

## Zinc Nutrition and HIV Infection

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*The trace element zinc is involved in many important immune processes. A number of immunologic impairments owing to zinc deficiency are also evident in HIV disease, most notably a reduction in the number of circulating T lymphocytes. Observational epidemiologic studies have provided conflicting results on the role of zinc status in HIV disease progression. Randomized, placebo-controlled trials are needed to resolve this controversy. Studies must also address the role of zinc in vertical transmission of HIV from mother to child and its role in reducing the risk of adverse pregnancy outcomes, both of which are of considerable public health importance in developing countries.*

**Key Words:** zinc deficiency, immune processes, T lymphocytes, vertical transmission of HIV

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### Introduction

The AIDS pandemic is estimated to affect approximately 36 million people worldwide.<sup>1</sup> Whereas highly active antiretroviral therapy has considerably facilitated the management of HIV disease where it is available, this costly treatment remains unobtainable in most parts of the world. Even as more affordable antiretroviral drugs are developed and the pharmaceutical industry provides drugs free of charge in certain instances, the improvement of the nutritional status of people living with HIV/AIDS remains an integral part of enhanced clinical care.

Various studies have investigated the role of the trace element zinc in HIV disease progression. Known to be essential for human growth and sexual maturation since the early 1960s, zinc is increasingly recognized for its critical role in immune function.<sup>2</sup> The immunologic ramifications of zinc deficiency are manifold and due to the ubiquitous involvement of zinc in catalytic, struc-

tural, and regulatory processes.<sup>3</sup> Specifically, zinc performs important functions in basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation that are indispensable for immunologic mechanisms.<sup>4</sup> Consequently, zinc deficiency is associated with increased susceptibility to infectious diseases such as diarrhea, pneumonia, and malaria.<sup>5</sup> Zinc deficiency can be acquired, as in the case of decreased dietary intakes or increased requirements of zinc. It may also have genetic origins, as in acrodermatitis enteropathica, a rare disorder caused by the malabsorption of zinc.<sup>6</sup>

The importance of proper zinc nutriture for immunity and its demonstrated benefit in various infectious diseases has motivated investigations of zinc nutrition in HIV disease. This paper reviews the existing epidemiologic evidence on the relation between zinc status and HIV disease; potential mechanisms of action are described and the implications of the existing evidence for management of HIV-infected individuals are discussed.

### Zinc Status and HIV Disease Progression

Descriptive and analytic epidemiologic study designs have been used to examine the role of zinc status in HIV/AIDS. Descriptive studies employing a cross-sectional design have yielded varying results on the prevalence of zinc deficiency in HIV-infected individuals. In those studies, zinc status was evaluated either by measuring dietary intake or by using serum zinc as a biochemical marker. Normal mean levels of serum zinc (as defined by levels  $\geq 10.7 \mu\text{mol/L}$ ) were reported in studies with HIV-infected individuals at asymptomatic and advanced stages of disease, regardless of zinc supplement use.<sup>7,8</sup> Decreased levels of serum zinc were uncommon (overall prevalence of 3%) in a cross-sectional study of HIV-infected patients with and without wasting syndrome who reported no use of micronutrient supplements at the time of assessment.<sup>9</sup> Likewise, decreased levels of plasma zinc were not prevalent in a study of afebrile HIV-infected children with or without growth retardation.<sup>10</sup> By contrast, several other studies showed low plasma zinc values in up to 26% of HIV-infected asymptomatic subjects without clinical evidence of nu-

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tritional deficiencies; low plasma zinc was seen in up to 96% of AIDS/AIDS-related complex patients.<sup>11,12</sup>

Some investigators reported a significant correlation between plasma zinc levels and immune parameters such as CD4+ T counts and HIV-RNA levels when data from HIV-positive subjects were evaluated cross-sectionally.<sup>13,14</sup> Correlations between serum zinc levels and CD4+ T counts were not seen in a group of AIDS patients, yet an increased susceptibility to bacterial infections was noted in subjects with low serum zinc levels.<sup>15</sup>

Using dietary intake as a status assessment method, a study in HIV-infected drug users in Florida showed that mean zinc intakes from food and supplements were 11.15 and 8.98 mg zinc/day for men and women, respectively.<sup>16</sup> The authors reported that the mean intakes of the study participants were significantly lower than the 1989 RDA levels (15 mg zinc/day for men and 12 mg/day for women).<sup>17</sup> Curiously, however, the reported intakes exceed the 2001 Estimated Average Requirement (EAR) levels for zinc (9.4 mg/day for men and 6.8 mg/day for women)<sup>18</sup> and may thus be largely sufficient based on the latest evidence from populations not infected with HIV.

Even though the evidence from cross-sectional studies is mixed, it appears that low blood levels of zinc are a common manifestation in HIV disease and may be correlated with stage of HIV disease and possibly other immune parameters. It is important to note that serum and plasma concentrations of zinc may not be valid indicators of tissue status in HIV disease. Both HIV activity and the presence of opportunistic infections can depress serum and plasma zinc levels even in the presence of adequate tissue levels.<sup>19-21</sup> Furthermore, serum and plasma zinc levels can vary widely as a result of circadian variation, meals, and stress, among other factors.<sup>22</sup> Varying circumstances of biochemical measurements may thus distort results and may limit comparisons of zinc levels between studies.

A limitation of cross-sectional studies is that they cannot ascertain the temporal relation between HIV infection and zinc status. In studies describing a state of zinc deficiency, HIV and opportunistic infections may have led to reductions in nutrient intakes and biochemical zinc parameters rather than the opposite (i.e., zinc deficiency contributing to faster progression of HIV disease).

Analytic epidemiologic studies, such as cohort, case-control, and intervention studies, are better suited to examine the role of zinc in HIV disease progression. In the Multicenter AIDS Cohort Study (MACS) and the San Francisco Men's Health Study (SFMHS), two cohorts of HIV-infected men were followed longitudinally to assess the relationship between dietary intake and the course of

HIV disease.<sup>23-25</sup> In both studies, dietary intake was assessed at baseline by means of a food-frequency questionnaire. In the MACS cohort, intakes of zinc greater than 11.6 mg/day were associated with an increased risk of developing AIDS in a dose-dependent manner and adjustment for several confounding factors did not alter this finding (Table 1).<sup>23</sup> Zinc intakes in excess of 20 mg/day were associated with a statistically significant twofold increased risk for progression to AIDS. In a later publication, the same group of researchers reported that dietary zinc intakes of 14 mg/day or greater were significantly and monotonically associated with poorer survival.<sup>24</sup> Any intake of zinc supplements was also significantly related to poorer survival. No association between increased dietary intakes of zinc (from food and supplements) and time to progression to AIDS was observed in the SFMHS cohort.<sup>25</sup>

Other observational analytic studies used biochemical markers to determine zinc status. A study of HIV-seropositive homosexual men examined the relationship between parameters of HIV disease progression, such as CD4+ T cell counts, and micronutrient deficiencies.<sup>26</sup> The prevalence of low plasma zinc levels rose considerably over the course of the 18-month follow-up period and development of low plasma levels was associated with a nonsignificant decrease in CD4+ T cell counts. Conversely, normalization of plasma zinc levels was associated with a significant increase in CD4+ T cell counts. In a study of 125 HIV-seropositive drug-using men and women, low plasma zinc levels over time were associated with an approximately twofold increase in HIV-related mortality even after accounting for low CD4+ T cell counts.<sup>27</sup> Low plasma zinc levels were not significantly associated with mortality in a final statistical model that included other predictors of survival such as CD4+ T cell counts over time and other nutrient deficiencies. Similarly, low plasma levels of zinc were not predictive of mortality among HIV-infected children.<sup>28</sup> Using a nested case-control design, Graham et al.<sup>19</sup> compared 54 asymptomatic HIV-seropositive people who later progressed to AIDS with 54 HIV-seropositive nonprogressors and 54 HIV-seronegative people. The study participants were part of the MACS cohort described above. The asymptomatic HIV-seropositive group had lower serum zinc levels than the seronegative control group, whereas those who progressed to AIDS had the lowest serum zinc levels of all three groups. In a logistic regression model, low serum zinc levels were a significant predictor for progression to AIDS among the HIV-seropositive people. The study also assessed dietary zinc intakes but did not find a significant difference in the mean zinc intake between HIV-seropositive progressors (24.1 mg/day) and nonprogressors (20.9 mg/day).

Longitudinal studies employing dietary and bio-

**Table 1. Observational Analytic Studies of Zinc Status in Relation to Progression of HIV Disease**

Author	Study Type	Population	Endpoint	Zinc Status Measurement	Relative Risk (95% CI)	Variables Adjusted For:
Tang et al. <sup>23</sup>	Prospective cohort study	281 HIV+ homosexual and bisexual men	Progression to AIDS	Dietary intake: 11.7–14.2 versus <11.7 mg/day 14.2–20.2 versus <11.7 mg/day >20.2 versus 11.7 mg/day	1.49 (0.83–2.68) 1.85 (1.03–3.31) 2.97 (1.59–5.56)	Age, symptoms, CD4+ T cell count, energy intake, use of antiretroviral medications, use of prophylaxis against <i>Pneumocystis carinii</i> , and intake of vitamin A, niacin, and vitamin C
Tang et al. <sup>24</sup>	Prospective cohort study	281 HIV+ homosexual and bisexual men	Mortality	Dietary intake: 14.0–20.0 versus <14 mg/day >20.0 versus <14 mg/day Supplements: yes versus no	1.84 (1.16–2.93) 2.44 (1.51–3.95) 1.49 (1.02–2.18)	Age, symptoms, CD4+ T cell count, energy intake, use of antiretroviral medications, use of prophylaxis against <i>P. carinii</i> , and intake of $\beta$ -carotene and vitamin B <sub>6</sub>
Abrams et al. <sup>25</sup>	Prospective cohort study	296 HIV+ homosexual and bisexual men	Progression to AIDS	100% increase (doubling) in dietary intake	0.85 (0.65–1.11)	Age, smoking, energy intake, symptoms, CD4+ T cell count
Baum et al. <sup>26</sup>	Prospective cohort study	108 HIV+ homosexual men	Change in CD4 cell count	Development of deficiency in plasma zinc* Normalization of plasma zinc <sup>†</sup>	-111 cells/mm <sup>3</sup> , <i>P</i> = 0.09 <sup>‡</sup> +61 cells/mm <sup>3</sup> , <i>P</i> = 0.01 <sup>‡</sup>	AZT use, CD4+ T cell count
Baum et al. <sup>27</sup>	Prospective cohort study	125 HIV+ drug-using men and women	Mortality	Plasma levels: <0.75 $\mu$ g/mL versus $\geq$ 0.75 $\mu$ g/mL Plasma levels: <0.75 $\mu$ g/mL versus $\geq$ 0.75 $\mu$ g/mL	2.91 (1.04–8.18) 1.5 (0.47–4.63)	Baseline CD4+ T cell count <200 mm <sup>3</sup> , time-dependent CD4+ T cell count Baseline CD4+ T cell count <200 mm <sup>3</sup> , time-dependent CD4+ T cell count, and deficiencies in vitamin A, vitamin B <sub>12</sub> , selenium, and prealbumin
Campa et al. <sup>28</sup>	Prospective cohort study	24 HIV+ children	Mortality	Plasma levels: $\leq$ 0.75 $\mu$ g/mL versus >0.75 $\mu$ g/mL	1.16 (0.23–5.74)	Baseline CD4+ T cell count <200 mm <sup>3</sup>
Graham et al. <sup>19</sup>	Nested case-control study	Homosexual men: 54 HIV+ progressors to AIDS 54 HIV+ nonprogressors 54 HIV- individuals	Progression to AIDS	Among HIV-seropositive people: 10 mg increase in dietary intake 20 $\mu$ g/dL increase in serum levels	1.03 (0.85–1.25) 0.30 (0.14–0.66)	Percent CD4+ T cell levels, energy intake, age, toenail zinc/copper levels

\* Decline from  $\geq$ 0.75  $\mu$ g/mL to <0.75  $\mu$ g/mL.

<sup>†</sup> Increase from <0.75  $\mu$ g/mL to  $\geq$ 0.75  $\mu$ g/mL.

<sup>‡</sup> Association was measured as change in CD4 cell count between baseline and follow-up times.

CI = confidence interval, AZT = zidovudine.

chemical zinc status assessment techniques provide valuable information on the role of zinc in HIV disease progression. However, several caveats exist for their interpretability. Cohort and case-control studies are prone to reverse causality if HIV infection has resulted in

altered dietary intakes and plasma levels, in which case it will be impossible to determine if low zinc status is a cause of HIV disease progression or simply a marker of it. By contrast, if dietary intake is assessed in an asymptomatic state or when HIV status was not known, studies

**Table 2. Zinc Supplementation Trials and HIV Disease Progression\***

Author	Study Population	Treatment†	Main Findings
Libanore et al. <sup>31</sup>	Intervention: 1 HIV+ infant with AIDS	2 mg Zn/day intravenously for 3 weeks	CD4+ T cell percent: +21%; CD4+/CD8+ T cell ratio: +0.46
	1 HIV+ drug user with AIDS-related complex	10 mg Zn/day intravenously for 3 weeks	CD4+ T cell percent: +12%; CD4+/CD8+ T cell ratio: +0.39
	1 HIV+ drug user with lymphadenopathy	10 mg Zn/day intravenously for 3 weeks	CD4+ T cell percent: +9%; CD4+/CD8+ T cell ratio: +0.97
Mathe et al. <sup>32</sup>	Intervention: 42 patients with AIDS-related complex or malignancy in remission‡	36 mg Zn/day orally for 3 weeks	↑ CD8+ T cell counts in subjects with low initial counts ( $P = 0.01$ ) <sup>§</sup> ↓ CD8+ T cell counts in subjects with high initial counts ( $P = 0.0228$ ) <sup>§</sup>
Zazzo et al. <sup>33</sup>	Intervention: 5 HIV+ subjects with AIDS-related complex	19 mg Zn/day orally for 15 days, then 13 mg Zn/day orally for 8 days	After 21 days of zinc treatment: CD4+ T cell counts (cells/mm <sup>3</sup> ): +27.7 <sup>  </sup> CD4+/CD8+ T cell ratio: -0.01 <sup>  </sup> Chemiluminescence index: +246.7, $P < 0.01$ Human leukocyte antigen-DR cells (cells/mm <sup>3</sup> ): +272.8 <sup>  , #</sup> Lymphocyte transformation index [concanavalin A (10 µg/mL)]: +28.5, $P < 0.01$
Cabotin et al. <sup>34</sup>	Intervention: 7 HIV+ asymptomatic homosexual males	91 mg Zn/day orally for 3 to 12 months	CD4+ T cell counts (cells/mm <sup>3</sup> ): +152, $P =$ not significant <sup>  </sup> CD8+ T cell counts (cells/mm <sup>3</sup> ): +157, $P =$ not significant <sup>  </sup> Serum p24 levels: unmodified (4 positive, 3 negative) <sup>  </sup>
Isa et al. <sup>35</sup>	Intervention: 11 HIV+ drug-using males with AIDS	1 mg Zn kg <sup>-1</sup> day <sup>-1</sup> orally for 10 weeks	CD4+ T cell counts (cells/mm <sup>3</sup> ): +110, $P < 0.05$ CD4+/CD8+ T cell ratio: +0.04, $P =$ not significant <sup>  </sup> Body weight (kg): +7 kg
Reich and Church <sup>36</sup>	Intervention: 13 HIV+ children (1 asymptomatic, 7 with AIDS-related complex, and 5 with AIDS)	1.8–2.2 mg Zn kg <sup>-1</sup> day <sup>-1</sup> orally for 3–4 weeks	CD4+ T cell counts (cells/mm <sup>3</sup> ): -76, $P =$ not significant <sup>  </sup> Serum p24 levels (pg/mL): +63, $P =$ not significant <sup>  </sup>
Mocchegiani et al. <sup>37</sup>	Intervention: 17 HIV+ subjects in CDC Stage** III	45.5 mg Zn orally for 1 month plus AZT	CD4+ T cell counts (cells/mm <sup>3</sup> ): <sup>††</sup> +49, $P < 0.05$ Body weight (Δ%): <sup>††, ‡‡</sup> 1.54, $P < 0.01$ Number of infections per person: <sup>§§</sup> 1.0 <sup>  </sup>
	Controls: 18 HIV+ subjects in CDC Stage III	AZT	CD4+ T cell counts (cells/mm <sup>3</sup> ): <sup>††</sup> -111, $P < 0.05$ Body weight (Δ%): <sup>††, ‡‡</sup> -2.80, $P < 0.01$ Number of infections per person: <sup>§§</sup> 2.17

**Table 2. Zinc Supplementation Trials and HIV Disease Progression\* (Cont'd)**

Author	Study Population	Treatment <sup>†</sup>	Main Findings
Mocchegiani et al. <sup>37</sup>	Intervention: 12 HIV+ subjects in CDC Stage IV C1	45.5 mg Zn orally for 1 month plus AZT	CD4+ T cell counts (cells/mm <sup>3</sup> ): <sup>††</sup> +40, <i>P</i> < 0.01 Body weight (Δ%): <sup>††,§§</sup> +0.04, <i>P</i> = not significant <sup>  </sup> Number of infections per person: <sup>§§</sup> 1.18 <sup>   </sup>
	Control subjects: 10 HIV+ subjects in CDC Stage IV C1	AZT	CD4+ T cell counts (cells/mm <sup>3</sup> ): <sup>††</sup> -30, <i>P</i> < 0.05 Body weight (Δ%): <sup>††,‡‡</sup> -3.30, <i>P</i> < 0.01 Number of infections per person: <sup>§§</sup> 2.5

\* Findings are based on comparisons before and after the intervention unless otherwise noted.

<sup>†</sup> All doses expressed as amounts of elemental zinc.

<sup>‡</sup> HIV infection was confirmed in three patients only.

<sup>§</sup> No estimates of cell counts provided in original paper.

<sup>||</sup> No *P* value provided.

<sup>#</sup> Human leukocyte antigen-DR is a major histocompatibility complex class II antigen and an indicator of in vivo T-cell activation.

<sup>\*\*</sup> According to the 1987 CDC case definition, HIV-positive subjects with persistent generalized lymphadenopathy are classified in CDC Stage III; subjects with at least one of 12 specified secondary infectious diseases listed in the surveillance definition of AIDS are classified in CDC Stage IV C1.<sup>39</sup>

<sup>††</sup> Evaluated 120 days after the beginning of treatment.

<sup>‡‡</sup> Calculated as individual variance (Δ%) of the body weight.

<sup>§§</sup> Evaluated over a 24-month period after the beginning of treatment.

<sup>|||</sup> *P* < 0.01 as compared with respective control group.

AZT = zidovudine.

may only be at limited risk for reverse causality. In addition, the cohort studies described excluded early AIDS cases from their analyses and thus HIV disease is unlikely to have affected zinc intake substantially in the study subjects. All cohort and case-control studies discussed were carried out among prevalent cohorts of seropositive persons at baseline. Therefore, the duration of infection was not known and could not be adjusted for in the analyses. Different lengths of follow-up time among zinc-deficient and zinc-sufficient groups could bias the association with disease progression. Seroincidence cohorts, as opposed to seroprevalence cohorts, would be able to assess these associations more reliably. Onset confounding also provides a threat to studies that use prevalent cohorts. This type of confounding occurs when the unknown calendar date of infection is associated with both the risk of disease progression and the exposure variable of interest.<sup>29</sup> Factors related to both risk of infection and disease progression, such as sexual behavior or drug abuse, cannot be adequately adjusted for in prevalent cohorts. In the studies discussed, CD4+ T cell counts were adjusted for in an attempt to account for different stages of disease progression. Although CD4+ T cell count is considered a sensitive surrogate marker of HIV disease progression, residual confounding may persist because the CD4+ T cell count alone may

not completely reflect the clinical status of an HIV-infected individual.<sup>30</sup> Finally, caution must be taken when generalizing the findings from the observational analytic studies to women and individuals in developing countries because most studies have been conducted in male subjects living in the United States.

Intervention trials, most preferably in the form of randomized-controlled trials, are best suited to examine the causal relationship between zinc status and HIV-related outcomes. However, information from intervention trials is scarce at present. In a series of case reports from Italy, intravenous zinc administration resulted in increases in CD4+ T cells and a normalization of CD4+/CD8+ T cell ratios (Table 2).<sup>31</sup> A better clinical picture of these patients paralleled the improvements in these immune parameters. Mathe et al.<sup>32</sup> supplemented 42 patients with cancer in remission or with AIDS with a daily dose of 36 mg zinc for 3 weeks and observed no significant change in CD4+ T cells. By contrast, CD8+ T cells increased in patients with low starting levels of these cells but decreased in patients with high starting levels of these cells. The significance of these changes in CD8+ T cells is unclear because their precise role in HIV infection remains controversial.<sup>38</sup> In addition, only three out of the 42 study participants had confirmed HIV-related symptoms, which limits the relevance of the

findings for HIV-infected groups. An increase in the neutrophil oxidative burst, which is part of the phagocytic defense function, as well as improvements in lymphocyte activation were signs of immune reconstitution in a small trial of zinc conducted by Zazzo et al.<sup>33</sup> The immune-enhancing effect of zinc appeared to be dose dependent but did not induce changes in CD4+ or CD8+ T cell levels. In a study with seven asymptomatic HIV-infected homosexual males, a high daily dose of 91 mg zinc for 3 to 12 months increased mean CD4+ and CD8+ T cell levels, albeit not significantly.<sup>34</sup> Owing to the high dose of zinc given, the authors were cognizant of potential detrimental consequences of the zinc treatment but did not note any effects on antigenic responses and viral levels as determined by p24 antigenemia. A trial with 11 drug users with AIDS reported a significant progressive weight gain and small elevations in CD4+ T cell counts after 10 weeks of zinc supplementation.<sup>35</sup> These findings were not replicated by Reich and Church<sup>36</sup> in a cohort of HIV-infected children because improvements in clinical parameters were largely absent after 3 to 4 weeks of zinc treatment. In the largest HIV and zinc trial to date, Mocchegiani et al.<sup>37</sup> investigated the effect of 45.5 mg of zinc/day for 1 month as an adjunct to zidovudine (AZT) in 57 subjects who were either at risk of developing AIDS or who had already developed AIDS.<sup>39</sup> The researchers demonstrated a significant benefit of zinc supplements on body weight and active thymulin levels. Furthermore, the supplemented group showed increases in CD4+ T cell counts and a reduced number of opportunistic infections compared with the control group. These findings are in agreement with findings of an earlier study on immune function and zinc status in AZT-treated HIV-positive subjects.<sup>40</sup> Following AZT treatment, individuals with adequate plasma zinc levels showed an increase in immune function as evidenced by a significant increase in the lymphocyte response to mitogens, whereas those with low plasma zinc levels did not.

Supplementation trials have the potential to provide a level of assurance about the role of zinc in HIV disease progression that is difficult to achieve with any observational design. The evidence from these supplementation trials suggests that zinc status can be improved because all studies that assessed biochemical indicators of zinc nutriture found them to be elevated after zinc treatment.<sup>31,33-37</sup> None of the studies reported adverse effects of the zinc supplements, yet sensitive indicators of zinc toxicity, such as measures of copper status, were not employed.<sup>41</sup> The clinical outcomes reported in these studies remain complex and did not always parallel improvements in zinc status. This may be due to the numerous interactions of zinc with HIV and immunologic proficiency of the host in addition to various dos-

ages and durations of the study designs. Nevertheless, the potential for zinc supplements to improve immunologic and anthropometric indicators is promising and warrants investigation in larger, placebo-controlled trials of longer duration.

Additional beneficial effects of zinc supplementation may lie in the treatment and prophylaxis of various AIDS-related opportunistic infections that have so far been largely unexamined. Respiratory infections account for a large number of the approximately two million deaths that occur each year in association with HIV disease.<sup>42</sup> Zinc supplementation trials in HIV-untreated children around the world demonstrated decreased incidences of upper and lower respiratory infections.<sup>43-45</sup> Diarrhea occurs frequently among persons with AIDS, possibly owing to impaired intestinal mucosal immunity.<sup>46</sup> A pooled analysis of randomized controlled trials in children in developing countries whose HIV status was not known demonstrated a significant benefit of zinc on diarrheal incidence and prevalence.<sup>47</sup> Concurrent malaria infection places HIV-infected individuals at double jeopardy and zinc supplementation may reduce this danger through its preventive effects on malarial diseases.<sup>5,48</sup>

### **Zinc, Pregnancy Outcomes, and Transmission of HIV**

Adverse pregnancy outcomes, such as fetal loss, low birth weight, preterm birth, and intrauterine growth retardation, are major problems for HIV-infected women.<sup>49,50</sup> Importantly, women infected with HIV can also transmit the virus to their children through transplacental, intrapartum, or breastfeeding routes. Research on the role of zinc in pregnancy outcomes is warranted because proper zinc nutriture may be important for optimal pregnancy outcomes both in HIV-infected and noninfected populations. For example, zinc deficiency has been associated with low birth weight, intrauterine growth retardation, preterm delivery, premature rupture of membranes, and the need for assisted or operative delivery.<sup>51</sup> Whereas most of this evidence stems from observational studies, several supplementation trials are reported in the literature. Zinc supplements were associated with a significant increase in birth weight among low-income women with low plasma zinc levels who participated in a placebo-controlled trial in the southeastern United States.<sup>52</sup> In the same geographic area, improved fetal growth was reported as a result of zinc supplementation from a study among pregnant adolescents who were presumed to be at risk of zinc deficiency.<sup>53</sup> In the United Kingdom, zinc supplements during the last trimester of pregnancy reduced the incidence of intrauterine growth retardation among pregnant women at risk of delivering small babies.<sup>54</sup> By contrast, several studies reported no beneficial effect of zinc supplements on pregnancy out-

**Table 3. Potential Mechanisms for the Relationship between Zinc Deficiency and HIV-related Outcomes**

<u>Immunologic Factors</u>
↓ Thymulin activity
↑ Apoptosis of CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells
↑ Apoptosis of CD4 <sup>+</sup> T cells
T <sub>h</sub> 1/T <sub>h</sub> 2 imbalance
↓ Activity of natural killer cells
↓ Differentiation/activation of CD8 <sup>+</sup> T cells
<u>Virologic Factors</u>
↑ HIV-1 transcription
↑ Production of viral capsid and core proteins
↑ Activation of nuclear factor κB
↓ Antiviral activity of zidovudine
<u>Clinical Factors</u>
↑ Occurrence of opportunistic infections
<u>Mother-to-Child Transmission</u>
↑ HIV disease progression
↑ Risk of premature rupture of the membranes
↓ Fetal membrane stability
↑ Risk of prematurity
↑ Risk of low birth weight
↓ Mucosal immunity/integrity of breastfeeding child

comes. A trial from Peru showed no significant treatment effect of zinc on infant size or gestational age at birth.<sup>55</sup> Likewise, zinc supplements did not improve birth outcomes among the Bangladeshi urban poor.<sup>56</sup>

Evidence on the role of zinc in maternal and fetal outcomes in HIV-infected women is limited to one study from Haiti.<sup>57</sup> HIV-seronegative and -seropositive infants born to HIV-infected mothers had impaired growth, lower CD4<sup>+</sup> T cell counts, and lower serum zinc levels than children born to HIV-negative women. Although statistically nonsignificant (possibly owing to small sample size), there was evidence that HIV-infected women who transmitted the virus to their children had lower serum zinc levels than nontransmitters.

### Potential Mechanisms of Action

Immunologic dysfunction during HIV infection is associated with the loss of critically important CD4<sup>+</sup> T cells (Table 3). There is evidence that zinc deficiency can accelerate this process via its effect on the hormone thymulin. CD4<sup>+</sup> T cells are dependent on thymulin for proper differentiation and maturation and zinc in turn is indispensable for the action of this hormone.<sup>58</sup> Zinc deficiency may account for the thymic defect in thymulin production that is commonly observed in advanced stages of HIV.<sup>59</sup> Zinc may also prevent the programmed cell death (apoptosis) of precursor T cell populations and mature CD4<sup>+</sup> T cells through various enzymatic mechanisms and through chronic production of glucocorticoids.<sup>60</sup>

In response to antigenic activation, CD4<sup>+</sup> T cells can differentiate into subsets of effector cells that are distinguished most clearly on the basis of the cytokines they produce. Th1 cells secrete interleukin-2 (IL-2) and interferon-γ (IFN-γ), which activate macrophages and are involved in delayed-type hypersensitivity responses. Th2 cells secrete IL-4, IL-5, IL-10, and IL-13, which are responsible for antibody responses and inhibition of several macrophage functions.<sup>61</sup> Progression from asymptomatic HIV infection to AIDS is characterized by a loss of IL-2 and INF-γ production from Th1 cells, paralleled by increases in IL-4 and IL-10 from Th2 cells.<sup>62</sup> Low levels of IL-2 caused by a Th1/Th2 imbalance may also decrease the activity of natural killer cells that are important in nonspecific killing of tumor cells and the differentiation and activation of CD8<sup>+</sup> T cells.<sup>63</sup> Based on these observations, it has been proposed that an imbalance in the Th1-type and Th2-type responses contributes to the immune dysregulation associated with HIV infection.<sup>62</sup> Prasad's group<sup>64,65</sup> demonstrated a Th1/Th2 imbalance in HIV-negative volunteers whose zinc intake was depressed under experimental conditions. The imbalance was corrected by zinc repletion and it is therefore possible that an adequate zinc status will delay clinical and immunologic symptoms that constitute the disease progression to AIDS.

Mechanisms that control the *in vitro* replication of HIV may be zinc dependent. Zinc may have strong anti-HIV activity through inhibition of HIV-1 RNA transcription.<sup>66</sup> At high concentrations, zinc inhibits the activity of HIV-1 protease, which is the virus' own protein-splitting enzyme needed for the creation of new infectious particles.<sup>67</sup> Gag precursors and the zinc finger protein KOX-1 are other molecules that inactivate HIV-1 replication *in vitro*.<sup>68</sup>

In the presence of a weakened antioxidant defense system, reactive oxygen species escape neutralization and can stimulate the secretion of abnormally high levels of the inflammatory cytokines tumor necrosis factor α (TNF-α), IL-1, and IL-6 that have been implicated in AIDS-related cachexia and wasting.<sup>20,69</sup> Reactive oxygen species can induce HIV-1 viral replication by activating the HIV-1 transcription factor nuclear factor κB either directly or by inducing of high levels of TNF-α.<sup>70,71</sup> Zinc regulates the expression of metallothionein and metallothionein-like proteins with documented antioxidant properties.<sup>72,73</sup> Zinc also protects several enzyme sulfhydryls from oxidation by reactive oxygen species and limits the production of free radicals by transition metals.<sup>74,75</sup> As a structural component of the enzyme Cu-Zn superoxide dismutase (Cu-Zn SOD), zinc contributes to a reduction of HIV-1 replication in TNF-α-activated cell lines.<sup>76</sup> These mechanistic considerations corroborate the importance of good zinc nutrient

status. However, there is little correlation between zinc status and Cu-Zn SOD activity and pharmacologic levels of zinc are needed to increase its antioxidant function by increasing the zinc-metallothionein pool in certain tissues.<sup>75</sup> It is therefore unknown whether zinc supplements can lead to a reduction in oxidative damage and decreased HIV-1 replication rate via the described mechanisms.

As stated earlier, Tang et al.<sup>23,24</sup> observed a significantly positive relationship between dietary zinc intake and HIV disease progression. This association may be due to the fact that HIV is a zinc-dependent retrovirus and that heightened availability of zinc may facilitate HIV replication. Zinc stimulates the activity of the viral enzyme integrase, which integrates viral DNA into host DNA.<sup>77</sup> Likewise, zinc is essential for the HIV-nucleocapsid protein p7 and the HIV-Tat protein that are involved in HIV replication.<sup>78,79</sup> An alternative explanation for findings by Tang et al.<sup>23,24</sup> is the immunosuppressive effect of zinc at high doses, which could have facilitated faster HIV progression.<sup>80,81</sup>

With regard to vertical transmission of HIV from mother to child, zinc deficiency may have detrimental effects on important predictors of vertical transmission, such as the clinical, immunologic, or viral stage of HIV disease among pregnant women. Zinc may influence the maintenance of fetal membrane integrity, which could lower a woman's risk of transmitting the virus to the fetus.<sup>82,83</sup> Maternal zinc deficiency is a risk factor for premature rupture of the membranes, which has been associated with higher risk of perinatal HIV transmission, probably related to increased duration of fetal exposure to infected cervicovaginal secretions.<sup>84,85</sup> By contributing to intestinal mucosal integrity and natural immunity, adequate zinc status may protect the breast-feeding child from contracting the virus from the mother.<sup>4,86</sup> Lastly, zinc may prevent transmission by decreasing the risk for prematurity and low birth weight, which are intermediate risk factors for HIV transmission.

## Comment

Zinc deficiency is highly prevalent in the developing world, especially among children and premenopausal women.<sup>87</sup> For example, a survey of schoolchildren from a northeastern province in Thailand found a 70% prevalence of low serum zinc.<sup>88</sup> In Malawi, between 60 and 90% of pregnant women are thought to be at risk of inadequate intakes of bioavailable zinc.<sup>89</sup> The majority of people infected with HIV reside in parts of the world where zinc deficiency is particularly prevalent and it is likely that HIV infection further precipitates zinc deficiency in those areas by adversely affecting the intake, absorption, and metabolism of zinc-containing foods. Because antiretroviral therapy is not commonly available

in these regions, the beneficial effects of improving zinc nutrition in both HIV-infected and noninfected populations may be immense and must be evaluated.

Evidence from industrialized countries suggests that zinc deficiency is likely to occur in HIV-infected populations and is more pronounced at advanced stages of HIV disease. It is of interest that the antiretroviral drug AZT may induce decreases in plasma zinc levels, which may limit the effectiveness of AZT treatment because the intracellular AZT mechanism is zinc dependent.<sup>90</sup> Newer highly active antiretroviral drugs such as HIV protease inhibitors, on the other hand, have been shown to increase plasma zinc levels and such increases in zinc levels may contribute to the effectiveness of these drugs in the prevention of recidivistic opportunistic infections.<sup>59</sup>

Based on the existing evidence, it is difficult to find consensus and establish causality between zinc nutrition and adverse HIV-related outcomes; findings have ranged from detrimental effects of zinc intakes above the RDA to no effect to potential beneficial effects. A considerable portion of the available information has come from the study of small numbers of patients, often investigated cross-sectionally, and sometimes when they have advanced and complicated end-stage disease. Several large observational studies have been conducted but are limited by many HIV-related confounding factors that are difficult to control for. In addition, low serum zinc levels in AIDS patients may not be indicative of zinc deficiency, but may rather be markers of disease progression.<sup>19</sup> At present, intervention studies are few with only one of them including HIV-positive controls and none of them being placebo-controlled.

In view of the role played by zinc in the immune response, zinc supplements may be essential in the management of HIV-infected individuals. However, sound scientific evidence is still not available to support a beneficial effect of zinc therapy in HIV infection and to formulate it into a treatment approach. Randomized, controlled trials are needed to examine the role of zinc nutrition in HIV disease. Such trials should also attempt to determine optimal doses of zinc supplementation because quantities beyond a certain threshold may impair immune function.<sup>80,81</sup>

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