within a fairly short period of time (9). Thus, despite the difficulties in using plasma zinc as an indicator of zinc status for individual subjects, it can reliably indicate recent low zinc intakes by groups of individuals if the samples are collected under controlled conditions and processed and analyzed with appropriate care.

Zinc is transported in plasma bound to albumin and, to a lesser extent, α_2 -macroglobulin and oligopeptides (10). Factors other than zinc intake that influence plasma zinc concentration are hypoalbuminemia, which influences absorption and transport of zinc (11); infection (12, 13) and other forms of stress, such as organ failure (13); tissue injury imposed by surgery (14) and strenuous physical exercise (15); pregnancy (16); and intestinal diseases that interfere with zinc absorption (6).

ZINC METABOLISM DURING INFECTION

Several experimental studies, both in laboratory animals and adult human volunteers, have consistently found a decline in plasma zinc concentration shortly after the onset of a broad range of febrile infections (13) or administration of bacterial endotoxin (12). These changes are associated with simultaneous hypoferrremia, hypercupremia, and elevations of selected plasma proteins, such as C-reactive protein (CRP), α_1 -antitrypsin, haptoglobin, and α_1 -acid glycoprotein. This predictable set of metabolic reactions to infection or tissue injury is now known as the acute phase response.

More recent studies have found that the acute phase response is mediated by cytokines such as interleukin 1 (IL-1) and tumor necrosis factor α , which are secreted primarily by monocytes and activated macrophages in response to infection or injury (17, 18). IL-1, in turn, stimulates secretion of interleukin 6 (IL-6) and glucocorticoids, both of which activate hepatic synthesis of metallothionein (MT), an intracellular metal-binding protein (19). Radiolabeling studies after IL-1 injection in rats showed increased uptake of zinc by the liver, bone marrow, and thymus and decreased uptake by bone, skin, and intestine compared with control animals (17). Subsequent studies in mice with and without the capacity to express the MT gene found that hepatic zinc concentrations increased and plasma zinc concentrations decreased only in the MT+ animals following injection with endotoxin. These studies confirmed the importance of MT in altering the hepatic uptake of zinc and consequent reduction in plasma zinc during inflammation (20).

Despite the fairly consistent occurrence of transient hypozincemia both during acute, febrile, experimentally induced infec-

tions and after the administration of endotoxin or cytokines, several factors can modify the effect of natural infections on plasma zinc concentrations. For example, there is some evidence from clinical studies that the magnitude of change in plasma zinc concentration is related to the severity and stage of infection (21). Likewise, in an animal model of parasite-induced hypozincemia, the decrement in plasma zinc was related to the level and duration of parasitemia (22). Also, the degree of depression of plasma zinc concentration was related to the dose of endotoxin administered in an experimental model in rats (23) and to the amount of IL-1 injected during a study of the bovine acute phase response (24). Thus, infections may induce hypozincemia only when they are severe enough to produce a clinically detectable cytokine response, and the extent of depression of plasma zinc appears to vary in relation to the magnitude of that response. It is conceivable that the appearance of fever might be a useful marker of whether or not a particular infection is affecting the plasma zinc concentration, although this hypothesis must be subjected to empirical testing.

As indicated above, not only the severity but the stage of infection can influence the magnitude of change in plasma zinc concentration. In a series of studies of experimentally infected human volunteers, Beisel (21) found that the fall in plasma zinc concentrations began during the prodromal phase of both bacterial and viral infections, even before fever or other symptoms were present. In their studies in rats, plasma zinc concentrations returned to normal within 24 h of a single dose of endotoxin.

The nutritional status of the host is another issue that must be considered when interpreting the effect of infection on the plasma zinc concentration. Several studies have shown that the cytokine response to infection is reduced in malnourished experimental animals (25) and in humans with protein-energy malnutrition (25, 26). Thus, infection may exert less of a confounding effect on plasma zinc concentration in malnourished individuals.

INFLUENCE OF INFECTION ON PLASMA ZINC CONCENTRATION IN POPULATION STUDIES

Concentrations of zinc and other micronutrients in blood plasma were compared in infected and noninfected individuals in several cross-sectional, community-based studies in selected developing countries (Table 1). Interpretation of these studies is complicated by the possibility that an association between hypozincemia and infection could be due to an increased susceptibility of zinc-deficient individuals to infection rather than to the metabolic responses to infection. Nevertheless, these studies are of interest in determining whether the 2 conditions are related in free-living populations in low-income settings.

Brown et al (27) measured the concentrations of selected trace elements and CRP in blood samples obtained from 153 Peruvian children 11–19 mo of age who were enrolled in a study of prospective surveillance for infectious diseases. About one-third of the children had clinical evidence of infection (they reported diarrhea, respiratory infection, fever, or anorexia) on the day their blood samples were drawn, and one-fourth had either leukocytosis or elevated serum CRP concentrations. Nearly one-half of the children had either clinical or laboratory evidence of infection. The mean serum zinc concentration of children with clinical signs of infection ($6.9 \pm 2.1 \mu\text{mol/L}$) was not significantly different from that of children without these signs ($7.5 \pm 2.1 \mu\text{mol/L}$). By contrast, the serum zinc concentrations

TABLE 1

Plasma zinc concentration by health status of young children in 3 community-based studies in developing countries¹

Study, country	Number of subjects	Age	Indicator of infection	Plasma zinc concentration	
				Infected	Noninfected
		<i>y</i>		<i>μmol/L</i>	
Brown et al (1993), Peru (27)	153	1.2 ± 0.2	Reported symptoms ²	6.9 ± 2.1	7.5 ± 2.1
Brown et al (1993), Peru (27)	153		Elevated CRP or leukocytosis	6.7 ± 2.4	7.5 ± 2.0 ³
Ruz et al (1995), Guatemala (28)	74	2.6 ± 1.4	Reported symptoms ⁴ or elevated ESR or leukocytosis	14.6 ± 2.9	15.2 ± 1.9
Friis et al (1996), Zimbabwe (29)	313	11.2 ± 2.1	Elevated CRP, neutrophilia, or history of fever or diarrhea during past week	11.9 ± 1.9	11.9 ± 2.5

¹ $\bar{x} \pm SD$. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.²Diarrhea, fever, or anorexia.³After adjusting for age and length-for-age *z* score, these results were NS by health status.⁴Acute respiratory infections, dermal infections, conjunctivitis, viral exanthems, otitis media.

of children with positive laboratory indicators of infection ($6.7 \pm 2.4 \mu\text{mol/L}$) were significantly lower than those of the subjects with normal laboratory results ($7.5 \pm 2.9 \mu\text{mol/L}$, $P < 0.05$). However, when these latter results were adjusted for differences in the ages and length-for-age *z* scores of the 2 subgroups of infected and noninfected children, the differences in their plasma zinc concentrations were no longer statistically significant. Thus, the types of infection generally encountered in this group of nonhospitalized children had, at most, only a marginal effect on their plasma zinc concentrations, possibly because the illnesses identified were not severe enough to perturb zinc metabolism.

Ruz et al (28) examined the plasma zinc concentrations of 74 anemic and stunted, but nonwasted, Guatemalan preschool children in relation to their current health status. About one-third of the children had evidence of infection, which was defined as the presence of clinical signs of acute respiratory infections, dermal infections, conjunctivitis, diarrhea, exanthematous rash, or otitis media on physical examination or elevation of their erythrocyte sedimentation rate or leukocyte count. There were no significant differences in the serum zinc concentrations of children with ($14.6 \pm 2.9 \mu\text{mol/L}$) or without ($15.2 \pm 2.7 \mu\text{mol/L}$) these indicators of infection.

A third study was conducted by Friis et al (29) in 313 rural Zimbabwean primary school children. About 20% of the children had a history of fever or diarrhea within the past week, 16% had elevated serum CRP concentrations or neutrophil counts, and >80% had urine or stool samples positive for schistosomiasis. None of the children tested positively for malaria. The mean plasma zinc concentration for all children was $11.9 \mu\text{mol/L}$ (range: $6.3\text{--}24.3 \mu\text{mol/L}$), and this mean value was identical for children with or without elevated serum CRP or any of the other indicators of infection.

In summary, none of these 3 cross-sectional, community-based studies detected significant relations between the presence of infection and the children's serum or plasma zinc concentrations. The discrepancy between these results and the findings of the experimental trials and clinical studies of adults described above may be due to methodologic differences in the research protocols or to differences in the severity of the infections. For example, the experimental studies analyzed differences within subject, thereby increasing the statistical power of the research design, whereas the community studies monitored each subject only once. Also, the studies of experimentally induced infections

(12) and the clinical studies of hospitalized patients (13) were conducted in adults, whereas the community-based studies were carried out in children. Thus, it is conceivable that the distinct age ranges of the study populations may have also contributed to the different outcomes. Finally, it is likely that on average the children included in the community studies had less severe infections than the adult subjects, thereby reducing the effect of the children's infections on their plasma zinc concentrations. Whatever the explanation, the results of the small number of community studies that are currently available suggest that common, acute infections encountered in community settings do not have a major effect on the mean plasma zinc concentrations of children in low-income countries.

In one community-based study, serum zinc concentrations were measured in lactating women with and without acute infections, including diarrhea ($n = 3$) or febrile infections, such as pneumonia and other respiratory tract infections ($n = 14$); urinary tract infections ($n = 13$); skin infections ($n = 2$); and fever of unknown origin ($n = 1$) (30). The mean serum CRP concentration was elevated in the infected women, but was negative in all of the noninfected control subjects. The mean serum zinc concentration of infected women ($9.3 \pm 0.2 \mu\text{mol/L}$) was significantly lower than that of the control subjects ($11.0 \pm 0.2 \mu\text{mol/L}$, $P = 0.01$). The difference between these results and the preceding reports on children may be due to differences in age and physiologic status or to the type or severity of the infections that were accompanied by fever in all but 3 women.

USE OF PLASMA ZINC CONCENTRATIONS FOR POPULATION ASSESSMENT


Several pieces of evidence suggest that the mean plasma zinc concentrations of groups of individuals provide useful information about their zinc status. First, in a recently completed meta-analysis of the effects of zinc supplementation on children's growth, one of the factors that predicted whether a particular population had a significant growth response to zinc was the baseline mean plasma zinc concentration (31). Studies of populations with lower initial mean plasma zinc concentrations found significantly greater increments in weight and length in response to zinc supplementation. Second, in the same analysis, there were clear and sizeable increases in the mean plasma zinc concentrations after supplementation. Thus, this assessment tool appears to be effective both in predicting whether a population is

likely to have a growth response to zinc supplementation (indicating that they are zinc deficient) and in monitoring intakes of zinc supplements.

TECHNICAL CONSIDERATIONS OF THE MEASUREMENT OF PLASMA ZINC CONCENTRATIONS

For plasma zinc concentrations to be useful indicators of a population's zinc status, care must be taken to avoid technical problems that may interfere with interpretation of the results. Many of these problems, which are related to either blood sampling or specimen processing, were discussed previously (32, 33). As noted above, plasma zinc concentrations vary according to the time of day, proximity of meals, and occurrence of recent exercise or other forms of stress. Ideally, these conditions should be standardized, to the extent possible, in field settings. The magnitude of the postprandial decline in plasma zinc concentration argues for particular attention to this factor. Once the blood sample is obtained, the serum or plasma should be separated as quickly as possible to prevent contamination with zinc released from cells. Interestingly, one study has found that the differences in serum and plasma zinc concentrations measured in the same subjects were relatively small compared with the significant increase in zinc concentrations that occurred with greater duration of time between collection of the blood specimens and separation of the serum or plasma (34). The authors speculated that, because most laboratories wait longer to separate serum than plasma, this might account for previous reports of higher serum than plasma values. Likewise, results from hemolyzed samples should not be considered reliable because of the loss of cellular zinc to serum or plasma. Other issues, such as contamination of specimens with zinc from needles, syringes, anticoagulants, transfer pipettes, rubber stoppers, dirty glassware, and impure reagents were reviewed previously (32).

CONCLUSIONS

In summary, there is clear evidence that severe infections and other forms of stress, particularly when encountered in hospitalized adults or accompanied by fever or other indicators of an acute phase response, produce a drop in plasma zinc concentrations. Nevertheless, infections do not seem to affect the mean plasma zinc concentrations of groups of children examined in community-based studies. Because the mean plasma zinc concentration has been found to be a useful predictor of children's growth response to zinc supplementation, this laboratory test should be considered a potentially useful indicator of the zinc status of populations of children, even in settings where there is a high prevalence of common childhood infections. 

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